The Effects of Oxytocin on Appetite in Humans

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Abstract

The nine-amino acid peptide oxytocin is synthesised mainly in the hypothalamus and secreted directly into the brain but also released via the posterior pituitary gland to regulate a range of physiologic and metabolic eating processes. Previous research suggests that oxytocin is an anorectic with selective effects on sweet-tasting food. The influence of oxytocin on palatable food intake not initiated by deprivation-induced hunger has not yet been investigated. There is evidence that oxytocin modulates attention from food to social stimuli in females with anorexia but, to date, this has not been researched in people without eating disorders. This thesis used the bogus taste-test paradigm but employed a more rigorously disguised experimental protocol than previous research in the field to examine palatable food intake in men and women individually. Using a similar protocol, it also tested the anorectic effects of oxytocin on group eating. To assess how oxytocin modulates attention in healthy adults not reporting disordered eating, a dot-probe test with food and neutral pictures was carried out. The dot probe also assessed the effects of oxytocin on romantic and social images as these domains are also influenced by oxytocin. The thesis results reported that in the male-only experiment showed that intranasal administration of 24 IU of oxytocin increased sweetness ratings for sweet food and attenuated both sweet and salty snack consumption, 15 minutes after lunch, with a particularly large reduction on sweet food of 63%. In fed females, 24 IU of intranasal oxytocin inhibited only sweet-snack intake but with a similarly large effect of 57%, but had no effect on taste ratings or cortisol, suggesting that reductions in snack eating may not be mediated by cortisol. In the group experiment, 24 IU of intranasal oxytocin facilitated increased self-reported sociality and social modelling of food intake, but there was no overall anorectic effect of the peptide on eating. In the dot probe task, an attentional bias to food was demonstrated and this was reduced by oxytocin. However, oxytocin did not affect reaction times to romantic or social stimuli, suggesting that effects were food specific. An additional online study in this thesis demonstrated that two domains related to oxytocin function, loneliness and parental bonding, predict higher scores on a measure of food "addiction". High loneliness scores and low maternal care predicted high food "addiction", but age, gender, hours of sleep, anxiety, and activity levels did not. The experiments of this doctoral thesis are the first

experiments to demonstrate anorectic effects of oxytocin on eating not initiated by deprivation-induced hunger. This thesis also shows, for the first time, the anorectic capacity of oxytocin in females, and its independence from cortisol levels. The dot-probe experiment of this thesis provides important evidence that oxytocin reduces attentional bias to food across a range of body mass indexes but does not alter the attention paid to romantic or social stimuli. The final experiment provided evidence that oxytocin increases social modelling of food intake, which is consistent with previous research into oxytocin's social effects. Future work could extend this thesis in a number of important research directions, for instance by using a wider range of snack foods and palatability measures to account for different tastes, improve generalisability and test oxytocin's efficacy with different foods. Another important direction that this thesis suggests would be useful to investigate is oxytocin's influence on the snack-food intake and sociability of different compositions of group, varied, for instance, by gender, size and familiarity. Taken together, the impact of these findings could have potential benefits for people with obesity or disorders of overeating and indicate that oxytocin might be useful to research as a therapeutic drug to target overeating.

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CHAPTER 1 - GENERAL INTRODUCTION

1.1 History and Overview of Oxytocin

The term 'oxytocin', derived from the Greek 'oxys' meaning quick and 'toxos' meaning birth, was not popularized until chemists separated it from the chemically similar vasopressin in 1928 (Kamm, Aldrich, Grote, Rowe, & Bugbee, 1928). However, twenty years earlier, Sir Henry Dale had discovered that extracts from the posterior-pituitary lobe induce strong uterine contractions in domestic animals (Dale, 1908). In 1973, the term 'neuropeptide' was coined to describe the hormone-like peptides operating independently of the endocrine system (de Wied, 1973). Oxytocin was found to affect not only physiological maternal events via circulatory release but also induced maternal behaviour through central actions in virgin rats (Pedersen & Prange, 1979), which led to a wave of research and the identification of diverse effects, particularly anxiolytic and affiliative. In 1984, the oxytocin gene was isolated on human chromosome 20 and sequenced (Ivell & Richter, 1984). The structure and DNA of the oxytocin receptor encoding region located on chromosome 3, was elucidated in the following decade (Kimura, Tanizawa, Mori, Brownstein, & Okayama, 1992).

Oxytocin is found exclusively in mammals (Insel, 1997), and is the most abundant neuropeptide expressed in the hypothalamus, in contrast to circulating quantities of oxytocin, which are low (Gautvik et al., 1996). However, plasma levels of oxytocin do not differ between the sexes, suggesting that oxytocin has physiological functions beyond its well-known prevention of postpartum haemorrhage and stimulation of milk ejection, currently the only two circumstances in which oxytocin is medically administered (Gimpl & Fahrenholz, 2001). Over half a century of research into the central effects of oxytocin has illuminated its role in regulating fluids, modulating memory, and hyperthermia. However, within the last decade the implications of central oxytocin's actions for motivated and social behaviours are becoming clear (Carter, 1992; Carter, & Altemus, 1997; Carter, 1998; Love, 2014; Marazziti & Canale, 2004; Riem et al., 2013; Scheele et al., 2013).

Both oxytocin and species-dependent analogues are produced in the phylogenetically ancient hypothalamus, are highly conserved through evolution, and common to all vertebrates (Gimpl & Fahrenholz, 2001). Sarnyai and Kovács' proposal that oxytocin as a neuromodulator has an adaptive function promoting learning and memory within the brain, even if only partly correct, would accord with its preservation across millennia (Sarnyai & Kovács, 1994). It is known that an oxytocin-like peptide exists in the freshwater hydra—the most primitive of species that has existed since the Precambrian period about 600 million years ago (Villarreal, 2008). The hydra lacks blood, so the peptide is thought to have originally functioned as a neurotransmitter (Acher, Chauvet, & Chauvet, 1995; Cruz et al., 1987). Development of the peptide's function came about through diversification of its target cells and their protein signals. Oxytocin operates across all evolutionary layers of the brain, from the ancient hindbrain to the recent neocortex, yet its effects

depend upon the part of the brain in which it acts (Knobloch & Grinevich, 2014). Evolutionary selection has also "invested" heavily in sophisticated neural reward circuitry (Berridge & Kringelbach, 2015), in which oxytocin is now known to play a key role (Aron, A. et al., 2005b; Love, 2014; Scheele et al., 2013).

1.2 Peripheral Physiological Actions

Oxytocin is best known as a hormone involved in maternal physiological processes. Despite its important role, however, oxytocin is not necessary for normal delivery (Winslow & Insel, 2002). Oxytocin is also released in response to suckling via nerve fibres extending from the nipple to the hypothalamus that facilitate the milk 'let-down' response (Svennersten-Sjaunja & Olsson, 2005). For both sexes, oxytocin is a key neuropeptide facilitating successful coitus. Both lordosis frequency and duration are increased by oxytocin and reversed by an oxytocin receptor antagonist (Benelli et al., 1994). In women, plasma oxytocin concentrations fluctuate around menstrual cycles, positively correlate with genital lubrication during arousal (Salonia et al., 2005), and elevate significantly at orgasm (Blaicher et al., 1999). In men, neurohypophysial oxytocin stimulates penile tumescence and reduces latency from intromission to ejaculation (Carter, 1992). Oxytocin-containing cells have also been found in the pancreas, the retina (Gauquelin et al., 1983), the thymus (Amico, Finn, & Haldar, 1988; Geenen et al., 1986), and the adrenal medulla (Ang & Jenkins, 1984). Owing to the presence of oxytocin receptors, several peripheral organs are known to be sensitive to oxytocin—including the heart and kidneys (Lim & Young, 2006; Veening & Olivier, 2013)—and oxytocin is involved in the diurnal fluctuations of renal glomerular filtration rates (Brooks & Pickford, 1958; Ezrin, Loach, & Nicholson, 1962). Oxytocin also affects myriad physiological processes such as reversing osteoporosis (Elabd et al., 2008), building muscle mass, promoting bone healing (Park et al., 2014), and lowering blood pressure (IsHak, Kahloon, & Fakhry, 2011; Petersson & Uvnas-Moberg, 2008; Taylor et al., 2006); these actions, in turn, have their own psychological consequences.

1.3 Structure, Synthesis, Pharmacology and Interactions

Oxytocin is a hydrophilic nine-chain polypeptide (Ren et al., 2015). Centrally, about two-thirds of oxytocin is synthesised by paravocellular and magnocellular cells of the hypothalamic paraventricular (PVN) and supraoptic nuclei (SON). The remainder is produced in accessory hypothalamic neurons situated between these two main oxytocin-producing nuclei, as well as the bed nucleus of the stria terminalis (BNST), medial preoptic area (MPOA), and the surrounding hypothalamic blood vessels (Rhodes, Morriell, & Pfaff, 1981; Saper, Loewy, Swanson, & Cowan, 1976; Swanson & McKellar, 1979; Veening, de Jong, Waldinger, Korte, & Olivier, 2015).

Magnocellular neurons of the PVN and SON project to form the hypothalamoneurohypophysial tract, along which oxytocin is transported by its carrier, neurophysin, at the rate of 1-3 mm per hour (Zeeman, Khan-Dawood, & Yusoff Dawood, 1997). The hypothalamoneurohypophysial tract targets the posterior pituitary gland via the internal lamina of the median eminence to release oxytocin directly into the circulation (Swanson & Sawchenko, 1980; Vandesande, Dierickx, & De Mey, 1977; Veening & Olivier, 2013). Both

oxytocinergic and chemically similar vasopressin neurons contribute to the hypothalamoneurohypophysial tract, but cellular organisation and morphological evidence of the hypothalamic nuclei and their projections, have established multifarious roles for oxytocin, including autonomic and allostatic functioning (Swanson & Sawchenko, 1980). The magnocellular SON also projects to extrahypothalamic sites (Veening & Olivier, 2013), and magnocellular PVN neurons project long varicose fibres to the nucleus accumbens (NAc, Ross, Heather E. & Young, 2009).

Aside from classical axonal and neurohaemal transmission, cell bodies and dendrites—which contain most of the oxytocin content of magnocellular neurons (Leng, Caquineau, & Sabatier, 2005; Ludwig & Leng, 2006)—release into the extracellular space, stimulating further oxytocin release by proximal cells that results in coordinated neural responses (Moos & Richard, 1989; Morris & Ludwig, 2004; Yamashita et al., 1987). Dendritic and somatic release mechanisms in the PVN and SON can result in a thousand-fold increase of extracellular oxytocin in the local region that, in turn, significantly raises cerebrospinal fluid (CSF) levels (Wright, 1982). Dendritically released PVN oxytocin is thought to travel via the CSF of the third ventricle to caudal hypothalamic and brainstem termini, whereas in the SON, dendritic oxytocin is likely to be released into the bordering arachnoid space (Veening & Olivier, 2013). The oxytocin-producing BNST is an integral regulator of the hypothalamic-pituitary-adrenal stress axis (HPA). The BNST relays both external stress events and systemic stress signals, such as hypotension, directly to the PVN, bypassing higher-order processing (Egli & Winder, 2003).

Oxytocin binds to the oxytocin receptor, a seven transmembrane G-protein coupled receptor widely expressed in the brain, spinal cord, and periphery (Gimpl & Fahrenholz, 2001; Kimura et al., 1992). Oxytocin receptors functionally couple to the alpha subunit (q or 11 subtype) of the G-protein complex that activates phospholipase C, leading to the generation of inositol trisphosphate that releases Ca^{2+} from intracellular stores, and 1,2-diacylglycerol that stimulates protein kinase C (Gimpl & Fahrenholz, 2001; Nishimori et al., 2008). A variety of events is instigated in response to an increase of intracellular $Ca^{2+,}$ including the production of nitric oxide, which results in smooth muscle contraction, regulation of neurosecretory cell firing rates, and gene transcription (Gimpl & Fahrenholz, 2001). The oxytocin receptor is relatively unselective and binds oxytocin with only about a ten-fold higher affinity than arginine vasopressin (AVP), which can also signal through oxytocin receptors (Chini, Bice et al., 1996). The main downstream signalling pathway is the phospholipase inositol pathway that is central to the process of contraction of the cervical myometrium, but other downstream pathways are also active that elevate prostaglandin during parturition (Zeeman et al., 1997). In the cardiovascular system, oxytocin receptors are associated with pathways that increase vasodilation, and slow the force and rate of contraction (Petersson, 2002a).

The pattern of oxytocin receptor distribution does not rely on presynaptic oxytocin-production levels (Insel & Young, 2001), is species specific, and corresponds to the sociability of the species, see section 1.3.3 for

further details (Young, L. J., Lim, Gingrich, & Insel, 2001). In mice, oxytocin receptors have been visualised in disparate brain regions, including those associated with mnemonic processing, such as the hippocampus and bed nucleus of the stria terminalis (Ferguson et al., 2000), and key mesocorticolimbic regions, including the ventral tegmental area (VTA), NAc, subthalamic nucleus, amygdala, and hypothalamus (Baracz et al., 2012; Love, 2014).

Cerebrospinal fluid is no longer viewed as a simple clearance mechanism, but a dynamic transporter of neuroactive substances (Sakka, Coll, & Chazal, 2011). Most CSF is secreted by choroid plexuses—with extracellular fluid (ECF) contributing about a quarter, and exhibits circadian changes; varies in flow speed and composition dependent on anatomical zone; and is susceptible to age-related changes (Devarajan, Marchant, & Rusak, 2005; Edsbagge, Tisell, Jacobsson, & Wikkelso, 2004; Veening & Barendregt, 2010). CSF transfer from the lateral ventricles to the ventral medullar surface takes only one to two minutes in rat and cat brains (Veening & Barendregt, 2010; Wright, 1982). Given both the extended 28 minute half-life of oxytocin in CSF together with the numerous oxytocin-receptive areas surrounding the arachnoid spaces and ventricular system (Vigh et al., 2004), CSF transport of oxytocin is likely to dominate central endogenous oxytocin circulation (Sewards & Sewards, 2003), but whether the neuropeptide is still in its active form in CSF is disputed (Landgraf, Rainer & Neumann, 2004). Though animal studies demonstrate the CSF's rapid and free access to extracellular fluid, Wright (1982) demonstrated how large-scale dendritic oxytocin release in the hypothalamus was mirrored by similar CSF rises of the neuropeptide, throwing doubt on the suggested upper limit for ECF to CSF drainage of fifteen percent. So, the correlation between the CSF and brain oxytocin levels remains in question, and studies relying on CSF oxytocin as a proxy for brain levels (e.g. Averbeck, 2010), may not be accurate.

1.3.1 Monoamine Interactions

Following a rise in intracerebral oxytocin, dopaminergic activity increases or decreases according to anatomical region. After acute doses of oxytocin, mesencephalic dopamine cell bodies decrease dopamine production, and dopaminergic usage in the striatum increases but decreases in the hypothalamus (Sarnyai & Kovács, 1994). However, oxytocin antisera had a tonic regulatory role on the VTA and substantia nigra dopamine neurons (Sarnyai & Kovács, 1994). Accordingly, oxytocin-induced allosteric receptor-receptor interactions in dopamine receptor-oxytocin receptor (D2R-oxytocin receptors) heteromers are found in the dorsal striatum and the neuropil of the NAc; these make more D2R available for dopamine binding (Romero-Fernandez, Borroto-Escuela, Agnati, & Fuxe, 2013). At particular oxytocin concentrations, increases in D2R affinity are also seen (Romero-Fernandez et al., 2013). Melis et al. (2007) found that in male rats, oxytocin stimulation of the caudal, but not rostral, VTA increased dopamine release in the NAc shell and hypothalamic PVN, which themselves express dopamine receptors and are modulated by dopamine. In vivo, exogenous oxytocin stimulates nitric oxide synthase—the enzyme that catalyzes production of nitric oxide from L-arginine—in mesolimbic dopamine neurons, suggesting oxytocin can limit dopamine release (Succu et al, 2008).

The effects of oxytocin on the motivational and behavioural effects of addictive drugs shows oxytocin reduces conditioned place preference (CPP) and lever pressing for drug fixes. Co-administration of oxytocin and dopamine into the subthalamus prevents CPP in rats and oxytocin antagonist dose-dependently reversed this, with low doses being ineffective and high doses blocking oxytocin's effects (Baracz & Cornish, 2013) Administration of oxytocin by micro-injection into the rat NAc core or subthalamic nuclei attenuates methamphetamine-induced place preference (Baracz et al., 2012). Similarly, turnover of amphetamine-evoked accumbal dopamine is reduced (Qi, Yang, Song, Wang & Wu, 2009). Interestingly, lesions to the subthalamic nuclei decrease alcohol and drug consumption but increase the motivation to eat (Baunez, Dias, Cador, & Amalric, 2005; Baunez, Amalric, & Robbins, 2002), suggesting that oxytocin's impact on reward reduction in the subthalamus might differ between drugs and food. Exogenous oxytocin inhibits rat self-administration of alcohol and stimulants, altering drug-induced changes in dopamine, glutamate and Fos expression (McGregor & Bowen, 2012). Administration of an oxytocin receptor antagonist, per contra, significantly diminishes dopamine agonist-stimulated dopamine release in the NAc (Melis et al, 2007), and stimulation of oxytocin in both the amygdala and the hippocampus results in increases of extracellular dopamine in the NAc via glutamatergic projections to the VTA (Succu, 2011). The neural interactions of oxytocin and dopamine are nuanced, and variability might be accounted for by activity that corresponds to the micro-circuitry it acts on within the framework of the corticolimbic-striatopallidal-hypothalamic-thalamocortical circuitry (Kenny, 2011).

1.3.2 Serotonin, Noradrenaline, Opioid, and Prolactin Interactions

Despite the importance of oxytocin's interaction with 5-hydroxytryptamine (5-HT, serotonin), little work has been carried out on the nature of the exchange. The discovery that serotonin 1A, 2A and 2C receptor agonists boost plasma oxytocin levels has led to the claim that oxytocin mediates selective serotonin reuptake inhibitor-induced antidepressant effects (Jørgensen, Riis, Knigge, Kjaer, & Warberg, 2003; Saydoff, Rittenhouse, van de Kar, & Brownfield, 1991; Van de Kar et al., 2001). However, peripheral levels of oxytocin do not correlate with central levels, and many selective serotonin reuptake inhibitors (SSRIs) have little affinity to either 5-HT1A or 2A receptors, being effective only at 2C receptors and at very high doses (van Roekel, Verhagen, Engels, Goossens, & Scholte, 2013; Zhang, Qiang et al., 2013; Zhou, Ligang et al., 2007). Fluoxetine (Prozac), for instance, targets 5-HT2B receptors, with only weak affinities at 2A and 2C receptors (Ni & Miledi, 1997; Wong, Threlkeld, & Robertson, 1991). Intriguingly, fluoxetine was found to block 5-HT agonist-induced oxytocin release (Li, et al., 1993). Serotonin interactions with oxytocin are thought to underlie some of the anxiolytic properties of oxytocin and social reward (Dölen, Darvishzadeh, Huang, & Malenka, 2013; Saydoff et al., 1991). Elevated plasma oxytocin from 5-HT agonists involves neurohypophysial discharge of magnocellular oxytocin cells from the SON (Saydoff et al., 1991) resulting in anxiolytic effects. About half of the pontine raphe nucleus 5-HT neurons express oxytocin receptors and local administration of oxytocin triggers 5-HT release (Yoshida et al., 2009). The mesocorticolimbic system encodes social reward and this is mediated by PVN oxytocin neurons terminating on 5-HT fibres that elevate

5-HT release in the NAc (Dölen, Darvishzadeh, Huang, & Malenka, 2013). A great deal of empirical evidence supports the opioid theory of social attachment that posits opioid reinforcement of social contact, attenuation of the reaction to social separation, and motivation to seek out social contact (Machin & Dunbar, 2011). Given that oxytocin promotes sociality, it would be expected to stimulate opioids in the brain, but a number of experiments reveal a rather nuanced situation dependent on opioid receptor type and dose (Tops, Koole, IJzerman, & Buisman-Pijlman, 2014). In rats, oxytocin treatment augments noradrenaline release and lesioning noradrenaline neurons limit the effects of oxytocin on social recognition and memory (Dluzen, Muraoka, Engelmann, Ebner, & Landgraf, 2000; Dluzen, Muraoka, Engelmann, & Landgraf, 1998). Dendritic oxytocin stimulates noradrenaline in the SON, is reversible by oxytocin antagonists, and in addition, facilitates activation of oxytocin neurons, at least in part by increasing noradrenaline release. Oxytocin reduces lactation surges of the peptide prolactin in rats, but its effect on pulsatile nonlactation release has not yet been illuminated (Samson, Lumpkin, & McCann, 1986). However, in the neurohypophysis, oxytocin acts in opposition to dopamine's inhibitory effects on lactotrophs to stimulate prolactin release, and oxytocin activity correlates with prolactin release (Samson, Lumpkin, & McCann, 1986).

1.3.3 Central Gonadal Steroid Interactions

The promoter for the oxytocin gene contains an oestrogen-responsive element, and oxytocin receptor levels are sensitive to gonadal hormone levels (Carter, 1998). In the hypothalamus, oestrogens stimulate the release of oxytocin (Akaishi & Sakuma, 1985), and in the amygdala oestrogens increase oxytocin receptor binding (Young, L. J., Wang, Donaldson, & Rissman, 1998). Oestrogens also regulate the number of oxytocin receptors in a few discrete nuclei including parvocellular cells of the PVN (Shughrue, Dellovade, & Merchenthaler, 2002) and upregulate central oxytocin receptor binding (Acevedo-Rodriguez, Mani, & Handa, 2015). Moreover, the onset of maternal behaviour coincides with an increase in oxytocin receptor expression in two key limbic regions at parturition (De Geest, Thiery, Piron-Possuyt, & Driessche, 1985). The oxytocin system continues to develop postnatally, and its development and functioning are sensitive to social environment, stress and illness, especially after birth (Buisman-Pijlman et al, 2014, p28). Prenatal oxytocin is mainly affected by stress and drug exposure but with sex differences (Buisman-Pijlman et al., 2014); see Chapter 6 for further details on sex differences. Although their function remains unknown, androgen receptors are expressed in about half of the parvocellular oxytocin cells in the PVN, although a role in the development of aspects of social reward has been hypothesised and their dysfunction in autism spectrum disorders is being investigated (Carter, C. S., 2007; Churchland & Winkielman, 2012; Insel, 1997; Young, LJ, Pitkow, & Ferguson, 2002; Zhou, L., Blaustein, & De Vries, 1994).

There is evidence that sex differences exist for selective features of the oxytocin system, such as oxytocin and oxytocin receptor synthesis, which are partially oestrogen dependent and result in higher female oxytocin levels (Carter, 2007). However, sex-dependent differences in cellular activity of oxytocin cells have not been found (Ishunina & Swaab, 1999). The size of oxytocin cells delimits activity level, and

autopsy data show that PVN oxytocin cell size is not sexually dimorphic, although other measures of cell activity, such as the oxytocin messenger ribonucleic acid (mRNA), may differ between the sexes (Ishunina & Saab, 1999). Several sex differences in oxytocinergic action have been demonstrated in taxa according to their mating patterns (Aragona et al., 2006; Smeltzer, Curtis, Aragona, & Wang, 2006). Promiscuous male montane voles, for instance, have fewer oxytocin receptors compared to their monogamous relatives. One study has demonstrated that higher plasma oxytocin is associated with greater attachment anxiety in women than in men, and that oxytocinergic anxiolysis was present for men but not women, see Chapter 9 (section 9.1) for further details (Weisman, Zagoory-Sharon, Schneiderman, Gordon, & Feldman, 2013).

1.3.4 Clearance

Oxytocin is rapidly cleared from the blood with a half-life of about 2 to 3.4 minutes (Veening & Olivier, 2013), and metabolised by the liver and kidneys, or proteolytically degraded by oxytocinases in gravid women into biologically inactive particles. Oxytocin has an extended half-life in CSF of 28 minutes (Robinson, I. C. A. F., 1983). Whole body clearance of oxytocin is primarily accomplished by the liver and kidneys via peritubal clearance (McEwen, 2004), a small study of four men found that less than one percent is cleared by the kidneys as urine (Amico, Ulbrecht, & Robinson, 1987). There is some evidence that synthetic peptides are more resistant to degradation than endogenous ones (Claybaugh & Uyehara, 1993), but further research is needed to confirm this. Proteolysis of oxytocin may also occur in the brain, as fragments of the peptide have been identified in extrahypothalamic regions that may, in addition, be biologically active (Claybaugh & Uyehara, 1993), for instance, regulating melanocyte-stimulating hormone release (Celis, Taleisnik, & Walter, 1971).

1.3.5 Routes of Administration for Oxytocin; Safety and Clearance

Oxytocin has featured in the World Health Organisation's list of essential medicines for over 40 years (World Health Organisation, 2015a). Intravenous and inhaled forms of oxytocin are well-tolerated obstetric treatments in parturient women (Burri, Heinrichs, Schedlowski, & Kruger, 2008). Intranasal oxytocin is also considered safe with no exclusion criteria of its own. A systematic review of thirty-eight trials across twenty years, found up to 40 international units (IU) of intranasal oxytocin was undetectable by participants and not associated with side effects or adverse outcomes (MacDonald et al., 2011). Given that the swift clearance of oxytocin from plasma at 18-22 ml/kg/minute is consistent across males, pregnant and nonpregnant females (Zeeman et al., 1997), and accelerates in response to external infusion, the time-frame for nasally applied oxytocin to be cleared from blood is likely to be under three minutes (Amico et al., 1987). However, one study concluded that exogenous oxytocin might be detectable in plasma seventy-five minutes post inhalation (Striepens et al., 2013), but the possibility that oxytocin degradation products were measured in this study cannot be ruled out. Given the complete renewal of CSF every five to six hours, this would constitute the upper boundary for CSF clearance (Wright, 1982).

There are many effective methods of administering oxytocin. Medically, peripheral oxytocin, or homologues

such as pitocin, assist in parturition or abortion, usually via continuous intravenous infusion (Blanks & Thornton, 2003; De Geest et al., 1985; Zeeman et al., 1997). Infrequently, intranasal oxytocin is provided to induce lactation (MacDonald et al., 2011). In animal research, oxytocin is delivered subcutaneously, intravenously, and intraperitoneally to the periphery; and via ICV or nasal routes to central nervous tissue (MacDonald et al., 2011; Macdonald & Feifel, 2013). Animal and human studies have also revealed that nasal oxytocin reaches the olfactory bulb and the cervical CSF (Born et al., 2002; Freeman et al., 2016; Modi, Connor-Stroud, Landgraf, Young, & Parr, 2014). In humans, intranasal oxytocin administration is an effective means of delivering exogenous oxytocin (Striepens et al., 2013) that bypasses the gastrointestinal tract and the blood-brain barrier. Two doses of intranasal oxytocin are commonly used in the literature: 24 and 40 IU, typically inhaled in 4 IU doses via a mechanical pump spray.

Although intranasal delivery of oxytocin is now established in research, neither its mechanism of action, nor its temporal frame, is agreed upon. Born et al. (2002) found that nasally applied peptides were detectable in CSF 10 minutes after administration. However, in endogenous stimulation paradigms where the stress-attenuating effects of oxytocin are tested during lactation, lactation has to begin 30 to 60 minutes before testing in order that the anxiolytic effects of oxytocin are detectable (Altemus, Magaret, Deuster, Galliven, Carter, & Gold, 1995; Altemus, Margaret et al., 2001; Heinrichs et al., 2001; Heinrichs, Neumann, & Ehlert, 2002). This suggests that clinical or behavioural effects of oxytocin are unlikely to occur before 30 minutes post administration. Striepens et al. (2013), however, claimed CSF oxytocin increases are relatively modest and not significant until 75 minutes after intranasal administration. As effects of oxytocin have been found at 30 minutes (e.g. Savaskan et al., 2008), the disparity between CSF levels and testable effects in the laboratory, suggests that peak CSF is not necessary for oxytocin to be effective and that CSF oxytocin is not a proxy for central oxytocin.

1.3.6 Limitations of Non-Nasal Routes of Administration

The blood-brain barrier (BBB) constitutes a substantial challenge for peripheral delivery of oxytocin to the brain. The epithelial cells which line the blood vessels of the brain are connected by tight junctions and are traditionally thought to be permeable only to very small molecules of less than 500 daltons molecular weight, such as the lipophilic molecule dopamine (Butt, Jones, & Abbott, 1990); oxytocin is lipophobic and has a molecular weight of 1007 daltons (Leake, Weitzman, & Fisher, 1980).

Multiple brain sites surrounding the third and fourth ventricles lack a blood-brain barrier, structures collectively known as the circumventricular organs (CVO). In mammals, the CVO comprise the neurohypophysis, subfornical organ, median eminence, the pineal, organum vasculosum lamina terminalis, the subcommissural organ, and the area postrema. CVOs are peptide-releasing neurohaemal structures that maintain homeostatic balance, allowing penetration of select substances not otherwise able to permeate, and by regulating hormone levels via negative feedback (Ganong, 2000). The fenestrated capillaries of the CVO permit free movement of solutes from blood to interstitial fluid via aqueous pathways (Begley, 1996); a

potential route, therefore, for polar peptides.

In addition to the CVO, the blockade of peptide entry to the brain may not be maintained by the CSF-producing choroid plexuses located in brain ventricles. The discontinuous tight junctions of fenestrated capillaries within the choroid plexus allow diffusion of selected molecules from the blood into its connective tissue (Johansson, P. A., Dziegielewska, Liddelow, & Saunders, 2008). According to Begley (1996), however, the relative surface area of fenestrated to tight junction capillaries is 1:5000, which renders the permeable areas uninfluential; furthermore, peptides have been found to cross the BBB in locations distinct from CVO (Mens, Witter, & Van Wimersma Greidanus, 1983). Begley argues that the most likely process by which oxytocin traverses the blood-brain barrier is by attaching to active-transport proteins that usually carry constituent amino acids (Begley, 1992).

Alterations to the permeability of the blood-brain barrier due to environmental, pathological, and mental states might account for the conflicting results of researchers. Hypertension, eating, diabetes, and weight disorders are notable conditions that alter the dynamic interface of the BBB and medication, for example sildefil (sold as Viagra®) and tricyclic antidepressants, can also open the BBB (Witt & Davis, 2006). Whilst a review by Dhuria et al (2010) propounded the orthodox view advanced by Partridge's (2001) that virtually 100% of large peptides were blocked and, moreover, that nearly 98% of small molecules that readily perfuse across porous capillaries are also blocked by the tight junctions of the BBB. On balance, however, the debate over BBB permeability to peptides now favours penetration (Almutairi, Gong, Xu, Chang, & Shi, 2016; Stalmans et al., 2015). Despite only 0.002% of the injected peptide reaching the central nervous system, it is a quantity sufficient to induce central actions (Kastin & Pan, 2008; Mens et al., 1983).

Although dismissed as a viable pathway and ignored by reviewers (e.g. Veening & Olivier, 2013), an alternative process by which oxytocin effects central change, is afferent peripheral feedback. In rats, intraperitoneal injection of oxytocin causes significant c-Fos expression where the vagal nerve terminates in the dorsal vagal complex (Maejima et al., 2011; Rhoades & Bell, 2012) and elevated c-Fos expression is also found in hypothalamic nuclei following peripheral dosing (Maejima et al., 2011), suggesting that afferent vagal feedback from oxytocin triggers central change. Animal research stretching back to 1974 has consistently demonstrated altered central oxytocinergic functioning, including anorexia, from peripherally administrated oxytocin (Arletti, Benelli, & Bertolini, 1989; Maejima et al., 2011; Morton et al., 2012; Zhang, G. & Cai, 2011). Viscera to central nervous system (CNS) vagal feedback is in the order of milliseconds (Duclaux, Mei, & Ranieri, 1976), quick enough to explain raised CSF concentrations of oxytocin after parenteral administration that are seen within two minutes and significantly elevated by five minutes (Mens et al., 1983).

1.3.7 Nose-to-Brain Pathways

Whilst the clinical data is strong, the mechanisms by which inhaled oxytocin achieves its effects are not fully

understood, with no accepted theory yet established. There are three possible methods of brain entry after intranasal administration: (1) intra-axonal and transneuronal, (2) peripheral bloodstream, (3) perivascular and perineuronal spaces. In addition to brain entry, the possibility that intranasal oxytocin may effect change centrally via afferent peripheral feedback cannot be excluded. Although prolonged effects have been demonstrated via peripheral routes, this remains to be addressed by reviews, which ignore the evidence of a potential fourth vagal afferent feedback signal and assume CSF delivery means brain delivery (Averbeck, 2010).

Although certain nasally applied substances have been traced to the olfactory bulb and brainstem where olfactory and cranial nerves terminate, respectively (Charlton et al., 2008; Veening & Olivier, 2013), intra-axonal and transneuronal transport via olfactory and trigeminal nerves has limited viability as a candidate for oxytocin transport mechanism due to oxytocin's large molecular size and hydrophilicity. However, Levasseur et al. (2004) have located AVP1a receptors on olfactory sensory neurons and, given that oxytocin can bind to AVP1a receptors (Love, 2014), neural activation patterns will alter (Veening & Olivier, 2013). The highest brain concentrations of oxytocin are thought to occur about 30 to 45 minutes after inhalation, and transport times of less than a minute have been demonstrated with several intranasally applied substances (Charlton et al., 2008; Illum, 2004; Thorne, Pronk, Padmanabhan, & Frey, 2004). Intra-axonal absorptive routes typically take hours and, therefore, are unlikely to play a major role in the effects seen after intranasal administration of oxytocin (Veening & Olivier, 2013).

Entrance sites for peptides in the blood, such as the CVO, have specific neural connections and distributive patterns that are markedly different from the olfactory and brainstem sites used by neuronal transport routes (Veening & Olivier, 2013). Visualisation of nasally applied substances is seen in cranial and olfactory areas, and lipophilic drugs with a low molecular weight have been visualised in orofacial and brainstem areas, following trigeminal nerve distribution (Ross, Heather & Young, 2009). However, similar evidence at typical peripheral entrance portals is lacking (Veening & Olivier, 2013). According to Mens et al. (1983), peripheral oxytocin has a negligible influence over brain levels of oxytocin, which means that absorption of intranasal oxytocin via circulation, is virtually impossible. Recently, Neumann et al. (2013) found that intranasal oxytocin reached the brain without the involvement of plasma or peripheral oxytocin, meaning direct nose-to-brain transfer can be achieved.

Nose-to-brain pathways that do not travel axonally or systemically use the spaces surrounding vasculature and neurons and can be called 'direct pathways' (Veening & Olivier, 2013). Olfactory sensory neurons are bipolar nerve cells that extend dendrites into the mucosal layer of the olfactory epithelium (Dhuria et al., 2010) and terminate in the olfactory bulb. Due to environmental toxin exposure, olfactory sensory neurons are short-lived and regenerate about every 20 days from basal cells in the nasal epithelium (Dhuria et al., 2010). Olfactory ensheathing cells surround and support olfactory sensory neurons through cycles of regrowth, creating fluid-filled channels that remain open irrespective of neural degeneration (Dhuria et al.,

2010). Many of these direct paths have been studied as drainage routes for CSF, but evidence shows that intranasal delivery is also possible via the same channels (Dhuria et al., 2010). Substances travelling along perineuronal and perivascular spaces would be expected to reach the rostral part of the cranial cavity, and whenever visualisation has been possible, the highest concentrations of labelled substances are found in this area (Veening & Olivier, 2013). The nasal clearance rate is between three and 25 mm per minute, which limits the residence time of inhaled substances to about 15 minutes. It is clear, therefore, that spacing out each nasal spray dose will maximise the overall nasal residence time.

Different species have evolved diverse olfactory capacities. The sensitive canine olfactory system, for example, is generated by a large olfactory mucosal area to receive odorous cues and constitutes about 30% of the overall nasal lining. In humans, the olfactory epithelium has an area of only one square centimetre–less than one-tenth of the overall mucosal covering, which measures about 150 square centimetres (Chaturvedi, Kumar, & Pathak, 2011). An additional neuronal pathway, known as the vomeronasal organ, whose putative function is to detect pheromones, exists in some animals arising from the nasal mucosa and extending to the secondary olfactory bulb in the brain. Pheromonal communication in humans is debated in the literature. There is mounting evidence that a number of chemical messages are transferred between humans. For example, ostensibly biologically inactive axillary steroids that affect the behaviour or mood of the recipient are produced by the testes, ovaries, adrenal and apocrine glands (Hays, 2003), and sex-dissociated hypothalamic activation occurs after smelling sex hormones (Savic, Berglund, Gulyas, & Roland, 2001). Babies respond to chemical signals from the mother, such as oxytocin, and foetal vomeronasal pathways in humans are undisputed (Boehm & Gasser, 1993; Meredith, 2001), but the existence of vomeronasal pathways in adults is uncertain (Meredith, 2001). In humans, chemical or pheromonal communication does occur (Monti‐Bloch, Jennings‐White, & Berliner, 1998). Emotional qualities associated with odours are known to activate the amygdala and the orbitofrontal cortex (Royet et al., 2000; Zald & Pardo, 1997), and physiological responses such as heart rate and blood pressure have been recorded to chemical stimuli that the participant was not able to smell (Monti-Bloch & Grosser, 1991). In adults it is not yet clear, though, whether pheromones use a vomeronasal route that receives information low down in the nose, or whether chemoreceptors embedded in the olfactory epithelium high up in the nose serve this purpose (Meredith, 2001).

In rodents, the primary sense for conspecific recognition is olfaction, whereas in man, it is vision. Large differences in downstream processing of these disparate stimuli may undermine the translational validity of rodent to human oxytocin models. Afferent visual information is processed by the gyrus and superior temporal sulcus, whereas incoming olfactory signals are routed through the amygdala and piriform cortex (Lim & Young, 2006). However, functional brain imaging has demonstrated that the amygdala mediates oxytocin-generated gazes to the eyes (Gamer, Zurowski, & Buchel, 2010), suggesting a role in attentional processing.

1.3.8 Methodological Issues

Although 24 and 40 IU are the usual doses in intranasal studies, other doses are sometimes used; this lack of standardisation also applies to testing latencies and the administration procedure itself. Although researchers are now recommending standard protocols (Guastella et al., 2013), there remains much variation in research and theory (Campbell, Morimoto, Nenciu, & Fox, 2012; Chaturvedi et al., 2011; Clary-Meinesz, Cosson, Huitorel, & Blaive, 1992; Hardy, Lee, & Wilson, 1985; Lansley, 1993; Marttin, Schipper, Verhoef, & Merkus, 1998; Ugwoke, Verbeke, & Kinget, 2001). Researchers are divided on the most absorptive head position, and practical considerations overrule certain head tilts, such as the 'praying to Mecca' position and supine head tilt (Dhuria et al., 2010; van Den Berg, Romeijn, Verhoef, & Merkus, 2002). An upright forty-five-degree backwards head tilt allows sprays to reach the olfactory mucosa that contain the nerves and vasculature extending to the brain (Hardy et al., 1985), and is most commonly seen in intranasal research. The rest time between each inhalation can also impact uptake, and at least 30-second breaks limit the possibility that oxytocin is swallowed. In addition, the alternation of nostrils during dosing, might avoid saturating one nostril beyond absorptive capacity (Jones, N. S., Quraishi, & Mason, 1997).

Research in this area often uses animals, models, or cadavers, so may not be generalizable (Illum, 1996). Nasal clearance rates as represented by cilia beat frequency are not related to age or sex but are, on average, much faster in animals than humans at 17 Hz and 10 Hz respectively. However nasal clearance rates are temperature sensitive and slow down under 20 ºc (Clary-Meinesz et al., 1992; Marttin et al., 1998). About 50% of an exogenous compound is cleared after 15-20 minutes (Illum, 1996), and the slow human clearance suggests that nasally applied substances might remain for longer in the human mucosa than in animal mucosa. Mucosal surface area is also important in determining absorptive capacity, and in many animals, especially rodents and dogs, the mucosa have a hundredfold greater surface area than those of humans (Illum, 1996). Despite this, peptides and proteins are absorbed through the nasal epithelium in 5-15 minutes.

1.4 Peripheral Measures of Oxytocin

1.4.1 Blood

Oxytocin is released from the posterior pituitary directly into the circulation, where it has a short half-life (see 1.3.4) before being metabolised by the liver and kidneys (De Geest et al., 1985; Fabian, Forsling, Jones, & Pryor, 1969; Veening & Olivier, 2013). Typical levels in human plasma range from 3.8 ± 1.1*f*Êu/ml 4.8 ± 0.5*f*Êu/ml.

There are two different methods commonly in use today to assess oxytocin in plasma: radioimmunoassay (RIA) and enzyme immunoassay (EIA), both of which can use extracted or diluted samples. Since EIA techniques were developed about twenty years ago (Prakash, Metten, Schams, & Wuttke, 1998) they have become the preferred measurement system due to low costs and the elimination of risks of radioactive substances. Unextracted EIA measurement yields oxytocin levels over one-hundredfold higher than standard RIA extraction methods (Mccullough, Churchland, & Mendez, 2013). However, such assay methods have

been criticised for measuring not only the target analyte, but other substances with oxytocin-like immunoreactivity (Szeto et al., 2011). The validity of studies using EIA to measure oxytocin has been questioned (Mccullough et al., 2013).

Another issue with measuring plasma oxytocin is that in order to detect oxytocin before it degrades, it is necessary to chill the samples immediately to lower enzymatic activity. Whilst lowering sample temperatures to 4 degrees Celsius is commonplace, the time interval between collecting and testing varies widely, which is likely to affect the accuracy of measured levels of oxytocin.

Historically, the issue of whether plasma levels of oxytocin correlate with brain CSF has divided investigators, but more recently, the debate has favoured the functional independence of central and peripheral systems with only 0.01% of CSF oxytocin levels deriving from blood oxytocin levels (Amico, Challinor, & Cameron, 1990; Kagerbauer et al., 2013; Mens et al., 1983; Striepens et al., 2013). Although experimental research on rats has shown oxytocin, but not vasopressin, is released simultaneously in the brain and periphery (Ross, Heather, & Young, 2009; Wotjak et al., 1998), this only indicates context-specific release synchronicity, not correlated levels. In man, exogenous oxytocin administered nasally raised both blood and CSF levels, but blood levels had already returned to baseline by the time CSF levels had changed significantly at 75 minutes (Striepens et al., 2013). However, clinical effects of oxytocin are typically seen at forty-five minutes and oxytocin may be delayed in reaching distal lumbar sample regions. Oxytocin concentrations in monkeys are significantly higher at thirty-five minutes when measured at the cervical level (Chang, Barter, Ebitz, Watson, & Platt, 2012). Magnocellular cell bodies and dendrites release oxytocin independent of their neurohypophysial circuits, so it remains possible that oxytocin release patterns are inconsistent across anatomical regions and fluctuate with functional requirements. However, given the wide array of behaviours influenced by oxytocin, the smallest contextual and procedural differences among studies are likely to result in different oxytocin responses.

Due to the attendant risks of spinal puncture, sample size presents a challenge to collecting unbiased data. For instance, a recent study by Carson et al. (2014) involving patients undergoing medically necessary lumbar puncture, demonstrated a strong association between blood and CSF oxytocin. However, participants qualifying for lumbar puncture are likely, as in the Carson et al. (2014) study, to have serious health problems such as cancer, pituitary abnormalities, and subarachnoid haemorrhage, conditions that, in and of themselves or via treatments or mood alterations, are likely alter blood to brain permeability, oxytocin baselines, and oxytocin release patterns. A further problem with the Carson et al. (2014) study was that the already small and unrepresentative sample was further undermined by non-random participant selection.

Given the short half-life of plasma oxytocin, extended elevation of blood oxytocin following intranasal application has been accounted for by centrally acting oxytocin driving neurohaemal release (Veening & Olivier, 2013). Whilst central and peripheral oxytocin levels may not correlate—and the two systems may

be functionally independent—central manipulation of peripheral oxytocin would imply coordination between the two systems (Veening & Olivier, 2013). Further evidence of peripheral oxytocin being controlled centrally is the variable rate of oxytocin decline, which at certain times during parturition speeds up, suggesting that the mechanism for removal or proteolysis of oxytocin is not passive but centrally regulated (Veening & Olivier, 2013). Unlike other neuropeptides that, after intranasal application, cause slow rises in blood pre-empted by raised CSF levels, vasopressin and oxytocin cause simultaneous rises in blood and CSF levels (Veening & Olivier, 2013); Veening and Olivier (2013) hypothesise that peripheral-central co-ordination mechanisms for vasopressin and oxytocin are, therefore, organised differently from other neuropeptides.

1.4.2 Other fluids

Salivary measurements of oxytocin vary greatly in outcome. This is due, in part, to the variation of assay methods used to measure oxytocin in saliva, and the difficulties of isolating the hormone. Although a number of researchers use salivary oxytocin as a proxy for brain oxytocin in intranasal studies, the possibility that elevated salivary concentrations simply reflect post-nasal contamination from swallowed oxytocin escaping into the mouth, cannot be ruled out (Chaturvedi et al., 2011). Almost all oxytocin passing through the kidneys is inactivated by two specific peptidases and, consequently, very little oxytocin excreted in urine is biologically active, although some oxytocin metabolites do remain biologically active (Amico et al., 1987). Meaningful assays of urinary oxytocin are, therefore, difficult.

After nasal inhalation, it is estimated that CSF takes in excess of 40 minutes to reach the lumbar region from the nasal mucosa travelling at a speed of 2 cm/min—a factor not always considered in investigations (Robinson, I. C. & Coombes, 1993). However, in a study using spinal punctures made at the lumbar, nasally applied peptides were detectable within 10 minutes (Born et al., 2002). CSF levels of oxytocin do not correspond with blood measures, with CSF peaking much later than blood levels (Freeman et al., 2016; Striepens et al., 2013).

1.5 Stress and Oxytocin

Oxytocin is an anxiolytic regulating the HPA axis to reduce blood pressure (Uvnas-Moberg, 1997) and levels of adrenocorticotropic hormone (ACTH), cortisol, adrenaline, and noradrenaline (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003). Relief from stress is generally considered to affect eating and can increase or decrease intake depending on intrapersonal factors (Wardle & Gibson, 2016), whilst intranasal oxytocin reduces HPA activity (Weisman, 2013b) and lowers amygdala activation (Fuxe et al., 2012; Kirsch et al., 2005; Riem et al., 2011). There is also evidence that oxytocin mediates the protective effect of social support on stress responsiveness (Taylor et al., 2006). In response to unpredictable behaviour from unfamiliar individuals, oxytocin is anxiogenic; this may explain oxytocin's differential stress response to in- and out-group members (Cardoso, Ellenbogen, Orlando, Bacon, & Joober, 2013; Carter, & Altemus, 1997; Carter, & Lightman, 1987a; Grillon et al., 2013; Grimm et al., 2014; Heinrichs et al., 2003; Kumsta & Heinrichs, 2012;

Neumann, Torner, & Wigger, 1999; Onaka, Takayanagi, & Yoshida, 2012; Pedersen, Cort A. & Boccia, 2002; Taylor et al., 2006).

As an anxiolytic, oxytocin reduces not only acute stress but also chronic stress (Cardoso et al., 2013; Carter, & Altemus, 1997; Chini, Bice, Leonzino, Sala, & Braida, 2014; Harbuz & Lightman, 1992; Qi et al., 2009; Tops et al., 2014; Wotjak et al., 1998). On the basis that oxytocin is phylogenetically ancient, it has been suggested that anxiolysis underlies all of oxytocin's behavioural effects, as only higher order processes would be affected by oxytocin (Churchland & Winkielman, 2012). Impaired oxytocin function causes increased stress (Carson et al., 2014) and there is evidence that childhood separation may, in adulthood, reduce oxytocin's capacity to lower cortisol levels, indicating that adverse developmental events could result in long-lasting changes to oxytocinergic function (Carson et al., 2014; Grimm et al., 2014; Meinlschmidt & Heim, 2007; Pedersen, Cort & Boccia, 2002). However, individual differences, such as emotionality (Carter & Lightman, 1987a), neurogenetics (Kumsta & Heinrichs, 2012), and amount of social support (Heinrichs et al., 2003) are also likely to influence endogenous oxytocin and anxiety (Averbeck, 2010; Domes et al., 2010; Kim, Kim, Park, Pyo, & Treasure, 2014; Kim, Eom, Yang, Kang, & Treasure, 2015; Kirkpatrick, Lee, Wardle, Jacob, & de Wit, 2014; Kirsch et al., 2005; Klump et al., 2012; Neumann et al., 1999; Strathearn, Iyengar, Fonagy, & Kim, 2012; Turner, Altemus, Enos, Cooper, & McGuinness, 1999; Wismer Fries, Ziegler, Kurian, Jacoris, & Pollak, 2005). Furthermore, administration of exogenous oxytocin is an effective anxiolytic (Lancaster et al., 2017; Uvnäs-Moberg, 1998).

1.6 Affiliative Effects of Oxytocin

Initial investigations into the affiliative effects of oxytocin focussed on its ability to promote infant and maternal bonding behaviours, and reduce infanticide in rodents (Lim & Young, 2006; McCarthy, 1990). During parturition, oestrogen-driven increases in hypothalamic and MPOA oxytocin receptors are theorised to prime dams for maternal-infant bonding (Francis, Champagne, & Meaney, 2000; Francis, Young, Meaney, & Insel, 2002; Lim & Young, 2006), and both partial and full receptor knockout mice exhibit severe deficits in maternal behaviour (Li et al., 1999; Takayanagi et al., 2005). Vagino-cervical stimulation induces central oxytocin release and maternal behaviour in virgin ewes (Kendrick, Keverne, & Baldwin, 1987; Keverne, Levy, Poindron, & Lindsay, 1983). Spontaneous maternal behaviour in juvenile prairie voles is shaped by oxytocin, and time spent crouching over pups positively correlates with oxytocin receptor density in the NAc; accordingly, non-monogamous rodents display very low baseline oxytocin binding in the NAc (Olazabal & Young, 2006). Oxytocin is also involved in instigating healthy attachment behaviours and maternal odour conditioning in mice pups (Nelson, Eric & Panksepp, 1996). The quiescent force of oxytocin is clear from oxytocin knockout pups that fail to show separation distress and have a reduced inclination to crawl to the mother (Insel & Shapiro, 1992). Similarly, mu-opoid knockout infant mice have reduced ultrasonic vocalisations and preference for mother's scent upon separation, suggesting opioid systems interact with oxytocin and underlie infant bonding (Moles, Kieffer, & D'Amato, 2004).

Maternal behaviour is also influenced by mesolimbic dopamine transmission (Francis et al., 2000; Takayanagi et al., 2005). Although dopamine was originally thought to be solely responsible for facilitating partner preferences in monogamous prairie voles, both dopamine and oxytocin together drive pair-bonding via stimulation of accumbal D2 receptors (Aragona et al., 2006). Whilst dopamine is also released in the NAc of nonmonogamous rodents (Mermelstein & Becker, 1995), accumbal oxytocin receptor densities are low (Love, 2014), and oxytocin has been identified as necessary to bond formation. Oxytocin antagonism prevents dopamine-induced pair-bonds and supports the current view that pair-bonding is mostly regulated by interactions between oxytocinergic and dopaminergic systems (Liu & Wang, 2003; Young et al., 2001), with blockade of either chemical, abolishing pair-bond formation. Oxytocin's effects on defensive maternal aggression are unclear. Oxytocin peaks during lactation, leading to the suggestion that oxytocin regulates this behaviour (Love, 2014). However, the neuropeptide prolactin also increases in response to suckling, and prolactin inhibits D2 receptor functioning. Interestingly, prolactin has been found to be higher in expectant fathers compared to un-mated males, and so may also play a role in pair-bonding induced aggression (Nelson, 2011).

There is some evidence that oxytocinergic behaviour may be sex dependent. Whilst microinjection of an oxytocin receptor antagonist into the NAc prevents bonding in female prairie voles, a vasopressin receptor antagonist in the ventral pallidum blocks male pair-bonding (Lim & Young, 2006; Young et al., 2001). The concentration of oxytocin receptors in the NAc also determines the speed of female partner preference formation in monogamous voles (Ross et al., 2009). In juvenile female rats, endogenous but not exogenous oxytocin enhances short-term olfactory memory with an oxytocin antagonist producing amnesia (Engelmann, Mario, Ebner, Wotjak, & Landgraf, 1998), whereas in male rats, oxytocin dose-dependently promotes social memory (Dluzen et al., 1998). Englemann et al. (1998) proposed that during ontogeny, sexually dimorphic development results in male territorial behaviour mediated by AVP, and exogenous oxytocin reinstates social behaviours in a system dominated by AVP. This theory is supported by evidence that castrated male rats, and intact male rats treated with AVP antagonist, both show female social recognition behaviour (Bluthé, Schoenen, & Dantzer, 1990; Bluthé & Dantzer, 1992).

Sociality has been shown in rodents to have biological underpinnings related to oxytocin. In the brain reward areas of promiscuous and antisocial montane voles the quantity of oxytocin receptors is low (Love, 2014) and during the mating season, oxytocin receptor density increases to facilitate the social interactions necessary to create a partner preference and breed. However, in the monogamous and social prairie vole, increasing oxytocin receptors density alone does not facilitate partner preference, nor stimulate alloparental behaviour (Ross et al., 2009) and blocking oxytocin receptors prevents pair bonding (Insel & Hulihan, 1995). In monogamous voles, per contra, oxytocin can induce partner preferences in the absence of mating when injected into the NAc or delivered intracerebroventricularly, suggesting an affiliative role for oxytocin in partner preference (Cho, DeVries, Williams, & Carter, 1999).

Although sociality in a number of animal species is associated with increased oxytocin signalling (Rosenblum et al., 2002), oxytocin's effects may not be uniform across and within species. In rodents, exogenous oxytocin induces affiliative behaviour such as approach and social contact (Witt, Winslow, & Insel, 1992), but specific regional distribution of oxytocin receptors within the brain is also vital (Young et al., 2001). Postpartum, the pattern of oxytocin receptor distribution in the antisocial and non-monogamous montane vole matches to that of the social and monogamous prairie vole, and maternal care is observed (Insel & Shapiro, 1992). Human oxytocin receptors are located in different areas from voles, though, (Loup, Tribollet, Dubois-Dauphin, & Dreifuss, 1991) raising the issue of the generalisability to humans of animal research. For example, in humans, oxytocin's negative social effects are greatest in individuals who are chronically predisposed to view the social milieu in uncertain or negative terms (Olff et al., 2013).

Whilst oxytocin's effects on memory are not yet fully known, and anomalies remain unexplained, current opinion favours a nuanced role in social memory functioning. Transgenic mice with no oxytocin production and oxytocin receptor knockout mice that have intact olfaction, learning, and memory, are unable to recognise familiar conspecifics despite repeated exposure (Choleris et al., 2003; Ferguson et al., 2000; Nishimori et al., 2008)—a deficit restored by oxytocin injection before, but not after, a social encounter (Ferguson, Aldag, Insel, & Young, 2001). However, although the oxytocin receptors in accessory olfactory regions are thought to mediate social recognition in rodents, olfactory oxytocin receptors have not been found in humans (Ferguson et al., 2000), so rodent social memory research may have a limited relevance to humans.

The emergence of cognitive neuroscience of social behaviour has spawned arguments that aspects of our sociality, such as attraction and theory of mind, are the defining behavioural aspect of humans (Adolphs, 2003). However, sociality is not monolithic and studies of human social bonds address different aspects of affiliation such as trust (Baumgartner, Heinrichs, Vonlanthen, Fischbacher, & Fehr, 2008; Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005), prosociality (Naber, van Ijzendoorn, Deschamps, van Engeland, & Bakermans-Kranenburg, 2010; Zak, Stanton, & Ahmadi, 2007), and social cognition (Kirsch et al., 2005). In psychology, prosocial behaviour is voluntary behaviour intended to benefit another (Eisenberg, Fabes, & Spinrad, 2007), and many studies claim oxytocin is a "prosocial peptide". However, whilst oxytocin does promote prosociality within certain contexts, it is not specifically prosocial, and can predict hostile behaviour in mothers protecting their young or in normally social male prairie voles that, after pair-bonding, attack unfamiliar conspecifics, including other receptive females (Shamay-Tsoory et al., 2009). It seems studies claiming oxytocin has prosocial effects, such as increased trust and closeness towards others, are, in fact, misusing the term to represent empathogenic behaviour (see McGregor & Bowen, 2012). The function of oxytocin is better defined as promoting social receptivity and enhancement of sensitivity to the salience of social cues, alongside the promotion of defensive behaviour to safeguard such relationships.

