The Role Of Oxytocin In Distress-Motivated Social Support Seeking

Christopher Cardoso

A Thesis
In The Department
Of
Psychology

Presented in Partial Fulfillment of the Requirement
For the Degree of
Doctor of Philosophy (Psychology, Research and Clinical Training Option) at
Concordia University
Montreal, Quebec, Canada

July 2016

CONCORDIA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared

By: **Christopher Cardoso**

Entitled: **The Role Of Oxytocin In Distress-Motivated Social Support Seeking**

and submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY (Psychology)

complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the final examining committee:

_____Chair
Dr. I. Ferguson

_____External Examiner
Dr. F. Chen

_____External to Program
Dr. P. Darlington

_____Examiner
Dr. K. Dunfield

_____Examiner
Dr. J. Pfaus

_____Thesis Supervisor
Dr. M. Ellenbogen

Approved by: _____
Dr. A. Arvanitogiannis, Graduate Program Director

July 8, 2016 _____
Dr. A. Roy, Dean, Faculty of Arts & Science

ABSTRACT

The Role Of Oxytocin In Distress-Motivated Social Support Seeking

Oxytocin is a hormone and neuropeptide that is traditionally known for its role in parturition and lactation in women. In the last few decades, researchers have theorized that oxytocin is involved in the human stress response. This theory, known as *tend-and-befriend*, posits that oxytocin promotes social support seeking in humans during stressful situations by inhibiting the traditional *fight-or-flight* response, promoting attention to socio-emotional stimuli, and enhancing feelings of trust. This theoretical framework, however, has received little attention by way of supporting evidence in human experimental research. In this dissertation, four studies are presented that investigate the role of oxytocin in (1) regulating the cortisol response to stress, (2) enhancing the perception of emotion in human faces, and (3) promoting trust and social-support seeking in distressed people, particularly women. Across these studies, oxytocin is experimentally manipulated using a nasal spray to examine its effects in humans in a double blind, placebo-controlled fashion. In the first study, a 24IU dose of intranasal oxytocin was shown to inhibit cortisol rise during physical stress. In the second study, oxytocin enhanced the perception of emotion in human faces. In the third study, oxytocin selectively improved dispositional trust in distressed participants following a stress induction, but not in those whose mood was euthymic in response to stress. In the final study, oxytocin increased perceived support in women during negative memory recall in the company of an experimenter, and decreased perceived support while recalling such memories in social isolation. Taken together, the results of this thesis support the role of oxytocin in stress-regulation and social cognition in humans. These results also suggest that the effect of oxytocin on social bonding (i.e. trust) may be dependent on the experience of distress, and that oxytocin-motivated social support seeking may have positive and negative consequences in both sexes, particularly in women, depending on the availability of social contact. This oxytocin-induced facilitation of social support seeking might explain why humans, particularly women, are more susceptible to stress-related mental illnesses such as major depressive disorder in the face of social conflict or in the absence of supportive social relationships.

Christopher Cardoso, Ph.D.

Concordia University, 2016

Acknowledgements

I would like to express my deepest respect and appreciation for my research mentor, Mark Ellenbogen. You gave me the opportunity to pursue my interests in oxytocin and clinical psychology, and without your trust, patience, and guidance, I would have never reached my potential as a scientist. I would also like to thank Anne-Marie Linnen and Jim Pfaus for taking an interest in oxytocin long before my graduate education began; without your inspired endeavour, encouragement, and support, I would have never stumbled upon a fascination for the subtle yet profound importance of hormones in the field of clinical psychology.

I would like to thank my parents, Jose and Filomena, for putting my interests before their own and for encouraging me to pursue higher education before I realized its importance. I would also like to thank my partner Danielle for being an essential source of emotional support and intellectual stimulation, and for keeping me grounded throughout graduate school; I couldn't have done this without you, and it wouldn't have been worth doing without you. I would like to also thank Kiran for giving me a profound appreciation for elegant complexity, as well as Erik for believing in me before I had the courage to believe in myself.

I am grateful to the countless graduate students, staff members, and volunteers of the Ellenbogen laboratory for their contributions to this body of research and for their friendship. I am particularly grateful to Christopher B., Mark Anthony, Christopher K., and Lisa for giving me their time and interest in exchange for young mentorship. Finally, I would like to thank my committee members, Jim Pfaus and Kristen Dunfield, for their feedback and guidance throughout the writing of this dissertation.

Throughout graduate school, I have been supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Fonds de la Recherche en Santé du Québec. I am extremely grateful for this support.

Contribution of Authors

This thesis consists of four studies. The relative contributions of my colleagues to each study is outlined below:

Study 1: Cardoso, C., Ellenbogen, M.A., Orlando, M.A., Bacon, S. L. & Joober, R. (2013). Intranasal oxytocin attenuates the cortisol response to physical stress: A dose-response study. *Psychoneuroendocrinology, 38,* 399-407.

Christopher Cardoso, Mark A. Ellenbogen, and Simon L. Bacon designed the study. Christopher Cardoso and Mark Anthony Orlando collected the data. Christopher Cardoso conducted the statistical analyses and wrote the first draft of the manuscript. Mark A. Ellenbogen extensively revised the manuscript. Ridha Joober supervised the use of oxytocin, and Simon L. Bacon supervised the use of exercise equipment. All authors contributed to and approved of the final manuscript.

Study 2: Cardoso, C., Ellenbogen M.A. & Linnen, A-M. (2014). The effect of intranasal oxytocin on perceiving and understanding emotion on the Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT). *Emotion, 14,* 43-50.

Christopher Cardoso, Mark A. Ellenbogen, and Anne-Marie Linnen designed the study. Christopher Cardoso and Anne-Marie Linnen collected the data. Christopher Cardoso conducted the statistical analyses and wrote the first draft of the manuscript. Mark A. Ellenbogen extensively revised the manuscript. All authors contributed to and approved of the final manuscript.

Study 3: Cardoso, C., Ellenbogen, M.A., Serravalle, L., & Linnen, A-M. (2013) Stress-induced negative mood moderates the relation between oxytocin administration and trust: Evidence for the *tend-and-befriend* response to stress? *Psychoneuroendocrinology,* 38, 2800-2804.

Christopher Cardoso, Mark A. Ellenbogen, and Anne-Marie Linnen designed the study. Christopher Cardoso and Anne-Marie Linnen collected the Data. Christopher Cardoso and Lisa

Serravalle conducted the statistical analyses and wrote the first draft of the manuscript. Mark A. Ellenbogen extensively revised the manuscript. All authors contributed to and approved of the final manuscript.

Study 4: Cardoso, C., Valkanas, H., Serravalle, L., & Ellenbogen, M.A. (Under Review). Oxytocin and social context moderate social support seeking in women during negative memory recall. *Manuscript under 2nd revision in Psychoneuroendocrinology.*

Christopher Cardoso and Mark Ellenbogen designed the study. Lisa Serravalle collected the data. Christopher Cardoso and Helen Valkanas conducted the statistical analyses. Christopher Cardoso and wrote the first draft of the manuscript, and Lisa Serravalle and Mark Ellenbogen provided revisions to the manuscript. All authors contributed to and approved of the final manuscript.

Table of Contents

List of Figures

List of Tables

List of Relevant Abbreviations

ANOVA ..... Analysis of variance

ACTH ..... Adrenocorticotropin releasing hormone

AuCi ..... Area under the curve with respect to increase

AuCg ..... Area under the curve with respect to ground

AVP ..... Arginine vasopression

BBB ..... Blood-brain barrier

CNS ..... Central nervous system

CRH ..... Corticotropin releasing hormone

CSF ..... Cerebrospinal fluid

HPA ..... Hypothalamic-pituitary-adrenal

ICV ..... intracerebroventricular

IU ..... International units

M ..... Mean

MANCOVA ..... Multivariate analysis of co-variance

MDD ..... Major Depressive Disorder

MET ..... Multifaceted Empathy Task

MSCEIT ..... Mayer-Salovey-Caruso Emotional Intelligence Test

OT/OXT ..... Oxytocin

OXTR ..... Oxytocin receptor

PL/PLC ..... Placebo

RMET ..... Reading the Mind in the Eyes Test

SD ..... Standard deviation

Chapter 1: General Introduction

Carter (2014) theorizes that the mammalian hormone and neuropeptide oxytocin is one of the fundamental molecules that helped shape the evolution of modern humans from an ancestral, small-brained, solitary organism to a large-brained, interdependent social species. This peptide originates from an evolutionarily conserved molecule that existed nearly 700 million years ago. The critical interconnected roles of oxytocin in the development of the neocortex, skull formation, lactation, parturition, and social cognition are theorized to be an impetus for the development of sophisticated social abilities (Carter, 2014). The role of oxytocin in the regulation of social behavior in humans is largely understood using inference from experimental research in non-human animal models including rats, voles, and non-human primates. Similar evidence using experimental research in humans, however, is lacking, and important gaps in our understanding of the role of oxytocin in the regulation of social behavior in humans remain. In this thesis, my goal is to improve the evidence base for the role of oxytocin in the regulation of social behavior in humans, which holds the promise of clarifying our understanding of how human relationships are shaped by biological processes. This understanding can improve our conceptualization of social dysfunction and its treatment in human populations, which is an important area of development in the field of mental health research.

The overall goal of this thesis is to delineate the evolutionarily conserved role of oxytocin in (1) stress regulation, (2) social information processing, and (3) social support seeking in humans. This goal is achieved through a series of experimental studies using pharmacological manipulations of the endogenous oxytocin system in humans. The guiding theoretical framework for the research delineated in this thesis is based on evidence from research in non-human animal models. This framework is called the *tend-and-befriend* theory, which posits that oxytocin is the biological impetus by which women engage in social-support seeking under stress more frequently than men (Taylor et al., 2000; Tamres et al., 2002). A primary tenet of this theory posits that oxytocin increases attention to socio-emotional information, down-regulates stress, and promotes social support seeking in distressing circumstances, which is a sexually differentiated alternative to the traditional *fight-or-flight* response to stress. Exploring the tend-and-befriend model using experimental manipulations of oxytocin will help substantiate its validity. The primary goal of this thesis is to provide support for the role of oxytocin as the

biological driver of the *tend-and-befriend* response to stress, which may have important implications for understanding the relation between sex, stress-regulation, and social support.

Oxytocin, maternal care, and social bonding

Oxytocin is produced in magnocellular neurons of the paraventricular and supraoptic nucleus of the hypothalamus, and it is released into the central nervous system (CNS) through neuronal soma, axons, and dendrites. In the CNS, oxytocin is a neuromodulator that acts on G-coupled receptors encoded by the OXTR receptor gene throughout regions of the brain stem, hypothalamus, and cortical areas (Carter, 2014). Oxytocin is also released into the blood stream via the posterior pituitary gland, where it effects change in visceral, metabolic, and smooth motor systems, including the mammary glands and uterus in females (Gimpl & Farenholz, 2001).

In addition to its vital role in parturition and maternity (Gimpl & Farenholz, 2001), oxytocin is involved in the regulation of maternal care and alloparental behaviours in experimental research using rats. This role is best illustrated in studies using virgin female rats. In one such study, intracerebroventricular (icv) infusion of synthetic oxytocin increased maternal behaviours toward foster rat pups, including grouping and regrouping pups, couching over grouped pups, pup licking, nest building, and retrieving pups (Pedersen & Prange, 1979). These effects were shown to be greater in magnitude in other studies using virgin female rats primed with estrogen, as well as in studies exploring the role of oxytocin in maternal behavior in virgin female rats in the last day of diestrus, proestrus or estrus (Farbach, Morrell, & Pfaff, 1985; Pederson, Ascher, Monroe, & Prange, 1982; Pederson & Prange, 1979). These results suggest that the effect of oxytocin on maternal behaviours is dependent on hormones that increase in circulation during the perinatal period, where maternal behavior increases naturally in rats. In support of this contention, natural variation in maternal behaviours in the post-partum period of female rats has been shown to decrease following icv infusion of an oxytocin receptor antagonist (van Leengoed, Kerker, & Swanson, 1987). These pharmacological manipulation studies support the role of oxytocin in maternal behaviour in rats.

The effect of oxytocin on maternal behaviour is further supported by studies that show that such behaviours in the rat are correlated with endogenous variations in oxytocin receptor expression in the CNS (Francis, Champagne, & Meany, 2000). These associations are shown to be responsible for the inter-generational transmission of maternal behaviours in rats from parent

to offspring, which is mediated through epigenetic changes in oxytocin receptor expression (Champagne, Diorio, Sharma, & Meaney, 2001). In these studies, the link between epigenetic changes in oxytocin receptor expression and maternal behaviour is shown to be greatest in adult female rats (Francis, Young, Meany, & Insel, 2002). The link between maternal behaviour and oxytocin is also shown in other mammals, including sheep and prairie voles (Bales, Kim, Lewis-Reese, & Carter, 2004; Bales, Pfeifer, & Carter, 2004; Kendrick, Keverne, & Baldwin, 1987; Keverne, & Kendrick, 1992). Many scientists have shown that interactions between oxytocin and reward systems in the brain are critical to the development of positive maternal behaviours in mammals (Champagne et al., 2004; Shahrokh, Zhang, Diorio, Gratton, & Meaney, 2010). Taken together, these findings support the role of oxytocin in promoting maternal behaviours in non-human animals across a number of different animal models and experimental paradigms.

In addition to the role of oxytocin in maternal behavior outlined above, the oxytocinergic system has also been shown to play a role in the formation of monogamous bonds between opposite-sex partners (Carter et al., 2014). In support of this contention, the oxytocinergic system has been shown to promote affiliative behaviours and pair ponding between opposite-sex partners in mammals across a variety of experimental paradigms (Insel & Young, 2001; Young & Wang, 2004). For example, early studies examining monogamous prairie voles found that icv infusion of oxytocin promotes partner preference in females (Cho, DeVries, Williams, & Carter, 1999; Williams, Insel, Harbaugh, & Carter, 1994). Specifically, these studies show that female prairie voles are more likely to spend time in proximity of a familiar opposite-sex partner relative to novel mates when under the influence of exogenous oxytocin, relative to a placebo. These effects are also supported by studies that show that icv infusion of an oxytocin antagonist inhibits the oxytocin-induced partner preference effect in female prairie voles (Insel & Hulihan, 1995; Williams et al., 1994).

The effect of oxytocin on pair bonding has also been shown to extend to other species including non-human primates. For example, in well-established marmoset pairs, exogenous oxytocin increases socio-sexual behaviour between established sexual partners and decreases this behaviour towards strangers of the opposite sex (Cavanaugh, Mustoe, Taylor, & French, 2014; Smith, Agmo, Birnie, & French, 2010). These effects show mediation by activation of reward-related circuits, as has been shown in the studies of oxytocin and maternal care described previously. Specifically, a number of studies have identified reward centers in the brain as critical

mediators of the effect of oxytocin on social bonding in both male and female prairie voles (Aragona, Liu, Curtis, Stephan, & Wang; Liu & Wang, 2003). The effects of oxytocin on partner preference and social bonding have also shown to be mediated by reward pathways in the brain in rats, monogamous songbirds, as well as in non-human primates (Klatt & Goodson, 2013; Triana-Del Rio, 2015; Witt, Winslow, & Insel, 1992). In short, the link between oxytocin and the formation of monogamous bonds is well established in non-human animal models and shares a mechanism that is common to the role of oxytocin in the promotion of maternal behaviour.

