OXYTOCIN AND GENETIC LINKS WITH EATING BEHAVIOURS AND RELEVANT ENDOPHENOTYPES

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ABSTRACT

Oxytocin (OXT) is an ancestral neuropeptide hormone essential in the regulation of human behaviours vital for sustaining life such as food intake and reproduction. Importantly, these behaviours have strong associations with the brain's reward circuitry. To date, there has been little OXT-genetic research in the field of eating behaviors. However, a recent study by Davis et al. (2017) identified several single nucleotide polymorphisms (SNPs) on the OXT receptor gene (*OXTR*), associated with overeating and relevant behaviours. The present study expanded on this work by analyzing SNPs of the *LNPEP, CD38,* and *OXTG* genes. Additionally, sex differences were also examined. A pre-existing data set consisting of 460 healthy volunteers was used in the study. A series of two-way MANOVA's were employed and results indicated nonsignificant main effects for genotype on all 12 SNPs. However, multivariate tests for sex were significant in each analysis. Possible explanations for the current findings are discussed.

DEDICATION

To my father,
Ronald Edward Shepherd.

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CHAPTER ONE: INTRODUCTION

1.1 Rational and Purpose

Oxytocin (OXT) is a well preserved ancestral neuropeptide hormone which has adapted and evolved with the changing environment (Feldman, Monakhov, Pratt, & Ebstein, 2016; Gimpl, Fahrenholz, & Gene, 2001). Although OXT is well known and established for its role in child birth and lactation, current research has also identified its role in regulating social and survival behaviours vital for sustaining life such as social affiliation, empathy, attachment, pair-bonding, and food intake – and their important role in stress reduction (Davis, Patte, Zai, & Kennedy, 2017; Fineberg & Ross, 2017; Lee, Macbeth, Pagani, & Scott Young, 2009; Olszewski, Klockars, & Levine, 2016). Importantly, these behaviours also have strong associations with the brain's reward circuitry (Davis et al., 2017; Ott et al., 2013; Spetter & Hallschmid, 2017).

Both animal and human research has provided good evidence that increases in OXT have anorexigenic properties, especially for sweet carbohydrates (Leng & Sabatier, 2017; Olszewski et al., 2016; Olszewski, Allen, & Levine, 2015; Spetter & Hallschmid, 2017). This suggests that low basal OXT levels may foster increased food consumption (Davis & Moghimi, 2017; Spetter & Hallschmid, 2017). Employing diverse methodologies such as intranasal administration, imaging genomics, receptor autoradiography, and pharmacology, research is developing an understanding of the extent to which OXT regulates eating behaviours (Ebstein, Knafo, Mankuta, Chew, & Lai, 2012; Gimpl et al., 2001; Lee et al., 2009; Lockard, Ebert, & Bargmann, 2017). However, much of what has been learned about the functions of OXT has derived from *exogenous* manipulation of OXT levels and animal-model experimentation (Lockard et al., 2017;

Spetter & Hallschmid, 2017). As such, in order to understand better the *endogenous* levels of OXT, research has begun to investigate genetic markers of the OXT system (Davis et al., 2017; Ebstein et al., 2012; Hovey et al., 2014).

To date, however there has been little of such research in the field of eating behaviours (Davis et al., 2017). A recent study from our laboratory identified several single nucleotide polymorphisms (SNPs) on the oxytocin receptor gene (*OXTR*), which were associated with overeating, and endophenotypes of overeating such as reward sensitivity, punishment sensitivity, and food preferences (Davis et al., 2017; Davis, Zai, Adams, Bonder, & Kennedy, 2019). The primary purpose of the present study is to expand on this work by analyzing SNPs of the *LNPEP, CD38,* and *OXTG* genes – all of which contribute to the management of OXT availability in the brain. Such information will contribute to a better understanding of neural targets of over-consumption and addictive tendencies towards highly palatable foods.

The present study involves the secondary analysis of a pre-existing data set for which funding was obtained recently to do additional genotyping of OXT genetic markers from DNA obtained in an earlier study (see e.g. Davis et al., 2009; Davis et al., 2008). A sample of 460 healthy community-based adults ranging from 24-50 years of age and representing a broad range of BMI values were recruited for the original study. The current study is entirely exploratory. While many studies have identified OXT markers and their association with prosocial behaviours such as social affiliation (Chong et al., 2017; Feldman et al., 2016; Lerer et al., 2010), empathy (Feldman et al., 2016; Kasperek-Zimowska, Zimowski, Biernacka, Kucharska, & Rybakowski, 2016), social recognition (Higashida, Yokoyama, Kikuchi, & Munesue, 2012), and pair-bonding (Ebstein et al., 2012), none has investigated links between genetic markers of the *OXTG, CD38,* and the *LNPEP* genes and overeating. This study represents the first attempt to examine *OXTG,*

CD38, and *LNPEP* SNPs in relation to overeating, reward sensitivity, punishment sensitivity, and food preferences.

1.2 Review of the Literature

1.2.1 Evolution and Oxytocin

Charles Darwin is renowned for his contribution to the science of evolution by his theory of natural selection (Dimech, 2017; Hill & Newlin, 2002). Darwin's theory arose from observations he made while travelling around the coast-line of South America aboard the H.M.S. Beagle (as cited in Johnson, 2018). For 5 years, Darwin collected fossils and made observations regarding species and their natural habitat. He concluded that, in response to changing climate, trait and behavioural variations unique to each species, and that serve to support their survival, continuously adapt and gradually reform overtime (Beatty, 2016; Dimech, 2017; Hill & Newlin, 2002; Johnson, 2018; Lockard et al., 2017). Specifically, the genotypes that best fit particular environments have been inherited over generations from reproduction success ensuring the survival of species, while eventually replacing the weakest fit genotypes (Beatty, 2016; Dimech, 2017; Hill & Newlin, 2002; Packard & Delafield-Butt, 2014; Partridge, 2018). This provided the foundation that sparked the interest of researchers to investigate the theory in order to further understand the process and the extent in which variations of traits and behaviours are inherited.

Following years of research, it has emerged that mammals have been evolving for at least 600 million years and this process has resulted in the survival of thousands of species as a result of adaptations to their changing environment (Beatty, 2016; Feldman et al., 2016; Lockard et al., 2017; Partridge, 2018). However, it was the discovery of genetics and DNA that ultimately allowed researchers to further understand the process of inherited genotypes (Partridge, 2018). What is most imperative for survival, are the innate behaviours that are hard-wired in all species,

such as feeding and reproductive, and what is interesting is the significant role OXT plays in regulating all survival behaviours (Lockard et al., 2017). OXT has been implicated in brain development and plasticity, and it assists in shaping pathways within the brain which are crucial for brain growth (Feldman, 2017; Theofanopoulou, 2016). As such, there is now good evidence that OXT is a central regulator of all the basic human behaviours vital for sustaining life, such as pair-bonding, reproductive behaviours, attachment, prosocial behaviours, learning, memory, feeding behaviours, and stress reduction (Feldman et al., 2016; Lockard et al., 2017). In other words, this neuropeptide is acknowledged as one of the vital hormones supporting the process of evolution, and making possible the survival of species over centuries (Feldman et al., 2016).

Not surprisingly, the behaviours described above have strong associations to the brain's reward circuitry, which has served as a vital survival mechanism throughout evolution (Carter, 2014; Damiano et al., 2014; Davis, 2014; Feldman, 2017; Kirsch, 2005; Lee et al., 2009; Lockard et al., 2017; Ranaldi, 2014). The reward system regulates the human drive to engage in activities that are life-sustaining such as eating, drinking, and reproductive behaviours (Carlier, Marshe, Cmorejova, Davis, & Müller, 2015; Davis, 2014; Sullivan, Hagen, & Hammerstein, 2008). The hedonic impact of an environmental stimulus is a result of neural mechanisms that are interconnected and responsible for: incentive salience (e.g., craving for a reward); associative learning (e.g., positive reinforcement); and positive emotions, specifically the ones that involve pleasure (e.g., joy and euphoria) (Carlier et al., 2015; Davis, 2014; Sullivan et al., 2008). The reward pathway is largely regulated by the dopamine neurotransmitter, whose activation is also signalled by other neurotransmitters and other hormones like OXT (Damiano et al., 2014; Ranaldi, 2014). It is well-established that dysregulation in the processing of the brain's reward

circuitry can increase the risk for conditions such as post-traumatic stress disorder (PTSD), addiction disorders, and eating disorders (Nawijn et al., 2016; Wu et al., 2016).

1.2.2 Molecular Structure and Function of Oxytocin

OXT was the first neuropeptide to have its molecular structure established (Gimpl et al., 2001). OXT is a nine-amino acid neuropeptide that functions either as a neurohormone, neurotransmitter, or neuromodulator, and is primarily synthesized within two areas of the hypothalamus: the paraventricular (PVN) nucleus and the supraoptic (SON) nucleus (Barengolts, 2016; Chaves, Tilelli, Brito, & Brito, 2013; Kiss & Mikkelsen, 2005). The nine-amino acid is composed of Cysteine-Tyrsine-Isoleucine-Glutamine-Asparagine-Cysteine-Proline-Leucine-Glycinamide (Vrachnis, Malamas, Sifakis, Deligeoroglou, & Iliodromiti, 2011). OXT is very similar to vasopressin (VP), differing by only two amino acids, and genes for both neuropeptides are found on the same chromosome (Stoop, Hegoburu, & van den Burg, 2015). It is interesting to note that OXT and VP originated from a single ancient peptide until a gene duplication event occurred (Feldman et al., 2016; Lockard et al., 2017; Stoop et. al, 2015). Gene duplication is described as a process whereby new genetic material is produced to support the enhancement of evolutionarily important survival functions (Feldman et al., 2016).

OXT is stored in the pituitary gland where it awaits activation by its receptor and then it is secreted in circulation in response to various stimuli (Barengolts, 2016; Gimpl et al., 2001; Kasperek-Zimowska et al., 2016; Uvnäs-Moberg, 1998). Although OXT is released by the pituitary gland, due to the blood-brain barrier, it is impossible for OXT to re-enter the brain (Leng & Ludwig, 2016). As such, there are also neurons in the PVN that project OXT to various brain regions such as the striatum and limbic sites, which comprise the hippocampus, hypothalamus, and the amygdala (Campbell, 2008; Carter, 2014; Fineberg & Ross, 2017; Leng

& Ludwig, 2016; Stoop et al., 2015). The limbic system is vital in supporting motivational, emotional, and behavioural functions (Fineberg & Ross, 2017). OXT neurons also send projections to the ventral tegmental area (VTA) which is the origin of the brain's reward circuitry (Damiano et al., 2014). Therefore, OXT plays an important role in the regulation of many reward-driven behaviours (Damiano et al., 2014).

OXT is released both centrally and peripherally giving it actions that have hormonal and behavioural effects (Valstad, Alvares, Andreassen, Westlye, & Quintana, 2016). Central release of OXT is restricted to the cerebral spinal fluid (CSF) of the central nervous system (CNS) (Barengolts, 2016; Valstad et al., 2016). We have learned that central secretion of OXT has effects that are fundamental to the development of social and affiliative behaviours such as bonding (Valstad et al., 2016), parenting, and social attachment, and has served as a vital survival mechanism throughout the evolution of mammals (Feldman et al., 2016; Gimpl et al., 2001; Grinevich, Knobloch-Bollmann, Eliava, Busnelli, & Chini, 2016). However, since the study of central OXT levels involves invasive procedures, much research has assessed the peripheral circulation of OXT and its effects via blood plasma, urine, or saliva samples (Campbell, 2008; Valstad et al., 2017).

The peripheral action of OXT is mainly the result of secretion from the pituitary gland to peripheral tissues such as the kidney, heart and cardiovascular system, pancreas and the thymus (Kiss & Mikkelsen, 2005). OXT is also produced in specific peripheral tissues such as the mammary tissues, testis, uterus, adrenal glands, and thereby aids in the development of physiological responses such as lactation, uterine contraction, reduction of urine secretion and feeding (Gimpl et al., 2001; Kiss & Mikkelsen, 2005; Toepfer et al., 2017). While there is overlap in the behavioural and physiological effects of OXT secretion along the central and

peripheral pathways there is not a perfect correlation between the two (Gimpl et al., 2001; Grinevich et al., 2016; Valstad et al., 2017). Valstad et al. (2017) concluded that the association found between central and peripheral levels of OXT seems to be related to 'experimental context'. For example, when OXT is administered intranasally a positive correlation in central and peripheral OXT concentration levels are typically found. However, the two levels are consistently found to differ in other situations suggesting that basal levels of peripheral OXT should not be used to reflect central levels of OXT - and vice versa (Valstad et al., 2017).

1.2.3 Oxytocin and Prosocial Behaviour

In recent years, one of the principal areas of OXT research interest has been its regulating and modulating properties on many facets of prosocial behaviours (Lee et al., 2009). For example, OXT facilitates social learning and memory by enhancing social recognition, decreasing attention to negative social cues (Kanat, Heinrichs, & Domes, 2014), and promoting positive social interactions (Fineberg & Ross, 2017; Lee et al., 2009; Leppanen et al., 2017). It also aids social behaviours by increasing positive communication (Ditzen et al., 2009), and social bonding (Kasperek-Zimowska et al., 2016; Lerer et al., 2010). The amygdala, in particular, is recognized for its role in the processing of memory, social recognition, and emotional reactions, such as fear and reward (Pham et al., 2009; Stoop et al., 2015). For instance, the Pavlovian fear-conditioning response is a mechanism, which involves oxytocin receptors (*OXTR*) and gamma-aminobutyric acidergic (GABA) neurons within the amygdala (Stoop et al., 2015). Specifically, OXT activates GABA neurons, which serves as an inhibitory neurotransmitter, reducing behavioural responses to fear (Fineberg & Ross, 2017; Stoop et al., 2015). Additionally, OXT activity within the amygdala has been associated with trust and attachment behaviours (Kirsch, 2005), interpretation of social cues, and social reward circuitry (Fineberg & Ross, 2017).

Affiliation is one of the primal social behaviours underpinned by the desire for attachment or connection (Feldman, 2017). Also known as 'social bonding', affiliation is crucial in the development of parental attachment, maternal care, and sexual behaviour (Campbell, 2008; Feldman et al., 2016). In order to understand the effects of OXT on social behaviours, over the years, research has focused on manipulating the levels of OXT in animal subjects. To illustrate, an early study conducted by Insel (1992) the effects of OXT on social isolation were examined by observing 6-day-old rat pups who were administered OXT through ICV injections into the non-calcified skulls. Insel (1992) observed that pups with greater levels of OXT demonstrated positive social affiliation, even after social isolation, by engaging in fewer stress responses compared to pups with lower levels of OXT. The findings supported the idea that the level of OXT is positively correlated with affiliation and also suggests its implications in prosocial behaviours due to OXT's involvement in early social interactions.