Oxytocin may also contribute to shifting attributions of motivational value toward social stimuli (Love,

2014) and has been posited to mediate the empathogenic effects of 3,4-methylenedioxymethamphetamine (MDMA) via the 5-HT_{1A} receptors (McGregor & Bowen, 2012). Although an increase in expression of dopamine D1 receptors in the NAc mediates aggressive behaviour and blocks bond formation with unfamiliar females, oxytocin is thought to generate a shift in value assignment towards strangers, probably via interactions with D1 receptors (Love, 2014).

1.7 Motivated Behaviours and Oxytocin

Although hormone systems underlie goal-directed behaviour (Veening & Barendregt, 2010), with central paracrine transmission coordinating and influencing neuronal activity in multiple brain regions (Veening & Barendregt, 2010), mesolimbic dopamine is thought to code for the motivation to attend to and consume rewards (Sabatier, Leng, & Menzies, 2014). Long, mostly unmyelinated, neuropeptidergic fibre systems that lack synaptic contact are found in behaviour-relevant brain areas, so are, thus, in a favourable position to alter neural network weight from one behavioural state to another (Veening & Barendregt, 2010). Although the neural mechanisms of oxytocin's effects on motivated behaviours are not fully known, oxytocin receptors are liberally distributed throughout mesocorticolimbic dopamine circuits, and it is theorised that central oxytocin influences a wide range of motivated behaviours by increasing the salience of social cues (Averbeck, 2010; Young, 2013). Other evidence, though, indicates a more nuanced role for oxytocin in modulating motivated behaviours, as oxytocin increases the appetitive valence of sexual behaviour (Love, 2014). However, the potential conflict of oxytocin's opposing effects could be explained by the inherently affiliative nature of sexual behaviour, which is not a quality of other motivated behaviours in oxytocin studies.

1.7.1 Romantic Love

Pair-bonding has been likened to romantic relations in humans (Carter, 1998); however, a paucity of studies has investigated neural correlates of romantic love (Love, 2014). Romantic love has been characterised as a primary drive and motivational state focused on a biological instinct to reproduce (Pfaff, 1999), which is distinct from sex drive (Aron, A. et al., 2005b). Neuroimaging studies show that the brain regions activated human sexual arousal differ from the areas activated by romantic love (Aron, E. & Aron, 2014; Fisher, 1998). Romantic love has been described as having a "cocaine effect" that produces exhilaration, excessive energy, sleeplessness and loss of appetite (Fisher, Brown, Aron, Strong, & Mashek, 2010). Consistent with this, key dopaminergic sites, such as the right VTA, ventral pallidum, and right caudate nucleus, are involved with the coding of romantic love in those self-identifying as strongly in love (Aron, A. et al., 2005b; Fisher, Aron, & Brown, 2005), which accords with areas identified in animal studies that promote pair bonding (Liu & Wang, 2003). Desire for chocolate reward diminishes in proportion to the degree to which individuals self-identify as being in love (Small, 2001). Bartels and Zeki (2000) also demonstrated an overlap between romantic and maternal love, which activates comparable brain areas to those mobilised by romantic relationships. Apart from dopamine, other neurochemicals promoting romantic passion and bonding have been identified as phenylethylamine, 5-HT and oxytocin (Fisher, Aron, Mashek, Li, & Brown, 2002). The

reward sites involved in coding for romantic love are rich in oxytocin receptors, which are thought to play a central role in motivational and learning processes involved in romantic attachment (Zeki, 2007). The role of oxytocin in later romantic love phases is not yet well characterised, although the balance of dopamine reception is believed to shift from D2 to D1 receptors (Tops et al., 2014).

Individuals with high romantic attachment anxiety have higher plasma oxytocin levels than those with low romantic attachment anxiety (Marazziti et al., 2006). This finding is consistent with a study on social anxiety that found higher levels of social anxiety were associated with higher plasma oxytocin (Hoge, Pollack, Kaufman, Zak, & Simon, 2008). However, raised plasma oxytocin has been observed in new couples and found to predict relationship success six months later (Schneiderman, Zagoory-Sharon, Leckman, & Feldman, 2012). Whilst romantic love seems to be a universally potent mental state (Jankowiak & Fischer, 1992), individual differences in endogenous oxytocin and reactions to exogenous oxytocin have been identified (Buisman-Pijlman et al., 2014; Engelmann, Mario, Ebner, Wotjak, & Landgraf, 1998; Tops et al., 2014; Wismer Fries, Ziegler, Kurian, Jacoris, & Pollak, 2005). The highest endogenous oxytocin levels occur in conative dyadic mother-infant and romantic, or pair-bonds: relationships of equal 'Darwinian' evolutionary importance and, thus, phylogenetically similar shaping of their neural reward organisation. New romantic relationships result in high oxytocin levels (Bartels & Zeki, 2000; Love, 2014; Marazziti & Canale, 2004; Zeki, 2007), and intranasal oxytocin increases both reciprocity between consorting partners (Schneiderman, Zagoory-Sharon, Leckman, & Feldman, 2012) and positive communication during couple conflict (Ditzen et al., 2009). Romantic preference formation for people encountered after oxytocin administration has not been demonstrated, oxytocin did induce participants to find out more information about strangers relative to placebo (Liu, Guastella, & Dadds, 2013). Intranasal oxytocin caused avoidance behaviour towards attractive females in heterosexual monogamous men but had no effect on heterosexual single men in a series of experiments (Scheele et al., 2012), and had empathogenic effects on individuals with borderline personality disorders, but not controls (Bartz, Zaki, Bolger, & Ochsner, 2011).

1.7.2 Substance Misuse

Oxytocin has three main effects on addictive drug use: it reduces appetitive motivation, alters consummatory experiences, and inhibits certain side effects (McGregor & Bowen, 2012). Although research is limited, oxytocin has been shown to diminish the motivational drive and hedonic experience associated with the use of stimulants, opiates, empathogenics, marijuana, and depressants in animals (Kovács, Sarnyai, & Szabó, 1998). In humans, intranasal oxytocin has had drug-dependent effects on a range of drug-taking behaviours, withdrawal, and tolerance. It has reduced the craving for marijuana and cocaine in dependent adults (McRae-Clark, Baker, Moran-Santa Maria, & Brady, 2013; Stauffer et al., 2016) and reduced tolerance to cocaine (Stauffer et al., 2016). In animals, oxytocin attenuates alcohol reward, craving, and symptoms of withdrawal (Jodogne, Tirelli, Klingbiel, & Legros, 1991; Puciklowski, Kostowski, & Trzaskowska, 1985; Rigter, Dortmans, & Crabbe Jr, 1980; Szabó, Kovács, Székeli, & Telegdy, 1985), but in humans only oxytocin's reduction in alcohol's side effects has so far been reported (Pedersen et al., 2013). Preclinical

studies have reported that oxytocin inhibits dopamine in the ventral striatum to reduce drug reward (McGregor & Bowen, 2012) and a number of studies have now demonstrated oxytocin's attenuation of methamphetamine-induced dopaminergic activity in reward areas. For example, Qi et al (2008) found that oxytocin reduces accumbal dopamine efflux, whereas Baracz and Cornish (2013) demonstrated the same result in the subthalamic nucleus. Carson et al. (2010) showed that oxytocin significantly diminishes Fos expression in the accumbens core, and Lee et al. (2005) reported that the oxytocin agonist lithium, also prevents Fos expression in the NAc.

A striking illustration of oxytocin's inhibition of reward motivation is provided by rat dams, high in postpartum oxytocin, that exhibit preferences for cues associated with pups over those associated with drugs of abuse and prefer suckling infants to cocaine (Ferris et al., 2005). Furthermore, oxytocin activates the dopamine, serotonin (5-HT), and opioid neuromodulatory systems involved in the shift from ventral to dorsal striatal control seen in addiction (Tops et al., 2014). This oxytocin-induced change involves D1-like receptors becoming dominant targets over D2 receptors in the ventral striatum (Tops et al., 2014). The oxytocin-driven switch to D1 receptor dominance has been theorised to foster increased attachment, familiarity-processing and socialisation, all of which reduce stress and addiction, whereas ventral dopamine is associated reward-driven activity and novelty (Tops et al., 2014).

1.8 Eating

1.8.1 Psychobiological Aspects of Eating

Eating is a natural reward and motivation to eat does not rely on conditioning or reinforcement. Interoceptive signals motivate eating by stimulating reward pathways; in this sense, all eating can be characterised as reward driven. However, eating not initiated by deprivation-induced hunger (reward-driven eating or "hedonic eating") occurs in the absence of an immediate biological imperative. Hunger signals that constitute this biological imperative can themselves be learned behaviours. In animals, the secretion of pre- and postprandial ghrelin that signals hunger, is triggered by anticipation of a meal as well as calorie deficit (Drazen, Vahl, D'alessio, Seeley, & Woods, 2006; Teff, 2006). A range of non-homeostatic stimuli can facilitate eating. Both positive and negative affective states can prompt eating and are susceptible to experimental manipulation (Köster & Mojet, 2015; Macht & Mueller, 2007; Macht, 2008), situational cues, such as group facilitation of eating (De Castro & Elmore, 1988; De Castro, Brewer, Elmore, & Orozco, 1990; De Castro, 1997), or appetite odorants (Bragulat et al., 2010), stress (Adam & Epel, 2007), and impression management (Robinson, Eric, Kersbergen, Brunstrom, & Field, 2014). There is also some evidence to suggest a genetic component to hunger (De Castro & Lilenfeld, 2005).

1.8.2 Disordered Eating

Disordered eating takes many forms, which themselves may have different aetiologies. Oxytocin is currently being researched both as an agent to reduce food cravings and motivation to binge, and to reduce anxiety and promote sociality in people with anorexia. There are four common umbrella categories under which

abnormal eaters fall: people with bulimia, anorexia, pica, and binge eating. Eating disorders have been classified as serious mental psychiatric illnesses and individuals with eating disorders can exhibit a combination of decreased sensitivity to homeostatic control and motivating factors unrelated to appetite (Gale, Castracane, & Mantzoros, 2004; Klump, Bulik, Kaye, Treasure, & Tyson, 2009; Polivy & Herman, 2002). A distortion of these homeostatic systems arising in restrained eaters, stress eaters, and some obese individuals is evident (Stroebe, Papies, & Aarts, 2008; Yau & Potenza, 2013). Palatable foods can also cause disordered eating, and sugar can be as motivationally potent as some drugs of abuse (Ahmed, Guillem, & Vandaele, 2013). Oxytocin's effectiveness for both these disorders suggests that one possible mechanism through which oxytocin achieves its effects is to adjust reward systems to enhance motivation for the social domain.

1.8.2.1 Disorders of Overeating

All disordered overeating is characterised by a loss of control over consummatory habits that can range from infrequent, as in disinhibited eating episodes in restrained eaters, to near-continual behaviour cycles and potentially life-threatening syndromes (Yagi et al., 2012). There is evidence that episodes of fasting and acute overeating cause cravings that are difficult to control (Moreno-Domínguez, Rodríguez-Ruiz, Fernández-Santaella, Ortega-Roldán, & Cepeda-Benito, 2012; Stice, Eric, Davis, Miller, & Marti, 2008). In typical bulimia, individuals purge after food consumption and this group includes a range of individuals, from those who purge infrequently to those who binge and purge continually and report uncontrollable cravings for food. It is likely that homeostatic maintenance of energy needs is lost in prolonged and frequent bulimic behaviour (Torsello et al., 2007), and that a shift in neural reward circuits will have occurred (Avena & Bocarsly, 2012; Frank, Shott, Hagman, & Mittal, 2013; Wagner et al., 2010). Bulimia is often a secretive activity that can result in the decline of social activity as the desire to binge escalates (Cardi, Leppanen, & Treasure, 2015; Jonas, 1990; Reid, 2012).

In common with bulimia, compulsive eating is characterised by uncontrolled binging underpinned by strong desire-driven urges and enhanced pleasure from food (Davis, C. & Carter, 2009). Although there is evidence that stretch receptor signalling is dulled in compulsive eaters, capacity limits still prevent unbroken binging that can occur in bulimia (Davis, 2013; Wang et al., 2001). Ensuing obesity often plunges compulsive eaters into a psychological and physical decline (Jonas, 1990; Marcus & Wildes, 2009). Like compulsive eating, binge eating disorder involves consumption of large quantities of food, usually high calorie foods, but it is episodic and intermittent, so does not necessarily trigger long-term changes to neural reward circuitry that result in addictive compulsions (Davis, 2013).

In the same way that, typically, about forty percent of university students binge drink yet only six percent meet the criteria for alcohol dependence, binge eating disorder does not, necessarily, imply food addiction (O'Malley & Johnston, 2002). In common with substance abuse, environmental associations play an important role in determining binge-related behaviour (Davis & Carter, 2009). Cravings can be contextually

and temporarily triggered or inhibited in individuals. When viewing pictures of food, satiated obese women showed more activation in brain reward areas than normal weight controls (Frankort et al., 2012) and, even when satiated, obese individuals respond more strongly to food cues than lean people (Higgs, 2015).

1.8.2.2 Definitional Difficulties

Within each category of abnormal eating, cases can vary in their severity or form rendering their comparison difficult or meaningless. Potential treatments may differ across subtypes, and umbrella terms are crude instruments for identifying specific patterns of behaviour. The degree and frequency of disordered eating is an especially important issue, as persistent and severe abnormal behaviour is likely to result in long-term neural adaptations, such as hypersensitisation, which reinforce dysfunctional behaviour (Robinson & Berridge, 1993).

Food addiction is regarded by some research teams as a classic substance use disorder (Gearhardt et al., 2011; Ifland et al., 2009) that is driven, at least initially, by pleasure and "serves to motivate an individual to pursue rewards necessary for fitness, yet in modern environments of abundance … induces maladaptive pursuits such as addictions." (Berridge & Kringelbach, 2015). The behaviour of individuals who compulsively overeat and drug abusers is also similar (Gearhardt et al., 2011; Ifland et al., 2009; Long, Blundell, & Finlayson, 2015) and a widely used scale for assessing food addiction is the Yale Food Addiction Scale, based on substance abuse criteria from the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2013). A lack of agreement among the DSM-5 Board of Trustees and DSM-5 Substance-Related Disorders Work Group on the use of the term "addiction" is reflected in the research literature which regards addiction as a general term and dependence as a more specific condition involving tolerance and/or withdrawal (Hasin et al., 2013).

1.8.2.3 Neural Substrates of Overeating

In common with drugs of abuse, eating can become compulsive and has neural correlates similar to substance misuse (Gearhardt et al., 2011). Dopamine signalling is central to the formation of associations between cues and rewards (Schultz, Tremblay, & Hollerman, 1998). In anticipation of palatable food, regions associated with cue-related craving and disinhibition, such as the ACC, striatum, insula, and DLPFC are activated to a greater degree in obese overeaters relative to lean individuals (Gearhardt et al., 2011; Rothemund et al., 2007; Stice, Spoor, Bohon, & Small, 2008; Stoeckel et al., 2008). Activation of dopamine mesolimbic pathways occurs in all cases of substance dependence but is also stimulated by natural rewards, such as eating and drinking (Cannon & Palmiter, 2003). Refined carbohydrates, like drugs of abuse, mobilise mesolimbic and mesocortical pathways (Davis & Carter, 2009). Although dopamine responses to intake of food in obese overeaters have been shown to reflect dopamine responses in substance users (Marcus & Wildes, 2009; Volkow et al., 2010; Volkow et al., 2006; Wang et al., 2001), it is difficult to draw conclusions about the significance of this, as the role of dopamine in reward remains unclear (Berridge & Robinson, 1998).

The original hedonic hypothesis of dopamine encoding is no longer tenable (Love, 2014). Robinson and Berridge (1993) showed that large fluctuations of dopamine in the NAc did not affect hedonic pleasure and argued that the neural states of 'liking' and 'wanting' can be separated. 'Liking'-with quotation marks-is characterised as behavioural and encephalic reactions to hedonic impact, in contrast to subjective liking, which does not necessarily coincide with core 'liking' reactions (Berridge & Kringelbach, 2015). Similarly, 'wanting' (in quotes) is a mesolimbically active motivational process of incentive salience, distinct from conscious wanting and driven by dopamine (Berridge & Kringelbach, 2015). Although the states of 'wanting' and 'liking', which dominate the initial appetitive and subsequent consummatory phases respectively, can be characterised as distinct from each other, the extent of their neural separateness is not yet fully established (Berridge & Kringelbach, 2015). Classical and instrumental learning, together with cognitive representations, are also important central processes that drive addictive behaviour; such processes are not limited to consummatory or appetitive phases and occur at any stage of the reward cycle (Berridge & Kringelbach, 2015). Bromberg-Martin et al. (2010) propose that contradictory roles of dopamine can be explained by distinct populations of neurons that respond to unexpected events, appetitive and aversive salient events, and motivational value, resulting in excitation to appetitive stimuli and inhibition to aversive stimuli.

Affective taste reactions in rodents, such as licking patterns, orofacial expressions, and the voluntary consumption of food, closely reflect how much the taste is liked (Kelley et al., 2002). This is not always the case with general affective reactions, which may not rely on the degree to which something is liked (Berridge & Kringelbach, 2015). Consummatory reactions, therefore, are heterogeneous and insights into one form of hedonic impact may not generalise to other forms (Berridge & Kringelbach, 2015). Additionally, subjective ratings of hedonic impact depend on individual past experience and induce subjective experiences that differ from objective measures (Berridge & Kringelbach, 2015). Presently, brain imaging techniques are too limited in spatial or temporal resolution to examine the complex neural encodings of pleasure, and attendant confounds, such as the unique movement patterns associated with particular rewards (Berridge & Kringelbach, 2015). The general areas that have so far been identified as responsible for pleasure and its consummatory reactions are the mid-anterior orbitofrontal cortex, which monitors and predicts pleasure, the insula cortex, and the ventromedial regions of the prefrontal cortex (Berridge & Kringelbach, 2015). In spite of the differences in subjective pleasure experiences and heterogeneity of pleasure reactions, the same regions encode all types of pleasure, and there is extensive overlap among the different processes that mediate reward (Berridge & Kringelbach, 2015). Operational difficulties have made replicating the results of these animal studies in humans difficult and a recent review has proposed that expected pleasantness also needs to be accounted for (Pool, Sennwald, Delplanque, Brosch, & Sander, 2016). While opioid activation results from all food intake, palatable food generates especially powerful opioid responses, particularly with foods that are already preferred (Ackroff, Yiin, & Sclafani, 2010; Barbano & Cador, 2007; Oliveira-Maia et al., 2011)

1.8.2.4 Effects of Eating Disorders on Oxytocin

Two studies have found that underweight people with anorexia secrete less oxytocin than normal weight individuals both in plasma and CSF (Chiodera, Volpi et al., 1991; Demitrack et al., 1990). As oxytocin is released during eating, reduced levels in individuals who undereat might be expected. Although less oxytocin is released in undereaters, the studies do not explain why tonic oxytocin levels might also be reduced. The two studies have small sample sizes, and the conclusions reached that low weight triggers inhibition of oxytocin might be premature as control studies that eliminate noise variables such as declining sociality in people with anorexia and examine the effects of fasting or starvation on non-disordered eaters are required.

1.8.3 Eating and Oxytocin

Oxytocin is an anorexiant (Olszewski, Klockars, Schiöth, & Levine, 2010) with multifarious hypophagic controls, including homeostatic satiety-related signalling between the peripheral and central nervous system, metabolic modulation, SON metabolic feedback, and the reduction of feeding reward. The topography of oxytocin neurons reflects feeding-related functions (Olszewski et al., 2010). In all animals, the hypothalamus controls autonomic functioning and endocrine demands, together with regulating energy balance. The hypothalamic paraventricular nucleus (PVN) is pivotal to metabolism and a target for many neurons involved in appetite regulation, such as ghrelin- and leptin-receptive neurons of the arcuate nucleus, neuropeptide Y and agouti-related peptide, as well as cocaine- and amphetamine-regulating transcript, and α-melanocyte-stimulating hormone (Valassi, Scacchi, & Cavagnini, 2008). Parvocellular oxytocin fibres extend from the PVN to the brainstem for reciprocal peripheral-central nervous system exchange, which alters the pattern of vagally controlled gastrointestinal hormones (Fuxe et al., 2012), and to midbrain and limbic reward areas (Baracz & Cornish, 2013). Bidirectional connections from the PVN to ventromedial and other hypothalamic regions maintain homeostatic control of feeding alongside dendritic extracellular release (Sabatier, 2014). Magnocellular oxytocin neurons of the PVN and SON have a key role in appetite regulation but are co-localised with a number of other anorexigenic factors, such as cholecystokinin that acts on magnocellular cells to release oxytocin from the posterior pituitary gland (Sabatier et al., 2014; Vanderhaeghen, Lotstra, Vandesande, & Dierickx, 1981).

The metabolic hormone leptin is produced in adipose tissue and circulating levels regulate appetite via the melanocortin pathway by activating a sub-population of oxytocin neurons in the PVN (Blevins, Schwartz, & Baskin, 2004; Friedman, 2009), and by modulating mesolimbic dopamine through leptin receptors expressed in the VTA (Figlewicz, Evans, Murphy, Hoen, & Baskin, 2003). Accordingly, leptin infusions into the rat VTA alone decrease food intake (Hommel et al., 2006). However, obesity elevates leptin, so it is unlikely that leptin alone is sufficient in inhibiting hedonic eating. Sustained elevation of leptin in obesity results in reduced brain sensitivity to leptin—leptin resistance—leading to detrimental effects on appetite control (Kettner et al., 2015). The lack of a leptin ‘brake’ can be overcome by oxytocin-sensitive neurons in the

hindbrain that suppress appetite (Morton et al., 2012), as seen by the anorexigenic effects of ICV oxytocin in leptin-resistant Zucker fatty rats. Conversely, blockade of oxytocin receptors in mice results in hyperphagia (Kublaoui, Gemelli, Tolson, Wang, & Zinn, 2008).

Cholecystokinin (CCK), a peptide hormone of satiety, is produced in the duodenum and the general enteric nervous system and evokes central oxytocin release via the vagal nerve (Carter & Lightman, 1987b; Olson, Beatriz, Hoffman, Sved, Stricker, & Verbalis, 1992; Renaud, Tang, McCann, Stricker, & Verbalis, 1987; Verbalis, Stricker, Robinson, & Hoffman, 1991; Verbalis, McCann, McHale, & Stricker, 1986). Together, peripheral and central CCK stimulate the digestion of protein and fat in the small intestine, and signal satiety, milk ejection, and maternal behaviours, presumably via downstream oxytocin engagement (Little, Horowitz, & Feinle-Bisset, 2005; Uvnäs-Moberg, Björkstrand, Hillegaart, & Ahlenius, 1999). In the reverse situation when no food is present in the intestinal tract, production and ejection of milk is blocked (Uvnäs-Moberg et al., 1999). In young male rats, non-gravid female rats, and obese rats, CCK hypophagic effects are reduced (Fink, Rex, Voits, & Voigt, 1998). One study found raised plasma CCK following intranasal oxytocin suggesting a reciprocal oxytocin-CCK mechanism (Hienel et al., 2001) but inhibition of feeding with CCK has also been demonstrated in oxytocin null mice (Mantella, Rinaman, Vollmer, & Amico, 2003), suggesting that oxytocin-CCK pathways are not mandatory in attenuating food consumption.

As expected, exogenous oxytocin is an effective anorectic both via systemic administration (Arletti, Benelli, & Bertolini, 1990; Arletti et al., 1989; Benelli, Bertolini, & Arletti, 1991; Benelli et al., 1994) and intracerebroventricular routes (Olson, B. R., Drutarosky, Stricker, & Verbalis, 1991), which is reversed by an antagonist (Arletti et al., 1989; Maejima et al., 2011; Zhang, G. & Cai, 2011). Manipulating oxytocin in the rat subthalamic nucleus also reduces food intake (Fuxe et al., 2012). One study has linked compulsive eating to abnormal EEG activity and reported that the anti-convulsive drug phenytoin resulted in behavioural improvements in the compulsive eaters (Rau, Struve, & Green, 1979). Phenytoin is an anti-convulsant that acts mainly on the motor cortex, but also reduces potassium-induced neurophysial release of oxytocin in vitro (Mittler & Glick, 1972). Phenytoin's mechanism of action in compulsive eaters, therefore, remains unknown; further, the study itself suffered from no control group, selection bias and poor experimental controls, which raise questions over its validity.

The links between obesity, appetite, and oxytocin are reinforced by genetics. For example, the number and size of PVN oxytocin neurons are reduced in obese hyperphagics with the genetic Prader-Willi syndrome (Swaab, Purba, & Hofman, 1995). Similarly, mice with haploinsufficiency of the single-minded 1 gene that influences PVN development, are susceptible to diet-induced obesity and develop extreme hyperphagia. (Kublaoui et al., 2008). However, knocking out the single-minded 1 gene also reduces the expression of melanocortin-4 receptors, which may promote obesity independent of the oxytocin system (Fani, Bak, Delhanty, van Rossum, & van den Akker, 2014). Expression of the synaptotagmin gene results in a synaptotagmin-4, a protein that negatively regulates oxytocin release by exocytosis in the PVN. Dietary

obesity is associated with increased vesicle binding of synaptotagmin-4 in oxytocin neurons and mice overexpressing synaptotagmin-4 are obesogenic in contrast to mice deficient in synaptotagmin-4 that are resistant to diet-induced obesity (Zhang, Guo et al., 2011).

Toxins, hormones, and dehydration are powerful influences on ingestive behaviour that give rise to the pituitary excretion of oxytocin, suggesting that oxytocin has an important generalised anorexigenic function irrespective of the initial trigger or caloric homeostasis (Olson et al., 1991). Given oxytocin's wide-ranging metabolic involvement, it is unsurprising that oxytocin neurons are themselves the target of most satiety-related pathways (Olszewski & Levine, 2007). This interactive milieu is reflected by oxytocin's co-ordination between enteric and central nervous systems.

The satiety signals from lithium chloride, gastric distension, and hyperosmolality all induce oxytocin release and are inversely proportional to food intake (Flanagan, Verbalis, & Stricker, 1989; Holmes et al., 2013; Olson et al., 1991; Verbalis, Blackburn, Olson, & Stricker, 1993). One study concluded that oxytocin is a central neuromodulator of food intake, most particularly noncaloric-induced mediation, such as occurs in illness, stress, or from noxious stimuli via chemoreceptors in the area postrema of the brainstem that detect toxins in the circulation (Olson et al., 1991).

The anorectic effects of chronic oxytocin administration have not been widely studied. However, intracerebroventricular (ICV) injections of oxytocin agonist (e-L-β-MePhe^2)OT, resulted in tolerance to oxytocin's inhibition of feeding in rats and by day three food intake returned to baseline levels (Olson, Beatriz R. et al., 1991). In contrast, chronic oxytocin across a four-week period reduces body mass in diet-induced obese rhesus monkeys without tolerance developing by decreasing food intake, elevating energy expenditure, and lipolysis (Blevins et al., 2014). The development of tolerance could be attributable to the properties of the agonist used in the mice experiment or might have occurred because mice were not overweight and were eating standard chow, so a continued drop in food intake and weight loss would have resulted in adverse health consequences, and conservation or survival motivation became dominant. In another study examining chronic oxytocin effects on fear in mice across five days, no tolerance was reported and reductions in body mass were not noted, suggesting that the agonist was effective, and that oxytocin does not cause organisms to lose weight beyond their limits (Pisansky, Hanson, Gottesman, & Gewirtz, 2017). In obese humans, (Zhang, Hai et al., 2013) chronic oxytocin over an 8-week period reversed obesity and prediabetic changes.

Hormones that regulate eating also interact with oxytocin. Elevated circulating levels of leptin exert a long-term anorectic influence, partly via moderation of acute gastrointestinal satiety pathways (Ahima & Osei, 2004; Valassi et al., 2008), but also by stimulating both magnocellular SON and parvocellular PVN oxytocin neurons (Perello & Raingo, 2013; Velmurugan, Russell, & Leng, 2013). There is no evidence that oxytocin stimulates leptin in the reverse direction, though (Chaves, Fau, Fau, & Brito, 2013). Whilst there is a

spectrum of sensitivity to leptin across strains of rodents, obese rodents fed a cafeteria-style diet are minimally responsive to leptin, or leptin resistant—a condition commonly seen in obese humans. In fact, Friedman (2009) claimed that most obese individuals are leptin resistant: they have high levels of circulating leptin but an absence of evident hormone efficaciousness. This suggests that western diets, and not adipose tissue mass alone, influence leptin functioning. Consistent with this a study by Morton et al. (2012) showed that oxytocin still exerts sustained hypophagia and weight loss in rats without leptin receptors. Ghrelin signals hunger and suppresses hypothalamic oxytocin function. Two studies examining the effects of oxytocin on serum ghrelin in men reported opposite results (Coiro et al., 2008; Vila et al., 2009). However, oxytocin was administered intravenously in one study and intranasally in the other, with different assay methods also undertaken in each, so direct comparison of the effects of oxytocin on ghrelin is not possible.

Bottom-up vagal feedback may also be involved in regulating the oxytocin responses to fasting. In rats, oxytocin mRNA in the paraventricular nucleus decreases in response to fasting and is restored upon refeeding, and a similar decrease of oxytocin has been observed in people with anorexia, which increases upon leptin-mediated weight restoration (Kublaoui et al., 2008). According to Verbalis et al. (1993), central oxytocin inhibition of food and salt ingestion—a mechanism for intake regulation of solute homeostasis—did not find peripheral oxytocin reduced sodium intake, so a potential peripheral oxytocin feedback loop is not likely to be generalised.

1.8.3.1 Metabolic Effects

Knowledge of oxytocin's interaction with the metabolic system is growing, but conflicts in emerging information have yet to be reconciled. Peripheral and central administration of oxytocin results in weight loss not accounted for through diet, by blocking concomitant reductions in energy expenditure that normally occur with weight loss (Morton et al., 2012; Zhang, G. & Cai, 2011). Conversely, in oxytocin receptor-deficient male mice, catabolic increases have been demonstrated without accompanying alterations to food intake , although, the reverse has been demonstrated in oxytocin receptor knockout male mice that became obese without alterations in food intake (Camerino, 2009; Takayanagi et al., 2008). During calorie-demanding lactation, efferent vagal activation driven by central oxytocin activity also results in energy conservation by the inhibition of anabolic metabolic processes (Uvnäs-Moberg, 1994). Alongside contextual cues, the opposite effects of oxytocin may be explained by dose-dependent actions. For instance, the release of growth hormone, which contributes to glucose regulation by altering carbohydrate metabolism and triggering uptake of lipid and protein, is inhibited by high oxytocin doses and stimulated by low dosage (Björkstrand, Anna-Lena Hulting, & Kerstin Uvnäs-Moberg, 1997). Oxytocin's influence on metabolism also involves the glucocorticosteroid responsive element of the oxytocin gene that influences levels of glucose produced by the adrenal cortex (Herman et al., 2003; Winslow & Insel, 2002). Oxytocinergic metabolic changes depend on which hypothalamic nuclei are activated. As SON oxytocin cells function as metabolic sensors, oxytocin has a second route for direct homeostatic regulation of appetite (Sabatier et al., 2014). On the other hand, parvocellular PVN oxytocin cells are stimulated by cold exposure and connect

postsynaptically to brown adipose tissue via stellate ganglia (Kasahara, Takayanagi, Kawada, Itoi, & Nishimori, 2007). In high fat diet-induced obese mice, chronic peripheral oxytocin injections resulted in an initial period of significantly decreased food intake compared to controls, followed by intake normalisation, without a return to overeating; fat metabolism was also promoted, and glucose tolerance raised (Maejima et al., 2011).

1.8.3.2 Oxytocin and Taste Preferences

Diverse research has demonstrated a role for oxytocin in modulating intake of sweet-tasting food. Three research groups have shown that oxytocin knockout mice show a preference for sweet carbohydrates but not palatable lipid meals (Amico, Vollmer, Cai, Miedlar, & Rinaman, 2005; Billings, Spero, Vollmer, & Amico, 2006; Miedlar, Rinaman, Vollmer, & Amico, 2007), suggesting that oxytocin inhibits the intake of sweet-tasting food. This finding is supported by Sinclair et al. (2015) who demonstrated that oxytocin reduces sensitivity to sweet tastes and that oxytocin receptors are expressed in murine taste buds (Sinclair et al., 2010), and further by Olszsewski, Klockars and Levine (2016) who found that oxytocin neurons are preferentially activated by and inhibit the ingestion of sweet-tasting carbohydrates. In rats, centrally administered oxytocin inhibits rat feeding, particularly for sucrose (Mullis, Kay, & Williams, 2013; Noble, Billington, Kotz, & Wang, 2014); scheduled feeding of a high sucrose diet increases oxytocin gene expression (Olszewski et al., 2009); and inhibition of the PVN increases motivation to obtain sugar (Atasoy, Betley, Su, & Sternson, 2012). Furthermore, oxytocin knockout mice have a greater preference for sucrose and sweet-tastes, consume significantly more of sweet-tasting food than lipid-based food, and develop late-onset obesity (Amico et al., 2005; Billings et al., 2006; Miedlar, 2007; Miedlar et al., 2007; Nishimori et al., 2008; Sclafani, Rinaman, Vollmer, & Amico, 2007; Sinclair et al., 2015). Chronic sucrose consumption downregulates the anorexigenic oxytocin system (Mitra et al., 2010). However, the dampening effect of sucrose on oxytocin neurons reverses at high sugar-intake levels, when oxytocin neurons are thought to act as a homeostatic 'brake' preventing homeostatic imbalances such as an excess osmotic load rather than altering satiation (Mitra et al., 2010). Whilst sustained elevation of sucrose reduces oxytocin production, diet-induced obesity rats respond more robustly to systemic oxytocin, theorised to be due to reduced endogenous oxytocin tone from overfeeding and increased receptor affinity (Deblon et al., 2011; Morton et al., 2012; Zhang et al., 2011). Administration of a hyperphagic dose of the opioid receptor agonist butorphanol tartrate, decreases sucrose intake-driven activity of oxytocin neurons (Olszewski, Shi, Billington, & Levine, 2000).

Although a growing body of research suggests that the oxytocin system is sensitive to sweet-tasting carbohydrates, oxytocin gene-deletion mice also overconsume non-sweet carbohydrate solutions (Sclafani et al., 2007), implicating oxytocin in broader macro-nutrient specific regulation. The relationship between macronutrients and oxytocin is poorly understood. Whilst consumption of sweet-tasting products is associated with oxytocin depression, high fat liquid formulations that are known to alter striatal activity and induce withdrawal symptoms, do not bring about oxytocin deficits (Miedlar et al., 2007; Teegarden & Bale,

2007). However, attributing these reward-system changes and withdrawal effects to fat alone as Lutter and Nestler (2009), and Epstein and Shaham (2010) do, may be misleading because the effects of fat in the rodent studies these authors cite—Teegarden & Bale (2007) and Cottone et al. (2009), respectively—are confounded by inclusion of sucrose in the high-fat food pellets. In the Cottone et al. (2009) study the diet only contained 12.7% fat with 50% of the calories from sucrose. In the Teegarden and Bale experiment rats were fed 17% sucrose and 39% fat mixed with corn starch–which is converted to sugar after digestion (Barclay, Brand-Miller, & Wolever, 2005)–and maltodextrin that has a glycaemic index double that of sucrose (Whelan, 2004). Kenny demonstrated that rats fed on fat alone would eat very limited amounts, and rats fed on sugar alone would do similarly (Johnson & Kenny, 2010; Tulleken, 2014), whereas a fat-sugar combination produced extreme over-consumption. It is noteworthy that fat pellets containing sucrose did not alter oxytocin systems. One explanation could be that the pellets were not perceived as sweet; this suggests that taste buds are involved in signalling and there is evidence that food reward can be provoked in the absence of taste receptor signalling (de Araujo et al., 2008). In obese humans, palatable energy-dense foods trigger different areas of the limbic system from normal weight individuals, suggesting that neither fat nor sugar alone triggers over-consumption, rather it is palatability or net energy load (Stoeckel et al., 2008).

Stress is associated with eating in excess of homeostatic needs (Kelley, Baldo, Pratt, & Will, 2005; Mathes, Brownley, Mo, & Bulik, 2009; Vicennati, Pasqui, Cavazza, Pagotto, & Pasquali, 2009). Corticotropin-releasing factor mediates the withdrawal symptoms of hyper-palatable food (Epstein & Shaham, 2010), a process modulated by oxytocin (Jamieson, Nair, & Iremonger, 2017). Likewise, McGregor and Bowen (2010) showed that exogenous oxytocin in rats prevents stress- and priming-induced relapse to drug seeking.

1.8.4 Oxytocin and Attention to Food

Unsurprisingly, hunger increases the attention paid to food stimuli. However, food stimuli might be sui generis because, in sated individuals, food stimuli generate an initial suppressive response, followed by a positive attentional bias; this attentional bias increased with body mass index (BMI) score (Kumar, Higgs, Rutters, & Humphreys, 2016). In common with people with bulimia, those with anorexia fear food and becoming fat, and food issues dominate daily life with increased awareness and attention to food cues and stimuli. However, acting on compulsive dopamine-driven cravings that people with severe bulimia experience is not part of classical anorexia, and the pleasure derived from food is likely to be subdued in people with anorexia (Tost et al., 2010).

Although self-monitoring alone results in altered eating patterns and weight loss (Burke, Wang, & Sevick, 2011), dietary restrainers self-impose dietary limitations in order to prevent weight gain or lose weight. One experiment comparing dietary restrainers with non-dieters found that when self-imposed limits were exceeded by restrainers, then sensitivity to homeostatic signals was impaired and moderation was abandoned, whereas in nondieters, homeostatic cues were self-limiting and prevented excessive food consumption. Dietary restraint can be defined as the inclination to consciously restrict food intake to prevent

weight gain and relies on high level cognitive control to inhibit appetitive responses to palatable foods (Polivy & Herman, 1985). Food-related stimuli have privileged access to working memory, which means that inhibition of food thoughts might be problematic (Higgs, 2015). Attentional biases towards unhealthy energy dense foods predict hyperphagia and increases in BMI.

1.8.5 Oxytocin and Eating Not Initiated by Deprivation-Induced Hunger

Being essential for survival, eating is a fundamental motivational drive: a natural reward that activates neurological pleasure centres for which a dissociation between homeostatic and nonhomeostatic has been established (Kenny, 2011). Although a distinction between homeostatic and nonhomeostatic eating can be drawn, hunger enhances food reward (LaBar et al., 2001), so the distinction between hedonistic and homeostatic eating is invalid. Unlike the use of drugs of abuse, food consumption cannot be stopped, and the nature of a dependence on hedonic rewards from food means that consuming reduced quantities of palatable food is extremely difficult. Anorexic agents addressing only the homeostatic mechanisms of hunger are unlikely to be effective against hedonic craving that overrides homeostatic signals (Narayanaswami & Dwoskin, 2017). The drive to obtain pleasure from calorie-free saccharin solution was shown to be more powerful than the drive to obtain cocaine in rats (Lenoir, Serre, Cantin, & Ahmed, 2007), highlighting the importance of a solution to the problem of hedonic eating in societies where escaping palatable food is very difficult, if not impossible.

Apart from limbic regulation, cortical control is also involved in motivated behaviour such as the impulsivity and compulsivity that characterise addiction. Although hunger activates midbrain and corticolimbic systems to enhance subsequent eating (LaBar et al., 2001), studies show that just imagining food via visual or olfactory stimuli activates corticolimbic circuits, irrespective of hunger (Bragulat et al., 2010; Schur et al., 2009; Simmons, Martin, & Barsalou, 2005). Such corticolimbic activation is thought to encode the value of specific rewards (Man, Clarke, & Roberts, 2009; Rolls, 2008; Sescousse, Redoute, & Dreher, 2010) and converging lines of research suggest that oxytocin also quells this motivational reward circuit.

The conflation of obesity with food addiction has diluted the strength of the argument for a biologically supported disorder of “food addiction”. Changes in dopaminergic functioning in people with obesity, such as long-term downregulation of accumbal D2 receptors that lead to tolerance and drive pathological dependence, are thought to arise from excess eating (Volkow, Fowler, & Wang, 2002). However, obesity is not a necessary condition for pathological overeating, and very modest increases in daily caloric intake over time result in obesity without the necessity for compulsive behaviour or a lack of control over food; moreover, obesity can result from physiologic dysfunction, such as a congenital lack of leptin (Pelleymounter et al., 1995). Some people with bulimia experience uncontrollable food cravings, and binge for extended periods by purging, thus avoiding obesity but clearly demonstrating compulsive eating behaviour. So, despite arguments that obesity should be considered a brain disorder (Devlin, 2007; Volkow, & O’Brien, 2007), it is clear that obesity alone is not a necessary condition of food “addiction”, and studies

should account for addictive-like eating behaviour that is not based on BMI alone.

Although the concept of food "addiction" is not universally accepted in the scientific literature or, indeed by the American Psychiatric Association, which does not acknowledge it as a psychiatric condition in their Diagnostic and Statistical Manual of Mental Disorders (DSM 5; American Psychiatric Association, 2013). Five pathological components of food "addiction" that persist in the literature were identified by Sussman and Sussman (2011). These components are (1) engagement in the behaviour to achieve appetitive effects; (2) preoccupation with the behaviour; (3) temporary satiation; (4) loss of control; and (5) suffering negative consequences. However, these criteria could also lead to a diagnosis of binge eating. Davis's (2013) research goes further and acknowledges that different overeating patterns involve distinct behaviours. Davis (2013) draws the distinction between food "addicts" and individuals with binge eating disorder by describing differing personality traits, different hedonic responses to food, and different motivations driving eating. In presenting all overeating on a continuum, though, with passive eating and food addiction at either ends, Davis (2013) is suggesting that overeating conditions are different only by degrees, or severity, at the expense of distinct aetiologies: one does not, necessarily, lead to the other. Clearly, then, not all overeaters are compulsive eaters, in much the same way that not all people with bulimia binge compulsively. Frequency and quantity are the two key factors that determine the extent of neural changes, with pathological patterns driving more extreme behaviour.

The motivation to eat, however, can be blocked with consummatory hedonia preserved. Typical neuroleptics, for instance, dampen neural motivation systems by antagonism of striatal D2Rs and partial deactivation of the right VTA, inducing a general motivational anhedonia without loss of hedonic responses (Kirsch, Ronshausen, Mier, & Gallhofer, 2007; Wise, 1982). Such neuroleptics, however, also cause metabolic disturbances which result in weight gain (Popovic et al., 2007). A similar outcome is seen in mice when the dopamine precursor tyrosine hydrolase is deactivated, resulting in dopamine deficiency but no reduction in sucrose or saccharine preference (Cannon & Palmiter, 2003). Conversely, the motivation to eat can be preserved and consumption of preferential foods abolished by opioid receptor agonists infused into the NAc of rats (Kelley, Bless, & Swanson, 1996).

In recent years, oxytocin's inhibitory effect on hedonic eating has been elucidated (Sabatier et al., 2014). Consuming palatable food results in the release of dopamine from the VTA to mesolimbic reward areas, which is theorised to increase both the motivation and subsequent reward associated with consuming pleasurable food, a process in rats inhibited by oxytocin (Sofroniew, 1980; Succu et al., 2008). Oxytocin has been shown to reduce consumption of palatable food in participants free of eating disorders (Ott et al., 2013; Lawson, 2015). However, only the two aforementioned studies have so far been conducted to examine oxytocin's effect on consumption of palatable food, although both found that oxytocin significantly reduces hedonic eating in satiated participants.

Like conditioning paradigms and addictive behaviour, long-term pathogenic consumption of food is context-driven (Davis, R., Freeman, & Garner, 1988; Koob & Volkow, 2010; Nakahara, Itoh, Kawagoe, Takikawa, & Hikosaka, 2004), and, in addition, often a secretive activity. One study has looked at the effects of intranasal oxytocin on people with anorexia and found that it attenuates the attentional bias towards food (Kim et al., 2014). In a follow-up study using food, the same research group found that oxytocin had no effect on food intake in people with anorexia (Kim et al., 2015). However, according to food diaries, females with bulimia did eat significantly less after oxytocin than after placebo, and emotion recognition sensitivity in this group was also improved (Kim et al., 2015). No effect of oxytocin was seen for immediate food intake in the laboratory, but poor study design and the tendency of people with bulimia to eat in private may explain this.

1.9 Summary and Research Questions

The pharmacological aspects of oxytocin are complex, highly interactive, and unclear. With a molecular weight of 1007 Daltons, oxytocin is over the threshold for transfer across lipid cell boundaries, and its ability to be absorbed into cells remains undecided. Similarly, oxytocin's solubility limits its ability to cross the BBB and this action is not established. Factors such as how intact the BBB is, the CVO, and proteolysis, are also important determinants of the central availability of peripherally administered oxytocin. Oxytocin might also enter the CNS via the blood-CSF barrier, or via vagal afferent signals but further research is needed to determine the feasibility of these routes. It is synthesised in parvocellular and magnocellular neurons with divergent downstream effects but the extent of crosstalk between the two systems remains elusive. It is released axonally to disparate brain regions but also released dendritically and somatically into extracellular fluid to act locally, either for self-regulation or for neuromodulation of proximal and/or distal targets.

Experimentally, oxytocin is usually administered intranasally as it can enter the CNS via the nasal cavity, although the mechanism remains speculative. It has a half-life of about 20 minutes in the CNS compared with its short 2-3-minute half-life in plasma and oxytocin responses in these fluids after nasal application do not correspond. The measurement of oxytocin in blood is, therefore, contentious, although widespread.

Oxytocin is a highly interactive peptide and its impact on psychological and behavioural outcomes is partly reliant on its neurochemical interlocutors. Oxytocin receptors are expressed in neurons, notably 5-HT, DA, oestrogen, noradrenaline, and opioid systems. Oxytocin receptors are widely distributed across the brain in limbic brain structures (including the NAc, ventral pallidum and medial preoptic nucleus), the hippocampus, olfactory processing regions (including olfactory nuclei and piriform cortex), the hypothalamus, and the brainstem (including the vestibular nuclei and autonomic centres-including the NTS). Oxytocin's main psychological influences are attention, reward, motivation, social memory, and social bonding. Behaviourally, it is known to reduce food intake and facilitate social contact, empathy, romantic attachment, and alloparental behaviour.

Oxytocin is anxiolytic and a number of central systems contribute independently to achieve this. Oxytocin reduces activity in the HPA axis to limit the release of ACTH and downstream, cortisol. Oxytocin also modulates fear and anxiety responses in the amygdala in a context-dependent way. Oxytocin also interacts with serotonin to reduce anxiety. Physiologically, oxytocin dilates blood vessels to lower blood pressure and reduces heart rate. Given its extensive role in reducing anxiety, some commentators have postulated that many of oxytocin's higher order effects are due to its anxiolysis.

Oxytocin reduces reward for food and increases social reward. The primary pathway that oxytocin modulates to achieve this is the mesocorticolimbic circuit, and oxytocin manipulates the motivation and craving for food at midbrain and limbic sites. Interactions with the opioid pleasure systems are also altered by oxytocin to attenuate the pleasure derived from food consumption. These two aspects of reward that oxytocin influences could be reframed as "wanting" and "liking". Attentional processes are altered by oxytocin, including attentional biases and this also supports its role in reducing food reward.

The anorectic properties of oxytocin are activated not only by perceived food reward but also by physiologic events such as toxins and via interactions with hormones of satiety. Preferential inhibition by oxytocin of sweet-tasting food has emerged from investigations, although effects on salty carbohydrates have also been demonstrated in rodents. Oxytocin also causes postprandial increases of glucose metabolism, possibly by insulin sensitisation. The chronic effects of exogenous oxytocin on food consumption are not yet well studied, but early indications are that oxytocin is well tolerated and effective at reducing caloric intake in humans.

A number of strands of research converge to justify studying oxytocin's effects on overeating. Oxytocin exhibits metabolic control of appetite via strong satiation signals that are independent of potential calorie deficits (Sabatier et al., 2014) but also reduces snacking on palatable food. However, previous human research has not adequately controlled for hunger, so hunger may have contributed to the motivation to snack eat. This thesis will address whether oxytocin still reduces snack eating when adequate controls are in place to minimise deprivation-induced hunger. Previous research into oxytocin's inhibition of palatable food intake has not fully disguised experimental aims and may have introduced bias via a self-selected sample or via demand characteristics. This thesis will correct this by disguising the bogus taste test as one of a number of sham sensory tests to fulfil the cover story that oxytocin's effects on sensory perception are being investigated.

The anorectic capacity of oxytocin has not yet been examined in women. This research will replicate the experimental work in men by investigating oxytocin's effects on snack intake not initiated by deprivation-induced hunger, using an all-female cohort. Given that oxytocin reduces attentional food biases in people with anorexia, this thesis will investigate whether the attentional bias to food is impacted by intranasal oxytocin in normal eaters. As oxytocin also confers increases in sociability and engenders romantic bonds,

the attention to these stimuli will be examined under placebo and oxytocin conditions. Social situations alter eating patterns and are strongly influenced by oxytocin; looking at oxytocin's effects on social eating will be the final research area of this thesis.

CHAPTER 2 - GENERAL METHODS AND MATERIALS

This section provides a rationale for and details of key methods and materials common to the three covert laboratory experiments investigating oxytocin's effects on eating: Experiment 1, investigating oxytocin's anorectic effects in men (Chapter 4); Experiment 2, examining oxytocin's effects on stress eating in females (Chapter 6); and experiment 4 looking at the effects of oxytocin in a social setting (Chapter 8). Experiment 3 of this thesis (Chapter 6) employed a different protocol and full details of the method and materials for that experiment are provided within the chapter itself.

2.1 Ethics and Participant Recruitment

All experiments involving adult human volunteers were approved by the Faculty Ethics Committee at Kingston University in accordance with the principles of the Declaration of Helsinki, 2013. Opportunity samples comprising students from Kingston University who participated in return for course credits, and adults in the local community who were approached directly and met the selection criteria were recruited. Experimental aims were concealed across the three covert experiments by describing them as investigations into oxytocin's sensory effects: first in males, then under stress conditions in a mixed sex sample (primarily female), and finally in a social setting. No upper age limits were stipulated. For laboratory-based experiments, participants were debriefed after the data from the last experiment (the social study) had been collected, to keep the experimental aims of this thesis concealed.

2.1.1 Inclusion and Exclusion Criteria

Inclusion and exclusion criteria were included on an information sheet that each participant was required to read through and confirm compliance to the researcher. All participants were required to be healthy and free from psychoactive drugs, including antidepressants because these might have affected the efficacy of intranasal oxytocin (Dölen et al., 2013). Expression of oxytocin receptors is determined by inflammatory cytokines, and the anti-inflammatory properties of non-steroidal anti-inflammatories (NSAIDs) block trigeminal oxytocin receptors, so use of NSAIDs was not allowed within 12 hours of testing (Yeomans, Angst, Mechanic, & Jacobs, 2013). Only non-smokers and social smokers were allowed, as smokers can control their food intake using cigarettes/vapes and may have altered taste perception (Audrain-McGovern & Benowitz, 2011; Gromysz-Kalkowska, Wojcik, Szubartowska, & Unkiewicz-Winiarczyk, 2002). As diabetic and vegan diets precluded eating the experimental lunch and snacks, these groups were excluded together with individuals following a restrictive diet or suffering from food allergies. Pregnant and breastfeeding women (by self-report) were also excluded from participation due to their altered oxytocin function and sensitivity (Crowley, Parker, Armstrong, Spinolo, & Grosvenor, 1992; Fenelon, Poulain, & Theodosis, 1993; Gimpl & Fahrenholz, 2001; Russell & Leng, 1998; Svennersten-Sjaunja & Olsson, 2005). Altered endogenous oxytocin function is associated with high emotional arousal or stress (e.g. bereavement, financial windfall) so participants reporting such events were excluded (Engelmann, Ebner, Landgraf, Holsboer, &

Wotjak, 1999; Kovacs, 1986). Participants were asked to refrain from alcohol and caffeine for 12 hours before testing. Participants fasted for two hours before the experiment on the day to prevent them being full and unable to eat the sandwiches at lunch: about 15% of solid food is retained in the stomach two hours after a meal (Bowen, 2018). A reminder email to avoid solid food and sugary drink was sent by the researcher to each participant the evening before a session to increase compliance. A record of both the contents and time of each participant's last ingestion of food and drink was made by the researcher, and participants who had not complied were excluded from further participation. To corroborate the sham experimental aims, inclusion criteria also included conditions consistent with the sensory tasks, so individuals who were hard-of-hearing were excluded and participants were required to confirm normal or corrected-to-normal vision.

2.2 Establishing an Effective Protocol

2.2.1 The Use of a "Bogus" Taste Test: The Covert Snack-Eating Test

It was necessary to establish an experimental protocol for studying oxytocin's effects on eating-related variables that facilitated snacking in a laboratory setting. A validated method to assess eating in the absence of deprivation-induced hunger is the covert 'snack test' (Robinson et al., 2017). The test begins with participants being asked to rate the taste of a range of snack foods. After the rating session, the experimenter leaves the participant alone with the snack food under some false pretext and invites the participant to help themselves to the snacks which they are told will be discarded after the experiment. The snack food is weighed before and after the snack test, and any difference in mass constitutes the measure of eating. Despite the pretext of a taste test, the covert snack test has been criticised for the transparency of its aims as studies often do not further disguise the bogus taste test within a cover story (Robinson et al., 2017). In this thesis, participants were told prior to participation that lunch was required to be eaten to "normalise blood sugar levels across participants", the bogus taste test was presented alongside a range of sham sensory tasks, for instance smell tests and touch tests, to provide a credible cover story this was considered particularly important in repeated-measures designs where the purposes of the study might become apparent when participants have two identical sessions to wonder about the experimental aims. Additionally, the experiments were conducted within the general psychology department and supervised by biological psychologists, not a specialist eating laboratory or team. No details of the author's research interests were published internally within the university and no articles were published in journals until after data collection had finished.

A review of 31 experiments that used the bogus taste test to measure snack eating identified measures that predicted subsequent snack intake in the covert test and recommended elements to maximise the effectiveness of the protocol (Robinson et al., 2017). Apart from serving as a covert means of measuring the hedonic component of eating, the snack test was appropriate because it had been used successfully in two previous experiments measuring oxytocin's effects on food intake (Ott et al., 2013; Thienel et al., 2016). Self-rated hunger prior to the taste test and a measure of liking for the snack foods both predicted snack food

intake in the covert test, and these features were implemented in this thesis by visual analogue scale (VAS) for hunger (see 2.2.2 Measures of Satiety) and VAS ratings of the palatability of the snacks (see 2.2.3 "Mood" VAS). Within this thesis, all covert measures of snack eating were disguised as sensory studies exploring hearing, sight, and other domains of perception of which the last element to be tested was always taste, affording the subsequent unobserved eating; this protocol is unique to these experiments and has not previously been adopted.

2.2.2 Measures of Hunger

Self-perceived hunger and self-perceived thirst were measured via VAS as part of a raft of checks supporting the assertion that snack eating was not initiated by deprivation-induced hunger . Although hunger and food intake do not always align, there is consistent evidence across two decades, that self-rated hunger measured before eating, predicts subsequent food intake (De Castro & Elmore, 1988; Horner, Byrne, & King, 2014; Sadoul, Schuring, Mela, & Peters, 2014) and even in the bogus taste-test protocol (Robinson et al., 2017). Although the array of measures varied across this set of experiments with additional measures such as fullness ratings, blood glucose, and baseline VAS being introduced, the main approach to supporting the claim that snack eating was not initiated by deprivation-induced hunger was the ingestion of a meal 15-25 minutes before the snack test itself; this was kept consistent across all three snack-test experiments. If participants left a small quantity of food, such as a crust or tomato slice, this was allowed by the researcher; however, if the participant failed to eat the test lunch, they were excluded from further participation.

2.2.3 Visual Analogue Scales of "Mood"

A variety of VAS measures was provided in both sessions of each experiment as quick self-report methods that offer a flexible and continuous grading rather than pre-defined categorical or Likert-type responses. Participants marked their responses along a VAS of 100 mm length that was presented on one sheet of paper referred to by the experimenter as a 'mood' questionnaire; this provided a foil for asking the participant to rate their hunger and thirst. As outlined in the introductory chapter of this thesis oxytocin is a well-known anxiolytic (see chapter 1, section 1.5), so a VAS estimate of anxiety was included in the mood questionnaire along with the other dimensions of happiness, excitement, and alertness. The content of this basic "mood" questionnaire varied a small amount to account for each experiment's requirements. Any changes are described further in the relevant chapters.