The role of oxytocin in parental behaviour and romantic bonding has also been demonstrated in humans, although the evidence base in this research area is smaller and less established than the literature on non-human animals. Examples of such evidence include studies in humans that have shown links between the OXTR receptor gene, which is posited to regulate oxytocin receptor expression, and paternal sensitivity (Feldman et al., 2012; Strathearn, Fonagy, Amico, & Montague, 2009). The role of endogenous oxytocin and paternal behavior is also supported by studies showing that endogenous levels of oxytocin measured in plasma and saliva are both elevated in response to positive paternal behaviours in humans (Feldman, Gordon, Schneiderman, Weisman, & Zagoory-Sharon, 2010; Feldman, Gordon, & Zagoory-Sharon, 2011; Weisman, Zagoory-Sharon, & Feldman, 2012b). One such study showed a robust association between paternal touch, which is an index of sensitive parenting, and plasma oxytocin in both mothers and fathers (Feldman et al., 2012). The role of oxytocin in romantic bonding has been demonstrated in studies administering exogenous oxytocin in a nasal spray to human participants, which increases central levels of oxytocin (Striepens et al., 2013). Examples of this research include studies that show that intranasal oxytocin administration, relative to a placebo, increases social distance between men in romantic relationships and attractive strangers of the opposite sex (Scheele et al., 2012). This effect was not found in single men, which suggests that oxytocin decreases the willingness of partnered men to have close proximity with a single female. In partnered men, intranasal oxytocin has also been shown to increase activity in reward pathways in the brain when viewing pictures of a current romantic partner (Scheele et al., 2013). This role of oxytocin in romantic bonding has been supported by studies showing that both intranasal oxytocin administration and endogenous concentrations of oxytocin in the periphery are associated with positive communication behaviours and close proximity in couples (Ditzen et al., 2009; Schneiderman, Zagoory-Sharon, Leckman, & Feldman, 2012). These findings are further

supported by studies that show that variations in the oxytocin receptor gene (OXTR) predict relationship quality and romantic separation over time in naturalistic settings (Thompson, Hammen, Starr, & Najman, 2014; Walum et al., 2012). Taken together, these results tentatively suggest that the roles of oxytocin in parental behavior and the formation of romantic attachments are replicable across non-human and human populations, which further substantiates the role of oxytocin in social bonding.

Aside from the well-established role of the reward system in mediating the effects of oxytocin on social bonding in human and non-human animals, the related cognitive, behavioural, and physiological mechanisms driving these effects in humans are not well understood. Some of these mechanisms do have an evidence base, however, in non-human animal research. Such mechanisms include (1) the effect of oxytocin on social cognition, and (2) the effect of oxytocin on stress-regulation. Specifically, in non-human animal models, there is evidence that oxytocin mediates social bonding in part through its effects on enhancing social cognition and increasing attention to socio-emotional information (Taylor et al., 2000). There is also evidence that oxytocin exerts its effect on social bonding by inhibiting activation of the hypothalamic-pituitary-adrenal (HPA) axis, a biological system involved in promoting the stress-response (i.e. *fight-or-flight;* Taylor et al., 2000). These mechanisms do not have a strong evidence base in human research, however. The existing evidence for these bodies of work is described next.

Social cognition and oxytocin

Oxytocin is posited to promote maternal care and social bonding by enhancing social cognition. In rodents, such effects have been repeatedly demonstrated. For example, studies have shown that exogenous oxytocin facilitates social recognition in rats and mice (Ferguson, Aldag, Insel, & Young, 2001; Popik, Vetulani, & Van Ree, 1992). In other words, these animals show more familiarity to peers that they have been previously introduced to under the influence of oxytocin relative to a placebo. In support of these findings, oxytocin gene knockout mice show deficits in social recognition, highlighting the role of oxytocin in facilitating this ability (Ferguson et al., 2000; Winslow & Insel, 2002). In humans, however, the interpretation of the role of oxytocin in social cognition is more complex because the multiple facets of human empathy are functionally distinct.

Human empathy is a multidimensional construct that involves a number of major functional components, including *affective sharing* and *emotional understanding* (Decety, 2011). Affective sharing refers to the automatic, bottom-up process of emotional contagion (Gallese, Keysers, & Rizzolatti, 2004). In other words, this phenomenon reflects the automatic process by which humans develop a neural representation of the emotions of others. Activation and coupling of the mirror-neuron system and limbic pathways is necessary to develop a neutral representation of the emotions of others, which allows people to feel what others are feeling. Emotional understanding, in contrast, is a top-down, effortful process that is mediated by prefrontal brain regions that allows us to make inferences about the beliefs, intentions, and emotional state of others (Lev-Ran, Shamay-Tsoory, Zangen, & Levkovitz, 2012; Shamay-Tsoory et al., 2005). These facets of empathy are posited to have distinct neural pathways (Decety & Jackson, 2004).

There is evidence in the literature to suggest that oxytocin increases affective sharing in humans. For example, it has been shown that intranasal oxytocin enhances the perception of human biological motion (Keri & Benedek, 2009), and that this effect is associated with suppression of Mu and Alpha beta band brain wave frequencies recorded using electroencephalography (Perry et al., 2010). Mu and Alpha brain wave rhythm suppression has been documented in response to observed actions, and evidence from parallel findings using fMRI technology suggests that this suppression reflects activity in the mirror neuron system (Perry & Bentin, 2009). In support of this contention, greater suppression of Mu and Alpha brain wave rhythms during the observation of intentional actions is correlated with greater measures of empathy (Perry, Troje, & Bentin, 2010)— a finding previously described in a study using functional neuroimaging (Pfeifer, Iacoboni, Mazziotta, & Dapretto, 2008). Riem and colleagues (2011) have shown that women administered intranasal oxytocin show enhanced activation of the insula and pars triangularus of the inferior frontal gyrus in response to infant crying— a stimulus that evokes emotional contagion in humans as young as a few days old (Sagi & Hoffman, 1976). The pars triangularus of the inferior frontal gyrus is an area of the motor neuron system that is highly responsive to observed actions (Molnar-Szakacs, Iacoboni, Koski, & Mazziotta, 2005), suggesting that oxytocin may enhance the neural representation of infant crying in women. Depressed people also show greater activation of the superior frontal gyrus and insula while viewing affective facial stimuli following intranasal oxytocin administration, indicating a greater neural representation of those emotions in these individuals (Pincus et al., 2010).

A number of tasks have been developed to examine how well individuals make inferences about the emotional state of others. For example, the Reading The Mind in The Eyes Task (RMET; Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001) was developed to determine how well participants can identify emotional expressions in stimuli that only reveal the eye region of the face. Successful completion of the RMET appears to be critically mediated by the amygdala (Adolphs, Baron-Cohen, & Tranel, 2002; Stone, Baron-Cohen, Calder, Keane, & Young, 2003). Research on oxytocin and the RMET suggests that oxytocin enhances affective sharing, or the automatic process of developing a neural representation of the emotions of others. Intranasal oxytocin has been shown to improve accuracy on the RMET in individuals with elevated alexithymia scores (Luminet, Grynberg, Ruzette, & Mikolajczak, 2011). In people suffering from autism spectrum disorders, intranasal oxytocin improves performance on the easy items of the RMET (Guastella et al., 2010), and in healthy individuals, oxytocin improves accuracy for the difficult items on this measure (Domes, Heinrichs, Michel, Berger, & Herpertz, 2007).

Intranasal oxytocin is hypothesized to moderate the visual saliency of socio-emotional stimuli, which is important for social communication (Averbeck, 2010; Bartz, Zaki, Bolger, & Ochsner, 2011a). Improved visual saliency can partly explain the aforementioned improvements on the RMET that are observed following oxytocin administration. Consistent with the social saliency hypothesis, intranasal oxytocin increases gaze to the eye region of the face (Guastella, Mitchell, & Dadds, 2008), an effect that is associated with an increased functional coupling of the superior colliculi and the amygdala (Gamer, Zurowski, & Büchel, 2010)— a circuit whose links to the pulvinar nuclei of the thalamus have been hypothesized to be important for moderating visual saliency (Robinson & Petersen, 1992).

Research on the ability to read the emotions of others is typically complimented by studies that examine how well individuals can recognize emotions in facial stimuli with varied degrees of emotional intensity— stimuli that are often digitally manipulated to express a very wide range of emotional intensities (Blair, Colledge, Murray, & Mitchell, 2001). These tasks often use pictures from standardized picture sets. A number of studies have shown that intranasal oxytocin enhances the detection of emotion in faces at lower emotional intensities across a number of methodologies (Leknes et al., 2012; Lischke et al., 2012; Marsh, Yu, Pine, & Blair, 2010; Prehn et al., 2013)— an effect that is often associated with increased pupil dilation (Leknes

et al., 2012; Prehn et al., 2013). Intranasal oxytocin also enhanced the detection of emotion in faces at an early level of perceptual processing in one study (Schulze et al., 2011) but not in another (Ellenbogen et al., 2012). These findings support the role of oxytocin in enhancing the salience of socio-emotional information in humans. The tasks employed in this research to date, however, have only examined the effect of oxytocin on affective sharing. The role of oxytocin in emotional understand, however, remains poorly understood.

In the only study of its kind, intranasal oxytocin administration has been shown to improve performance on the empathic accuracy task, but only in individuals with lower social competence (Bartz et al., 2010). The empathic accuracy task purportedly boasts the most ecologically valid test of the ability to infer the emotions of others (i.e. emotional understanding). In this task, participants rate the emotion of a target person in a video. In this video, the target person is describing a memory for an event that happened to them. An important component of this task occurs before the video is used in the experiment. Specifically, after the target person is finished the recording, they are asked to view their own video and to rate the emotions that they experienced while recalling the memory using a scale ranging from very negative to very positive at pre-determined intervals. Participant ratings of the target person's emotions are then compared with the target person's own rating of their emotions during the video, and the correlation between these two ratings is considered to be a measure of the participant's accuracy for making emotional inferences (Ickes, 2001). Performance on the empathic accuracy task is associated with activation in the medial and ventral medial prefrontal cortex as well as the inferior frontal gyrus (Zaki, Weber, Bolger, & Ochsner, 2009). This task has only been used in the context of oxytocin administration in one study, however, and the role of oxytocin in more complex forms of social cognition (i.e. emotional understanding) is not well supported.

Going forward, it will be important to establish three important effects that concern the role of oxytocin in social cognition and empathic processes in humans to better understand how this construct might inform the role of oxytocin in social bonding. First, it will have to be determined whether the effect of oxytocin on emotion perception is specific to social stimuli, as there have been no published studies comparing the effect of oxytocin on emotion perception in social and non-social stimuli. Second, it will be important to determine whether oxytocin increases attention to specific emotions (i.e. positive facial expressions) or whether oxytocin enhances the perception of all emotions. The specificity of this effect has not yet been

substantiated using experimental designs that employ a comprehensive set of varied emotions, and such evidence will be important for making predictions about the behavioural consequences of exogenous oxytocin administration. A meta-analytic review has shown an overall effect of oxytocin on increasing attention to happy, sad, and angry faces, however, more diverse facial expressions have not been examined within a single study (Shahrestani, Kemp, & Guastella, 2013). Third, it will be important to establish whether oxytocin influences more complex forms of social and emotional cognition that are independent of visual processes. In other words, it is not yet clear whether the effects of oxytocin on social cognition are solely consistent with the visual saliency hypothesis, or whether additional processes might be driving these effects. In summary, the reviewed evidence suggests that oxytocin enhances the affective sharing aspect of empathy, but more specific questions concerning its role in empathy must be addressed to clarify our understanding of how the behavioural effects of oxytocin might be moderated by individual differences (i.e. baseline levels of empathic abilities) and contextual factors (i.e. the intensity and type of emotions being expressed, or the availability of social stimuli in the environment; Bartz et al., 2011a). It may be that the role of oxytocin in increasing attention to socio-emotional information is one mechanism by which oxytocin facilitates social information processing and promotes social bonding. This proposal, however, will require more specific delineation using experimental research before predictions can be made about how this mechanism might influence behavior in a person and context dependent manner (Bartz et al., 2011).

Stress physiology and oxytocin

Before exploring the role of oxytocin in stress-regulation, a brief explanation the body's stress system is required. This system has evolved to promote environmental adaptation in response to challenges in the environment, which is necessary for human survival. The ability to adapt to an environmental challenge is partly made possible by the hypothalamic-pituitary-adrenal (HPA) axis, which is a biological system that is implicated in the *fight-or-flight* response to challenge in the environment. In response to perceived threat in the environment, the hypothalamic-pituitary-adrenal (HPA) axis initiates a cascade of biological events that is intended to increase energy mobilization so that an individual can respond to environmental challenge. At the onset of stress, the brain releases noradrenaline from widely distributed synapses, and adrenaline is released from the adrenal glands in the periphery as part of the response of the

sympathetic nervous system to increase heart rate and blood pressure, respiration, sweating, and pupil dilation and consequently enhance arousal, vigilance and attention (de Kloet, Joëls, & Holsboer, 2005). The HPA-axis then initiates a series of additional biological events if the perceived threat persists for longer than a few minutes. As part of this response, corticotropin-releasing hormone (CRH) is released from the paraventricular nucleus of the hypothalamus into the anterior lobe of the pituitary gland, where it stimulates the systemic release of adrenocorticotropic hormone (ACTH) into the blood stream. ACTH then binds to receptors on the adrenal cortex of the kidney, stimulating the release of glucocorticoids (e.g. cortisol) into the bloodstream. Glucocorticoids increase the metabolism of energy in the body, and its levels peak between 15 and 30 minutes following the initiation of the stress response, and return to basal levels 60 to 90 minutes later (de Kloet, Joëls, & Holsboer, 2005). At lower concentrations, glucocorticoids further stimulate the stress system via binding to mineralocorticoid receptors distributed widely throughout the limbic system, particularly in the hippocampus. Once glucocorticoids reach higher concentrations, however, they bind to glucocorticoid receptors and initiate the termination of the stress response as part of a negative feedback loop. This occurs because glucocorticoid receptors are co-located with mineralocorticoid receptors, but have a 10-fold lower affinity for glucocorticoids (de Kloet, Joëls, & Holsboer, 2005).

An important mechanism by which oxytocin is posited to promote social bonding in animals is by lowering physiological response to stress via its effects on the HPA-axis (DeVries, Craft, Glasper, Neigh, & Alexander). Studies in this research area used non-human animal models to highlight the role of oxytocin in the regulation of the HPA-axis response to stressful tasks and have identified number of important regions of the HPA-axis that serve as sites of action for oxytocin (e.g. Smith & Wang, 2013). For example, it has been shown that oxytocin receptors co-localize with CRH and glucocorticoid receptors across various regions of the HPA-axis, and it is speculated that its vigilance-lowering and stress-dampening effects are conducive to normalizing energy balance and increasing social bonding and prosocial behaviours (Carter, 1998; Unvas-Moberg & Francis, 2003). One such study demonstrated that the effect of oxytocin on stress-regulation is partly mediated by its effects in the hippocampus (Cohen et al., 2010; Neuman & Landgraff, 2012; Windle et al., 2004). In keeping with this contention, both acute and chronic stress increase oxytocin receptor binding in the hippocampus (Liberzon, & Young, 1997). In this brain region, exogenous oxytocin increases neurogenesis (Leuner, Caponiti, &

Gould, 2012) and alters glucocorticoid function by increasing mineralocorticoid receptor expression and decreasing glucocorticoid receptor expression (Cohen et al., 2010; Petersson, & Uvnäs-Moberg, 2003). These effects are thought to promote stress-regulation.