However, effects of the OXT hormone in animals does not extrapolate perfectly to human research. Therefore, recent research has shifted focus towards understanding the influence of OXT in human participants. Ditzen et al. (2009) demonstrated that when couples were disputing a topic, intranasal administration of OXT - compared to a control substance - increased positive communication and pair-bonding behaviour. In a recent review, Leppanen et al. (2017) also concluded that intranasal OXT increased the recognition of basic emotions among clinical populations, such as participants diagnosed with autism spectrum disorder, anorexia nervosa, bulimia nervosa, frontotemporal dementia, and depression (Leppanen et al., 2017). Additionally, among healthy individuals, recognition in basic emotions such as sadness, anger, happiness, and fear, also significantly improved after administration of OXT (Leppanen et al., 2017).

An increase in OXT can impact the effects of neural and perceived pain. Specifically, OXT can reduce neural activity in regions of the brain associated with pain, and can also decrease the subjective experience of pain (Lea et al., 2018). For instance, a current study conducted by Lea et al. (2018) investigated the influence and interaction effects of OXT and social support on the neural mechanisms involved in the subjective experience of induced pain. Lea at al. (2018) administered intranasally 24 IU of either OXT or a placebo to participants who then endured brief electric shocks. The participants were then randomly placed in one of three groups: social support from their partner; social support from a stranger; or no social support (Lea et al., 2018). Social support was provided by handholding - a common form of emotional support (Lea et al., 2018). Through observing the scanned neural activity with a functional magnetic resonance imaging (fMRI) during the electric shocks, it was discovered that partner and stranger support reduced neural activity associated with pain in the Anterior Insula (AI) compared to no support (Lea et al., 2018). Furthermore, Lea et al. (2018) measured the participants subjective experience and found that participants in the partner support group reported significantly less unpleasantness from the electric shocks indicating that receiving support from a romantic partner was more effective in providing some pain-relief compared stranger support or no support.

In summary, it is well-established that OXT has positive influence on prosocial behaviours (Fineberg & Ross, 2017; Lee et al., 2009; Leppanen et al., 2017; Nawijn et al., 2016). As such, research continues to demonstrate that low levels of OXT are associated with interpersonal deficits such as distrust, attachment avoidance, relationship difficulties (Quirin, Kuhl, & Düsing Rainer, 2011), poor coping mechanisms (Toepfer et al., 2017), and low emotional expressivity (Leppanen et al., 2017). In other words, the OXT hormone is positively

associated with behaviours such as empathy, pair bonding, and social affiliation, and importantly, it facilitates the use of these behaviours to buffer against stress (Damiano et al., 2014).

1.2.4 Oxytocin and Stress

Human survival is dependent on the physiological mechanisms that prepares the body for fight-or-flight in the face of danger. However, frequent stressful challenges result in the body continuously producing cortisol, which can have detrimental effects such as sleep deficiency and loss of cognitive flexibility (Stanić et al., 2017; Swaab, Bao, & Lucassen, 2005). Cortisol - also known as the stress hormone - is part of the glucocorticoid class of hormones and plays a fundamental role in the development of stress responses due to its regulation of the hypothalamic-pituitary-adrenal (HPA)-axis, which is well established for its role in the body's reaction to stress (McQuaid et al., 2016; Swaab et al., 2005). Research has discovered that OXT is another hormone essential in the regulation of the HPA-axis because it serves as a built-in mechanism to counter stress-related responses by indirectly inhibiting the release of cortisol and the adrenocorticotropic hormone (ACTH) (Ditzen et al., 2009; Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003; Kirsch, 2005; Lee et al., 2009; McQuaid et al., 2016; Swaab et al., 2005).

There is good evidence that OXT has stress-reducing effects because it enhances the desire for social contact and social interaction, which in turn decreases behavioural and physiological stress responses (Ditzen et al., 2009; Heinrichs et al., 2003; Stanić et al., 2017; Walker & McGlone, 2013). As previously mentioned, in a study conducted by Ditzen et al. (2009), intranasal administration of OXT decreased couple conflict by increasing positive communication and behaviour during a discussion on an opposing topic. In addition, OXT

reduced cortisol levels among couples during the conflict scenario (Ditzen et al., 2009). In another study, in response to a stressor, when children received social support from their mother an increase in OXT levels and a decrease in cortisol levels were observed (Seltzer, Ziegler, & Pollak, 2010). Low levels of OXT have been associated with interpersonal difficulties such as emotional dysregulation and sensitivity to social rejection, and these traits have, in turn, been linked to an individual's diminished ability to cope with stress (Quirin et al., 2011). Furthermore, stress is known to promote overeating tendencies (Capello & Markus, 2014). For example, in animal research, rodents choose highly palatable foods when under chronic stress (Egan et al., 2018). Importantly in this regard, there is a developing research interest in links between OXT and food intake (Davis et al., 2017; Spetter & Hallschmid, 2017).

1.2.5 Oxytocin and Food Intake

As previously mentioned, OXT is primarily synthesized in the hypothalamus, which is an area of the brain known for its preeminent involvement in the regulation of food intake and other reward-driven behaviours (Olszewski et al., 2016). Importantly, the ventromedial hypothalamus (VMH), is where OXT-expressing receptors are found and this area has also been linked to appetite and the management of glucose levels (Leng & Sabatier, 2017; Stoop et al., 2015). It is well-established that increases in OXT serve as a powerful anorexigenic agent (Olszewski et al., 2016; Striepens et al., 2016). In particular, OXT has been found to decrease the consumption of sweet carbohydrates (Herisson et al., 2016).

In earlier research, Arletti et al. (1989) demonstrated in fasting rats, that central administration of OXT by intracerebroventricular injection (ICV), inhibited feeding behaviours by significantly reducing the amount of food consumed and the duration of time spent feeding. More recently, OXT knockout mice were found to consume significantly more sweetened

solutions and saccharin relative to wild-type mice (Billings, Spero, Vollmer, & Amico, 2006). Further evidence in animal research continues to support OXT as a powerful anorexigenic. For instance, ablation of the PVN in rodents resulted in an excessive appetite and extreme weight gain, and an increase in OXT, through injection, demonstrated a significant decrease in food consumption in free-feeding rats (Olszewski et al., 2016). Chaves et al. (2013) also concluded that OXT plays a vital role in regulating metabolic homeostasis based on their evidence that OXT-deficient mice developed later-onset obesity without any change in diet or food consumption. OXT has also been linked to leptin which is another important hormone in the regulation of food intake (Chaves et al., 2013). When OXT is deficient, it tends to reduce the signaling of leptin-effecting energy expenditure, which can lead to diet-induced obesity (Chaves et al., 2013; Spetter & Hallschmid, 2017).

Since OXT has been recognized for its role in food intake, studies have also examined its involvement and influence in aspects of disordered eating such as in the *Prader-Willi Syndrome* (PWS), which is characterized by extreme overeating, an insatiable appetite, and obesity (Kuppens, Donze, & Hokken-Koelega, 2016; Swaab & Hofman, 2014). Swaab et al. (2014) studied five subjects diagnosed with PWS by investigating OXT neurons in the PVN using a staining solution designed to bind to acid proteins and nucleic acids. These investigators discovered that the volume of the PVN was 28% smaller in PWS cases compared to control subjects. In addition, there was a 42% reduction in the number of OXT neurons in the PVN in the patient group (Swaab & Hofman, 2014). It appears that the extreme food intake which characterizes PWS may possibly be the result of an "OXT deficiency" (Swaab et al., 2014).

Although OXT is recognized for its anorexigenic influence on food intake, Olszewski et al. (2016) examined the evidence that OXT is a 'conditional anorexigenic' for which the

anorexigenic properties of OXT depend largely on social, physiological, and behavioural contexts. In other words, OXT does not always decrease the appetite for sweet carbohydrates and this is becoming increasingly evident in human studies. For instance, Olszewski et al. (2016) discusses a study conducted by Ott et al. (2013) where participants were administered OXT intranasally 45 minutes before eating breakfast after fasting the night before. Contrary to expectation, OXT did not reduce the amount of food intake among healthy male participants, including the intake of sweet carbohydrates (Ott et al. 2013). However, after the meal when they were asked to choose a snack with the choices being salty crackers, rice waffles, or sweet chocolate cookies, there was an observable decrease in the consumption of the chocolate cookies, which is consistent with past research linking higher OXT levels to a decrease in appetite for sweet carbohydrates (Ott et al., 2013). Sweet carbohydrates have been found to activate the OXT system (Leng & Sabatier, 2017), whereas energy in food derived from fats does not (Olszewski et al., 2016).

In a recent review, Davis and Moghimi (2017) proposed a "*OXT-deficiency* behavioural model" for compulsive overeating. Specifically, they drew several theoretical parallels between low OXT levels and the characteristics of those with binge eating disorder (BED) and addictive tendencies towards overeating. For instance, low levels of OXT are correlated with interpersonal problems such as poor emotional regulation, relationship difficulties, and sensitivity to stressful events. As such, overeating may be a coping mechanism where readily available palatable foods serve as a reward strategy or as a means of "self-medication" (Davis & Moghimi, 2017). Further research indicates that low levels of OXT are correlated with many of the pathological symptoms associated with eating disorders such as anxiety, increased emotional reactivity, and social impairments (Plessow, Eddy, & Lawson, 2018). Those with interpersonal deficits are also at a

greater risk of developing addictive behaviours (Davis & Moghimi, 2017). Interestingly, Davis and Moghimi (2017) discuss the evidence that there appears to be a bidirectional relationship between low levels of OXT and interpersonal deficits. Specifically, not only are low levels of OXT associated with poor emotional regulation, stress responsiveness, and increased risk of using addictive behaviours; but chronic overeating and repeatedly overusing addictive behaviours can foster a downregulation of OXT levels that ultimately perpetuates a vicious pathological cycle (Davis & Moghimi, 2017). Mitra et al. (2010) provided evidence for this concept in animal research when they discovered that rats who excessively consumed foods high in sugar for prolonged periods of time, showed diminished anorexigenic effects of OXT. They concluded that the decrease of anorexigenic signaling within the OXT system, paired with consumption of high sugar foods, may serve as a mechanism fostering excessive overeating, which could lead to weight gain (Mitra et al., 2010).

Research also continues to demonstrate that addictive tendencies towards over-consumption are influenced by OXT's regulating role in the brain's reward system (Spetter & Hallschmid, 2017). High OXT activity is found in the brain's ventral tegmental area (VTA) and nucleus accumbens, which are regions that regulate food-reward behaviours (Ranaldi, 2014; Spetter & Hallschmid, 2017). Administration of OXT has been found to selectively inhibit hedonic eating by weakening the dopamine signaling in the brain's reward pathway (Ott et al., 2013). For instance, Ott et al. (2013) demonstrated that OXT decreased the consumption of chocolate cookies three hours after intranasal administration and 45 minutes after the intake of breakfast. As previously stated, there is much evidence that excessive overeating is a result of "neural adaptations" that have occurred over time as a consequence to chronic and prolonged

consumption of highly addictive palatable foods (Alsiö, Olszewski, Levine, & Schiöth, 2012; Mitra et al., 2010; Wu et al., 2016).

Another area of interest in this field of research is understanding how genes play a role in affecting the brain systems involved in the processing of reward-driven behaviours (Davis et al., 2019). Genetic research is becoming of great interest in understanding the mechanisms involved in food-reward eating behaviours. For instance, a very recent study by Davis et al. (2019) investigated whether genetic markers on the oxytocin receptor (*OXTR)* and the cluster of differentiation (*CD38)* genes are linked to reward-related personality traits, such as reward sensitivity and novelty seeking. Results indicated that six of the 13 single nucleotide polymorphisms (SNPs) that were genotyped – four *OXTR* and two *CD38* SNPs – were significantly associated with the reward-based traits (Davis et al., 2019). In this study, the six SNPs were combined in a quantitative multilocus genetic profile (MLGP) score since a SNP on its own typically only accounts for a small proportion of phenotypic variance (Davis et al., 2019). In other words, genotypic variation may be better understood in quantitative terms than in qualitative terms because individual genetic markers contribute only a small proportion of the behavioural and characteristic diversity in traits associated with reward (Davis & Loxton, 2013; Nikolova, Ferrell, Manuck, & Hariri, 2011).

1.2.6 Oxytocin and Sex Differences

The differing effects of OXT in males and females is well documented, which strongly suggests that this hormone is sexually dimorphic (Borland, Rilling, Frantz, & Albers, 2018; Bredewold & Veenema, 2018; Lee et al., 2009; Ma et al., 2018). While OXT is a social hormone that bonds males and females for reproductive purposes, it also serves as a sex-dependent regulator that takes situational-context into account (Borland et al., 2018; Rubin et al., 2017). In

other words, OXT facilitates different neural and behavioural responses in males and females (Borland et al., 2018; Rubin et al., 2017).

In animal studies, it was discovered that OXT is differentially involved in social recognition and social memory in males and females. For example, when rodents were introduced to a new animal, females were found to focus and investigate much less than males, supporting the idea that OXT influences more guarded behaviours in males. In addition, it was found that females seemed to retain social memory longer than males (Lee, 2009). In human studies, research has also demonstrated that females tend to find positive social interactions with their friends more rewarding than males do (Borland et al., 2018). Furthermore, intranasal administration of OXT decreased fear in males but the opposite was observed in females (Bredewold & Veenema, 2018). Interestingly, the differing sex effects of OXT has been explained by research suggesting that testosterone regulates the effects of OXT in males and estrogen regulates the effects of OXT in females (Lee, 2009).

Sex differences are also evident in social bonding behaviours. For instance, Gao et al. (2016) suggested that OXT may facilitate an increase in bonding or maternal behaviours in females. By contrast, males tend to demonstrate more protective, or aggressive behaviours that serve to ensure a safe environment for their partner and offspring. To test this theory, these authors had male and female participants self-administer intranasally 24 IU of either OXT or a placebo, and then exposed them to faces, each of which was accompanied by characteristic statements that reflected either positive, negative, or mixed (consisted of positive and negative statements) social judgements. Using fMRI, Gao et al. (2016) found that there was a significant increase of activity in the amygdala in females compared to males in the OXT group who observed individuals praising others in the positive statement group. In contrast, neural activity

in the amygdala increased in males when they were exposed to the negative statements, which consisted of criticizing others (Gao et al., 2016). These findings supports the view that OXT increases activation in the amygdala to emotional stimuli, but in a context- and sex-dependent manner (Gao et al., 2016). In addition, this research demonstrated that there are sex differences in social and reproductive behaviours such as aggression and communication where increases in OXT in females focuses more on positive social stimuli, whereas males tend to focus more on the negative social stimuli (Gao et al., 2016).