2.2.4 Additional Participant Information

Further measures and participant information were collected relating to situational and lifestyle factors thought to affect endogenous oxytocin levels or that affect eating patterns. Researchers propound that central oxytocin levels are substantially higher when individuals are 'in love' (Aron, A. et al., 2005b; Bartels & Zeki, 2000; Carter, 1998; Marazziti & Canale, 2004; Zeki, 2007). To assess the degree to which participants were 'in love', Rubin's widely validated 'Romantic Love Scale' (1970) was completed during both visits to account for possible inter-sessional changes but also to maintain an identical procedure across visits. Details

of both the content and time of the participant's last food intake were recorded to ensure compliance and to correlate with the snack-food outcomes.

2.2.5 Sham Tasks

To encourage snack eating that was uninhibited by participant awareness of their food intake being scrutinised, the experiments were disguised as investigations into the effects of oxytocin on sensory tasks; this, of course, facilitated a 'taste test' that then enabled snack food to be left with the participant during the break. To support the purpose of the study as it had been described to the participants, a range of sham sensory tests was presented that were chosen to be quick to administer and unlikely to bias results. An online pitch test was conducted with the participant seated and responding via a computer keyboard to a series of diminishing pitch intervals in the range 96-500 Hz presented through the computer's speakers (Schlaug, 2017). The pitch test lasted about four minutes and required participants to decide whether the second tone sounded higher than the first tone. A two-point touch discrimination test on the right index finger was conducted to identify the smallest gap, in millimetres, that could be sensed. Using a two-point probe starting with a distance of 7 mm between the points and progressing to 1 mm and then one point, participants had to decide with their eyes shut whether they could feel two points or one point. The smallest interval they could discriminate was noted. A smell test with smelling bottles containing synthetic soap or almond scents, depending on session, was then presented once and participants were asked to guess what the odour was, their answer was noted but they were not informed here, or elsewhere, of their performance or whether they were correct. Next was a timed balance test that required participants to stand on one leg while simultaneously 'drawing' counted numbers in the air, with their eyes shut, until they lost balance. There is no theoretical reason why oxytocin would affect any of the sham tasks, which were analysed after the first experiment and performance did not differ between drug conditions. Participants were also informed in advance via the recruitment literature, the information sheet, and a reminder email to fast for two hours before the experiment and that the consumption of the lunch (which was specified) was required "to stabilize blood sugar levels across participants". Further questions from the participant on the experimental lunch were answered with a version of this same neutral reason.

2.3 Administration of Oxytocin

The nasal sprays were supplied by Victoria Pharmacy, Zürich, Switzerland. The active ingredient of the Syntocinon nasal spray was oxytocin and the excipients were the preservatives E 216, E 218 and chlorobutanol hemihydrate; the placebo spray contained only vehicle. The dose of 24 IU was administered to remain consistent with previous research (Ott et al., 2013). As oxytocin is an endogenous circulating hormone in the blood and a profuse neuropeptide, the only foreign substances being introduced via the nasal spray were the two preservatives: polyparaben (E216) and methylparaben (E218), commonly found in food (Soni, Carabin, & Burdock, 2005), and chlorobutanol hemihydrate (Nair & Lach, 1959), commonly used in mouthwashes and medicines.

In the first session, the experimenter carefully explained how to use the nasal spray and participants were given a test bottle filled with water and invited to familiarise themselves with the pump action by squirting into mid-air. To self-administer the spray, participants tilted their head back to about a 45-degree angle. This head position maximises the chance of the liquid reaching the olfactory epithelium (Hardy et al., 1985). Participants used alternating nostrils and every 30 seconds they inhaled one puff deeply, then kept sniffing to keep the liquid in the nostrils. Alternating the nostrils avoids flooding one nostril beyond its absorptive capacity, thus maximising the residence time of the spray (Jones, N. S. et al., 1997). In total, 24 IU were administered across 6 puffs. If the participant reported that the spray had not worked, or if the researcher observed that the participant did not inhale properly or operate the spray correctly, it was assumed that the actualisation of that puff had not been made sufficiently well for the liquid to be delivered to the nasal cavity, so the participant repeated that inhalation. The competence of participants at self-administering the spray was recorded by the researcher and participants who did not effectively inhale the spray were excluded from further participation.

2.4 Food

The experimental lunches provided varied across experiments, but all were selected to be suitable for vegetarians and to deliver sufficient bulk and calories to provide participants with a sense of satiation. Snacks were also vegetarian and presented in separate bowls filled to the top, so a considerable amount could be eaten without the bowl appearing empty. The snacks for the female (chapter 6) and male (chapter 4) experiments were matched to the snacks used in a previous experiment that had found anorectic effects of intranasal oxytocin in men (Ott et al., 2013) and the group experiment (Chapter 8), used different snacks. Neither the brand of chocolate biscuit nor the “rice waffles” used in Ott et al.’s study was available in the UK, so British equivalents were sourced. Table 2.1 summarises the full nutritional content and calories of biscuits in the German study (Edeke) and contrasts this with the seven British brands that a preliminary survey found closest aligned with Edeke for calories and macronutrients. Although Ott et al. (2013) reported only macronutrients for snack foods, chocolate biscuits were the only category of snack food affected by oxytocin in the Ott et al. study, so chocolate biscuits were matched for all common nutritional elements.

Table 2.1

Nutritional Breakdown of Top Seven UK Matches for 'Edeka' Chocolate Biscuits

Nutritional Category	Edeka Double Choc per 100g	Differences by nutrient from 'Edeka Double Chocolate' (g)						
		Waitrose Choc Chunk	Waitrose Triple Choc	Fox's Choc Chunk	Pepperidge Soft	Pepperidge Crispy	Maryland Gooey	Maryland Big Chunk
kCalories	500	-7	1	6	-35	9	10	12
Protein	6	-0.3	0.1	0.2	-0.7	-2.2	-0.6	-0.5
Carbohydrate	57.2	6	2.3	4.8	10.1	9.3	1.2	2.2
of which sugars	38.8	-2.3	-2	1.8	3	-4	-5.7	-3.8
Fat	26.6	-3	-0.8	-0.3	-7.4	-1.3	1	1.4
of which saturates	15.3	-0.4	-0.1	-1.5	-4.7	-1.4	0.9	0.4
Fibre	3.9	-1.3	-0.6	-1.3	1.8	1.9	-0.9	-1.5
Sodium	0.21	0.08	0.14	0.05	0.29	0.59	0.09	0.12

The UK brand of chocolate biscuit that correlated most highly with German Edeke biscuits was Waitrose's 'Triple Choc Chip', both for overall nutritional content [$r > .99$, $p < .001$], and for calories and macronutrients only [$r > .99$, $p < .001$]. Ott et al. (2013) used TUC 'Classic' crackers for the salty category of snack food. The British equivalent of TUC 'Classic' is TUC 'Original', which has a slightly different nutritional profile (see Table 2.2) and this was used instead. The food was not broken up into pieces because, in the pilot phase, participants ate the food without needing it chopped up into smaller units to disguise its volume, and it was felt that unbroken snacks were more palatable than broken versions which crumbled easily.

Table 2.2

Comparison of Nutritional Contents of TUC 'Original' and TUC 'Classic' Crackers

	Nutritional values per 100 g	
	TUC Original	TUC Classic
kCal	518	463
Fat (g)	29.9	19
of which saturates (g)	13.7	8.4
Carbohydrate (g)	54.2	64
of which sugars (g)	6.9	0.3
Fibre (g)	2.6	2.3
Protein (g)	6.9	5.3
Salt (g)	2.4	2.5

No rice-based equivalent with sufficient fat content was found for the 'rice waffles' used in the Ott et al. (2013) snack test. Equivalent bland-tasting oatcakes with a closely matched nutritional profile (see Table 2.3) were used instead. In pilot studies, participants neglected the oatcakes in favour of the more palatable chocolate biscuits and crackers, suggesting that it was a suitable substitute neutral-tasting snack. Switching

to oatcakes also had the advantage of equalising the calories across the snack foods, which were not consistent in Ott et al.'s experiment, as illustrated in Table 2.4.

Table 2.3

Nutritional Comparison of Neutral-Tasting Snacks

	Continental Bakeries Rice Waffles	Sainsburys Oatcakes
Calories (kcal/100g)	390	488
Protein (g/100g)	8.6	10.7
Carbohydrate (g/100g)	63	56.9
Fat (g/100g)	22	23.5

Table 2.4

Nutritional Values of the Snack-Test Food used in Thesis Replications and by Ott et al. (2013)

	Neutral		Sweet		Salty	
	Thesis	Ott	Thesis	Ott	Thesis	Ott
Calories (kcal/100g)	488	390	501	500	518	486
Protein (g/100g)	10.7	8.6	5.7	6.0	6.9	7.8
Carbohydrate (g/100g)	56.9	63.0	59.5	57.2	54.2	63.0
Fat (g/100g)	23.5	22.0	25.8	26.6	29.9	22.0

2.5 Materials

A Salter electronic food scale was used to weigh food and professional floor dial scales (Healthometer, Bridgeview, Illinois, USA) were used to weigh participants. Blood glucose levels were obtained using Accu-Chek's 'Aviv' hand-held monitor (Roche, Dublin, Ireland). Sham tasks used the following: Exacta Healthcare's 'Two Point Discriminator Touch-Test' (Northcoast Health care, Morgan Hill, California, USA); synthetic almond- and soap-scented smelling bottles (Caravansons LLP, Bury, UK); and a stopwatch. The pitch discrimination test from Music and Neuroimaging Laboratory was presented online (Schlaug, 2017). Bespoke materials for the sham visual tasks were A4-sized laminated colour prints of famous paintings and A6-sized laminated black-and-white pictures of castles for a 'spot-the-difference' test. Self-report scales varied across experiments so are, therefore, reported in the relevant chapter.

2.6 Time of Testing

Participants were tested at lunch time between 12:00 and 14:30 as oxytocin production has a diurnal circadian rhythm that is stable through the middle of the day, and lunch time assessments enabled comparisons to be made with Ott et al. (2013), who tested participants at 12:40 hours, and participant hunger could be minimised with the provision of lunch. Oxytocin has a well-documented role in control of cortisol (Cardoso et al., 2013; Ditzen et al., 2009; Heinrichs et al., 2003; Kumsta & Heinrichs, 2012; Legros, Chiodera, & Geenen, 1988; Parker, Buckmaster, Schatzberg, & Lyons, 2005), which itself exhibits a diurnal rhythm. Circadian cortisol release drops rapidly from morning maxima of 12.5 µg/dL at 8 o'clock and continues dropping through the day to a nadir of 2 µg/dL at about 4 am (Chung, Son, & Kim, 2011). The small difference in cortisol levels between midday and 4 pm is about 2 µg/dL, still less between 12:00 and 14:30. Therefore, cortisol levels of participants being tested at midday and 2.30 pm were unlikely to vary greatly. The large testing window beginning at midday allowed more than one participant to be tested; however, this generated variance in pre-experimental fasting times. Some participants fasted from dinner the previous evening to testing, and other participants—particularly those attending later—ate an early lunch.

2.7 Hypotheses

The main hypotheses together with any exploratory hypotheses, for each study are provided below.

2.7.1 Nasal Spray Pilot

The hypothesis being tested was whether there was any difference in the sensory qualities of the oxytocin nasal spray compared to the placebo nasal spray that might alter food intake. Additional exploratory hypotheses were whether the oxytocin nasal spray had an effect on mood compared to the placebo nasal spray, which might in turn affect appetite, and whether there were any effects of gender.

2.7.2 Male Snack-Test Study

The main hypothesis was that 24 IU of intranasal oxytocin reduces snack eating 15-20 minutes after ingestion of a sandwich and packet of crisps. The exploratory hypotheses were whether the oxytocin affected the amount of lunch consumed or the taste and mood VAS ratings, and additionally whether BMI related to snack intake.

2.7.3 Female Pilot Study

The hypothesis being tested in the female snack-test pilot was whether the bogus-taste test facilitated snack eating in females. The first set of exploratory hypotheses examined whether scores on the DEBQ, the STAI, the BIDR-S impression management, and the Synder's Self-Monitoring Scale might correlate with the amount of food consumed. The next exploratory hypothesis assessed whether self-report mood VAS differed between males in the male snack-test experiment (Chapter 4) and this pilot study.

2.7.4 Female Snack-Test Study

The two main hypotheses were that 24 IU of intranasal oxytocin (1) reduces snack eating 15-20 minutes after ingestion of a low-protein pasta meal and a packet of crisps, and (2) reduces salivary cortisol concentrations. Exploratory hypotheses relating to consumption were whether the oxytocin affected the amount of lunch consumed or the taste VAS ratings; whether there were differences in snack intake in participants who reported stress eating from those who did not report stress eating; and whether BMI related to snack intake. Exploratory hypotheses were whether 24 IU oxytocin affected scores on the STAI, the mood VAS ratings, and the biomarkers of blood glucose, heart rate, and blood pressure.

2.7.5 Attentional Study

The main hypotheses tested in the attentional study were whether there was (1) a bias to palatable food pictures presented in a dot-probe task and (2) whether 24 IU of intranasal oxytocin reduced vigilance to food pictures in this same task. Exploratory hypotheses were whether attention to stimuli might be driven by initial orientation or by disengagement from the stimuli, whether gender affected attention to stimuli and whether there were differences between salty and sweet food stimuli. Further exploratory hypotheses relating to social and romantic stimuli were whether oxytocin would affect reaction times to social and to romantic images presented together, and whether the attention to social or romantic images would change when presented alongside food images. Finally, exploratory hypotheses relating to participant characteristics were whether scores on the STAI, DEBQ, Rubin's Romantic Love Scale, PBI, FCQ, age, BMI and frame size would be related to oxytocin's effects on the attention paid to food, and whether high scores on the UCLA Loneliness Scale would be associated with increases in attention to food images.

2.7.6 Social Study

The two main hypotheses were whether 24 IU of intranasal oxytocin would affect (1) snack eating 15-20 minutes after ingestion of a sandwich and packet of crisps and (2) and alter any effect of group membership on snack eating. Exploratory hypotheses were whether (1) gender, (2) the number of people in each group, or (3) the group composition being mixed- or single-sex affected snack intake in either drug condition. Additional exploratory hypotheses were whether there would be selective effects of oxytocin on sweet and salty snacks and whether oxytocin affected VAS taste and mood ratings.

2.7.9 Online Survey

The main hypothesis was whether scores on the Yale Food Addiction Scale were predicted by age, number of hours spent sitting, gender, hours of sleep, and scores on the STAI (trait only), UCLA Loneliness Scale, and PBI. Exploratory analyses were whether scores on the Yale Food Addiction subscales had equal response frequencies and whether subscales were predicted by age, number of hours spent sitting, gender, hours of sleep, and scores on the STAI (trait only), UCLA Loneliness Scale, and PBI.

2.8 Analyses

Data were analysed using IBM's Statistical Package for the Social Sciences (SPSS) version 24. The eating patterns for bland oatcakes, salty crackers, and chocolate biscuits were expected to be different, with oatcakes not being perceived or eaten as a treat, and salty crackers being more palatable but perhaps not eaten in great quantities on their own and without a topping. Chocolate cookies, on the other hand, were regarded as a highly palatable treat designed to be eaten on their own, and a snack that some participants might indulge in. Given the expected difference in data distributions for the consumption of each snack, an analysis of variance using snack-food consumption as a single entity, and each type of food as level within that variable, was not considered theoretically suitable. Indeed, analyses revealed uneven distribution patterns and sphericity was violated. This violation of sphericity occurred in previous research using these three very different foods, and adjusted degrees of freedom were used to compensate for this.

CHAPTER 3 – PRELIMINARY NASAL SPRAY PILOT

3. 1 INTRODUCTION

Although oxytocin nasal sprays have been used successfully in previous eating research, no previous study has examined their direct effect on eating and appetite. Experiments using oxytocin delivered by nasal sprays with participants who were then free to choose as much or as little to eat from a selection have had mixed results. Ott et al. (2013) used oxytocin nasal sprays in a cohort of males who had fasted overnight and found that 45 minutes post administration, nasally applied oxytocin did not reduce food consumption in a free buffet. Lawson et al. (2015) found 60 minutes after intranasal oxytocin food intake was reduced.

The sprays are not tasteless or odourless and contain preservatives (see Chapter 2, section 2.3 for details of excipients), so when inhaling these nasal sprays, the two senses of smell and taste are affected, as both the nasal epithelium and the back of the tongue are coated. In addition, small quantities of liquid are swallowed. Their use, therefore, may interfere with subsequent eating or produce a negative psychological reaction, such as anxiety, that could influence an experimental outcome. A small between-groups pilot study was conducted to assess the experience and effects on eating of using the nasal sprays. Participants self-administered oxytocin or placebo and completed brief bespoke VAS questions about the nasal sprays and their mood before eating a sandwich.

3.2 METHODS AND MATERIALS

3.2.1 Participants

In exchange for course credits, 18 adults (3 male, 15 female) were recruited to a short session that was advertised as a pilot study examining the experience of administering oxytocin or placebo spray. Inclusion and exclusion criteria were consistent with those detailed in the General Methods (see Chapter 2, section 2.1.1), and one female participant was excluded from analysis due to noncompliance, leaving 17 participants. Participant ages ranged from 18 to 36 years [M = 24 years, SD = 5.78 years] and BMI ranged from 18.73 to 31.10 kg/m^2 [M = 23.79 kg/m^2, SD = 3.72 kg/m^2] with 27% (five participants) being overweight or obese.

3.2.2 Materials

The scales and nasal sprays containing oxytocin or placebo were as detailed in the General Methods (Chapter 2) as was the romantic love questionnaire and the "mood" VAS. A second VAS examining the nasal spray experience was used, with the same format of 100 mm lines anchored by "not at all" and "very" or "a lot" depending on the question, and with higher scores indicating more positive responses. The first two questions of the nasal spray VAS asked about how the participant was feeling, both physically and mentally. The remaining eleven VAS assessed the spray's comfort, pleasantness of smell, irritability, ease of use, dripping from nose, effect on throat, taste, effect on appetite, and how stressful or pleasant the overall experience was, see Appendix I for the full nasal spray experience VAS sheets.

3.2.3 Procedure

The study was approved by Kingston University's Ethical Committee. The experiment was double-blind and participants visited once and were required to abstain from eating food or drinking sugary drinks for two hours before the session to maximise the likelihood that they would eat the sandwich, which they were informed was to equalise blood sugar levels across participants. After providing informed consent, participants confirmed their compliance with the study inclusion and exclusion criteria and provided the demographic information of age and gender. Participants self-administered the nasal spray in accordance with the method described in the General Methods. This was overseen by a researcher who noted how well the spray was administered. Participants then completed the VAS questions along 100 mm lines anchored by "not at all" and "very" or "a lot" depending on the question, with higher scores indicating more positive responses. Participants then completed the romantic measure and mood VAS described in Chapter 2 General Methods, then at 30 minutes post spray administration, participants were provided with a Sainsbury's 'Cheese and Tomato on Malted Bread' sandwich to eat. At the end of the session, participants had their height measured and were weighed, and before leaving were asked whether they thought they had been given oxytocin or placebo.

3.3 RESULTS

The means and standard deviations of scores of the 17 participants (14 female) aged 18-36 years (M = 24.0 years, SD = 5.78 years) on the VAS are presented in Table 3.1. Participant BMI ranged from 18.73 kg/m^2 to 31.10 kg/m^2 (M = 23.79 kg/m^2, SD = 3.72 kg/m^2). Participants who were not in a relationship did not complete the romantic measure and were assigned a score of zero but excluded from analysis. Scores on the romantic love scale, therefore, varied from 0 to 63% representing the degree to which participants felt in love. The mean romantic love scores of participants (not including participants who had a romantic love score of zero) was 52.75% (SD = 6.23%). A chi-square goodness-of-fit test was performed to determine whether the 17 participants were better than chance at guessing which spray contained oxytocin. Participant guesses were equally distributed in the sample, $\chi^2(1)$, = 0.06, p = .81 indicating that participants were not able to guess which spray was oxytocin. There was no difference between conditions in an independent t test on scores for romantic love $t(16) = 1.36$, p = .19.

An independent-samples t test was run to see whether the consumption of food differed between participants receiving oxytocin (M = 146.13, SD = 69.12) and participants receiving placebo (M = 142.67, SD = 74.64), which showed that oxytocin did not affect consumption of food, $t(16) = 0.99$, p = .90. The effect of oxytocin on the nasal spray experience VAS and the mood VAS was also examined via independent t tests and there were no significant differences between drug conditions on any of the VAS measures (ts < 1).

Table 3.1

Means and Standard Deviations (mm) for VAS scores in the Nasal Spray Pilot

		Placebo		Oxytocin	
		M	*SD*	*M*	*SD*
Nasal VAS	1. Do you feel in a good mood today?	65.78	13.15	69.63	13.03
	2. Do you feel ill (headache, blocked nose, stomach ache, etc.)?	11.44	7.21	13.13	9.42
	3. How comfortable was the nasal spray?	24.11	16.00	30.00	18.46
	4. Did the nasal spray irritate your nose?	46.11	10.08	43.88	6.08
	5. How pleasant was the taste of the nasal spray?	18.33	11.65	27.50	11.20
	6. Was the nasal spray itchy?	15.00	15.90	20.25	15.93
	7. How easy was the nasal spray pump?	52.13	12.31	52.13	12.31
	8. Did your nose drip after inhaling the nasal spray?	86.00	21.04	90.25	6.34
	9. Did you feel liquid flowing through your throat?	55.78	9.48	56.88	9.48
	10. Does your throat feel irritated?	23.33	13.50	17.75	14.43
	11. Is your appetite negatively affected?	67.00	13.27	72.50	12.19
	12. How pleasant was the overall experience?	27.56	10.67	25.38	12.35
	13. How stressful was the overall experience?	54.44	17.91	53.38	17.68
Mood VAS	1. How happy are you feeling right now?	63.33	15.48	69.50	11.51
	2. How excited are you feeling right now?	44.33	16.32	41.38	15.15
	3. How anxious are you feeling right now?	44.78	6.78	49.75	16.05
	4. How hungry are you feeling right now?	61.11	25.90	67.63	25.01
	5. How thirsty are you feeling right now?	49.56	14.90	35.00	21.00
	6. How alert are you feeling right now?	66.56	17.05	63.50	15.93

The mean amount of lunch eaten after the sprays was 144.29 grams (*SD* = 69.85 grams). However, eight participants did not eat all their sandwich, so to explore the relationship of the nasal spray VAS and the amount of lunch eaten, the quantity of lunch eaten was regressed onto the VAS questions about nasal experience. Preliminary analyses were conducted to ensure no violation of the assumptions of normality, linearity, and homoscedasticity. There were no correlations greater than .8 among the predictor variables indicating heteroscedasticity was unlikely, but only three questions correlated significantly with the outcome variable, see Appendix VIII for correlation table, and the model with all 13 predictor variables was not statistically significant $F(13, 3) = 5.45$, $p = .09$.

The three questions that correlated with the amount of lunch eaten were Q7 about ease of use [Pearson's $r = .44$, $p = .04$], Q11 about negative impact of the nasal spray on appetite [Pearson's $r = -.76$, $p < .001$], and Q13 that measured how stressful the experience was [Pearson's $r = -.65$, $p = .002$]. These three questions

were retained, and the multiple regression was repeated. The trimmed model was significant and explained 74% of the variance in the amount of lunch eaten: $F(3, 13) = 15.93$, $p < .001$, $r^2 = .74$, and all three of the predictors contributed significantly to the model. Ease of use [(Q7) $\beta = .31$, $t(2) = 2.37$, $p = .034$] positively predicted food intake, thus, the easier the spray was to use, the more lunch was subsequently eaten. The effects of the sprays on self-rated appetite negatively predicted food intake [(Q11) $\beta = -.51$, $t(2) = 3.42$, $p = .005$], such that the higher the score for self-rated loss of appetite, the lower the amount of food eaten afterwards. The last question about the stressfulness of the nasal spray experience (Q13) also negatively predicted how much food participants ate [$\beta = -.41$, $t(2) = 2.80$, $p = .015$], with higher stress ratings leading to lower food intake.

Given the potential cuing effect on eating of asking a participant about whether the nasal spray might affect their appetite (Q11) and the obvious association between appetite and food intake, the regression was repeated with a hierarchical entry method. Model 1 with only Q11 about the effect of the nasal spray on appetite was significant [$F(1, 15) = 20.52$, $p < .001$], and model 2 that included the remaining questions on ease of use (Q7) and stress (Q13) was also significant [$F(2, 13) = 6.34$, $p = .012$]. Table 3.2 presents the individual coefficients in each model.

Table 3.2

Coefficients from the Nasal Spray Experience VAS Predicting the Lunch Intake

		b	*SE b*	*β*
Model 1	Constant	435.44	65.28	
	Q11 - Is your appetite negatively affected?	-4.18	0.92	-0.76***
Model 2	Constant	349.96	65.96	
	Q11 - Is your appetite negatively affected?	-2.79	0.82	-0.51***
	Q7 - How easy was the nasal spray pump?	1.59	0.67	0.31*
	Q13 - How stressful was the overall experience?	-1.64	0.59	-0.41*

Note. $R^2 = .58$ in model 1 and $\Delta R^2 = .74$ in model 2. * $p < .05$, *** $p < .001$

To check whether a low score on ease of use (Q7) was related to stress (Q13) and whether stress was associated with estimates of subsequent appetite (Q11) correlations were performed. There were no significant correlations between Q7 and Q11 [Pearson's $r = -.21$, $p = .42$], nor Q7 and Q13 [Pearson's $r = -.05$, $p = .81$]; however, the relationship between Q11 and Q13 approached significance [Pearson's $r = .46$, $p = .061$].

Next, the amount of food eaten was regressed onto the VAS mood questions. Only levels of excitement correlated significantly with the outcome variable: Pearson's $r = .50$, $p = .02$ and the model was not statistically significant $F(6,10) = 1.43$, $p = .29$. The VAS for levels of excitement was retained and the

regression was repeated. The updated model explained a significant amount of variance in lunch eaten: $F(1, 15) = 5.05$, $p < .04$, $r^2 = .20$, $\beta = .50$, $t(2) = 2.25$.

Questions 7, 11, and 13 of the nasal spray experience VAS were then correlated with the excitement VAS scores, which also predicted the amount of sandwich eaten, but no significant relationships were found.

3.4 DISCUSSION

The amount of food eaten after self-administration of oxytocin or placebo did not differ between conditions, and this is in line with Ott et al. (2013), who found that oxytocin did not reduce hunger-driven eating in fasted participants. Lawson et al. (2015) did find anorectic effects on hunger-driven eating in men and suggested that the divergent results of their study and Ott et al.'s (2013) could be their inclusion of overweight and obese participants as Ott et al. (2013) examined only lean men. In this mixed cohort 27% were overweight or obese but no effects of oxytocin on the amount of lunch eaten by participants were demonstrated. However, the small sample size of the between-groups analysis may have limited the statistical power of the analyses, so differences in food consumption between the oxytocin and placebo sprays cannot, therefore, be ruled out. However, the participant's overall excitement levels, the nasal spray's ease of use, how much the nasal spray affected appetite, and how stressful the experience was, all predicted the amount of lunch eaten. Neither participant age, BMI, nor how in love they felt affected results, and no differences on the VAS or romantic measures were observed between conditions.

Unsurprisingly, the participants' view that the nasal spray affected appetite was a strong negative predictor of actual food intake at lunch with low self-estimates of the nasal spray affecting appetite predicting higher food intake at lunch. This finding is supported by previous studies which show that the amount individuals feel they can eat closely aligns with subsequent eating (de Castro & Elmore, 1988; Horner et al., 2014; Sadoul et al., 2014). Overall, the self-rated effect of the nasal spray on appetite accounted for in excess of 70% of the variance in lunch eaten. However, the overall stress generated also negatively predicted food intake, which is in line with previous research showing that stress is a factor predicting food intake (Leslie, Silva, Paloyelis, Blevins, & Treasure, 2018). Participants' ratings of how easy the nasal sprays were to use positively predicted the amount of subsequent lunch eaten; therefore, ensuring that participants understand how to use the sprays by prior demonstration will be included in following experiments.

The results show that the experience of nasal sprays was negatively associated with the consumption of a sandwich 10-15 minutes later in a cohort who had fasted for two hours prior to the experiment and rated themselves as moderately hungry. Experimental aims were not disguised, though, and participants were aware that reporting changes in appetite that resulted from the nasal spray was important and desired by the researcher. This may have had a priming effect and prompted participants to look for, or perhaps generate, issues to fulfil their role successfully.

The three nasal spray questions that positively predicted the amount of sandwich participants subsequently ate addressed how easy the sprays were to use, how the sprays might have negatively affected appetite, and how stressful the experience was. It is not clear why ease of use was related to subsequent eating, although problems administering the spray may have generated negative affect, frustration or embarrassment. The association between ease of use and stressfulness of the nasal spray experience was not significant, nor was there an association between ease of use and the excitement mood parameter, which also predicted how much food was eaten. However, there was a near-significant relationship between how the sprays negatively affected appetite and stress, suggesting that levels of stress are related to estimates of appetite. Excitement positively predicted the amount of lunch consumed and it might be that the lunch itself was exciting to hungry participants.

Stress negatively predicted the amount of food consumed. The literature on the relationship between stress and eating is complex but an overarching finding is that about a third of the population reduce eating in response to stress (Wardle & Gibson, 2016). The type of stress is a factor that in determining an eating response to stress. The general VAS question is not nuanced enough to provide further information on how the participants might have experienced stress. For example, some participants may have felt physically stressed by the spray, whilst for other individuals a dripping nose and the need to sniff might have been experienced as embarrassing or an ego challenge. However, females are more likely to stress eat (Gibson, 2012). In this pilot study, however, participants were hungry and the effects of oxytocin on appetite were being examined, so the eating that the stress influenced was not stress eating, per se.

The solution employed in this thesis to minimise the potential negative impact of intranasal oxytocin on appetite and eating that is available to studies that do not examine hunger-driven appetite, is to make the consumption of the sandwich after the nasal spray administration a compulsory study requirement. This also regulates the food intake of participants, ensuring all participants are tested after eating a set number of calories, which previous studies into oxytocin and eating have failed to do.

CHAPTER 4 - OXYTOCIN'S INHIBITION OF SNACK EATING NOT INITIATED BY DEPRIVATION-INDUCED HUNGER IN MEN

A published article based on this chapter can be found here:
https://www.sciencedirect.com/science/article/pii/S0195666318301806

4.1 INTRODUCTION

In recent times, palatable foods high in sugar, salt, and fat have become commonplace in western diets, and in the UK, two-thirds of the general population is overweight or obese (UK Government, 2017), a proportion that is reflected in the BMI ranges of the experimental cohorts of this thesis. The corollary of sugary, fatty food is widespread overconsumption that leads to obesity-related disease and, for some, difficulties controlling intake (World Health Organisation, 2015b). Highly palatable foods interfere with the normal eating cycle in which anticipation of eating and hunger activate reward circuits (Berridge & Kringelbach, 2015; Sescousse et al., 2010), and satiety hormones and gastric stretch receptors, for example, inhibit eating (Valassi et al., 2008; Woods, 2004).

As the initial experiment of this PhD, the following study set out to establish an effective experimental protocol for testing eating not initiated by deprivation-induced hunger using the snack-test protocol outlined in section 2.2.1 of the General Methods Chapter. The experiment aimed to replicate the snack-test component of Ott et al's (2013) study that also examined oxytocin's effects on eating, blood hormones, and metabolism. Twenty men who were recruited to a study assessing oxytocin's effects on metabolism were first assessed as clinically fit to participate. The study was a double-blind crossover procedure and participants attended twice, ten days apart. Before each session the men fasted overnight then attended a hospital at 8 am where a cannula was inserted in their arm to collect blood; their mood, hunger and thirst were assessed; then their metabolic rate and calorie expenditure were measured via indirect calorimetry. The participants then self-administered 24 IU of intranasal oxytocin or vehicle and underwent another assessment of calorie expenditure plus three further blood samples before being assessed for mood, hunger, and thirst again. Forty-five minutes post-intranasal administration, after 14.5 hours fasting, the men were provided with an ad libitum breakfast buffet and instructed to help themselves. After breakfast, a series of four further blood tests were administered, together with a smell test, another indirect calorimetry session, and an assessment of mood, hunger, and thirst. The next 45 minutes is not accounted for in the procedure, but at lunch time (12:40), three hours after oxytocin administration and 100 minutes after breakfast, a bogus taste test was conducted using chocolate biscuits (sweet taste), salty crackers (salty taste), and rice waffles (bland taste). The experimenters left the room for ten minutes and invited the participants to help themselves as the snacks would be discarded; this constituted the snack test. The only effect on intake of 24 IU intranasal oxytocin that Ott et al.'s study found on food intake, was a reduction of chocolate biscuit consumption in the snack test.

A replication of the snack test component of Ott et al. (2013) study enabled two outstanding issues to be addressed: (1) the anorectic effectiveness of intranasal oxytocin at 45 minutes post inhalation and (2) whether any anorectic effects occur in participants provided with lunch 15-20 minutes beforehand. Although a standard procedure for oxytocin nasal insufflation has yet to be established, the most usual latency in human oxytocin experiments between administration and testing is 45 minutes. In Ott et al.'s experiment (2013), participants were tested almost three hours after receiving oxytocin. Establishing the same clinical outcome with a shorter time lapse of 45 minutes would suggest that different pharmacokinetic pathways can alter eating behaviour. Intranasal absorptive pathways have not yet been established but Dhuria et al. (2010) proposed intracellular transmission was likely. Given that intracellular transmission is a slow process, it is unlikely to effect clinical change by 45 minutes. The second main issue in Ott et al.'s 2013 experiment that this semi-replication addressed, was whether Ott et al.'s participants were food-satiated when tested on the snack food. In Ott et al.'s 2013 study participants fasted overnight before being given a breakfast in the laboratory and waiting a further 100 minutes for the snack test. The possibility that participants in the Ott et al. 2013 experiment were hungry cannot be ruled out and the studies of this thesis implemented additional controls for hunger to specifically address eating in the absence of deprivation-induced hunger. Fasting from eight in the evening and delaying breakfast till half-past ten in the morning could have resulted in changes to mood and decreased alertness (Chtourou et al., 2011; Fond, Macgregor, Leboyer, & Michalsen, 2013; Roky, Iraki, HajKhlifa, Lakhdar Ghazal, & Hakkou, 2000), creating a confound in Ott et al.'s measurement of mood and alertness that was not addressed.

Other methodological aspects of Ott (2013) were altered to tighten experimental controls. The experimental aims were fully concealed from the participants, the calorie content of the three snacks was equalised and, unlike the study by Ott and his colleagues, there were no periods of the experiment that were unaccounted for in the procedure. A positive control test was included in this first experiment to check that oxytocin was active—should the effects on eating be non-significant—through testing its actions on a different process: memory for faces. The memory test from a separate oxytocin study that measured performance 30 minutes post administration was incorporated (Savaskan, Ehrhardt, Schulz, Walter, & Schachinger, 2008).

A small but growing number of studies have examined the influence of oxytocin on human food consumption. In a longitudinal design, Zhang et al. (2013) reported a decline in body mass in people with prediabetes using a fixed schedule of 24 IU oxytocin administered 20 minutes before eating, four times per day, over eight weeks. This body mass reduction was attributed to oxytocin's reductions of postprandial glucose and insulin levels that moved participants towards healthier profiles within the normal ranges. Ott et al. (2013) investigated oxytocin's effects on an unusually wide range of measures: energy expenditure; post-fast food intake; snack intake; heart rate; blood pressure; olfaction; self-rated mood, self-rated thirst, trust in experimenter; and serum levels of insulin, C-peptide, cortisol, growth hormone, leptin, plasma glucose, glucagon, total glucagonlike peptide-1 (GLP-1), active GLP-1, adrenocorticotropic hormone (ACTH), and total ghrelin. Ott et al. (2013) found that oxytocin had no effect on the amount of breakfast eaten after an

overnight fast but significantly reduced postprandial intake of chocolate biscuits and significantly increased the palatability of bland food in the same male cohort. In a hospital setting, Lawson et al. (2015) also measured food intake in fasted men with an emphasis on medical measurements: calories eaten; heart rate; blood pressure; fasting respiratory exchange ratio and respiratory quotient; plasma glucose, oxytocin, cholecystokinin, ghrelin, insulin, leptin, and peptide YY (PYY); self-assessed hunger, desire to eat preferred foods, satisfaction, fullness, quantity one could eat, desire for specific types of foods (sweet, salty, savoury, and fatty), and nausea. Lawson et al. (2015) provided double portions of food ordered by fasted men from a menu and found that 24 IU intranasal oxytocin significantly reduced breakfast consumption of both regular and highly palatable food relative to placebo, though no other effects were seen.

Consumption of food can be divided into eating initiated by hunger and eating not initiated by hunger (Kenny, 2011), or an interaction between both systems (Berthoud, 2011). Unlike eating initiated by hunger, which is regulated by homeostatic cues, eating not initiated by hunger has no immediate biological imperative. However, such binary categorisation of eating is difficult to justify as metabolic needs and the hormones that contribute to hunger modulate taste and reward systems to increase incentive salience and motivate eating (Saper, Chou, & Elmquist, 2002; Zheng & Berthoud, 2007). Although absolute definitions within this research area such as 'hedonic eating' and 'hunger-driven eating' are still widely used (Alblas, Mollen, Fransen, & van den Putte, 2019; Feig, Piers, Kral, & Lowe, 2018; Gupta et al., 2018; Iven et al., 2018; Lawson et al., 2015; Ott et al., 2013; Thienel et al., 2016), eating research is protean and labels which reflect the more complex reality that most eating is hedonic to some degree, are now favoured (Higgs et al., 2017).

Although oxytocin has previously been tested on palatable food intake in the laboratory, it has not been tested on eating that is unlikely to have been initiated by deprivation-induced hunger. In the Ott et al. (2013) and Thienel et al. (2016) experiments, the 14.5 hour overnight fast was broken with a breakfast buffet from 10:30 – 11:00, snack eating was not tested until lunch time at 12:40, and self-report hunger was not assessed after 11:50. Menu choices at the breakfast buffet included high calorie liquid options, meaning blood glucose spikes may have resulted and gastric emptying may have been quicker than 120 minutes to process 85% of solid food (Bowen, 2018). It is, therefore, possible that participants felt hungry again and that oxytocin's effects could predominantly reflect its anorectic action on homeostatic mechanisms (Maejima et al., 2009). Lawson et al. (2015) also fasted participants overnight for 12 hours, before serving double portions of the food requested by participants, thereby creating the opportunity for participants to overeat. However, whether participants were eating in line with their metabolic needs or overeating was not controlled for by Lawson et al. (2015). Since half of the Lawson et al. participants had BMI scores above 25 kg/m^2 and since caloric requirements are proportional to body mass in mammals (Hayssen & Lacy, 1985; Kleiber, 1932), larger caloric requirements may be associated with greater body mass, so eating part or all of a second portion may not constitute overeating (Hayssen & Lacy, 1985; Kleiber, 1932). In the current experiment, results were independent of fasting times which varied from 2 to 17 hours, and two measures

were included to achieve participant satiation before eating snack-test food: a meal of sandwiches and crisps was consumed by all participants 20 minutes before a snack test, and participants provided ratings of their hunger before the snack test. If participants indicated that they were hungry on the VAS, did not eat lunch, or did not eat all the lunch and had glucose levels below 4 mmol/L, they were excluded from further participation.

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As described in the introduction (Chapter 1), in animal models, oxytocin is a powerful anorectic that acts in the hypothalamus to inhibit appetite (Leng et al., 2008; Maejima et al., 2009; Sabatier et al., 2014; Valassi et al., 2008) and its effects are reversed by oxytocin antagonists (Arletti et al., 1989; Olson, B. R. et al., 1991; Olson, Beatriz R. et al., 1991; Sabatier et al., 2014). Oxytocin also reduces rodent preferences for sweet-tasting foods and prevents overconsumption of hyper-palatable food pellets via reward pathways in the limbic system (Amico et al., 2005; Miedlar et al., 2007; Mullis et al., 2013; Sclafani et al., 2007). In human studies, however, oxytocin's effects on food consumption have been inconsistent.

A number of experimental differences might account for oxytocin's inconsistent effects in the American (Lawson et al., 2015) and German studies (Ott et al., 2013). Eating is an intrinsically rewarding activity, especially when hungry; at the same time, irrespective of hunger, highly palatable foods that are rich in sugar, salt, and oil are more rewarding than bland food. Varying menu choices, therefore, might account for oxytocin's disparate effects on food intake in fasted men. In the Lawson et al. (2015) experiment, participants had about an hour in a food-deprived state to anticipate eating the food they had ordered from a menu, whereas the Ott et al. (2013) study was a free-choice buffet and subjects ate without this delay. Sampling differences may also have contributed to the disparate findings, as Lawson et al. (2015) used a male cohort comprising both normal-weight and obese participants, whereas the Ott group used only a normal-weight sample. Recent findings from Thienel et al. (2016) showed that oxytocin's effects on eating were stronger in an obese subgroup of an otherwise similar sample, suggesting that participant body mass or associated eating behaviour may affect sensitivity to the effects of oxytocin.

A range of studies has shown that oxytocin reduces anxiety (Grimm et al., 2014; Mccullough et al., 2013; Neumann et al., 1999), and biological markers of stress were significantly lower after oxytocin administration in the Ott et al. experiment (2013). However, self-reported anxiety was not measured in the previous experiments that examined oxytocin's impact on a covert snack test, so it is not yet known whether changes in perceived anxiety that contributes to stress or comfort eating, might also contribute to oxytocin's reductions in palatable food intake (Gibson, 2012; Wardle & Gibson, 2016). The present experiment, therefore, incorporated a self-report measure of anxiety.

Although neuropeptides have been found in cerebrospinal fluid just 10 minutes after administration (Born et al., 2002), peak effects of oxytocin are theorised to occur between 30- and 90-minutes post intranasal administration, and this therapeutic window is usually adopted in human experiments (Gossen et al., 2012).

However, the Ott et al. (2013) investigation of oxytocin's acute effects on postprandial snack intake employed a three-hour post administration interval. In addition to the rationale presented in the General Methods chapter of this thesis, a three-hour drug to testing latency makes results difficult to compare directly with studies showing clinical effects of oxytocin at 45 minutes post-administration, so the present study employs the more typical 45-minute latency for the first time in this context (Gossen et al., 2012).

Although this experiment replicated the key features of Ott et al.'s snack test (2013), a number of parameters were altered to build on that study. Ott et al. (2013) allowed participants to help themselves to each of the snack foods during the taste test, meaning that the amount of snack food eaten also included food that did not constitute snacking. In this experiment, to ensure that the amount of food consumed in the taste test did not impact the snack test, a laboratory assistant provided only small sample pieces of food for tasting that weighed about a gram. The snack food was provided in deep bowls rather than on plates, to allow participants to eat substantial quantities without the bowl appearing empty. The experimental aims of this study were fully concealed to avoid undesirable demand characteristics and self-selection bias, participants were likely to have been unaware that the study had anything to do with eating reward, or the related areas of metabolism and energy expenditure. In this experiment, self-perceived hunger was measured a few minutes before snack eating was assessed, whereas Ott et al. (2013) assessed self-rated hunger 50 minutes before the snack test. As a positive control, a partial replication of a study by Savaskan, Ehrhardt, Schulz, Walter, and Schächinger's (2008) that tested memory for happy, angry, sad, and neutral faces at 30 minutes after oxytocin administration was incorporated. Because oxytocin reduces preferences for sweet-tasting food in rodents and also non-specifically reduces overall food intake in men (Ott et al., 2013; Thienel et al., 2016), it was predicted that oxytocin would reduce the consumption of snack foods, particularly sweet-tasting foods, despite adopting a much shorter latency between drug administration and testing than in previous related studies.

4.2 METHOD AND MATERIALS

4.2.1 Design

A double-blind, placebo-controlled, randomised, and counterbalanced crossover protocol was implemented using a within-subjects repeated measures design comprising two drug tests scheduled about a week apart. Participants were informed that the study investigated the effects of oxytocin on sensory perception across a range of modalities.

4.2.2 Participants

One participant was excluded from analysis due to poor spray inhalation, leaving twenty healthy men aged 18 to 38 years (M = 23.5 yrs, SD = 6.5 yrs). BMI scores ranged from 19.41 kg/m^2 to 30.84 kg/m^2 (M = 25.44 kg/m^2, SD = 3.07 kg/m^2) and 45% of the sample were overweight or obese. Ethical approval and participant inclusion and exclusion criteria were consistent with the General Methods Chapter of this thesis (section

2.1.1), with the additional requirement that participants were heterosexual in order to avoid potential biases on the task involving images of male faces. One participant was also excluded for non-compliance with pre-experimental fasting but replaced by a new recruit during the testing period.

4.2.3 Materials

The sprays, romantic love questionnaires, and scales used were consistent with those detailed in the General Methods Chapter.

For lunch, each participant was provided with a meal of 546 kcal that was low in readily catabolisable sugars and consisted of a pre-packaged supermarket sandwich (Sainsburys 'Cheese and Tomato on Malted Bread', 424 kcal, 173 g) followed by a packet of plain crisps (Hula Hoops, 121 kcal, 25 g). Snack-test food used in this experiment was consistent with the General Methods Chapter.

The facial recognition test (acquisition phase) employed a set of 60 colour pictures of Caucasian men (age range 20-65 years) with happy, angry, and neutral expressions on a white background. A further set of 50 colour pictures of Caucasian men (age range 20-65 years) with neutral expressions and a white background was used for the recall test, 20 of which featured in the acquisition set but with angry or happy expressions, and 10 faces with neutral expressions were repeated from the acquisition set. Pictures were adapted from three databases: 'A lifespan database of adult facial stimuli' (Minear & Park, 2004), 'The NimStim set of facial expressions' (Tottenham et al., 2009) and the 'Stirling ESRC Face Database' (Psychological Image Collection Stirling University, 2017).

4.2.4 Procedure

Participants were tested individually between 12 and 2 pm. After providing informed consent and confirming compliance with the exclusion criteria, participants began the session with the facial recognition task. They familiarised themselves with 60 face pictures (20 neutral, 20 happy, and 20 angry) presented in random order on a computer screen approximately 60 cm away for 10 seconds each with a 3 second break between stimuli. Immediately afterwards, participants self-administered either 24 IU of oxytocin or placebo under the supervision of the researcher with six puffs alternated by nostril every 30 seconds. Height and body mass were measured, and participants completed a measure of their romantic love status (Rubin, 1970), then 10 minutes later they were asked to eat the lunch provided (a study requirement). Because thirst can sometimes be experienced as hunger and oxytocin is involved in its regulation (Balleine, 1994; Ryan, Ross, Campos, Derkach, & Palmiter, 2017), all participants were offered water in a 200 ml disposable cup with their lunch and indicated their thirst level on a VAS. The first set of sham tasks was then completed, see timeline (Figure 4.1) and the general methods chapter (Chapter 2, section 2.2.5) for further details.

At 30 minutes post oxytocin/placebo administration, a memory recognition test of the faces was undertaken that constituted the positive control. Further sham tasks were conducted and just before the snack test, a

finger-prick blood glucose test was conducted as a third and final check to ensure that participants did not have low blood glucose. Participants then completed a VAS measuring levels of 'happiness', 'excitement', 'alertness', 'anxiety', 'hunger', and 'thirst'. A few minutes before the covert snack test, a researcher provided the participants with small tasting samples of about a gram, one by one, from each of three tasting bowls containing 300g of neutral snacks, 230g of salty crackers, and 450g of chocolate cookies. Participants rated each snack in turn on a 100 mm VAS line anchored with 'Not at all' and 'Very palatable', 'Very sweet' or 'Very salty' for sweet, salty, and bland snacks respectively. The final sham task involved presenting one of two near-identical spot-the-difference pictures for 30 seconds. Then the critical snack test occurred: at 45-minutes post administration, the participant was instructed to enjoy a 10 minute 'cognitive break'; to let his mind 'relax'; and to select his preferred picture from a choice of five A4-sized prints of famous paintings. The experimenter announced that she would leave the room during the participant's 'cognitive break', and it was mentioned that the snack foods would be thrown away after the experiment due to health and safety regulations, so the participants were free to help themselves to as much of the snack foods as they wished. The snack test period lasted 10 mins, after which the second spot-the-difference picture was presented for 30 seconds, and the participant was asked to identify the differences between the two pictures. Before participants left, they were asked whether they thought they had been given oxytocin or placebo. The lunch foods and the snack test foods were weighed before and after testing, and the test sessions lasted about 70 mins in total. Figure 4.1 presents a visual timeline of the procedure. As the consumption of salty, sweet and bland snack food has different consumption patterns, it was expected and found that the distributions of sweet, salty, and neutral snacks reflected these different eating styles. In short, snack eating was not considered as one entity, so different results were expected, and they were treated separately. Ott et al. (2013), on the other hand, treated snack eating as one entity but found sphericity was violated and corrected the degrees of freedom to adjust the *p* value.

4.2.5 Statistical Analyses

Paired-sample *t* tests were conducted to test for differences between drug conditions and for order effects between sessions; results were divided by three to minimise false positives. Binary logistic regression was used to test for any effects of oxytocin on performance in the positive control (memory) task.

Event sequence	Time relative to drug administration (mins)
1. Positive control task (memory test)	-15
2. Oxytocin or Placebo	0
3. Height and weight measured	+5
4. Time of last food recorded	+8
5. Romantic Love Scale	+11
6. Set lunch	+15
7. Auditory pitch test	+20
8. Touch discrimination test	+24
9. Smell test	+26
10. Balance test	+28
11. Positive control task (memory test)	+30
12. VAS self-report measures	+34
13. Blood glucose measure	+38
14. Taste test	+40
15. Spot-the-difference picture 1	+44
16. SNACK TEST (“cognitive break”)	+45
17. Spot-the difference picture 2	+55

Figure 4.1

Timeline (mins) of an Experimental Session for Oxytocin’s Effects on Male Snack-Eating

4.3 RESULTS

4.3.1 Food Intake and Taste Test Ratings

A paired-samples *t* test was conducted to evaluate whether food intake differed between the oxytocin and placebo conditions. Table 4.1 presents mean scores for the eating measures and BMI. Eating lunch was a study requirement and, as expected, oxytocin did not affect the amount of food consumed during the lunch that occurred 15 minutes after oxytocin administration ($t(19) = 1.06$, $p = .30$). However, intranasal oxytocin significantly reduced consumption of two of the palatable snack types. Oxytocin significantly reduced the amount of chocolate biscuits eaten by about 63% compared to placebo ($t(19) = 3.51$, $p = .002$), and oxytocin also significantly reduced salty cracker consumption ($t(19) = 3.52$, $p = .002$) but only by 2.8%. However, there was no significant difference between oxytocin and placebo conditions for the consumption of bland oatcakes ($t(19) = 1.44$, $p = .167$). The effect of oxytocin on consumption of the chocolate biscuits and the salty crackers was sufficient to produce a reduction in the total amount of snack food consumed ($t(19) = 4.15$, $p = .001$). Sweetness ratings for chocolate biscuits were significantly higher in the oxytocin condition

($t(19) = 2.14$, $p = .046$), but taste ratings for salty crackers and oatcakes were not significantly different between drug conditions (respectively: $t(19) = 0.42$, $p = .68$; $t(19) = 1.79$, $p = .09$). To examine whether consumption of chocolate biscuits was related to sweetness ratings in the oxytocin condition, change scores were formulated by subtracting chocolate biscuit sweetness ratings in the placebo condition from ratings in oxytocin condition, and chocolate biscuit intake after placebo from chocolate biscuit intake after oxytocin. A correlation analysis was conducted on change scores and there was no significant association between participant ratings of sweetness and chocolate biscuit consumption after oxytocin (Pearson's $r = .01$, $p = .97$). The amount of salty or sweet snack food consumed by each participant in the placebo condition might indicate baseline taste preferences. To test whether potential pre-existing snack preferences in the placebo condition influenced the amount of snack food eaten, the amount of salty or sweet food after placebo was regressed on salty and sweet ratings after placebo, respectively. The placebo salty-snack intake was not significantly correlated with saltiness ratings Pearson's $r = .25$, $p = .15$ and the linear regression results showed that the model was not significant $F(1,18) = 1.18$, $p = .29$ and saltiness ratings did not significantly predict salty-snack intake $\beta = .25$, $p = .29$. The placebo sweet-snack intake was not significantly correlated with sweetness ratings Pearson's $r = -.17$, $p = .46$ and the linear regression results showed that the model was not significant $F(1,18) = 0.05$, $p = .83$ and sweetness ratings did not significantly predict sweet-snack intake $\beta = -.05$, $p = .83$

As would be expected, BMI did not change between tests ($t(19) = 0.96$, $p = .35$). However, in the oxytocin condition, BMI was significantly related to sweet snack intake: Pearson's $r = .56$, $p = .005$ and in a linear regression predicting sweet snack intake from BMI in the oxytocin condition, the model was significant $F(1,18) = 8.23$, $p = .01$ and sweetness ratings did not significantly predict sweet-snack intake $\beta = .56$, $p = .01$. In the placebo condition, though, BMI was not significantly correlated with sweet-snack food intake Pearson's $r = .24$, $p = .15$ and the linear regression results showed that the model was not significant $F(1,18) = 1.14$, $p = .30$ and BMI did not significantly predict sweet-snack intake $\beta = .24$, $p = .30$. There were no significant linear regressions or correlations in either drug condition for salty snack or bland snack intake.

Table 4.1

Means (*SD*) for BMI, Taste Measures, and Food Intake after Oxytocin/Placebo in Males

	Placebo		Oxytocin	
MEASURE	*M*	*SD*	*M*	*SD*
Lunch eaten (g)	187.6	6.3	181.6	25.2
Chocolate biscuits eaten (g)	68.7	60.1	25.1	20.8
Salty crackers eaten (g)	19.4	18.0	5.7	6.8
Bland oatcakes eaten (g)	5.6	7.5	3.1	1.9
Combined snack food eaten (g)	93.7	67.4	33.9	23.3
Sweetness of chocolate cookies (VAS)	77	18	85	9
Saltiness of crackers (VAS)	58	18	57	19
Palatability of oatcakes (VAS)	35	23	25	19
BMI (kg/m^2)	25.5	3.1	25.4	3.0

4.3.2 Other Measures

No differences were found between oxytocin and placebo conditions on measures of mood, blood glucose or romantic attachment. Table 4.2 below presents the means and standard deviations for levels of anxiety [$t(19) = 0.32$, $p = .76$], happiness [$t(19) = 0.99$, $p = .34$], hunger [$t(19) = 0.56$, $p = .58$], alertness [$t(19) = 0.37$, $p = .71$], excitement [$t(19) = 0.23$, $p = .82$], and thirst [$t(19) = 0.67$, $p = .51$]. Blood glucose levels did not differ between placebo ($M = 5.41$ mmol/l, $SD = 0.83$ mmol/l) and oxytocin ($M = 5.73$ mmol/l, $SD = 0.82$ mmol/l) conditions [$t(19) = 1.20$ mmol/l, $p = .25$ mmol/l]. To check whether levels of romantic love might have biased scores, they were compared in the placebo ($M = 62.20$, $SD = 45.52$) and oxytocin ($M = 63.60$, $SD = 43.12$) conditions but did not differ, $t(19) = 1.19$, $p = .25$. Romantic scores were also analysed using correlation with the intake of each of the snack types in each condition and significance levels were altered for multiple comparisons by dividing the alpha of .05 by 3. After placebo, romantic scores did not significantly correlate with chocolate biscuit intake (Pearson's $r = .08$, $p = .73$), or cracker intake (Pearson's $r = .01$, $p = .95$), or oatcake intake (Pearson's $r = .20$, $p = .40$). Similarly, after oxytocin romantic scores did not correlate with the grams of chocolate biscuit (Pearson's $r = -.12$, $p = .63$), or grams of cracker (Pearson's $r = -.63$, $p = .09$), or grams of oatcake (Pearson's $r = -.12$, $p = .63$) consumed.