Oxytocin also exerts its effects on stress-reactivity in the amygdala, the paraventricular nucleus of the hypothalamus, and in the periphery (Neuman & Landgradd, 2012). Both endogenous and exogenous oxytocin in the central amygdala can decrease activation of this brain region (Huber, Veinante, & Stoop, 2005; Knobloch et al., 2012) and inhibit efferent projections to related fear-circuitry (Ebner et al., 2005; Viviani et al., 2011). These effects can decrease physiological arousal and allow animals to behave flexibly in response to stress (Ebner et al., 2005, Viviani et al., 2011). An additional central region of the HPA-axis that is modulated by oxytocin is the paraventricular nucleus of the hypothalamus (Neuman & Landgraff, 2012). In this brain region, endogenous release of oxytocin is observed in response to stress (Bosch, Krömer, Brunton, & Neumann, 2004; Neumann, Wigger, Torner, Holsboer, & Landgraf, 2000). In support of this effect, CRF neurons express mRNA for co-localized oxytocin receptors in the paraventricular nucleus (Dabrowska et al., 2011), lending further evidence for the role of oxytocin in regulating the HPA-axis. The evidence for these endogenous effects has been further complimented by studies using exogenous icv infusion of oxytocin in the paraventricular nucleus, which consistently shows attenuation of the CRF, ACTH, and corticosterone response to stress (Windle et al., 2004). Studies in humans have also demonstrated an effect of oxytocin on the HPA-axis, with robust effects in the periphery. For example, intravenous administration of oxytocin lowers plasma cortisol levels in response to exercise and pharmacological challenge (Legros, Choidera, Geenen, & von Frenckell, 2001; Legros, 2001). In short, the role of oxytocin in the regulation of the HPA-axis is well documented in non-human animals and evidences multiple sites of action throughout the brain and periphery. However, studies in humans have been limited to the study of the role of oxytocin on peripheral targets of the HPA-axis.

Research on the role of oxytocin in regulating the HPA-axis in humans has been limited because peripheral oxytocin does not cross the blood-brain-barrier (BBB). Because of this, the BBB represents an obstacle to investigating the central effects of exogenous oxytocin in humans. Researchers have tried to overcome this obstacle by administering oxytocin to humans using a nasal spray. Using this methodology, researchers have shown that participants who sniff oxytocin show elevated levels of oxytocin in cerebrospinal fluid (CSF) 90 minutes later (Striepens et al.,

2013). Similar results have been shown in individuals who sniffed a nonapeptide that was structurally similar to oxytocin (Born et al., 2002). Because sniffing oxytocin increases its levels in the CSF, it may also increase bioavailability in the CNS. Rats administered oxytocin using a pipette to deliver intranasal oxytocin show increased levels of oxytocin in the amygdala and paraventricular nucleus within minutes (Neumann, Maloumby, Beiderbeck, Lukas, & Landgraf, 2013). We cannot demonstrate evidence for a similar effect in humans, however, because we do not currently have the technology to measure oxytocin in specific brain regions in-vivo without using invasive techniques. A great number of studies, however, have shown that intranasal oxytocin dampens amygdala activation and the startle response while viewing emotional stimuli (Cardoso, Payne, & Ellenbogen, unpublished observations; Ellenbogen, Linnen, Cardoso & Joober, 2014). These studies have led some to speculate that the CNS mediates the stress-dampening effects of oxytocin. Guastella and colleagues (2013) propose a number of different pathways for oxytocin to reach the CNS after nasal inhalation. Specifically, inhaled oxytocin may enter the CNS through two pathways: the first pathway is via the olfactory bulb pathway, which leads directly to the CSF and CNS including surrounding lymphatic fluid, and the second pathway is the trigeminal nerve pathway, which leads directly to the brain stem. Inhaled oxytocin also enters peripheral circulation through nasal vasculature and oral mucosa, as well as gastroenterally, where it can directly effect change in peripheral sites of action for oxytocin.

The method of administering oxytocin using a nasal spray in laboratory challenge studies could theoretically lower the cortisol response to various stressors simply via the effect of oxytocin on the inhibition of cortisol release from the adrenal glands (Legros, 2001). Many such studies now exist, and show that intranasal oxytocin lowers the cortisol response to psychosocial stress, including the Trier Social Stress Test (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003; McRae-Clark, Baker, Marie, & Brady, 2013; Quirin, Kuhl, & Dusing, 2011; Simeon et al., 2011), couple conflict (Ditzen et al., 2009), the Yale Interpersonal Stressor (Linnen et al., 2012), and the Montreal Imaging Stress Test (Grimm et al., 2014). The authors of these studies argue that intranasal oxytocin lowers the cortisol response to stressful tasks via its effects on the central regions of the HPA-axis (for a review, see Cardoso, Kingdon, & Ellenbogen, 2014), in spite of the fact that similar effects could be achieved through peripheral pathways. This line of reasoning is used to justify long post-administration wait-times, which range from 30 minutes to 50 minutes (Guastella et al., 2013). These wait times are based on research showing that elevations of

vasopressin and oxytocin in CSF only peak at 75-80 minutes post administration, while plasma levels peak at approximately 15 to 20 minutes post-administration (Born et al., 2002; Striepens et al., 2013). While animal studies show that nasal inhalation of oxytocin increases its bioavailability in the amygdala and paraventricular nucleus (Neumann et al., 2013), modulation of these regions is not required to observe the effect of intranasal oxytocin on cortisol (Cardoso, Kindgon, & Ellenbogen, 2013; Legros, 2001). Consistent with this contention, intranasal oxytocin lowers cortisol at rest (Meinlschmidt, & Heim, 2007), in the absence of cortisol rise (Ditzen et al., 2009; Linnen et al., 2012), and in response to non-psychological stressors (Ott et al., 2013). The reviewed evidence suggests that intranasal oxytocin lowers the cortisol response to stress in humans, which parallels findings that implicate oxytocin in rodent stress-regulation (Cardoso, Kindgon, & Ellenbogen, 2013; Neuman & Landgraff, 2012). This evidence base, however, is less precise with respect to the proposed mechanism of action.

The next steps in this research area include the establishment of two important effects that concern the role of oxytocin in stress-regulation in humans. This will help facilitate our understanding of how the role of oxytocin in stress-regulation informs its effect on social bonding. First, it must be determined whether the effect of intranasal oxytocin on the inhibition of the HPA-axis is specific to social contexts, or whether this effect can be produced in the absence of psychosocial stress. The specificity of this effect has not yet been established in human research, and it has important implications for the interpretation of physiological data in this research area. For example, researchers in this area have assumed that intranasal oxytocin acts directly on the CNS to down-regulate stress, even though peripheral sites of action that do not respond exclusively to psychosocial stress (i.e. the adrenal glands) are equally plausible candidates for the observed effects of oxytocin on HPA-axis activity (Guastella et al., 2013). Second, it must be determined whether the effect of intranasal oxytocin on HPA-activity is dose-dependent. Researchers in this area have employed a wide range of doses of intranasal oxytocin ranging from 16 IU to 60 IU with no empirically grounded rationale (Fehm-Wolfsdorf et al., 1988; Riem et al., 2012). These doses may have differing effects on HPA-axis activity, and selecting the most effective inhibitory dose will be important for designing studies in this research area.

In summary, the reviewed evidence suggests that oxytocin inhibits activation of the HPA-axis during stress, but more specific studies concerning the specificity of this effect to central

sites of action and social context, as well as determining the effective dose range, will help inform future research in this area. In addition, it is not known whether oxytocin's dampening of HPA activity is moderated by individual differences (i.e. sensitivity to psychosocial distress) and contextual factors (i.e. whether the stressor is interpersonal in nature; Bartz et al., 2011a). It may be that the role of oxytocin in regulating the physiological stress system is one mechanism by which oxytocin facilitates social bonding during periods of distress. This proposal, however, will require more specific delineation using experimental research before predictions can be made about how this mechanism might influence behavior in a person and context dependent manner (Bartz et al., 2011).

Distress-motivated social support seeking: Beyond fight-or-flight

Taylor and colleagues (2000) posit that the effects of oxytocin on social cognition, stress, and social bonding work in conjunction to promote an alternate biobehavioral response to stress known as the *tend-and-befriend* response. This theory was developed using evidence from research on non-human animals, and posits that the *fight-or-flight* response may be a maladaptive response to environmental challenge in women across contexts that have historically been unique to women from an evolutionary perspective. Specifically, it is theorized that because women are smaller in stature and historically provided more care for offspring than men, the traditional fight-or-flight response may be maladaptive when women are pregnant, rearing offspring, or defending against aggression from men. In these instances, rather than fighting or fleeing, tending to offspring could increase the chances that they survive and reproduce, and befriending peers in a social group could serve as protection against in-group aggression from physically dominant men. This increased flexibility in the biobehavioural response to stress (i.e. not just fight-or-flight, but also tend-and-befriend) is hypothesized to confer increased evolutionary fitness. Taylor and colleagues (2000) posit that hormones that vary across stages of the menstrual cycle and maternity, and oxytocin in particular, coordinate the multiple biological systems involved in the tend-and-befriend response to stress via its effects on social cognition, stress, and social bonding.

The tend-and-befriend theory offers researchers a framework for understanding why men and women often respond differently to stress across the lifespan (Kudielka et al., 2009; Tamres et al., 2002), as well as across phases of the menstrual cycle and maternity (a question that is

often ignored for its cost and complexity in stress research). The role of oxytocin in the tend-and-befriend response has not received a great deal of attention in experimental research on stress in humans to date; women were underrepresented in experimental research on stress when Taylor and colleagues developed the tend-and-befriend theory 16 years ago (17% female; Taylor et al., 2000), and women are still underrepresented in experimental research on stress and oxytocin today (32% female; Cardoso et al., 2014b). Going forward, substantiating the role of oxytocin in the *tend-and-befriend* response has the potential to shed light on important sex differences in the stress-response that have eluded scientists in this underdeveloped research area for decades (Kudielka et al., 2009).

There is currently a dearth of experimental research on the *tend-and-befriend* response; however, there is preliminary evidence available to support this hypothesis. For example, as reviewed previously, a number of published experimental studies support the effects of oxytocin on enhancing attention to socio-emotional stimuli and down-regulating stress. These effects are in keeping with the *tend-and-befriend* theory (Taylor et al., 2000). There is also evidence that oxytocin increases cognitive and behavioral indices of trust (Cardoso et al., 2012; Kosfeld et al., 2005) and social bonding (Buchheim et al., 2009). The interdependence of these effects has been examined in a few notable non-human animal studies. For example, studies on pair bonding in the monogamous prairie vole have shown that oxytocin enhances social bonding in distressed females relative to those who are not distressed (DeVries, DeVries, Taymans, & Carter, 1995; DeVries, DeVries, Taymans, & Carter, 1996; DeVries, Guptaa, Cardillo, Cho, & Carter, 2002). While the interdependence of the effects of oxytocin on stress and social bonding has not been examined in experimental research in humans, there is published correlational evidence consistent with such effects. For example, a few studies have shown that endogenous oxytocin concentration is elevated in the periphery of women who report relationship distress (Taylor et al., 2006; Taylor et al., 2010). Studies corroborating these correlational findings have shown that distressed girls evidence increased peripheral oxytocin following contact with a caregiver (Seltzer et al., 2010). Similarly, increased homogeneity for the G allele on the OXTR receptor gene is correlated with social support seeking in distressed people from western culture (Kim et al., 2010). Taken together, the *tend-and-befriend* theory is supported by promising experimental evidence in non-human animals and correlational evidence in humans. Experimental support for the *tend-and-befriend* theory, however, is lacking in human research.

The sexually differentiated effect of oxytocin on social support seeking that is outlined by the *tend-and-befriend* theory requires experimental evidence in humans. This theory has the potential to elucidate what individual and contextual factors influence the effects of oxytocin outlined in the extant literature on this subject (Bartz et al., 2011). For example, increasing social sensitivity and trust, particularly in distressed people, could have negative or positive effects depending on the social context, and it is unclear how the promotion of social support seeking in distressed people might influence them in circumstances where social support is withheld or unavailable. Experimental research consistent with this contention shows that oxytocin produces both anxiolytic effects (Cardoso, Linnen, Ellenbogen & Joober, 2012; Heinrichs et al., 2003) and anxiogenic effects (MacDonald et al., 2013; Simeon et al., 2011) in stressful circumstances, but these studies do not explicitly examine how context modulates such outcomes. Going forward, it will be important to substantiate the *tend-and-befriend* theory using experimental research to validate this theory as a framework for understanding the role of oxytocin in social behaviour in humans. Such a framework will be useful for predicting how oxytocin influences stress and social bonding across different contexts and individual differences, and it will also provide a framework for understanding sex differences in the stress-response from a biopsychosocial perspective.

Objectives and study hypotheses

The objective of this thesis is to provide experimental evidence for the role of oxytocin in (1) stress-regulation, (2) perception of socio-emotional stimuli, and (3) distress-motivated social support seeking and social bonding in humans. This objective will be accomplished by experimentally manipulating oxytocin in humans by administering it using a nasal spray across different contexts. **Chapter 2** will establish a dose-response curve for the effect of intranasal oxytocin on the cortisol and mood response to physical stress using two doses: a 24IU dose, which is the most common in the literature, and a 48IU dose. This study will also examine whether the effect of oxytocin on the inhibition of cortisol generalizes to non-social contexts. **Chapter 3** will use a 24IU dose of intranasal oxytocin to determine whether oxytocin specifically enhances the perception of different emotions using subtle, more ecologically valid facial expressions, consistent with the social saliency hypothesis. This chapter will also examine whether this effect is specific to visual stimuli or whether oxytocin also enhances linguistic

representations of emotion. This will be a particularly important study because the literature has shown mixed, and at times opposite findings on the effect of oxytocin on social cognition (Schulze et al., 2011; Shahrestani et al., 2013; Radke & de Brujin, 2016). **Chapter 4** will directly examine whether the effect of oxytocin on dispositional trust is dependent on the experience of distress during a live social rejection paradigm. This chapter will also examine whether this effect is greater in women. Finally **Chapter 5** will evaluate whether the effect of oxytocin on perceived social support depends on the experience of distress and on the availability of social partners. This chapter will also evaluate whether this effect is sex-dependent, as is predicted by the *tend-and-befriend* theory. Across these chapters, it is predicted that oxytocin will influence stress-regulation, emotion perception, and social-support seeking in a manner that is consistent with the *tend-and-befriend* theory.

In the chapters that follow, it is important to note that the use of a nasal spray to administer oxytocin in humans has been criticized because the pathway by which the peptide reaches central and peripheral targets is poorly understood (Guastella et al., 2013) and contested (Walum, Hasse, Irwin, & Young, 2015). This method is still being used in spite of this issue because it is a non-invasive technique that consistently shows modest effect sizes relative to placebo across a wide variety of indices (Hedges g = .20—.30; Bakermans-Kranenburg and van IJzendoorn, 2013; Cardoso, Kingdon, & Ellenbogen, 2014; Cardoso, Payne, & Ellenbogen, unpublished observations; Shahrestani et al., 2013). The studies presented in this thesis are well powered to detect effect sizes in this range (Walum et al., 2015), and the limitations of this methodology are discussed in the next chapters.

Chapter 2: Intranasal Oxytocin Attenuates the Cortisol Response to Physical Stress: A Dose-Response Study

Cardoso, C., Ellenbogen, M.A., Orlando, M.A., Bacon, S. L. & Joober, R. (2013). Intranasal oxytocin attenuates the cortisol response to physical stress: A dose-response study. *Psychoneuroendocrinology, 38,* 399-407.

Abstract

Intranasal oxytocin attenuates cortisol levels during social stress inductions. However, no research to date has documented the dose-response relation between intranasal oxytocin administration and cortisol, and researchers examining intranasal oxytocin have not examined the cortisol response to physical stress. We therefore examined the effects of 24 and 48 IU of intranasal oxytocin on the cortisol response to vigorous exercise. Seventeen males participated in a randomized, placebo-controlled, double-blind, and within-subject experiment. Participants engaged in vigorous exercise for sixty minutes following the administration of placebo or intranasal oxytocin on three occasions. Saliva samples and mood ratings were collected at eight intervals across each session. Salivary cortisol concentrations changed over time, peaking after 60 minutes of exercise (Quadratic: $F(1,16) = 7.349$, $p = .015$, partial $\eta^2 = .32$). The 24IU dose of oxytocin attenuated cortisol levels relative to placebo ($F(1,16) = 4.496$, $p = .05$, partial $\eta^2 = .22$) and the 48IU dose, although the latter fell just short of statistical significance ($F(1,16) = 3.054$, $p = .10$, partial $\eta^2 = .16$). There was no difference in the cortisol response to exercise in participants who were administered 48IU of intranasal oxytocin relative to placebo. Intranasal oxytocin had no effect on mood. This is the first study to demonstrate that the effect of intranasal oxytocin on salivary cortisol is dose-dependent, and that intranasal oxytocin attenuates cortisol levels in response to physical stress. Future research using exogenous oxytocin will need to consider the possibility of dose-response relations.