OXT regulates the neural mechanisms involved with sensitivity to reward (Damiano et al., 2014; Davis et al., 2019; Spetter & Hallschmid, 2017). As previously mentioned, an increase in OXT levels has been found to decrease the hedonic impact of palatable foods (Arletti et al., 1989; Herisson et al., 2016; Lawson et al., 2015; Olszewski et al., 2016; Ott et al., 2013; Spetter & Hallschmid, 2017; Striepens et al., 2016). However, it is clear that sex differences also play an influential role in the perception and rewarding properties of food (Legget et al., 2018; Reynaert et al., 2016). For instance, in females there was an observed increase of brain activity in regions associated with reward, such as the nucleus accumbens, the insula, and the anterior cingulate cortex, compared to males when both groups were exposed to visual food cues in a fasted state (Legget et al., 2018). Increased neural responses to palatable foods in the nucleus accumbens and the insula, in females, has also been linked to weight gain and reduced weight-loss success (Egan et al., 2018; Legget et al., 2018). And, a greater insula response to food cues in a neutral hunger state was found in females compared to males (Legget et al., 2018). Importantly, females tend to report greater problematic eating behaviours, such as stress and emotional eating, which is not surprising given that obesity is more prevalent in females (Egan et al., 2018; Jääskeläinen et al., 2014; Legget et al., 2018).

The underlying mechanisms are not fully understood, however there is evidence that the gonadal hormones and differences in hormonal levels between males and females plays an important role (Caldú & Dreher, 2007; Egan et al., 2018; Jääskeläinen et al., 2014; Legget et al., 2018). Expression of OXT tends to be higher in females compared to males (Love et al., 2012). And, it has been well-established that estrogen influences the regulation of food intake, energy expenditure, and body weight control (Legget et al., 2018; Reynaert et al., 2016; Richard, López-Ferreras, Anderberg, Olandersson, & Skibicka, 2017). For instance, estrogen plays a role in signaling beta estrogen receptors within the nucleus accumbens – an area of the brain linked to the processing of rewarding stimuli - and those receptors are linked to increased hedonic eating in females (Legget et al., 2018; Richard et al., 2017). Increases in emotional and stress-eating found in females is also a result of the influence of the estrous cycle phase and increased estrogen levels - when estrogen levels are high, an increase in reward-seeking behaviours were observed (Egan et al., 2018; Legget et al., 2018). It is important to note that estrogen regulates the effects of OXT within the brain which in turns regulates many survival behaviours, such as reproduction and food intake (McCarthy & Altemus, 1997). As such, this has evolutionary significance because increases in reward-seeking behaviour and neural responses found in females has served, for centuries, as a survival mechanism to ensure reproductive success (Becker, 1999; Becker & Chartoff, 2018; Egan et al., 2018; Legget et al., 2018).

1.2.7 Measurement of Oxytocin

Over the years, much of what has been learned about OXT and its influence on behavioural and physiological responses in humans has been derived from animal-model experimentation (Francis, Champagne, & Meaney, 2000; Lockard et al., 2017). Pavlovian classical conditioning experiments, which are forms of associative learning that play a vital role

in the reward system, was developed using a canine model (Pavlov, 1932; as cited by Eckstein et al., 2016). In addition, using an animal model of PTSD allowed Eskandarian et al. (2013) to suggest that OXT should be cautiously used in treating PTSD because they discovered that increasing levels of OXT, through intraperitoneal administration, delayed fear extinction conditioning in rats. For instance, one of the main symptoms of PTSD is impaired extinction of traumatic memory. Fear extinction is used in exposure therapy for the treatment of traumatic memories to reduce the conditioned fear responses (Eskandarian et al., 2013). Research suggests that administration of OXT could serve as a pharmacology treatment for PTSD and decrease its debilitating symptoms (Eskandarian et al., 2013). As such, Eskandarian et al. (2013) investigated the effects of OXT on fear extinction in rats and, contrary to expectations, found that increasing OXT levels actually delayed fear extinction conditioning in rats. However, animal studies do not generalize perfectly to the human condition. For example, in contrast to the results of Eskandarian et al. (2013), Eckstein et al. (2015) found that an increase in OXT levels facilitated the extinction of fear conditioning in the human condition. Inconsistent findings like these have motivated researchers to try and better understand the role of OXT via human experimentation.

To date, investigations of OXT and its effects have been inferred from exogenous manipulation OXT levels (Spetter & Hallschmid, 2017). A popular method has been the use of intranasal administration (Spetter & Hallschmid, 2017). This procedure is the only method in which OXT is able, non-invasively, to pass through the blood-brain barrier thereby allowing investigators to observe its effects directly on the central nervous system oxytocin receptors (Spetter & Hallschmid, 2017). Currently, research on disorders that are characterized by social impairments, such as autism and schizophrenia, have emphasized the importance of focusing on endogenous OXT levels in order to understand the physiological development and maintenance

of the social deficits associated with these disorders (Bartz & Hollander, 2006; Ebstein et al., 2012). Bartz and Hollander (2006) found a significant correlation between OXT levels in the blood and the social deficits associated with disorders such as autism. This relationship highlights the crucial role of OXT in adaptive behaviours, and how disruptions on social processes can severely impact important human interactions (Bartz & Hollander, 2006). However, social behaviours are not the only survival mechanism that is effected and, as such, OXT's involvement in reward-driven eating is an important new area of research interest (Spetter & Hallschmid, 2017).

While much research has focused on using intranasal administration and brain-imaging techniques to study OXT, recent research has also investigated genetic markers to understand better the role of endogenous levels of OXT (Davis et al., 2019). For instance, Davis et al. (2019) found, as previously mentioned, six OXT SNPs associated with various aspects of reward responsiveness. The primary target of genetic studies has been SNPs of the oxytocin receptor gene (*OXTR*) (Feldman et al., 2016). Another recent study by Davis et al. (2017) was the first to investigate variants on the *OXTR* in the context of overeating and risk factors for overeating such as sensitivity to both reward and punishment, and preference for palatable foods. Seven genetic *OXTR* polymorphisms were genotyped, and results identified links between four of the markers and overeating, and relevant endophenotypes of overeating. Specifically, the rs2268493 TT genotype group reported greater overeating and higher reward sensitivity compared to the C allele carriers. The rs2268494 A allele carriers reported a greater preference for sweet and fatty foods. For the rs2268498 SNP, the homozygous C genotype group indicated greater sensitivity to both reward and punishment and the SNP rs227885 homozygous G genotype reported greater sensitivity to reward compared to the T allele carriers. While these findings have successfully

identified SNPs of the *OXTR* associated with overeating, it is important to note that the *OXTR* is not the only gene involved in the regulation and expression of OXT (Ebstein et al., 2012).

1.2.8 Genetic Markers of Oxytocin in the Present Study

1.2.8.1 Oxytocin Peptide Gene (OXTG)

The oxytocin peptide coding gene (*OXTG),* located on band 20p13, is a structural gene encoding a precursor protein that is processed to produce mature OXT (Ebstein et al., 2012; Kasperek-Zimowska et al., 2016). It is also known as the human *OXT-neurophysin I (OXT-NPI)* because it includes the carrier protein neurophysin I (Zhang, Zhang, Han, & Han, 2017). OXT is synthesized in the hypothalamus as an inactive precursor, along with neurophysin I, and is then transported along the axon to the pituitary gland where it is stored and secreted in response to various stimuli (Zhang et al., 2017). The *OXTG* has received relatively little research attention compared to the *OXTR*, with respect to its influence on behavioural and physiological responses.

One study by Mileva-Seitz et al. (2013) investigated variants on the *OXTG* in the context of maternal behaviours such as maternal vocalization, which is infant directed speech, and maternal caretaking behaviours such as bathing and feeding. These investigators found that the A-allele of the rs2740210 SNP and the A-allele of the rs4813627 SNP served as a protectant allele. Mothers who carried the protectant allele demonstrated an increase in maternal caretaking behaviours and greater maternal vocalization compared to the rs2740210 CC genotype and the rs4813627 GG genotype (Mileva-Seitz et al., 2013). In addition, mothers with the rs2740210 CC genotype discontinued breastfeeding much earlier than those who carried the A-allele (Toepfer et al., 2017).

There is growing evidence that the rs2740210 SNP is also significantly associated with prosocial behaviours (Love et al., 2018; Wade, Hoffmann, Knafo-Noam, O'Connor, & Jenkins,

2016). Some research has identified this SNP in relation to behavioural and impulsivity deficits found in a youth population (Wade et al., 2016). In a recent study, this OXT SNP was also investigated in an alcohol-dependent population to determine if it mediated the relationship between social support and psychiatric distress (Love et al., 2018). Increased access to social support encourages better mental health outcomes and plays a crucial role in recovery and maintenance of substance dependence by promoting positive psychological states (Love et al., 2018). Results indicated that there was a significant association between social support and psychological distress of the rs2740210 SNP. Specifically, those who carried the G-allele demonstrated the negative relationship between social support and psychological distress (Love et al., 2018). The investigators suggested that the *OXTG* rs2740210 SNP plays an important role and mediates the effectiveness of social support on mental health and psychological states.

Another variant of interest is the rs4813625 SNP - implicated in relation to social behaviours and stress responsivity (Feldman et al., 2016; Love et al., 2012; Olofsdotter, Åslund, Furmark, Comasco, & Nilsson, 2018). For instance, females carrying at least one copy of the C-allele, reported lower emotional well-being along with higher trait and attachment anxiety compared to homozygous GG females (Love et al., 2012). In addition, greater stress-induced dopamine release was found in C-allele carriers in an induced pain-stress condition (Love et al., 2012). The rs4813625 SNP was most recently investigated in a study to determine if there is a significant association between this *OXTG* variant and perceived parenting styles on social anxiety in adolescents (Olofsdotter et al., 2018). Adolescents completed a series of self-report questionnaires on parenting style and social anxiety symptomology. Results indicated that C-allele carries who reported receiving a low supportive parenting style, also reported higher than normal social anxiety levels compared to the G-allele carriers. Similarly, C-allele carriers who

reported receiving a high supportive parenting style, reported lower than normal social anxiety levels (Olofsdotter et al., 2018). These results support previous findings that the C-allele of the rs4813625 SNP may serve as a risk allele in the development of social deficits.

Research continues to implicate the *OXTG* in prosocial-behaviour deficits and, as a consequence, recent studies on autism spectrum disorder (ASD) have also investigated regions along the *OXTG* in connection with the risk for developing this condition (Ebstein et al., 2012; Kasperek-Zimowska et al., 2016; Yrigollen et al., 2008; Zhang et al., 2017). For example, Yrigollen at al. (2008) found significant associations between the rs2740204 GG genotype and stereotypic ASD behaviours such as hand-flapping, body rocking, and spinning objects. Those carrying the GG genotype also scored higher on questions pertaining to autistic like traits that reflected the three central symptom dimensions of ASD: language impairment; impairment of communication and poor social interaction; and restrictive and repetitive behaviours (Hovey et al., 2014; Zhang et al., 2017). Other genetic markers of the *OXTG* – such as the rs877172 SNP (Francis et al., 2016) and rs3761248 SNP (Malik, Zai, Abu, Nowrouzi, & Beitchman, 2012) - have been linked to social withdrawal and anti-social behaviours.

In addition, it is important to note that *OXTG* has been linked to dopaminergic signaling in humans – a process which plays a major role in the regulation of stress responses and reward-driven behaviours (Feldman et al., 2016; Love et al., 2012). Importantly, reward-seeking behaviours are implicated in food preferences, and human attraction to palatable foods, which are high in sugar, fat, and salt (Klockars, Levine, & Olszewski, 2015). OXT gene expression also plays a mediating role in the production of leptin, which in turn is linked to risk for obesity suggesting that *OXTG* plays a role in the regulation of body weight and energy expenditure

(Altirriba, Poher, & Rohner-Jeanrenaud, 2015; Klockars et al., 2015; Perello & Raingo, 2013). To date, however, no research has directly examined its links with eating behaviours.

1.2.8.2 Cluster of Differentiation 38 (CD38)

The cluster of differentiation (*CD38)* gene is a ADP-ribosyl cyclase type II transmembrane protein located on band 4p15 and is involved in the central regulation and secretion of OXT (Kasperek-Zimowska et al., 2016; Tabak et al., 2016.; Zhang et al., 2017). As such, research has linked the *CD38* gene to levels of OXT availability in the brain (Jin et al., 2007; McInnis, McQuaid, Matheson, & Anisman, 2017). *CD38* has been the focus of recent genetic investigations in the field of social processes and behaviours (Feldman et al., 2016). Jin et al. (2007) investigated the protein by examining *CD38* knock-out (KO) mice and found they had significantly lower plasma concentration levels of OXT and low levels of OXT in the cerebral spinal fluid (CSF) relative to their wild-type littermates who carried the *CD38* gene.

CD38 has also been linked to interpersonal deficits such as social withdrawal and social anxiety (Jin et al., 2007; Tabak et al., 2016). Jin et al. (2007) detected deficits in maternal nurturing and prosocial behaviours in *CD38* KO mice compared to their wild-type littermates who carried the *CD38* gene (Jin et al., 2007). More recently, Tabak et al. (2016) examined the rs3796863 SNP and found that C-allele carriers exhibited decreased social sensitivity, as indicated by reactivity and responsiveness to social cues, compared to those who carried the A-allele. Evidence also suggests that the C-allele of the rs3796863 appears to serve as a risk allele for the severity of high functioning autism while the more common A-allele serves as a buffer (Munesue et al., 2010). Specifically, autistic patients who were rs3796863 homozygous C-allele carriers presented severe symptoms such as restrictive and repetitive behaviours, and had lower levels of *CD38* gene expression compared to A-allele carriers (Munesue et al., 2010).

Interestingly, while the rs3796863 SNP was significantly associated with autism among the U.S. participants this was not the case among the Japanese participants (Munesue et al., 2010; Zhang et al., 2017). These findings emphasize that polymorphic variations are sensitive to ethnicity. Further, in autistic studies, a deficit in the processing of social stimuli was detected even in healthy participants who carried the CC genotype (Sauer, Montag, Wö Rner, Kirsch, & Reuter, 2012).