Table 4.2

Means (*SD*) for Mood VAS (mm) in Placebo and Oxytocin Conditions

	Placebo		Oxytocin	
	M	*SD*	*M*	*SD*
Anxiety	13.0	18.0	13.9	14.6
Happiness	67.7	17.1	64.4	16.5
Hunger	34.7	24.6	38.9	23.0
Alertness	57.6	27.9	55.2	24.8
Excitement	48.2	23.8	49.6	22.6
Thirst	42.2	21.4	46.4	23.8

For the binary logistic regression in the positive control task (face recognition), error/no error was used as the outcome variable. Drug condition and picture type (new face, previously neutral, previously happy, previously angry) were categorical predictors, and the neutral was used as the reference category for picture type. The results indicated that picture type was a significant predictor of face recognition error rates: *Wald*(3) = 81.69, $p < .001$. The odds of correctly identifying the new faces presented in the test phase were almost three times greater when the other predictor variables in the model were accounted for [*Exp(B)* = 2.90] and this was significant: *Wald*(1) = 39.92, $p < .001$. Participants were more likely to respond correctly to a new face under the influence of oxytocin, compared with placebo, but only when oxytocin was administered in the first session: *Wald*(1) = 12.66, $p < .001$, *Exp(B)* = .30. Thus, the findings on the memory test did not fully replicate those of Savaskan et al. (2008).

4.3.3 Order Effects

Mean self-rated anxiety scores (VAS) in the first session (*M* =15.72, *SD* = 19.00) were lower than in the second session and close to significance [*M* = 11.15, *SD* = 12.86, *t*(19) = 1.785, $p = .090$]. All other measurements showed no significant differences or trends between sessions.

4.4 DISCUSSION

In a double-blind, crossover, placebo-controlled experiment, 24 IU of oxytocin significantly decreased snacking on chocolate biscuits and salty crackers in 20 healthy men, 45 minutes after drug administration. Self-report measures, such as hunger, anxiety and other aspects of mood, did not differ between conditions. The results are strikingly consistent with those of Ott et al. (2013) and Thienel (2016), which also found significant and pronounced effects of oxytocin on sweet-tasting carbohydrates specifically. In contrast, Lawson et al. (2015) only identified an effect of oxytocin when taking a combined macronutrient measure, with no selective effects on fat or carbohydrate subgroups, after controlling for multiplicity. Unlike previous human studies, the present experiment additionally identified a significant reduction in salty snack

consumption following oxytocin, a novel finding but consistent with animal data (Blackburn, Samson, Fulton, Stricker, & Verbalis, 1995; Puryear, Rigatto, Amico, & Morris, 2001; Verbalis et al., 1993). Importantly, the reduced snack consumption occurred straight after having eaten lunch and in the absence of self-reported hunger: previous findings typically reflect the effects of oxytocin on hunger-driven eating rather than on eating in the absence of deprivation-induced hunger.

Given that central oxytocin has a number of central hypophagic actions (for example, reward-reducing effects, stress-linked changes, and homeostatic 'stop switches'), characterising the type of eating, and accounting for procedural differences could be important in explaining oxytocin's effects on food intake. The principal difference between this experiment and the German group is the tightening up of measures that ensure the assessment of eating without deprivation-induced hunger without the participant being too full to snack-eat. There were a range of enhancements that this thesis made to the Ott et al. design to assess eating not derived from deprivation-induced hunger. In the Ott et al. experiment, there was about an hour (11:45 to 12:40) that was unaccounted for in the procedure. Given that there are a number of activities that could speed up calorie expenditure, food metabolism, affect stress, or otherwise contribute to explaining results, providing information on this part of the experiment is important. In this thesis, all time points are accounted for and none confound the outcomes investigated. In the German groups, participants were free to eat different foods, so without controlling the calorie and macronutrient intake, as was done in this experiment with a fixed lunch, claims of satiation and subsequent 'hedonic eating' are problematic. The calories consumed in the German studies comprised liquids. In Ott et al.'s study, four out of the twenty three breakfast buffet items were liquid (e.g. strawberry milk, whole milk, orange juice) and a further nine were semisolid (e.g. vanilla pudding, lemon curd) and, therefore, likely to have emptied from the stomach in 15-20 minutes (Camilleri et al., 1989; Charles, Camilleri, Phillips, Thomforde, & Forstrom, 1995; Hunt & Stubbs, 1975; Vist & Maughan, 1995). As stated in Chapter 1 (section 1.9), two hours after ingesting solid food, typically only 15% remains in the stomach (Bowen, 2018). Many of the calories offered in the breakfast buffets of the German experiments (14 out of 23 items) were in the form of readily catabolisable sugars, meaning that participants may well have felt hungry again due to the insulin response to a blood sugar spike (Sabatier, Leng, Menzies, 2013). The snack test in the German studies, which was 100 minutes later, also occurred at 12.40, a time when conditioned hunger is likely to occur that anticipates lunch. In this thesis, all the calories from lunch were from solid food that was deliberately chosen to be low in readily catabolisable sugars (Camilleri et al., 1989; Proano, Camilleri, Phillips, Brown, & Thomforde, 1990). In the German experiments, no VAS hunger assessment was made within 50 minutes of the snack test but in this experiment, a VAS hunger measure was completed within minutes of completion of the snack test. Lastly, the effects of oxytocin were tested in line with other experiments in the oxytocin literature at 45 minutes post administration.

The consumption of chocolate biscuits in the placebo condition of this experiment (68.7 g) was almost double that of Ott et al. participants (37 g). A luxury brand of chocolate biscuits was selected for this study

and the brand selected by Ott et al. may not have appealed to participants to the same degree. The large amount eaten in this experiment and oxytocin's reduction of it might suggest a reward diminishing effect of oxytocin, which was not as effective in Ott et al.'s study as the baseline reward of the chocolate biscuits was not as great in the placebo condition. The presentation of food may also have contributed to the differences in intake. In the German group, all snacks were broken up by the researchers beforehand. The TUC crackers, particularly, were brittle and it is likely that many crackers will have crumbled making the snacks less appetising.

Sampling differences could also account for some variation in food intake within the oxytocin literature. This experiment's 63% reduction in chocolate biscuit consumption was much greater than Ott et al.'s 25% drop (see Table 4.3) and an effect on salty crackers was also found. In Lawson et al.'s 2015 experiment, 'obese[1]' men form a large subset (12/25 were obese) of a group that includes normal-weight men and is analysed as a whole. In the present study, oxytocin's effects were found in a predominantly lean group. Both the age range (25 to 27 years) and BMI range (22.3 to 23.2 kg/m^2) of the German group were unusually narrow whereas the ranges of the American group were far wider: age range 18 to 45 years and BMI range 18.5 to 40 kg/m^2. Despite the inclusion of BMI scores as high as 40 kg/m^2, Lawson et al. (2015) stated that their sample was healthy and free from eating disorders. To screen participants, Lawson et al. (2015) used the structured clinical interview for DSM disorders-IV (SCID), which has a limited capacity to diagnose eating disorders, particularly at the mild end of the spectrum, and has been superseded by improved instruments based around the DSM 5 (American Psychiatric Association, 2013; Call, Walsh, & Attia, 2013; Sysko et al., 2015; Thomas et al., 2015). It is unknown how the Ott et al. (2013) study was promoted, but the small ranges of age and BMI, would suggest that the study either targeted or attracted a very specific population. Cultural differences between German and American attitudes to, for instance, volunteering for experiments, eating habits, food tastes, and compliance, may also explain some of the variance in results but have not yet been explored.

Thienel et al. (2016) found that obese individuals are more sensitive to the anorectic effects of oxytocin relative to normal-weight participants. However, in this experiment, BMI positively predicted sweet-snack intake only after oxytocin, suggesting that higher BMI did not result in increased sensitivity to the anorectic effects of oxytocin. A more nuanced model of oxytocin's anorectic effects, therefore, and one that is not based on a simple split between obese and normal weight, may be necessary, particularly since all of the healthy cohorts used in the current and previous eating experiments, apart from Ott et al. (2013), have included a proportion of participants with obese BMI scores, which reflects the population at large (UK Government, 2017).

[1] Lawson et al. (2015) include overweight participants in their obese group

Table 4.3

Comparison of Snack Consumption in this Experiment and in Ott et al. (2013)

	Placebo		Oxytocin	
MEASURE	*M*	*SD*	*M*	*SD*
Chocolate biscuits eaten (kcal)	344.19	301.10	125.76	104.21
Chocolate biscuits Ott et al. (kcal)	185	41	138	38
Crackers eaten (kcal)	100.49	93.24	29.53	35.22
Crackers Ott et al. (kcal)	81	19	75	16
Oatcakes eaten (kcal)	27.33	36.6	15.13	9.28
Rice Waffles Ott et al. (kcal)	18	3	13	2

Unexpectedly, chocolate biscuits were rated as sweeter in the oxytocin condition than in the placebo condition, which is not consistent with the animal literature outlined in Chapter 1 (section 1.8.3.2) that shows oxytocin-driven decreases in the sensitivity to sweet tastants (Sinclair, 2015). Typically, increased sweetness might be expected to correlate with increased palatability and, therefore, intake; however, oxytocin reduced intake. This finding is in line with an *f*MRI imaging study, though, that found oxytocin enhanced brain activity in the ventro-medial prefrontal cortex (vmPFC; Spetter et al., 2018). Activation of the vmPFC is positively correlated with the rewarding value of food (Gottfried, O'Doherty, & Dolan, 2003; Hare, O'Doherty, Camerer, Schultz, & Rangel, 2008), suggesting that oxytocin may enhance this process. Unfortunately, the parameter of general palatability was not included for the sweet and salty snacks, so it cannot be determined whether palatability was adversely affected by the elevated sense of sweetness, although reduced intake suggests it was. The absence of a general palatability measure makes it difficult to conclude that the intake of the more palatable snack foods – chocolate biscuits and salty crackers—was specifically reduced by oxytocin regardless of whether the palatability was conferred by sweetness or saltiness. However, no correlation was found between the change in sweetness ratings and change in sweet snack consumption across oxytocin and placebo conditions Pearson's $r = .01$, $p = .97$ and it could be argued that increased perceived sweetness of a snack leads to reduced consumption because of stronger habituations. Ott et al. (2013) tested saltiness, sweetness, and palatability for each of the snack groups (bland, salty, and sweet), and only found bland to be rated more palatable after oxytocin. In contrast, we found no effect of oxytocin on oatcake palatability. However, the calories contained in the bland food (390 kcal/100g) used by Ott et al. (2013) were substantially lower than the calories found in the salty and sweet foods (486 and 500 kcal/g respectively), meaning that the increased palatability rating in Ott et al.'s study could be a reflection of a preference for low calorie foods in the oxytocin condition. In the present study, snack food was also matched for calorie content, see Table 2.1 of the General Methods (Chapter 2).

A mediating role for anxiety-relief in explaining the effects of oxytocin on eating was not identified. Oxytocin administration is often associated with resultant decreased anxiety (Mccullough et al., 2013) and in both the Ott and Thienel groups, biological markers of anxiety were significantly lower in the oxytocin

condition (Ott et al., 2013; Thienel et al., 2016). However, in this experiment, self-reported anxiety did not differ between conditions. The inability to detect oxytocin-induced changes in self-reported anxiety may reflect the insensitivity of the VAS measure used, but the outcome accords with the findings of a review of the safety and side effects of oxytocin, which concluded that participants were unable to detect the presence of oxytocin either physically or through its behavioural effects (Mccullough et al., 2013). Participants in the present study were no better than chance at guessing on which session oxytocin was administered. Together, these data suggest that individuals are unaware of measurable changes in their mood, behaviour, and anxiety brought about by oxytocin in a covert laboratory snack test. It is noteworthy that previous studies have not fully disguised their research aims, introducing the possibility of undesirable demand characteristics. In the present study, researcher effects on mood or anxiety may have been generated by a female experimenter and a heterosexual male cohort.

Perhaps surprisingly, the pharmacokinetics of oxytocin are still not fully understood (see Chapter 1, particularly section 1.3). A number of mechanisms have been proposed to explain how intranasal oxytocin might reach the brain (Dhuria et al., 2010), none of which have yet been ruled out (Madara, 2000). Neuropeptides have been detected in cervical CSF just 10 minutes after nasal application (Born et al., 2002), and measurable effects from intranasal oxytocin have been reported in humans at 30 minutes post drug (De Dreu et al., 2010; Rupp et al., 2012; Savaskan, Ehrhardt, Schulz, Walter, & Schachinger, 2008). The contrasting latencies between drug administration and first eating, which were 15 minutes (to lunch) in this experiment and 45 minutes in Ott et al.'s study (2013), made no difference: neither latency was associated with changes to initial (presumably hunger-driven) appetite. The drug-to-snacking latencies also differed substantially between the two studies: 45 minutes here and 175 minutes in Ott et al. (2013); hence we demonstrated similar anorectic effects with a much shorter post-drug latency than reported previously. The speed of response in the current study would seem to rule out intracellular transmission as a mode of drug transport to the relevant receptors. More generally, the broad temporal range of oxytocin's efficacy at reducing food intake in the laboratory suggests that oxytocin's efficacy is not tightly reliant on time of administration. This could be important, given the potential difficulties for overeaters in maintaining a fixed eating regime (Zhang et al., 2013). However, a study in mice has shown the rapid development of tolerance to the anorectic effects of an oxytocin receptor agonist administered intranasally: the effects were negligible by day three of daily exposure (Olson et al., 1991). The possibility of tolerance in humans is yet to be explored. Nevertheless, the potential for oxytocin to be an effective component of dietary control with a practically achievable dosage regimen supports the need for further research, particularly given the difficulties involved in maintaining fixed drug and eating schedules.

In summary, the current study identified significant anorectic effects of oxytocin on palatable snack-test food consumption, effects that are consistent with those of various previous studies. However, the findings also demonstrated that such effects can be obtained in the absence of deprivation-induced hunger and within a much shorter timeframe post-drug (45 minutes) than has been reported previously.

CHAPTER 5 - PILOT STUDY: SNACK INTAKE IN FEMALES WITHOUT DEPRIVATION-INDUCED HUNGER

Intranasal oxytocin's effect on mainly hedonic appetite has not yet been investigated in a normal-eating female cohort, which, given oxytocin's sexually dimorphic central expression (Ochedalski, Subburaju, Wynn, & Aguilera, 2007; Patisaul, Scordalakes, Young, & Rissman, 2003), is an important research area that still needs to be addressed. In female mice, oxytocin reduces appetite for sweet-tasting foodstuffs and curtails feeding in response to hyperosmotic and lithium-induced toxicity (Flanagan et al., 1989; Verbalis et al., 1993). However, oxytocin has a regulatory osmotic role unique to rodents that may make translational inferences unreliable (Blackburn et al., 1995). More research with female participants is essential; hence the next main study of the thesis will test females.

5.1 INTRODUCTION

Research into demand characteristics and experimenter effects has found that less food is consumed in an environment where intake is measured or perceived to be measured (Robinson, Eric et al., 2014). The effect of social desirability reducing palatable food intake in laboratory experiments is far greater in females than males (Hebert, Clemow, Pbert, Ockene, & Ockene, 1995; Hebert et al., 1997; Robinson, Eric, Proctor, Oldham, & Masic, 2016). As inducing laboratory snacking in women can be challenging (Laessle, Lehrke, & Dückers, 2007; Petty, Melanson, & Greene, 2013; Pudel & Oetting, 1977), a pilot snack-test experiment was conducted to examine whether the bogus taste test used for the male snack-test study described in Chapter 4 facilitated snack eating in women.

As the aims of the pilot were not to examine oxytocin's effects on food intake, a number of changes were made to the experimental protocol of the general methods of this thesis described in Chapter 2. Participants attended for just a single session and, although they were told by the researcher that the nasal spray might contain oxytocin or placebo, in fact, only placebo was used. Lunch was not provided but participants were required to eat lunch before attending, as it was only necessary to ensure participants had eaten, but not provide a standardised lunch. Romantic love status was not assessed as oxytocin's effects were not being measured, and a number of exclusion criteria were removed (detailed in methods section below).

Social desirability, as originally conceived and measured, was a unidimensional concept captured by a 60-item questionnaire (Crowne & Marlowe, 1960). Whilst the length of the Marlowe–Crowne Social Desirability Scale has been addressed with shortened forms, the outdated language remains problematic. In addition, research shows that social desirability can be divided into at least two further constructs; most commonly these are impression management and self-deceptive enhancement. The Balanced Inventory of Desirable Responding Short Form (BIDR-S; Hart, Ritchie, Hepper, & Gebauer, 2015) was constructed to address the two-dimension model of social desirability, the language, and the length issues of the Marlowe–

Crowne Social Desirability Scale. The BIDR-S contains two sections, each comprising eight questions. The first set is concerned with impression management and second set relates to concern for self-deceptive enhancement. To address social desirability bias that pertains to monitored eating, only the impression management items from the BIDR-S were assessed in this pilot.

To some degree, the concept of self-monitoring identified by Snyder (1974) overlaps with impression management. Snyder partitioned individuals into those with predispositions to use situational cues to guide their behaviour and are, therefore, more concerned with the image of themselves that they present to others (high self-monitors), and those who use their inner values and beliefs to determine their behaviour (low self-monitors). A laboratory-based experiment by Cavazza, Graziani, and Guidetti (2011) examining self-monitoring and eating, found that the eating behaviour of high self-monitors was more influenced by situational factors than low self-monitors. In a mock restaurant, the amount of food that high self-monitors ordered varied according to the implicit norms of the situation.

The social pressure on females in Western cultures to conform to slender body shapes, together with the negative associations of overeating, overlap with the concerns of dieters and restrained eaters (Klesges, Bartsch, Norwood, Kautzrnan, & Haugrud, 1984). Dietary restraint (see Chapter 1, section 1.8.4) has been characterised as the norm in young women and is associated with early regulation of energy intake (Stunkard, 1981). The tendency to limit consumption of food in restrained eaters can be lost and result in disinhibited eating; evidence points to the unintended consequence that is disinhibition being more predictive of laboratory food intake than restraint itself (Johnson, Pratt, & Wardle, 2012; Stroebe, Van Koningsbruggen, Papies, & Aarts, 2013). In addition, restrained and emotional eating are most commonly found in females (Kenardy, Butler, Carter, & Moor, 2003; Wardle, Jane, Steptoe, Oliver, & Lipsey, 2000). The Dutch Eating Behaviour Questionnaire (DEBQ) not only includes the factors of emotional and restrained eating apposite to women, but also covers external eating, which assesses the extent that external influences determine eating.

5.2 MATERIALS AND METHODS

5.2.1 Participants

Fifteen healthy women aged between 18 and 36 years, *M* (*SD*) = 26.6 (8.6) years, participated, in exchange for university course credits. Their body mass indices ranged from 18.65 to 26.61 kg/m^2, *M* (*SD*) = 22.65 (2.15) kg/m^2, with one participant reaching the threshold for overweight and the remainder having body masses in the healthy range. Exclusion and inclusion criteria relating to food conformed to those set out in the General Methods Chapter of this thesis, and participants were required to eat lunch before attending and the importance of this for equalising blood sugar levels before oxytocin was stressed.

5.2.2 Materials

Snack foods, VAS, and sham materials were as specified in the General Methods Chapter. The instruments as described below were used to investigate the psychological characteristics of the participants.

The State Trait Anxiety Inventory (STAI: Spielberger, Gorsuch, & Lushene, 1970) is a reliable instrument developed to provide operational measures of state and trait anxiety and validated cross-culturally using different age groups. Spielberger, Vagg, Barker, Donham, and Westberry (1980) reported an improved psychometric structure in the STAI Form Y (STAI-Y) and this version, therefore, was used. The STAI-Y consists of two sections, each comprising 20 questions: the trait anxiety scale and the state anxiety scale. The state anxiety scale assesses the intensity of anxious feelings at a particular moment with statements such as "I feel calm" and "I feel tense" with a four-part Likert-type response: (1) not at all; (2) somewhat; (3) moderately so; (4) very much so. The trait anxiety scale reflects a predisposition to anxiety and asks to participants to describe how they generally feel by reporting the frequency of specified symptoms of anxiety, in response to statement such as "I feel self-confident" and "I feel inadequate". Response categories for the trait scale are (1) almost never; (2) sometimes; (3) often; (4) almost always. Both scales contain items that describe symptoms of anxiety as well as items that indicate the absence of anxiety and high STAI scores represent higher anxiety. The STAI has also been widely used in oxytocin research (Gordon et al., 2008; Heinrichs et al., 2003; Tops, Van Peer, Korf, Wijers, & Tucker, 2007), thus facilitating comparisons with previous oxytocin research.

Snyder's Self-Monitoring Scale: The Self-Monitoring Scale evaluates the degree to which an individual uses situational signals to manage their behaviour. The scale has high validity and a high test-retest reliability, which was of .83 across four weeks (Snyder, 1974). The 25 questions are scored as true or false and typical examples are "I rarely need the advice of my friends to choose movies, books, or music" and " I can only argue for ideas I already believe".

BIDR-S: The impression management dimension from the BIDR-S, which has good reliability across large and diverse populations, was used (BIDR-S: Hart et al., 2015). It is formed of eight short statements, such as "sometimes tell lies", "said something bad about a friend", "avoid listening", and "don't gossip", which are rated on 8-point scales from 1 "totally disagree" to 7 "totally agree".

DEBQ: The DEBQ assesses levels of restrained (10 items), emotional (13 items), and external (10 items) eating, with each scale having a range of mean scores from 1 to 5. A higher score reflects higher levels of the eating dimension. Typical questions on the restrained scale include "Do you deliberately eat foods that are slimming?" and "How often do you try not to eat between meals because you are watching your weight?". The emotional scale includes questions such as "Do you have a desire to eat when you have nothing to do?" and "Do you have a desire to eat when you are depressed or discouraged". The external subset includes "Can you resist eating delicious food" and "If you see others eating do you also want to eat?". The DEBQ has high construct validity and test retest reliability.

5.2.3 Procedure

The study was approved by the Faculty Research Ethics Committee at Kingston University. Upon arrival, exclusion and inclusion criteria were confirmed and participants provided details verbally of the lunch that had eaten. The components of the reduced procedure shown in Figure 5.1 followed the detailed outline provided in Chapter 2 General Methods. Sessions lasted about 50 mins.

Event sequence	Time relative to drug administration (mins)
1. Placebo administered	0
2. Height and weight measured	+5
3. Auditory pitch test	+10
4. Touch discrimination test	+16
5. Smell test	+18
6. Balance test	+20
7. VAS self-report measures	+24
8. Taste test	+30
9. Spot-the-difference picture 1	+33
10. SNACK TEST ("cognitive break")	+34
11. Spot-the difference picture 2	+44
12. Questionnaires	+45

Figure 5.1

Timeline (mins) of Experimental Procedure in Female Pilot Study

5.3 RESULTS

5.3.1 Participant Characteristics

For the dietary restraint component of the DEBQ, participants scored higher [*M* (*SD*) = 3.43 (0.80)] than the mean of normative scores from the British validation of the DEBQ using healthy women (Wardle, J., 1987): [Normative: *M* (*SD*) = 2.75 (0.79)], using a one-sample *t* test:, $t(14) = 3.32$, $p = .005$. Participants scored higher [*M* (*SD*) = 3.79 (0.93)] than the average of normative data on the DEBQ external eating scale, [Normative: *M* (*SD*) = 3.19 (1.1)], one-sample *t* test:, $t(14) = 2.52$, $p = .025$. For emotional eating, participant scores were not significantly different [*M* (*SD*) = 2.99 (0.92)]; and from normative data [Normative: *M*(*SD*) = 2.65(0.72)], one-sample *t* test:, $t(14) = 1.43$, $p = .17$. Responses on the BIDR-S impression management factor [*M* (*SD*) = 4.38 (0.94)] were not significantly different from the normative mean score [Normative: *M* (*SD*) = 4.50 (1.24)] in a one-sample *t* test: $t(14) = 0.48$, $p = .64$. Scores on the STAI were significantly below normative averages [Normative: *M* (*SD*) = 40.40 (10.10)] for trait anxiety [*M*

(*SD*) = 32.47 (4.63)], using a one-sample *t* test: $t(14) = 4.80$, $p = < .001$. State anxiety scores [*M* (*SD*) = 32.73 (6.19)] were also significantly lower than normative means [Normative: *M* (*SD*) = 38.76 (11.95)] via a one-sample *t* test: $t(14) = 5.27$, $p = < .001$. Self-monitoring scores [*M* (*SD*) = 16.13 (3.24)] were high in this cohort according to self-monitoring guidelines provided by Ickes and Barnes (1977) that are based on data from 207 undergraduate subjects: high scores are from 15 to 22, intermediate scores are from 9 to 14, and low scores are from 0 to 8.

5.3.2 Food

Snack-food consumption was low. Nevertheless, snack intake was analysed with one-way *t* tests using comparator snack-intake means from Chapter 4's male snack-test study. Intake of chocolate biscuits ($M = 6.20$g, $SD = 5.19$g) was low compared to the male group [$t(14) = -46.68$ $p < .001$]. Intake of salty crackers was also low ($M = 1.07$g, $SD = 1.10$g) compared to the male STS levels [$t(14) = -64.39$ $p < .001$] and oatcake consumption ($M = 1.27$g, $SD = 1.53$g) was significantly lower than men [$t(14) = -10.94$ $p < .001$].

5.3.3 Self-Report VAS

VAS scores for alertness, anxiety, happiness, excitement, fullness, and thirst, were compared to the male VAS scores in the placebo condition (see Chapter 4, Table 4.2) using one-way *t* tests, see Table 5.1 below for the means and standard deviations.

Table 5.1

Means (*SD*) for VAS (mm) in Females after Placebo Administration

	M	*SD*
Happiness	63.88	14.96
Alertness	44.07	16.90
Anxiety	17.60	13.31
Excitement	34.73	14.00
Hunger	23.73	8.45
Thirst	28.93	7.69

There were no differences in VAS scores between male participants in the previous study and female participants in this pilot in levels of happiness [$t(14) = .14$, $p = .90$] and anxiety [$t(14) = 1.34$, $p = 20$]. However, on the remaining VAS, women had lower self-rated levels of alertness [$t(14) = 3.09$, $p = .008$], thirst [$t(14) = 4.38$, $p = .001$], excitement [$t(14)= 3.73$, $p = .002$], and hunger [$t(14) = 5.00$, $p < .001$] than men in Chapter 4.

5.4 DISCUSSION

The pilot experiment had great difficulty in facilitating eating in women. Many participants ate nothing, and the upper boundary weight of snack food consumed amounted to a mouthful. However, the results of the

questionnaires showed that the cohort was high on the restrained and external eating dimensions of the DEBQ, and also high self-monitors, suggesting that laboratory eating may have been inhibited.

Hunger levels generally determine food intake (De Castro, 1988), and the results of this pilot accord with this observation because the low levels of snack eating in this female cohort were anticipated by low hunger ratings. It is possible that lower hunger ratings in this female cohort than found in the male participants (Chapter 4) might reflect a greater number of calories consumed at lunch, but the details of food eaten at lunch that were provided to the researcher in this pilot do not suggest high calorie intakes among the female pilot participants. The female hunger ratings were significantly lower than male ratings in the snack-test experiment (Chapter 4). There is evidence that resting metabolic rate positively predicts hunger and subsequent food intake and that males have significantly higher resting metabolic rates than females (Caudwell et al., 2012). Given that males typically have more lean body mass than females and that lean body mass determines high resting metabolic rates, this finding is, perhaps, not surprising (Hasson, Howe, Jones, & Freedson, 2011). Lower self-rated alertness and excitement were also reported than males in the snack-test study (Chapter 4), but whether alertness and snack-eating or excitement and snack-eating are associated is not known, and potential links are not apparent. Lastly, elevated thirst is sometimes mistaken for hunger (Balleine, 1994; Ryan et al., 2017). However, scores on the thirst VAS in this female cohort were not high.

The low STAI state scores revealed that participants were not feeling stressed by the study, and this may have impacted their food intake. In a laboratory experiment examining palatable food intake that exposed women to a stressor in one session and no stressor in a control session, high-stress reactors consumed significantly more calories on stress days than low-stress reactors (Epel, Lapidus, McEwen, & Brownell, 2001; Kandiah, Yake, & Willett, 2008). Additionally, high-stress reactors ate more sweet food in both sessions, which accords with other research that stress can increase consumption of sweet food (O'Connor, Jones, Conner, McMillan, & Ferguson, 2008; Oliver, Wardle, & Gibson, 2000). As a consequence, mild stressors were included in the main female snack-test study of this thesis with the intention of facilitating snack eating in women. To measure the effect of stressors in the next experiment, and any changes after oxytocin, the biomarkers of cortisol and heart rate were included. As lunch was not controlled in this study, this may have contributed to the lack of snack eating, in the main female study, as with the male snack-test study, lunch was provided and a two-hour fast required.

CHAPTER 6 - OXYTOCIN'S EFFECTS ON SNACK INTAKE IN FEMALES WITHOUT DEPRIVATION-INDUCED HUNGER

6.1 INTRODUCTION

The first experiment of this thesis (Chapter 4) demonstrated that administration of 24 IU of intranasal oxytocin markedly decreased palatable snack intake in males 15 minutes after eating lunch. The reductions in total snack intake were primarily driven by a 63% reduction in chocolate biscuit consumption, but a significant attenuation of salty cracker intake was also seen. In the same experiment, bland oatcake intake was not affected by oxytocin. A comparable reduction of 25% with sweet-snack intake in men was found by Ott et al. (2013) 100 minutes after a meal. As described in the introductory chapter of this thesis (Chapter1, sections 1.3.1, 1.8.3, 1.8.5), oxytocin's dose-dependent reductions in sucrose intake occur in rats via the VTA (Mullis, 2013), an area known to influence palatable food consumption, reward, and motivated behaviour (Lutter and Nestler, 2009), so oxytocin may alter the motivation to eat in the absence of hunger. The oxytocin knockout animal experiments on feeding described in detail in the introduction (Chapter 1, sections 1.6 and 1.8.3.2), highlighted oxytocin's inhibition of overconsumption of sweet-tasting sucrose and noncaloric saccharin that persisted during periods of induced stress. Oxytocin's effects on palatable food intake have not yet been tested in females, however.

Although about equal numbers of the population overeat, under eat, or do neither (Wardle, J. & Gibson, 2016), a gender divide exists, with women engaging in stress eating more frequently than men (Gibson, 2012). Eating energy-dense foods relieves the negative consequences of stress and suppresses the HPA axis (Buwalda, Blom, Koolhaas, & van Dijk, 2001; Dallman et al., 2003; Foster et al., 2008; Pecoraro, Reyes, Gomez, Bhargava, & Dallman, 2004) and, as mentioned in the previous chapter's discussion, laboratory-induced stress in women causes high cortisol reactors to eat (Epel, Lapidus, McEwen, and Brownell, 2001). Eating in response to stress typically involves choosing highly palatable and calorie-dense foods (Pliner & Mann, 2004). The severity of the stressor may influence these patterns (Charmandari, Tsigos, & Chrousos, 2005). Acute stressors can activate the sympathetic nervous system's 'fight or flight' to suppress appetite, or can leave eating unchanged (Wardle & Gibson, 2016), but may also depend on chronic stress levels, as chronically stressed women who comfort eat have been shown to have a blunted cortisol response to an acute stressor (Tomiyama, Dallman, & Epel, 2011). A suppressed cortisol response may also be attributable to visceral fat accumulation (Tomiyama et al., 2011). However, using acute stressors such as public speaking and mathematical tasks, laboratory studies have produced robust stress-eating responses irrespective of BMI (Epel et al., 2001).

The sex steroids, in particular the oestrogens, are important regulators of the tightly regulated HPA axis and oxytocin system, resulting in a gender disparity in oxytocin synthesis and receptor expression. Oestrogen

receptors are expressed by hypothalamic oxytocin neurons and enhance the anxiolytic effects of oxytocin, in addition they influence the transcription of both oxytocin and its receptors (McCarthy, McDonald, Brooks, & Goldman, 1996; Olszewski, Waas, Brooks, Herisson, & Levine, 2013; Young et al., 1998). To a lesser extent, testosterone influences oxytocin receptor expression and its effects vary according to brain region (Insel, Young, Witt, & Crews, 1993). Relatedly, the sex hormones exert sex-dependent stress effects that result in higher stress levels in women than men. Premenopausal women, for example, are vulnerable to monthly variations in oestrogen and progesterone that regulate central levels of serotonin and allopregnanalone, two endogenous "anxiolytics" (Li & Graham, 2017).

The sex hormones also affect body weight. Broadly, the energy demands of living organisms are subject to allometric scaling and the energy demands of large bodies are greater than small ones (Labra, Marquet, & Bozinovic, 2007). However, research into human BMI and food intake is inconsistent, with some studies linking higher food intake with higher BMI and others finding no association or an inverse relationship (Howarth, Huang, Roberts, Lin, & McCrory, 2007; Nijs, Muris, Euser, & Franken, 2010; Togo, Osler, Sørensen, & Heitmann, 2001). Under-reporting of dietary intake, especially by females and obese individuals, together with impression management are significant challenges for this research area that can invalidate results (Heitmann & Lissner, 1995; Klesges et al., 1984; Poppitt, Swann, Black, & Prentice, 1998; Vartanian, Herman, & Polivy, 2007). However, BMI remains reliably associated with overeating (Carter, Baker, & Brownell, 2000; Geller, Cassin, Brown, & Srikameswaran, 2009; Howarth et al., 2007; Sung, 2005).

Oxytocin regulates the HPA axis and is released centrally and peripherally in response to both psychological (Carter & Altemus, 1997; Onaka et al., 2012) and physical stress (Cardoso et al., 2013). Experimental rats given intracerebral oxytocin were exposed to emotional and physical stressors, which resulted in the inhibition of basal HPA axis activity but potentiated adrenocorticotropic hormone release under stress conditions, indicating a differential involvement of central oxytocin on HPA axis functioning (Neumann, Krömer, Toschi, & Ebner, 2000), reversible by antagonist (Neumann et al., 1999; Neumann, Wigger, Torner, Holsboer, & Landgraf, 2000). In a further experiment, the Neumann research group found that both the tonic reductions in HPA axis activity of brain oxytocin and stress-induced release of intracerebral oxytocin are gender independent (2000). In rat dams and women, lactation-related rises in oxytocin reliably reduce adrenocorticotropic hormone (ACTH) and cortisol (Amico, Johnston, & Vagnucci, 1994; Chiodera, Salvarani et al., 1991). Oxytocin and cortisol are bidirectionally interactive (Tops et al., 2007) and cortisol is so tightly linked to oxytocin that it has been used as an experimental proxy for oxytocin (Meinlschmidt & Heim, 2007). In previous studies showing oxytocin's attenuation of palatable food intake, a concomitant decrease in cortisol was found after oxytocin but not after placebo, suggesting that cortisol levels might influence palatable snack intake.

Given the prevalence of stress eating in women and the central elevation of oxytocin by oestradiol, the

inhibition of snack eating by oxytocin may be enhanced in females. For the first time, the effect of oxytocin on snack intake not initiated by deprivation-induced hunger in women was investigated. As in the male snack-test experiment, to minimise the possibility of deprivation-induced hunger, participants were provided with lunch 20 minutes before a covert snack-eating test. Oxytocin reduces tonic stress but potentiates cortisol release under acute stress and salivary cortisol was measured for the first time in this experimental paradigm, and to avoid a cortisol increase from protein, the protein content of the lunch was kept below 10 mg. The results of the pilot DEBQ showed that the women tended towards restrained and emotional eating, so this questionnaire was included in the experiment to examine whether these characteristics influenced the outcome. The STAI used in the female pilot study was also integrated together with Snyder's Self-Monitoring Scale, as pilot participants who did not eat scored below average on the trait stress component and above average on the self-monitoring behaviour. It was expected that, given oxytocin's attenuation of palatable snack intake in males, oxytocin would decrease snack consumption. It was also expected that, in line with previous studies, oxytocin would lower cortisol levels.

6.2 MATERIALS AND METHODS

6.2.1 Design

A double-blind, placebo-controlled, randomised, and counterbalanced crossover protocol was implemented using a within-subjects design comprising drug or placebo tests scheduled about a week apart. The aims of the experiment were disguised to minimise undesirable impression management, and participants were informed that the study investigated the effect of oxytocin on stress and sensory perception. The study was approved by the Faculty Research Ethics Committee at Kingston University.

6.2.2 Participants

Thirty-eight healthy women aged between 19 and 51 years, *M* (*SD*) = 26.6 (8.6) years were recruited both by word-of-mouth and in exchange for university course credits. Their body mass indices ranged from 18.4 to 35.0 kg/m^2, *M* (*SD*) = 24.8 (4.2) kg/m^2, indicating a range from low to obese. Although participants were not selected on the basis of their stress eating responses, a bespoke questionnaire on stress-response habits included a question on stress and eating that categorised participants as stress eaters and non-stress eaters, as these two groups might react differently to oxytocin given its anxiety-reducing effects. Individuals with self-declared food allergies, diabetes, pregnant, breastfeeding, on prescription medicines or a vegan diet, were excluded. Altered endogenous oxytocin function is associated with high emotional arousal or stress (e.g. bereavement, financial windfall) so participants reporting such events were also excluded (Engelmann et al., 1999; Kovacs, 1986). Due to possible changes in taste sensitivity and suppressed eating (Audrain-McGovern & Benowitz, 2011; Gromysz-Kalkowska et al., 2002), regular smokers were also excluded. Participants were asked not to consume alcohol or non-steroidal anti-inflammatory drugs for 24 hours beforehand and to abstain from consuming food and sugary drinks for two hours before the experiment.

6.2.3 Materials

During the enrolment process, the participant's status as a stress or non-stress eater was obtained using a bespoke questionnaire that asked about a range of stress responses in order to disguise the experimental aims. The questionnaire asked participants to indicate how they react to a number of stress scenarios by rating their top three responses from "sleep", "disturbed sleep", "exercise", "chat with someone", "work/study", "eat", "reduced eating", "smoke/vape", "read/watch TV or film", "cry", "drink alcohol", "let your hair down", "drugs of abuse", and "none of the options"; a full copy is included in Appendix VI. The sprays, romantic love questionnaires, VAS, blood glucose, food scales, sham task materials and snack food used were all consistent with those detailed in the General Methods Chapter. Koogeek's biometric impedance scales were used to weigh participants. The Omron handheld M2 model measured blood pressure, and Biopac's SC-Flex Pro was used with a fingertip sensor that measured heart rate. Saliva was collected in 'Salivettes' (Sarstedt UK, Leicester, England). Mood parameters were measured using a bespoke visual analogue scale (VAS) measuring levels of happiness, excitement, fullness, thirst, anxiousness, and alertness with 100 mm lines anchored at 'not at all' and 'as much as I can imagine'. For the low-protein lunch, each participant was provided with a pre-packaged supermarket lunch (Waitrose 'Pesto and Spinach Salad', 376 kcal, 190g). Stress was examined with the STAI-Y (Spielberger et al., 1970) described in Chapter 5 (section 5.2.2). Snyder's Self-Monitoring Scale (1974) was used to measure self-monitoring behaviour, and the DEBQ was used to assess eating behaviour, both described in Chapter 5 (section 5.2.2). The neutral film viewed by participants was David Attenborough's "Life of Plants".

6.2.4 Procedure

The timeline of events in a test session is shown in Figure 6.1. After providing informed consent and confirming compliance with the inclusion and exclusion criteria, participants provided the first of two baseline saliva samples by drooling into the collection tube; at least 1 ml was collected with each sample. Salivettes were stored in fridge during the experiment and then transferred to a freezer and stored at -20 ºC until centrifuging. Participants next completed the first of three mood VAS scales. Blood pressure was taken, and blood glucose was then measured. Participant height, age, time since last meal, and content of last meal were recorded, and the second baseline cortisol sample was given. Next, the participant self-administered either 24 IU of oxytocin or placebo under the supervision of the researcher with six puffs alternated by nostril every 30 seconds. A measure of their romantic love status (Rubin, 1970) was obtained and they were asked to eat the lunch provided. As thirst can sometimes be experienced as hunger (Balleine, 1994), participants were offered water and a maximum of 100 ml was provided with their lunch; participants were also required to rinse out their mouth with a small sip of water after food to reduce salivary sample contamination. To support the purpose of the study as it had been described to the participants, the first two sham tasks were presented: A two-point discrimination touch test and an olfactory test, refer to Chapter 2 (section 2.2.5) for details. On completion, saliva was again drooled into the salivettes and their left hand was rested on the table-top and a heart rate sensor placed on the middle finger. The participant then watched a neutral film and kept their hand still while a 10-minute baseline recording was made at the end of which they

were allowed to move their arm briefly and re-position it if uncomfortable.

The two stress tests were then carried out. The first stress test was a modified version of the "Sing-a-Song" stress test (SSST: Brouwer & Hogervorst, 2014) and chosen as an ego stressor, in other words an experimental manipulation that inherently threatens an individual's self-image (Polivy & Herman, 1999). The SSST consists of a set of timed instructions presented on a computer screen that instruct the participant to sit still and relax then ask the participant to perform neutral thinking tasks such as, "think about different animals that begin with the letter 'P'; these neutral questions allow baseline stress to be assessed. The sixth command is to think about a song to sing out loud for 30 seconds and the seventh instruction is to sing a song for 30 seconds. As participant baseline was measured for ten minutes previously in this experiment when the participants viewed a neutral video, only the singing component was used, and this was extended to 60 seconds as this generated more stress in preparatory pilots (not reported in this thesis). After adjusting their position to be comfortable, the participant was instructed to sing a song for a minute and heart rate recording was resumed. The participant was given no further advice on song selection or style and to generate more stress the researchers stopped all activity and watched the participant whose 60 seconds of singing was timed by a stopwatch. At the end of the singing, recording was paused, and the participant was instructed on how to perform the second of the stress tests: the serial sevens task (Smith, 1967), which was selected as a mainly cognitive stressor. Bio recording of heart rate was resumed and a random starting number between 700 and 800 was given and the participant began 60 seconds of subtracting seven from the running total.

Immediately following the second stress test, a saliva sample was taken, and blood pressure was measured. Next, a taste test was conducted with the three snack foods. Small pieces of each snack, weighing about a gram, were provided for the participant to taste and rate on a VAS. Another mood/hunger VAS was then completed, and the sham pitch test was completed online. After the pitch test, another saliva sample was taken. The final sham task involved presenting one of two near-identical spot-the-difference pictures for 30 seconds for the participant to memorise the details. Then the critical snack test occurred: at 45-minutes post drug/placebo administration: the participant was instructed to enjoy a 10 minute 'cognitive break', let his or her mind 'relax', and select a preferred picture from a choice of five A4-sized prints of famous paintings. The experimenters announced that they would leave the room during the participant's 'cognitive break', and it was mentioned that the snack food would be thrown away after the experiment due to health and safety regulations so they were free to help themselves to as much of the snack food as they wished. The snack test period lasted 10 mins, after which the second spot-the-difference picture was presented for 30 seconds, and the participant was asked to identify the difference between the two pictures. After the break, the participant provided another saliva sample and completed a mood/hunger VAS. Next, the STAI was completed then blood pressure and blood glucose were measured. One last saliva sample was provided; then, before participants left, they were asked whether they thought they had been given oxytocin or placebo. At the end of the second visit, participant body mass and fat ratio were measured. Lunch and snack test foods were weighed before and after testing, and each session lasted about 95 mins. After the experiment ended,

participants were emailed a link to complete an online survey comprising the DEBQ, and Snyder's self-monitoring scale.

Event sequence	Time relative to drug administration (mins)
1. Cortisol-1	-20
2. Mood, hunger VAS-baseline	-17
3. Blood pressure-1	-10
4. Blood glucose-1	-7
5. Cortisol-2	-5
6. Oxytocin or placebo	0
7. Sensory tests: touch, smell	+5
8. Romantic scale and lunch	+10
9. Cortisol-3	+15
10. Baseline heart rate	+17
11. Stress test 1: sing-a-song	+28
12. Stress test 2: serial 7s	+29
13. Cortisol-4	+30
14. Blood pressure-2	+31
15. Taste test	+32
16. Mood, hunger VAS-sample 2	+36
17. Sensory test: pitch	+39
18. Cortisol-5	+43
19. Snack test	+45
20. Cortisol-6	+59
21. Mood, hunger VAS-sample 3	+61
22. STAI	+64
23. Blood pressure-3	+69
24. Blood glucose-2	+72
25. Cortisol-7	+74

Figure 6.1

Timeline (mins) of Experimental Session in the Female Snack-Test Study

6.2.4.1 Saliva Assays for Cortisol

Samples were centrifuged at 1000 × g for 2 min, and supernatant was stored at −20 ºC for 1 to 6 days and then -80 ºC until assay. Saliva cortisol levels were measured all in the same day in duplicate by Surrey Assays Ltd by radioimmunoassay adapted from Riad-Fahmy et al. (1979). Limit of detection was 0.44 +/-

0.15 nmol/L and quality controls were low = 3.2 +/- 0.4 nmol/L CV = 11.6%; medium = 17.7 +/- 1.7 nmol/L CV = 9.6%, and high = 48.2 +/- 3.2 nmol/L CV = 6.7%.

6.3 RESULTS

6.3.1 Participant Characteristics

For the dietary restraint component of the DEBQ, a one-sample *t* test showed that the mean of participant scores [*M* (*SD*) = 3.19 (1.1)] did not differ from the mean of normative scores [Normative: *M* (*SD*) = 2.75 (0.79)] in the British validation of the DEBQ using healthy women: $t(37) = 1.21$, $p = .24$, although normative data presented here and elsewhere, must be treated with caution as direct comparisons may not be valid. Participants scores on the DEBQ external eating scale [*M* (*SD*) = 3.33 (0.59)] did not differ from normative data in a one-sample *t* test [Normative: *M* (*SD*) = 3.19 (1.1)], $t(37) = 1.51$, $p = .14$. However, a one-sample *t* test found that participants' scores [*M* (*SD*) = 1.93 (0.76)] were significantly lower than normative data [Normative: *M* (*SD*) = 2.65 (0.72)] for emotional eating, $t(37) = 8.62$, $p < .001$. In contrast to the pilot (see Chapter 5, section 5.4.1), a one-sample *t* test found that scores on the self-monitoring scale [*M* (*SD*) = 8.89 (2.65)] were low in this cohort in contrast to the average score of 11.5 from the authors, $t(37) = 6.04$, $p < .001$. Scores on the STAI-Y trait scale [*M* (*SD*) = 45.50 (4.60)] were above the normative average for working adults $t(37) = 12.64$, $p < .001$, and for university students $t(37) = 6.83$, $p < .001$, in a one-sample *t* test, see Chapter 5 (section 5.2.2) for STAI-Y trait normative data. For working females and females at university, normative means on the STAI-Y state were 35.72 and 35.20 (*SD* = 10.40 and 10.61) respectively (Spielberger et al., 1970), which were significantly below participant scores [*M* (*SD*) = 44.91 (3.04)] when analysed using one-sampled *t* tests: $t(37) = 18.62$, $p < .001$ and $t(37) = 19.67$, $p < .001$, respectively. Scores on the romantic love scale were measured in both sessions and were low-average *M* (*SD*) = 76.65 (14.86) and were no different between placebo and oxytocin conditions: $t(37) = 0.14$, $p = .89$.

6.3.2 Snack Food Intake

Oxytocin significantly reduced the intake of chocolate biscuits [$t(37) = 5.10$ $p < .001$] but not salty crackers [$t(37) = .262$ $p = .80$] or oatcakes [$t(37) = 1.18$ $p = .25$], see Figure 6.2. As expected, there was no significant difference in lunch eaten (a study requirement), between the oxytocin condition [*M* = 179.79 g, *SD* = 31.94 g] or the placebo condition [*M* = 177.68 g, *SD* = 25.35 g] $t(37) = 0.74$ $p = .47$.

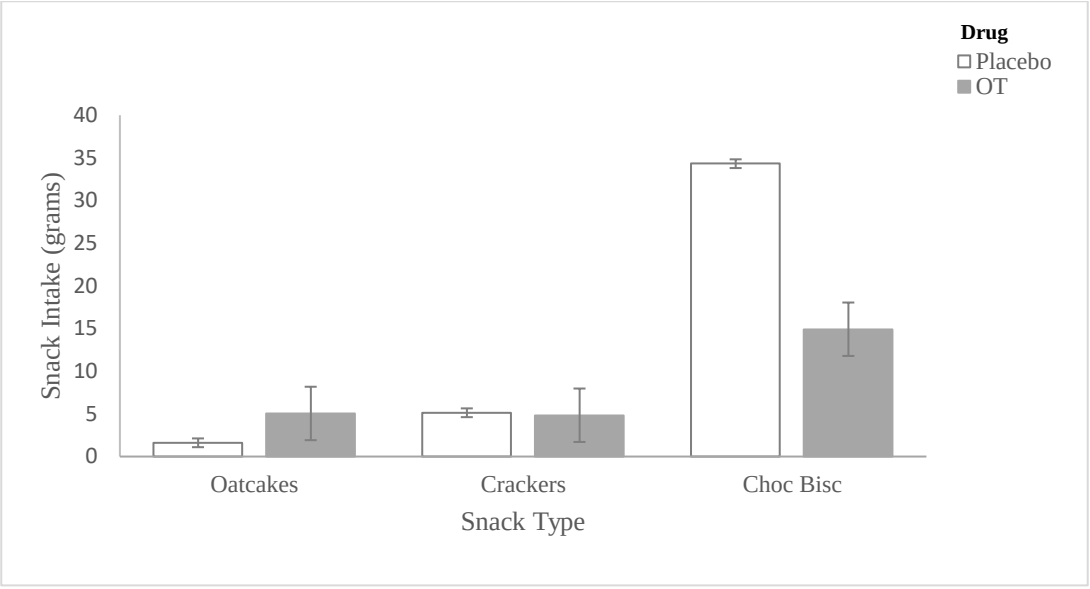


Figure 6.2

Effects of Oxytocin vs. Placebo on Intake (mean (g) ± SEM; n = 38) of Different Snacks

6.3.3 Snack Consumption in Stress-Eaters and Non-Stress Eaters

The cohort was divided by the answers to the screening questionnaire into stress eaters and those who did not stress eat. Differences in chocolate biscuit consumption, salty cracker consumption, and oatcake consumption for self-rated stress eaters and non-stress eaters were analysed using separate 2 (drug: oxytocin or placebo) x 2 (stress eater or non-stress eater) mixed model ANOVAs for each of the three snacks. Table 6.1 presents the means and standard deviations by snack type overall (n = 38), for stress eaters (n = 15), and non-stress eaters (n = 23) in each condition.

Table 6.1

Means (*SD*) (g) of Snack Food Consumption by Stress Eaters and Non-Stress Eaters

		Stress Eater		Non-Stress Eater		Total	
		M	*SD*	*M*	*SD*	*M*	*SD*
Oxytocin	Sweet	13.80	13.03	15.65	28.68	14.92	23.54
	Salty	6.87	14.04	3.52	5.62	4.84	9.80
	Bland	2.07	3.69	7.00	24.65	5.05	19.30
Placebo	Sweet	39.80	33.99	30.74	32.86	34.32	33.16
	Salty	7.80	15.38	3.39	6.49	5.13	10.93
	Bland	1.27	2.52	1.83	3.56	1.61	3.17

There was no main effect of stress eating status on consumption of chocolate biscuits: $F(1,36) = 0.17$, $p = .69$. There was a significant main effect of oxytocin on chocolate biscuit consumption [$F(1,36) = 28.60$, $p < .001$], but no significant interaction, $F(1,36) = 2.02$, $p = .16$. There was no main effect of stress eating status

on consumption of salty crackers: $F(1,36) = 1.44$, $p = .24$. There was no main effect of drug on salty cracker consumption [$F(1,36) = 0.12$, $p = .73$], and no significant interaction, $F(1,36) = 0.21$, $p = .65$. There was no main effect of stress eating status on consumption of oat snacks: $F(1,36) = 0.62$, $p = .44$. There was no main effect of drug on oatcake consumption [$F(1,36) = 0.99$, $p = .33$], and no significant interaction, $F(1,36) = 0.53$, $p = .47$.

6.3.4 Snack Food Taste Tests

There were no significant differences in the taste test when analysed with paired-sample *t* tests. Mean sweetness ratings for chocolate biscuits were 77.50 mm in the placebo condition and 77.76 mm after oxytocin, standard deviations were also similar: 12.76 mm and 13.50 mm respectively, $t(37) = 0.11$ $p = .91$. The mean saltiness ratings for salty crackers were similar in both the placebo (55.79 mm, $SD = 19.52$ mm) and oxytocin conditions (56.26 mm, $SD = 17.41$ mm), $t(37) = 0.17$ $p = .87$. Oatcakes were rated similarly for palatability in the placebo condition ($M = 34.92$ mm, $SD = 22.46$ mm) as in the oxytocin condition ($M = 37.63$ mm, $SD = 26.53$ mm) $t(37) = 0.62$ $p = .54$. To assess whether pre-existing food preference—as represented by amount consumed without the influence of oxytocin in the placebo condition—was associated with taste ratings for those foods, the placebo chocolate biscuit intake was compared with sweetness ratings for chocolate biscuits and the placebo salty cracker intake was compared with saltiness ratings. The results revealed that neither sweet nor salty snack consumption correlated with sweet or salty ratings, respectively: Pearson's $r = .04$, $p = .81$ for sweet snacks, and Pearson's $r = .19$, $p = .24$ for salty snacks. Taste ratings did not differ between stress eater and non-stress eater groups, see Appendix IX for further details.

6.3.5 Self-Report Data

Scores on the STAI-S were compared after placebo ($M = 45.16$, $SD = 3.87$) and after oxytocin ($M = 44.66$, $SD = 3.35$) via a t test, but no significant difference was found $t(37) = .79$, $p = .44$. Similarly, the STAI-T scores were compared after placebo ($M = 45.66$, $SD = 6.00$) and after oxytocin ($M = 45.34$, $SD = 4.50$) via a t test, and again no significant difference was found $t(37) = .38$, $p = .71$.

Fullness scores significantly dropped between the VAS measure for the baseline sample and sample 2 that was taken after lunch, in both the placebo [$t(37) = 8.70$ $p < .001$] and the oxytocin [$t(37) = 6.90$ $p < .001$] conditions, see Table 6.2 below for the means and standard deviations.

Baseline-adjusted scores were calculated by subtracting scores from the first baseline VAS before administration of oxytocin or placebo from scores on the second VAS and also from the scores on the third VAS. A 2 (Drug: oxytocin or placebo) x 2 (Time: Time: post stress or post stress + 30') repeated-measures analysis of variance (ANOVA) was conducted for each of the VAS using baseline-adjusted scores. The group means and standard deviations of the ANOVAs for the 38 participants are presented in Table 6.2.

Table 6.2

Means (*SD*) for VAS Measures in Oxytocin and Placebo Conditions in Females

		Placebo		Oxytocin	
		M	SD	M	SD
Alert	Baseline	51.50	23.13	53.39	23.61
	Post-stress	49.58	22.45	47.74	24.38
	Post-stress+30'	42.58	23.14	48.87	22.81
Anxious	Baseline	20.74	20.38	22.21	22.87
	Post-stress	19.34	18.70	17.21	16.97
	Post-stress+30'	18.68	18.94	17.87	15.68
Excited	Baseline	36.32	19.76	38.21	22.49
	Post-stress	38.71	24.38	37.55	22.08
	Post-stress+30'	31.76	19.77	35.45	23.36
Full	Baseline	23.45	19.78	31.92	24.59
	Post-stress	60.39	22.59	60.71	20.44
	Post-stress+30'	64.53	22.45	64.84	23.30
Happy	Baseline	58.68	18.11	56.29	21.12
	Post-stress	62.89	16.06	57.29	19.02
	Post-stress+30'	58.18	18.86	58.24	19.83
Thirsty	Baseline	56.45	27.44	56.16	24.84
	Post-stress	68.47	20.64	59.29	23.23
	Post-stress+30'	68.18	22.76	66.58	24.88

Happiness: There was no main effect of treatment condition [$F(1,37)$ 0.23, $p = .89$] or sample time [$F(1,37)$ 1.84, $p = .12$] in the happiness VAS but there was a significant interaction between treatment and sample time [$F(1,37)$ 5.13, $p = .029$, partial $\eta^2 = 0.11$], such that self-rated happiness declined from a slight rise immediately post-stress by the end of the session in the placebo condition but did not drop in the oxytocin condition.

There were no further main effects between treatment conditions nor were there any interactions in the remaining VAS data. However, self-rated excitement levels decreased significantly by the last session in both treatment conditions [$F(1,37)$ 9.07, $p = .005$, partial $\eta^2 = 0.51$] and ratings of fullness increased significantly through both sessions [$F(1,37)$ 6.80, $p = .013$, partial $\eta^2 = 0.39$].