Introduction

Oxytocin is a mammalian hormone that is produced in both magnocellular and parvocellular neurons of the paraventricular and supraoptic nuclei of the hypothalamus. Oxytocin is released in the central nervous system through terminating axonal projections within select brain areas that originate from the paraventricular and supraoptic nuclei, as well as in the periphery through the posterior pituitary gland (Gimpl and Farenholz, 2001). While oxytocin is traditionally known for its role in stimulating uterine contractions during parturition and milk let-down during breast feeding, there is increasing evidence that oxytocin regulates affiliative behavior in humans (Young and Wang, 2004; Bartz and Hollander, 2006; Ross and Young, 2009; Campbell, 2010).

It has been hypothesized that one way oxytocin promotes affiliative behavior is through actions on the hypothalamic-pituitary-adrenal (HPA) axis. Taylor and colleagues (2000) contend that oxytocin facilitates approach behavior in a social context by lowering arousal levels. This hypothesis was originally put forth in the context of a review of animal research, where the effect of oxytocin on the attenuation of HPA-activity is well established. For example, adult virgin female Wister rats display increased ACTH and corticosterone levels following swim stress after being treated with central infusion of an oxytocin antagonist (Neuman et al., 2000b), and this effect has been replicated in male rats (Neuman et al., 2000a; Ebner et al., 2005). It has also been shown that exposure to swim and restraint stress upregulates the expression of oxytocin receptors in the amygdala (Liberzon et al., 1997), and that exposure to repeated swim stress upregulates the expression of oxytocin receptors in the hippocampus (Liberzon et al., 1997; Leuner et al., 2012). This research has led many to speculate that oxytocin may play a key role in the regulation of HPA-activity (for a comprehensive review, see Engelmann et al., 2004; DeVries et al., 2007; Neuman, 2009).

The effect of oxytocin on the HPA-axis in humans has only recently been investigated. Cortisol, a hormone that is released by the adrenal glands during strenuous physical or psychological challenge (de Kloet et al., 2005), is a reliable biomarker of the stress response in experimental research (Foley and Kirschbaum, 2010). Consistent with the hypothesis that oxytocin lowers physiological arousal in humans, intranasal administration of oxytocin— a method that is widely used on the basis of a study that shows intranasal administration of vasopressin, which is similar in structure to oxytocin, increases its concentration in the cerebrospinal fluid (Born et al., 2002) — decreases cortisol levels during interpersonal stress

(Ditzen et al., 2009; Quirin et al., 2011; Linnen et al., 2012) and during the recovery phase following a public speech stressor (Heinrichs et al., 2003). Interestingly, oxytocin infused in the periphery also attenuates basal levels of cortisol in response to exercise, suggesting possible inhibitory action at the level of the adrenal gland, since oxytocin administered in the periphery does not cross the blood-brain barrier (Legros et al., 1982; Legros et al., 1984; Legros et al., 1987; Coiro et al., 1988). Thus, it appears oxytocin can attenuate HPA activity in humans, even in response to non-psychological stressors.

An important limitation of the research on intranasal oxytocin administration in humans is that few studies have examined dose-response relations. Dose-dependent effects of oxytocin on cortisol levels have been observed in animal studies. For example, centrally administered oxytocin in animals dampens the corticosterone response to white noise stress in a quadratic fashion (i.e. small doses lower cortisol, but doses much larger than 10ng/h do not increase the magnitude of this effect; Windle et al., 1997). Similarly, administration of large doses of intranasal oxytocin (200µg) fails to attenuate activation of the HPA-axis in primates, while there is evidence for attenuation with chronic smaller doses (50µg; Parker et al., 2005). In humans, the administration of oxytocin in the periphery dampens cortisol in a linear fashion (i.e. cortisol concentrations in the plasma decrease as oxytocin concentration increases, up to a dose as high as 128mIU/minute; Legros et al., 1984). Despite documented dose-response relations, human research studies have utilized a wide range of doses of intranasal oxytocin, from 20IU (Bruins et al., 1992) to as high as 60 IU (Fehm-Wolfsdorf et al., 1988), with no apparent rationale or theoretical justification. Two recent studies have even published work on intranasal oxytocin in humans using doses as low as 16IU (van Ijzendoorn et al., 2011; Riem et al., 2012). Importantly, dose-response relations for the effect of intranasal oxytocin in humans cannot currently be studied using meta-analysis (van Ijzendoorn and Bakermans-Kranenburg, 2012) because there are too few studies published on intranasal oxytocin in humans, and thus there is a need for further experimental work in this area.

In the current study, we investigated whether the effect of intranasal oxytocin administration on the cortisol response to vigorous exercise is dose-dependent. We utilized a dose of 24IU because it is the most commonly reported dose in the literature, and then doubled it for our higher dose. Moreover, the 48IU was large enough to capture the upper range of doses most commonly reported in the literature (Macdonald and Macdonald, 2010). We investigated both

doses of intranasal oxytocin relative to placebo in a double-blind, randomized, placebo-controlled, and within-subject design. We employed a physical stress paradigm in the current study for two reasons. First, there is no habituation when participants repeatedly exercise at high intensity, as occurs when participants are repeatedly exposed to psychosocial laboratory stressors (Foley and Kirschbaum, 2010). This makes the current investigation amenable to a within-subject design, which is a more robust test of dose-response effects than a between-subject design. Second, while oxytocin has previously been shown to attenuate cortisol levels during exercise when administered intravenously (Legros et al., 1987; Coiro et al., 1988), this has never been demonstrated following intranasal administration of oxytocin. We hypothesized that intranasal oxytocin would attenuate cortisol rise in response to exercise consistent with the literature reviewed above. However, since there are no published data comparing the effect of different doses of intranasal oxytocin on cortisol in healthy humans, we had no specific hypotheses concerning the direction of the dose-response effects. As an additional consideration of our hypotheses, we measured participants' mood in response to exercise to rule out the possibility that oxytocin-induced changes in cortisol were elicited by putative effects of oxytocin on mood.

Method

Participants

Seventeen men, aged 18-30 (Mean ± SD; 23.1 ± 3.5) years, were recruited from the community through online classified ads to participate in this study. The following were used as criteria for excluding participation: Current medical illness, existing physical injury, current recreational drug use or past history of drug use (with the exception of cannabis, which required 1 year abstinence), use of current medications including psychotropic medication, history of psychiatric disorders, and prior or current use of psychological treatment services. Exclusion criteria were assessed using an in-house structured interview protocol prior to participation. Participants were queried about current or past substance use, mental disorders, use of anti-depressants, anxiolytics, or any psychotropic medications and psychological treatments. Only participants in athletic physical condition were recruited to participate in this study to ensure participants could complete the exercise paradigm. Participants were asked how frequently they exercised and if they felt confident enough to complete one hour of vigorous exercise on a treadmill. If participants did not exercise on a weekly basis, or expressed uncertainty in their

ability to complete the exercise paradigm, they were not admitted to participate in this study. One hundred and two individuals were screened for the study, and approximately half (48) of these participants were excluded based on our pre-screening criteria. Of the 54 participants remaining in our pool, the first 17 were successfully scheduled and completed the study. Participants in the current study exercised on average 6.89 ± 2.96 hours a week, and had a resting heart rate of 64 ± 6.9 beats per minute. This project was approved by the Human Research Ethics Committee at Concordia University (Montréal, Canada). Informed consent was obtained for all participants.

Salivary cortisol sampling

Saliva was expressed directly into polypropylene 6 ml vials. Samples were frozen at minus 20 °C until assayed, in duplicate, for cortisol using a sensitive commercial enzyme immunoassay kit from Salimetrics (State College, Pennsylvania). After thawing, samples were centrifuged at 3000 RPM for 10 minutes to separate debris from saliva. The sensitivity of the assay was set at 0.012 µg/dl. The inter- and intra-assay coefficient of variation for the assays were 2.2% and 4.6% (on a range 0.01-10 µg/dl dose), respectively. Assays were conducted in the laboratory of Dr. C.-D. Walker at the Douglas Mental Health University Institute (Montreal, Canada). The test-retest reliability for salivary cortisol collected at the beginning of each session (before drug administration) was as follows: Placebo and 24IU oxytocin condition ($r(17) = .67$, $p = .004$), Placebo and 48IU oxytocin condition ($r(17) = .48$, $p = .051$), 24IU oxytocin condition and 48IU oxytocin condition ($r(17) = .58$, $p = .015$).

Mood ratings

Mood state was measured using a Visual Analogue Scale (VAS), which consisted of ten 10 cm lines, marked 0 (not at all) and 100 (extremely) at the extremities. Each line corresponds to a descriptor of mood state (*calm, anxious, energetic, tired, elated, depressed, agreeable, hostile, challenged, defeated*) and participants were asked to mark the place on each line that best represented how they felt in the moment.

Procedure

Participants were told that the purpose of the study was to examine the relation between hormones and the physiological response to exercise. Participants were provided with instruction

on how to measure their own heart rate from home and were told to report this information to the experimenter before their first test session. This was done to avoid measuring participant heart rate at the beginning of their first test session, where the novelty of the laboratory could have influenced this measure. This measure of heart rate was used throughout the study to set exercise intensity. Sessions were held in the afternoon after 1300h, and sessions were scheduled a week apart keeping the day of the week and time of day consistent across laboratory visits to control for diurnal rhythms in cortisol. Participants were advised to refrain from eating food or drinking liquids (with the exception of water) for 1 hour before each test session. They were also advised not to consume caffeine and not to exercise for the entire day before each test session, and they were told to abstain from consuming alcohol the day before each test session. Upon arrival to the laboratory, participants provided informed consent and a saliva sample (S1), and completed a rating of their current mood state (VAS1). Participants were taught how to use the nasal spray and subsequently self-administered a placebo, 24IU, or 48IU dose of intranasal oxytocin under the supervision of the experimenter. The order of administration was counterbalanced across test sessions and across participants. Each spray (4IU) was separated by a 30 second interval according to the instructions for intranasal administration of neuropeptides documented in previous research (Born et al., 2002), for a total of 6 sprays in the 24 IU oxytocin condition, 12 sprays in the 48 IU oxytocin condition, and either 6 or 12 sprays in the placebo condition (to prevent the experimenter from deciphering what drug condition the participant was completing). Participants rested for 10 minutes, provided a second saliva sample (S2) and mood rating (VAS2), and then engaged in vigorous exercise (i.e., running on a treadmill). The intensity of the exercise was set at 70% maximum heart rate using the Karvonen formula, a level known to increase cortisol in humans (Davies and Few, 1973; Few, 1974; Buckley and Eston, 2007). The experimenter paused the treadmill exercise every twenty minutes to collect saliva and mood data. Participants provided saliva samples (S3, S4, S5) and mood ratings (VAS3, VAS4, VAS5) every twenty minutes during the exercise paradigm. Participants were provided with water that they could only consume immediately after providing a saliva sample. Participants then relaxed for 40 minutes following exercise, providing saliva samples (S6, S7) and mood ratings (VAS6, VAS7) every twenty minutes during this period. Participants subsequently completed the autobiographical memory test (Williams and Scott, 1988) as part of a separate study component during the last 20 minutes of the test session, which is reported elsewhere (C. Cardoso, M. A.

Orlando, and M. A. Ellenbogen, unpublished observations). Finally, at the end of the study, participants provided an additional saliva sample (S8) and completed a mood rating (VAS8).

Two male research assistants completed the data collection for this study, and the same research assistant tested all three sessions of any given participant. Further, participants were monitored from a distance of approximately 15 feet away while they exercised, and the experimenter was out of view during this time. Participants were remunerated Can$100 for their participation following their third session. Thirteen participants completed the study in 14 days, 3 participants completed the study in 21 days, and 1 participant completed the study in 35 days.

Statistical analyses

The distribution of cortisol values was positively skewed (+3), with substantial positive kurtosis (+10), and was therefore $\log^{10}$ transformed. Four data points that were considered outliers (± 2.5 SD) were transformed to the next most extreme score in the data to further normalize the distribution of the data. Analyses were conducted on the transformed data unless otherwise specified, and the raw cortisol values can be found in Table 1. Omnibus statistical tests were adjusted using the Greenhouse-Geisser correction for violations of sphericity.

A Drug (placebo, oxytocin 24IU, oxytocin 48IU) x Time (8 samples) within-subject repeated measures ANOVA was conducted on salivary cortisol concentrations across test sessions. Statistically significant effects of time were followed up with an analysis of polynomial trends, and a significant drug main effect or Drug X Time interaction were followed up with simple comparisons of cortisol reactivity from baseline using AUCi (Area under the Curve with reference to increase; Pruessner et al., 2003) computed using the transformed data. As secondary analyses, we also examined drug effects on total cortisol output during the experiment, using AUCg (Area under the Curve with reference to ground; Pruessner et al., 2003). We added a constant sum of 3 to the transformed data to render all values positive in order to calculate AUCg values. To assess whether order of administration (24IU before 48IU one week apart; 48IU before 24IU one week apart; two weeks between oxytocin sessions) had an effect on our findings, we ran the Drug x Time within-subject repeated measures ANOVA described above using order of administration as a between-subject variable.

In order to evaluate the possibility that psychological factors (i.e., mood state) contributed to the cortisol response to exercise in this study, and to evaluate the effect of oxytocin on mood,

we ran additional Drug x Time within-subjects repeated measures ANOVAs on mood ratings on the VAS. Statistically significant effects were followed up in the manner described above.

Table 1. Descriptive statistics for raw cortisol values (ug/dl) across time and drug condition

	Placebo		Oxytocin 24		Oxytocin 48	
Variable	M	SD	M	SD	M	SD
Sample 1 (T - 20)	0.186	0.113	0.179	0.077	0.150	0.089
Drug Administration						
Sample 2 (T)	0.157	0.101	0.152	0.060	0.127	0.060
Sample 3 (T + 20)	0.178	0.083	0.183	0.116	0.139	0.053
Sample 4 (T + 40)	0.219	0.122	0.206	0.169	0.183	0.096
Sample 5 (T + 60)	0.280	0.228	0.259	0.334	0.219	0.117
Sample 6 (T + 80)	0.287	0.183	0.183	0.133	0.209	0.117
Sample 7 (T + 100)	0.280	0.250	0.152	0.120	0.175	0.113
Sample 8 (T + 120)	0.219	0.154	0.154	0.102	0.147	0.088

Note: N = 17. T = Beginning of a 60 minute of exercise period.

Results

The effect of intranasal oxytocin and exercise on cortisol

A Drug x Time within-subjects ANOVA revealed a statistical trend for the main effect of drug condition on cortisol levels ($F(1.5, 24.1) = 2.857$, $p = .089$, partial $\eta^2 = .15$). The analysis also revealed a statistically significant change in salivary cortisol concentrations across time ($F(3.1, 49.6) = 3.755$, $p = .016$, partial $\eta^2 = .19$). An analysis of polynomial trends revealed that, as expected, a statistically significant quadratic pattern best fit the cortisol data (Quadratic: $F(1,16) = 7.349$, $p = .015$, partial $\eta^2 = .32$). Cortisol concentrations increased in response to exercise, and decreased following exercise (see Figure 1A).