Research continues to identify strong associations between the rs3796863 variant and prosocial behaviours. For instance, a recent study investigated the rs3796863 SNP and its association between unsupportive social interactions and current affective states (McInnis et al., 2017). Results indicated a significant association between negative affective state and greater perceived unsupportive social interactions from peers among those who carried the CC genotype compared to A-allele carriers (McInnis et al., 2017). Interestingly, there was a modest association between the A-allele carriers and perceived unsupportive social interactions from peers. In previous research the A-allele has been linked to higher levels of plasma OXT availability in the brain which, in turn, is linked to an increase in sensitivity to social cues thus, possibly accounting for the moderate association found between those who carried the A-allele and perceived unsupportive social interactions found in the study (McInnis et al., 2017; Tabak et al., 2016). To further support these findings, in another study examining empathy as a mediator, it was found that those who carried the A-allele of the rs3796863 variant made a greater donation in response to a compelling letter that was meant to increase altruistic behaviour (Liu, Gong, Li, & Zhou, 2017). Recent studies have also linked the A-allele to an increased risk for suicide ideation and attempt (McQuaid, McInnis, Matheson, & Anisman, 2016; Parris et al., 2018).

It is important to note that although there is evidence of an association between *CD38* and prosocial behaviours, and the social deficits found in disorders such as autism, no prior research has examined the *CD38* gene and its influence on eating behaviours. The only known link between *CD38* and food-related intake has been its influence on impaired insulin release associated with non-insulin-dependent type II diabetes (Lerer et al., 2010; Malavasi et al., 2008). For instance, it was found that deficiencies in the expression of *CD38* effects glucose-induced insulin by impairing the release of insulin (Malavasi et al., 2008).

1.2.8.3 Leucyl-cystinyl Aminopeptidase (LNPEP)

LNPEP is a leucyl-cystinyl aminopeptidase protein coding gene that metabolizes and inactivates OXT, vasopressin, and other peptide hormones within brain tissue (Ebstein et al., 2012; Zhang et al., 2017). *LNPEP* is located on band 5q15 and has been established as an oxytocinase due to its disintegrating properties on OXT (Ebstein et al., 2012; Zhang et al., 2017). The enzyme has been found within specific areas of the brain such as the cerebellum, cerebral cortex, and the basal ganglia (Ebstein et al., 2012; Zhang et al., 2017). It is becoming increasingly recognized for its role in the maintenance of homeostasis for a variety of different physiological functions such as maintaining a normal pregnancy, (Ebstein et al., 2012; Hill et al., 2011; Johnson et al., 2009), and energy expenditure (Niwa et al., 2015). However, to date, little attention has been given to *LNPEP* and its relationship with behavioural phenotypes and relevant brain functions.

LNPEP expression is vital in the circulation of OXT levels during pregnancy in order to assist in the prevention of the onset of preterm labour (Pham et al., 2009). OXT is well known for its role in maternal-related physiological functions such as uterine contraction, milk production and ejection, and also its influence on maternal behaviours, such as feeding and

nurturing (Elkins, Walti, Newberry, & Lema, 2017; Pham et al., 2009). Pham et al. (2009) investigated the *LNPEP* gene on nonhuman pregnancies by manipulating the genotype in KO mice and found no observable difference between them and wildtype dams on reproductive maternal behaviours, such as pup retrieval and feeding. Pham et al. (2009) suggested that this may indicate that the influence of the *LNPEP* gene is specific to human pregnancies.

In human studies, research provided evidence that the *LNPEP* gene is significantly associated with an increased risk for developing preeclampsia, which is a pregnancy-specific disorder that, if left untreated, can lead to eclampsia which is a serious condition that puts mom and baby at risk, and in some cases can lead to death (Farzaneh, Tavakolikia, & Soleimanzadeh Mousavi, 2018; Hill et al., 2011; Johnson et al., 2009). Preeclampsia is characterized by high blood pressure and, what is interesting to note, is that those who have a history of obesity are at greater risk of developing preeclampsia during pregnancy (Farzaneh et al., 2018; Hill et al., 2011; Johnson et al., 2009). Research is also linking increased risks to poor diet and higher body fat among other risk factors (Farzaneh et al., 2018). It was found that *LNPEP* levels are crucial in maintaining a healthy pregnancy and those with preeclampsia demonstrated lower than normal levels of *LNPEP* in the maternal serum (Johnson et al., 2009). Furthermore, in a recent longitudinal study, it was found that the rs4869317 SNP was significantly associated with hypertensive, the symptom associated with preeclampsia (Zee et al., 2018).

The *LNPEP* gene is well-established for its influence in maternal-related behaviours, however recent studies have begun focusing on other potential roles (Elkins et al., 2017). For instance, in non-human studies, it has been linked to stress regulation (Hernández et al., 2015). Specifically, Hernández et al. (2015) found that when rats were under acute restraint stress, *LNPEP* activity decreased in the amygdala and increased in the hypothalamus suggesting its

potential role in stress regulation. In human participants, some research has identified genetic markers and possible links to social cognition (Ebstein et al., 2012; Kasperek-Zimowska et al., 2016). Regarding disorders such as ASD and anorexia nervosa, research has suggested that the rs4869317 SNP may be linked to the development and maintenance of their social-behavioural impairments (Ebstein et al., 2012; Kasperek-Zimowska et al., 2016). However, in that regard, and to our knowledge, there is very little research on the *LNPEP* gene, therefore there is very little evidence linking the gene to many facets of human behaviours. Additionally, no research has linked this gene with reward-driven in general and eating behaviours specifically.

CHAPTER 2: THE PRESENT STUDY

The present study is an extension of the recent study by Davis et al. (2017) and has investigated links between a total of 12 markers of the *OXTG, CD38,* and *LNPEP* genes, and overeating and relevant endophenotypes of overeating such as such as sensitivity to reward, sensitivity to punishment, and palatable food preferences. This study is entirely exploratory since the genetic polymorphisms that will be investigated have not been studied in the context of overeating behaviours. However, they have been associated with prosocial behaviours that are theoretically connected with overeating and relevant endophenotypes of overeating such as a reward sensitivity, punishment sensitivity, and food preferences (Ebstein et al., 2012; Feldman et al., 2016; Francis et al., 2016; Kasperek-Zimowska et al., 2016; Lerer et al., 2010; Liu et al., 2017; Love et al., 2012; Love et al., 2018; McInnis et al., 2017; McQuaid, et al., 2016; Mickey et al., 2013; Olofsdotter et al., 2018; Parris et al., 2018; Zhang et al., 2017). It is important to note, that since research continues to provide evidence of the significant differences between males

and females in overeating and related behaviours, sex differences were also examined in the current study.

CHAPTER 3: METHODOLOGY

3.1 Participants

The sample consists of 460 healthy community-recruited adults (female = 346) with a mean age of 33.8 and a range from 24–50 years of age. Participants were recruited from posters, newspaper advertisements and online sites such as Craigslist and Kijiji. All assessments took place at the Centre for Addiction and Mental Health (CAMH) in central Toronto. The majority of the sample is Caucasian (79%), with the remainder identifying as African-Canadian (15%) and "Other" (6%). Inclusion criteria consisted of residency in North America for at least five years prior to study enrollment and English fluency. Exclusion criteria consisted of any serious medical illnesses, disabilities, or those with an Axis I diagnosis, with the exception of unipolar depression and binge eating disorder, and any women with a pregnancy in the previous six months, or who were lactating.

3.2 Procedures

In the original study, participants were contacted by telephone and then screened for eligibility. If a participant was eligible, an appointment was made for them to visit the CAMH laboratory, in-person, where they were then given a questionnaire package to complete. Physical measurements of height and weight were recorded with participants standing in their stocking feet and wearing light clothing. Body Mass Index (BMI) was recorded with values representing a broad range among the sample (17.8–75.2 kgm^{-2}). DNA was extracted from a venous blood

sample taken at the on-site medical laboratory. Aspects of the protocol irrelevant to this genetic analysis have been reported elsewhere (see Davis, Levitan, Kaplan, Carter-Major, & Kennedy, 2016; Patte et al., 2016).

3.3 Selection of Genetic Markers

Twelve SNPs were selected for inclusion: four SNPs on the *OXTG* gene (rs4813625, rs877172, rs3761248, rs2740210); two SNPs on the *CD38* gene (rs3796863, rs3756242); and six SNPs on the *LNPEP* gene (rs4869317, rs13175726, rs18059, rs316206, rs3849749, rs4869315). These SNPs were selected based on their prior associations with reward/punishment related concepts, and/or with various aspects of prosocial behaviours.

3.4 Questionnaire Measures

The four dependent variables were operationally defined as separate composite variables which were each derived from a *Principal Components Analysis* (PCA) to reflect the multidimensionality of the construct.

3.4.1 Reward Sensitivity included three separate questionnaires designed to assess the variation in *approach motivation,* which is an evolutionary brain system that regulates the hedonic capacity and the incentive to seek out rewarding environmental stimuli (Berridge & Kringelbach, 2013).

The *Barratt Impulsivity Scale* version 11 (BIS-11) is a 30-item, 4-point Likert scale with scores ranging from 1 (rarely/never) to 4 (almost always/always) (Patton, Stanford, & Barratt, 1995). It was designed to identify three facets of impulsiveness: motor impulsiveness, “I buy things on impulse”; non-planning impulsiveness, “I plan tasks carefully”; attentional impulsiveness, “I am restless at the theatre or lectures” (Patton et al., 1995). The total scores

range from 30-120 with higher scores indicative of greater impulsivity (see Appendix A for a copy of the full scale).

The *Reward Subscale* (RS) of the *Sensitivity to Punishment and Sensitivity to Reward Questionnaire* (SPSRQ) consists of 24 forced-choice items (see Appendix B for a copy of the subscale) measured on a dichotomous scale of yes-no responses with scores ranging from 0-24 by summing all of the "yes" responses (Torrubia, Ávila, Moltó, & Caseras, 2001). The RS subscale was designed to assess an individual's approach responses during circumstances involving exposure to all kinds of rewards such as money, sex, pleasure-inducing substances, as well as social rewards such as appraisal and approval from others (e.g. "Do you need people to show their affection for you all the time?") (Torrubia et al., 2001).

The *Novelty Seeking Scale* (NS) of the 100-item *Tridimensional Personality Questionnaire* (TPQ; see Appendix C for a copy of the full scale) was designed to assess the tendency to impulsive decision making, quick loss of temper, disorderliness, and extravagance approach to reward cues (e.g. "When nothing new is happening, I usually start looking for something that is thrilling and exciting") (Cloninger, 1987). The NS consists of 34 forced-choice items measured on a dichotomous scale of yes-no responses. All of the "yes" responses are summed where a higher score is indicative of a greater novelty-seeking tendency (Cloninger, 1987). To avoid response bias, 20 items have been reverse-scored.

3.4.2 Punishment Sensitivity included three questionnaires designed to assess *avoidance motivation,* which is another evolutionary brain system that regulates the incentive to avoid or retreat from threatening or punishing situations and other aversive events (Hahn et al., 2010).

The *Behavioural Inhibition Scale* (BIS) of the *BAS/BIS* questionnaire contains 7-items (see Appendix D for a copy of the full scale) measured on a 4-point Likert scale with scores ranging from 0 (not true) to 4 (very true) and total scores ranging from 0-28. The BIS was designed to assess a respondents' dispositional sensitivity to both fear and anxiety responses to aversive social and environmental situations (e.g. "I usually get very tense when I think that something unpleasant is going to happen"). To avoid response bias, 1 item has been reverse-scored (Carver & White, 1994).

The *Harm Avoidance Scale* (HA) of the TPQ consists of 34 forced-choice items (see Appendix E for a copy of the full scale) designed to assess the tendency to inhibit behaviour in response to aversive or punishing stimuli and the fear of uncertainty (e.g. "I often feel tense and worried in unfamiliar situations, even when others feel there is little to worry about") (Cloninger, 1987). The HA is measured on a dichotomous scale of yes-no responses. All of the "yes" responses are summed, and a higher score is indicative of a greater harm-avoidance tendency (Cloninger, 1987). To avoid response bias, 19 items have been reverse-scored.

The *Punishment Subscale* consists of 24 forced-choice items of the SPSRQ (see Appendix F for a copy of the subscale) and is designed to assess an individuals' avoidance responses to the worry and threat of mistreatment, failure, and the anticipation of non-reward in typical situations (e.g. "Are you afraid of new or unexpected situations?") (Torrubia et al., 2001). The PS is measured on a dichotomous scale of yes-no responses with scores ranging from 0-24 by summing all of the "yes" responses.

3.4.3 Food Preferences comprised three subscales of the *Food Preference Questionnaire* (FPQ) designed to assess the desire or preference for specific foods (Geiselman et al., 1998).

The FPQ provides hedonic ratings for 72 common food items and was developed as a 2 (FAT: High Fat vs Low Fat) x 3 (Carbohydrate (CHO) Factor: High Simple Sugar, High Complex CHO, and Low CHO/High Protein) matrix with 12 foods listed randomly in each cell (see Appendix G for an example of foods for each of the 6 subscales scale). The three subscales of the FPQ used in this study are i) *High Sugar and High Fat Foods*, which includes the 12 items from the high fat/high simple sugar cell; ii) *High Sugar Foods* includes the 12 items from the High Simple Sugar/Low Fat cell; and iii) *High Fat Foods* includes the 12 items of the High Fat/High Carbohydrates and the 12 items of the High Fat/High Protein subscales (24 items in total). Respondents rated their preference for each of the foods on a 9-point Likert scale: 1 = dislike extremely; 5 = neutral, neither like nor dislike; 9 = like extremely. Higher scores are indicative of a greater liking or preference for a food item (Geiselman et al., 1998). The foods listed on the FPQ vary systematically and significantly with respect to their macronutrient content and are considered to be common sources of macronutrient in Western diets.

3.4.4 Overeating comprised three scales representing different facets of overconsumption towards highly palatable foods.

The subscale of the *Binge Eating Questionnaire* (BEQ; see Appendix H for a copy of the full scale) consists of 5-items where scoring was measured by a dichotomous scale of yes-no responses (Halmi, Falk, & Schwartz, 1981). The item content is used to identify the frequency and severity of symptoms such as loss-of-control, overeating, and negative affect following an episode of binge eating. A higher score is indicative of the severity of the symptoms (Halmi, Falk, & Schwartz, 1981).