6.3.6 Order Effects

Mean self-ratings of anxiety on VAS were significantly lower in the second session $t(37) = 2.34$, $p = .025$, see Table 6.3 for means and standard deviations. There were no order effects for self-reported state [$t(37) = 1.30$, $p = .20$] or trait [$t(37) = 0.89$, $p = .38$] anxiety on the STAI, however. All other measurements showed no significant differences or trends between sessions.

Table 6.3

Means (*SD*) for VAS Ratings of Anxiety in the First and Second Sessions

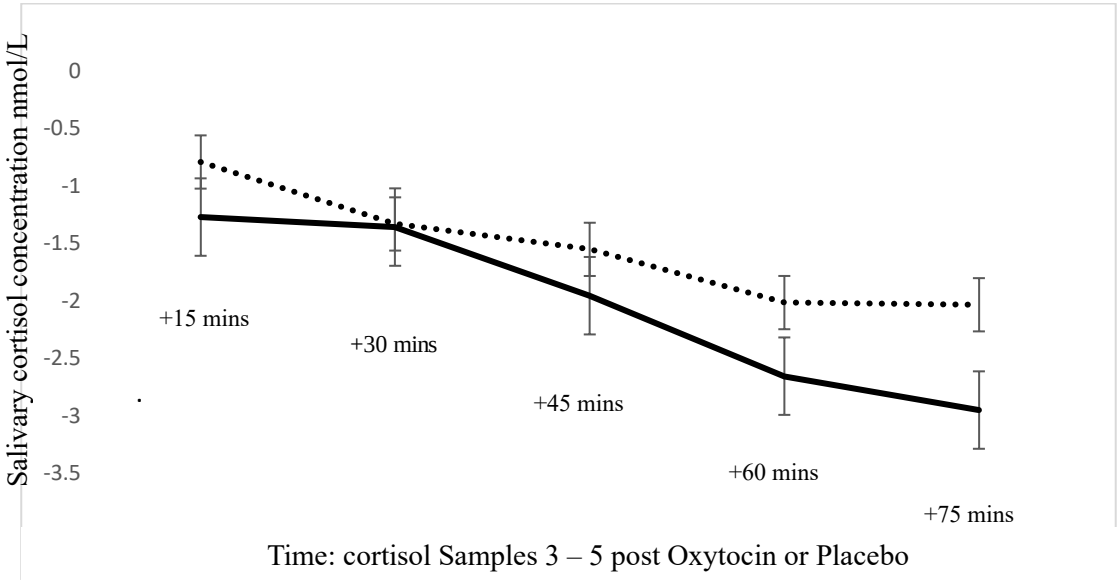
		M	SD
First Session	Baseline	24.97	24.88
	After oxytocin	19.82	19.31
	Endpoint	20.21	19.22
	Overall	21.67	18.37
Second Session	Baseline	17.97	17.18
	After oxytocin	16.74	16.21
	Endpoint	16.34	15.10
	Overall	17.02	15.13

6.3.7 Cortisol

The means of the two baseline samples (sample 1 and sample 2) were subtracted from each of the remaining samples of the same session (samples 3 to samples 7) to create baseline-adjusted data. A repeated-measures ANOVA was used to compare baseline-adjusted cortisol scores for salivary cortisol levels of samples 3 to 7 taken at 15-, 30-, 45-, 60-, and 75-minutes post administration (see Figure 6.3) between treatment conditions.

The administration of oxytocin did not significantly change cortisol levels [$F(1,37) = .85$, $p = .36$]. Mauchly's test indicated that the assumption of sphericity had been violated for the sample time factor [$\chi^2(9) = 72.00$, $p < .001$] and the interaction between sample and drug [$\chi^2(9) = 36.29$, $p < .001$], so degrees of freedom were estimated using the Greenhouse-Geisser estimates of sphericity ($\varepsilon = 0.50$ for sample, $\varepsilon = 0.65$ for interaction). As expected, there was a main effect of sample time with salivary cortisol quantities dropping over time [$F(2.0, 74.1) = 7.76$, $p = .001$, partial $\eta^2 = 0.23$] but there was no interaction with drug administered [$F(2.6, 96.0) = 0.82$, $p = .47$].

Area under the curve using the trapezoid method is a useful means of simplifying data from multiple repeated measures and maximising statistical power to detect significant differences without increasing alpha errors. A number of formulae can be used to calculate the area under the curve, each focusing on a different aspect of change. Area under the curve with respect to increase (AUCi) was used because in experimental designs where the baseline errors are minimised, it is the most effective formula to measure change over time (Fekedulegn et al., 2007). In this experiment, two baseline samples were taken and the mean of the two baselines was used, thus minimising baseline error. When the AUCi was compared between drug conditions in a repeated-measures ANOVA, however, no differences were seen, $F(1,37)$ 0.74, $p = .40$. Paired t tests on peak cortisol were also not significant between conditions using baseline-adjusted data, $t(37) = .74$, $p = .47$, and t tests on the baseline-adjusted final samples (60 and 75 minutes) found no significant differences either. There was no significant correlation between AUCi data and chocolate biscuit snack intake $r = -.01$.



Note. oxytocin condition indicated by a solid line and placebo condition shown with a dotted line. Error bars are standard error of the means.

Figure 6.3

Baseline-Adjusted Mean Salivary Cortisol Concentration at 15-, 30-, 45-, 60-, and 75-mins post drug in Females

6.3.8 Blood Glucose

A 2 (drug: oxytocin and placebo) x 2 (sample: first and second) repeated-measures ANOVA was used to analyse blood glucose samples in both drug conditions. The mean and standard deviation of the baseline sample in the oxytocin session taken before administration (M = 5.10, SD = 0.77) was lower than the second blood glucose sample taken 70 minutes post administration of oxytocin and 25 minutes after snack intake (M = 6.20, SD = 0.74). The mean and standard deviation of the baseline sample in the placebo session taken before administration (M = 5.04, SD = 0.68) was lower than the second blood glucose sample taken 70 minutes post administration of placebo and 25 minutes after snack intake (M = 6.15, SD = 0.78). There was no main effect of drug on blood glucose levels $F(1,37) = 0.28$, $p = .60$. There was, however, a main effect of sample time $F(1,37) = 83.95$, $p < .001$, with samples taken after eating being significantly higher than before eating. There was no interaction between blood sample time and drug condition $F(1,37) = 0.00$, $p = .98$.

6.3.9 Blood Pressure

Two separate 2 (drug: oxytocin and placebo) x 2 (sample: first and second) repeated-measures ANOVAs were conducted for systolic and diastolic measurements to assess the effect of oxytocin on blood pressure and the stress of the experimental procedure on blood pressure. Baseline blood pressure readings taken 10 minutes before administration of drug were subtracted from the readings taken before and after the stress tests to form baseline-adjusted data, the means and standard deviations are provided broken down by drug (Table 6.4).

Table 6.4

Means (*SD*) of Baseline-Adjusted Systolic and Diastolic Blood Pressure (mmHg) in Females

		Systolic		Diastolic	
		M	*SD*	*M*	*SD*
Oxytocin	Before Stress	0.50	7.74	-0.66	5.43
	After Stress	-1.03	7.32	-1.26	5.72
Placebo	Before Stress	1.21	6.61	-1.89	7.27
	After Stress	-0.11	6.25	-1.95	6.67

There were no main effects of oxytocin on either systolic [$F(1,37) = 0.31$, $p = .58$] or diastolic [$F(1,37) = 0.58$, $p = .45$] blood pressure. Neither systolic [$F(1,37) = 3.36$, $p = .08$] nor diastolic blood pressure [$F(1,37) = 0.23$, $p = .63$] changed from pre to post stress tests during the sessions and there were no interactions.

6.3.10 Heart Rate

Due to procedural error, the heart-rate data from 16 participants was not analysable. The heart-rate data for the remaining 23 participants was analysed by a 2 (drug: oxytocin and placebo) x 2 (sample: first and second) repeated-measures ANOVA to assess the effect of oxytocin on heart rate. Baseline heart-rate data

measured in beats per minute (BPM) was recorded for 10 minutes, then trimmed by the first and last two minutes to create a reliable baseline rate. Mean (± *SD*) heart-rate readings in the baseline placebo condition [82.79 ± 19.12 bpm] were below mean (± *SD*) readings obtained during the sing-a-song stress test [95.60 ± 15.57 bpm]. The same pattern was observed in the oxytocin sessions, where mean (± *SD*) heart-rate readings at baseline [82.38 ± 8.98 bpm] were below mean (± *SD*) readings obtained during the sing-a-song stress test [99.91 ± 17.33 bpm]. ANOVA results showed no main effect of drug [$F(1,22) = 0.37$, $p = .55$], but heart rate increased significantly in the sing-a-song test period with a large effect: $F(1,22) = 29.13$, $p < .001$, partial $\eta^2 = .57$; no interactions emerged.

To ensure that increases in heart rate during the SSST were not due to any physiological effects of singing, the anticipatory phase was calculated from 5 to 20 seconds after the researcher's start instructions and separated from the test phase, which was calculated from the end of the recording backwards for 20 seconds. Results were examined via a *t* test and the mean heart rate during the anticipatory phase ($M = 15.64$, $SD = 3.26$) was higher than the mean heart rate during the singing stress test ($M = 15.75$, $SD = 3.28$), but there were no statistically significant differences, $t(22) = 1.62$, $p = .12$. These results indicate that the rise in heart rate during the SSST was not driven by the potential physiological impact of singing.

6.4 DISCUSSION

This study demonstrated for the first time that intranasal administration of 24 IU of oxytocin significantly reduced postprandial intake of chocolate biscuits after stress tests in women, regardless of their status as stress eaters or non-stress eaters. Unlike previous studies in men (Ott et al., 2013), oxytocin had no significant effect on cortisol levels, suggesting that oxytocin's attenuation of palatable snacking in the absence of deprivation-induced hunger is not driven by oxytocin's stress suppressive effect on the HPA axis. This is the first study to show oxytocin reduces non-deprivation induced eating in women and unlike the previous study in Chapter 4, no reductions were seen on salty snack intake. Through the session, self-reported levels of happiness, excitement, and alertness declined significantly in the placebo condition but remained stable in the oxytocin condition, indicating that oxytocin positively affected mood and arousal by preventing a decline over the course of the session. In contrast to previous studies (Ott et al., 2013; e.g. Petersson, 2002b; Uvnas-Moberg, 1997), oxytocin did not affect blood glucose or blood pressure and it is likely that procedural differences account for this. Heart rate was significantly elevated after the stress tests but not affected by oxytocin and it might be that a more nuanced analysis of heart rate such as heart rate variability might reveal differences (Petersson, 2002a); since the purpose of the heart rate recording was to establish that the stress tests worked, this was not undertaken.

Although female oestradiol is highest peri-ovulation and in the luteal menstrual phase, in this experiment, we did not control for menstrual phase or hormone-based contraceptive usage. However, reductions in palatable snack intake occurred across different menstrual phases, suggesting that oestrogen-related interactions did

not drive attenuation of palatable snack intake (Asarian & Geary, 2006; Buffenstein, Poppitt, McDevitt, & Prentice, 1995). Further, oestradiol increases oxytocin levels but menstrual peaks of oestradiol result in increased appetite and food intake, rather than the expected reductions that oxytocin might bring about. Endogenous and exogenous oxytocin have different effects in rat pups (Engelmann, Mario, Ebner, Wotjak, & Landgraf, 1998). It is possible, therefore, that an exogenous oxytocin boost has a different effect from endogenous rises of oxytocin, which may explain how the data here are unaffected by menstrual fluctuations. Aulinas et al. (2018) distinguish between the patterns of postprandial peripheral oxytocin release, which remain stable across menstrual phases, and fasting levels of peripheral oxytocin recorded before food intake, which vary, to explain the discrepancy. However, previous studies rely on peripheral and central oxytocin measurements, which are not tightly correlated, so further research is needed (Mccullough et al., 2013).

Unlike the results of the male snack-test experiment (Chapter 4), there was no effect of oxytocin on salty food intake. The levels of salty cracker consumption were low in both the placebo and oxytocin conditions—4 .84 and 5.18 grams, respectively, which equate to about one cracker (cracker weight is 4.9 grams). Given that on average only one cracker was eaten in the control condition, a floor-effect might have prevented reductions in cracker consumption in the oxytocin condition. Stress can preferentially increase consumption of sweet and fatty food (O'Connor et al., 2008; Oliver et al., 2000), and it is possible that the stress potentiated sweet, but not salty, snack intake. In mice, oxytocin reduces consumption of sweet-tasting food including saccharin (Sclafani, Rinaman, Vollmer, & Amico, 2007), but its effects on salt intake are not directly comparable to humans, as oxytocin regulates osmolality in rodents (Verbalis, Blackburn, Olson, & Stricker, 1993). Pre-existing preferences for salty versus sweet food in participants were not examined and may have affected results, although placebo levels of snack intake were four times greater for chocolate biscuits than for crackers, suggesting that participants preferred sweet snacks. However, it also possible that the crackers supplied were not regarded as palatable, particularly in comparison to the chocolate biscuits. Animal research has shown that oxytocin's anorectic effects are mediated by VTA changes that dampen reward (Mullis, Kay, & Williams, 2013). Unlike the male snack-test experiment (Chapter 4), here, reductions in sweet-snack eating after oxytocin did not coincide with changes in sweet-taste sensitivity, suggesting that changes in sweet sensitivity did not contribute to attenuation of intake. Oxytocin's reduction of sweetness sensitivity is in line with previous research that found oxytocin significantly affected taste ratings (Ott et al., 2013; Thienel et al., 2016). The addition of acute stress promotes sweet food preference as sweet food reduces HPA axis activity (Pecoraro, Reyes, Gomez, Bhargava, & Dallman, 2004), so it is possible that oxytocin's relation to sweet-taste perception is specific to a stress paradigm.

Oxytocin's inhibition of chocolate snack intake in this experiment and Chapter 4's male experiment are of comparable magnitude: consumption by dropped by 57% in females and 63% in males. On the other hand, the German research groups, Ott and Thienel saw only 25% in normal weight men and 19.5% reductions respectively using a similar protocol (Ott et al., 2013 & Thienel, 2016). The Ott group tested participants on a wide array of eating-related measures, but the only significant effect of oxytocin on intake was with

chocolate biscuits. As described in section 4.4 of Chapter 4, it is possible that deprivation hunger played a role in snack eating for the German group participants. The neural mechanisms underlying oxytocin's anorectic capacity are complex but involve motivational and physiological changes, see Chapter 1 for details. In situations that demand an urgent need to stop eating, such as in the presence of poisons, oxytocin can bring about quick motivational changes to cease eating, but this is not true in non-urgent circumstances. For example, oxytocin levels rise upon onset of feeding, but the motivational "brakes" in the absence of urgency are not as powerful, as evidenced by the ubiquitous phenomenon of overeating, and it has been theorised that feeding-induced rises in oxytocin may be specific to rodents whose salt balance is regulated by oxytocin and simply reflect the anticipation of a solute load from feeding (Mandelblat-Cerf et al., 2017). It might be the case that the difference in the magnitude of oxytocin's effect (19.5% in Thienel et al., 2016 and about threefold in this experiment at 57%), is that palatable food intake in the presence of hunger is modulated differently by oxytocin from palatable food intake that is not initiated by deprivation-induced hunger.

There were differences in the self-report measures obtained in the male snack-test experiment (Chapter 4) and the female experiment described here. Men did not report a drop in their levels of happiness, excitement or alertness after placebo, whereas women in this experiment showed a significant decline in all three, which was prevented by oxytocin. Procedural differences may explain this difference as the female snack-test spanned 95 minutes and was designed to induce stress, whereas the male snack-test was shorter at 70 minutes and did not incorporate stress tests. There may also have been an experimenter effect that influenced male and female VAS ratings, as heterosexual males were tested by one female researcher and women were tested by two female researchers meaning dyadic intensity was reduced. Although the men completed only one VAS during the session whereas the women completed three, the male stress scores on the VAS were lower than any of the female stress scores, reflecting the fact that the female snack-test study was more stressful. However, in the male experiment there was a trend towards reduced anxiety in the second session but in the female experiment, the difference was significant. The stress of the male test was not as great as the female snack test study, meaning measurements for the initial session were higher for females who were then able to anticipate the stress in the second session and benefit from a level of preparedness that they did not have in the first session.

In this experiment cortisol levels fell, as expected, in line with circadian rhythms, whereas in both the Ott et al. (2013) and Thienel et al. (2016) studies, cortisol levels rose in both conditions after a free-choice breakfast buffet, although they were significantly lower in the oxytocin condition at 45 minute and two hours post administration. It is likely that the relatively high protein intake (36 g) from their breakfast buffet induced a metabolic processing rise in cortisol (Gibson et al., 1999), which was avoided in our study by limiting the protein content of lunch; this could also be related to the reduced postprandial glucose rise after oxytocin treatment (Ott et al., 2013). There may be alternative mechanisms involved that oxytocin acts on to reduce stress eating, such as the reward system or the suppression of the amygdala (Bale, Davis, Auger, Dorsa, & McCarthy, 2001; Huber, Veinante, & Stoop, 2005).

Nevertheless, Ott et al. (2013) reported that the oxytocin-induced suppression of cortisol (AUC) correlated with the reduction in chocolate cookie intake, suggesting some mechanistic link. However, oxytocin's regulation of the HPA axis is context dependent in rats, where oxytocin attenuates tonic HPA activity but not stress-induced activity. The serial sevens subtraction stressor employed here is widely used to trigger a stress-related cortisol response (Gotlib, Joormann, Minor, & Hallmayer, 2008), so oxytocin's effects may have a similar tonic-only effect in humans. The measurement of cortisol may also explain the differences between these findings and previous studies. The Gotlib, Ott and Thienel groups examined total plasma cortisol, only a small percentage of which is active (Gibson et al., 1999) and in this study the more active form of salivary cortisol was collected. Another possibility could be that males and females react differently to exogenous oxytocin (Weisman, Zagoory-Sharon, Schneiderman, Gordon, & Feldman, 2013).

The timing of cortisol collection may have affected results. The experiment took place in the late morning, which avoided the cortisol awakening response, but the individual sample points may not have captured potential cortisol fluctuations and resulted in a significant effect of oxytocin on acute stress remaining obscure. An inherent difficulty with salivary cortisol sampling and eating research is salivary contamination from food and fluid intake. In this study, fluid intake was limited to small sips directly after lunch to clean the palate in order not to dilute saliva, and assays were taken as far away from food intake as possible. An alternative possibility is that a combination of higher circadian oxytocin in the early light phase (Perlow et al., 1982) and the feed-forward mechanism of oxytocin (Van Ijzendoorn, Bhandari, Van der Veen, Grewen, & Bakermans-Kranenburg, 2012) resulted in higher oxytocin levels in participants tested during the morning phase in the Ott and Thienel groups compared with the lunch-time procedures in this experiment. Increased oxytocin levels may induce lowering of the HPA axis and cortisol release that were not possible at lower levels.

In this experiment, oxytocin significantly reduced postprandial snacking of palatable sweet biscuits in females without deprivation-induced hunger without concomitant reductions in salivary cortisol levels, heart rate, or blood pressure. This study did not measure oestrogen levels or control for menstrual fluctuations, and this would be a useful future research direction. The findings suggest that the inhibitory effect of oxytocin on stress-induced palatable food intake may not be mediated by an HPA axis mechanism, and that nasally administered oxytocin may reduce palatable food reward in women and contribute to tackling comfort eating and obesity. In contrast to Chapter 4, no effect of oxytocin was found for salty food. However, stress can preferentially increase consumption of sweet food and since the participants did not eat the salty snack in the control condition, a "floor" effect may have confounded the results. Alternatively, the salty snack may not have been sufficiently palatable and low consumption may have reflected this. A general palatability measure was not taken, and this will be addressed in the next snack-test experiment (Chapter 8) by introducing a general palatability rating for each snack food.

Oxytocin may achieve anorectic outcomes via converging or related brain pathways that work together, independently or both. In addition to reward modification, another possible route by which oxytocin might inhibit eating that has not yet been examined by this thesis, is the modulation of attention. Given the strong inhibitory effects of oxytocin demonstrated in the male and female studies, it is likely that the attentional processes that support consumption will be altered, and the next experiment of this thesis will address this.

CHAPTER 7 - OXYTOCIN'S REDUCTION OF ATTENTIONAL BIAS TO FOOD

7.1 INTRODUCTION

Although eating is an essential process and a natural reward generator, surprisingly little research demonstrates that attentional biases towards food exist in normal eaters who are not hungry. Attentional biases for food in the general population that arise from hunger, however, have been demonstrated in an array of attentional paradigms (Piech, Pastorino, & Zald, 2010; Tapper, Pothos, & Lawrence, 2010). Piech, Pastorino and Zald (2010) used the emotional attention-blink paradigm in which participants search for a target in a rapid stream of images that include distractors. They found that when participants were hungry, food stimuli acquired increased attentional power and ability to distract participants from targets. Tapper, Pothos and Lawrence (2010) used pictures of appetising and bland food alongside neutral objects in a visual dot-probe task and demonstrated that hunger impaired disengagement from bland and palatable food cues. In a word dot-probe design, Mogg, Bradley, Hyare, and Lee (1998) found that hungry participants had attentional biases to stimuli presented for 500 ms but not 14 ms.

Oxytocin's effects on hypothalamic activation to high-calorie food pictures in hungry participants has also been investigated. In an *f*MRI study, oxytocin significantly attenuated BOLD responses to high versus low calorie food images in the hypothalamus (van der Klaauw, Agatha et al., 2017). No other brain regions were significantly affected by oxytocin in the van der Klaauw et al. study, and hypothalamic suppression by oxytocin did not coincide with decreased food intake in an ad libitum breakfast buffet. The significant but local effects of oxytocin that decrease hypothalamic activation suggest that limbic reward centres were not affected by oxytocin. This may be attributable to the fasted state of participants, as hunger enhances food reward, and in the absence of aversive signalling suggesting poison ingestion or similar urgent cessation signals, oxytocin may not influence reward pathways when there is an energy deficit.

Oxytocin-driven attentional shifts away from food-related stimuli in anorexic women biased to food, have been demonstrated in the laboratory. Anorexic women who had eaten lunch viewed food-, shape-, and weight-related pictures, together with "neutral" body-related images, such as eyebrows. After oxytocin, the attentional bias towards food-related stimuli observed in the placebo condition was reduced (Kim et al., 2014). Importantly, in the same study, Kim and colleagues demonstrated the same attentional bias to food in the healthy control group after placebo administration; this bias was not reduced by oxytocin. In a separate group of anorexic women, intranasal oxytocin also attenuated a bias towards food-related images (Leppanen et al., 2017). Leppanen et al.'s reductions in attentional bias were preceded by decreases in salivary cortisol levels, which may suggest that food-related anxiety was lowered by oxytocin in people with anorexia.

Recent evidence has extended the findings of research to demonstrate an attentional bias towards food that is

independent of hunger. Apart from the baseline attentional bias towards food pictures in healthy women who had eaten were incidentally demonstrated by Kim et al. (2014) and discussed above, Kumar et al. (2016) used electrophysiological measures of attention to assess performance on a word-search paradigm that was conducted in satiated participants who had eaten breakfast. Participants either had to remember, or to hold in working memory, a food or non-food cue, before searching for the target. Results showed that participant attention was strongly affected by food stimuli and correlated with BMI indicating that attention and eating behaviour are linked.

Neural sensitivity to food reward and attentional bias to food stimuli are both greater in the overweight and in people with obesity (Brignell, Griffiths, Bradley, & Mogg, 2009; Stice, Eric, Spoor, Ng, & Zald, 2009; Volkow, Wang, & Baler, 2011). Using eye tracking and a visual dot-probe, obese participants were found to have a bias towards food pictures in a satiated state that persisted in the fasted state (Castellanos et al., 2009). In a similar visual dot probe paradigm using eye tracking, Werthmann et al. (2011) found that overweight participants showed increased initial orientation towards food stimuli which also correlated with increased self-rated cravings. Whether increased food reward sensitivity precedes obesity as a causal factor, however, remains unknown but in an obesogenic society, reducing food intake is clearly challenging for the overweight and obese. Neural activity associated with hunger and satiety has also been shown to differ between males and females (Del Parigi et al., 2002; Führer, Zysset, & Stumvoll, 2008). The patterns of neural activation were also shown to differ in two studies using pictorial food stimuli, whereby stronger neural activation was only observed in women (Frank, S. et al., 2010; Uher, Treasure, Heining, Brammer, & Campbell, 2006). However, although the calorie contents of the food pictured and participant satiety also influenced results, both these studies used small samples, with only six and eight female participants, respectively.

In humans and animals, the phylogenetically highly conserved neuropeptide oxytocin is involved in promoting a range of behaviours and might, therefore, divert attentional resources towards them. As demonstrated in the preceding snack-test studies (Chapters 4 and 6), oxytocin reduces palatable food intake in food-satiated men and women (Burmester, Higgs, & Terry, 2018; Leslie et al., 2018). Central and peripheral oxytocin release is triggered by eating (Blevins & Ho, 2013), and multiple mechanisms subserve its anorectic actions, described in detail in Chapter 1 of this thesis. These dual roles for oxytocin of promoting bonding and anorectic functioning are supported by neural evidence that the ventromedial nucleus of the hypothalamus, a site known to play an important role in energy balance and behaviour related to reproduction, has very high levels of oxytocin receptors that reciprocally regulate these two functions (Leng & Ludwig, 2008; Leng et al., 2008; McCarthy, Kleopoulos, Mobbs, & Pfaff, 1994; Tribollet, Barberis, Jard, Dubois-Dauphin, & Dreifuss, 1988). The motivational transfer from food-related incentives to reproductive ones mediated by ventromedial hypothalamic neurons (Leng et al., 2008; McCarthy, M. M. et al., 1994) cannot wholly explain motivational or attentional shifts mediated by oxytocin. Eating is one of a small number of primary reinforcers that stimulate mesolimbic reward circuits without conditioning, and

extrahypothalamic accumbal and striatal dopamine is restrained by oxytocin (Blevins & Ho, 2013) to reduce motivation for hedonic consumption (McGregor & Bowen, 2012).

The motivational shift from food to sexual targets also fails to explain how the administration of intranasal oxytocin creates an attentional bias towards social stimuli (Domes et al., 2013; MacDonald, K. & MacDonald, 2010; Shamay-Tsoory & Abu-Akel, 2016). Although this bias is robust and has been demonstrated in other species after nasally applied oxytocin, such as in the rhesus monkey that had reduced attention to negative facial expressions in the dot-probe paradigm, sex differences exist (Parr, Modi, Siebert, & Young, 2013). Oxytocin attenuates amygdala activity in men (Domes et al., 2007) but selectively enhances amygdala reactivity to fearful faces in women (Domes et al., 2010), and in the "Interpersonal Perception Task" (Costanzo & Archer, 1989) increases of perception of kinship in women and perception of competition in men after intranasal oxytocin are observed (Fischer-Shofty, Levkovitz, & Shamay-Tsoory, 2012). Tactile and vocal social contact also trigger oxytocin release in the central nervous system, which leads to the formation or maintenance of conspecific affiliation (Seltzer, Ziegler & Pollak, 2010).

The range of body masses of the participants used in this thesis reflects the prevalence of obese and overweight individuals in the normal population, which in 2015 was estimated to be about two-thirds of adults in England (UK Government, 2017). BMI is associated with food-related cue-responsivity, with people with obesity exhibiting greater attentional biases to food-related cues relative to lean individuals (Rothemund et al., 2007; Stoeckel et al., 2008). These changes are attributed to increased craving and thought to result from changes to the mesolimbic dopaminergic reward system and subsequent hedonic pleasure (Berridge, 1996; Robinson. & Berridge, 1993; Volkow. & Wise, 2005; Volkow et al., 2011). Craving may also contribute to attention to food in healthy weight participants. In an eye-tracking study that measured perceived availability of chocolate, increased craving was associated with higher attentional biases to food (Werthmann, Roefs, Nederkoorn, & Jansen, 2013). As an individual's cravings for food indicate the increased salience or preoccupation with food, vigilance towards food in a dot probe task is likely to be affected (Jones, Nicola & Rogers, 2003; Timmerman & Gregg, 2003).

Increased preoccupation with food is a consequence of restrained eating, too (Herman & Polivy, 1975), and a number of studies link restrained eating to attentional bias for food-related stimuli, although the direction of results is not consistent, as some studies find restrained eaters have increased avoidance of food stimuli whilst other experiments reveal elevated attention. Using a range of attentional tasks, high restraint scores have been associated with greater attention towards food stimuli. For example, using the Stroop test, restrained eating in a nonclinical population was related to greater attention towards food words, irrespective of the dieting status of the participant (Green & Rogers, 1993). A similar association between restrained eaters and enhanced attention to food stimuli using a Stroop test was found by Boon, Vogelzang, and Jansen, (2000) but the same experiment could not replicate the association using a word-based dot probe. Using a word-search task where participants located a food-related word from a matrix of neutral words, restrained

eaters were significantly faster than non-restrained eaters at detection (Hollitt, Kemps, Tiggemann, Smeets, & Mills, 2010). The relationship between dietary restraint and food-related attentional bias has, therefore, been found across an array of attentional tasks and so the type of task is unlikely to solely explain the inconsistent findings. An array of parameters, such as cultural norms, procedural differences, and individual traits, are likely to influence the relationship between restrained eating and attentional bias to food-related stimuli.

The instrument used to measure restrained eating, however, may also contribute to differing results. Restrained eating is commonly measured using one of two scales: The Restraint Scale (Polivy, Herman, & Howard, 1988) and the restraint subscale of the DEBQ (DEBQ-R). However, the Restraint Scale conflates restrained eating with disinhibited eating whereas the DEBQ captures successful restraint and separates the constructs of restrained and external eating into separate subscales (Calitri, Pothos, Tapper, Brunstrom, & Rogers, 2010; Pothos, Calitri, Tapper, Brunstrom, & Rogers, 2009; Tapper, Pothos, Fadardi, & Ziori, 2008; Wardle et al., 2000).

Research has also linked restrained eating and loneliness. Experimentally induced loneliness caused restrained eaters to eat more food, whereas non-restrained eaters ate less (Rotenberg & Flood, 1999). In the same study, the food consumed by individuals high in restraint increased as a function of loneliness but decreased for participants who were low in restraint. Obese women's scores of loneliness using the UCLA Loneliness Scale (UCLA) were higher than normal-weight women, and loneliness was interpreted as causing overeating (Schumaker, Krejci, Small, & Sargent, 1985). Loneliness is included as a potential factor that triggers eating in the Three-Factor Eating Questionnaire (Stunkard, 1981) and the Emotional Eating Questionaire (Arnow, Kenardy, & Agras, 1995). Loneliness has also been identified as contributing to and fuelling a range of eating disorders, including compulsive eating (Levine, 2012) – this relationship will find further support in Chapter 9. As loneliness scores are higher in individuals who are not in romantic relationships relative to individuals in high-quality romantic partnerships (Diener, Gohm, Suh, & Oishi, 2000; Qualter et al., 2015), romantic attachment in participants was assessed.

Altered oxytocin function is also associated with loneliness, although whether isolation reduces oxytocin, or whether reduced central levels of oxytocin precipitate alterations to sociality is unknown. Two populations at risk of social isolation—substance abusers and the elderly—both have lower plasma oxytocin levels (Elabd et al., 2014; McGregor & Bowen, 2012; Sannino, Chini, & Grinevich, 2017) although there is evidence that age-related socioemotional changes produce loneliness in the elderly (Ebner, Natalie C., Maura, MacDonald, Westberg, & Fischer, 2013). Interestingly, depression caused by loneliness is ameliorated by oxytocin administration (Chagnon, Potvin, Hudon, & Préville, 2015). Loneliness levels on the UCLA also moderated oxytocin's beneficial cardiac control with higher levels of loneliness being associated with diminished parasympathetic cardiac reactivity to intranasal oxytocin (Norman et al., 2011). As early-life infant-parent separation modifies the oxytocin response to social stress, as measured by

sympathetic cortisol release in adult men (Meinlschmidt & Heim, 2007), oxytocin is implicated in autonomic as well as genetic resilience to loneliness and was, therefore, measured in this experiment.

Oxytocin is an important part of the dyadic bonding process between mother and infant, and disruptions to this process may affect later oxytocin levels in the child (Apter-L., Feldman, Vakart, Ebstein, & Feldman, 2014; Feldman et al., 2012). Lower CSF levels of oxytocin have been recorded in women with a history of childhood abuse compared with controls (Heim et al., 2009). Wismer Fries, Ziegler, Kurian, Jacoris, and Pollak (2005) showed that adverse childhood experience alters the oxytocin system and may lead to extensive difficulties later in life. Attempts have been made to link attachment styles to oxytocin levels and it is theorised that poor attachments to caregivers result in lower long-term oxytocin levels (Insel & Young, 2001; Nelson & Panksepp, 1998; Strathearn, 2011; Wismer Fries et al., 2005). After intranasal oxytocin, adults who experienced early life stress were found to have blunted stress responsivity and limbic deactivation (Grimm et al., 2014); parental bonding, therefore, was assessed in this experiment.

Anxiety not only contributes to adverse childhood experiences, loneliness, and overeating but also affects attentional processes. For example, stress-induced rises in cortisol altered preconscious selective attention in a Stroop task (Roelofs, Bakvis, Hermans, van Pelt, & van Honk, 2007), and the HPA axis plays a key role in facilitating an organism's threat response (Aston-Jones, Rajkowski, Kubiak, & Alexinsky, 1994) by influencing attention allocation. As detailed in Chapter 1 (section 1.5), oxytocin is anxiolytic and suppresses the HPA axis (Churchland & Winkielman, 2012), so the relevance of oxytocin to attentional mechanisms, therefore, is multifold.

The potential of oxytocin to alter attentional food biases in normal eaters has not been yet explored. Given the distinct pathways in the medial prefrontal cortex for feeding and bonding that oxytocin regulates and the transfer from food-related incentives to reproductive ones via oxytocin receptors in the ventromedial nucleus of the hypothalamus (Leng et al., 2008; McCarthy et al., 1994), attention experiments using pictures representing food, social, and sexual bonding, would enable comparisons of oxytocin's effect across the three stimuli. This is the first time that three types of stimuli relating to motivations that are independently modulated by oxytocin have been examined alongside each other. Contrasting the two affiliative domains of romantic and social with eating related stimuli will enable any differences in attention caused by oxytocin to be explored, thus providing tentative evidence of preferential enhancement of one type of bonding over another by oxytocin.

In line with previous research (Kim et al., 2014; Kumar et al., 2016), an overall bias towards food-related stimuli was expected. Experimental aims were concealed and the potential influences on endogenous oxytocin function that also link to food-related biases were measured, and it was predicted that participants high in loneliness would be more responsive to oxytocin than individuals who were less lonely. Levels of restraint on the DEBQ, high food cravings, and high trait stress were expected to correlate with high

vigilance to food.

7.2 METHOD AND MATERIALS

7.2.1 Participants

Forty-two well-rested, healthy adults aged between 19 years and 38 years (*M* = 24.02 yrs; *SD* = 4.78 yrs) with BMIs ranging from underweight at 15.43 kg/m^2 to obese at 35 kg/m^2 (*M* = 23.73 kg/m^2; *SD* = 4.39 kg/m^2) were recruited in exchange for course credits. Two participants were excluded as they did not complete the online surveys, leaving forty participants. By gender, there were 11 men aged 21 years to 38 years (*M* = 25.67 yrs; *SD* = 5.52 yrs) with BMIs ranging from 19.81 kg/m^2 to 33.03 kg/m^2 (*M* = 24.73 kg/m^2; *SD* = 4.61 kg/m^2) , and 29 women aged 19 years to 37 years (*M* = 23.55 years; *SD* = 4.53 years) with BMIs ranging from 15.43 kg/m^2 to 35.00kg/m^2 (*M* = 23.44 kg/m^2; *SD* = 4.36 kg/m^2). Five participants (one male) were lefthanded. Inclusion and exclusion criteria were scaled-back from those presented in the General Methods (Chapter 2, section 2.1.1) as food intake was not measured. Individuals on a vegan diet or with diabetes were excluded due to the food provided and exclusion criteria from the General Methods (Chapter 2) germane to oxytocin research were applied, so pregnant or breastfeeding women, and individuals taking psychoactive drugs were also excluded. To participate, individuals had to confirm on the day to the researcher that they had abstained from alcohol and nonsteroidal anti-inflammatories for 12 hours.

7.2.2 Materials

Details of nasal sprays were consistent with the General Methods (Chapter 2, section 2.3). All participants consumed a readily catabolisable chocolate bar (Kitkat®, 40g, 233 kcal) and either a carton of apple or orange juice (200ml, 91 kcal or 84 kcal respectively). This food was designed to boost blood sugar levels during the attention task to minimise fatigue and deprivation-induced hunger that might have increased attention to food pictures. The measurement of height was made using a wall ruler and Koogeek biometric impedance scales were used to weigh participants. Images were displayed using a 20-inch monitor, table-mounted at head height with a fixed keyboard for responses. For task presentation and recording of the responses, Superlab presentation software (Version 5.0, Cedrus Corporation, CA, USA) was used.

7.2.2.1 Questionnaires

Rubin's Romantic Love Scale (details provided in Chapter 4) was used together with the STAI-Y and the DEBQ (both described in Chapter 5).

The Parental Bonding Instrument (PBI: Parker, G., Tupling, & Brown, 1979) was also used. The 25 items are assessed separately for mother and father, resulting in 50 responses. In this survey, the PBI was prefaced with options to complete only the father or mother section, as relevant. The PBI examines two separate dimensions: a care-neglect continuum and an estimate of overprotection. Thus 13 statements, such as "spoke to me in a warm and friendly voice" constitute the parental care-neglect construct, and the remaining 12 statements investigate parental overprotection, for example, "Did not want me to grow up". For each

statement, respondents select the most appropriate response from: “Very like”, “Moderately like”, “Moderately unlike”, and “Very unlike”. Low PBI scores on the care dimension indicate a lack of care and predict adult anxiety and high care scores correlate with altered oxytocin function (Feldman et al., 2012) The validity and reliability of the PBI are high, and in one cohort, scores remained stable across an extended 20-year period (Wilhelm, Niven, Parker, & Hadzi-Pavlovic, 2005).

Lastly, 20-item Revised UCLA Loneliness Scale version 3 (Russell, Paplau & Cutrons, 1980), was used, which measures subjective feelings of loneliness as well as feelings of social isolation. Version 3 was shown by the scale’s authors to have high internal consistency and re-test reliability over the period of one year and is a prominent measure of loneliness within psychology, including oxytocin research (Russell, D. W., 1996). The scale contains 20 positive and negative statements about social relations and respondents are required to say how often they feel the way described by statements such as “There is no one I can turn to” and “There are people who really understand me”. There are four possible responses ranging from (1) "never" to (4) "often". The ratings of all 20 statements are averaged and high scores represent greater loneliness.

To assess trait levels of food craving, the Food Cravings Questionnaire trait subscale (FCQ-T) was used, which is the most often used instrument for the assessment of food cravings and was developed to assess cravings that relate to palatable food intake in addition to physiological hunger. The FCQ-T consists of 39 statements, such as “Eating what I crave makes me feel better” and “I daydream about food”. The instructions of the FCQ-T ask participants how frequently each statement “would be true for you in general” using a 6-point scale that ranges from 1 (never or not applicable) to 6 (always). Scores on the FCQ-T have been found to be positively associated with BMI, eating pathology, low dieting success, and increases in state food craving during cognitive tasks involving appealing food stimuli. Subscales are highly inter-correlated and internal consistency of the total FCQ-T is $\alpha > .90$ (Cepeda-Benito, Gleaves, Williams, & Erath, 2000; Meule & Kübler, 2012).

7.2.2.2 Picture Stimuli

Forty pictures of palatable salty and sweet food were paired with forty neutral objects of common household objects, such as a ruler or metronome, matched carefully for colour and shape (stimuli were from Birmingham University’s food research database, see Appendix III for examples of each type of stimuli used). Social and romantic pictures from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 1999) were validated by 215 respondents for typicality within their category, and the top 40 pictures of each category were used and randomly allocated into two sets of 20 pictures each for counterbalancing. All pictures were three inches high and four inches wide.

7.2.3 Procedure

The experiment conformed to the Declaration of Helsinki and ethical approval was given by Kingston University’s Research Ethics committee. To disguise experimental aims, descriptions on the online course

credits portal were for recruitment to a general attention study using oxytocin and provided the inclusion and exclusion criteria. Before the experiment began, exclusion criteria were confirmed, and participants provided informed consent. Using alternating nostrils, participants self-administered 24 IU of oxytocin or placebo, depending on session, supervised by a researcher. Participants provided their age and were then blind weighed by the researcher by facing away from the scales. Height and weight were measured by the experimenter and the participants age, gender, and handedness were recorded. Participants then watched a neutral video (*Life of Plants*, David Attenborough) for about half an hour. At 40 minutes post inhalation, participants consumed the snack and drink before undertaking the dot probe task. After completing both laboratory sessions, participants completed the questionnaires online via a Qualtrics link.

7.2.3.1. Dot Probe Task

Participants were seated in front of a monitor and were told that their reaction times to a dot probe would be measured. The dot probe task design was based on two studies that showed attentional effects via oxytocin with the same dot-probe procedure: Leppanen et al. (2017) that showed oxytocin reduced attentional food bias in people with anorexia, and by Simplicio, Massey-Chase, Cowen, and Harmer, (2009) that showed attentional effects of oxytocin on emotional stimuli. Participants were instructed to indicate the dot probe position by responding as quickly as possible without compromising accuracy, by pressing one of two keyboard 'home' keys with raised haptic dashes, which had also been marked in different coloured tape. The importance of looking at the fixation cross in between trials was emphasised, to avoid reaction times reflecting the previous set of pictures. At the beginning of each trial a central fixation cross was presented for 500 ms, which was immediately followed by a picture pair with the two pictures of the pair being presented in the right and the left part of the screen for 500 ms, as a positional bias can be created when stimuli are displayed above one and other (Brosschot, de Ruiter, & Kindt, 1999; Johansson, Ghaderi, & Andersson, 2004; Mogg, Bradley, & Hallowell, 1994; Mogg, Bradley, Hyare, & Lee, 1998). Upon picture offset, a dot probe (solid circle, Arial, size 50, black on a white background) appeared in either the location of the right or the left picture and remained until the participant pressed one of the response keys. After a valid response, a 500 ms break occurred, then the fixation cross of the next trial was presented. Practice trials were undertaken by each participant until they were satisfied that they understood the task. A total of 180 trials per session were administered and two sets of pictures were used: Set A and Set B, which were presented in a counterbalanced design. Table 7.1 shows the pairing scheme and position of the probe behind the picture used for both sets. The picture locations (left or right), dot probe locations (left or right) and presentation order were randomised for each participant.

Table 7.1

Dot Probe Stimuli Presentation Scheme and their Organisation Across Right/Left Visual Fields

Left	Right
10 food	• 10 neutral
10 neutral	• 10 food
10 romantic	• 10 food
10 social	• 10 food
10 neutral	• 10 neutral
10 romantic	• 10 social
10 social	• 10 romantic
10 food	• 10 social
10 food	• 10 romantic
• 10 food	10 neutral
• 10 neutral	10 food
• 10 romantic	10 food
• 10 social	10 food
• 10 neutral	10 neutral
• 10 romantic	10 social
• 10 social	10 romantic
• 10 food	10 social
• 10 food	10 romantic

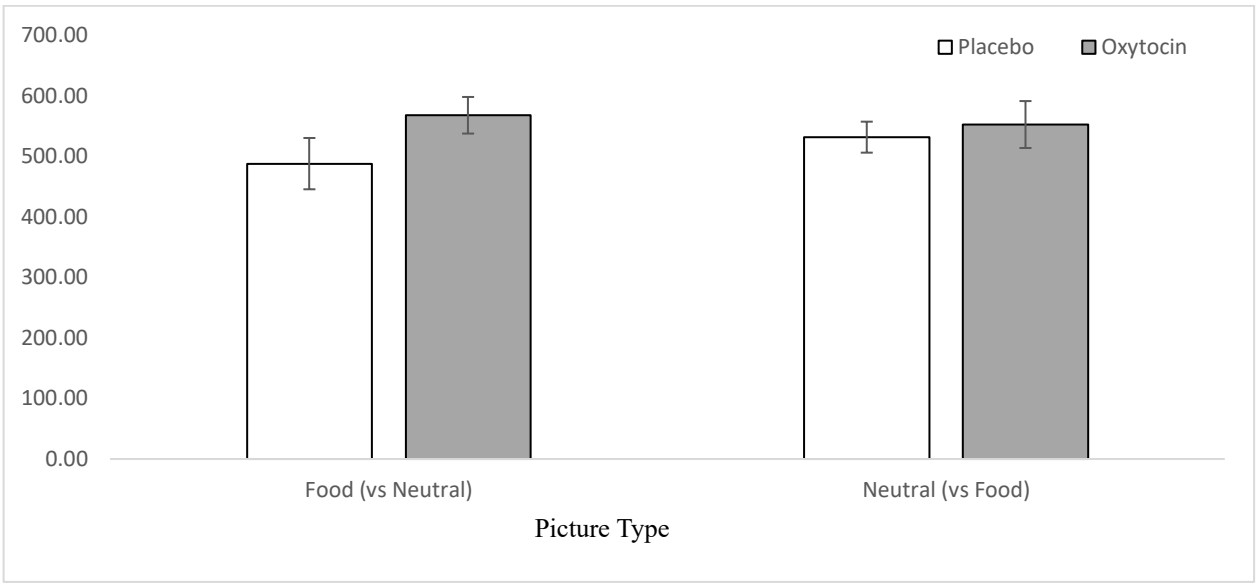
7.3 RESULTS

7.3.1 Data preparation

All trials with errors or response latencies below 200 ms were removed from the results, as values below 200 ms might indicate that a response was not based on the stimulus (Ehrman et al., 2002). Previous research has commonly eliminated latencies either over 1000 ms and 1500 ms, or on the basis of their standard deviation from the mean. In this data set, there were no outliers between 1000 ms and 1300 ms, but thirty-three cases were greater than 1300 ms, some six standard deviations above the mean RT. The boundary for exclusion justified by the data was, therefore, 1300 ms, a set point also used in previous research as a suitable compromise between 1000 ms and 1500 ms (Kujawa et al., 2011). Overall, fewer than 1% of responses were discarded.

7.3.2 Analysis of Attentional Bias

The means and standard deviations for congruent responses to probes associated with neutral and food picture types, in both the placebo and oxytocin condition are shown in Figure 7.1 (detailed statistics are provided in Appendix IV). A 2 (Drugs: oxytocin or placebo) x 2 (Picture: food or neutral) repeated-measures ANOVA was conducted that revealed a main effect of drug [$F(1,39) = 76.89$, $p < .001$, partial $\eta^2 = .66$] whereby administration of oxytocin had a significant effect on response times in the dot probe. There was also a significant interaction between drug and picture type [$F(1,39) = 20.14$, $p < .001$, partial $\eta^2 = .34$] that indicated oxytocin reduced attention to food pictures only.



Note. Error bars are standard deviations

Figure 7.1

Mean (*SD*) Reaction Times (ms) of Food Congruent vs Neutral Congruent Picture Probes in Placebo and Oxytocin Conditions of Dot Probe Task

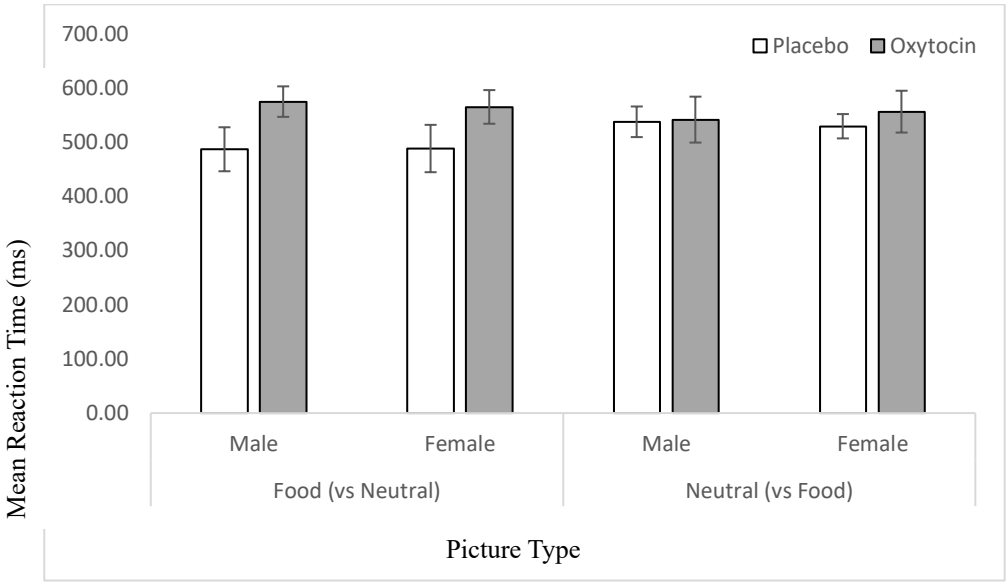
7.3.3 Initial Orientation versus Disengagement

To examine whether the attentional bias was due to initial orientation or to difficulties in disengagement from the stimuli, congruent food stimuli versus neutral pictures in the oxytocin condition were compared with neutral versus neutral pictures in the oxytocin condition via a *t* test, and then incongruent food stimuli versus neutral pictures in the oxytocin condition were compared with neutral versus neutral pictures in the oxytocin condition via a second *t* test. Results were non-significant for both: $t(39) = 1.63$, $p = .11$ and $t(39) = 0.30$, $p = .77$, respectively, meaning neither initial orientation nor disengagement were identified as dominant.

7.3.4 **Gender**

The means and standard deviations for the drug conditions and neutral or food pictures are broken down by gender and presented in Figure 7.2 (detailed statistics are provided in Appendix IV). In a 2 (drug: oxytocin vs placebo) x 2 (picture: food vs neutral) x 2 (gender: male vs female) mixed-model ANOVA the administration of oxytocin had a significant effect on mean response times regardless of picture type or

gender [$F(1, 38) = 63.54$, $p < .001$, partial $\eta^2 = .60$]. The picture type was also a significant factor impacting reaction times independent of the drug condition or gender of person viewing it [$F(1, 38) = 5.81$, $p = .02$, partial $\eta^2 = .13$] with responses to dot probe replacing neutral pictures being greater than for food pictures, indicating increased attention towards food pictures. There was no main effect of gender [$F(1, 38) = .01$, $p = .93$], however. There was no significant interaction between drug and gender [$F(1, 38) = .21$, $p = .65$], or picture choice and gender [$F(1, 38) = 0.52$, $p = .47$]. There was also no three-way significant interaction between drug and picture choice by gender [$F(1, 38) = 1.34$, $p = .25$]. There was, however, a significant interaction between drug and picture type [$F(1, 38) = 20.67$, $p < .001$, partial $\eta^2 = .35$], showing that oxytocin reduced attention to food pictures.



Note. Error bars are standard deviations

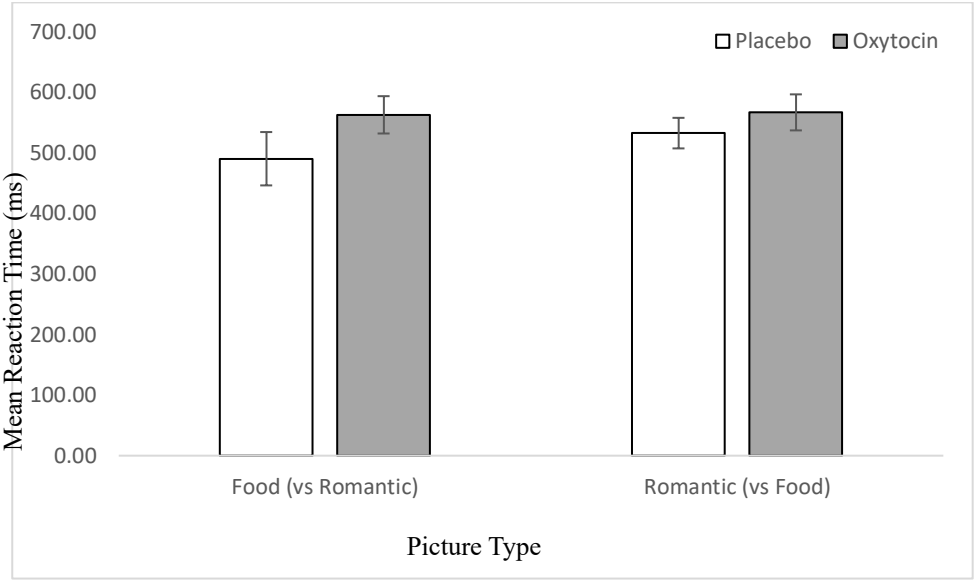
Figure 7.2

Mean (*SD*) Reaction Times (ms) of Food Congruent and Neutral Congruent Picture Probes by Gender in Placebo and Oxytocin Conditions of Dot Probe Task

To further check the impact of gender specifically on food stimuli, food bias scores were calculated with the formula used in previous studies, see for example MacLeod (1988) and Mathews and Mogg, Bradley, Brendan and Williams (1995). This was calculated by subtracting the mean reaction time when the probe was in the same position as food picture (congruent trials) from the mean reaction time when the probe was in a different position to food pictures (incongruent trials) in both drug conditions. Food bias scores were compared between men and women in a mixed-measures ANOVA. Mean male bias scores (M = -425.18 ms, SD = 294.46 ms, n = 11) were below those of females (M = -379.43 ms, SD = 308.63 ms, n = 29), but ANOVA results showed this was not significant: $F(1, 38) = 0.18$, $p = .67$. In line with other results, there was a main effect of drug condition on bias scores: $F(1, 38) = 21.39$, $p < .001$, partial $\eta^2 = 0.41$ that indicated oxytocin reduced attention to food picture probes.

7.3.5 Food versus Social Pictures and Food versus Romantic Pictures

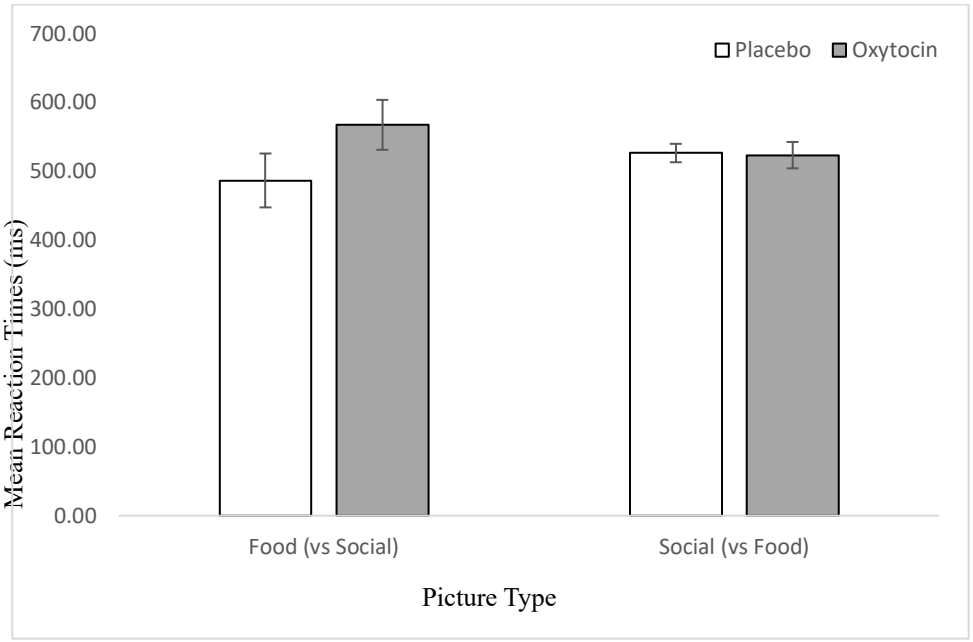
The means and standard deviations for congruent responses to probes associated food, romantic and social picture types are presented in Figures 7.3 and 7.4 for both the drug conditions (see Appendix IV for the detailed statistics). To compare food and romantic images, a repeated-measures 2 (drug: oxytocin vs placebo) x 2 (picture: food vs romantic) was conducted. The main effect of drug [$F(1,39) = 54.77$, $p < .001$, partial $\eta^2 = .57$] was significant meaning that administration of oxytocin lengthened reaction times irrespective of the type of picture viewed. There was no main effect of picture type [$F(1, 39) = 0.63$, $p = .43$]. Figure 7.3 illustrates the significant interaction between drug and picture type, which revealed that oxytocin only reduced attention to food stimuli [$F(1, 39) = 47.53$, $p < .001$, partial $\eta^2 = .55$]. There was a significant interaction [$F(1,39) = 50.45$, $p < .001$, partial $\eta^2 = .55$], such that oxytocin reduced attention to food stimuli only.