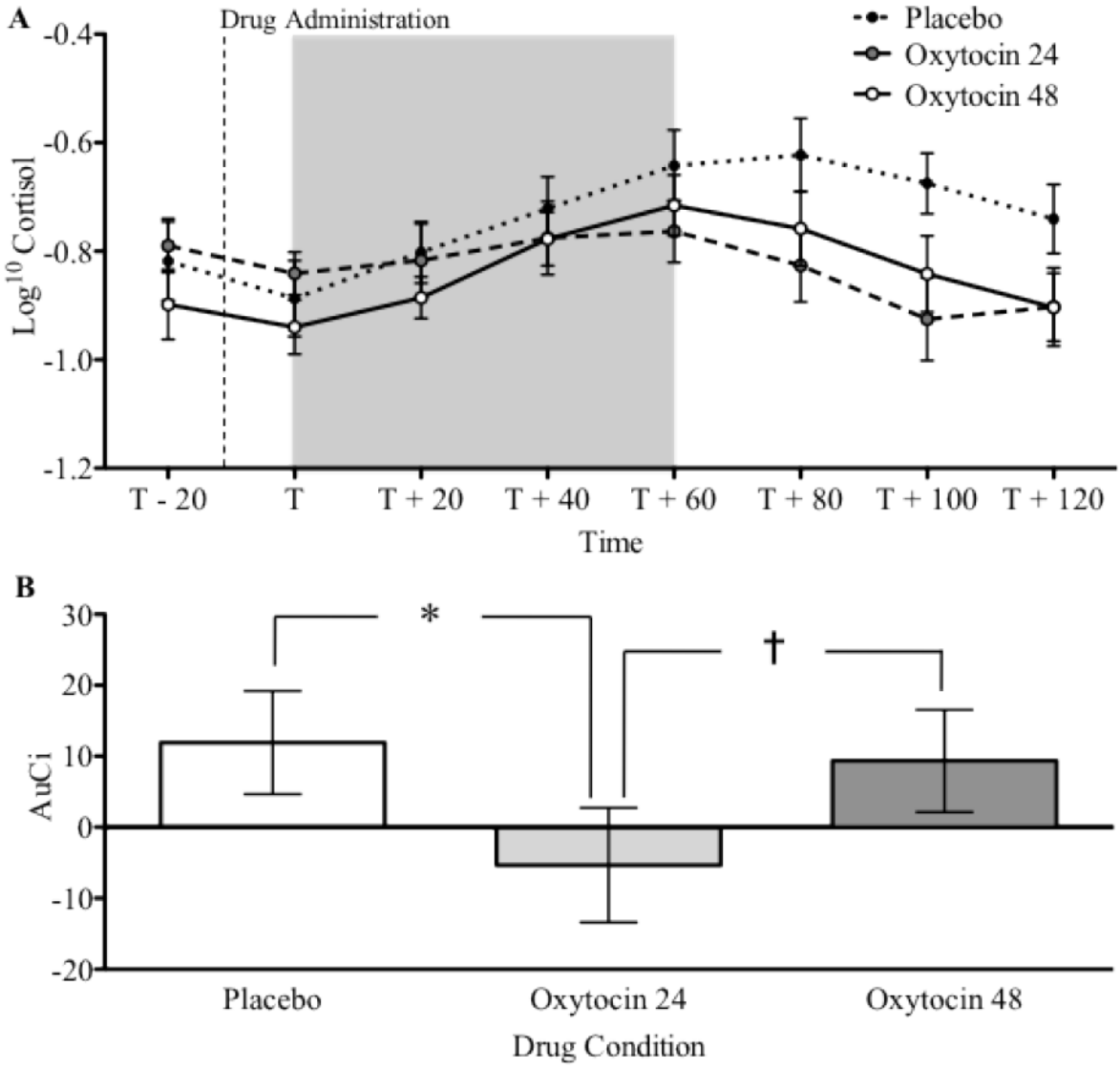

Figure 1. Error bars represent 1 SEM. * $p < .05$. † $p < .10$. **(A)** The shaded area indicates the time period when participants were exercising. Data points represent changes in salivary cortisol concentrations over time in participants following a placebo, a 24IU dose of intranasal oxytocin, and a 48IU dose of intranasal oxytocin. **(B)** Changes in cortisol concentrations over time were calculated using Area under the curve with reference to increase (AUCi; Pruessner et al., 2003). This formula was calculated for all sampled time points, using the first sample collected as a baseline. Participants showed an attenuation of the cortisol response to vigorous exercise following administration of the 24IU dose of intranasal oxytocin relative to placebo ($F(1,16) = 4.496$, $p = .05$, partial $\eta^2 = .22$), and the 48IU dose ($F(1,16) = 3.054$, $p = .10$, partial $\eta^2 = .16$),

although the latter fell short of conventional statistical significance.

A statistically significant Drug X Time interaction was also detected ($F(4.6, 74.3) = 2.903$, $p = .022$, partial $\eta^2 = .15$). To explore this interaction, we conducted simple comparisons of change in cortisol concentrations from baseline for each drug condition using AUCi. Participants showed a decrease in cortisol reactivity after the administration of the 24IU dose of intranasal oxytocin relative to placebo ($F(1,16) = 4.496$, $p = .05$, partial $\eta^2 = .22$) and relative to the 48IU dose ($F(1,16) = 3.054$, $p = .10$, partial $\eta^2 = .16$), although the latter fell short of conventional statistical significance. Importantly, cortisol AUCi did not differ in participants following the administration of the 48IU dose of intranasal oxytocin relative to the placebo ($F(1,16) = 0.083$, $p = .776$). These results are presented in Figure 1B. As a secondary analysis, we also examined the effect of drug on total cortisol concentration across the experiment using AUCg. A one-way repeated measures ANOVA revealed a statistical trend for the main effect of drug condition on total cortisol concentration ($F(1.5, 23.6) = 2.817$, $p = .093$, partial $\eta^2 = .15$).

To ensure the order of administration across sessions did not influence our results, we repeated the Drug x Time within-subjects ANOVA described above with the addition of order as a between subjects factor. We did not detect a statistically significant effect of order of administration across sessions on cortisol variation over time ($F(14, 18) = 1.037$, $p = .463$), the main effect of drug condition on cortisol ($F(4, 28) = 1.846$, $p = .148$), or the interaction between drug condition and cortisol variation over time ($F(28, 4) = 2.513$, $p = .192$).

Taken together, the pattern of cortisol variation following exercise was comparable in both the 48IU oxytocin condition and the placebo condition, whereas cortisol did not rise in response to exercise in the 24IU oxytocin condition. This effect was specific to the stress response to physical exercise: no robust effects of drug were found for total cortisol output during the experiment.

The effect of intranasal oxytocin and exercise on positive mood states

To evaluate the effect of oxytocin on mood, we repeated the above statistical analyses on the ratings of positive mood during the exercise stressor. Changes in positive mood states over time are presented in Table 2. A Drug X Time within-subject ANOVA revealed a statistically significant change (main effects of time) in participants' ratings of how calm, energetic,

agreeable, and challenged they felt [calm: $F(2, 32.4) = 10.651$, $p < .001$, partial $\eta^2 = .40$; energetic: $F(2.9, 49) = 4.686$, $p = .008$, partial $\eta^2 = .21$; agreeable: $F(2.7, 47) = 3.56$, $p = .024$, partial $\eta^2 = .17$; challenged: $F(2.4, 40.9) = 14.776$, $p < .001$, partial $\eta^2 = .47$]. Follow-up analyses of polynomial trends revealed a quadratic trend best fit the data, which demonstrated that participants reported feeling less agreeable and more challenged during exercise (agreeable: $F(1,16) = 5.399$, $p = .033$, partial $\eta^2 = .24$; challenged: $F(1,16) = 27.791$, $p < .001$, partial $\eta^2 = .62$). A linear polynomial trend was observed for ratings of energy levels, revealing that these ratings gradually decreased throughout the testing session (energetic: $F(1,16) = 8.556$, $p = .009$, partial $\eta^2 = .34$). A cubic polynomial trend was also observed; participants' ratings of calmness decreased during exercise, but increased following the exercise paradigm ($F(1,16) = 22.559$, $p < .001$, partial $\eta^2 = .59$). Feelings of elation did not change over time ($F(2.7, 46) = 1.754$, $p = .174$).

Table 2. Descriptive statistics for VAS mood scores across time collapsed across drug condition.

	Mean (Standard Deviation)							
Variable	T – 20	T	T + 20	T + 40	T + 60	T + 80	T + 100	T + 120
Calm	75 (19)	73 (22)	61 (24)	59 (26)	64 (26)	80 (17)	81 (17)	79 (18)
Energetic	58 (18)	54 (22)	62 (19)	54 (24)	55 (25)	48 (24)	44 (25)	49 (25)
Elated	47 (25)	45 (27)	53 (26)	52 (29)	56 (28)	51 (29)	48 (28)	48 (28)
Agreeable	72 (19)	73 (20)	70 (21)	65 (24)	71 (20)	73 (20)	72 (19)	73 (19)
Challenged	18 (21)	17 (23)	32 (26)	34 (26)	30 (28)	17 (22)	14 (21)	17 (23)
Anxious	23 (25)	24 (27)	19 (24)	22(27)	13 (19)	13 (15)	15 (22)	14 (19)
Tired	29 (22)	26 (22)	30 (24)	36 (26)	43 (28)	39 (24)	41 (26)	37 (26)
Depressed	8 (12)	7 (11)	6 (8)	6 (8)	5 (6)	6 (7)	5 (7)	6 (7)
Hostile	7 (10)	5 (7)	7 (13)	9 (14)	7 (12)	4 (8)	5 (8)	5 (9)
Defeated	7 (10)	5 (8)	7 (11)	9 (13)	8 (14)	5 (8)	6 (10)	6 (13)

Note: N = 17. Scores range from 0 (*not at all*) to 100 (*extremely*). T = Beginning of a 60 minute of exercise period. Scores were collapsed across drug condition because no effect of drug on mood was detected in our analyses. Participants felt less agreeable, less calm, and more challenged during exercise, however, participants felt calmer following exercise relative to their

arrival at the laboratory. Participants also felt less energetic, more tired, and less anxious during and following exercise relative to their arrival at the laboratory.

Neither the main effect of drug condition nor the interaction between drug and time were statistically significant predictors of ratings of positive mood. In short, participants felt less agreeable, less calm, and more challenged during exercise, but they felt calmer following exercise relative to when they arrived at the laboratory. Participants also felt less energetic during and following exercise relative to when they arrived at the lab. However, oxytocin had no impact on ratings of positive mood during the experiment.

The effect of intranasal oxytocin and exercise on negative mood states

The above analyses were repeated on ratings of negative mood state. Changes in negative mood over time are presented in Table 2. A Drug X Time within-subject ANOVA revealed a statistically significant change (main effect of time) in participants' ratings of how anxious and tired they felt (anxious: $F(2.5, 39.6) = 3.545$, $p = .030$, partial $\eta^2 = .18$; tired: $F(3, 47.2) = 4.646$, $p = .007$, partial $\eta^2 = .23$). Polynomial trend analysis revealed a linear trend, where participants reported feeling less anxious and more tired over time (anxious: $F(1,16) = 6.954$, $p = .018$, partial $\eta^2 = .30$; tired: $F(1,16) = 9.859$, $p = .006$, partial $\eta^2 = .38$). In contrast, ratings of depression, hostility, and defeat did not change across the test session (depressed: $F(2, 33.4) = 2.281$, $p = .116$; hostile: $F(2.3, 36.6) = 1.982$, $p = .147$; defeated: $F(3, 47.5) = 1.665$, $p = .188$).

Neither the main effect of drug condition nor the interaction between drug and time were statistically significant predictors of ratings of negative mood. In short, participants felt less anxious and more tired during and following the exercise stressor relative to their baseline ratings. However, oxytocin had no impact on ratings of negative mood during the experiment.

Discussion

In the current study, intranasal oxytocin attenuated the cortisol response to vigorous exercise following a 24IU dose of intranasal oxytocin, but not a 48IU dose, relative to placebo. Importantly, the cortisol response to exercise in this study did not appear to be modulated by psychological factors, as participants did not subjectively report significant psychological distress during exercise as measured with self-reported mood. In fact, participants felt calmer and less

anxious following exercise, even though their cortisol levels remained elevated during recovery in the placebo condition. More importantly, there was no evidence that oxytocin had a main effect on mood ratings, consistent with previous studies (Kirsch et al., 2005; Linnen et al., 2012). However, it should be noted that there is some evidence to suggest that the effect of oxytocin on mood is modulated by sex (Heinrichs et al., 2003; Kubzansky et al., 2012) as well as individual factors (Cardoso et al., 2012b), and thus the relation between oxytocin and mood is still not well understood and merits further investigation.

The implications of these findings are twofold. First, previous studies have demonstrated that oxytocin attenuates cortisol levels during interpersonal and social stressors (Ditzen et al., 2009; Quirin et al., 2011; Linnen et al., 2012). The present study indicates that the effect of intranasal oxytocin on the attenuation of HPA activity is robust (i.e. replicable) and independent of the type of stressor, as oxytocin appears to reduce HPA output during both physical and different types of psychological stress inductions. Second, the effect of intranasal oxytocin on cortisol levels appears to be dose-dependent, and doses higher than 24IU may be less effective in lowering salivary concentrations of cortisol in humans. The only other studies to investigate dose-dependent effects of intranasal oxytocin in humans found evidence of a greater effect of a 20IU dose relative to a 10IU dose on indices of emotion recognition using chronic administration in healthy controls and individuals suffering from schizophrenia (Goldman et al., 2011). In a sample of 8 adolescent and adult males with Fragile X syndrome, researchers found evidence for a greater effect of a 24IU dose relative to a 48IU dose on indices of social cognition in a within-subject design (Hall et al., 2012). Interestingly, these researchers also found that the 48IU dose of intranasal oxytocin was associated with lower salivary cortisol levels before and after a social challenge relative to the placebo, and not the 24IU dose. However, baseline levels of salivary cortisol prior to oxytocin administration were not collected in this study, and the results may have been influenced by baseline differences across test sessions (Hall et al., 2012). Clearly, more research on dose-dependent effects of intranasal oxytocin in humans is warranted. There is currently no consensus in the human literature on the optimal dosage for intranasal administration studies, and the present results suggest that different dosages may have different effects in humans.

The present finding that oxytocin dampens the cortisol response to vigorous exercise is consistent with a growing body of literature suggesting that oxytocin promotes prosocial

approach behavior by, in part, dampening biological reactivity in stress-sensitive fear-related circuitry. For example, in another study, we demonstrated that oxytocin dampens the startle response to an acoustic probe while viewing emotional pictures (A-M. Linnen, M. A. Ellenbogen, C. Cardoso, and R. Joober, unpublished observations), and other studies have demonstrated that intranasal oxytocin dampens activation of the amygdala in humans in response to affective pictures (Kirsch et al., 2005) and betrayal (Baumgartner et al., 2008). These effects parallel corroborating findings in animal studies that suggest oxytocin dampens the fear response through actions in the amygdala (Huber et al., 2005; Vivianni et al., 2011; Knobloch et al., 2012). Interestingly, arginine-vasopressin (AVP) appears to have an opposite effect on stress reactivity and the HPA system, at least in certain contexts. For example, at least two studies demonstrated that intranasal AVP increases cortisol in response to a public speaking laboratory stressor (Ebstein et al., 2009; Shalev et al., 2011). Thus, the results of this study are consistent with the hypothesized contrary effects of oxytocin and AVP on stress-reactivity, with oxytocin attenuating HPA-activity and vasopressin increasing it (Legros, 2001).

Despite the compelling link between dampened amygdala activation and reduced stress reactivity, the present research suggests that there are equally plausible pathways by which intranasal oxytocin influences HPA activity beyond its actions in the amygdala. For example, it is known that changes in oxytocin levels in the periphery, which cannot cross the blood-brain barrier, also reduce HPA activity during stress. Legros and colleagues (1982, 1984, 1987) have demonstrated that infusion of oxytocin in the periphery dampens the adrenal response to exercise, as well as during rest and recovery from exercise. In a study of parent-child dyads, endogenous increases in peripheral oxytocin in children paralleled decreases in salivary cortisol in response to physical and vocal comfort from their mother following a stressor (Seltzer et al., 2010). Individuals who are vulnerable to stress do not exhibit this relation between plasma oxytocin and cortisol in the blood, suggesting that plasma concentrations of oxytocin and its variation may be an important modulator of HPA-axis activity (Pierrehumbert et al., 2010). However, as we did not collect plasma in this study, we can only speculate on alternative sites of action for the effect we observed. Because oxytocin receptors are distributed widely throughout the brain (Loup et al., 1991; Gimpl and Farenholz, 2001), exogenous oxytocin likely has widespread effects in a variety of different brain regions. Clearly, further investigation of these pathways warrants consideration in human research.