Two subscales of the *Eating Behaviour Patterns Questionnaire* (EBPQ) were also included. The *Emotional Eating* subscale consists of 10 items used to assess eating behaviours

during negative moods and/or distressed states and whether the respondent seeks comfort through the intake of palatable foods (Schlundt, Hargreaves, & Buchowski, 2003). The *Snacking on Sweets* subscale consists of 6 items designed to assess how often the respondent snacks on sugary sweets throughout the day and whether on occasion the snacking replaces a regular meal. Both subscales were rated on a 5-point Likert scale rating from 1 (strongly disagree) to 5 (strongly agree) with higher scores indicative of unhealthy eating behaviours (Schlundt et al., 2003). To avoid response bias, 1 item in the *Emotional Eating* subscale has been reverse-scored (see Appendix I for a copy of the Emotional Eating and Snacking on Sweets subscales).

3.5 Genotyping

Genotyping was performed in the Neurogenetics laboratory at CAMH. Each SNP was coded into the *Statistical Package for the Social Sciences Version 24* (SPSS-24) program for analysis with the dependent variables and the relevant demographic variables.

3.6 Statistical Analysis

All analyses were carried out using SPSS-24. The first initial step was to create the four composite variables using the *Principle Component Analysis* (PCA). Second, sex group differences were assessed by employing a one-way multivariate analysis of variance (one-way MANOVA). Due to fact that some DNA resists being genotyped, 18% of the SNP data had missing values.

A series of two-way multivariate of variance analysis (two-way MANOVA) were carried out to determine main effects for each genotype group and sex. Two-way MANOVA considers several dependent variables simultaneously and has the power to detect effects because it takes into account the correlations between dependent variables (Field, 2009; Tabachnick & Fidell, 2007). The independent variables were the genotype group – that is, the major allele

homozygous, minor allele homozygous, and heterozygous – and sex. The separate dependent variables were the PCA- derived factor scores for the reward sensitivity, punishment sensitivity, food preferences, and overeating composite variables. Much research has provided evidence strongly suggesting that OXT influences sex-specific roles in prosocial behaviours. (Borland et al., 2018; Gao et al., 2016). Specifically, increase in OXT in females demonstrate more positive social interactions such as an increase in social communication. Whereas, in contrast, increase of OXT in males tend to demonstrate more protective and aggressive behaviours (Borland et al., 2018; Gao et al., 2016; Shang, Wu, & Su, 2017).

CHAPTER 4: RESULTS

4.1 Composite Variables

As described before in the Methods section, the PCA was used to create the four composite variables. The total scores for each scale, not individual items, were entered into the PCA. Each analysis extracted only one component with an eigenvalue > 1. The component matrix for each composite variable with factor loadings, and the percentage of variance accounted for by the extracted component, is presented in Table 1.

Table 1: *Factor loadings and explained variance for the four composite variables*

Latent variable	*Factor loadings*	*% Explained variance*
Reward Sensitivity (n=420)		64.61
1. Impulsivity	.84	
2. Sensitivity to Reward	.72	
3. Novelty Seeking	.85	
Punishment Sensitivity (n=423)		79.42
1. Behavioural Inhibition	.87	
2. Sensitivity to Punishment	.90	
3. Harm Avoidance	.90	
Food Preference (n=432)		87.76
1. High Fat and High Sugar Foods	.96	
2. High Fat Foods	.92	
3. High Sugar Foods	.94	
Overeating (n=427)		69.69
1. Binge Eating Severity	.84	
2. Emotional Eating	.89	
3. Snacking on Sweets	.77	

4.2 Descriptive Statistics and Chi-Squared Test

Descriptive statistics for all the composite-variable factor scores, including BMI and age, recorded separately for males and females, are presented in Table 2. Results from a one-way MANOVA indicated that females reported a greater tendency to overeat and also greater sensitivity to punishment compared to males. In addition, males reported a greater sensitivity to reward than did females. These findings are consistent with previous research (e.g. Davis et al., 2017). All composite-variable factor scores were screened for normality and were approximately normally distributed and within the acceptable limits of ±2 skewness and kurtosis. A chi-squared (x^2) test was conducted between sex and each SNP to determine the independence - or if a relationship exists - between the two variables. Null results were found for all SNPs except for

the *OXTG* SNP rs4813625. There was a statistically significant association between sex and allele group. The x^2 results for the *OXTG* SNP rs4813625 are presented in Table 3.

Table 2: *Means and standard deviation for all quantitative variables, listed separately for male and female participants*

Variables	*Females*		*Males*			
	N	Means (SD)	N	Means (SD)	*F*	*p-value*
BMI	346	32.42 (9.53)	114	32.16 (8.8)	.07	.799
Age	346	33.34 (6.6)	114	34.34 (7.02)	1.92	.166
Reward Sensitivity Factor Score	315	-.07 (.96)	105	.22 (1.08)	7.16	**.008**
Punishment Sensitivity Factor Score	317	.09 (.98)	106	-.27 (1.0)	10.68	**.001**
Food Preference Factor Score	324	-.04 (1.0)	108	.13 (.98)	2.43	.120
Overeating Factor Score	320	.06 (.98)	107	-.19 (1.04)	5.02	**.026**

Note. BMI, body mass index.

Table 3: *Crosstabulation of OXTG SNP rs4313625 and sex*

Allele Group	**Sex**		***x²***	***p-value***
	Females (%)	*Males (%)*		
CC	20.6 (2.7)	3.7 (-2.7)	8.866	**.012**
CG	31.9 (-2.4)	14.0 (2.4)		
GG	22.3 (.1)	7.4 (-.1)		

Note. Adjusted standardized residuals appear in parentheses beside group percentages.

4.3 Genotype Frequencies

Table 4 lists the genotype frequencies for the 12 SNPs used in the following analyses. In the present study, the homozygous minor-allele group of the following four SNPs: *LNPEP* rs4869317*; CD38* rs3756242*; OXTG* rs2740210 and rs3761248, were combined with the

heterozygous to form a binary genotype variable group due to the very low frequency of the minor allele. The purpose was to create a more dominant model for analysis and to keep consistent with previous research which has done the same (Davis et al., 2017; Jonas et al., 2013; Rujescu, 2012). The remaining 8 SNPs have 3 genotype categories.

Table 4: *Genotype frequencies for the 12 SNPs included in the analyses*

LNPEP	***rs13175726***	**GG**	**AG**	**AA**	**Total**
Frequency		57	201	239	497
	rs18059	**CC**	**CT**	**TT**	
		135	207	152	494
	rs316206	**AA**	**AG**	**GG**	
		59	219	218	496
	rs3849749	**AA**	**AT**	**TT**	
		74	207	216	497
	rs4869315	**AA**	**AG**	**GG**	
		119	227	151	497
	rs4869317	**AA**	**AT**	**TT**	
		31	192	268	491
CD38	***rs3796863***	**TT**	**GT**	**GG**	
Frequency		68	230	199	497
	rs3756242	**AA**	**AC**	**CC**	
		12	154	331	497
OXTG	***rs2740210***	**AA**	**AC**	**CC**	
Frequency		41	201	255	497
	rs877172	**GG**	**GT**	**TT**	
		76	211	200	487
	rs3761248	**CC**	**CT**	**TT**	
		21	124	353	498
	rs4813625	**CC**	**CG**	**GG**	
		115	232	151	498

Note. LNPEP, leucyl-cystinyl aminopeptidase; CD38, cluster of differentiation 38; OXTG, oxytocin peptide gene.

4.4 Two-way Multivariate Analysis of Variance (two-way MANOVA)

A series of two-way MANOVAs were carried out to assess group differences for each of the 12 OXT SNPs across the four dependent variables; reward sensitivity, punishment

sensitivity, food preference, and overeating. The use of two-way MANOVA procedures requires nine assumptions: (1) two or more dependent variables are measured at either the ratio or interval level; (2) the two independent variables in the current study consists of two or more categorical groups; (3) no relationship exists between the observations in each group of the independent variables or between the group themselves; (4) there must be a sufficient sample size; (5) there are no univariate or multivariate outliers; (6) there must be multivariate normality; (7) a linear relationship should exist between each pair of dependent variables within each group of the two independent variables; (8) should have a homogeneity of variance-covariance matrices; (9) no multicollinearity problems must be present.

The distribution of the data satisfied the assumptions for the univariate and multivariate analyses. The first four assumptions cannot be tested. These assumptions are about understanding the study design and making sure the independent and dependent variables meet the requirements of a two-way MANOVA. Specifically, assumption 1 satisfied the two-way MANOVA procedures because the dependent variables in the current study were measured at a continuous level. Assumption 2 was met because the two independent variables - genotype group and sex - consisted of at least two levels. For assumption 3, genotyping results indicated which genotype group the participant was assigned too. No participant could be placed into more than one genotype group per SNP. Similarly, for sex the participant either indicated male or female, therefore assumption 3 was also met. In order to satisfy assumption 4, the sample size was deemed sufficient because there were more cases in each group then the number of dependent variables being analyzed in the current study. To test for the remainder assumptions, SPSS was employed to determine whether the assumptions 5 through to 9 satisfied the two-way MANOVA procedures. Specifically, the 5th assumption was tested by employing boxplots to detect

univariate outliers and the Mahalanobis distance to detect multivariate outliers. No outliers were found at the $p < .05$ level. Assumption 6 was tested using the Shapiro-Wilk test of normality. Histogram and boxplots were created and upon visual inspection, all were deemed satisfactory. The food preference variable was negatively skewed, however was still within the ±2 skewness and deemed acceptable. Assumption 7, scatterplots of the residuals were generated where visual inspection of the plots deemed the assumption satisfactory. For assumption 8, the homogeneity of the variance-covariance matrices, were tested and the homoscedasticity of samples was confirmed by Box-M and Levene Tests (all p-values > 0.05). The 9th assumption was confirmed by employing a series of multiple regressions where the variance inflation factors (VIF) scores were all below the cut-off (all VIF values < 3.0). It is important to note that Wilks' Lambda was selected as the multivariate test because it is the most widely-used multivariate statistic when the independent variable(s) forms more than two groups (Field, 2009; Tabachnick & Fidell, 2007).

4.4.1 Two-way MANOVA for the *LNPEP* SNPs

Separate analyses were carried out for the six SNPs of the *LNPEP* gene. Wilks' Lambda indicated nonsignificant main effects for genotype on all six SNPs. As anticipated from the univariate analyses, the multivariate test for sex was significant in each analysis. Univariate tests revealed that males reported a greater reward sensitivity. However, females reported greater punishment sensitivity and overeating. Nonsignificant sex effects were found for food preference. Additionally, no significant interactions were found (all p-values > .05). Table 5 presents the summary statistics for the multivariate and univariate analyses found for each of the *LNPEP* SNP.

Table 5: *Multivariate and univariate effects for the LNPEP SNPs and sex with the four composite variables as dependent variables*

rs13175726						
			Multivariate Effects			
Independent Variable	**Wilks' Λ**	***df* (effect)**	***df* (error)**	***F***	***p***	***ηρ²***
rs13175726	.977	8	794	1.178	.309	.012
Sex	.947	4	397	5.567	**< .0001**	.053
			Univariate Effects			
Independent Variable	**Dependent Variable**	**Type III Sum of Squares**	**df**	***F***	***p***	***ηρ²***
Sex	RS	6.969	1	7.142	**.008**	.018
	PS	4.409	1	4.432	**.036**	.011
	FP	2.203	1	2.163	.142	.005
	OE	4.964	1	4.964	**.026**	.012
rs18059						
			Multivariate Effects			
Independent Variable	**Wilks' Λ**	***df* (effect)**	***df* (error)**	***F***	***p***	***ηρ²***
rs18059	.966	8	794	1.734	.087	.017
Sex	.945	4	397	5.749	**< .0001**	.055
			Univariate Effects			
Independent Variable	**Dependent Variable**	**Type III Sum of Squares**	**df**	***F***	***p***	***ηρ²***
Sex	RS	4.934	1	5.118	**.024**	.013
	PS	9.254	1	9.310	**.002**	.023
	FP	1.200	1	1.204	.273	.003
	OE	6.003	1	6.012	**.015**	.015
rs316206						
			Multivariate Effects			
Independent Variable	**Wilks' Λ**	***df* (effect)**	***df* (error)**	***F***	***p***	***ηρ²***
rs316206	.980	8	792	.980	.417	.010
Sex	.940	4	396	6.359	**< .0001**	.060
			Univariate Effects			
Independent Variable	**Dependent Variable**	**Type III Sum of Squares**	**df**	***F***	***p***	***ηρ²***
Sex	RS	6.387	1	6.513	**.011**	.016
	PS	6.948	1	6.968	**.009**	.017
	FP	2.306	1	2.279	.132	.006
	OE	6.473	1	6.434	**.012**	.016
rs3849749						
			Multivariate Effects			
Independent Variable	**Wilks' Λ**	***df* (effect)**	***df* (error)**	***F***	***p***	***ηρ²***
rs3849749	.982	8	796	.910	.508	.009
Sex	.946	4	398	5.700	**< .0001**	.054

Table 5 (continued).

Univariate Effects						
Independent Variable	**Dependent Variable**	**Type III Sum of Squares**	**df**	***F***	***p***	***ηρ²***
Sex	RS	6.116	1	6.165	**.013**	.015
	PS	6.134	1	6.160	**.013**	.015
	FP	4.083	1	4.069	.054	.010
	OE	3.700	1	3.719	**.044**	.009
rs4869315						
Multivariate Effects						
Independent Variable	**Wilks' Λ**	***df* (effect)**	***df* (error)**	***F***	***p***	***ηρ²***
rs4869315	.987	8	796	.632	.752	.006
Sex	.930	4	398	7.466	**< .0001**	.070
Univariate Effects						
Independent Variable	**Dependent Variable**	**Type III Sum of Squares**	**df**	***F***	***p***	***ηρ²***
Sex	RS	6.508	1	6.628	**.010**	.016
	PS	10.844	1	10.832	**.001**	.026
	FP	2.718	1	2.686	.102	.007
	OE	7.224	1	7.305	**.007**	.018
rs4869317						
Multivariate Effects						
Independent Variable	**Wilks' Λ**	***df* (effect)**	***df* (error)**	***F***	***p***	***ηρ²***
rs4869317	.988	4	397	1.214	.305	.012
Sex	.937	4	397	6.657	**< .0001**	.063
Univariate Effects						
Independent Variable	**Dependent Variable**	**Type III Sum of Squares**	**df**	***F***	***p***	***ηρ²***
Sex	RS	5.426	1	5.556	**.019**	.014
	PS	11.195	1	11.246	**.001**	.027
	FP	2.142	1	2.130	.145	.005
	OE	6.208	1	6.259	**.013**	.015

Note. RS: Reward Sensitivity; PS: Punishment Sensitivity; FP: Food Preference; OE; Overeating.