Note. Error bars are standard deviations

Figure 7.3

Mean (*SD*) Reaction Times (ms) to Food Congruent and Romantic Congruent Picture Probes in Oxytocin and Placebo Conditions of the Dot Probe Task



Note. Error bars are standard deviations

Figure 7.4

Mean (*SD*) Reaction Times (ms) to Food Congruent and Social Congruent Picture Probes in Oxytocin and Placebo Conditions of the Dot Probe Task

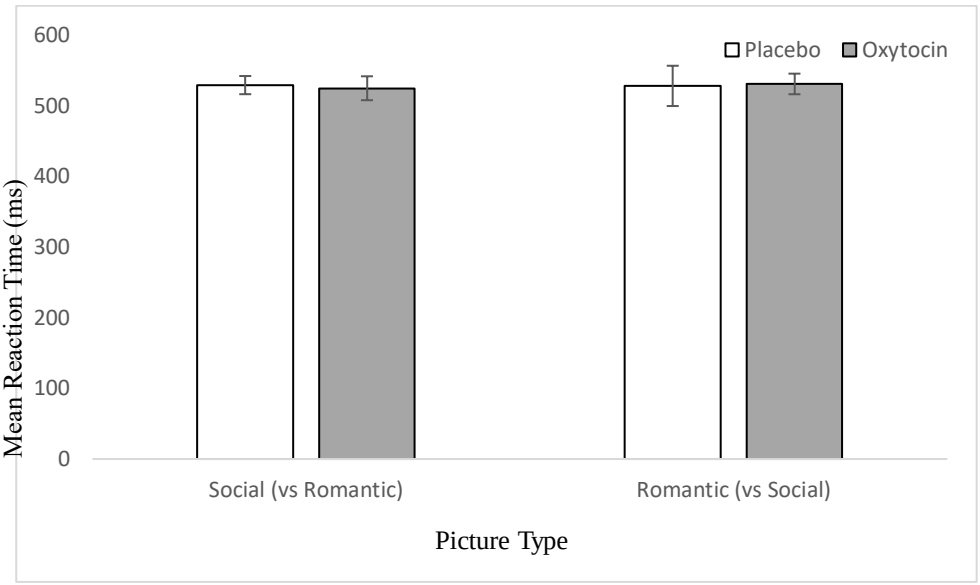
A repeated-measures 2 (drug: oxytocin vs placebo) x 2 (picture: food vs social) ANOVA was conducted to compare food and social stimuli. Results showed that administration of oxytocin increased mean response times to pictures [$F(1,39) = 63.77$ $p < .001$ partial $\eta^2 = .62$], but that there was no main effect of picture type on response times [$F(1, 39) = 0.08$, $p = .78$]. Figure 7.3 shows that there was a significant interaction between drug and picture type [$F(1, 39) = 81.33$, $p < .001$, partial $\eta^2 = .68$] such that oxytocin reduced attention paid to food stimuli only.

7.3.6 Sweet- Versus Salty Food Pictures

A repeated-measures 2 (oxytocin vs placebo) x 2 (sweet vs savoury) ANOVA was conducted to examine whether participants responded differently to sweet and savoury pictures in oxytocin and placebo conditions. Average reaction times were slower with oxytocin for both sweet food ($M = 540.87$ ms, $SD = 19.24$ ms) and savoury food ($M = 539.43$ ms, $SD = 16.90$ ms) than they were for sweet ($M = 527.03$ ms, $SD = 17.77$ ms) and savoury food ($M = 527.64$ ms, $SD = 15.31$ ms) in the placebo condition. There was a main effect of drug on reaction times to sweet and savoury picture probes: $F(1, 39) = 69.49$, $p < .001$, partial $\eta^2 = .64$. However, there was no main effect of type of food used in the pictures [$F(1, 39) = 0.20$, $p = .65$] and no interaction, $F(1, 39) = .92$, $p = .34$.

7.3.7 Social and Romantic Pictures

To assess whether oxytocin impacted attention to social and romantic picture probes, placebo and oxytocin conditions were compared using *t* tests. The means and standard deviations for romantic and social pictures are displayed in Figure 7.5 (and Appendix IV). The results of the related *t* test for romantic pictures revealed no significant difference between mean scores for romantic pictures in the oxytocin or placebo conditions, $t(39) = 0.29$, $p = .78$. The results of the related *t* test for social pictures also revealed no significant difference between mean scores for social pictures in the oxytocin or placebo conditions, $t(39) = 1.63$, $p = .11$. Similarly, oxytocin did not significantly change in attention to social pictures.



Note. Error bars are standard deviations

Figure 7.5

Mean (*SD*) Reaction Times (ms) to Social Congruent and Romantic Congruent Picture Probes in Oxytocin and Placebo Conditions of the Dot Probe Task

High scores on Rubin's Love Scale might indicate higher endogenous oxytocin levels arising from romantic love. To test whether higher scores on Rubin's Love Scale [$M = 77.53$, $SD = 17.22$] affected attention to romantic pictures, the mean response times to romantic pictures in oxytocin and placebo conditions were re-examined in a repeated-measures ANOVA using Rubin's romantic love scores as a covariate. Response times to romantic picture probes in the oxytocin condition were compared to response times for romantic pictures in the placebo condition (see Appendix IV for means and standard deviations). There was no main effect of drug condition on mean reaction time to romantic pictures [$F(1, 38) = 0.11$, $p = .74$]. There was also no interaction with mean reaction times to romantic pictures and scores on Rubin's Romantic Love Scale [$F(1, 38) = 0.16$, $p = .69$], indicating that attention to romantic stimuli in relation to food picture probes was not linked to self-rated individual romantic love status.

7.3.8 Participant Characteristics

To assess whether participant characteristics influenced the attenuating effect of oxytocin on attentional bias towards food, demographic and questionnaire data were analysed as covariates in a repeated-measures ANOVAs with food bias scores in both drug conditions as the repeated-measures factor (see Appendix IV for the means and standard deviations). Results are presented in subsections below.

7.3.8.1 Age

When age was included as a covariate in a repeated-measures ANOVA using food bias scores, there was no main effect of drug on food bias scores [$F(1, 38) = 0.15$, $p = .90$] and no interaction: $F(1,38) = 0.89$, $p = .35$.

7.3.8.2 Body Mass and Frame Size

When BMI was included as a covariate in a repeated-measures ANOVA using food bias scores, there was a main effect of oxytocin on food bias scores [$F(1, 38) = 5.93$, $p = .02$, partial $\eta^2 = .11$] but no interaction: $F(1, 38) = 2.04$, $p = .16$. Mean elbow width was 6.50 cm ($SD = .69$ cm) and correlated significantly with BMI: Pearson's $r = .32$, $p = .04$, $n = 40$. However, elbow width did not correlate with food bias scores in either oxytocin conditions [Pearson's $r = -.07$, $p = .66$, $n = 40$] or placebo conditions: Pearson's $r = .05$, $p = .77$, $n = 40$.

7.3.8.3 State-Trait Anxiety Index

The mean STAI-trait score (see Appendix IV), was significantly higher than normative averages (see Chapter 5, section 5.2.2 for normative data) in a one-sampled t test: $t(39) = 14.44$, $p < .001$. When the STAI was included as a covariate in a repeated-measures ANOVA using food bias scores, there was a main effect of oxytocin on food bias scores [$F(1, 38) = 4.93$, $p = .03$, partial $\eta^2 = .14$] and a significant interaction: $F(1,38) = 8.85$, $p = .005$, partial $\eta^2 = .19$, that showed trait anxiety covaried with food bias scores. STAI scores significantly and negatively correlated with food bias scores such that as trait stress increased, attention to food pictures under oxytocin decreased: Pearson's $r = -.43$, $p = .005$, $n = 40$.

As discussed in the previous chapter, stress levels were higher in women, so the effect of gender on food bias was covaried with STAI scores. A one-way ANCOVA was used to examine whether there were gender differences in food bias scores between men and women after accounting for STAI. The means and standard deviations were provided in the analysis of gender (Figure 7.2). Food bias scores were not different between men and women after accounting for STAI-trait levels: $F(1, 37) = 0.13$, $p = .72$. However, the STAI-trait scores significantly covaried with food bias scores $F(1, 37) = 7.98$, $p = .01$, partial $\eta^2 = .18$.

7.3.8.4 DEBQ

One-sample t tests showed that the mean scores for restraint [$t(39) = 3.54$, $p = .001$], external [$t(39) = 10.10$, $p < .001$], and emotional eating [$t(39) = 8.77$, $p < .001$] were all different from normative data (see Chapter 5, section 5.2.2 for normative means and Appendix IV for experimental means) with restrained eating in the

normative samples being lower than this cohort, but the normative scores for external and emotional eating were higher than in this experiment. When the DEBQ restraint subscale was included as a covariate in a repeated-measures ANOVA using food bias scores, there was a main effect of drug on food bias scores [$F(1, 38) = 10.11$, $p = .003$, partial $\eta^2 = .21$] but no interaction: $F(1,38) = 2.77$, $p = .10$. When the DEBQ external eating subscale was included as a covariate in a repeated-measures ANOVA using food bias scores, there was a main effect of drug on food bias scores [$F(1, 38) = 5.26$, $p = .02$, partial $\eta^2 = .13$] but no interaction: $F(1,38) = 0.38$, $p = .54$. When the DEBQ emotional eating subscale was included as a covariate in a repeated-measures ANOVA using food bias scores, there was no main effect of drug on food bias scores [$F(1, 38) = .25$, $p = .62$] and no interaction: $F(1,38) = 1.54$, $p = .22$. No dimensions of the DEBQ correlated with food bias scores: restrained eating - Pearson's $r = .19$, $p = .24$, n = 40, external eating - Pearson's $r = -.03$, $p = .88$, n = 40, and emotional eating - Pearson's $r = -.13$, $p = .44$, n = 40. Therefore, scores on the DEBQ were not associated with food bias scores.

7.3.8.5 Romantic Attachment

Possible scores on Rubin's love scale range from 0 to 113. The mean romantic score for romantic love in this cohort was 77.53, which a one-sampled *t* test showed was below mean normative scores of 89.46 [$t(39) = 4.38$, $p < .001$]. When romantic scores were included as a covariate in a repeated-measures ANOVA using food bias scores, there was no main effect of drug on food bias scores [$F(1, 38) = 1.65$, $p = .21$] and no interaction: $F(1, 38) = 0.12$, $p = .73$.

7.3.8.6 Parental Bonding Index

The scale's authors do provide a range of normative data that relate to either the mother or the father (or both), and these scores vary according to characteristics of the respondents, such the social class, age, and sex. However, general means are provided, and both the care-neglect dimension [$t(39) = 0.02$, $p = .98$] matches normative data (Normative: 24.9), and the overprotection subscale is no different [$t(39) = 0.99$, $p = .34$] from the PBI's normative mean (Normative: 13.3), see Appendix IV for experimental means. The care dimension of the PBI did not correlate with food bias scores, $r = -.23$, $p = .29$, $n = 40$, indicating that the levels of care participants perceived that they received as children did not influence their bias towards food pictures in the dot probe task. Similarly, the overprotection questions on the PBI did not significantly relate to participant food bias scores in this dot probe task: Pearson's $r = -.25$, $p = .24$, n = 40. When PBI care scores were included as a covariate in a repeated-measures ANOVA using food bias scores, there was no main effect of drug on food bias scores [$F(1, 38) = 3.66$, $p = .07$] and no interaction: $F(1, 38) = 0.57$, $p = .46$. When PBI overprotection scores were included as a covariate in a repeated-measures ANOVA using food bias scores, there was no main effect of drug on food bias scores [$F(1, 38) = 3.25$, $p = .09$] and no interaction: $F(1, 38) = 0.01$, $p = .97$.

7.3.8.7 Food Cravings Questionnaire

Scores on the FCQ-T shown in Table 7.2 were higher than normative scores when tested with one-sample *t* tests. Because two sets of normative data were provided by the scale authors that represented pre-breakfast and post-breakfast cravings, results were instead compared to two sets of normative scores distributed as online surveys and completed by respondents at non-specified times (Meule & Kluber, (2012); Ulian et al., (2017). Food craving scores were generally higher in this sample than in the two normative samples. However, the scores between the two normative samples themselves also varied. The FCQ score was included as a covariate in a repeated-measures ANOVA using food bias scores, there was a main effect of oxytocin on food bias scores [$F(1, 38) = 9.95$, $p = .003$, partial $\eta^2 = .21$] and a significant interaction: $F(1,38) = 5.36$, $p = .026$, partial $\eta^2 = .12$. To investigate the components of food craving the subscales were correlated separately with the difference between placebo and oxytocin food bias scores, and the results are in Table 7.3. The alpha of 5% was divided by nine to correct for multiplicity ($p = .006$).

Table 7.2

Experimental and Normative Mean (*SD*) Scores and *t*-Test Significance for Food Cravings

	Thesis Sample		Normative Samples					
	M	*SD*	Meule & Kluber *M(SD)*	*t(df)*	*p*	Ulian et al. *M(SD)*	*t(df)*	*p*
FCQ total	147.60	28.29	98.29(27.69)	11.02(39)	< .001	113.3(17.8)	7.67(39)	<.001
Subscale 1 (intentions)	13.18	3.32	8.33(2.90)	9.23(39)	< .001	9.6(2.9)	6.8(39)	< .001
Subscale 2 (positive reinforcement)	22.58	4.86	13.83(4.48)	11.39(39)	< .001	18.3(5.8)	5.57(39)	< .001
Subscale 3 (relief from negative affect)	9.28	3.38	7.14(2.92)	3.99(39)	< .001	8.5(3.1)	1.44(39)	.16
Subscale 4 (lack of control)	20.50	6.49	14.84(5.72)	5.51(39)	< .001	13.0(3.7)	7.31(39)	< .001
Subscale 5 (preoccupation with food)	20.95	7.15	12.94(5.54)	7.09(39)	< .001	17.7(6.0)	3.43(39)	.001
Subscale 6 (as result of physiological need)	21.80	3.67	11.67(3.29)	17.47(39)	< .001	13.7(3.4)	13.97(39)	< .001
Subscale 7 (emotions connected to it)	11.45	4.01	10.00(4.38)	2.29(39)	.028	11.6(3.5)	.24(39)	.81
Subscale 8 (guilt from/giving in to craving)	13.23	4.12	13.06(3.92)	.25(39)	.80	15.0(4.6)	2.73(39)	.010
Subscale 9 (cues from environment)	13.25	4.11	6.48(3.11)	12.93(39)	< .001	5.9(2.3)	13.85(39)	< .001

Table 7.3

Pearson Product Moment Correlations of FCQ and Food Bias Change Scores

	r	*p*
Subscale 1 (intentions)	.21	.57
Subscale 2 (positive reinforcement)	.19	.25
Subscale 3 (relief from negative affect)	.14	.38
Subscale 4 (lack of control)	.25	.12
Subscale 5 (preoccupation with food)	.16	.33
Subscale 6 (as result of physiological need)	.14	.38
Subscale 7 (emotions connected to it)	.30	.54
Subscale 8 (guilt from/giving in to craving)	.26	.11
Subscale 9 (cues from environment)	.14	.40

7.3.8.8 Loneliness

Scores on the loneliness were above the normative scores for females provided by the scale's authors [normative: 36.06 (*SD* = 10.11)] but a one-sample *t* test showed that this difference was not significant $t(39) = 1.07$, $p = .29$, see Appendix IV for means and standard deviations. When loneliness scores were included as a covariate in a repeated-measures ANOVA using food bias scores, there was no main effect of drug on food bias scores [$F(1, 38) = 0.98$, $p = .33$] and no interaction: $F(1, 38) = 0.01$, $p = .93$. A correlation analysis compared loneliness scores with food bias scores to check the prediction that individuals with high loneliness would have higher food bias than individuals with low scores on loneliness, but no significant relationship was found: Pearson's $r = .27$, $p = .09$, n = 40, meaning that levels of loneliness did not significantly relate to food bias scores in the dot probe task.

7.4 DISCUSSION

In a dot probe experiment using neutral and food pictures, for the first time, intranasal oxytocin was demonstrated to reduce an attentional bias to food stimuli by lengthening reaction times to food picture probes relative to neutral pictures in a sample of 40 healthy adults who had consumed a readily catabolisable snack and drink to reduce fatigue and minimise attention to food stimuli from deficit-induced cravings. The oxytocin-led shift away from food stimuli was independent of BMI, age, gender, parental bonding, salty or sweet picture types, loneliness, and eating behaviour as measured by the DEBQ, but trait stress and food cravings covaried with food bias scores. Response times to food-picture probes in relation to romantic- or social-stimuli probes were greater after oxytocin than after placebo, meaning participants' attention was less focused on the food stimuli after oxytocin when presented alongside affiliative stimuli. The finding that oxytocin reduced an attentional bias to food stimuli but did not alter attention to romantic or socially salient stimuli suggests a food-specific attentional effect. The results also indicate that oxytocin's established

anorectic effects on palatable food intake not initiated by deprivation-induced hunger might be mediated to some degree by an attentional shift or by an oxytocin-driven reduction in the reward value of food.

This finding draws together the work using oxytocin to modify attentional bias and research uncovering an attentional bias towards food in the general population. It is also in line with previous research demonstrating oxytocin's effectiveness at 500 ms and above for changing attentional bias using food and face pictures (Brüne et al., 2013; Kim et al., 2014). However, in the previous experiments of the Brüne and Kim groups, the cohorts were anorexic whereas in this study an attentional bias to food was found in normal eaters whose BMI were predominantly in the normal weight-range (12/40, or 30%, were overweight or obese). Food craving scores were related to response times for bias scores, suggesting that oxytocin's modification of vigilance to food may not be independent of self-assessed food cravings in a healthy sample. Although attentional bias to food may have a different motivation in people with anorexia, nonetheless oxytocin removes the attentional bias to food in the dot probe independent of BMI, gender, loneliness, age, DEBQ scores or whether the cohort is anorexic.

According to Loeber, Grosshans, Herpetz, Kiefer and Herpetz (2013), two psychological processes are important to consider in relation to food consumption. The first involves the perception of palatable food that initiates cognitive and motivational approach processes, such as attention allocation to food cues that enhances salience attribution. The second process is the self-control required to resist food consumption, which would be problematic in an anorexic cohort and difficult to assess in any event using pictorial stimuli and not food. It appears that oxytocin may modulate the perceptive, motivational, and cognitive processes to alter attentional bias to food, rather than inhibitory mechanisms, since oxytocin has not been successful in potentiating food intake in people with anorexia (Kim et al., 2014). This is consistent with preclinical studies and brain imaging research showing that the anorectic effects of oxytocin on ingestive behaviour downregulate reward circuitry including the ventral tegmental area, putamen, caudate nucleus, NAc and amygdala (Baracz & Cornish, 2013; Sabatier et al., 2014; Striepens et al., 2016). However, since oxytocin has also been shown to control the cognitive control of inhibitory processes mediated by prefrontal regions (Striepens et al., 2016), further attentional research into oxytocin's modulation of self-control processes using paradigms such as go-no go is warranted.

The lack of correspondence in this experiment between BMI and an attentional food bias does not agree with previous research suggesting that being overweight or obese is associated with a greater attentional bias towards food (Brignell et al., 2009) or that oxytocin's effects are greater in obese than lean men (Thienel et al., 2016). It is likely that participants in the present study were unaware of experimental aims or that their weight was a consideration for the researcher, whereas in the Thienel et al. and Brignell et al. studies, obese participants specifically, were recruited via targeted adverts in local community specialist groups (e.g. slimming clubs) and hospitals. As part of the selection procedure, the participants with obesity completed eating questionnaires such as the DEBQ and the Three Factor Eating Questionnaire. The attentional

processing of naïve participants may be different from participants who self-select as obese. The relationship between food bias and BMI may be moderated by other factors, though. In a sample of overweight participants recruited to a study on slimming, the external eating component of the DEBQ and the personality trait of impulsivity were associated with a greater attentional bias towards food pictures (Bongers et al., 2015).

The association found between loneliness and eating described by Levine (2012) was not supported by a relationship between food bias or its modification and subjective loneliness in this study: Individuals with higher loneliness scores were not more responsive to oxytocin than low scorers. A disparity between the effects of loneliness on eating and attention may, therefore, exist, though it could also be the case that lonely people have reduced central oxytocin, resulting in exogenous oxytocin normalising endogenous levels.

Motivational states, such as hunger, can alter the magnitude of conditioned responses, moreover, the state of hunger increases attentional bias to food in the general population. To this end, participants were provided with a chocolate and juice snack before completing the dot probe task. However, it is possible that the palatable food beforehand primed participants to be vigilant towards food and the results reflect oxytocin's attenuation of the priming effect. Small amounts of food can stimulate the appetite and increase attention to food (Zheng, Lenard, Shin, & Berthoud, 2009) and Konorski termed the ensuing increased locomotor activity and attention to olfactory stimuli related to food a "hunger reflex" (Konorski, 1973; Pavlov, 1897). Cornell, Rodin, and Weingarten first demonstrated this priming effect, or cue reactivity, in humans (1989): Exposure to pizza or ice cream potentiated subsequent eating of that food in food-sated participants. Liu et al. (2016) proposed that consumption of palatable food, or mere exposure to it, induces long-lasting strengthening of excitatory synaptic transmission in mesolimbic dopamine neurons. A recent meta-analysis found that consuming palatable food prospectively and significantly alters subsequent eating behaviour (Boswell & Kober, 2016). However, it is not clear that attention to picture probes is a reliable proxy for eating behaviour and, thus, is altered by prior consumption of palatable food.

Methodological differences between the Kim et al. (2014) study and the present experiment may have contributed to the lack of attentional bias modification by oxytocin in normal eaters in the Kim group that was demonstrated in this experiment. Oxytocin's effects are dose dependent and the 40 IU used by Kim et al. (2014) that was effective at attentional bias modification in people with anorexia, may not be effective in a non-disordered cohort. Anxiety and fear in relation to eating and gaining weight are characteristic of people with anorexia (Steinglass et al., 2011). Increased attention in the dot-probe paradigm within the anorexic cohort may be, at least partly, due to increased threat processing, as has been demonstrated in individuals with anxiety disorders towards, for example, spiders (Öhman & Soares, 1994). However, trait anxiety was higher than normative averages in this cohort and covaried with food bias scores, so oxytocin may, therefore, be effective at attenuating attentional bias differently in people with anorexia than in healthy eaters. In people with anorexia, oxytocin may modulate the increased threat sensitivity present when

exposed to food-related cues, whereas in normal eaters, oxytocin may attenuate the reward processes. The food-related images used by the Kim group included items that were related to food (e.g. cooking utensils) and food that was not high valence (e.g. celery). Although both anorexic groups and healthy controls were found to have attentional bias to food-related stimuli, the three types of food-related stimuli were collapsed into one bias result. Low or medium valence stimuli used by Kim et al. (2014) may trigger increased attention in anorexic groups but not healthy controls, meaning that the stimuli were not of equal valence across groups. The food and neutral pictures in this dot-probe study were from the validated University of Birmingham database, and the romantic and social pictures were rated for typicality by a large number of people (189) compared to other studies that use just four 'judges' (e.g. Mogg et al., 2000). The high valence of all non-neutral stimuli used in this experiment may partially account for the significant findings.

In this study, the other valenced stimuli presented alongside food images were social and romantic images. The domains of romantic and social affiliation are also influenced by oxytocin, as of course is eating, meaning that placebo scores for these stimuli reflect the effects of endogenous oxytocin levels on attention. Reactions to the filler stimuli, however, were not likely to be influenced by endogenous oxytocin levels. The neutral pictures were designed to have low valence, so it is possible, therefore, that the inclusion of a valenced set of pictures not associated with oxytocin functioning (e.g. money) may have provided a more rigorous control in the oxytocin condition than unvalenced neutral pictures not influenced by oxytocin.

Although attentional biases to social and romantic stimuli were not explicitly examined in this dot probe since no direct comparisons were made with neutral stimuli, response times to romantic and social pictures were as quick as the food response times in the placebo condition, and these were also similar to the attentional bias scores observed when food pictures were viewed alongside neutral pictures. The similarly quick response times to the probes behind romantic, social, and food pictures in the placebo condition suggest that attention across these stimuli was equally distributed in the placebo condition. Given the body of literature showing that oxytocin influences attention toward social stimuli in males (Domes et al., 2013; Simplicio, Massey-Chase, Cowen, & Harmer, 2009), females (Kim et al., 2014; Kim et al., 2015), autistic individuals (Kanat et al., 2017; Xu et al., 2015), and rhesus monkeys (Parr, Modi, Siebert, & Young, 2013), the finding that social response times did not change after oxytocin is surprising. Previous attentional research on oxytocin's effect on romantic stimuli could not be found. However, Scheele et al. (2013) demonstrated that oxytocin's enhancing effects on men viewing female faces was specific to their romantic partners only. The generic romantic stimuli used in this experiment may not, therefore, convey sufficient valence. The computational demands of diverse social environments have driven the evolution of complex social and cognitive functioning in social animals (Dunbar, 2009) and, although oxytocin plays a key role in forming the social brain, as detailed in Chapter 1, measurable effects of oxytocin are highly sensitive to context. Given the tactile and vocal elicitation of oxytocin in bonding, the romantic pictures used in this may not have been an adequate substitute to generate an effect of oxytocin. Further research could extend this study by contrasting social and romantic images with neutral ones to examine whether attentional bias

towards them exists, and whether it persists under oxytocin.

Clearly there is an evolutionary advantage to threat recognition, one that underlies the classical dot-probe model. However, evolutionary benefits are not just confined to reaction toward negative stimuli, but the selective advantages to rapid identification of natural, life-sustaining rewards, such as food, are clearly important. One might also anticipate that reaction to potentially life-threatening events might be shorter than the reaction time to stimuli that are beneficial to the organism's survival. Failure to react promptly to, for example, visual cues of an imminent predator attack have far more significant implications than failure to quickly recognise the presence of food in the environment. Attentional research into food bias relies on the ability of rewarding stimuli to capture attention. In this experiment, the attentional bias to food stimuli that oxytocin modified is unlikely to represent early, or pre-attentive, covert orientation towards food. It has been argued that 500 ms does not capture early attention and might encompass avoidance reactions (Cooper & Langton, 2006). Some dot-probe experiments have employed shorter presentation times to avoid the effects of active avoidance behaviour (e.g. Noël et al., 2006). However, the main objection to durations of 500 ms upwards is that biases present at shorter durations are lost when the dot probe task is repeated at 500 ms (Cooper & Langton, 2006), and since a bias towards food was captured with 500 ms, the objection is invalidated.

To summarise, a significant attentional bias towards food-related stimuli was eliminated with 24 IU of intranasal oxytocin. These results are preliminary evidence of the potential of oxytocin as an agent for modification of attentional bias towards food.

CHAPTER 8 – OXYTOCIN'S EFFECTS ON SOCIAL EATING

The first two experiments of this thesis showed that oxytocin reduces snacking in men and women without deprivation-induced hunger. Concomitant cortisol reductions were not found with oxytocin's reduction of snack intake after stress tests, suggesting that cortisol reductions may not underpin anorectic actions in that experiment. Oxytocin was also shown to reduce an attentional bias to food stimuli and, at the same time, reduced attention to food when paired with affiliative pictures. Taken together, this suggests that oxytocin might alter the reward value of eating and increase the reward value for affiliative connection. The final study of this thesis examined the anorectic effects of oxytocin in a social setting.

8.1 INTRODUCTION

Social eating is a prominent activity across cultures, and a variety of group influences can alter food intake in social situations, such as social facilitation that augments intake (De Castro, 1997); modelling, which overrides hunger cues (Goldman, Herman, & Polivy, 1991); and impression management, which reduces intake. A recent meta-analysis found that, overall, group eating situations have a stronger inhibitory effect than augmenting effect on consumption (Vartanian, Spanos, Herman, & Polivy, 2015), but there is a large variation in experimental findings and contextual details matter greatly.

As a consequence of the types of food (e.g. healthy or unhealthy) and quantities eaten (e.g. large or small intake), stereotypes are attributed to individuals, and impression management involves individuals exploiting food consumption stereotypes to convey a desired impression (Vartanian et al., 2007). Whilst most people conform to explicit social norms, individuals high in self-monitoring behaviour are likely to be influenced by implicit norms, such as the amount of food appropriate to eat at a social gathering (Guidetti, Cavazza, & Conner, 2016). The type of food eaten influences how an individual is perceived by others, with consumption of high fat "junk" foods potentially signifying the individual is less intelligent, attractive, and studious than consumers of low-fat diets. Conversely, people who ate high-fat junk foods were seen as more easy-going and more likely to drink alcohol and attend parties than low-fat consumers who were regarded as unhappy, serious, highly-strung, and antisocial. Females are judged to be more feminine when they are slender, and small portion size reflects femininity (Mori, Chaiken, & Pliner, 1987). However, both sexes are regarded as being more feminine and less masculine when they eat low-fat foods than when they eat high-fat foods. Body weight is stereotypically regarded as inversely related to health and a large body size is perceived as a consequence of 'bad' dietary choices (Burke & Heiland, 2007).

Another process that can result in certain group eating situations, is the social facilitation of eating. This phenomenon results in higher intake in the presence or absence of pre-meal satiety, and irrespective of whether the group of eaters are strangers or socially connected (De Castro & de Castro, 1989; Herman, Roth, & Polivy, 2003; Vartanian et al., 2015). Social facilitation is also independent of the facilitatory effects of

alcohol (de Castro & Orozco, 1990; De Castro, 1991), prior snack intake, location, or timing of the meal (De Castro et al., 1990; De Castro, 1991). However, a repeated finding in the social facilitation literature, is that the impact of social facilitation on eating increases as a function of how many people are eating together, and the more people co-eating, the greater the amount consumed (De Castro & de Castro, 1989; De Castro & Brewer, 1992). However, intake varying as a function of the number of other people present does not apply when the group are strangers: increasing the group size of strangers does not increase per capita food intake (Herman et al., 2003). The effect of social facilitation on food intake is also greater for males than for females (De Castro, 1994).

As outlined in Chapter 1 (section 1.6), oxytocin affects social behaviour in both humans and animals. In humans, intranasal oxytocin can produce affiliative effects, such as improvements in social memory, face recognition, bonding, maternal behaviour, and empathy (Insel, 1997; Kosfeld et al., 2005; Love, 2014; Pedersen & Prange, 1979; Rodrigues, Saslow, Garcia, John, & Keltner, 2009) but the interpretation of oxytocin as a primarily prosocial agent is inadequate, as oxytocin can also increase envy, mistrust, and hostility towards out-group members (Bartz et al., 2011; De Dreu, Greer, Van Kleef, Shalvi, & Handgraaf, 2011; Declerck, Boone, & Kiyonari, 2010; Shamay-Tsoory et al., 2009). Negative affect associated with outgroup hostility may result in increased eating as a stress response, as implied in Chapter 6, and although oxytocin can reduce stress eating in women, it is not clear that it has the same impact on men. Ethnocentricity and hostility might influence eating behaviour. Given that oxytocin can promote negative behavioural responses, cultivating positive affect within the group in this study was important to ensure oxytocin's effects on social eating were examined.

Although oxytocin promotes affiliative behaviour and reduces food intake, group eating can increase or decrease food intake depending on the context. The anorectic effects of oxytocin in a social setting remain unknown as the two have not yet been tested together but given oxytocin's facilitation of social bonding and strengthening of in-group allegiance, it was expected that amount of snack food consumed would reduce in the oxytocin condition in line with previous research into its anorectic properties. It was also anticipated that participants' snack-food intake would be more similar to the other members of their group than out-group participants.

8.2 MATERIALS AND METHODS

8.2.1 Design

Double-blind, placebo-controlled, randomised, and counterbalanced crossover protocols were implemented using a within-subjects repeated measures design comprising two drug tests scheduled one to three weeks apart. The aims of the experiment were disguised, and participants were informed that the study investigated the effect of oxytocin on social collaboration.

8.2.2 Participants

Thirty-five healthy adults aged 19 to 52 years (*M* = 29.20 yrs, *SD* = 10.99 yrs) were recruited from Kingston University in exchange for university course credits or from the local community who were approached and met the inclusion criteria. There were 27 females (*M* = 29.20 yrs, *SD* = 10.99 yrs) and eight males (*M* = 29.20 yrs, *SD* = 10.99 yrs). When registering, participants were automatically assigned to a group, in which they remained for both sessions. Exclusion criteria were as per Chapter 2 General Methods (section 2.1.1), and four individuals were excluded from registration. All other participant criteria were consistent with the Chapter 2 General Methods inclusion and exclusion criteria.

8.2.3 Materials and Measures

The materials used in this experiment were in accordance with Chapter 2 General Methods, with the following exceptions. Sham tasks were pared back and altered for this experiment, so only the following materials were used: Elite Healthcare's 'Two Point Discriminator Touch-Test', synthetic pear-scented smelling bottles (Caravansons LLP, Bury, UK), a stopwatch, and 100-piece jigsaws with pictures of London (Ravensburger). The lunch provided for the groups differed from lunches provided in the other experiments as a large number of sandwiches needed supplying at a time, so Kingston University catering sourced the food to ensure a reliable supply. Each participant was provided with a pre-packaged sandwich (Elior 'Cheese Ploughmans', 405 kcal, 173g) and 200 ml water. As very little of the salty snack was eaten in the previous experiments, a more enticing cheese-pastry straw was chosen as the savoury snack for the group experiment (Sainsbury's mini cheese straws, 250g/1283 kcal) and sweet snack food was changed to be bite-sized (Sainsbury's mini chocolate millionaire bites, 200g/990 kcal). Both snack types were matched for calorie content, fat, and carbohydrate (see Table 8.1 below) and presented in separate 1.5 litre bowls for each participant and filled to the top, so a considerable amount could be eaten without the bowl appearing empty. All food was presented on individual trays covertly numbered on the underside to identify each participant.

A participant pack was provided on each tray pre-labelled with the date and the participant's number. Each pack contained a rating/score sheet, a sheet of visual analogue scales (VAS) for the taste test, and a sheet of VAS for mood. As per other thesis materials, the VAS response lines measured 100mm and were anchored by "not at all" and "as much as I can imagine" for the taste test. Two VAS were used to assess each snack type: one for the snack's palatability and a second to rate its sweetness or saltiness, depending on snack type. The mood VAS sheet was more comprehensive than in the previous experiments of this thesis, extending the VAS to include how bored and how sociable participants felt and asking about both their fullness and their hunger. A question asking how influenced the participant felt by the group was also included, and because in the pilot phase, VAS responses tended towards the centre on this question, it was presented instead as a Likert-type response, with four options: "not really" ("not at all" was avoided because there is always some influence), "not very", "somewhat", and "very". The score sheet required participants to note their scores on the sham tasks (see below for tasks), then rate the difficulty of each sham task and indicate whether they thought their performance on the task would improve or decline if it was performed individually (if the task

was a group one) or in a group (if the task was individual).

Table 8.1
Nutritional Values of the Food used in the Taste Test and Snack Test

	Chocolate Millionaires	Cheese Straws
Calories (kcal/100g)	495	513
Carbohydrate (g/100g)	55.2	51.6
Fat (g/100g)	27.9	27.8
Protein (g/100g)	4.9	12.9

8.2.4 Procedure

The study was approved by the Faculty Research Ethics Committee at Kingston University. Groups were tested one at a time between the hours of 12 and 2.30 pm. After providing informed consent and confirming compliance with the inclusion criteria of fasting on water for two hours before the experiment, and the exclusion criteria as per Chapter 2 General Methods (section 2.1.1), each group of participants was seated around a central coffee table in a group laboratory at Kingston University. Seating was arranged before the experiment to be too far apart for participants to share food. When seated, participants were each handed a personal tray with a pack of sandwiches, water, taste-test food in two covered 1.5 l bowls, pen, and a pack of scoring/rating sheets. The session began with an ice-breaker task designed to put participants at ease and help them get to know each other. The researcher and participants each presented three facts about themselves to the group—two true and one false—and the group had to decide which was the false fact. Next, participants self-administered 24 IU of oxytocin or placebo, depending on session, overseen by the researcher. After this, participants completed the first mood VAS.

The first sham task was an online pitch test (see General Methods for details), and this was conducted as a group exercise. Responsibility was given to the group to elect one person to use the computer and run the online test. Each group member, including the participant at the computer, had to agree each response. The second sham task was a timed group-balance test. All members of the group held hands, shut their eyes then lifted one leg as high in the air as possible when the researcher said ‘go’. The researcher timed how long the group were able to balance on one leg, and as soon as one participant lost balance, stopped the timer. After the balance test, participants were given five minutes to eat their sandwiches, see Figure 1, with the experiment’s timeline. The next sham sensory test was a group smell test. The researcher removed the lid to a smelling bottle and presented it to each participant in turn. The group task was to agree upon what the scent was and note it on the scoring sheet; the answer was not provided by the researcher. The final sensory test was the taste test. Using the snack bowls on their individual trays, participants were asked to rate each

snack for palatability, and assess how salty or sweet it was, depending on snack type. After the taste test, participants completed the second of their mood VAS. The jigsaw was the last sham task and was presented to participants as an exercise in collaboration. Before the researcher departed for 30 minutes to leave the group to complete the jigsaw, it was mentioned that participants were free to help themselves to the snack-test food left over; the snack-food was in individual bowls on their individual tray. Lunch and buffet foods were weighed before and after testing, and sessions lasted about 90 mins.

8.3 RESULTS

8.3.1 Effect of Treatment, Snack Type, and Group on Consumption

Each sweet snack weighed about 15 grams, and about two chocolate snacks were consumed in both the oxytocin and placebo condition across the cohort, see Table 8.2. The cheese snacks were about 2.25 grams each, and approximately 15 to 18 were eaten by the entire sample in oxytocin and placebo sessions. The means and standard deviations for snack intake broken down by group are provided in Table 8.3; these vary widely from group to group, but not between conditions.

Table 8.2

Overall Means (*SD*) (g) for Snack Food Consumption in Social Study

		M	SD
Salty Food	Oxytocin	35.43	31.86
	Placebo	41.83	36.68
	Total	77.26	68.54
Sweet Food	Oxytocin	40.20	34.03
	Placebo	39.49	35.06
	Total	79.69	69.09
Total Snacks	Oxytocin	75.63	59.23
	Placebo	81.32	66.86

As the snack foods in this experiment did not include oatcakes, snacks were considered palatable and liable to be consumed in a similar pattern. This was checked by comparing the mean palatability ratings for salty (M = 64.36, SD = 19.82) and sweet snacks (M = 69.57, SD = 23.22), and no differences were found, $t(34) = 1.30$, $p = .20$. The snack foods were, therefore, treated as different levels within a variable representing palatable snack food. A mixed 2 (drug: oxytocin and placebo) x 2 (snack: sweet and salty) mixed-measures ANOVA was conducted using group membership as a between-subjects factor. There was no main effect of treatment on snack intake $F(1,22) = .89$, $p = .36$, and no main effect of snack type $F(1,22) = .09$, $p = .77$, indicating that there was not a significant difference in snack preference or between drug conditions. Figures 8.2 and 8.3 illustrate the overall mean snack intake under oxytocin and placebo conditions. However, there was a main effect of group $F(1,22) = 5.55$, $p < .001$, partial $\eta^2 = 0.30$, see Figure 8.1.

To assess whether pre-existing food preference—as represented by amount consumed without the influence of oxytocin in the placebo condition—was associated with taste ratings for those foods, the placebo chocolate biscuit intake was compared with sweetness and palatability ratings for chocolate biscuits and the placebo salty straw intake was compared with saltiness and palatability ratings for salty straws. The results revealed that neither sweet nor salty snack consumption correlated with palatability ratings: Pearson's r = -.16, p = .36 for sweet snacks, and Pearson's r = .-.18, p = .32 for salty snacks. Nor was salty straw intake associated with how salty participants perceived the food to be [Pearson's r = .11, p = .51], but the relationship between chocolate snack intake and ratings for sweetness approached significance [Pearson's r = -.33, p = .053], with lower chocolate snack intake being associated with higher sweetness ratings, suggesting that overall participants may not have preferred sweet snacks.

Table 8.3

Means (*SD*) of Snack Food (g) Consumed in Social Experiment by Group

		Sweet Snack				Salty Snack			
		Placebo		Oxytocin		Placebo		Oxytocin	
Group	*N*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
1	3	10.67	1.53	8.33	1.53	10.67	10.69	9.67	3.21
2	3	11.67	8.96	8.33	1.53	14.00	8.72	8.67	1.53
3	3	43.00	23.81	28.67	12.74	71.67	25.15	39.67	22.75
4	2	12.50	3.54	9.50	2.12	9.50	0.71	8.50	0.71
5	2	20.50	12.02	13.50	4.95	15.50	3.54	13.00	1.41
6	3	83.00	17.06	66.33	15.50	94.00	54.67	83.33	49.17
7	3	11.00	6.08	8.33	1.53	14.67	13.61	12.67	9.07
8	3	79.67	33.86	73.00	4.00	52.33	21.83	52.67	22.50
9	2	31.50	20.51	9.00	1.41	12.00	2.83	10.50	3.54
10	3	24.33	21.46	79.00	42.04	55.67	20.98	53.33	36.30
11	3	69.00	57.66	66.00	13.45	56.00	40.84	21.33	3.21
12	3	56.67	45.76	89.33	16.80	65.00	45.90	71.33	22.50
13	2	43.00	29.70	30.50	2.12	44.00	41.01	59.00	2.83

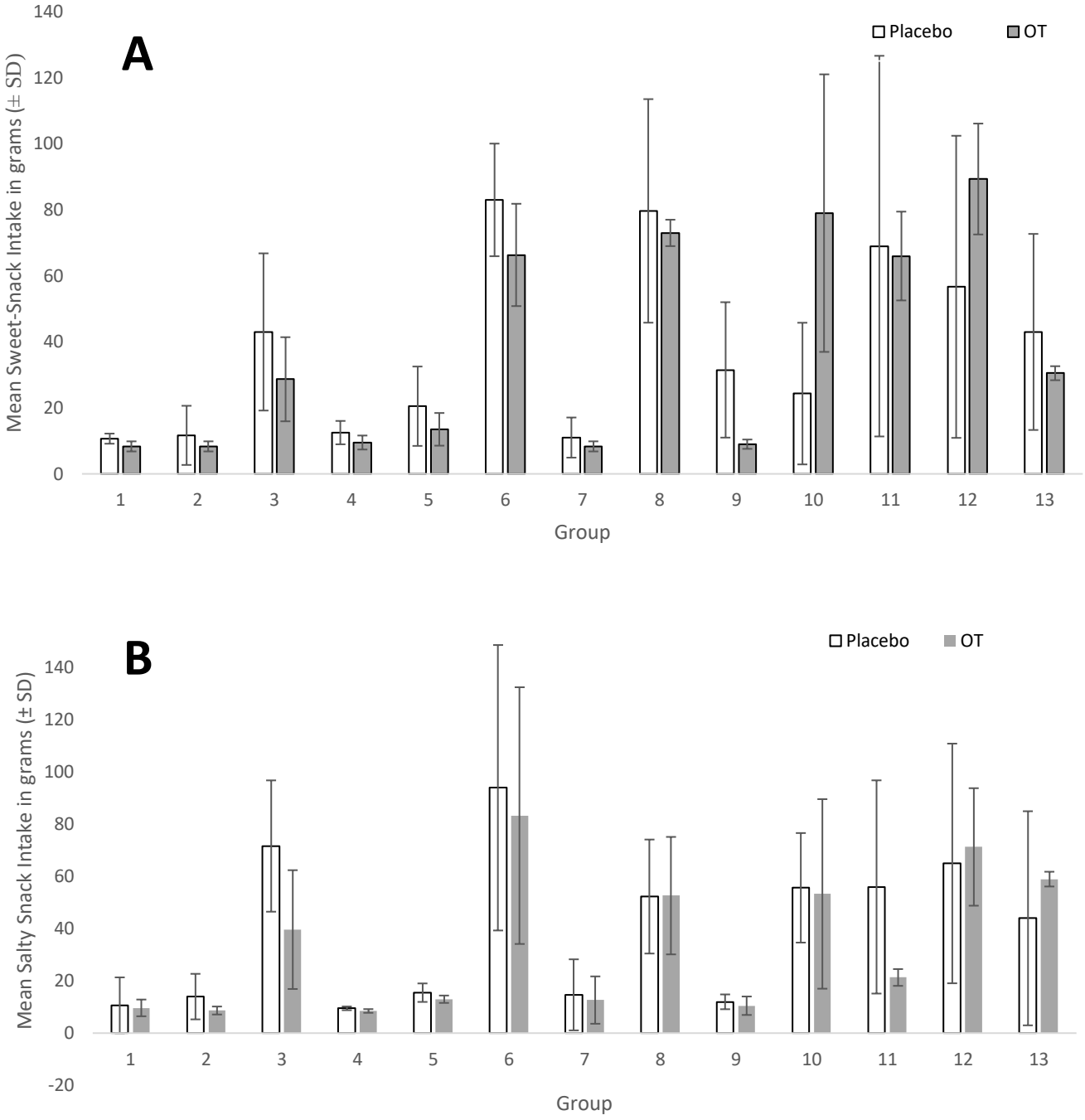


Figure 8.1

Total Means for Sweet (A) and Salty (B) Snack Intake (g) in Oxytocin and Placebo Conditions for each Group

8.3.2 Group Size and Sex

There were seven female-only groups: 1, 2, 4, 7, 8, 9, and 11; one male-only group: 13; and four mixed groups: 3, 5, 6, 10. A mixed-methods 2 (drug: oxytocin and placebo) x 2 (snack: sweet and salty) x 2 (Group type: 2-person and 3-person) ANOVA was conducted using group size as a between-subjects factor (see

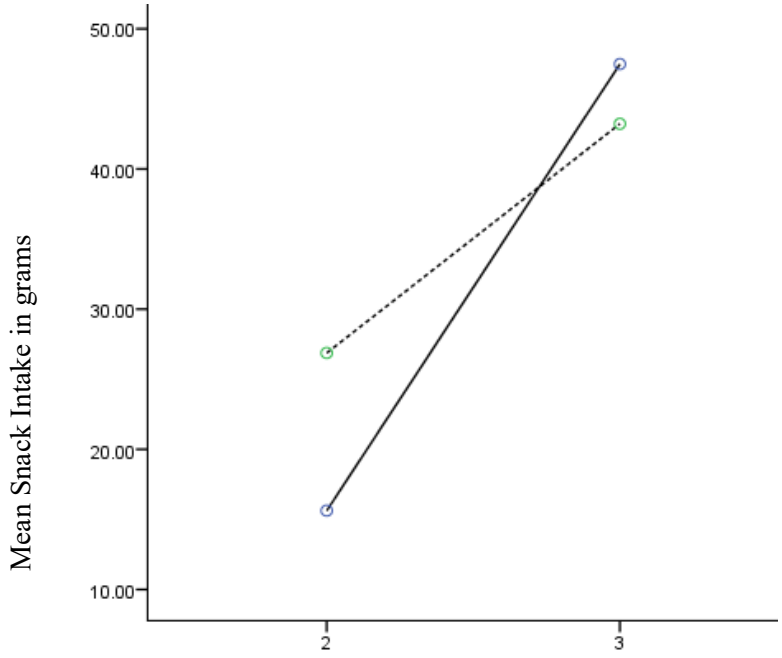
Table 8.4 for means and standard deviations).

Table 8.4

Total means (*SD*) for Sweet and Salty Snack Intake (g) in Oxytocin and Placebo Conditions by Group Type

		Sweet				Salt			
		Oxytocin		Placebo		Oxytocin		Placebo	
Grp Type	N	M	SD	M	SD	M	SD	M	SD
2-person grp	8	15.63	9.64	26.88	18.96	23	22.34	20.25	21.53
3-person grp	27	47.48	35.33	43.22	38.04	40.48	35.79	48.22	38.07
Total	35	40.2	34.03	39.49	35.06	36.49	33.73	41.83	36.68

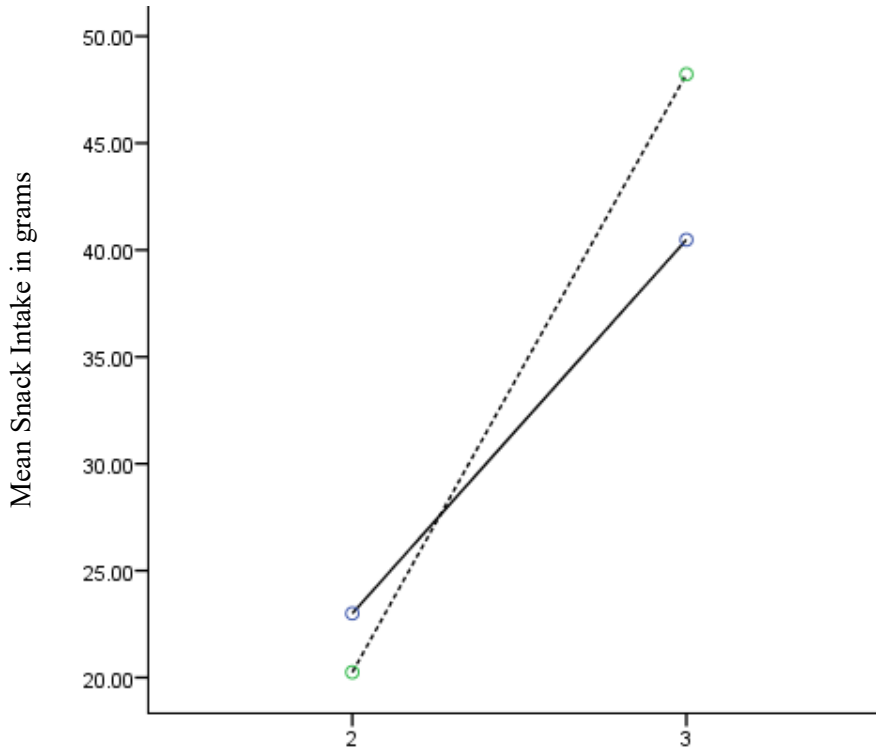
There was no main effect of treatment on snack intake $F(1,33) = .56$, $p = .46$, and no main effect of snack type $F(1,33) = .02$, $p = .89$ indicating that there was not a significant difference in snack preference or drug condition. The type of the group, however, was a near-significant factor in snack-food consumption $F(1,33) = 3.97$, $p = .0547$ partial $\eta^2 = .11$. There was no interaction between drug and group size [$F(1,33) = 0.76$, $p = .09$], nor drug and snack type [$F(1,22) = 0.02$, $p = .89$] and no interaction between group size and snack type [$F(1,33) = 0.03$, $p = .86$], but there was a significant three-way interaction involving drug, snack, and group size, [$F(1,33) = 5.72$ $p = .02$], illustrated in Figures 8.2 and 8.3.



Note. Solid line is oxytocin, dotted line is placebo

Figure 8.2

Overall means (g) of Sweet Snack-Food Intake in the Social Experiment, n = 35



Note. Solid line is oxytocin, dotted line is placebo

Figure 8.3

Overall means (g) of Salty Snack-Food Intake in the Social Experiment, n = 35

A mixed-measures ANOVA examined the effect of gender on snack intake. There were 8 men and 27 women, and the means and standard deviations are provided in Table 8.5. There were no main effects or interactions in the model (*p*-values > .05). However, results must be interpreted cautiously due to the small male sample and uneven sample sizes.

Table 8.5

Means (*SD*) for Snack Intake (g) by Condition and Gender, n = 35

	Gender	*M*	*SD*
Sweet Snacks oxytocin	Male	45.75	26.51
	Female	38.56	36.23
	Total	40.20	34.03
Salty Snacks oxytocin	Male	58.25	33.99
	Female	28.67	28.44
	Total	35.43	31.86
Sweet Snacks Placebo	Male	45.25	27.02
	Female	37.78	37.39
	Total	39.49	35.06
Salty Snacks Placebo	Male	50.88	42.72
	Female	39.15	35.15
	Total	41.83	36.68

A mixed model repeated-measures ANOVA examined whether single- or mixed-sex groups impacted on snack intake. There were only four groups comprising mixed sexes (total of 11 participants) and nine groups made up of women only (total of 24 participants), see Table 8.6 for the means and standard deviations.

Table 8.6

Means (*SD*) for Snack Intake in Mixed and Single-Sex Groups

	Sex of Group	*M*	*SD*
Sweet Snacks oxytocin	Single	35.75	33.71
	Mixed	49.91	34.23
	Total	40.20	34.03
Salty Snacks oxytocin	Single	28.54	26.35
	Mixed	50.46	38.64
	Total	35.43	31.86
Sweet Snacks Placebo	Single	37.08	37.15
	Mixed	44.73	30.99
	Total	39.49	35.06
Salty Snacks Placebo	Single	32.04	31.29
	Mixed	63.18	39.90
	Total	41.83	36.68

There were no main effects of drug [$F(1,33) = 0.38$, $p = .54$] or snack [$F(1,33) = 0.00$, $p = .99$], but there was a borderline significant interaction between snack type and whether the group was single or mixed sex, $F(1,33) = 4.12$, $p = .054$, partial $\eta^2 = .11$. Estimated marginal means showed that snack intake for sweet (M =36.42 g, SE 6.42 g) food in the female-only groups was greater than salty food (M = 30.29 g, SE = 6.17 g) and that this was reversed in mixed-sex groups that also had a higher overall intake than female groups: the sweet food mean was 47.32 g (SE = 9.5 g) and for salty food the mean was 56.82 g (SE = 9 .12 g).

8.3.3 Self-Report & Taste-Test Ratings

The ranked individual scores of how influenced each participant felt by their group were examined using a Wilcoxon Signed Rank Test and the median oxytocin ranks were not statistically different from the median placebo ranks, $Z = 0.92$, $p = 36$. Hunger and fullness VAS were considered interchangeable and, as results showed the same outcome, only fullness is reported below.

To assess how participants felt at the beginning of the session compared to the end of the session, and to examine the results between drug conditions, a 2 (Drug: placebo vs oxytocin) by 2 (Time: baseline vs sample 2) for each of the VAS questions. Table 8.7 provides the means and standard deviations for all the VAS.

Alertness: there was no main effect of oxytocin on VAS ratings of alertness [$F(1,34) = 1.25$, $p = .27$] and no main effect of sample time through the session on VAS ratings of alertness [$F(1,34) = 0.01$, $p = .94$]. There was also no significant interaction [$F(1,34) = 0.03$, $p = .87$].

Anxiety: there was no main effect of oxytocin on VAS ratings of anxiety [$F(1,34) = 0.83$, $p = .37$] and no main effect of sample time through the session on VAS ratings of anxiety [$F(1,34) = 1.42$, $p = .24$]. There was also no significant interaction [$F(1,34) = 0.63$, $p = .43$].

Boredom: there was no main effect of oxytocin on VAS ratings of boredom [$F(1,34) = 0.79$, $p = .38$] but there was a main effect of sample time on VAS ratings of boredom [$F(1,34) = 25.27$, $p < .00$, partial $\eta^2 = .43$] such that boredom decreased through the session. There was also no significant interaction [$F(1,34) = 1.17$, $p = .29$].

Excitement: there was no main effect of oxytocin on VAS ratings of excitement [$F(1,34) = 0.15$, $p = .70$] and there was no main effect of sample time on VAS ratings of excitement [$F(1,34) = 3.08$, $p = .09$]. There was also no significant interaction [$F(1,34) = 0.07$, $p = .79$].

Fullness: there was no main effect of oxytocin on VAS ratings of fullness [$F(1,34) = 0.01$, $p = .91$] but there was a main effect of sample time on VAS ratings of fullness [$F(1,34) = 779.05$, $p < .001$, partial $\eta^2 = .96$] such that fullness increased through the session. There was also no significant interaction [$F(1,34) = 0.32$, p

= .58].

Happiness: there was no main effect of oxytocin on VAS ratings of happiness [$F(1,34) = 0.03$, $p = .87$] and no main effect of sample time through the session on VAS ratings of happiness [$F(1,34) = 0.80$, $p = .38$]. There was also no significant interaction [$F(1,34) = 0.19$, $p = .67$].