It is not known why intranasal oxytocin attenuated cortisol levels in response to exercise at the low dose (24IU) but not at the high dose (48IU). Speculatively, oxytocin may partially occupy arginine-vasopressin (AVP) receptors at higher doses. Since AVP heightens HPA reactivity (Legros, 2001), it is possible that AVP cross-binding "cancelled out" the oxytocinergic attenuation of the cortisol response to exercise. This has been previously conjectured in a study of primates, where higher doses of intranasal oxytocin did not result in attenuation of HPA-activity in response to a laboratory stressor (Parker et al., 2005). It will be important to determine if this dose-response pattern replicates using other measures of stress reactivity in humans. Of note, we have observed a similar dose-response pattern for the effect of intranasal oxytocin on autobiographical memory (C. Cardoso, M. A. Orlando, and M. A. Ellenbogen, unpublished observations).

A number of study limitations warrant consideration. First, participants in this study were males in excellent physical condition. It is possible that our results will not generalize to individuals who are less physically active or to the general population. Thus, the present findings should be interpreted with caution, and future studies in more diverse and representative samples, including female participants, are needed. Second, most studies have examined the relation between oxytocin and indices of cognition and behavior 30 minutes following administration, and the present study examined the acute effects of intranasal oxytocin on cortisol and mood up to 130 minutes after administration. A report by Born and colleagues (2002) suggests that neuropeptides administered intranasally remain stable and elevated 80 minutes after administration, and studies from our laboratory have documented effects of intransal oxytocin on cognition 90 minutes (Cardoso et al., 2012a; Cardoso et al., 2012b) and 115 minutes (Ellenbogen et al., 2012; Ellenbogen et al., In Press) after administration. Further, at least two studies have documented higher salivary oxytocin concentrations at least two hours following intranasal oxytocin administration (Huffmeijer et al., 2012; Weisman et al., 2012). Thus, it appears plausible that intranasal oxytocin has long lasting effects in the central nervous system. Finally, the placebo used in the current study contained saline, and did not contain the inactive ingredients contained in the oxytocin solution. It is possible that the findings observed in the present study could be due to the effects of a non-active compound administered with oxytocin.

In conclusion, this study lends further support to the hypothesis that intranasal oxytocin lowers physiological biomarkers of stress, which may be one mechanism by which oxytocin

promotes affiliative approach behavior (Taylor et al., 2000). Although speculative, intranasal oxytocin may be attenuating the cortisol response to different laboratory paradigms through a common action on the adrenal glands. Moreover, it appears that the effect of oxytocin on the cortisol response to a physical stressor is dose-specific, as it was not observed when participants were administered 48IU of oxytocin. Further research is needed to delineate dose effects of intranasal oxytocin in humans, as current practice dictates no concensus on dosage in experimental research. Clearly, understanding dose-response relations will become increasingly important, as attempts to use oxytocin clinically are currently underway (Guastella et al., 2009).

Transition paragraph

The first dissertation study had the central aim of examining the impact of two doses of intranasal oxytocin on the cortisol response to stress. This effect was shown to be inhibitory, similar to research in non-human animals. This inhibitory effect is posited to be central to inhibiting the *fight-or-flight* response to stress, which might mobilize an individual to seek social support instead of fighting or fleeing during a stressful situation (i.e. *tend-and-befriend*, not *fight-or-flight*). Although the glucocorticoid response is not considered to be the central immediate *fight-or-flight* neurohormonal response to stress (i.e. adrenaline, noradrenaline), it is central to the long-term adaptation to a stressful challenge. Thus, oxytocin may act to shift the stress response from *fight-or*-flight to seeking social support during stressful situations (i.e. *tend-and-befriend*) by dampening the HPA response over time. The results of this study also show that oxytocin inhibits cortisol rise during physical stress, which may suggest that this effect generalizes across social and non-social contexts. This effect was further shown to be dose-dependent, such that a 24IU dose of intranasal oxytocin had the strongest inhibitory effect on cortisol. The results of this study support the notion that endogenous release of oxytocin in response to various stressors could effectively inhibit cortisol rise and thus inhibit *fight-or-flight*. Inhibiting this response is only one component effect of oxytocin outlined in the *tend-and-befriend* theory, however, and additional mechanisms require support to substantiate the role of oxytocin in distress-motivated social support seeking in humans. For example, this response includes the effect of oxytocin on promoting attention to socio-emotional stimuli as a means of enhancing social support seeking and social bonding, in addition to the established inhibitory effect of oxytocin on the *fight-or-flight* response. Such a selective and specific effect of oxytocin on attention to socio-emotional stimuli is the central aim of the study presented next. In this study, we use a 24IU dose of intranasal oxytocin, which was shown in the preceeding chapter to have the greatest effects. The study presented next will help to bolster support for a component mechanism of the *tend-and-befriend* response in humans, namely increasing attention to socio-emotional cues in the environment.

Chapter 3: The Effect of Intranasal Oxytocin on Perceiving and Understanding Emotion on the Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT)

Cardoso, C., Ellenbogen M.A. & Linnen, A-M. (2014). The effect of intranasal oxytocin on perceiving and understanding emotion on the Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT). *Emotion, 14,* 43-50.

Abstract

Evidence suggests that intranasal oxytocin enhances the perception of emotion in facial expressions during standard emotion identification tasks. However, it is not clear whether this effect is desirable in people who do not show deficits in emotion perception. That is, a heightened perception of emotion in faces could lead to "oversensitivity" to the emotions of others in non-clinical participants. The goal of this study was to assess the effects of intranasal oxytocin on emotion perception using ecologically-valid social and non-social visual tasks. Eighty-two participants (42 women) were administered a 24IU dose of intranasal oxytocin or a placebo in a double-blind, randomized experiment, and then completed the perceiving and understanding emotion components of the Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT). In this test, emotion identification accuracy is based on agreement with a normative sample. As expected, participants administered intranasal oxytocin rated emotion in facial stimuli as expressing greater emotional intensity than those given a placebo. Consequently, accurate identification of emotion in faces, based on agreement with a normative sample, was impaired in the oxytocin group relative to placebo. No such effect was observed for tests using non-social stimuli. The results are consistent with the hypothesis that intranasal oxytocin enhances the salience of social stimuli in the environment, but not non-social stimuli. The present findings support a growing literature showing that the effects of intranasal oxytocin on social cognition can be negative under certain circumstances, in this case promoting "oversensitivity" to emotion in faces in healthy people.

Introduction

There is a growing agreement among researchers that oxytocin is an important regulator of social behavior in humans (Bartz & Hollander, 2006; Campbell, 2010). It is posited that one way oxytocin modulates social behavior is through its effect on emotion recognition (Guastella & Macleod, 2012). A number of studies have demonstrated that the administration of oxytocin using a nasal spray— a method that has been shown to reliably increase the bioavailability of structurally related nonapeptides (i.e., vasopressin) in the cerebrospinal fluid (CSF; Born et al., 2002)— alters the perception of emotion using facial stimuli. Intranasal oxytocin has been shown to improve accuracy on the Reading the Mind in the Eyes Test (RMET; Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001) in individuals with elevated alexithymia scores (Luminet, Grynberg, Ruzette, & Mikolajczak, 2011). The RMET is a measure of the ability to identify the mental state of others solely from expressions in the eye region of the face. In people diagnosed with autism spectrum disorders, intranasal oxytocin improves performance on the easy items of the RMET (Guastella et al., 2010), and healthy individuals show improvement on the difficult items of the RMET following intranasal oxytocin administration (Domes et al., 2007).

According to the social salience hypothesis (Averbeck, 2010; Bartz et al., 2011b), intranasal oxytocin increases the perceived visual salience of socially-relevant stimuli in the environment, regardless of valence, which could partly explain the aforementioned improvements on the RMET. Consistent with the social salience hypothesis, intranasal oxytocin increases gaze to the eye region of the face (Guastella, Mitchell, & Dadds, 2008), an effect that is associated with an increased functional coupling of the superior colliculi and the amygdala (Gamer, Zurowski, & Büchel, 2010)— a circuit whose links to the pulvinar nuclei of the thalamus have been hypothesized to be important for moderating visual saliency (Robinson & Petersen, 1992). While the effect of oxytocin on the perception of different emotional expressions in faces has been inconsistent (Guastella & MacLeod, 2012), a number of studies have shown that intranasal oxytocin enhances the detection of emotion in faces at lower emotional intensities across a number of methodologies (Leknes et al., 2012; Lischke et al., 2012; Marsh, Yu, Pine, & Blair, 2010; Prehn et al., 2013)— an effect that is often associated with increased pupil dilation (Leknes et al., 2012; Prehn et al., 2013). Intranasal oxytocin has also been shown to enhance the detection of emotion in faces at an early level of perceptual processing (Schulze et al., 2011), and a recent meta-analysis confirms that emotion recognition is enhanced following oxytocin administration

(Shahrestani, Kemp, & Guastella, 2013). Taken together, these data suggest that intranasal oxytocin appears to enhance the ability to recognize emotion in faces under circumstances where it may be difficult to discern the presence of emotion (e.g., low intensity emotions, difficult items on the RMET), which compliments research that suggests intranasal oxytocin is beneficial primarily among those individuals who have difficulty identifying emotion in faces (Bartz et al., 2010; Guastella et al., 2010; Leknes et al., 2012; Pedersen et al., 2011).

Researchers have posited that, since we often feel what others are feeling in an effort to make ourselves sympathetic (Jackson, Rainville, & Decety, 2006), being able to identify a greater number of emotions in others may incur certain additional costs in resources, with respect to having to regulate those emotions (Hodges & Kline, 2001). While the effects of intranasal oxytocin are often interpreted as prosocial and beneficial (Unvas Moberg & Francis, 2003), enhanced emotion perception may not be desirable in persons who are already hypersensitive to the emotions of others and have difficulties regulating their own emotions, such as patients with borderline personality disorder (Fertuck et al., 2009; Flury, Ickes, & Schweinle, 2008). In healthy people, having an exceptional ability to infer the emotions of others may be characteristic of individuals who are labeled oversensitive by their peer groups (Schaller & Cialdini, 1988), and thus with respect to emotion perception, more is not necessarily better.

The methodologies employed in this research area have not directly addressed the question of whether intranasal oxytocin effects an adaptive change in emotion perception in healthy people who have no difficulties in this domain. Many studies in this area use paradigms with digitally manipulated facial stimuli from standardized picture sets to express an extreme range of emotional intensities (Blair, Colledge, Murray, & Mitchell, 2001), and the real-world implications of performance on these tasks cannot be readily translated. Further, it is unclear how oxytocin might influence the perception of ambiguous blends of emotion that are characteristic of real-world social interactions. More recently, studies have examined the effect of intranasal oxytocin on performance on the empathic accuracy task (Bartz et al., 2010; Zaki, Weber, Bolger, & Ochsner, 2009) and the multifaceted empathy task (MET; Hurlemann et al., 2010; Dziobek et al., 2008)— two tasks that have greater ecological validity than face morphing tasks. However, these tasks are not without limitation for addressing the effect of oxytocin on perceived saliency of emotion in faces. The empathic accuracy task is a measure of the *correlation* between the participants' rating of emotions in a target person and that target person's own rating of their

emotions on a scale from 1 (very negative) to 9 (very positive), but this task does not measure differences in average ratings (Zaki, Weber, Bolger, & Ochsner, 2009). In other words, if the participant rates the emotions of the target person over time the same way as the target person rates their own emotions (i.e. participant ratings = 4, 5, 6; target person's ratings = 4, 5, 6), or if the participant consistently "overshoots" the target person's ratings (i.e. participant ratings = 7, 8, 9; target person's ratings = 4, 5, 6), both result in the same *correlation*, or measure of accuracy. Further, while the MET obtains ratings of emotion identification, it does not measure ratings of perceived intensity of the emotion being rated. The MET does, however, ask participants how emotionally aroused they felt while viewing pictures of faces, which appears to be increased following intranasal oxytocin administration (Hurlemann et al. 2010).

Another important issue for understanding the effects of oxytocin on emotion perception is whether its effects are specific to social stimuli, relative to non-social stimuli. Few studies have compared the effects of oxytocin on social cognition using social and non-social stimuli. In a study of non-human primates (Parr, Modi, Siebert, & Young, In Press), the administration of oxytocin reduced attention to negative facial expressions in non-human primates, but had no effect on attention to neutral faces or non-social images. Human studies of memory (Rimmele, Hediger, Heinrichs, & Klaver, 2009; Unkelbach, Guastella, & Forgas, 2008) and social reinforcement (Hurlemann et al. 2010) have similarly demonstrated that oxytocin promotes the processing of social over non-social stimuli. In the present study, we will explicitly examine whether oxytocin influences emotion perception in social and non-social stimuli using faces and natural scenes. Artists have long understood that emotion is often evoked from natural scenes (e.g., an image of a plush rain forest may elicit different emotions than an image of an arid desert or an active volcano), and a task that measures this form of aesthetic empathy (Wispé, 1968) could serve to verify whether the effects of intranasal oxytocin on emotion perception are specific to social stimuli, which could help inform our prediction of how contextual factors moderate the effects of oxytocin (Bartz, Zaki, Bolger, & Ochsner, 2011b).

To investigate the influence of intranasal oxytocin the perception of emotion in faces using naturalistic stimuli, healthy male and female participants completed the *perceiving* (faces and designs task) and *understanding* emotion scales of the Mayer-Salovey-Caruso Emotional Intelligence Test, following the administration of a 24IU dose of intranasal oxytocin or a placebo. The administration of the "understanding emotion" scales of the MSCEIT allowed us to compare

the effects of intranasal oxytocin on emotion perception with its effects on participants' ability for abstract verbal reasoning about the emotions of others. Another novel aspect of the MSCEIT is that it assesses both the accuracy of detecting the emotion being expressed and its perceived intensity. Given that there is evidence that oxytocin may increase the salience of social stimuli, thereby increasing sensitivity to the emotion of others, the MSCEIT allows us to directly examine this hypothesis by comparing the drug effects on ratings of intensity of emotions in faces. There is some controversy in how "accuracy" is assessed in emotion identification tasks, particularly in the context of naturalistic stimuli (Batson, 2009). The MSCEIT and its corresponding subscales have often been criticized in the literature for defining accurate responses with respect to how well participant responses are in agreement with the responses of a normative sample (Maul, 2012). That is, if the participant responses are very consistent with the responses of a normative sample, they are considered to be more accurate, and thus more emotionally "intelligent" than individuals whose responses are not consistent with the normative sample. However, there is evidence to support the validity of this test (Mayer, Salovey, & Caruso, 2012)— standardized scores on the MSCEIT are positively related to a number of indicies of adaptive interpersonal functioning (Brackett, Mayer, & Warner, 2004; Brackett, Rivers, & Salovey, 2011; Lopes, Salovey & Straus, 2003). More importantly, the MSCEIT is a useful tool in this study to examine whether intranasal oxytocin increases participants' sensitivity to the emotions of others when compared with a normative sample. We put forth the following predictions: First, since intranasal oxytocin enhances the perception of emotion in faces, we predicted that individuals would perceive more emotional intensity in stimuli on the faces task of the MSCEIT, which would consequently *decrease* participants accurate identification of emotions on this task based on agreement with a normative sample. Second, we hypothesized that the effect of intranasal oxytocin on emotion perception would be specific to the perceptions of social stimuli, and would therefore have no effect on non-social emotional stimuli or on one's verbal understanding of emotion, both of which have rarely been examined in this area of research.