4.4.2 Two-way MANOVA for the *CD38* SNPs

Separate analyses were carried out for the two SNPs of the *CD38* gene. Wilks' Lambda indicated nonsignificant main effects on the two SNPs. As anticipated from the univariate analyses, the multivariate test for sex was significant in each analysis. Univariate tests revealed that males reported a greater reward sensitivity. However, females reported greater punishment

sensitivity and overeating. Nonsignificant sex effects were found for food preference. Additionally, no significant interactions were found (all p-values > .05). Table 6 presents the summary statistics for the multivariate and univariate analyses found for each of the *CD38* SNP.

Table 6: *Multivariate and univariate effects for the CD38 SNPs and sex with the four composite variables as dependent variables*

rs3796863						
		Multivariate Effects				
Independent Variable	**Wilks' Λ**	***df* (effect)**	***df* (error)**	***F***	***p***	***ηp²***
rs3796863	.973	8	796	1.382	.200	.014
Sex	.947	4	398	5.616	**< .0001**	.053
		Univariate Effects				
Independent Variable	**Dependent Variable**	**Type III Sum of Squares**	**df**	***F***	***p***	***ηp²***
Sex	RS	2.343	1	2.388	.123	.006
	PS	12.404	1	12.473	**< .0001**	.030
	FP	.466	1	.488	.503	.001
	OE	8.520	1	8.541	**.004**	.021
rs3756242						
		Multivariate Effects				
Independent Variable	**Wilks' Λ**	***df* (effect)**	***df* (error)**	***F***	***p***	***ηp²***
rs3756242	.984	4	399	1.649	.161	.016
Sex	.929	4	399	7.582	**< .0001**	.071
		Univariate Effects				
Independent Variable	**Dependent Variable**	**Type III Sum of Squares**	**df**	***F***	***p***	***ηp²***
Sex	RS	7.293	1	7.436	**.007**	.018
	PS	12.229	1	12.229	**< .0001**	.030
	FP	3.076	1	3.059	.081	.008
	OE	5.005	1	4.999	**.026**	.012

Note. RS: Reward Sensitivity; PS: Punishment Sensitivity; FP: Food Preference; OE; Overeating.

4.4.3 Two-way MANOVA for the *OXTG* SNPs

Separate analyses were carried out for the four SNPs of the *OXTG* gene. Wilks' Lambda indicated nonsignificant main effects for genotype on all four SNPs. As anticipated from the

univariate analyses, the multivariate test for sex was significant in each analysis. Univariate tests revealed that males reported a greater reward sensitivity. However, females reported greater punishment sensitivity and overeating. Nonsignificant sex effects were found for food preference. Additionally, no significant interactions were found (all p-values > .05). Table 7 presents the summary statistics for the multivariate and univariate analyses found for each of the *OXTG* SNP.

Table 7: *Multivariate and univariate effects for the OXTG SNPs and sex with the four composite variables as dependent variables*

rs2740210						
		Multivariate Effects				
Independent Variable	**Wilks' Λ**	***df* (effect)**	***df* (error)**	***F***	***p***	***ηρ²***
rs2740210	.996	4	400	.412	.800	.004
Sex	.933	4	400	7.148	**< .0001**	.067
		Univariate Effects				
Independent Variable	**Dependent Variable**	**Type III Sum of Squares**	**df**	***F***	***p***	***ηρ²***
Sex	RS	7.324	1	7.436	**.007**	.018
	PS	10.549	1	10.634	**.001**	.026
	FP	3.020	1	3.005	.084	.007
	OE	5.086	1	5.079	**.025**	.012
rs877172						
		Multivariate Effects				
Independent Variable	**Wilks' Λ**	***df* (effect)**	***df* (error)**	***F***	***p***	***ηρ²***
rs877172	.981	8	776	.919	.500	.009
Sex	.943	4	388	5.869	**< .0001**	.057
		Univariate Effects				
Independent Variable	**Dependent Variable**	**Type III Sum of Squares**	**df**	***F***	***p***	***ηρ²***
Sex	RS	3.747	1	3.766	.053	.010
	PS	9.380	1	9.604	**.002**	.024
	FP	3.490	1	3.499	.062	.009
	OE	5.595	1	5.573	**.019**	.014
rs3761248						
		Multivariate Effects				
Independent Variable	**Wilks' Λ**	***df* (effect)**	***df* (error)**	***F***	***p***	***ηρ²***
rs3761248	.980	4	400	2.071	.084	.020
Sex	.965	4	400	3.652	**.006**	.035

Table 7 (continued).

Univariate Effects						
Independent Variable	**Dependent Variable**	**Type III Sum of Squares**	**df**	***F***	***p***	***ηp²***
Sex	RS	4.084	1	4.177	**.042**	.010
	PS	5.147	1	5.235	**.023**	.013
	FP	.930	1	.919	.338	.002
	OE	2.802	1	2.800	.095	.001
rs4813625						
Multivariate Effects						
Independent Variable	**Wilks' Λ**	***df* (effect)**	***df* (error)**	***F***	***p***	***ηp²***
rs4813625	.987	8	796	.650	.735	.006
Sex	.939	4	398	6.483	**< .0001**	.061
Univariate Effects						
Independent Variable	**Dependent Variable**	**Type III Sum of Squares**	**df**	***F***	***p***	***ηp²***
Sex	RS	6.371	1	6.464	**.011**	.016
	PS	10.386	1	10.503	**.001**	.026
	FP	1.679	1	1.653	.199	.004
	OE	5.590	1	5.572	**.019**	.014

Note. RS: Reward Sensitivity; PS: Punishment Sensitivity; FP: Food Preference; OE; Overeating.

CHAPTER 5: DISCUSSION

The present study was a preliminary and exploratory investigation into the links between markers of the *OXTG, CD38,* and *LNPEP* genes and overeating and its relevant endophenotypes such as sensitivity to reward, sensitivity to punishment, and food preferences. This endeavor was an extension of the recent study by Davis et al., (2017) where SNPs of the *OXTR* were found to be significantly associated with the dependent variables listed above. Contrary to our expectations, none of the SNPs reached statistical significance across any of the four dependent variables. Possible explanations for the nonsignificant findings are discussed.

One consideration that may have contributed to the null findings relates to the *biological mechanisms* of the genes investigated in the current study. The OXT system is extremely complex and depends on many genes in order for the hormone to function properly (Ebstein et al., 2012; Kasperek-Zimowska et al., 2016; Zhang et al., 2017). In addition, the OXT pathway is extensive and exerts a wide range of central and peripheral effects, therefore the genes that assist with the production, synthetization, or secretion of OXT may not have a direct effect on the behaviours influenced by OXT, but instead a polygenic influence giving it a very small and indirect effect on human behaviours, such as eating and related behaviours (Ding et al., 2017; Konttinen et al., 2015; Rokicki et al., 2019; Solmi, Mascarell, Zammit, Kirkbride, & Lewis, 2019).

5.1 Biological Mechanisms of the *OXTG* Gene

The *OXTG* synthesizes the precursor protein – neurophysin-I – which is responsible for the production of mature OXT (Ebstein et al., 2012; Kasperek-Zimowska et al., 2016; Zhang et al., 2017). Previous research has suggested that the *OXTG* gene influence social behaviours, however, to our knowledge, there have been no such human studies directly investigating this link (Ebstein et al., 2012; Kasperek-Zimowska et al., 2016; Zhang et al., 2017). In a recent study, Rokicki et al. (2019) investigated OXT-gene pathway networks in the brain by examining the *OXTG, OXTR,* and *CD38* genes because of their association with social behaviours in human and animal research. Using six donor brains they collected a full dataset of 20,737 protein coding genes which included the genes of interest and other genes that co-express with OXT-pathway genes such as a dopaminergic set, muscarinic acetylcholine set, a vasopressin set, and a opioid set (Rokicki et al., 2019). The purpose of the study was to provide a better understanding of the OXT pathway system as it relates to cognitive processing. In addition, they examined the

influence of OXT pathways in the context of metabolic and feeding regulation. These authors found that although there was a strong presence of *OXTG* expression within hypothalamic regions, the *OXTG* did not demonstrate strong whole-brain correspondence with other genes, such as *OXTR* and *CD38*, and *OXTG* also did not demonstrate strong links to feeding regulation (Rokicki et al., 2019). Additionally, they found that expression patterns of the *OXTG* were less stable from donor to donor making it difficult to comment on the genes possible influence, if any, on human behaviours (Rokicki et al., 2019). These current results raise the possibility that the *OXTG* may not be on the frontline in influencing complex behaviours such as social and feeding behaviors, but instead its influence and crucial role is further up the brain network giving it a more distal effect. This might also explain the limited body of research on this gene and its associations with human behaviours vital for survival.

5.2 Biological Mechanisms of the *LNPEP* Gene

The *LNPEP* gene it is a leucyl-cystinyl aminopeptidase protein coding gene, whose protein metabolizes and inactivates OXT, vasopressin, and other peptide hormones within brain tissue (Ebstein et al., 2012; Zhang et al., 2017). *LNPEP* has been established as an enzyme and is recognized as an oxytocinase due to its disintegrating properties on OXT (Ebstein et al., 2012; Zhang et al., 2017). There are very few studies on *LNPEP,* therefore little research linking it to human behavioural phenotypes, such as eating and personality factors. Much of what is known about *LNPEP* surrounds its influence on maintaining healthy pregnancies (Ebstein et al., 2012; Hill et al., 2011; Johnson et al., 2009), and energy expenditure (Niwa et al., 2015). As such, *LNPEP* is becoming increasingly recognized for its role in maintaining homeostasis for a variety of physiological functions (Ebstein et al., 2012; Hill et al., 2011; Johnson et al., 2009). Although previous studies have speculated about the genes potential regulatory role on social behaviours -

including the social deficits associated with autism because of its influence on central OXT (Zhang et al., 2017) - there are no studies directly linking *LNPEP* to such human behaviours.

The reason there is very little research linking *LNPEP* to human behavioural phenotypes may be because the gene is more involved in the physiological mechanisms that keep the human body alive and functioning. For instance, the *LNPEP* gene encodes for the human insulin-regulated aminopeptidase (IRAP), the placental leucine aminopeptidase (P-LAP), leukocyte-derived aminopeptidase (L-RAP), and adipocyte-derived leucine aminopeptidase (A-LAP), which are considered to be part of the distinct group of the M1 family - oxytocinase subfamily (Ebstein et al., 2012; Hill et al., 2011; Johnson et al., 2009). There is strong evidence that this subfamily plays a vital role in maintaining homeostasis in the human body (Ebstein et al., 2012; Hill et al., 2011; Johnson et al., 2009). One of the important homeostatic roles is regulating the circulating levels of oxytocin during pregnancy by modulating the OXT neurotransmission (Ebstein et al., 2012; Hill et al., 2011; Johnson et al., 2009; Pham et al., 2009). In addition, there is evidence that the *LNPEP* gene regulates the body's energy metabolism (Niwa et al., 2015). For instance, in the gene knockout mice it was found that the deficiency of IRAP reduced weight gain in mice who consumed a high-fat diet compared to the wild-type mice (Niwa et al., 2015). Both groups consumed the same amount of high-fat foods and their observed feeding behaviours were also consistent – that is, no differences in feeding behaviours were observed (Niwa et al., 2015). This study demonstrated the *biological mechanisms* of the *LNPEP* gene in metabolic activity and suggests that an IRAP deficiency could lead to an increase in energy expenditure potentially preventing the development of obesity (Niwa et al., 2015). It is also important to note, that in a study of maternal behaviour, such as pup retrieval, feeding, and nurturing, results

indicated that there were no observed differences between the knockout mice and the wild type dams (Pham et al., 2009).

By contrast, a recent study conducted by Elkins et al. (2017) indicated a potential link between the *LNPEP* gene and behavioural phenotypes. These investigators found that the *LNPEP* gene was associated with social behaviours in the teleost fish, specifically regarding some aspects of social status (Elkins et al., 2017). However, these findings were obtained from non-human studies therefore may not extrapolate well to human studies. In human studies, research continues to support the gene's role in homeostasis. In addition, genetic variations in the *LNPEP* gene were significantly associated with increased 28-day mortality in patients with septic shock (Nakada et al., 2011). Variants of the gene were also associated with vasopressin clearance and serum sodium regulation (Nakada et al., 2011). Specifically, the rs4869317 TT genotype was significantly associated with increased 28-day mortality and accounted for 80% of the increased plasma vasopressin clearance (Nakada et al., 2011). In summary, the body of research investigated, to date, demonstrates that the *LNPEP* gene may not be directly involved in human behaviours. Instead, the gene may play an influencing role in the physiological mechanisms involved in maintaining homeostasis in the human body.

5.3 Biological Mechanisms of the *CD38* Gene

In the present study, neither of the *CD38* gene SNPs – rs3796863 and rs3756242 - had significant associations with any of the four dependent variables. The *CD38* gene expresses an ADP-ribosyl cyclase type II transmembrane protein and is involved in the central regulation and secretion of OXT (Higashida et al., 2012; Kasperek-Zimowska et al., 2016; Tabak et al., 2016.; Zhang et al., 2017). As such, research has linked the *CD38* gene to levels of OXT availability in the brain for which OXT is highly expressed in the hypothalamus, supraoptic nucleus, and the

lateral hypothalamic area (Higashida et al., 2012; Jin et al., 2007; McInnis et al., 2017; Rokicki et al., 2019). Although there is evidence suggesting that the *CD38* gene contributes to the individual differences found in social behaviours – specifically to symptoms of associated with ASD such as extreme avoidance - there is still much research needed to understand the mechanisms involved (Chong et al., 2017; Higashida et al., 2012; Zhang et al., 2017)

CD38 has been the focus of recent genetic investigations in the field of social processes and behaviours (Feldman et al., 2016). However, there is very little research specific to eating related behaviours. Though the current findings on the two CD38 SNPs were not significant, there are possible explanations that need to be considered. As previously mentioned with regard to the other two genes investigated in the current study – *OXTG* and *LNPEP* – concerning the evidence that they have distal effects on OXT availability, this is unlikely to be the case for the *CD38* gene. In a recent study, *CD38* was found to contribute to the expression of OXT in areas of the brain associated with feeding behaviours and its contribution was always paired with the expression of other genes (Rokicki et al., 2019). Specifically, *CD38* was highly co-expressed with *OXTR* (Rokicki et al., 2019). In previous research, there is evidence that the behavioural effects of OXT are critically dependent on *OXTR* suggesting that expression of *OXTR* directly modulates human behaviours (Rokicki et al., 2019; Toepfer et al., 2017). Therefore, this study may demonstrate that the influence of the *CD38* gene is dependent on the interaction and expression of other genes - specifically *OXTR* - thereby possibly explaining the null results found in the present study.