Thirst: there was no main effect of oxytocin on VAS ratings of thirst [$F(1,34) = 0.85$, $p = .36$] and no main effect of sample time through the session on VAS ratings of thirst [$F(1,34) = 1.13$, $p = .30$]. There was also no significant interaction [$F(1,34) = 2.19$, $p = .15$].

Sociality: there was a main effect of oxytocin on VAS ratings of sociality [$F(1,34) = 6.33$, $p = .02$, partial $\eta^2 = .16$] whereby sociality was higher after oxytocin than after placebo. There was also a main effect of sample time on VAS ratings of sociality [$F(1,34) = 23.58$, $p < .001$, partial $\eta^2 = .41$] such that sociality increased through the session. Drug and time significantly interacted [$F(1,34) = 4.68$, $p = .038$, partial $\eta^2 = .12$], with high sociality scores in the second sample of the oxytocin condition.

Table 8.7

Overall Means (*SD*) for "Mood" VAS Scores Before/After Nasal Spray Administration, n = 35

	Placebo				Oxytocin			
	Before		After		Before		After	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Alertness	60.03	15.83	59.63	19.78	54.89	23.62	54.97	29.03
Anxiety	21.40	16.10	19.43	17.80	19.94	17.77	14.63	21.76
Boredom	19.17	20.52	11.51	12.60	18.26	13.79	7.06	10.75
Excitement	54.17	19.37	56.91	19.86	56.11	21.07	58.17	22.69
Fullness	17.91	16.95	82.17	8.31	16.97	19.81	83.57	10.52
Happiness	61.89	17.54	62.63	18.02	60.91	22.09	62.43	22.21
Hunger	81.80	15.52	10.94	16.22	81.40	9.89	6.26	11.52
Sociality	49.77	20.18	56.91	19.61	52.74	18.77	72.34	18.33

Taste-Test Ratings: There were no differences between drug conditions in palatability ratings for salty [$t(34) = 0.23$, $p = .82$] or sweet snacks[$t(34) = 1.06$, $p = .69$], or sweetness ratings for sweet snacks [$t(34) = 0.38$, $p = .71$], or saltiness ratings for salty snacks [$t(34) = 0.41$, $p = .68$]. See Table 8.8 below for means and standard deviations of taste test scores.

Table 8.8

Means (*SD*) for Total Participant Taste-Test VAS Scores, n = 35

	Placebo		Oxytocin	
	M	*SD*	*M*	*SD*
Palatability of Salty Snack	64.09	20.42	64.63	21.66
Palatability of Sweet Snack	70.71	23.46	68.43	24.69
Saltiness of Salty Snack	56.43	27.76	54.91	25.58
Sweetness of Sweet Snack	73.83	21.50	72.54	19.56

8.3.4 Multilevel Modelling

Participant group was a significant factor determining snack intake, and because the data were collected within a nested structure, hierarchical linear modelling was used to isolate potential group effects. Separate analyses were conducted for oxytocin and placebo conditions, see Table 8.8. A model containing only the group and the outcome variables of sweet and salty food eaten in either oxytocin or placebo conditions was generated to determine whether group membership was a significant determinant of variability in the outcome variables requiring further investigation. A second model was built with the predictor variables of age, gender, and BMI.

8.3.4.1 Model 1 – Group Only

Hierarchical linear modelling (HLM) results for the first model (see Table 8.8) confirmed previous ANOVA results in showing that group membership was a significant predictor for snack intake. In the oxytocin condition, model one showed that group membership predicted the consumption of sweet, but not salty, snack food, meaning that participants' chocolate biscuit intake was determined by which group they were in. In the placebo condition, group membership was not a significant predictor for either salty or sweet snack consumption in the first model, meaning that the group a participant was in did not determine their food intake.

8.3.4.2 Model 2 – Group and Predictors

When the predictor variables were added in the second model (see Table 8.8), self-reported levels of sociality and ratings of sweetness for the chocolate biscuits each explained significant amounts of the inter-group variance of chocolate snack intake in the oxytocin condition. In model 2, no predictor variables significantly explained inter-group variability for salty snacks after oxytocin or for either snack after placebo.

Table 8.8

HLM for Group Level Only (Model 1) and Regression Predictors (Model 2) for Snack Intake by Group

Predictors	Sweet Snack Food in Oxytocin Condition		Sweet Snack Food in Placebo Condition		Salty Snack Food in Oxytocin Condition		Salty Snack Food in Placebo Condition	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
	E(SE)	*E(SE)*	*E(SE)*	*E(SE)*	*E(SE)*	*E(SE)*	*E(SE)*	*E(SE)*
Gender	0.79 (.81)*	0.90 (0.89) 6.70(12.68)	0.37 (0.57)	0.32(0.53) 9.84 (24.61)	0.47 (0.64)	.32(0.48) -7.43 (20.54)	0.35 (0.53)	0.11(0.48) -62.51 (40.63)
Age		0.27(0.33)		-0.14 (0.76)		-0.73 (0.68)		-2.23 (1.14)
BMI		-1.63(1.40)		-2.61 (2.29)		1.88 (1.87)		-2.63 (6.81)
Group influence		-10.89(5.40)		-17.72 (11.56)		-11.86 (9.55)		-28.70 (19.50)
Alert		0.16(0.21)		-0.16 (0.48)		-0.11 (0.41)		-1.25 (0.78)
Anxiety		0.07(0.14)		-0.02 (0.31)		-0.10 (0.27)		-1.01 (0.32)
Boredom		-0.51(0.43)		-0.87 (0.77)		0.04 (0.61)		-1.63 (1.54)
Excitement		-0.17(0.38)		-0.74 (0.81)		-0.07 (0.72)		-2.12 (1.47)
Fullness		0.08(0.19)		0.23 (0.40)		0.45 (0.33)		-0.29 (1.38)
Happiness		-0.94(0.35)		-0.35 (0.80)		0.11 (0.68)		-1.80 (1.59)
Hunger		-0.27(0.20)		-0.20 (0.41)		-0.41 (0.34)		-1.13 (0.58)
Sociality		-0.23(0.21)*		-0.23 (0.38)		0.22 (0.31)		-0.76 (0.83)
Palatability Salt Cracker		-0.15(0.16)		-0.34 (0.36)		0.11 (0.31)		-0.47 (1.08)
Saltiness of Salt Cracker		-0.03(0.16)		0.01 (0.29)		0.30 (0.24)		-0.28 (0.93)
Palatability Choc Bisc		0.08(0.16)		0.18 (0.37)		-0.11 (0.32)		-0.76 (0.83)
Sweetness Choc Bisc		-.76(0.23)**		-0.53 (0.43)		-0.73 (0.34)		-1.55 (0.21)

Note. * $p < .05$, ** $p < .01$, ** $P < .001$, *E* represents unstandardized coefficients. *SE* is standard error of the mean.

8.4. DISCUSSION

In a social setting where participants were assigned to groups and covertly tested on sweet and salty snack food intake after eating lunch, intranasal oxytocin did not reduce snack-food consumption or alter palatability of snacks. However, group membership was a significant factor determining sweet snack intake in the oxytocin condition but not in the placebo condition. This suggests that there is evidence for social modelling that was augmented by oxytocin, a finding that is consistent with oxytocin's empathogenic and ingroup favouritism effects (see Chapter 1, section 1.6). Oxytocin also significantly increased ratings of sociability across groups in comparison to placebo, but no further changes to mood were found following oxytocin.

Although oxytocin's effects on self-perceived sociability in group settings have not before been tested in a study, they have been measured indirectly. In an experiment that assessed the anxiolytic effects of both social support and oxytocin in participants who were preparing for the Trier Social Stress Test, salivary cortisol was significantly reduced by social support and oxytocin independently, but the interaction of the two suppressed salivary cortisol the most (Heinrichs et al., 2003). In the same experiment, however, no significant differences on self-rated anxiety or mood were found. In this group experiment, cortisol was not measured but changes to self-perceived stress were also not seen after oxytocin. The lack of awareness of

potential manipulation of mood states and anxiety by oxytocin despite experimental effects is consistent with the findings of the individual snack-tests in this PhD thesis (Chapters 4 and 6) and the wider literature. Despite the methodological differences in the collection of self-report anxiety data in the Henrich et al. (2003) study and this experiment, no effect of oxytocin on self-report anxiety in either study was found. Henrich et al. (2003) used the screening STAI results in lieu of a baseline, so baseline data from the STAI in the session itself were not compared to state stress levels of participants during the experiment. In this experiment, participants' anxiety was measured via VAS, female participants were included, and participants with high STAI scores were not excluded. The variability of stress scores, therefore, might be expected to increase, but oxytocin's experimental effects occurred in both studies without concomitant self-report stress changes. It is surprising, therefore, that participants recorded changes to how social they felt during the sessions but not to measures related to sociality, such as happiness or stress. Oxytocin-driven increases of self-rated sociability accord with oxytocin's wide-ranging promotion of social behaviour in animals and humans, including increases in trust, bonding, social memory, and emotional recognition (Kosfeld et al., 2005; Rimmele, Hediger, Heinrichs, & Klaver, 2009; Skuse et al., 2014; Striepens, Kendrick, Maier, & Hurlemann, 2011) and further research is needed to uncover whether and when oxytocin-induced changes lead to awareness.

Context is a critical factor in determining not only the effects of different group eating configurations on the likelihood of social modelling of eating occurring, but also on the effects of exogenous oxytocin. The oxytocin-driven increase in ratings of sociability suggests that the experimental procedure promoted increased affiliation among groups, positive group identities, and attenuation of outgroup hostility. The ice-breaker task required participants to share a personal fact with other members of the group that could not be guessed at, the pitch and smell tests required co-operation among group members to find appropriate answers, the balancing task transgressed social norms by participants holding hands and the jigsaw required strategic cooperation. Despite participants being automatically assigned to a group for the experiment, and the groups not being friendship alliances predisposed to affiliate (Cruwys, Bevelander, & Hermans, 2015), a strong sense of group identity may have been engendered. This was supported by within-group variation of food intake being lower than the between-group variation, which suggests that group affiliation and consequent social modelling occurred, and future studies could address this by measuring group cohesion.

The group demographics and profile were not tightly controlled, and this may have created excess noise in the data that obscured oxytocin's potential anorectic effects on sweet-tasking carbohydrate. In this experiment, no psychological profiling of personality characteristics, such as empathy or extraversion, or participant selection based on physical features, such as BMI, was undertaken. Dieters and restrained eaters were not excluded from participation because in identifying them, the experimental aims may have been obvious. Whilst controlling for noise variables may have impacted the patterns of consumption, doing so is a trade-off with ecological validity, and the results of the present experiment do reflect a representative cross-section of the population that brings with it the benefits of improved generalisability. Some groups in this

study were mixed gender and others single gender, so impression management and social facilitation of eating would have varied across groups. The effectiveness of group bonding given the higher endogenous levels of oxytocin in females than in males may also have differentially altered group dynamics. Preliminary results suggested that female-only groups ate less than mixed groups and that mixed groups ate more salty snacks. This finding is consistent with research showing that females avoid eating larger portions of food or foods that may make them seem gluttonous, and that healthier choices of food are considered more feminine. However, the results of gender-based analyses should be viewed with some caution as the sample sizes were not balanced and there was only one male-only group.

The effects of oxytocin were different in mixed groups than female-only groups. Oxytocin's reversal of the ratio of higher sweet to lower salty food intake in female-only groups to a higher salty to lower sweet ratio in the mixed groups, suggests that females ate less sweet food in the presence of men. This finding accords with previous research that females choose foods to reflect perceived "feminine" attributes of modesty and avoid high calorie foods in the presence of men (De Castro, 1997; Polivy, Herman, & Coelho, 2008; Vartanian et al., 2007).

However, in contrast to previous studies, oxytocin did not have an anorectic effect on snack intake. The first experiment of this thesis showed an anorectic effect of oxytocin on salty and sweet snack-food (see Chapter 4) and oxytocin reduced sweet-food consumption in the female-only experiment (see Chapter 6). One explanation might be the inconsistency of snack food used across studies. In the present experiment, sweet and salty snacks both had high palatability ratings and were eaten with equal enthusiasm in both conditions. The high palatability ratings were not correlated with the amounts of snack food eaten, though. Unfortunately, in the first two snack-test studies palatability ratings were not taken for the snacks, but a comparison of salty snack intake across the experiments suggests that the cheese straws provided in this study were more palatable than the TUC crackers in the others. In the control condition of the male snack test (Chapter 4) the mean TUC cracker intake was about 19g, which dropped to about 5g in the female study (Chapter 6). In the present snack test, mean salty snack consumption rose to about 42g. By contrast, mean sweet food intake in this experiment and the female-only snack test was similar (about 40g and 34g respectively), but in the male snack-test, almost double the amount of chocolate snack was consumed (69g). In the individual snack-test experiments, the chocolate snack was a full-sized biscuit that was bigger in size and weighed 10g, more than the bite-sized chocolate 'millionaires' used in this experiment. It may have been the case that participants felt that they wanted to finish the chocolate biscuit, so would automatically end up eating more than in this study with bite-sized food, and this could partly account for inconsistent baseline intakes.

As participants were left unmonitored at the end of the experiment whilst they completed the jigsaw, it is possible that snack sharing occurred. However, the participants were seated too far apart to reach each other's food without moving, and participants did not move. There was also no need to eat from other

participant's bowls as individual portions were generous and contained more than enough for one person and no bowls were completely empty, or even nearing emptiness. It seems unlikely, therefore, that snack sharing occurred.

To conclude, it is shown for the first time, that oxytocin resulted in participants' postprandial snack eating being predicted by the group they were in, and that group membership has a significant effect on snack intake, suggesting that social modelling of eating behaviour occurred. There was a trend towards reductions in salty snack consumption in the female-only groups and there appear to be systematic effects on affiliation and eating that future research using single sex cohorts might uncover. The findings suggest that oxytocin may increase social reward and influence eating behaviour in groups, but evidence was not found for a specifically an anorectic effect but rather one of enhanced social modelling of snack consumption.

CHAPTER 9 - MEASURES OF CHARACTERISTICS RELEVANT TO OXYTOCIN'S EFFECTS ON APPETITE: OVEREATING, CHILDHOOD BONDING, LONELINESS, AND STRESS

9.1 INTRODUCTION

Alongside the first experimental study in Chapter 4, an online survey was conducted to look at aspects of psychological functioning associated with oxytocin as an heuristic for examining the main aspects of oxytocin psychology research that might relate to overeating. As outlined in Chapter 1, the field of oxytocin research in psychology is wide and reflects oxytocin's multiple roles of fast-acting neurotransmitter, longer-lasting neuromodulator, and hormone. In the domains of overeating and oxytocin research, stress is a key focus, and its relationship to overeating was, therefore, measured in the survey via the STAI. Oxytocin functioning as an adult is partly determined by the levels of care received as a child, and this influence was accounted for in the survey by including the PBI. Loneliness can be regarded as a measure of successful affiliation, and is also positively correlated with overeating, so the online survey, therefore, included the UCLA Loneliness Scale.

A growing body of research highlights how compulsive consumption of refined foods high in sugar and fat can resemble substance addictions across a range of dimensions, such as phenomenology, aetiology, neural substrates, comorbidity and genetic dispositions (Davis, 2013; Davis & Carter, 2009; Gearhardt et al., 2011; Ifland et al., 2009; Long et al., 2015; Nestler, 2005). In a comparable way to substance misuse, disorders of overeating are persistent and difficult to overcome and, interestingly, oxytocin is associated with the attenuation of both overeating and substance misuse. To explore the similarities between food "addiction" and substance abuse, and to understand which aspects of overeating relate to the psychological domains that oxytocin influences, a scale measuring food "addiction" was used. The 'Yale Food Addiction Scale' (YFAS) is a measure that was modelled on the DSM-IV criteria for substance dependence and developed to identify individuals in clinical and nonclinical populations exhibiting markers of food "dependence" with the consumption of high fat/high sugar foods (Gearhardt, Corbin, & Brownell, 2009b). The YFAS was selected not only as the most representative and established measure of food "addiction", but also because its subcategories allowed a nuanced analysis of the components of compulsive eating that could be examined alongside other domains related to oxytocin functioning.

A growing body of drug-abuse research is revealing why some individuals become addicted and others do not—a conundrum that also applies to food: not everyone overeats. The causes and corollaries of substance misuse and the use of food as a self-medicating comfort by some, may overlap. A sequence of association can be formed with adverse childhood experience, reduced oxytocin function, and substance dependence in later life, and similar relationships can be formulated for adverse childhood experience, reduced oxytocin function, and compulsive eating. Endogenous oxytocin protects against addiction (Buisman-Pijlman et al.,

2014; Tops et al., 2014) and treatment with exogenous oxytocin prevents the neuroadaptations that can occur to a range of addictive substances (Kovács et al., 1998; McGregor & Bowen, 2012; Sarnyai & Kovács, 1994) and is presently being trialled to prevent relapse in opioid addicts (Bailey, 2014). Attachment and oxytocin have been found to protect against addiction. Differences in the endogenous oxytocin system can arise from individual or environmental factors and lead to susceptibility to addiction (Buisman-Pijlman et al., 2014; Tops et al., 2014). Additionally, social attachment and oxytocin together may confer resilience to addiction (Buisman-Pijlman et al., 2014; Tops et al., 2014).

Oxytocin is an important part of the dyadic bonding process between mother and infant, and disruptions to this process may affect later oxytocin levels in the child (Apter-L. et al., 2014; Feldman et al., 2012). Lower CSF levels of oxytocin have been recorded in women with a history of childhood abuse compared with controls (Heim et al., 2009). Wismer Fries, Ziegler, Kurian, Jacoris, and Pollak (2005) showed that adverse childhood experience alters the oxytocin system and may lead to extensive difficulties later in life. Attempts have been made to link attachment styles to oxytocin levels and it is theorised that poor attachments to caregivers result in lower long-term oxytocin levels (Insel & Young, 2001; Nelson, Eric E. & Panksepp, 1998; Strathearn, 2011; Wismer Fries et al., 2005). After intranasal oxytocin, adults who experienced early life stress were found to have blunted stress responsivity and limbic deactivation (Grimm et al., 2014). Anxiety is linked to oxytocin and is also a risk factor for drug misuse (Sinha, 2008) being frequently comorbid with addiction (Merikangas et al., 1996). Anxiety is also linked to childhood adversity, loneliness, adverse childhood experience, and overeating. As detailed in Chapter 1 (section 1.5), oxytocin is anxiolytic and suppresses the activity of the HPA axis (Churchland & Winkielman, 2012).

Altered oxytocin function is also associated with loneliness. In normal participants, Lucht et al. (2009) found that three different haplotypes of the A-allele containing oxytocin receptor gene scored significantly higher on the emotional loneliness component of the UCLA Loneliness Scale, compared with other haplotypes. Whilst the sample size was small, the results were consistent with the oxytocinergic system having a role in the regulation of loneliness. This research was extended to show that this is moderated both by sex, with girls possessing a particular allele reporting higher loneliness levels, and also the participant's genotype for a dopamine-gene (van Roekel et al., 2013). Loneliness is also implicated in stress eating and overeating. A review by Levine (2012) found that characteristics associated with loneliness are related to the emergence of disordered overeating and, further, that loneliness fuels overeating. Whether isolation reduces endogenous oxytocin functioning, or whether reduced central concentrations of oxytocin must precipitate alterations to sociality is unknown. However, two populations at risk of social isolation are substance abusers and the elderly, and both groups have lower plasma oxytocin levels (Elabd et al., 2014; McGregor & Bowen, 2012; Sannino et al., 2017). Evidence is also accruing that age-related socioemotional changes and loneliness in the elderly cause depression that is ameliorated by oxytocin administration (Chagnon et al., 2015; Ebner et al., 2013). Loneliness levels on the UCLA also moderated oxytocin's beneficial cardiac control with higher levels of loneliness being associated with diminished parasympathetic cardiac reactivity

to intranasal oxytocin (Norman et al., 2011). As early-life infant-parent separation modifies the oxytocin response to social stress, as measured by sympathetic cortisol release in adult men (Meinlschmidt & Heim, 2007), oxytocin is implicated in autonomic as well as genetic resilience to loneliness.

There are other participant characteristics not linked to oxytocin that affect food intake that are necessary to control for in this survey. Amount of sleep is also associated with food intake and sleep deficits can result in physiological changes that increase the desire to eat, body mass, and the loss of dietary restraint, particularly in women (Greer, Goldstein, & Walker, 2013; Knutson, 2012; Markwald et al., 2013; Prinz, 2004; Van Cauter & Knutson, 2008). Physical activity levels reliably explain some of the variance in amount of food consumed (Crews-III, William-Fuge, Oscai, Holloszy, & Shank, 1969; Epstein, Paluch, Consalvi, Riordan, & Scholl, 2002). In addition, sedentary behaviour is associated with unhealthy food choice (Pearson & Biddle, 2011).

The online survey drew together measures of loneliness, parental neglect and overprotection, anxiety alongside a scale examining propensity for food addiction in the YFAS to examine the inter-scale relationships in a non-clinical sample. In line with the literature, it was expected that higher levels of loneliness, parental neglect and anxiety—all related to oxytocin function—would predict overeating on the YFAS.

9.2 METHOD

9.2.1 Participants

An international sample of 205 adults (75 male, 130 female) aged from 18 years to 71 years (M = 33.02 years; SD = 13.83 years) was recruited via a survey link shared on social media. The majority of respondents (71.22%) were from the UK or the USA and Table 9.1 provides information on age variability for these two nationalities (details of the frequencies for nationalities other than British or American can be found in Appendix V). To ensure, as far as was possible, that a high YFAS score only reflected overeating, the following potential confounds that might drive eating in excess of hunger were identified and excluded or measured as covariates. Exercise impacts eating patterns, and a committed exerciser may eat in excess of normative levels, so participants identifying as bodybuilders or exercisers who undertake regular heavy-weight training to increase muscle mass, were excluded to avoid false positive results on the YFAS. People who identified themselves as daily smokers—as opposed to occasional social smokers—were also excluded as their smoking habits may suppress eating. As people with diabetes follow a regime that limits overeating, this group were excluded from participation. People with problem drinking may have altered eating habits, so this was screened for and individuals meeting the criteria were excluded from further participation. Individuals confirming that they were using psychotropic substances or medication were also excluded from participating, as these could alter responses on the survey.

Table 9.1

Age Frequencies by Nationality in the Online Survey Cohort

	Age			
	UK	USA	Other	Total
18-25 years	59	3	24	86
26-45 years	29	17	31	77
46-64 years	8	23	4	35
Over 65 years	2	5	0	7
Total	98	48	59	205

9.2.2 Materials

Qualtrics survey software (Qualtrics, Provo, UT) was used to present the questionnaires anonymously online. To assess problem drinking the four-question drinking screen CAGE was used, which is a validated screening technique for alcoholism with a high sensitivity (Bernadt, Taylor, Mumford, Smith, & Murray, 1982; Ewing, 1984; Mayfield, McLeod, & Hall, 1974). A screening section was presented comprising bespoke questions about exercise, smoking and psychoactive drug use. The first question asked participants whether they were bodybuilders, the second asked whether participants considered themselves to be regular, as opposed to occasional or social-only smokers, and the last screening question asked participants whether they are currently taking any psychoactive medication such as anti-depressants or recreational drugs.

The YFAS was designed to assess the construct of "food addiction" and has been validated using university populations, weight-loss surgery patients, and non-bariatric binge eaters (Clark & Saules, 2013; Gearhardt et al., 2009b). The YFAS is a reliable 25-item measure divided into the eight subscales of symptoms for substance dependence as stated in the Diagnostic and Statistical Manual of Mental Disorders IV-R and operationalized in the Structured Clinical Interview for DSM-IV Axis I Disorders (Gearhardt, Corbin, & Brownell, 2009). The eight scales are: (1) Substance taken in larger amount and for longer period than intended [items 1, 2, and 3]; (2) Persistent desire or repeated unsuccessful attempts to quit [items 4, 22, 24 and 25]; (3) Much time/activity to obtain, use, recover [items 5, 6, and 7]; (4) Important social, occupational, or recreational activities given up or reduced [items 8, 9, 10, and 11]; (5) Use continues despite knowledge of adverse consequences - e.g., failure to fulfil role obligation, use when physically hazardous [item 10]; (6) Tolerance - marked increase in amount/marked decrease in effect [items 20, and 21]; (7) Characteristic withdrawal symptoms; substance taken to relieve withdrawal [items 12, 13, and 14]; and (8) Use causes clinically significant impairment or distress [items 15 and 16]. The scale uses a mixture of Likert type and dichotomous response categories and can be scored in two ways: as a "symptom" count that totals scores on subscales 1 to 7 only resulting in a symptom count from 0 to 7, or as a diagnostic tool that also uses subscale 8 as a determining clinical factor to make a diagnosis of "food addiction".

The trait component of the STAI-Y (Spielberger, 1983) was used (see Chapter 5, section 5.2.2 for details of

the scale), and the questions relating to state anxiety were not used, as situational stress was not being measured in the online survey.

To assess participants' subjective experiences of their parental bond during childhood, the Parental Bonding Instrument (PBI: Parker, G. et al., 1979) was used, see Chapter 7 (section 7.2.2.1) for full details. The 25 items are assessed separately for mother and father, resulting in 50 responses. In this survey, the PBI was prefaced with options to complete only the father or mother section, as relevant.

The component of sitting time that is contained within the International Physical Activity Questionnaire (IPAQ) was used to estimate activity levels (Craig et al., 2003). The IPAQ is a cross-national measure of physical activity, which includes the calculation of sedentary time by totalling questions asking for an estimated average daily sitting time during the weekdays and at weekends. The sitting estimate from the IPAQ has been widely used on its own as a proxy for activity in research spanning 20 countries (Bauman et al., 2011). A preliminary analysis of survey data showed that there was a strong, positive correlation between the total IPAQ score and sitting time component [$r = .69$, $p = .003$, n = 103], so the sitting time score was used.

A large body of research suggests that sleep deprivation can lead to increased food intake and increased desire to eat (Greer et al., 2013; Knutson, Spiegel, Penev, & Van Cauter, 2007; Knutson, 2007; Knutson, 2012; Van Cauter & Knutson, 2008). A question asking participants to estimate how many hours of sleep they had had over the past week, therefore, was included

The Revised UCLA Loneliness Scale version 3 (Russell, Paplau & Cutrons, 1980) was used, refer to Chapter 7 (section 7.2.2.1) for full details.

9.2.3 Procedure

The study was approved by the ethics committee of Kingston University. Participants were supplied with an online link to the survey. The survey was prefaced with a section asking respondents to confirm compliance with the exclusion criteria (see section 9.2.1 for full details of exclusion criteria). Informed consent was then provided online via a check box. Respondents were unaware of the research aims and were informed that the research was "looking at parental bonding/attachment and aspects of adult health, such as eating behaviour, anxiety, sleep and physical activity habits". Participants first provided their date of birth, country of birth, and country of residence. The participant was informed that all sections and questions required responses, but that they could stop at any point; if the survey was terminated before completion, the debrief page was displayed. Next, the screening section containing questions about exercise, smoking, and psychoactive drug use, together with the CAGE was presented. If respondents indicated that did not meet the inclusion criteria, the survey was terminated, and participants viewed the debrief page. The questionnaires were presented in the following order: The PBI, IPAQ sitting questions, UCLA Loneliness Scale, sleep question, YFAS, and

STAI. Respondents were then thanked for their time and debriefed.

9.3 RESULTS

There were initially 292 participants but only 205 provided data suitable for analysis. Incomplete surveys where the respondent had not answered all questions through to the end were discarded (n = 35). Pilots of the survey showed a wide variation in completion times. However, the mean response time was 44.2 minutes (SD = 146.56 mins), and the fastest completion time achieved with coherent responses was eight minutes. Surveys under 10 minutes, therefore, were checked for accuracy and the threshold eight minutes was set as a lower limit for completing the survey. Surveys completed in under eight minutes, which included respondents who were automatically rejected by the online survey screening process, were ignored and not analysed (n = 52).

The means and standard deviations for each of the scales used in the survey are presented in Table 9.2. Scores on the STAI-Y trait (range from 0-80) were above normative means from the US samples upon which the inventory was developed: For working females and females at university, normative means were 34.79 and 40.40 (SD = 9.22 and 10.10) respectively; and for working males and males at university, means were 34.89 and 38.30 (SD = 9.19 and 9.18) respectively (Spielberger et al., 1970). The normative means for the UCLA Loneliness Scale provided by the scale's authors, are 37.06 (SD = 10.91) and 36.06 (SD = 10.11) for males and females respectively. The loneliness scores for men in this survey were significantly greater than normative data [$t(74) = 4.28$, $p < .001$] and, similarly, females in this survey were significantly higher than normative means [$t(129) = 6.88$, $p < .001$]. As stated previously in the attention experiment (see section 7.3.8.6), the authors of the PBI provide a range of normative data and these scores vary according to characteristics of the respondents. One-sample t tests with the general normative means available, showed that scores on the care-neglect dimension in this survey were not statistically different from normative data [$t(204) = 1.45$, $p = .15$] but scores on the overprotection subscale were significantly lower than normative means [$t(204) = 63.76$, $p < .001$], meaning that respondents in this survey did not rate their parents as overprotective.

Table 9.2

Means and Standard Deviations for Predictor Variables Measured, n = 205

	M			*SD*		
	Female	Male	Total	Female	Male	Total
STAI-Y Trait	45.85	44.83	45.50	5.99	5.24	5.72
Hours of Sitting	19.24	16.49	18.31	18.12	11.87	16.24
Loneliness	42.25	42.75	42.43	10.27	11.59	10.71
Hours of sleep	7.11	7.10	7.07	1.41	1.13	1.40
PBI - care	25.64	25.14	25.38	4.87	4.38	4.78
PBI - overprotection	29.22	29.76	29.41	5.01	4.94	4.98

The criteria for each subscale or "symptom" were either met or not met on the basis of responses to the questions that comprised it. For the non-diagnostic scoring of the YFAS, a participant's total YFAS score was the summation of seven subscales that the individual met the threshold for; this total YFAS score is equivalent to a "symptom" count and scores range from 1 to 7. The prevalence of each subscale ("symptom") within the sample is given in Table 9.3.

Table 9.3

Response Frequencies for each YFAS Subscale Behaviour, n = 205

Subscale	N	Percentage
(1) Substance taken in larger amount and for longer period than intended	47	22.9%
(2) Persistent desire or repeated unsuccessful attempts to quit	168	82.0%
(3) Much time/activity to obtain, use, recover	63	30.7%
(4) Important social, occupational, or recreational activities given up or reduced	36	17.6%
(5) Use continues despite knowledge of adverse consequences	48	23.4%
(6) Tolerance - marked increase in amount/marked decrease in effect	33	16.1%
(7) Characteristic withdrawal symptoms; substance taken to relieve withdrawal	19	9.3%
(8) Use causes clinically significant impairment or distress	19	9.3%

9.3.1 Relationship between Total YFAS Score and Predictor Variables

Hierarchical multiple regression was performed to investigate the extent to which anxiety, loneliness, parental neglect, and parental overprotection predicted levels of predisposition to "food addiction", as represented by the total YFAS score. The correlations among the predictor and control variables (activity level, average sleep, anxiety, loneliness, parental neglect, parental overprotection) are presented in Appendix VII. All correlations were weak to moderate, ranging between Pearson's $r = .02$ and Pearson's $r = .56$, which indicates that multicollinearity was unlikely to be a problem. Parental overprotection and average sleep did not statistically correlate with the criterion variable of overeating, so were rejected as predictor variables. The remaining variables of anxiety, parental neglect, loneliness, and activity level were statistically correlated with overeating, which indicates that the data was suitably correlated with the dependent variable for examination through multiple linear regression to be reliably undertaken. The significant correlations between each predictor variable and the dependent variable of overeating were all weak to moderately strong, ranging from Pearson's $r = .15$, $p < .05$ to Pearson's $r = .56$, $p < .001$.

In the first step of the hierarchical multiple regression, activity level (hours of sitting) was entered. This model was statistically significant [$F(1, 203) = 4.34$, $p = .04$, partial $\eta^2 = .33$] but only explained 2% of variance in overeating. The control variable made a significant unique contribution to the model. After entry of anxiety, parental neglect, and loneliness at step 2, the total variance explained by the overall model was 32

% [$F(4, 200) = 24.90$, $p < .001$, partial $\eta^2 = .45$]. The introduction of these three predictor variables explained an additional 30% of variance in overeating, after controlling for activity levels (R^2 change = .32; $F(3, 200) = 31.12$, $p < .001$, partial $\eta^2 = 61\%$). In the final adjusted model, only loneliness and parental neglect were statistically significant, with loneliness recording a higher beta value ($\beta = .52$, $p < .001$) than parental neglect ($\beta = .13$, $p = .03$).

The scoring for each of the seven subscales of the YFAS resulted in each participant either meeting or not meeting the criteria for that subscale. A binary logistic regression, therefore, was conducted to test the power of the eight independent variables collected in the survey (age, anxiety, childhood neglect, childhood overprotection, loneliness, activity levels, and sleep) to predict whether the criteria for each subscale was met. Seven binary logistic regressions were conducted with the eight predictor variables entered nonhierarchically in one block for each regression.

9.3.2 Subscale 1: "Substance taken in larger amount and for longer period than intended"

The intercept-only model was 76.7% accurate and showed that the number of participants who met the criteria for subscale 1 was significantly different from those who did not, Wald $\chi^2(1) = 51.35$, $p < .001$, Exp. $\beta = 0.30$. When the eight predictor variables were entered, the predictive capacity of the model rose to 84.2%. Interestingly, the new model underestimated the number of respondents who met the criteria by more than half (22 were predicted and 47 participants met the criteria). Using Nagelkerke pseudo r^2 values, about 39.4% of the variance in whether subscale 1 criteria were met can be explained by the new model. Out of the eight predictor variables in the model, only loneliness was a significant predictor for meeting the criteria on subscale 1, Wald $\chi^2(1) = 28.91$, $p < .001$, Exp. $\beta = 1.13$.

9.3.3 Subscale 2: "Persistent desire or repeated unsuccessful attempts to quit"

The majority of respondents (166/202) met the criteria for Subscale 2. The subscale comprised four questions about the frequency and success of cutting back on certain types of food or trying to diet.

The intercept-only model was 82.2% accurate and showed that the number of participants who met the criteria for subscale 2 was significantly different from those who did not, Wald $\chi^2(1) = 69.12$, $p < .001$, Exp. $\beta = 4.61$. When the eight predictor variables were entered, the predictive capacity of the model did not change from 82.2%. Using Nagelkerke pseudo r^2 values, only about 17.6% of the variance in whether subscale 2 criteria were met was explained by the new model. Out of the eight predictor variables in the model, trait anxiety significantly predicted meeting the criteria on subscale 2, Wald $\chi^2(1) = 4.90$, $p = .027$, Exp. $\beta = 0.91$. The level of perceived neglect by parents also significantly affected meeting the subscale 2 criteria with higher scores associated with meeting the criteria, Wald $\chi^2(1) = 4.92$, $p = .027$, Exp. $\beta = 1.10$. Lastly, the younger participants were, the more likely they were to meet the subscale 2 criteria, Wald $\chi^2(1) = 3.77$, $p = .052$, Exp. $\beta = 0.97$.

9.3.4 Subscale 3: "Much time/activity to obtain, use, recover"

The intercept-only model was 68.8% accurate and showed that the number of participants who met the criteria for subscale 3 was significantly different from those who did not, Wald $\chi^2(1) = 27.15$, $p < .001$, Exp. $\beta = 0.45$. When the eight predictor variables were entered, the predictive capacity of the model rose to 75.2%. As in Subscale 1, the model with predictors underestimated the number of respondents who met the criteria by more than half (28 were predicted but 63 participants met the criteria). Using Nagelkerke pseudo r^2 values, about 27.3% of the variance in whether subscale 3 criteria were met can be explained by the new model. Out of the eight predictor variables in the model, higher loneliness scores significantly predicted meeting the criteria on subscale 3, Wald $\chi^2(1) = 20.22$, $p < .001$, Exp. $\beta = 1.09$. The level of perceived of neglect by parents also significantly affected meeting the subscale 3 criteria with higher scores associated with meeting the criteria, Wald $\chi^2(1) = 6.42$, $p = .011$, Exp. $\beta = 1.12$.

9.3.5 Subscale 4: "Important social, occupational, or recreational activities given up or reduced"

The intercept-only model was 82.2% accurate and showed that the number of participants who met the criteria for subscale 4 was significantly different from those who did not, Wald $\chi^2(1) = 69.12$, $p < .001$, Exp. $\beta = 0.22$. When the eight predictor variables were entered, the predictive capacity of the model rose slightly to 84.7%. Interestingly, the model underestimated the number of respondents who met the criteria by more than half (22 were predicted and 47 participants met the criteria). Using Nagelkerke pseudo r^2 values, about 34.7% of the variance in whether subscale 4 criteria were met can be explained by the new model. Out of the eight predictor variables in the model, only loneliness was a significant predictor for meeting the criteria on subscale 4, Wald $\chi^2(1) = 27.57$, $p < .001$, Exp. $\beta = 1.15$.

9.3.6 Subscale 5: "Use continues despite knowledge of adverse consequences"

The intercept-only model was 76.2% accurate and showed that the number of participants who met the criteria for subscale 5 was significantly different from those who did not, Wald $\chi^2(1) = 49.73$, $p < .001$, Exp. $\beta = 0.31$. When the eight predictor variables were entered, the predictive capacity of the model rose to 84.2%. The new model underestimated the number of respondents who met the criteria by more than half (25 were predicted and 48 participants met the criteria). Using Nagelkerke pseudo r^2 values, about 33.4% of the variance in whether subscale 5 criteria were met can be explained by the new model. Out of the eight predictor variables in the model, lower trait anxiety significantly predicted meeting the criteria on subscale 5, Wald $\chi^2(1) = 6.11$, $p = .013$, Exp. $\beta = 0.92$. Loneliness also significantly affected meeting the subscale 5 criteria with higher scores associated with meeting the criteria, Wald $\chi^2(1) = 18.62$, $p = < .001$, Exp. $\beta = 1.10$. Lastly, higher levels of sleep predicted participants were more likely to meet the subscale 5 criteria, Wald $\chi^2(1) = 3.77$, $p = .052$, Exp. $\beta = 0.97$.

9.3.7 Subscale 6: "Tolerance - marked increase in amount/marked decrease in effect"

The intercept-only model was 83.7% accurate and showed that the number of participants who met the criteria for subscale 6 was significantly different from those who did not, Wald $\chi^2(1) = 73.66$, $p < .001$, Exp.

$\beta = 0.20$. However, when the eight predictor variables were entered, the predictive capacity of the model fell to 82.2%, indicating that the predictor model was less effective, and no further analyses were made.

9.3.8 Subscale 7: "Characteristic withdrawal symptoms; substance taken to relieve withdrawal"

The intercept-only model was 90.6% accurate at predicting how many met and didn't meet the criteria as most respondents (183/202) did not meet the criteria for subscale 7, so the observed and expected values were similar. Unsurprisingly, there was a significant difference between the number who met the criteria and the number who did not from those who did not: Wald $\chi^2(1) = 88.31$, $p < .001$, Exp. $\beta = .10$. When the eight predictor variables were entered, the predictive capacity of the model rose slightly to 92.6%. Using Nagelkerke pseudo r^2 values, about 31.9% of the variance in whether subscale 7 criteria were met can be explained by the new model. Out of the eight predictor variables in the model, only loneliness was a significant predictor for meeting the criteria on subscale 7, Wald $\chi^2(1) = 16.58$, $p < .001$, Exp. $\beta = 1.15$.

9.3.9 Subscale 8: "Use causes clinically significant impairment or distress" and Diagnosis

Meeting the criteria for subscale 8 combined with meeting the criteria on a further three subscales results in a YFAS diagnosis of "food addiction". Interestingly, 18 out of 205 participants (8.7%) met the criteria for subscale 8 and the same 18 participants also met the threshold for a "food addiction" diagnosis. A binary logistic regression was conducted to see which predictors were associated with meeting the clinical subscale criteria. As only 18 individuals met the criteria, as expected, the intercept-only model's predictions were good, and per the previous subscale 90.6% of responses were correctly predicted from the null model, which rose to 92.6% with the inclusion of predictors in the second model. The difference between those who met the subscale 8 criteria and those who did not was, therefore, significant: Wald $\chi^2(1) = 88.31$, $p < .001$, Exp. $\beta = .10$. Using Nagelkerke pseudo r^2 values, about 31.9% of the variance in whether subscale 8 criteria were met can be explained by the second model. Out of the eight predictor variables in the model, both loneliness [Wald $\chi^2(1) = 16.58$, $p = .< .001$, Exp. $\beta = 1.15$] and gender [Wald $\chi^2(1) = 3.94$, $p = .047$, Exp. $\beta = .32$] were significant predictors for meeting the criteria on subscale 8, Wald $\chi^2(1) = 3.94$, $p = .047$, Exp. $\beta = .32$. The beta coefficient for gender was negative, and females were coded as 1 whereas males were 2; therefore, in this model, being female predicted meeting the criteria on subscale eight. Increased loneliness also predicted meeting the criteria.

The number of responses on the YFAS for each of the three types of response category are provided for all 205 participants in Tables 9.4, 9.5, and 9.6. Respondents stated that four behaviours occurred most frequently. These were question one about eating more of certain foods once they had started, question two concerning eating beyond hunger, question six about eating certain foods constantly, and question fourteen addressing elevated desire for food during restriction. In response to question six, 25% of the cohort stated that four or more times per week—or daily—they constantly eat certain foods through the day. Cutting down on foods and experiencing an elevated desire for them (question fourteen) and eating much more than planned of certain foods (question one) were both prevalent in 17% of the sample four or more times per

week, or daily. Question two was similar to the first question and, accordingly, its response profile was the same. On the other hand, avoiding social situations for food-related reasons (questions ten & eleven) was not prevalent in this sample with 86% and 91% (respectively) of participants stating these behaviours never occurred.

Table 9.4

Response Frequencies for Questions 1-16 of the YFAS, n = 205

	Never	Once month	2-4 times/ month	2-3 times/ week	4 + times/ daily
1. I find that when I start eating certain foods, I end up eating much more than planned	30	46	47	47	35
2. I find myself continuing to consume certain foods even though I am no longer hungry	34	48	49	42	32
3. I eat to the point where I feel physically ill	135	35	20	11	4
4. Not eating certain types of food or cutting down on certain types of food is something I worry about	76	30	37	33	29
5. I spend a lot of time feeling sluggish or fatigued from overeating	124	45	22	9	5
6. I find myself constantly eating certain foods throughout the day	60	26	31	37	51
7. I find that when certain foods are not available, I will go out of my way to obtain them. For example, I will drive to the store to purchase certain foods even though I have other options available to me at home.	103	43	41	14	4
8. There have been times when I consumed certain foods so often or in such large quantities that I started to eat food instead of working, spending time with my family or friends, or engaging in other important activities or recreational activities I enjoy.	151	21	19	10	4
9. There have been times when I consumed certain foods so often or in such large quantities that I spent time dealing with negative feelings from overeating instead of working, spending time with my family or friends, or engaging in other important activities or recreational activities I enjoy.	169	19	12	5	0
10. There have been times when I avoided professional or social situations where certain foods were available,	177	17	7	4	0

because I was afraid, I would overeat.					
11. There have been times when I avoided professional or social situations because I was not able to consume certain foods there.	187	10	6	1	1
12. I have had withdrawal symptoms such as agitation, anxiety, or other physical symptoms when I cut down or stopped eating certain foods. (Please do not include withdrawal symptoms caused by cutting down on caffeinated beverages such as soda pop, coffee, tea, energy drinks, etc.)	164	13	11	2	15
13. I have consumed certain foods to prevent feelings of anxiety, agitation, or other physical symptoms that were developing.	115	32	22	11	25
14. I have found that I have elevated desire for or urges to consume certain foods when I cut down or stop eating them.	90	44	24	13	34
15. My behaviour with respect to food and eating causes significant distress.	149	25	15	8	8
16. I experience significant problems in my ability to function effectively (daily routine, job/school, social activities, family activities, health difficulties) because of food and eating.	154	27	15	5	4

The two questions that relate to tolerance (20 & 21) were not relevant to this sample as 92% and 89% (respectively) of participants responded that the statements did not apply to them. The questions on cutting down (22, 23, & 24) divided the sample somewhat, with the majority of participants feeling that these statements applied to them. Participant responses on the question asking about the success of cutting back on palatable food in the last 12 months (Q24) were more evenly split than other dichotomous questions, with 55% feeling they had been successful and 45% thinking not.

Table 9.5

Response Frequencies for Questions 7-24 of the YFAS

	NO	YES
17. My food consumption has caused significant psychological problems such as depression, anxiety, self-loathing, or guilt.	169	36
18. My food consumption has caused significant physical problems or made a physical problem worse.	168	37
19. I kept consuming the same types of food or the same amount of food even though I was having emotional and/or physical problems.	156	49
20. Over time, I have found that I need to eat more and more to get the feeling I want, such as reduced negative emotions or increased pleasure.	189	16
21. I have found that eating the same amount of food does not reduce my negative emotions or increase pleasurable feelings the way it used to.	183	22
22. I want to cut down or stop eating certain kinds of food.	71	134
23. I have tried to cut down or stop eating certain kinds of food.	62	143
24. I have been successful at cutting down or not eating these kinds of food	92	113

Table 9.6

Response Frequency for Question 25 of the YFAS

25. How many times in the past year did you try to cut down or stop eating certain foods altogether?	1 or none	2 times	3 times	4 times	5 +
	80	29	33	16	47

9.4 DISCUSSION

In an online survey examining overeating and the psychological domains influenced by oxytocin of stress, parental attachment, and loneliness, a strong association linking childhood neglect and loneliness to levels of food “addiction” emerged. However, age, gender, BMI, trait anxiety, parental overprotection, exercise levels, and amount of sleep did not predict levels of food “addiction”. Strikingly, in separate analyses of the subscales, loneliness was a significant predictor the majority of subscales. Perceived parental neglect, anxiety, age, and amount of sleep were also significant predictors of overeating in some of the subscales. The frequency of the behaviours measured by the YFAS was measured and questions that related to DSM-IV criteria of withdrawal and tolerance had very low frequency, whereas participant responses to questions on behaviours involving restriction of food intake indicated that they occurred frequently. When answering the

YFAS, participants are instructed to think about the questions in relation to highly palatable foods such as chocolate or French fries, which suggests that consumption of highly palatable food is a unique problem rather than a part of general dysregulation of eating or metabolic dysfunction.

The lack of association between the predictor variables of sleep and exercise levels used as controls and the total YFAS score of overeating is not consistent with previous research showing the hormonal, motivational, and emotional impact that curtailed sleep has, which can result in overeating (Chaput, Després, Bouchard, & Tremblay, 2008; Markwald et al., 2013; Marshall, Glozier, & Grunstein, 2008; Patel & Hu, 2008). However, the method of measuring exercise and sleep may not have captured the information about each activity that was required. Another possibility is that a third factor, such as BMI or impulsivity, moderates the effects of sleep and activity levels. It is possible that a self-selection bias resulted in participants with eating problems that impact BMI, exercise and sleep. However, the prevalence of "food addiction" in this sample is 8.7%, which matches the prevalence in a French cohort, which was also 8.7%, is similar to a German sample, which was 8.8% (Meule, 2011), and is less than a US study that found a prevalence of 11.4% (Gearhardt, Corbin, & Brownell, 2009a).

The only online survey predictor related to oxytocin that was significantly associated to the YFAS overall score, was loneliness. Loneliness scores were higher than normative means provided by the scale's developers. Although the prevalence of loneliness is greater in the elderly than in younger populations (de Jong-Gierveld, Kamphuis, & Dykstra, 1987), only a small proportion of the sample were older adults in this survey, and only two respondents were over 70 years of age, so old age cannot account for higher mean loneliness scores. Romantic attachment was not recorded in this survey but has been shown to inflate scores of loneliness for individuals not in a relationship. Students not dating or romantically involved recorded mean loneliness scores of 43.01 (Peplau & Cutrona, 1980), which accord with the average scores from this survey of 42.43. Whilst the state of being lonely is situational not dispositional, loneliness itself is associated with a range of factors such as health, personality traits, and other psychosocial measures, such as depression (Bell & Daly, 1985; Creecy, Berg, & Wright, 1985; de Jong-Gierveld, 1978). However, as this study was concerned primarily with factors related to oxytocin, these were not measured.

Although the YFAS is validated for use in nonclinical samples as an instrument to assess "food addiction" symptoms, the relevance of the YFAS as an exploratory rather than diagnostic tool in nonclinical samples, is questionable. The thresholds for each of the seven subscales (the eighth was diagnostic) were only met for the majority of the sample in one of the seven subscales: subscale 2. Subscale 2 addressed the extent and success of restraint or "cutting back" in the sample, and the prevalence of this aspect of overeating is reflected in the response frequencies to the 25 items comprising the YFAS. Restraining food intake is clearly easier for some individuals than others, as reflected in responses in the YFAS, and was predicted by three variables that also relate to oxytocin: trait anxiety, parental care, and age. Interestingly, loneliness was not associated with restraining eating, but with the components of the YFAS that address the increase of

behaviours towards overeating.

The YFAS subscales of tolerance and withdrawal might be unreliable. Food is a natural reward and, as such, the concept of developing long-term tolerance towards food is problematic as hunger will always motivate eating, and the reward, at least when hungry, is unlikely to be susceptible to tolerance. The rewarding value of food does diminish with satiation (Siep et al., 2009) and eating beyond this state could have a decreased pleasurable effect (Thomas, JM et al., 2015), but this is temporary and lasts until hunger returns, and because of its transitory nature, by definition does not parallel the long-term tolerance that develops to drugs of abuse. Similarly, withdrawal effects derived from sugar and fat, such as anxiety, have been described in animals (Hoebel, Avena, Bocarsly, & Rada, 2009). However, likening these signs of agitation observed in some animals after sugar or fat restriction to symptoms of opiate withdrawal has been criticised, as opiate withdrawal is qualitatively different from the physical withdrawal symptoms resulting from sugar or fat withdrawal (Bocarsly, Berner, Hoebel, & Avena, 2011).

Given the predictive value of loneliness, childhood neglect, and anxiety in relation to aspects of overeating, together with their established links to oxytocin and eating, the results of the present study informed this thesis in a number of ways. These results were analysed after the female pilot study in Chapter 5, and so changes were incorporated from the female stress experiment (Chapter 6). The most prevalent aspect of overeating found in this online survey was restraint, an aspect of overeating not fully explored by the YFAS, so the DEBQ was added to measure restrained eating and examine other influences on eating. Since loneliness levels are higher in the romantically unattached, the UCLA Loneliness Scale was incorporated into experiments. As the social study (Chapter 8) analysed data by group, stable individual characteristics that related to how much an individual might eat by themselves were considered less important and so these scales were omitted from that experiment.

This was not a clinical sample and so the relative absence of "food addiction" is unsurprising. However, the sample may not be representative as the ages are not matched between the US and UK samples with the UK sample being predominantly younger adults and the US sample being mostly older adults. Despite this unevenness in ages, scores were nevertheless broadly in line with normative scores, indicating that the cohort used was not necessarily unrepresentative. The exception was the subscale of overprotection in the PBI, which in this sample was significantly lower than normative data, and higher scores may have had more predictive value to measures of overeating on the YFAS. It is clear, though, that behavioural aspects of "food addiction" occur in the general population and that the population of interest does not need to be one meeting the criteria for "food addiction".

CHAPTER 10 – GENERAL DISCUSSION

10.1 Overview of Key Findings

The overarching aim of the thesis was to investigate oxytocin's anorectic effect on postprandial snacking and explore oxytocin's alteration of attention to food as a possible mechanism for achieving this; significant effects were demonstrated in both of these domains. For the first time, oxytocin's anorectic effects were demonstrated to occur without deprivation-induced hunger in experiments employing a much shorter meal-to-snacking latency of 15-20 minutes than previous times of 100 minutes. The work of this thesis examines for the first time the effects of oxytocin on female snacking not triggered by deprivation-induced hunger. It shows that oxytocin inhibits palatable snack intake in this group without concomitant reductions of salivary cortisol. The bogus taste test paradigm has never before been fully disguised in oxytocin research, and in this thesis a credible cover story was provided to reduce demand characteristics and self-selection bias. The anorectic effects of oxytocin were explored for the first time in a social setting and an effect of oxytocin on social modelling of food intake was found but no overall reductions in snack intake were seen. Previous research on attentional biases to food in the normal population was extended and showed the successful reduction by oxytocin of bias to food in men and women using a dot-probe paradigm. The online survey of this PhD demonstrated that symptoms of "food addiction" could be predicted by loneliness, parental neglect, and trait anxiety, providing potential target populations for further research and suggesting participant characteristics that exogenous oxytocin administration might be a useful therapeutic.

10.2 Overview of Snack-Test Experiments (Chapters 4, 6, and 8)

The initial experiment described in Chapter 4 found that 24 IU of intranasal oxytocin reduced palatable food intake in men. Hunger was minimised by providing lunch to participants 15-20 minutes before testing snacking, thus shortening the 100-minute delay previously employed to test oxytocin's effects on snacking. Results in this experiment showed that oxytocin reduced sweet snack intake to an even greater degree than in the Ott et al. study it partially replicated: 63% versus 25% reductions, respectively. The caloric and macronutrient contents of the snacks were equalised across the snack types, contrary to Ott et al. (2013) and Thienel et al. (2016) who did not match the calories of all their snacks. Unlike the German group studies, the sham-sensory tests that were analysable were also compared to ensure they were not influenced by oxytocin and so the procedure would remain stable across experimental conditions.

In contrast to the studies by Ott et al. (2013) and Thienel et al. (2016), salty snack consumption was reduced by intranasal oxytocin, a finding that is in line with animal research showing that oxytocin reduced non-sweet carbohydrate and salt intake (Blackburn et al., 1995; Sclafani et al., 2007; Verbalis et al., 1993). However, animal research into inhibition of salty food intake by oxytocin has been conducted exclusively in rodents whose sodium excretion is regulated by endogenous oxytocin signals, such that dehydration or sodium loading increases oxytocin production (Sabatier et al., 2014). Osmoregulation in humans, however,

is not mediated by oxytocin, so direct comparisons between rodent and human studies may not be valid (Sabatier et al., 2014).

In the female-only snack-test experiment (Chapter 6), snacking was successfully promoted after two stress tests and was, for the first time, attenuated by prior inhalation of oxytocin. Although comfort eating is typically associated with females, monthly menstrual fluctuations and societal pressure to remain slim make investigating female eating problematic and this was illustrated by the negligible amounts of food eaten in the pilot female study (Chapter 5). However, in line with Epel, Lapidus, McEwen, and Brownell (2001) who demonstrated that eating can be generated in females in response to stress, in the main female experiment, snack eating after stress tests did occur, which was reduced by oxytocin. The results also accord with a previous female mouse study that found oxytocin was an important mediator of the hyperphagic effects of single-minded 1 gene (SIM-1) haploinsufficiency, the effects of which were reversible by oxytocin injection (Kublaoui et al., 2008). The different effects on salty food intake across genders in this thesis might be explained by the introduction of stress in the female procedure, because stress in females in known to preferentially facilitate sweet food consumption. Importantly, salivary cortisol was not reduced by intranasal oxytocin and oxytocin's hypophagic effects were, therefore, independent of oxytocin's cortisol inhibition in the female experiment. This was an unexpected finding as some previous studies have shown concomitant cortisol reductions (Ott et al., 2013; Thienel et al., 2016). However, previous experiments measured total plasma cortisol, which has very low concentrations of active cortisol, and is, therefore, not necessarily an accurate gauge of real-time cortisol fluctuations (Gibson et al., 1999). The lack of correspondence between cortisol and oxytocin's inhibition of stress eating, suggests that other processes that are affected by oxytocin—such as the activity of the amygdala or limbic reward centres—might underpin the effect. Future research might revisit this by contrasting plasma and salivary cortisol with reward eating during the early-light circadian phase when both cortisol and oxytocin concentrations are highest (Chung et al., 2011; Devarajan et al., 2005; Perlow et al., 1982; Reppert et al., 1984).