Method

Participants

One hundred and two men and women aged 18 to 30 years old were recruited to participate in the study through advertisements at local universities. Participants were excluded from participation if they met any of the following criteria: current smoking, regular consumption

of prescription, non-prescription, or illicit drugs, poor English language fluency, current illness, chronic medical condition or major sensory impairment, history of a mental disorder, or pregnancy.

Of the 102 students who participated in the study, data on the MSCEIT was collected in only 82 participants because the MSCEIT was omitted from the study protocol as a consequence of a clerical error for the first 20 participants in the study. Forty-two participants were administered oxytocin (21 females; 3/11 luteal phase, 5/10 follicular phase on oral contraceptives) and 40 were administered the placebo (21 females; 5/9 luteal phase, 4/12 follicular phase on oral contraceptives). The current project was approved by the Human Research Ethics Committee at Concordia University (Montréal, Canada). Informed consent was obtained for all participants.

The Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT)

The Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT) is a well-known objective measure of emotional intelligence (Mayer et al., 2000; Mayer et al., 2002). The internal consistency reliability for the Perceiving Emotion scale (r=.91) and Understanding Emotion scale (r=.80), as reported by the authors of the MSCEIT, range from good to excellent, and shows modest convergent validity with other scales of emotional processing (Mayer et al., 2002; Roberts et al., 2006), as well as discriminant validity from intelligence and personality measures (Orchard et al., 2009). Scores on the MSCEIT are meaningfully related to real-world outcomes (Brackett, Mayer, & Warner, 2004; Brackett, Rivers, & Salovey, 2011; Lopes, Salovey, & Straus, 2003; Salovey & Grewal, 2005) and are distinct from self-report ratings of empathy (Orchard et al., 2009). Tasks related to the perceiving emotion (*faces* and *designs* task) and understanding emotion (*changes* and *blends* task) scales were used in this study. While research on the MSCEIT does not typically examine individual sub-scales relative to the full-scale emotional intelligence quotient, there are studies that have meaningfully used the MSCEIT in this way (Lin et al., 2012), and sub-scales are often explored independently of the full-scale emotional intelligence quotient (Roberts et al., 2006). This method is partly supported by a recent study on the factor structure of the MSCEIT (Brannick, Wahi, & Goldin, 2011).

The *faces task* required participants to rate four facial expressions on the degree to which they expressed a combination of five of the following emotions from 1 (not at all) to 5

(extremely): sadness, fear, surprise, disgust, excitement, happiness, and anger. The *designs task* required participants to rate six pictures of non-social natural scenes (e.g., a picture of an arid desert, a picture of a calm lake) and designs in the same fashion as described in the faces task. The *changes task* assessed the conceptual understanding of how the intensification of certain emotions may lead to different emotions. It consisted of twenty questions, and participants selected an emotion from a list that best described the end of a progression of emotions. For example, one item asks participants "Majorie felt more and more ashamed, and began to feel worthless. She then felt _____(a. overwhelmed, b. depressed, c. ashamed, etc…). The *blends task* assessed the identification of complex emotional blends. It consisted of 12 questions, and participants determined how emotions combine to form other emotions. For example, one item asks participants "Combining the feelings of disgust and anger results in _____ (c. shame, d. hatred, e. contempt, etc…)

Procedure

Following informed consent and an assessment of medical history, participants self-administered 24IU of intranasal oxytocin or a placebo (saline) using a nasal spray in a double-blind, randomized fashion. After drug administration, participants underwent 35 minutes of relaxation, followed by an emotion-modulated EMG startle protocol, a spatial cueing task, and a negative priming task described elsewhere (Ellenbogen et al., 2012; Ellenbogen et al., 2013). At 120 minutes following administration of intranasal oxytocin or placebo, participants completed the MSCEIT (described above). Previous work has shown that the effects of neuropeptides in the central nervous system persist beyond 80 minutes (Born et al., 2002), and we have documented effects of oxytocin on cognition up to 115 minutes post-administration (Ellenbogen at al., 2012). Another study has demonstrated that the effects of intranasal oxytocin on social cognition in dementia patients last up to 8 hours post-administration (Jesso et al., 2011). Further, at least two studies have documented higher salivary oxytocin concentrations at least two hours following intranasal oxytocin administration (Huffmeijer et al., 2012; Weisman, Zagoory-Sharon, & Feldman, 2012) and a third study documented elevated levels 7 hours post-administration (van IJzendoom, Bhandari, van der Veen, Grewen, & Bakermans-Kraneburg, 2012). Participants were remunerated $60 for their participation.

Statistical analyses

Scores on the MSCEIT were calculated using the *consensus* method employed by the authors of this test (Mayer et al., 2002; Mayer, Salovey, & Caruso, 2012). Participants are awarded a score for each item proportional to the percentage of a normative sample (2,112 participants of ages ranging from 16 to 79 from a mixed sex/ethnicity sample) that endorsed their response. That is, if a participant rates an item "3" on a likert scale ranging from 1 to 5, and 32% of the normative sample endorsed "3" on that test item when the measure was normed, then the participant would be awarded a score of .32 for that item. It should be noted that the validity of this method for assessing emotional intelligence has been criticized (Maul, 2012), and this critique has been followed by a rebuttal from the authors (Mayer, Salovey, & Caruso, 2012). There is currently no consensus in the literature concerning the optimal method for assessing emotional intelligence (for a review, see Orchard et al., 2009).

We conducted a 2 (oxytocin, placebo) x 2 (male, female) MANOVA on the total standard scores (Mean = 100, SD = 15) of the four tasks of the MSCEIT (faces, designs, blends, changes). Multivariate effects were explored for interactions with oral contraceptive use and menstrual cycle phase in females using multivariate analysis of covariance (MANCOVAs), to determine if univariate tests should be further conducted to probe these interactions.

We followed up statistically significant effects with Univariate ANOVAs on participant accuracy scores. We transformed the raw accuracy scores from the different facets of each task (e.g., identification of fear, excitement, surprise, anger, happiness, sadness, disgust on the faces task) into a T-score transformed (Mean = 50, SD = 10) and between-subject value that reflects how well participants scored on each facet of a task in isolation (e.g., how accurately participants identified disgust on the faces task in the oxytocin condition relative to placebo). As an additional consideration of these data, it is important to note that rating an emotion as too high or too low results in lower absolute accuracy scores on the *faces* and *designs* tasks compared with a normative sample. Thus, we compared participant *intensity* ratings across drug condition (i.e., the degree to which each emotion was being expressed from 1, not at all, to 5, extremely). The following example illustrates the utility of intensity ratings: if there is consensus that the target emotion being expressed is a "3", a rating of both "2" or "4" will both result in lower absolute accuracy based on agreement with a normative sample. Thus, by comparing these ratings across drug condition, we can infer whether participants were rating emotions as *too high* or *too low*

following drug administration, relative to the normative sample. Since the MSCEIT is a protected test, we do not provide mean participant ratings for stimuli on the MSCEIT; however, the direction and magnitude of the effects can be inferred from the effect size and test statistics provided.

Results

The effect of oxytocin on perceiving and understanding emotion

A 2 x 2 MANOVA revealed that drug condition was a statistically significant predictor of total standardized scores on the 4 tasks of the MSCEIT [drug: Wilks' $\lambda = .884$, $F(4,75) = 2.457$, $p = .05$]. No effects of sex or the interaction between drug condition and sex were found [sex: Wilks' $\lambda = .961$, $F(4,75) = 0.765$, $p > .05$; drug X sex: Wilks' $\lambda = .938$, $F(4,75) = 1.245$, $p > .05$]. A follow-up analysis of the effect of drug condition revealed that standardized scores on the faces task of the MSCEIT were lower following oxytocin administration (Mean ± SD; 106.12 ± 23.92) relative to placebo (118.92 ± 19.54; $F(1,80) = 8.861$, $p < .01$, $\eta^2 = .10$). In other words, participants in the oxytocin condition were less accurate in identifying the emotional expression in faces on this task relative to placebo based on agreement with a normative sample. No statistically significant drug effects were detected on standardized scores for the remaining tasks on the MSCEIT [changes: placebo 95.75 ± 12.82 vs. oxytocin 96.78 ± 11.76, $F(1,80) = 0.572$, $p > .05$; designs: placebo 96.56 ± 9.25 vs. oxytocin 95.14 ± 10.34, $F(1,80) = 0.429$, $p > .05$; blends: placebo 97.36 ± 10.43 vs. oxytocin 97.82 ± 8.91, $F(1,80) = 0.149$, $p > .05$). A MANCOVA revealed no relation between drug condition and oral contraceptive use (yes, no) and menstrual phase (follicular, luteal) on standardized scores of the four tasks of the MSCEIT [drug X oral contraceptives controlling for menstrual phase: Wilks' $\lambda = .897$, $F(4,34) = 0.974$, $p > .05$; drug X menstrual phase controlling for oral contraceptives: Wilks' $\lambda = .767$, $F(4,34) = 2.579$, $p > .05$).

The effect of oxytocin on perceiving specific emotions on the faces task

We conducted analyses of participant accuracy scores on the faces task based on agreement with a normative sample to follow-up the main effect of oxytocin on standardized accuracy scores described previously. Accuracy scores were lower following oxytocin administration (47.10 ± 11.85) relative to placebo (53.05 ± 6.44) across all emotions ($F(1,80) = 7.874$, $p < .01$, $\eta^2 = .09$; Figure 1). The effect of oxytocin on emotion identification accuracy

based on agreement with a normative sample also varied depending on the emotion being rated (drug X valence; $F(6,75) = 2.689$, $p < .05$, $\eta^2 = .20$), such that the effect of oxytocin on lowered accuracy was greatest when rating surprise (46.47 ± 11.37) and disgust (46.63 ± 11.72) relative to placebo [surprise: 53.71 ± 6.65, $F(1,80) = 12.210$, $p < .001$, $\eta^2 = .13$; disgust: 53.54 ± 6.18; $F(1,80) = 11.283$, $p < .001$, $\eta^2 = .12$; Figure 1).

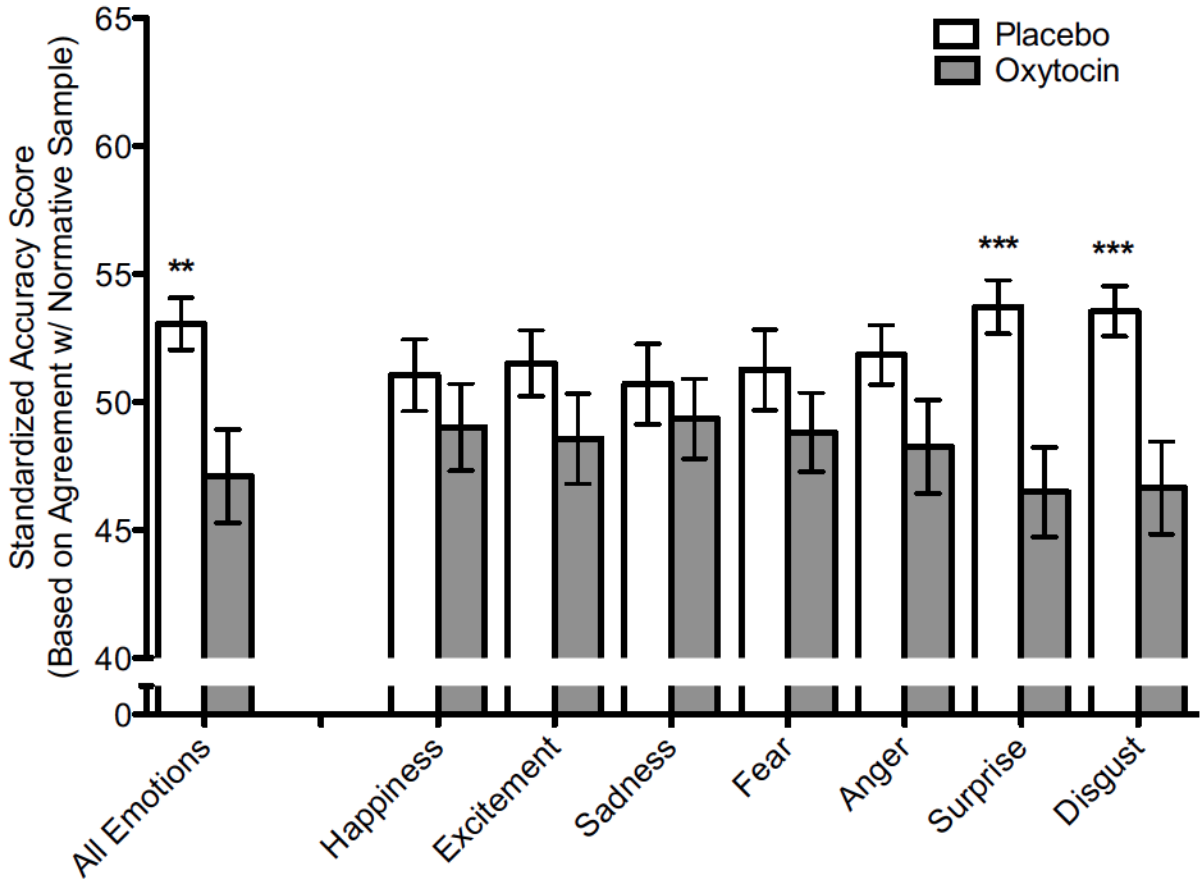

Figure 1. Oxytocin (OT) $N = 42$, Placebo (PL) $N = 40$. * $p < .05$, *** $p < .001$. All data in this graph represents participant performance when rating emotions on the faces task of the MSCEIT collapsed across all items. Error bars represent 1 SEM. The standardized accuracy score is a T-score transformed (Mean = 50, SD = 10) and between-subject value that reflects how well participants scored when rating each emotion on this task. Accurate identification was impaired for ratings of all emotions based on agreement with a normative sample, particularly disgust and surprise, following oxytocin administration relative to placebo.

The effect of oxytocin on intensity ratings of facial emotions

In order to determine the direction of the effects described above (as mentioned previously, rating an emotion too high or too low could result in lower accuracy based on agreement with a normative sample), we explored the raw intensity ratings that the participants in this study endorsed (Figure 2). Our results show that participants rated all facial expression with more intensity relative to ratings following the administration of placebo, particularly when rating surprise and disgust.

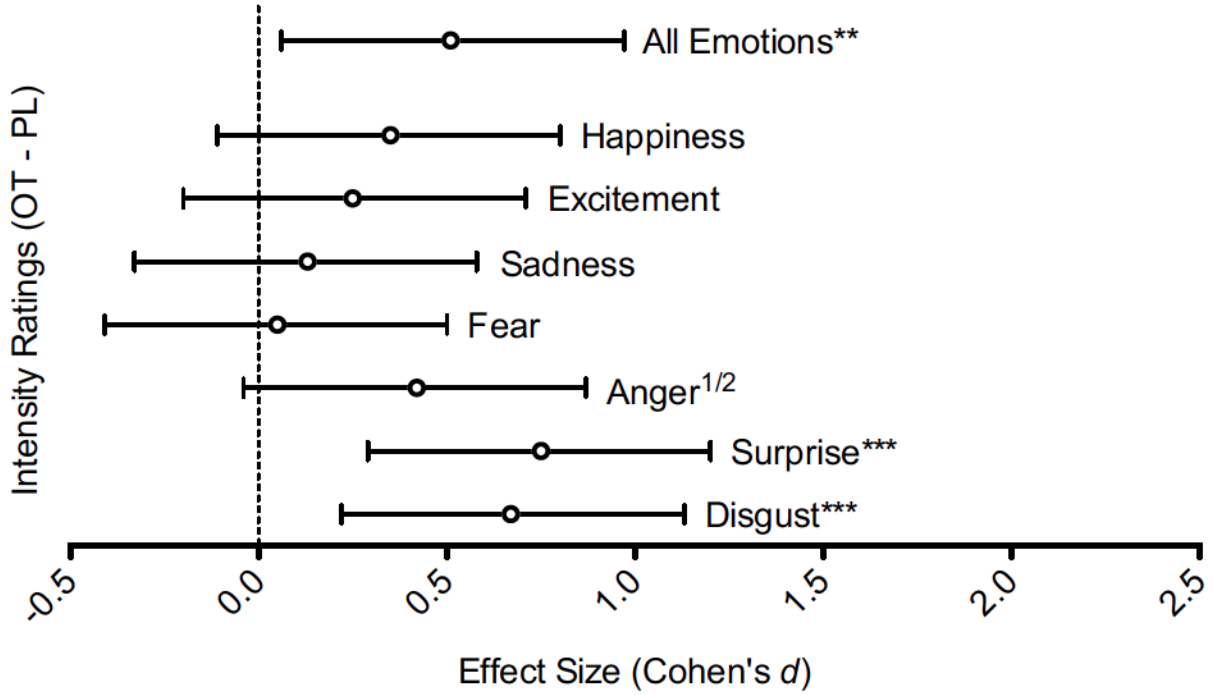

Figure 2. Oxytocin (OT) $N = 42$, Placebo (PL) $N = 40$. * $p < .01$, *** $p < .001$. All data in this graph represents participant performance when rating emotions on the faces task of the MSCEIT collapsed across all items. Error bars represent the 95% confidence interval. Positive *Cohen d* statistics reflect higher ratings of intensity for the oxytocin administration group relative to placebo. Participants rated all emotions with more intensity in the oxytocin condition relative to placebo, particularly for ratings of surprise and disgust.