5.4 Male-Female Differences

The present study indicated that males reported significantly greater sensitivity to reward while females indicated significantly greater punishment sensitivity and overeating. However, no

significant sex differences were detected for food preferences. Although the findings demonstrate that males reported a greater reward sensitivity compared to females, previous research has provided evidence suggesting that this is not always the case (Legget et al., 2018; Ma et al., 2018; Soutschek et al., 2017). Relative to males, females tend to report greater reward dependence and greater intensity of reward signals (Becker & Chartoff, 2018; Westbrook, Hankosky, Dwyer, & Gulley, 2018), whereas males report higher sensation seeking and tend to engage in riskier and more impulsive behaviours (Shulman, Harden, Chein, & Steinberg, 2014; Westbrook et al., 2018). As such, it is important to consider that the current findings may have been influenced by the composite nature of the four dependent variables, each of which was a factor score derived from a PCA of three independent measures reflecting the multifaceted aspects of the construct. As such, the reward sensitivity factor may been biased towards the impulsivity construct which relates to a greater extent to the reward-seeking behaviours found in males (Cross, Copping, & Campbell, 2011). If so, this could account for the results in the present study.

As stated before, in the present study it was found that males indicated greater sensitivity to reward. Relative to females, males have been found to engage in riskier behaviours and have indicated greater reward-seeking impulses (Becker & Chartoff, 2018; Cross et al., 2011; Eneva et al., 2017; Shulman et al., 2014; Westbrook et al., 2018). Testosterone is a vital hormone which interacts and regulates OXT to influence the aggressive behaviours in males and there is evidence that testosterone levels are positively correlated with sensation-seeking and impulsivity (Cross et al., 2011; Fragkaki, Cima, & Granic, 2018). From an evolutionary perspective, the greater reward drive found in males may reflect an underlying psychological mechanism in male-on-male aggression (Cross et al., 2011; Fragkaki et al., 2018). In our current environment,

this increased desire for risk now manifests itself as greater risk-taking behaviours, such as drug use and gambling (Becker & Chartoff, 2018; Cross et al., 2011). In other words, an evolutionary mechanism that has served for centuries to ensure survival, has now become disadvantageous in risky environments (Becker & Chartoff, 2018; Cross et al., 2011).

However, research indicates that females tend to report a greater sensitivity to the properties of reward related to reproduction (Legget et al., 2018; Ma et al., 2018; Soutschek et al., 2017). Relative to males, females are also found to have a greater sensitivity to the rewarding properties of food and pair-bonding (Legget et al., 2018; Richard et al., 2017). As such, confirming previous research, the current findings indicate that females reported greater overeating behaviours (Camilleri et al., 2014; Legget et al., 2018). Neural imaging of the reward-related region of the brain – viz. the nucleus accumbens - found that females demonstrated greater reactivity to hedonic food than their male colleagues (Legget et al., 2018; Richard et al., 2017). This suggests that females may be more sensitive to the rewarding properties of palatable foods influencing more overeating behaviours compared to males (Legget et al., 2018; Richard et al., 2017). Females also reported a greater tendency to engage in binge-eating behaviours during stressful situations (Calvez & Timofeeva, 2016; Legget et al., 2018). For instance, it was found that adolescent females reported stress-related eating behaviours significantly greater compared to adolescent males and that consuming palatable foods served as a coping mechanism for which aided in decreasing stress-like symptoms (Jääskeläinen et al., 2014). A possible explanation for the sex differences on stress-related and overeating behaviours is the estrous cycle in females (Egan et al., 2018). For example, during the luteal phase - the phase of the menstrual cycle that begins after ovulation and ends right before the period begins – is when the greatest amount of emotional eating is found in females (Egan et al., 2018). It is important to note, that this is the

phase of the menstrual cycle where estrogen levels are relatively high (Egan et al., 2018). Most reward and feeding-related brain regions – hypothalamus and nucleus accumbens - express estrogen receptors and when estrogen levels are high, an increase in reward-seeking behaviours are observed in females (Egan et al., 2018; Legget et al., 2018). A compelling body of research has indicated that estrogen regulates OXT and enhances dopamine signaling in the brain (Love et al., 2012). However, further research is needed to fully understand the mechanisms involved in how high levels of estrogen regulate OXT to influence overeating behaviours in females (Legget et al., 2018; Ma et al., 2018; Soutschek et al., 2017).

The current findings also found that females indicated a greater sensitivity to punishment compared to males. Such results are consistent with past research where sex differences in risk aversion have been well documented indicating that females are generally more risk-averse (Brand, Brown, & Cross, 2018; Cross et al., 2011; Davis, 2014; Lynn, Hoge, Fischer, Barrett, & Simon, 2014; Sapienza, Zingales, & Maestripieri, 2009). Evolutionary perspectives suggest this is because females reproductive success depends largely on avoiding injury or death (as cited in Cross et al., 2011). There is good evidence that punishment sensitivity is positively correlated with disordered eating (Dietrich, Federbusch, Grellmann, Villringer, & Horstmann, 2014). And, females who are sensitive to signals of punishment reported turning to food as an emotional coping mechanism (Bartkiene et al., 2019; Dietrich et al., 2014). Relative to females, males generally demonstrate stronger control over emotional events, and low punishment responsiveness and impulsive behaviours (Dietrich et al., 2014; Ding et al., 2017; Santesso, Dzyundzyak, & Segalowitz, 2011). In summary, the current findings are consistent with past research suggesting that females generally indicate a greater sensitivity to punishment relative to males (Bartkiene et al., 2019; Cross et al., 2011; Dietrich et al., 2014).

Despite some evidence suggesting there are sex differences in food preferences, (Abdella, Farssi, Broom, Hadden, & Dalton, 2019; Bartkiene et al., 2019; Wansink, Cheney, & Chan, 2003), the current findings did not support this. In previous research, it has been found that males reported greater habitual intake of fats and prefer hearty meal-related foods, such as steak (Padulo et al., 2017; Wansink et al., 2003). Relative to males, females reported lower intake of fats and a greater preference for sweet, snack-related foods, such as chocolate, ice cream and cookies (Bartkiene et al., 2019; Padulo et al., 2017; Wansink et al., 2003). Food preferences for sweet and salty foods have been positively correlated with sensitivity to reward (Leng & Sabatier, 2017; Olszewski, Wood, Klockars, & Levine, 2019; Stokes, Davies, Lattimore, Winstanley, & Rogers, 2019). The consumption of palatable foods increases dopamine signaling within brain reward regions, sequentially increasing the experience of pleasure (Kleinridders & Pothos, 2019; Olszewski et al., 2019) The experience of pleasure then becomes detrimental as it increases reward-seeking and overeating behaviours (Olszewski et al., 2019). Females typically report greater consumption of sweets compared to their male counterparts (Olszewski et al., 2019). However, the mechanisms involved to explain why females prefer and have enhanced cravings for sweet foods are poorly understood (Olszewski et al., 2019). Although the current findings found no significant sex differences regarding food preferences, these results could be explained by the composite nature of the food preference factor score derived from the PCA which included foods both high in fat and sugar.

5.5 Limitations and Future Directions

While this study is entirely exploratory, there was relative consistency across three of the four dependent variables with regard to sex differences. Specifically, males reported greater reward sensitivity and females reported greater punishment sensitivity and overeating. However,

although there were no significant relationships between any of the 12 SNPs and the dependent variables, these findings must be considered within the study's limitations. For instance, although there was an adequate sample size in the current study, genome-wide association studies (GWAS) may improve the identification of associations between genetic regions and behavioural phenotypes or traits because of its success in identifying thousands of SNPs and their associations with diseases and traits (Rokicki et al., 2019; Soiza, Donaldson, & Myint, 2018).

Future research would also benefit from the study of gene x gene interactions. For instance, it is becoming clear that a single gene only accounts for a small portion of phenotypic variance. It is possible that different OXT genes, such as *CD38, OXTG,* and *LNPEP,* may be dependent on each other in order for the phenotypic characteristics to become prominent. In addition, the OXT SNPs investigated in the current study are not the only SNPs located on the *CD38, OXTG,* or *LNPEP* genes. There are other SNPs that may be of interest that could allow for greater understanding into the associations of OXT genes with eating behaviours. For instance, the rs4813627 SNP of the *OXTG* has been linked to prosocial behaviours in females, such as maternal bonding (Mileva-Seitz et al., 2013). In addition, the *OXTG* SNP rs2770378 has been linked to social deficits found in Autism such as the language impairment (Hovey et al., 2014). The rs6449182 SNP of the *CD38* gene has also been associated with prosocial behaviours specifically the behavioural expression of gratitude (Algoe & Way, 2013). It may be of interest in research to investigate these SNPs to determine if they are significantly associated with other prosocial behaviours that are related to overeating behaviours.

Finally, it is also important to emphasize that this was the first study to examine relationships between the OXT SNPs and overeating and relevant endophenotypes such as

reward sensitivity, punishment sensitivity, and food preference. Replication in other samples is necessary to confirm our findings, especially since some of the SNPs investigated in the current study have been previously associated with prosocial behaviours that are theoretically connected with overeating and relevant endophenotypes of overeating (Ebstein et al., 2012; Feldman et al., 2016; Francis et al., 2016; Kasperek-Zimowska et al., 2016; Love et al., 2012; Love et al., 2018; McInnis et al., 2017; McQuaid, et al., 2016; Mickey et al., 2013; Olofsdotter et al., 2018; Parris et al., 2018; Zhang et al., 2017). In addition, a different sample may allow for greater insight by providing opportunity to assess environmental factors that may moderate the relationship between OXT and genetic associations with eating behaviours, such as cultural and economic factors.

5.4 Summary and Conclusions

The current study provides confirmation of the differences found between males and females on overeating and relevant endophenotypes such as reward and punishment sensitivity. In this study, females indicated greater overeating and punishment sensitivity while males reported greater sensitivity to reward. These results are consistent with past research highlighting sex differences on behaviours with a strong evolutionary component, such as eating and social interactions. The gonadal hormones, particularly estrogen and testosterone, are becoming increasingly evident for their role in the observed sex differences (Cross et al., 2011; Egan et al., 2018). In addition, while OXT is well-established for its influence on many survival behaviours, gonadal hormones are now recognized for their regulating role on the effects of OXT (Cross et al., 2011; Egan et al., 2018).

Although there were no significant associations between any of the 12 SNPs and the four independent variables, replication is necessary to confirm the results, especially since the SNPs

investigated have been found in previous studies to be significantly associated with prosocial behaviours. It is also important to consider that the effect of any particular gene is relatively small in the context of complex human behaviours. Therefore, it is important to examine the interaction of SNPs on such behaviours. In addition, it is also of great interest to consider other OXT genetic markers involved in the availability and influence of OXT levels in the brain in order to uncover further associations.

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Appendix A
Barratt Impulsivity Scale – 11

Items	***Rarely/Never***	***Occasionally***	***Often***	***Almost Always/Always***
1. I plan tasks carefully				
2. I do things without thinking.				
3. I make up my mind quickly.				
4. I am happy-go-lucky.				
5. I don't "pay attention."				
6. I have "racing" thoughts.				
7. I plan trips well ahead of time.				
8. I am self-controlled.				
9. I concentrate easily.				
10. I save regularly.				
11. I "squirm" at plays or lectures.				
12. I am a careful thinker.				
13. I plan for job security.				
14. I say things without thinking.				
15. I like to think about complex problems.				
16. I change jobs.				
17. I act "on impulses".				
18. I get easily bored when solving thought problems.				
19. I act on the spur of the moment.				
20. I am a steady thinker.				
21. I change residences.				
22. I buy things on impulses.				
23. I can only think about one problem at a time.				
24. I change hobbies.				
25. I spend or change more than I earn.				
26. I often have extraneous thoughts when thinking.				
27. I am more interested in the present than the future.				
28. I am restless at the theatre or lectures.				
29. I like puzzles.				
30. I am future oriented.				

Appendix B
Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ)
Reward Subscale

	Items	***Yes***	***No***
1.	Does the good prospect of obtaining money motivate you strongly to do some things?	**Y**	**N**
2.	Are you frequently encouraged to act by the possibility of being valued in your work, in your studies, with your friends or with your family?	**Y**	**N**
3.	Do you often meet people that you find physically attractive?	**Y**	**N**
4.	Do you like to take some drugs because of the pleasure you get from them?	**Y**	**N**
5.	Do you often do things to be praised?	**Y**	**N**
6.	Do you like being the center of attention at a party or a social meeting?	**Y**	**N**
7.	Do you spend a lot of your time on obtaining a good image?	**Y**	**N**
8.	Do you need people to show their affection for you all the time?	**Y**	**N**
9.	When you are in a group, do you try to make your opinions the most intelligent or the funniest?	**Y**	**N**
10.	Do you often take the opportunity to pick up people you find attractive?	**Y**	**N**
11.	As a child, did you do a lot of things to get people's approval?	**Y**	**N**
12.	Does the possibility of social advancement move you to action, even if this involves not playing fair?	**Y**	**N**
13.	Do you generally give preference to those activities that imply an immediate gain?	**Y**	**N**
14.	Do you often have trouble resisting the temptation of doing forbidden things?	**Y**	**N**
15.	Do you like to compete and do everything you can to win?	**Y**	**N**
16.	Is it easy for you to associate tastes and smells to very pleasant events?	**Y**	**N**
17.	Are there a large number of objects or sensations that remind you of pleasant events?	**Y**	**N**
18.	When you start to play with a slot machine, is it often difficult for you to stop?	**Y**	**N**
19.	Do you sometimes do things for quick gains?	**Y**	**N**
20.	Does your attention easily stray from your work in the presence of an attractive stranger?	**Y**	**N**
21.	Are you interested in money to the point of being able to do risky jobs?	**Y**	**N**
22.	Do you like to put competitive ingredients in all of your activities?	**Y**	**N**
23.	Would you like to be a socially powerful person?	**Y**	**N**
24.	Do you like displaying your physical abilities even though this may involve danger?	**Y**	**N**

Appendix C
The Tridimensional Personality Questionnaire (TPQ)
Novelty Seeking Scale

In this questionnaire you will find statements people might use to describe their attitudes, opinions, interests, and other personal feelings. Each statement can be answered YES or NO. Read the statement and decide which choice best describes you. Circle the Y or N after each question. Read each statement carefully but don't spend too much time deciding to answer. Please answer every statement, even if you are not completely sure of the answer. Remember, there are no right or wrong answers – just describe your own personal opinions and feelings.