The last experiment using the covert snack-test design (Chapter 8), showed that oxytocin promoted social modelling of snack-food intake in participants who had eaten lunch shortly beforehand. In contrast to the two snack-test experiments that tested participants individually, which showed that oxytocin influenced eating by reducing palatable snack intake, the group study demonstrated effects of oxytocin on the social modelling of eating. One of the main drivers for predominantly hedonic eating is anxiety but in the female snack-test experiment (Chapter 6) snacking decreased without reductions in cortisol. In the social study, an increase of social modelling without subjective changes in stress or consistent effects on intake was observed. Together, these findings may challenge the parsimonious theory that oxytocin achieves higher-order sociocognitive effects via anxiolysis alone (e.g. Churchland & Winkielman, 2012). Although oxytocin's anorectic effects were not salient in this experimental protocol, oxytocin still influenced eating by enhancing social modelling of eating either by increasing the social facilitation of snack consumption or by increasing social impression management concerns that resulted in inhibited snacking. Although this thesis

demonstrated no concomitant reduction of cortisol with oxytocin's inhibition of palatable food intake, cortisol was not measured in the group study (Chapter 8) and it is possible that the social modelling of food intake reflected an anxiolytic social facilitation that future research could explore. In this experiment, as in the rest of the thesis, participants were not aware of the motivational changes caused by oxytocin and did not report that they felt more influenced by the group in the oxytocin condition.

In order to retain ecological validity in the group study (Chapter 8), the groups' composition was not controlled; notwithstanding this, oxytocin still effected changes to social modelling of food intake. In line with previous research, oxytocin's effects were not perceived by participants, and significant changes in social conformity were not reflected in self-rated assessments of how influenced individuals felt by their group. As context is critical to oxytocin's effects, changes to the social dynamic would be likely to result in different outcomes, and this was evident from the divergent effects of oxytocin on snack intakes in each group. In mixed-sex groups, women select lower calorie food to eat than in single-sex groups, and the number of calories eaten by women is negatively predicted by the number of men in the group (Young, Meredith, Mizzau, Mai, Sirisegaram, & Wilson, 2009). In this social study there was a borderline effect of gender in line with research that found women-only groups consumed less than mixed groups. Both sexes eat less in the presence of a stranger than when eating with friends or family, and the amount eaten increases as the group size increases (Clendenen, Herman, & Polivy, 1994; De Castro & Brewer, 1992; De Castro, 1994; Hetherington, Anderson, Norton, & Newson, 2006; Salvy, Jarrin, Paluch, Irfan, & Pliner, 2007). Accordingly, the level of influence that participants reported was greater with three group members in this experiment and smaller groups ate less in both conditions. This suggests that the social facilitation of eating was stronger with the addition of an extra participant, which is consistent with de Castro's finding that the amount eaten was a function of the number of people present (De Castro & Brewer, 1992). Future experiments could explore the impact of oxytocin on both the number and familiarity of diners. The prosocial and anorectic effects of oxytocin on single sex eating groups, larger groups and groups of friends or family, are unknown. Further research could usefully extend the work of this thesis by replicating Chapter 8's social experiment using these different configurations of groups.

Social interactions and enjoyable sham task experiences during the experimental sessions, combined with exogenous oxytocin may have generated prosocial effects in participants. For example, there is evidence that social support and oxytocin interact to reduce stress levels (Chen et al., 2011; Heinrichs et al., 2003), and it is known that endogenous oxytocin is released by physical contact and conspecific proximity (Brosnan et al., 2015; Heinrichs, von Dawans, & Domes, 2009; Kosfeld et al., 2005; Scheele et al., 2014; Scheele et al., 2012). In addition, the combination of the administration of exogenous oxytocin and positive social interactions could encourage a "feed forward" release of endogenous oxytocin that prolongs and enhances its prosocial effects. Undoubtedly, the group study (Chapter 8) was a popular study that had a surplus of participant registrations resulting from its reputation within the university as an enjoyable experience. The dynamic interactions between group members in the social study contributed to social conformity after

oxytocin, suggesting a prosocial effect in this experiment, but it is difficult to disentangle the relative contributions of social interactions and oxytocin on prosociality. It is also possible that the escalated social interactions in the group study may have masked any measurable influence of oxytocin administration on food intake. The interactions between the researchers and participants, together with the inclusion of similar enjoyable sham tasks, may also have created a prosocial effect in the snack-test studies that assessed people individually (Chapters 4 and 6). Although the researcher's impact could potentially have been diluted in the social study by the additional group members, this was not relevant in the individual studies. However, in an analogous way to the social experiment, the influence of the social aspects of the researcher's presence may have involved an attentional deprioritising of food, as suggested by oxytocin's reductions in Chapter 7 on attentional food bias, thereby contributing to oxytocin's anorectic impact. Whilst not directly applicable to addressing the problems of overconsumption of palatable foods, oxytocin's promotion of social modelling and subjective sociability in the group study, have implications for eating disorders such as bulimia, which can result in concomitant reductions in sociality (see Chapter 1, section 1.8.2.1).

Although the experiments that assessed snack intake via the bogus taste test were in a laboratory setting, they were nonetheless successful in demonstrating effects of oxytocin on snack intake. Concealing experimental aims and providing large snack bowls with appealing and unbroken snacks, in contrast to the broken-up snacks in the Ott et al. (2013) and Thienel et al. (2016) experiments, allowed participants to eat a considerable amount without it being obvious, which may have assisted in generating the increased snack eating observed in this thesis. However, these studies lack some ecological validity and snack intake may have been greater if the participants were unmonitored and in a "natural" environment rather than in a laboratory where they perceive that eating a lot might be noticed. The anorectic effects of 96 IU of daily oxytocin across eight weeks have been demonstrated in a weight loss study using type II diabetes patients with BMIs above 28 kg/m^2 but because experimental aims were not concealed, it is possible that oxytocin may have increased compliance or motivation to conform, in line with its prosocial effects (Zhang, Hai et al., 2013). The efficacy of oxytocin in humans to reduce palatable food intake covertly across time in ecological settings remains unknown.

Unlike previous snack tests using oxytocin (Ott et al., 2013; Lawson et al., 2015; Thienel et al., 2016), participant self-selection, self-exclusion, and self-monitoring were minimised as these PhD experiments were not associated with an eating research department, professor, or hospital, and neither the experimental aims nor the researchers' interests were revealed or suggested by recruitment adverts or other materials. In line with the recommendations of Robinson et al. (2017), the aims of these experiments were rigorously concealed with decoy exclusion criteria, cover stories, and by the bulk of participant tasks being sham tests. Hospitals and food laboratories could attract participants interested in their health or diet and, conversely, could disincentivise potential participants with aversions to such places or the research suggested by them. Experiments took place in generic laboratories that provided no clues to the nature of the research and participants regularly posed questions about their scores on the sham sensory tests and how oxytocin could

affect their performance on them, which suggested that they were both engaged with and deceived by the cover story. Concealing experimental aims also reduced demand characteristics. Weber and Cook (1972) proposed that participants can assume different roles in an experimental situation, such as the "faithful" participant who is compliant. However, some of roles proposed by Weber and Cook that participants assume have the potential to undermine experimental aims if the participant can guess them: the "negative" participant who "trashes" experimental goals, the "good" participant who tries to "help", and the "anxious" participant who self-evaluates. Further, participants did not complete questionnaires on overeating and food craving until after experiments were completed, unlike, for instance, Thienel et al. (2016). The stringent concealment of experimental aims also facilitates eating because self-monitoring induced inhibition of eating is increased when self-impression concerns result in participants feeling that the amount they eat might be tracked.

The experimental design eliminated some of the potential criticisms that have been levied against previous papers (Field, A. & Hole, 2002). Experiments in this thesis did not expose the participants to the equivocal effects of fasting for 14.5 hours on alertness, cognition, and mood (Benau, Orloff, Janke, Serpell, & Timko, 2014; Chtourou et al., 2011; Fond et al., 2013; Roky et al., 2000), and then spend in excess of four hours in a laboratory, which was likely to have boosted recruitment, widened the recruitment pool, and reduced experimental fatigue. Additionally, the experiments were held at lunch time and at weekends to widen their demographic reach.

Another proposal of this thesis is that oxytocin's anorectic actions relate to snack intake not initiated by deprivation-induced hunger. In the previous research from the German groups the gap between eating a meal and the snack test was 100 minutes (e.g. Ott et al., 2013; Thienel et al., 2016), which is potentially too great a latency to claim that participants were food sated as the stomach is typically only 15% full 120 minutes after ingestion of solid food (Bowen, 2018). Additionally, a number of the high-calorie foods offered by the German research teams were liquid (e.g. strawberry milk) and will have emptied from the stomach more quickly than solid food and resulted in a blood sugar spike (Camilleri et al., 1989; Charles et al., 1995; Hunt & Stubbs, 1975; Vist & Maughan, 1995). An additional factor that might have generated hunger was that participants in the German studies were offered snack food at 12:40, and lunch time would have cued participants to eat. In this thesis, these confounds were corrected for as the participants were provided with lunch to eat, the food eaten was not readily catabolisable avoiding a blood sugar surge, the only liquid offered was water, participants rated themselves as not hungry immediately before the snack test, and lunch was eaten 15 to 20 minutes before the snack intake was assessed. Subjective hunger was measured by VAS after lunch in all snack-test experiments, and oxytocin had no effect on self-reported satiety levels in this thesis, which is in line with previous research (Lawson, 2017). Beyond satiety, some of the motivations behind eating not initiated by deprivation-induced hunger and hunger-driven are likely to differ, and this thesis has added to the literature by demonstrating that oxytocin's anorectic effects on eating that is not initiated by deprivation-induced hunger, are likely to be modification of a hedonic drive.

10.3 Overview of Other Experiments

For the first time, oxytocin was found to reduce attention to palatable food stimuli (Chapter 7) in participants who had consumed a readily catabolisable snack and drink to minimise hunger and fatigue. The attentional bias to food pictures that were displayed for half a second, was significantly reduced by 24IU intranasal oxytocin. This result was not related to the participant's BMI, age, subjective loneliness, parental bonding or score on the DEBQ, but trait anxiety and trait food craving did co-vary with food bias. Oxytocin also resulted in participants paying less attention to food pictures when paired to social and romantic stimuli but did not modify attention to romantic or social picture probes when paired with food or paired against each other.

The presentation time of the pictures used in the attention study was 500 ms, a standard duration used in research of this nature. An unresolved issue in the literature, however, is what type of attention is represented by responses to presentation durations. The presentation of stimuli for 500 ms has been variously described as measuring the initial allocation of overt attention to the stimuli (Cooper & Langton, 2006) and the maintenance of attention towards a stimulus (Field & Cox, 2008). However, Cooper and Langton (2006) argue that the shift of attention that precedes an eye movement, in other words covert attention (Posner & Petersen, 1990), can change more than once within the 500 ms time window. Bradley, Mogg, and Miller (2000), tested participants simultaneously with an eye tracker and dot-probe task and found evidence that participants' gaze shifted between the pictures presented during the 500 ms presentation. Aside from the question of which attentional processes might be captured by different durations, Bradley, Mogg and Miller (2000) suggest that measuring this covert attention is crucial because attentional biases found with short time presentations, such as 100 ms, are not captured when the stimuli are presented for 500 ms. There is evidence in the other direction, however, as Mogg, Bradley, Hyare, and Lee (1998) found attentional biases to food-related stimuli using a 500 ms presentation time that were not demonstrable with a shorter duration; as 500 ms was an effective duration in this thesis, the point is moot, therefore.

The final study of this PhD assessed whether symptoms of "food addiction" as measured by the YFAS, could be predicted by personal characteristics that relate to oxytocin. Age, gender, level of activity, and amount of sleep were also measured, and results showed that only loneliness and parental neglect predict the overall score on the YFAS that represents "food addiction". Loneliness also predicted meeting the criteria for five out of the eight YFAS subscales and parental neglect predicted meeting the criteria for two subscales. Trait anxiety was not related to an overall "food addiction" status but lower anxiety and greater sleep both predicted meeting the criterion for continuing to overeat despite adverse consequences, whereas higher anxiety and youth predicted meeting the criteria for persistent desire to quit overeating. Being female predicted meeting the criteria for significant distress and impairment as a result of overeating. The elements of the YFAS that respondents indicated were most relevant to them related to the persistent desire to quit overeating and the amount of time taken up trying to stop overeating.

The dominance of loneliness as a predictor for overeating on the YFAS was not supported by the findings of the attention study that found loneliness was not related to food bias or oxytocin's reduction of it. Although only a small number of respondents were elderly, still a substantial proportion of the online survey respondents were older adults over 50 years and, as loneliness increases as a function of increasing age, as such, they may be more likely than the younger cohort used in the attention study to feel lonely (Creecy et al., 1985; de Jong-Gierveld et al., 1987; Qualter et al., 2015). The predictive capacity of anxiety in the overeating subscales is not mirrored by associations between subjective anxiety and eating, or oxytocin's modulation of food intake, found in the main body of this thesis. However, females who did not eat in the pilot study (Chapter 5) had lower than normative average trait anxiety levels; further, trait anxiety negatively correlated with attention to food pictures in the oxytocin condition.

10.4 Oxytocin's Effect on Taste Ratings

Sweetness ratings for the chocolate snacks were unexpectedly higher in the oxytocin condition of the male snack-test study (Chapter 4), despite consumption of the chocolate biscuits being dramatically reduced, suggesting that the perceived increase in sweetness may have adversely affected palatability. Given that oxytocin acts on the satiety hormone leptin to reduce food intake, and that leptin can suppress the perception of sweet taste via leptin responsive lingual taste receptors (Shigemura et al., 2004), this is an unexpected finding, and one that suggests leptin-induced changes in sweetness perception may not contribute to oxytocin's anorectic effects. However, while the elevated sweetness ratings and reductions of chocolate biscuit intake in the male snack test study were in the opposite direction from those suggested by Shigemura et al.'s work, they were not significantly associated, suggesting that they may be independent effects. Although the preference for consuming readily catabolising sugars seems to be innate to all mammals (Mitra et al., 2010; Sabatier et al., 2014) and persists in transgenic mice lacking functional sweet taste receptors (de Araujo, 2011; de Araujo et al., 2008), central oxytocin has been shown to modulate sweet-taste preference. A motivational shift away from sweet food via the VTA may also be independent of the perception of how sweet the chocolate snacks were, but the brain mechanisms that underlie functional independence of taste and motivation are not yet established.

Although snack intake in the placebo condition indicated the type of snack that participants preferred to eat, pre-existing bias towards sweet or salty food was not measured, and the choice of snacks presented may not have appealed to some individuals independent of any sweet or salty predisposition. However, taste ratings themselves are likely to reflect personal food preferences, but taste ratings in the placebo conditions of the snack tests of this thesis were not significantly correlated with intake of the same food. This raises the possibility that the careful tasting of food during the snack test that resulted in taste ratings, did not reflect the participant's food preferences or eating style, that may have been less mindful, for example, when left alone with the snack-food and the task of choosing their favourite picture. Neonatal pleasure responses to sucrose (Steiner & Glaser, 1995) make sense from a survival perspective but these are superseded by

psychosocial conditioning over time that shapes petitive, paracrine, endocrine, and behavioural responses (Volkow et al., 2011). However, no further effects on taste were found in any of the snack-test experiments of this thesis, so intake reductions are unlikely to be generally attributable to a modulation of taste perception. A need for a more sophisticated characterization of taste properties in future studies is indicated in order to unpick the associations between sensation and behaviour. Currently, no study has reported associations between taste and intake that might logically underlie the effect of oxytocin, but studies are difficult to compare as they use different protocols and oxytocin dosages.

10.5 Oxytocin's Effect on Mood

The finding of this PhD that oxytocin's behavioural effects are not mirrored by any detectable changes in mood on the VAS is consistent with other research into the peptide's anorectic capacity. It is striking how little effect on self-reported VAS mood states, including anxiety, intranasal oxytocin engendered. In this thesis, oxytocin did not change self-reported levels of alertness, excitement, happiness or boredom in the snack-test experiments. In the German groups' snack tests, no effects on mood after oxytocin were found using bespoke 5-point scales (Ott et al., 2013; Thienel et al., 2016) but, apart from alertness, the three moods measured (good/bad mood, alertness/sleepiness, and calmness/agitation) differed from the six categories measured in this thesis, which reflected a more nuanced set of mood characteristics. The German groups' finding that oxytocin did not influence alertness was consistent with the findings in the equivalent male experiment of this thesis (Chapter 4).

However, the behaviour of the participants in the social study whose social conformity increased, suggested that mood was altered. One possibility is that the VAS and 5-point measures used do not ensure sufficient discriminative validity is recordable. Few studies have addressed oxytocin's effects on mood directly using comprehensive mood profiles and the results are, so far, inconsistent. In a study that examined oxytocin's effects on pain and mood, the shortened "Profile of Mood States" (Shacham, 1983) found that negative mood states were significantly lower after administration of oxytocin (Goodin et al., 2015). By contrast, oxytocin produced sex- and age-dependent effects using the "Trait Meta-Mood Scale" (Salovey, Mayer, Goldman, Turvey, & Palfai, 1995): mood scores in young women, but not men, improved after oxytocin, whereas older women's mood scores declined after oxytocin (Ebner, Natalie et al., 2015). One reason for this discrepancy may be that oxytocin's socio-affective influence is context dependent, but another explanation derives from instruments used to measure mood, which vary greatly to make comparisons across papers difficult.

In psychiatric disorders, research into oxytocin's effect on mood is more prolific and consistent in its findings than similar research in healthy populations. Oxytocin has been shown to stabilise mood and regulate emotions across a variety of neuropsychiatric conditions including schizophrenia, bipolar disorder, and autism (Averbeck, Bobin, Evans, & Shergill, 2012; Guastella & Hickie, 2016; Turan, Uysal, Asdemir, & Kılıç, 2013). Lower plasma oxytocin in depression has been found by a number of research groups (Bell, Nicholson, Mulder, Luty, & Joyce, 2006; Cyranowski et al., 2008; Scantamburlo et al., 2007; Zetzsche,

Frasch, Jirikowski, Murck, & Steiger, 1996), and oxytocin's lowering of depressive symptoms and promotion of mood improvements, particularly in postpartum women, is corroborated in different studies (Mah, Van Ijzendoorn, Smith, & Bakermans-Kranenburg, 2013; Scantamburlo, Ansseau, Geenen, & Legros, 2011; Scantamburlo, Hansenne, Geenen, Legros, & Ansseau, 2015; Slattery & Neumann, 2010). In preclinical disorders, oxytocin has also supported beneficial mood effects (Averbeck et al., 2012; Guastella & Hickie, 2016; Quirin, Kuhl, & Düsing, 2011; Turan et al., 2013). Oxytocin may, therefore, have a prophylactic role in preventing mood decline; this would account for enhancing mood effects not being detected by healthy participants. In line with this, self-rated happiness in the female snack-test experiment (Chapter 6) remained stable through the oxytocin sessions but declined in the control condition with placebo.

Linked to the development of mood disorders, gene variants in oxytocin have not been found to be associated with childhood-onset mood disorders (Strauss et al., 2010). Relatedly, oxytocin knockout—but not oxytocin receptor knockout—mice are more sensitive to exogenous oxytocin administration as demonstrated by increased grooming (Amico et al., 2004) and ICV oxytocin reverses impairments engineered by genetic alterations to the oxytocin system (Kublaoui et al., 2008). Taken together, this suggests that that individuals with low endogenous oxytocin levels, as may be the case in depressed individuals or populations with genetic oxytocin dysfunction, such as in Prader Willi syndrome, could be more sensitive to external oxytocin's modulation of mood than those with higher levels. However, as with other research into oxytocin's regulation of mood states, genetic research findings are preliminary, and cultural factors are thought to strongly influence phenotypical outcomes (Kim et al., 2011).

Experiments that have addressed states closely related to mood, reveal disparate findings. Stress is reduced by intranasal oxytocin leading to better emotional processing and increased trust in men (Kosfeld et al., 2005) and, as suggested in the discussion of Chapter 6, this may be related to reduced amygdala activity, but is not reflected in self-reported anxiety scores after oxytocin administration. Increases in ethnocentrism, outgroup hostility, envy, and gloating have all been observed in participants after administration of oxytocin (De Dreu et al., 2011; Pietrowsky, Braun, Fehm, Pauschinger, & Born, 1991; Shamay-Tsoory et al., 2009), reinforcing the importance of context and oxytocin's dual role as a "tend and defend" peptide. Another effect of oxytocin indirectly related to mood demonstrated in this thesis was in the group study (Chapter 8), where oxytocin increased ratings of sociability relative to placebo, consistent with oxytocin-induced increases in prosociality, but arising in groups organised by the researcher that began as strangers. However, though generalised effects on mood in healthy participants are not evident from oxytocin research so far, the research using this population is sparse and much more work on the influences of situational, intra- and inter-person contexts is required.

A key hub of the oxytocinergic circuitry involved in mood is the amygdala, a brain structure advanced as a substrate for human emotion (Domes et al., 2007; Huber et al., 2005; Schneider et al., 1997). However, evidence so far suggests that alterations to amygdala activation by oxytocin result in behavioural outcomes

but do not translate into changes to mood states (Domes et al., 2007) . These results are in line with the reporting in Chapter 4 of the inability of participants to guess whether they had received oxytocin or placebo, the undetectable VAS changes, and also accord with a review of oxytocin's safety that found that participants were unaware of intranasal oxytocin's effects, (MacDonald, E. et al., 2011). In laboratory experiments, the data so far suggest that oxytocin's behavioural effects occur outside of participant awareness.

10.6 The Influence of Participant Characteristics on Oxytocin's Effects

In the experiments reported here, oxytocin's effects on snack intake were associated with BMI in the male experiment but in the opposite direction to previous findings that obesity increased sensitivity to oxytocin (Thienel et al. 2016). However, the finding of Thienel et al. only related to hunger-driven eating, which this thesis was not examining, so the ability of oxytocin to preferentially inhibit the reward-seeking aspect of food intake in people with obesity remains unclear.

Low-trait anxiety as measured by the STAI was found in females who ate very little food in the pilot study (Chapter 5) and this was in line with research showing that women who were high cortisol responders ate more after a stress test than low cortisol responders (Epel et al., 2001). Conversely, trait levels of anxiety were negatively associated with food bias in the dot probe task (Chapter 7) such that individuals with higher stress levels paid less attention to the food pictures with oxytocin. This could suggest that high trait stress individuals employed avoidance when viewing food stimuli after oxytocin. In accordance with research showing that participants are unaware of the effects of oxytocin and the VAS data from this thesis, the STAI scores obtained in both conditions of the female snack-test study (Chapter 6) did not differ. The inclusion of stress tests in the female STS may have masked subjective awareness of anxiety reductions as the average anxiety levels were significantly higher than the mean. However, this would require experimental confirmation that a snack-test experiment without stress tests elicited a drop in STAI scores, and the VAS anxiety data from this male snack test experiment (Chapter 4) suggest participants do not report stress changes. In the online survey, trait anxiety was associated with the subscale "Persistent desire or repeated unsuccessful attempts to quit" but was not a statistical predictor of the overall propensity to overeat. A possible explanation might be that the ability to process situational or acute stress might have more predictive value than trait stress when assessing the drivers for overeating.

Brain imaging studies have shown that individuals reporting to be "in love" have greater activation of cerebral areas rich in oxytocin receptors (Aron, A. et al., 2005a; Bartels & Zeki, 2000; Carter, 1998; Fisher et al., 2005). Although romantic attachment was not associated with any experimental outcomes reported here, aspects of the procedure may have obscured the effects of potentially higher endogenous levels of oxytocin. Raised levels of endogenous oxytocin and other compounds, most notably phenylethylamine (Marazziti & Canale, 2004; Silverstone, 1992), may inhibit general appetite, and a reduced appetite for food might be most evident in appetite-driven consumption, not mainly hedonic snack consumption. All of the experiments required participants to consume food before testing, so the anorectic effects of endogenous oxytocin may

not have been evident. Furthermore, although snack-test intake did not correlate with self-assessed levels of romantic love, the method used to measure romantic attachment may have prevented this. The construct of romantic love is rooted in cultural and inter-individual factors and participants' scores on a questionnaire measuring love may not reflect the same universal concept. Aron et al. (2014) argued that early-stage romantic love captures a unique cross-cultural euphoria. However, participants in the Aron et al. (2005) study were in romantic relationships that ranged from a single month to seventeen months, so the temporal window, in itself, was insufficient to delineate early romantic love and a number of measures including a structured interview were used to define early romantic love instead. A common feature of intense new romantic relationships is the loss of appetite (Fisher et al., 2005), but the involvement of oxytocin in inhibiting appetite in this scenario remains hypothetical.

Age was not related to oxytocin's effects on eating in any of the experiments of this thesis. There is some evidence that sensitivity to oxytocin changes in old age (Sannino et al., 2017). In females, menopausal changes result in reductions of oestrogen and in males declines in testosterone and increases in oestrogen are seen in old age (Aulinas et al., 2018; Klump et al., 2012; Taylor et al., 2006); oestrogen-induced rises in oxytocin will change for both sexes, therefore, in old age. In this thesis, the upper bound of individuals participating in experiments was 52 years, which is below the threshold of old age, so the assessment of the role of old age in oxytocin's anorectic effects was not possible. At the other end of the age spectrum, levels of oxytocin in childhood are considered important and may be related to eating habits (Apter-L. et al., 2014; Heim et al., 2009; Wismer Fries et al., 2005), but this is beyond the scope of the present research.

Despite the preponderance of snack eating among females, it was male participants who ate more snack food in the laboratory (Chapters 4 and 6). Impression management concerns and societal expectations of female body size may have contributed to females eating less than males in this thesis. It is possible that the stressors used may not have invoked the type of ego-challenging or emotional stress that triggers eating (Lattimore, 2001). In the female pilot study (Chapter 5) where eating was difficult to provoke, impression management scores were low. However, in the same experiment, self-monitoring scores were above normative averages, but self-monitoring was not associated with snack intake in the female stress study (Chapter 6). The external eating component of the DEBQ also measures self-monitoring behaviour, but this was not related to snack intake in the female stress study either. The possibility that issues related to social desirability influenced the self-report measures was minimised in the female stress study as participants completed the scales online after the experimental surveys via a link and using a participant code. Alternatively, although the heart rate data showed that the stress tasks were effective, the stress tests may not have been of a sufficiently long duration to trigger measurable cortisol increases, which was a key factor identified in previous research as triggering non-deficit driven food intake (Epel, Lapidus, McEwen, & Brownell, 2001). In the Epel et al. study, stress eating was induced in women using the same serial subtraction task and by a public speaking task by Epel, Lapidus, McEwen, and Brownell (2001) similar to the singing task used in this PhD. However, the stress tasks in Epel et al's study spanned a much longer

period of 45 minutes than the stress tests in this experiment (Chapter 6), which lasted one minute each. A longer stress duration may have prompted even greater eating, perhaps instigated by cortisol release. In the research showing increased eating in females after stress (Epel, Lapidus, McEwen, & Brownell, 2001), it was only participants with high cortisol reactivity who were found to respond to the stress by eating. In the female experiment of this research, however, levels of cortisol were not associated with the amount of snack food eaten in the female experiment.

Neither measures of eating behaviour using the DEBQ nor parental bonding related to oxytocin's reductions of snack eating or food bias. Loneliness was a significant predictor of overall food "addiction" in the online survey (Chapter 9) but not related to food bias or oxytocin's reduction of it in the attention study (Chapter 7). The global construct of loneliness may not be sufficiently specific to explain its relationship to traits that predict food addiction. In other words, loneliness can result from a number of psychological states, such as depression, and although the general construct of loneliness was not associated with oxytocin's experimental outcomes, the subcomponents of loneliness, or the consequences of loneliness, such as boredom, may be more insightful. Similarly, levels of childhood neglect and their relation to the overeating components of food "addiction" in Chapter 9, but not to food bias, and whether food bias in a laboratory experiment reflects everyday eating habits is a question future research could address.

In summary, the findings of this PhD that oxytocin reduces eating which is predominantly hedonic in nature in both men and women has been demonstrated, alongside the reduction of food bias on a dot probe task. The percentage decrease in snack intake that oxytocin effected was sizeable, suggesting that oxytocin has potential as a therapeutic agent for overeating and obesity, currently intractable issues with no effective treatment presently available. Important methodological details in this set of thesis experiments may not only account for increased effects of oxytocin compared to similar studies but could provide a useful model for future eating research using oxytocin. Specifically, the components introduced in this thesis were enhanced concealment of experimental aims that included a rationale for eating lunch, calorie matching across snack foods, enhanced presentation of food, exclusion of participants on anti-depressants or on a hormone-based form of birth control and the collection of participants' characteristics that might relate to their endogenous oxytocin function.

10.7 Limitations and Future Directions

There are factors that contribute to appetite and oxytocin function that were not controlled in this thesis that might relate to the generalisability and clinical utility of oxytocin as an anorectic agent. Participants consumed food high in salt and sugar, and as glucose can promote diuresis in humans, in combination with salt, it is possible that osmolality was affected. High levels of glucose in the blood can lead to dehydration, which causes oxytocin release in rats, so anorectic outcomes in rodents cannot necessarily be attributed to the straightforward effects of oxytocin on reward-driven eating (Ohnishi, Horan, Levin, & Levin, 1999). In this thesis, water was made available in all the snack-test experiments, and fruit juice was provided in the

attention study. On the basis that subjective thirst ratings were not high and individuals who felt thirsty would drink, thirst was not considered an important influence. However, dehydration leads to appetite loss and changes in preference for salty food (Rowland, 2002) and since osmoregulation in humans is controlled by oxytocin's sister compound vasopressin that stimulates natriuresis and oxytocin has a weak affinity at vasopressin receptors (Robertson, 2001), research into the reciprocal relationship between oxytocin and hydration is necessary.

There are additional translational difficulties between rodent and human studies that procedural changes in future research could address. Franchini, Rubinstein and Vivas (2003) showed that β-endorphins, which can be released following palatable food ingestion, influence brain oxytocin systems. However, oxytocin released by endorphins may not be anorectic, given oxytocin's diverse effects. In the study of pain management, the release of oxytocin as an endogenous pain control agent has been demonstrated, and future research should explore this element in food studies. Acute stress can result in the adrenal glands producing aldosterone, which is known to reduce sensitivity to leptin and stimulate appetite (Solano & Jacobson, 1999). The release of appetite-stimulator aldosterone from acute stress might confound the effects of oxytocin but, conversely, decreased aldosterone can lead to the loss of sodium ions, which would affect rodents differently from humans (Ang & Jenkins, 1984). Decreased aldosterone also leads to the depletion of adrenal glucocorticoid hormones such as cortisol, leading to decreased gluconeogenesis, rapid hypoglycemia, and potassium retention (Ang & Jenkins, 1984; Diorio, Viau, & Meaney, 1993; Frankenhaeuser & Lundberg, 1985; Nielsen et al., 2004) and accounting for its effects in rodent oxytocin research might be important.

The gut microbiome has been implicated in appetite control and food intake (Cani et al., 2009; Fetissov, 2017; Holzer & Farzi, 2014; van de Wouw, Schellekens, Dinan, & Cryan, 2017) and the development of obesity (Poutahidis et al., 2013a). In addition, the bacteria Lactobacillus reuteri ATCC-PTA-6475 upregulates central oxytocin in mice (Poutahidis et al., 2013b). The importance, therefore, for clinicians to understand the role that the microbiome plays in the control of oxytocin synthesis is twofold. First, if central oxytocin can be elevated by Lactobacillus reuteri ATCC-PTA-6475 itself or a human gut equivalent, then this may provide a viable alternative to exogenous oxytocin that is potentially available through diet, prebiotics or probiotics. Second, emerging evidence suggests that peripheral oxytocin does affect central function, yet no explanation of how peripheral oxytocin might cross the BBB has been accepted. The afferent vagal feedback route that Lactobaceillus reuteri ATCC-PTS-6475 is known to use might provide an explanation for the efficacy of exogenous oxytocin that might lead to successful oral administration of the peptide. Genetic polymorphisms of the oxytocin receptor are associated with disordered eating, susceptibility to stress, depression, and emotionality (Feldman et al., 2012; Gimpl & Fahrenholz, 2001; Skuse et al., 2014). Moreover, experimental oxytocin KO mice show hyperphagia and an increased meal size (Leng & Ludwig, 2008; Olson et al., 1991; Swaab et al., 1995). In order to explain some of the variance in oxytocin's anorectic effects and refine the target populations who overeat and would most benefit from a potential oxytocin treatment, more genetic work is needed.

There are compounds within cocoa that could potentially suppress or mask oxytocinergic actions, thereby undermining its clinical usefulness. Cocoa contains both theobromine and theophylline, which are methylxanthines and act as nonselective antagonists for adenosine (Smit, 2011; Spiller, 1997). The stimulatory effects of these methylated xanthines increase alertness and heart rate (Smit, 2011), so could potentially confound oxytocin's anxiolytic effects. Additionally, chocolate is not enjoyed by everyone and responses to chocolate recruit different brain regions depending on whether participants are satiated or rate the chocolate as pleasant (Small, Zatorre, Dagher, Evans, & Jones-Gotman, 2001). The neural substrates supporting oxytocin's anorectic effects are largely unexplored in the brain imaging literature. However, one *f*MRI study distinguished different regional responses to a monetary reward task and to passive viewing of palatable food pictures after administration of 24 IU of intranasal oxytocin in fasted participants (Spetter et al., 2018). In the same study, though, no macronutrient-specific effects or sated-state effects of oxytocin on food intake or brain responses were found. Considering the differential brain responses to chocolate in the sated and fasted states, a snack test that avoided this confound might reveal differences in imaging studies. Future experiments could also examine the potential differences in effects of oxytocin among participants who rate the chocolate as pleasant and those who do not. Examining sweet food other than chocolate would be a logical next step, but the generalisability of oxytocin's anorectic effect to a wide range of foods might depend on the food, and this issue has important clinical implications.

Natural endocannabinoids, such as anandamide have been identified in cacao and linked to feeding behaviour, motivation, pleasure, and obesity (Maccarrone, 2017; Mahler, Smith, & Berridge, 2007; Nehlig, 2013; Osei-Hyiaman et al., 2005; Pacher, Batkai, & Kunos, 2006). Interestingly, Δ9-tetrahydrocannabinol (THC)-induced feeding is not blocked by the administration of oxytocin and chronic exposure to THC downregulates the transcription of oxytocin-neurophysin mRNA and expression of oxytocin in the VTA and NAc (Butovsky et al., 2006; Verty, McFarlane, McGregor, & Mallet, 2004). Experiments co-administering the two compounds could help to uncover whether oxytocin's actions in the VTA and NAc, which are blocked by THC, is the factor that prevents its anorectic actions. Further research on the interactions between the two cannabinoid and oxytocin systems that independently modulate feeding would, therefore, provide a valuable insight into oxytocin's mechanism of action.

Oxytocin did not affect all participants within the experiments of this thesis, and one possibility is that the absorption of oxytocin may have varied across participants. The pharmacokinetics of oxytocin are poorly characterised and a number of unexplored factors germane to this thesis could affect the absorption of oxytocin via intranasal routes. Head position, nasal cavity capacity, and mucocilliary clearance rates all influence absorption of oxytocin. Bowman's glands secrete water, mucins, and proteins from blood to the olfactory epithelium's surface, which protect against dehydration and infection, the quantity of liquid produced is likely to vary among individuals, which influences the absorptive capacity (Solbu & Holen, 2011). Vomeronasal chemoreceptors are potential targets for intranasal oxytocin transmission and have been

identified in the olfactory epithelium of the nose, and recent research from Zanos (personal communication, 2018) has identified amygdala activation via peripheral administration, reopening the debate on the capacity of oxytocin to cross the BBB. The integrity of the BBB in individuals is likely to vary considerably, too. Illness, eating disorders, addiction (Kovacs, 1986) and common over-the-counter medications such as Sildenafil (Viagra®) all cause changes in BBB permeability (see Chapter 1 for further examples), which in turn influence the central impact of exogenous oxytocin.

The pharmacodynamics of oxytocin are not well understood. For example, certain doses of oxytocin have no anorectic effect in rats who have fasted for six hours but reduce eating after 21 hours of fasting (Blevins et al., 2004), which may be explained by a moderating effect of leptin (Ahima et al., 1996). and explanations for the dose-dependent effects of oxytocin, therefore, are limited. A similar pattern in human studies has been found where 24 IU administered after a 14.5 hour overnight fast failed to inhibit eating (Ott et al., 2013), but inhibited food intake after a 12-hour fast in a separate study (Lawson et al., 2015). However, in a replica study of the Ott et al. protocol, 24 IU of oxytocin did reduce hunger-driven eating (Thienel et al., 2016), suggesting fasting length alone may be insufficient to explain whilst also not ruling out a role for oxytocin-leptin interactions. Further research is clearly needed to unpick the relative contributions of hunger and body mass on oxytocin's effectiveness.

Understanding the pharmacodynamics of oxytocin would help to explain the dose-dependent effects of oxytocin (Björkstrand et al., 1997), which have not yet been explored in relation to eating. In psychopathology, comparing oxytocin's effects across different psychiatric conditions may not be a valid objective as aetiology and symptoms across psychiatric categories are not equivalent, and some work with different doses of oxytocin has been carried out showing that the effective dose varies within the same psychiatric or research domain. In humans, for instance, doses of 32 IU in people with high-functioning autism are effective whereas lower doses are not (Kosaka et al., 2016), and in people with schizophrenia 20 IU, but not 10 IU, improved facial recognition capability (Goldman, Gomes, Carter, & Lee, 2011). In depression, work with people who have treatment-resistant depression has shown that 16 IU daily as an adjunctive to an antidepressant medication was effective at reducing depressive symptoms (Scantamburlo, Hansenne, Geenen, Legros, & Ansseau, 2015) but 24 IU did not reduce depression in post-natal women although the parent-infant relationship improved (Mah, Van Ijzendoorn, Smith, & Bakermans-Kranenburg, 2013).

Widely varying amounts of the peptide are used across the oxytocin eating-focussed literature (Leslie et al., 2018). Although intranasal oxytocin doses are not standardised, 24IU is the most frequently used dose in research (Guastella et al., 2013). Furthermore, up to 40 IU was found to be undetectable by participants (Huffmeijer et al., 2012; MacDonald, E. et al., 2011) and 480 IU daily across six weeks had no negative side effects (den Boer & Westenberg, 1992). The posterior pituitary gland of an adult human is estimated to contain 14 IU of oxytocin (Heller & Zaimis, 1949) and doses in excess of this amount are considered

supraphysiologic and quantities less than this may not have central effects (Lawson, 2017; Lee et al., 2018). In this thesis, the standard dose of 24 IU was consistently applied and achieved significant effects in every experiment. However, a meta-analysis of studies examining oxytocin's effects on eating in animals and humans, found a significant effect of dose (Leslie et al., 2018) reinforcing the relevance of further dose-dependent research. Oxytocin is a partial agonist at the vasopressin receptor (Chini & Manning, 2007) and further research is needed to separate the effects of the two sister compounds of oxytocin and vasopressin. However, oxytocin and vasopressin receptor distributions differ. The circumstances and ramifications of cross activation between oxytocin and vasopressin neurons is not known, and experiments still report that the neuropeptides activate distinct neuronal populations (Huber et al., 2005).

The oxytocin literature is inconsistent in its measurement of plasma and CSF oxytocin, and the relationship between the two. The disagreement between CSF and plasma oxytocin is also evident in tonic release. The circadian release of CSF oxytocin is highly organised and reliable with peaks in the early light phase and lows midway through the dark cycle (Van Ijzendoorn et al., 2012). Plasma oxytocin does not have the same consistent release schedule and one study found that its nadir in plasma was from 8 am to 4 pm, the opposite to CSF oxytocin (Landgraf, Hacker, & Buhl, 1982). Radioimmunoassay and enzymeimmunoassay techniques result in vastly different concentrations of oxytocin (see Chapter 1), and it is likely that the elevated oxytocin quantities found using enzymeimmunoassay result from that procedure's sensitivity to oxytocin degradation products. New proteomics methodology has found plasma oxytocin levels up to 1000 greater than traditional assay techniques, as it also measures protein-bound oxytocin (Brandtzaeg et al., 2016). However, the relevance of protein-bound oxytocin is not yet known. CSF oxytocin does not correlate with plasma oxytocin (see Chapter 1) and oxytocin quickly broken down by oxytocinases and has a half-life of only 2-3.2 minutes (De Geest, Thiery, Piron-Possuyt, Driessche, 1985; Fabian, Forsling, Jones, & Pryor, 1969; Veening & Olivier, 2013), yet many experiments (e.g. Lawson et al., 2013) form conclusions on the basis of blood oxytocin sampling. A related problem is that estimates of the apparent volume of oxytocin distribution that are based on CSF proxy are invalid, as ICV CSF clearance of oxytocin does not occur at the same rate as the rapid plasma clearance. More work is urgently needed to resolve these issues for example by measuring plasma and CSF in parallel in experiments to ascertain the relationship between the two and by standardising oxytocin assay procedures.

Although aberrant eating habits have serious consequences, stress-eating is more widespread, and can lead to obesity and related disease. The findings of this thesis suggest that cortisol may not be associated with oxytocin's reductions of palatable food intake, suggesting that a future extension to this thesis would be to examine whether anxiolysis via amygdala or serotonergic routes supports oxytocin's reductions in food consumption. Brain imaging could be incorporated into the protocol of a snack-test study to check the profusion cerebral blood flow in the amygdala, hypothalamus, and midbrain regions to distinguish among potential stress-reducing capacities of oxytocin. The dual anorectic-anxiolytic effects of oxytocin could be assessed in animal models using 5-HT antagonists alongside oxytocin, to record palatable food intake in the

fed state.

The last main area of future research suggested by this thesis is in understanding the effects of context on oxytocin's hypophagic effects, since fine-grained procedural differences are likely to have important consequences for clinical application. The anorectic effect of oxytocin is broadly consistent in the laboratory, but the interpretational complexities of varying procedures, populations, foods, and doses are hard to unpick, and standardising procedures could help. Factors such as the diurnal fluctuations of oxytocin, and oxytocin's feed-forward action are likely to mean the timing of experiments is an important consideration, and this is yet to be addressed (Perlow et al., 1982; Van Ijzendoorn, Bhandari, Van der Veen, Grewen, & Bakermans-Kranenburg, 2012). A series of elimination experiments using the same protocol, which alters one element in each replication (e.g. dose, time of day, food, participant characteristic), will advance the understanding of context to be attained. Given oxytocin's inconsistent success as a replacement therapy in hyperphagic Prader Willi children and in lactating mothers, measuring context-dependent effects would be valuable, and maximise its therapeutic potential beyond hormone replacement.

10.8 Conclusion

In addition to the drive to eat to maintain metabolic and nutritional homeostasis, animals and humans are motivated to eat for pleasure. This thesis has shown for the first time that oxytocin inhibits eating that is not likely to be deprivation induced. Given that oxytocinergic activity in the reward system has also been implicated in reward reductions for drugs of abuse, the findings of this thesis could suggest that intranasal oxytocin reduces the reward for palatable food via similar central mechanisms. Also for the first time in humans, oxytocin has been demonstrated to reduce palatable food intake in individuals without deficit-driven hunger. In this cohort, oxytocin's anorectic actions were achieved without concomitant reductions of cortisol, implying that attenuation of food intake was not due to HPA axis inhibition. Attentional bias to food was reduced by oxytocin, a finding that could also support a reward-related shift away from food stimuli. The final experiment assessed the impact of oxytocin on palatable food intake in a group setting and demonstrated an increase in social modelling of eating behaviour after oxytocin in the absence of an overall anorectic effect of oxytocin. Finally, alongside the core experimental studies of this thesis, an online survey found that overeating was predicted by loneliness and parental neglect during childhood, two domains which relate to sub-optimal endogenous oxytocin function.

The reward-limiting properties of oxytocin on palatable food consumption demonstrated in this PhD combined with its physiological anorectic effect, its attentional modification, and its prosocial enhancement, make oxytocin a promising agent for the treatment of disordered overeating, which has no consistently effective clinical treatment to date. Disorders such as compulsive eating or bulimia can result in long-term changes to mesolimbic reward pathways in much the same way as substance abuse, making their abolition difficult to achieve. Future research should address the effectiveness of oxytocin in treating compulsive eating in placebo-controlled trials that link outcomes to oxytocin genotypes across a range of foods.

Confirmatory fMRI studies that assess potential reward-reducing effects of oxytocin on food in target populations using food and non-food images, together with follow-up food-based experiments would also be helpful in distinguishing whether oxytocin reduces meal duration and inhibitory processes to terminate eating. These brain imaging results would help to unpick the opposite effects of oxytocin in people with anorexia and obese populations, and support oxytocin's potential as a method of restoring healthy eating in these groups.

Notwithstanding the inherent complexities in the interactions of the oxytocin molecule within the human body, this thesis has elucidated novel functions in relation to non-deprivation induced hunger and oxytocin's key role in appetite modification in humans. Oxytocin's potential for alleviating some of the world's most debilitating, and personally and economically damaging conditions is becoming clear. This thesis has suggested future avenues for research, whose attendant scientific and commercial potential is becoming abundantly clear.

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APPENDICES

Appendix I – Nasal Spray Experience

The following rating scales consist of a line with two end points. The line represents a continuum of possibilities between two statements. Above the line will be a rating question. Please mark a cross on the scale at the place that best describes your answer.

Participant no. _____

1. Do you feel in a good mood today?

Not at all ——————————— Very

2. Do you feel ill (headache, cold, blocked nose, stomach ache, etc.)?

Not at all ——————————— Very

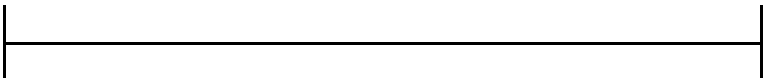
3. How comfortable was the nasal spray?

Not at all ——————————— Very

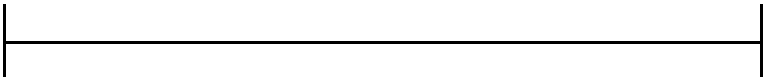
4. Did the nasal spray irritate your nose?

Not at all ——————————— A lot

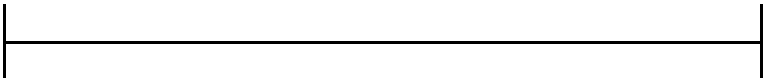
5. How pleasant was the taste of the nasal spray?

Not at all  Very

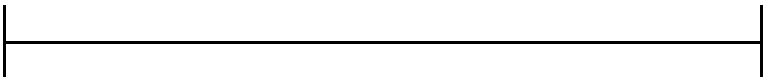
6. Was the nasal spray itchy?

Not at all  Very

7. How easy was the nasal spray pump?

Not at all  Very

8. Did your nose drip after inhaling the nasal spray?

Not at all  A lot

9. Did you feel liquid flowing through your throat?

Not at all — A lot

10. Does your throat feel irritated?

Not at all — Very

11. Is your appetite negatively affected?

Not at all — Very

12. How pleasant was the overall experience?

Not at all — Very

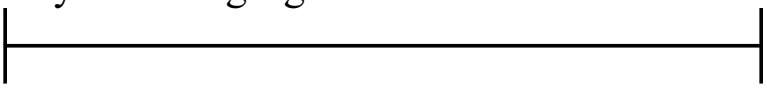
13. How stressful was the overall experience?

Not at all — Very

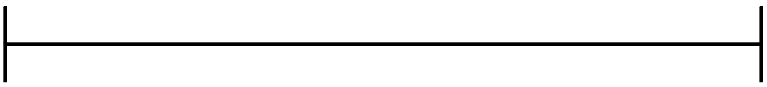
Appendix II – Mood Questionnaire

The following rating scales consist of a line with two end points. The line represents a continuum of possibilities between two statements. Above the line will be a rating question. Please mark a cross on the scale at the place that best describes your answer.

1. How happy are you feeling right now?

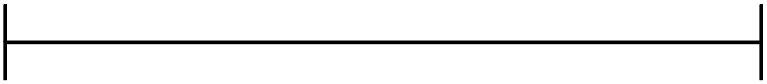
Not at all  Extremely

2. How excited are you feeling right now?

Not at all 

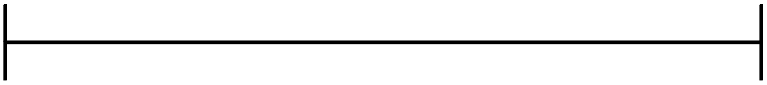
Extremely

3. How anxious are you feeling right now?

Not at all 

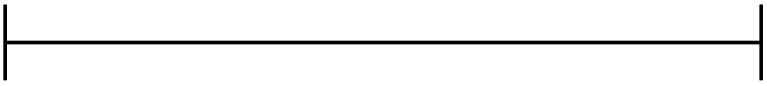
Extremely

4. How hungry are you feeling right now?

Not at all 

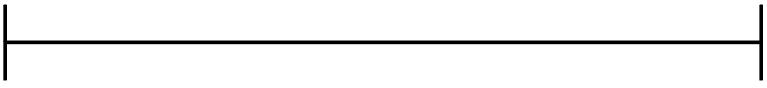
Extremely

5. How thirsty are you feeling right now?

Not at all 

Extremely

6. How alert are you feeling right now?

Not at all  Extremely

Appendix III – Examples of Stimuli in the Attention Study

1. Food Picture matched with neutral object (2)

2. Neutral object matched to food picture (1)

3. Romantic picture

4. Social Picture

Appendix IV – Supplementary Dot Probe Data

Means (SD) in ms for Congruent Probes Associated with Food, Romantic, Social, and Neutral Stimuli in the Dot Probe Task

	Placebo		Oxytocin	
	M	*SD*	*M*	*SD*
Food (vs Romantic)	490.31	44.06	562.80	30.81
Food (vs Social)	486.97	39.12	567.73	36.29
Food (vs Neutral)	487.74	42.35	567.58	30.21
Food (all)	488.66	39.75	565.67	27.84
Romantic (vs Social)	528.07	28.50	530.83	14.58
Romantic (vs Food)	532.60	25.23	566.83	29.76
Romantic (all)	530.34	24.16	529.81	10.44
Social (vs Romantic)	529.19	12.86	524.73	16.93
Social (vs Food)	526.82	13.37	523.76	19.18
Social (all)	528.00	8.64	524.24	16.02
Neutral (vs Food)	531.46	25.59	552.30	38.74
Neutral (vs Neutral)	531.82	32.84	553.85	43.32
Neutral (all)	531.97	23.83	553.12	36.92

Means (*SD*) Response Times of Food and Neutral Picture Probes by Gender in Dot Probe Task

	Neutral				Food			
	Male, n = 9		Female, n = 29		Male, n= 11		Female, n = 29	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Placebo	486.77	40.62	488.12	43.69	537.45	28.24	529.23	22.45
Oxytocin	574.68	28.12	564.89	31.01	541.43	42.37	556.16	38.58

Means (*SD*) of Questionnaire Data in the Dot-Probe Cohort

	M	*SD*
STAI-trait	46.23	5.01
BMI	23.73	4.39
Loneliness	37.68	9.57
PBI Care-Neglect	49.88	15.75
PBI Overprotection	29.63	15.05
Romantic Love	77.53	17.22
FCQ – total	147.60	28.29
FCQ – subscale 1 (intentions)	13.18	3.32
FCQ – subscale 2 (positive reinforcement)	22.58	4.86
FCQ – subscale 3 (relief from negative affect)	9.28	3.38
FCQ – subscale 4 (lack of control)	20.50	6.49
FCQ – subscale 5 (preoccupation with food)	20.95	7.15
FCQ – subscale 6 (as result of physiological need)	21.80	3.67
FCQ – subscale 7 (emotions connected to it)	11.45	4.01
FCQ – subscale 8 (guilt from craving/giving in to craving)	13.23	4.12
FCQ – subscale 9 (cues from environment)	13.25	4.11
DEBQ - Restrained	3.56	1.30
DEBQ - External	1.88	0.82
DEBQ - Emotional	1.95	0.75

Appendix V - Frequencies of Other Nationalities in Online Survey

Nationality	Frequency
Argentina	2
Australia	5
Belgium	1
Bulgaria	2
Canada	2
Eritrea	2
Germany	1
Greece	2
Guinea	1
India	3
Iran	1
Iraq	1
Italy	4
Jamaica	1
Jordan	1
Latvia	1
Madagascar	1
Mexico	1
Monserrat	1
The Netherlands	2
New Zealand	1
Nigeria	1
Pakistan	2
Poland	2
Portugal	2
Russia	3
Saudi Arabia	1
South Africa	2
South Korea	1
Sri Lanka	3
Sweden	1
Switzerland	1
Turkey	1

Uganda	1
UAE	1
Zimbabwe	1

Appendix VI Covert Screening for Stress Eaters

Appendix VI – Screening Questionnaire in Female STS

Stress Responses

Please rate your top three active responses to each of the six situations below or tick none of the options.. 1 = most likely

	Sleep	*Disturbed sleep*	*Exercise*	*Chat with someone*	*Work/study*	*Eat*	*Reduced eating*	*Smoke/vape*	*Read/Watch TV or film*	*Cry*	*Alcohol*	*Let your hair down*	*Drugs of abuse*	*None of the options*
1. Feeling lonely														
2. Bereavement														
3. Exams														
4. Anger directed towards you														
5. Romantic relationship breakdown														
6. Feeling depressed														

Appendix VII - Pearson Product Correlations among Predictors of Total YFAS Score

	Overeating	Activity Level	Average Sleep	Loneliness	Parental Neglect	Parental Overprotect.	Anxiety
Overeating	1.00						
Activity Level	0.15*	1.00					
Average Sleep	-0.05	-0.11	1.00				
Loneliness	0.56***	0.13	-0.20	1.00			
Parental Neglect	0.23***	0.16	0.06	0.18	1.00		
Parental Overprotection	0.08	0.03	0.07	0.08	0.38	1.00	
Anxiety	-0.21**	0.07	0.02	-0.36	-0.12	-0.08	1.00

Note. * $p < .05$, ** $p < .01$, *** $p < .001$

Appendix VIII - Pearson Product Correlations Among VAS Variables in Nasal Spray Pilot Study

	LE	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13
Lunch Eaten (LE)	1.00													
1. Do you feel in a good mood today?	0.01	1.00												
2. Do you feel ill (headache, blocked nose, stomach...)?	0.03	0.14	1.00											
3. How comfortable was the nasal spray?	0.37	-0.17	-0.25	1.00										
4. Did the nasal spray irritate your nose?	0.26	-0.19	-0.08	-0.08	1.00									
5. How pleasant was the taste of the nasal spray?	0.23	-0.50	-0.25	0.65***	-0.01	1.00								
6. Was the nasal spray itchy?	0.19	0.11	0.14	-0.26	0.11	-0.12	1.00							
7. How easy was the nasal spray pump?	0.44*	0.09	0.11	0.32	0.35	0.24	0.12	1.00						
8. Did your nose drip after inhaling the nasal spray?	-0.21	0.30	0.40	0.07	-0.04	-0.38	-0.04	0.15	1.00					
9. Did you feel liquid flowing through your throat?	-0.34	-0.15	0.52*	-0.44	-0.32	-0.23	0.19	-0.43*	0.36	1.00				
10. Does your throat feel irritated?	0.11	-0.17	-0.21	0.47*	-0.12	0.18	0.19	-0.06	0.17	-0.04	1.00			
11. Is your appetite negatively affected?	-0.76***	0.06	0.02	-0.06	-0.33	-0.14	-0.08	-0.21	0.38	0.11	0.00	1.00		
12. How pleasant was the overall experience?	0.25	0.15	0.26	0.34	-0.43*	0.01	-0.34	-0.06	0.04	-0.06	0.20	-0.11	1.00	
13. How stressful was the overall experience?	-0.65***	0.32	-0.20	-0.49*	-0.11	-0.48*	0.04	-0.05	0.40	0.19	-0.14	0.46*	-0.46*	1.00

Note. * $p < .05$, ** $p < .01$, *** $p < .001$

Appendix IX

Mean (*SD*) VAS Ratings (mm) for Snack Food in Oxytocin and Placebo Conditions for Stress and Non-Stress Eaters

	Placebo			Oxytocin		
	Stress Eater	Non-Stress Eater	Total	Stress Eater	Non-Stress Eater	Total
Chocolate Biscuits	79.33 (12.64)	76.30 (12.98)	77.5 (12.76)	73.87 (17.12)	80.30 (10.13)	77.76 (13.50)
Salty Crackers	64.60 (18.37)	50.04 (18.40)	55.79 (19.52)	61.60 (16.26)	52.78 (17.58)	56.26 (17.41)
Bland Oatcakes	38.27 (26.11)	32.74 (20.05)	34.92 (22.46)	35.33 (25.26)	39.13 (27.77)	37.63 (26.53)

Note. * $p < .05$, ** $p < .01$, *** $p < .001$