	Items	***Yes***	***No***
1.	I often try new things just for fun or thrills, even if most people think it is a waste of time.	**Y**	**N**
2.	When nothing new is happening, I usually start looking for something that is thrilling and exciting.	**Y**	**N**
3.	I usually demand very good practical reasons before I am willing to change my old ways of doing things. (R)	**Y**	**N**
4.	I hate to change the way I do things, even if many people tell me there is a new and better way of doing it. (R)	**Y**	**N**
5.	I like it when people can do whatever they want without strict rules and regulations.	**Y**	**N**
6.	I like to be very organized and set rules for people whenever I can. (R)	**Y**	**N**
7.	Even when most people feel it is not important, I often insist on things being done in a strict and orderly fashion. (R)	**Y**	**N**
8.	I often do things based on how I feel at the moment without thinking about how they were done in the past.	**Y**	**N**
9.	I often break rules and regulations when I think I can get away with it.	**Y**	**N**
10.	I lose my temper more quickly than most people.	**Y**	**N**
11.	I often react so strongly to unexpected news that I say or do things that I regret.	**Y**	**N**
12.	I am more reserved and controlled than most people. (R)	**Y**	**N**
13.	I almost never get so excited that I lose control of myself. (R)	**Y**	**N**
14.	I am slower than most people to get excited about new ideas and activities. (R)	**Y**	**N**
15.	It is difficult for me to keep the same interests for a long time because my attention often shifts to something else.	**Y**	**N**
16.	I like to think about things for a long time before I make a decision. (R)	**Y**	**N**
17.	I often follow my instincts, hunches or intuition without thinking through all the details.	**Y**	**N**
18.	I often have to change my decisions because I had a wrong hunch or mistaken first impression.	**Y**	**N**
19.	I usually think about all the facts and details before I make a decision. (R)	**Y**	**N**
20.	I nearly always think about all the facts in detail before I make a decision even when other people demand a quick decision. (R)	**Y**	**N**
21.	I am usually able to get other people to believe me, even when I know that what I am saying is exaggerated or untrue.	**Y**	**N**

22.	I can usually do a good job of stretching the truth to tell a funnier story or to play a joke on someone.	**Y**	**N**
23.	I have trouble telling a lie, even when it is meant to spare someone else's feelings. (R)	**Y**	**N**
24.	I am better at saving money than most people. (R)	**Y**	**N**
25.	I often spend money until I run out of cash or get into debt from using too much credit.	**Y**	**N**
26.	Because I so often spend too much money on impulse, it is hard for me to save money even for special plans like a vacation.	**Y**	**N**
27.	Some people think I am too stingy or tight with my money. (R)	**Y**	**N**
28.	It is hard for me to enjoy spending money on myself, even when I have saved plenty of money. (R)	**Y**	**N**
29.	I hate to make decisions based only on my first impressions. (R)	**Y**	**N**
30.	I like old "tried and true" ways of doing things much better than trying "new and improved" ways. (R)	**Y**	**N**
31.	I enjoy saving money more than spending it on entertainment or thrills. (R)	**Y**	**N**
32.	In conversations I am much better as a listener than as a talker. (R)	**Y**	**N**
33.	I like to stay at home better than to travel or explore new places. (R)	**Y**	**N**
34.	I like to pay close attention to details in everything I do. (R)	**Y**	**N**

Note. R = reversed item

Appendix D
Carver and White's BIS/BAS Scales – Revised
BIS Subscale

Items	***Not True***	***Somewhat True***	***True***	***Very True***
BIS:				
1. I usually get very tense when I think something unpleasant is going to happen.				
2. I worry about making mistakes.				
3. I am hurt when people scold me or tell me that I do something wrong.				
4. I feel pretty upset when I think that someone is angry with me.				
5. I do not become fearful or nervous, even when something bad happens to me. (R)				
6. I feel worried when I think I have done poorly at something.				

Note. BIS=behavioural inhibition system, R=reversed item

Appendix E
The Tridimensional Personality Questionnaire (TPQ)
Harm Avoidance Scale

In this questionnaire you will find statements people might use to describe their attitudes, opinions, interests, and other personal feelings. Each statement can be answered YES or NO. Read the statement and decide which choice best describes you. Circle the Y or N after each question. Read each statement carefully but don't spend too much time deciding to answer. Please answer every statement, even if you are not completely sure of the answer. Remember, there are no right or wrong answers – just describe your own personal opinions and feelings.

	Items	***Yes***	***No***
1.	I usually am confident that everything will go well, even in situations that worry most people. (R)	**Y**	**N**
2.	Usually I am more worried than most people that something might go wrong in the future.	**Y**	**N**
3.	I nearly always stay relaxed and carefree, even when nearly everyone else is fearful. (R)	**Y**	**N**
4.	I often have to stop what I am doing because I start worrying about what might be wrong.	**Y**	**N**
5.	I often stop what I am doing because I get worried, even when my friends tell me everything will go well.	**Y**	**N**
6.	I usually feel tense and worried when I have to do something new and unfamiliar.	**Y**	**N**
7.	I often feel tense and worried in unfamiliar situations, even when others feel there is little to worry about.	**Y**	**N**
8.	I often feel tense and worried in unfamiliar situations, even when others feel there is no danger at all.	**Y**	**N**
9.	I usually stay calm and secure in situations that most people would find physically dangerous. (R)	**Y**	**N**
10.	I am usually confident that I can easily do things that most people would consider dangerous (such as driving an automobile fast on a wet or icy road). (R)	**Y**	**N**
11.	When I have to meet a group of strangers, I am more shy than most people.	**Y**	**N**
12.	I often avoid meeting strangers because I lack confidence with people I do not know.	**Y**	**N**
13.	I usually stay away from social situations where I would have to meet strangers, even if I am assured that they will be friendly.	**Y**	**N**
14.	I would probably stay relaxed and outgoing when meeting a group of strangers, even if I were told they were unfriendly. (R)	**Y**	**N**
15.	I think I would stay confident and relaxed when meeting strangers, even if I were told they are angry at me. (R)	**Y**	**N**
16.	Most of the time I would prefer to do something a little risky (like riding in a fast automobile over steep hills and sharp turns) rather than having to stay quiet and inactive for a few hours. (R)	**Y**	**N**
17.	I try to do as little work as possible, even when other people expect more of me.	**Y**	**N**
18.	Most of the time I would prefer to do something risky (like hang-gliding or parachute jumping) rather than having to stay quiet or inactive for a few hours. (R)	**Y**	**N**
19.	I have less energy and get tired more quickly than most people.	**Y**	**N**

20.	I often need naps or extra rest periods because I get tired so easily.	**Y**	**N**
21.	I am more energetic and tire less quickly than most people. (R)	**Y**	**N**
22.	I usually can stay "on the go" all day without having to push myself. (R)	**Y**	**N**
23.	I recover more slowly than most people from minor illnesses or stress.	**Y**	**N**
24.	I need much extra rest, support and reassurance to recover form minor illnesses or stress.	**Y**	**N**
25.	It is extremely difficult for me to adjust to changes in my usual way of doing things because I get so tense, tired, or worried.	**Y**	**N**
26.	I usually feel much more confident and energetic than most people, even after major illness or stress. (R)	**Y**	**N**
27.	I recover more quickly than most people from minor illnesses or stress. (R)	**Y**	**N**
28.	I think I will have very good luck in the future. (R)	**Y**	**N**
29.	If I am embarrassed or humiliated, I get over it very quickly. (R)	**Y**	**N**
30.	I feel very confident and sure of myself in most social situations. (R)	**Y**	**N**
31.	I never worry about terrible things that might happen in the future. (R)	**Y**	**N**
32.	Regardless of any temporary problem that I have to overcome, I always think it will turn out. (R)	**Y**	**N**
33.	I usually have good luck in whatever I do. (R)	**Y**	**N**
34.	It is easy for me to organize my thoughts while talking to someone. (R)	**Y**	**N**

Notes: R = reversed items

Appendix F
Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSQR)
Punishment Subscale

	Items	***Yes***	***No***
1.	Do you often refrain from doing something because you are afraid of it being illegal?	**Y**	**N**
2.	Do you prefer not to ask for something when you are not sure you will obtain it?	**Y**	**N**
3.	Are you often afraid of new or unexpected situations?	**Y**	**N**
4.	Is it difficult for you to telephone someone you do not know?	**Y**	**N**
5.	Do you often renounce your rights when you know you can avoid a quarrel with a person or an organization?	**Y**	**N**
6.	As a child, were you troubled by punishments at home or in school?	**Y**	**N**
7.	In tasks that you are not prepared for, do you attach great importance to the possibility of failure?	**Y**	**N**
8.	Are you easily discouraged in difficult situations?	**Y**	**N**
9.	Are you a shy person?	**Y**	**N**
10.	Whenever possible, do you avoid demonstrating your skills for fear of being embarrassed?	**Y**	**N**
11.	When you are with a group, do you have difficulties selecting a good topic to talk about?	**Y**	**N**
12.	Is it often difficult for you to fall asleep when you think about things you have done or must do?	**Y**	**N**
13.	Do you think a lot before complaining in a restaurant if your meal is not well prepared?	**Y**	**N**
14.	Would you be bothered if you had to return to a store when you noticed you were given the wrong change?	**Y**	**N**
15.	Whenever you can, do you avoid going to unknown places?	**Y**	**N**
16.	Are you often worried by things that you said or did?	**Y**	**N**
17.	Would it be difficult for you to ask your boss for a raise (salary increase)?	**Y**	**N**
18.	Do you generally try to avoid speaking in public?	**Y**	**N**
19.	Do you, on a regular basis, think that you could do more things if it was not for your insecurity or fear?	**Y**	**N**
20.	Comparing yourself to people you know, are you afraid of many things?	**Y**	**N**
21.	Do you often find yourself worrying about things to the extent that performance in intellectual abilities is impaired?	**Y**	**N**
22.	Do you often refrain from doing something you like in order not to be rejected or disapproved of by others?	**Y**	**N**
23.	Generally, do you pay more attention to threats than to pleasant events?	**Y**	**N**
24.	Do you often refrain from doing something because of your fear of being embarrassed?	**Y**	**N**

Appendix G
Example of Food Items Listed in the Food Preference Questionnaire (FPQ)

	High Simple Sugar	**High Complex Carbohydrate**	**Low Carbohydrate/High Protein**
High Fat	*Donuts* *Muffins* *Cookies* *Candy Bars*	*Avocado* *Butter-Flavoured Popcorn* *Potato Chips* *Butter Crackers*	*Boiled Egg* *Sunflower Seeds* *Peanuts* *Cheddar Cheese*
Low Fat	*Angel Food Cake* *Cereal Bar* *Jam-filled Sponge Cake* *Fig Cookie*	*Baked Potato Chips* *Whole Wheat Bread* *Popcorn-Plain* *Tomato*	*Broccoli* *Canned Tuna in Water* *Sliced Turkey* *Fat-Free Mozzarella*

Appendix H
Binge Eating Questionnaire

Date of Birth: mm_____ / dd_____ / year_____

Present Age: _____ years

What is your PRESENT WEIGHT? _____ lbs

What is your HEIGHT? _____ ft _____ in

What is the LOWEST YOU HAVE WEIGHED since reaching your present height? _____lbs

What is the MOST YOU HAVE WEIGHED since reaching your present height? _____lbs

In your opinion, you are now (check one)

1	2	3	4	5
Very underweight	Underweight	Average	Overweight	Very Overweight

What was the MOST you have weighed during the past year? _____lbs

What was the LEAST you have weighed during the past year? _____lbs

Do you ever get uncontrollable urges to eat and eat until you feel physically ill? **Yes□ No□**

Are there times when you are afraid that you cannot voluntarily stop eating? **Yes□ No□**

Do you make yourself vomit after eating too much? **Yes□ No□**

Do you feel miserable and annoyed with yourself after an eating binge? **Yes□ No□**

Have you ever had an episode of eating an enormous amount of food in a short space of time (an eating binge)? **Yes□ No□**

Do you consider yourself a binge-eater? **Yes□ No□**

In order to control your weight, do you use…..

Diet pills

Never □ **0**
Less than once every four weeks □ **1**
1 to 3 times every four weeks □ **2**
Once every week □ **3**
2 to 6 times every week □ **4**
once every day □ **5**
more than once a day □ **6**

Laxatives

Never □ **0**
Less than once every four weeks □ **1**
1 to 3 times every four weeks □ **2**
Once every week □ **3**
2 to 6 times every week □ **4**
once every day □ **5**
more than once a day □ **6**

Diuretics or water pills

Never □ **0**
Less than once every four weeks □ **1**
1 to 3 times every four weeks □ **2**
Once every week □ **3**
2 to 6 times every week □ **4**
once every day □ **5**
more than once a day □ **6**

Other? _____ (please specify) How often? _____

(Note: If you use these aids for any other reasons, please specify reasons.)

What is the average number of days between your episodes of binge-eating? (If never, leave blank) _____ days

Have you ever vomited after eating? **Yes□ No□**

How frequently do you vomit after eating?

Never □ **0**
Less than once every four weeks □ **1**
1 to 3 times every four weeks □ **2**
Once every week □ **3**
2 to 6 times every week □ **4**
once every day □ **5**
more than once a day □ **6**

Do you have any other type of eating problem? **Yes□ No□**
(If 'yes', please describe the nature of the problem).

Appendix I
Eating Behaviours Patterns Questionnaire (EBPQ)
Subscales: Emotional Eating and Snacking on Sweets

Items	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
Emotional Eating:					
I eat when I am upset.					
When I am in a bad mood, I eat whatever I feel like eating.					
I eat for comfort.					
My emotions affect what and how much I eat.					
If I am bored, I will snack more.					
I sometimes snack even when I am not hungry.					
I am a snacker.					
I snack more at night.					
When I buy snack foods, I wat until I have finished the whole package.					
When I am upset, I tend to stop eating. (R)					
Snacking on Sweets:					
Sometimes I eat dessert more than once a day.					
I usually keep cookies in the house.					
I have a sweet tooth.					
I eat cookies, candy bars, or ice cream in place of dinner.					
I snack two to three times every day.					
To me, cookies are an ideal snack food.					

Note. R = reversed items