In sum, participants were less accurate in identifying emotions on the faces task of the MSCEIT following oxytocin administration relative to placebo, particularly when rating surprise and disgust. Decreased accuracy following oxytocin administration co-occurred with ratings of

increased intensity, suggesting that increased ratings of intensity impeded the accurate recognition of emotions on the faces task of the MSCEIT. Oxytocin did not influence emotional understanding or the perception of emotion in non-social stimuli (all Cohen *d* statistics for these variables ranged from -0.02— 0.15).

Discussion

In line with the social salience hypothesis concerning the effect of intranasal oxytocin on social cognition (Guastella & Macleod, 2012), we predicted that intranasal oxytocin would enhance the perception of emotion in faces, but not the perception of emotion in non-social stimuli. Consistent with our prediction, intranasal oxytocin increased ratings of intensity of all emotions perceived in faces. Consequently, this effect *decreased* the accurate identification of emotions in faces on the MSCEIT, where the identification accuracy of an emotion is based on agreement with a normative sample. We did not find an effect of intranasal oxytocin on ratings of emotion in non-social stimuli (designs and natural scenes), or on a task of abstract verbal reasoning about emotion. While it has been previously reported that oxytocin improves emotion recognition in individuals who have difficulty identifying emotion in faces (Bartz et al., 2010; Guastella et al., 2010; Leknes et al., 2012; Pedersen et al., 2011), our results show that intranasal oxytocin may actually impair emotion recognition in healthy individuals on tasks that contain natural, ecologically-valid facial stimuli when compared with agreement of a normative sample. These data suggest that increasing the salience of emotion in faces may not be beneficial in individuals who do not show impairments in emotion identification.

Considering that oxytocin has been implicated in socially-relevant information processing (Guastella & Macleoud, 2012), it is not surprising that intranasal oxytocin altered emotion recognition during the faces task of the MSCEIT, but not on tasks assessing emotions in non-social natural scenes or abstract verbal reasoning about emotion. The study has important implications regarding theoretical views of how intranasal oxytocin alters social cognition. For individuals who show deficits in emotion-perception, the administration of oxytocin has overwhelmingly elicited adaptive effects on their ability to recognize emotion in others (e.g., persons with autistic traits, poor social competence, schizophrenia; Bartz et al., 2010; Guastella et al., 2010; Leknes et al., 2012; Pedersen et al., 2011). However, in persons who have no deficits in this domain, oxytocin administration may be maladaptive in some situations, as an increase in

emotion perception may cause an overestimation of emotions that are not present. Further, for individuals who are too sensitive to emotion perception (i.e., Borderline Personality Disorder; Fertuck et al., 2009), oxytocin may be harmful. At least two studies have documented negative effects of oxytocin on trust and cooperation in individuals who suffer from borderline personality disorder (Bartz et at al., 2011a; Ebert et al., 2013), and another recent study has shown inhibitory deficits following oxytocin administration in persons with high depression scores (Ellenbogen et al., 2013). Thus, the results of the present study suggest that individual differences in emotion perception may represent an important determinant of whether intranasal can be used to positively enhance social cognition.

Although oxytocin administration worsened accuracy ratings of all emotions presented in this study, ratings of disgust and surprise were most affected by the drug administration. It is important to note that the specific effects of intranasal oxytocin on disgust and surprise were unexpected and unrelated to the study hypotheses, and therefore should be interpreted cautiously. One possible explanation for the effect of oxytocin on the perception of disgust in the current study could be related to the effect of intranasal oxytocin on the allocation of attention to the eye region of the face (Gamer, Zurowski, & Büchel, 2010; Guastella, Mitchell, & Dadds, 2008). It has been shown that disgust is difficult to identify by solely reading the eyes, and people more often allocate attention to both the eyes and the mouth to correctly classify disgust (Aviezer et al., 2008). Thus, differential allocation of attention to the eye region of the face when rating disgust could have led participants to over-rate the intensity of disgust in faces, more so than other emotions that can more easily be read in the eyes. The amplified effect of intranasal oxytocin on ratings of surprise, on the other hand, could be related to having participants rate multiple emotions in the same face. Fear and surprise share common characteristics (e.g., widening of the eyes), and the amygdala has been shown to play a crucial role in identifying surprise (Kim, Somerville, Johnstone, Alexander, & Whalen, 2003), particularly when rating blends of surprise and fear in facial expressions (Adolphs et al., 1994). In the current study, rating all facial expressions on a number of emotional dimensions may have led participants who were administered oxytocin to have particular difficulty deciphering which of the two similar emotions (i.e. fear, surprise) were present in the same face, leading them to rate faces as expressing comparable intensities of multiple emotions. In line with this interpretation of the data, a large percentage of participants in the placebo condition gave the most accurate response to ratings of

surprise across all faces based on agreement with a normative sample, while most individuals in the oxytocin condition did not (placebo: 60%, oxytocin: 36%, $p < .05$), and this effect was not found for ratings of fear. Clearly, there is a need to replicate these findings, and more research on the effect of intranasal oxytocin on emotion perception in ecologically-valid contexts (i.e., rating multiple emotions in the same face using naturalistic stimuli) is warranted.

A number of study limitations warrant consideration. First, the MSCEIT has been criticized for defining accuracy in terms of consensus, since consensus of a normative group of people on the expression of an emotion may not necessarily be the correct response (Maul, 2012). However, the authors of the MSCEIT have recently shown that there is strong correlation between expert responses and consensus responses on the MSCEIT using a multi-aged, multi-ethnic sample of 5,000 individuals (Mayer, Salovey, & Caruso, 2012), indicating that both experts and normative samples tend show convergent responses to the MSCEIT. Second, we analyzed the different facets of the MSCEIT in such a way as to render the measure less reliable (i.e., a smaller number of responses per category of emotion), and thus the results of the current work need to be replicated in the context of a more rigorous laboratory task with more reliable trials. However, there is empirical support for using the *faces tasks* (one of the measures of the "perceiving emotion" factor of the MSCEIT) as an independent measure (Brannick, Wahi, & Goldin, 2011; Lin et al., 2012), which partly supports the validity of our main findings. Third, the placebo used in the current work contained saline, and was not matched with oxytocin on inactive pharmacological compounds. Thus, it is possible that the observed effects were in part a consequence of the inactive compounds administered with oxytocin. Fourth, it should be noted that while we did not find an effect of menstrual cycle or contraceptive use in the current study, self-report methods are not entirely reliable, and more subtle hormonal interactions may only become evident with the use of better measures (e.g., hormone assays).

Finally, while most studies have examined the relation between oxytocin and indices of cognition and behavior 30 or 45 minutes following administration, the present study examined the acute effects of intranasal oxytocin on emotion recognition 120 minutes after administration. As noted previously, a report by Born and colleagues (2002) suggests that structurally related neuropeptides (i.e., vasopressin) administered intranasally remain stable and elevated 80 minutes after administration, and studies from our laboratory have documented effects of intransal oxytocin 90 minutes (Cardoso, Ellenbogen, & Linnen, 2012a; Cardoso, Linnen, Ellenbogen, &

Joober, 2012b) 115 minutes (Ellenbogen et al., 2012; Ellenbogen et al., 2013), and even 130 minutes (Cardoso, Ellenbogen, Orlando, Bacon, & Joober, 2013) after administration. Another study has demonstrated that the effects of intranasal oxytocin on social cognition in dementia patients last up to 8 hours post-administration (Jesso et al., 2011). Further, at least two studies have documented higher salivary oxytocin concentrations at least two hours following intranasal oxytocin administration (Huffmeijer et al., 2012; Weisman, Zagoory-Sharon, & Feldman, 2012) and a third study documented elevated levels 7 hours post-administration (van IJzendoom, Bhandari, van der Veen, Grewen, & Bakermans-Kraneburg, 2012). Thus, it appears plausible that intranasal oxytocin has long lasting effects on the central nervous system. However, even though the effects of intranasal oxytocin are long lasting on different indirect markers of oxytocinergic function (i.e. social behavior, saliva, CSF), it is not yet clear how oxytocin administered intranasally effects change on biological systems. Despite the documented evidence of neuropeptide changes in CSF following intranasal administration, there is no conclusive evidence that intranasal oxytocin actually reaches the brain, and if it does, whether it reach putative target areas in the limbic system and midbrain. That is, there are no ligand-binding positron emission tomography studies documenting that the molecule does in fact reach target areas in the brain directly. Indeed, the effects observed in this and other intranasal oxytocin studies could very well be a consequence of interactions between the oxytocinergic and other neurochemical systems, without synthetic oxytocin ever reaching specific targets in brain tissue (Churchland & Winkielman, 2012). Clearly, there is urgent need for animal models of the intranasal neuropeptide administration paradigm and human imaging studies assessing synthetic oxytocin binding to further clarify these important methodological concerns.

In conclusion, we demonstrate that intranasal oxytocin enhances the perception of emotional intensity in faces, which consequently impairs accurate identification of facial emotions in healthy young adults when compared with the responses of a normative sample. Importantly, this is the first study to demonstrate that intranasal oxytocin does not influence perception of emotion in non-social pictorial stimuli, nor does it influence complex abstract reasoning about the emotions of others, suggesting that the acute effects of oxytocin on emotion perception are most consistent with the hypothesis that intranasal oxytocin enhances the perceived salience of social cues in the environment (Averbeck, 2010; Bartz et al., 2011b), rather than promoting positive social cognition more generally. There is an urgent need to better

understand the component processes of social behaviour that are affected by intranasal oxytocin so that we can improve our predictions of how oxytocin will influence behaviour depending on characteristics of the individual as well as contextual factors (Bartz et al., 2011b), particularly since there is strong interest in the use of intranasal oxytocin in clinical populations (Andari et al., 2010; Feifel et al., 2010; Guastella, Howard, Dadds, Mitchell, & Carson, 2009; Hall, Lightbody, McCarthy, Parker, & Reiss, 2012; Jesso et al., 2011).

Transition paragraph

The second dissertation study was conducted to examine the impact of a 24IU dose of intranasal oxytocin on the perception of emotion in human faces. The results of this study clearly demonstrated that oxytocin specifically and selectively enhances the visual perception of emotion in humans faces irrespective of valence, consistent with research in non-human animals as well as the theorized effects of oxytocin in the *tend-and-befriend* theory. These results also indicated that increased bioavailability of oxytocin can lead to oversensitivity and misidentification of emotions under some circumstances, which could conceivably have different behavioral consequences depending on the social context (i.e. positive vs. negative social feedback). The results of the presented studies so far indicate that oxytocin can (1) inhibit cortisol rise during stress, and (2) selectively increase attention to socio-emotional stimuli. The results of this latter study suggest that the behavioral consequences of oxytocin could vary depending on the social context because oxytocin may be increasing the perception of all emotional signals, irrespective of valence.

Although the effects of oxytocin on social cognition are well known, one central tenet of the *tend-and-befriend* stress response theory, namely that oxytocin should promote increased bonding during stressful circumstances, has not been adequately examined. This effect is the subject of examination in the next study in this thesis which is presented in Chapter 4. This study examined whether the effect of a 24IU dose of intranasal oxytocin on dispositional trust is dependent on the experience of high levels of distress following a stressful event, namely social rejection. This is the first experimental study to examine whether the effect of oxytocin on indices of social bonding is dependent on the experience of distress, consistent with the *tend-and-befriend* theory.

Chapter 4: Stress-Induced Negative Mood Moderates The Relation Between Oxytocin Administration And Trust: Evidence For The *Tend-And-Befriend* Response To Stress?

Cardoso, C., Ellenbogen, M.A., Serravalle, L., & Linnen, A-M. (2013) Stress-induced negative mood moderates the relation between oxytocin administration and trust: Evidence for the tend-and-befriend response to stress? *Psychoneuroendocrinology, 38,* 2800-2804.

Abstract

Recent evidence suggests that oxytocin, a nonapeptide posited to underlie the affiliation-related "tend-and-befriend" behavioral response to stress (Taylor et al, 2000), may improve interpersonal functioning by facilitating the acquisition of social support during times of distress. The assertion, however, has not been explicitly tested in humans. Thus, we examined whether the effect of oxytocin on self-perceived trust is magnified in individuals who experienced higher ratings of negative mood following social rejection. In a double-blind experiment, 100 students (50♀) were subject to a live social rejection paradigm following random assignment to either a 24IU intranasal oxytocin or placebo administration. Mood and self-perceived trust were measured following social rejection. Multiple regression and simple slope analysis revealed that oxytocin administration increased self-perceived trust relative to placebo in participants reporting a negative mood response following social rejection [b=4.245, $t(96)=3.10$, p=.003], but not in those whose mood state was euthymic. These results demonstrate that oxytocin may promote the acquisition of social support in times of distress by increasing self-perceived trust. The findings provide empirical support that oxytocin promotes an affiliation-related behavioral response to stress, consistent with the tend-and-befriend theory.

Introduction

Oxytocin is a neuropeptide that is produced in magnocellular neurosecretory cells in the supraoptic and paraventricular nuclei of the hypothalamus. Oxytocin has long been described as a stress hormone, with increased release into the blood in humans and animals through the posterior pituitary gland (Lang et al., 1983) and actions as a neuromodulator in distinct brain regions in response to emotional and physical challenge (Engelmann et al., 2004; Neumann, 2009; Neuman and Landgraf, 2012). More recently, Taylor and colleagues (2000) proposed that oxytocin is involved in a female-specific "tend-and-befriend" behavioral response to stress. It was proposed that in response to threat, oxytocin motivates women to seek out social support to help respond to challenge and ameliorate the negative impact of stress (Taylor et al., 2000). The proposal was later extended to be relevant for men as well (Geary and Flinn, 2002), being an alternative to the traditional "fight-or-flight" view of the stress response. In animals, experimentally manipulated central oxytocin promotes social approach behaviors following social stress (Lukas et al., 2011), however, evidence for the tend-and-befriend hypothesis has only been examined in humans using correlational analysis. Taylor et al (2010) found a positive relationship between concurrent measures of relationship distress and high levels of plasma oxytocin in women. In a study of mother-daughter dyads, children evidenced high levels of urinary oxytocin following a laboratory stressor when presented with the opportunity to speak with their mother afterwards (Seltzer et al., 2010). These findings are consistent with the view that high oxytocin levels during stress serve as an impetus to seek out social contact during difficult circumstances. Unfortunately, these correlational findings do not directly assess Taylor et al.'s (2000) hypothesis that oxytocin promotes affiliation during stress. As expected, studies of the effects of intranasal oxytocin on the response to different laboratory challenges reveal a stress-dampening effect of exogenous oxytocin on the hypothalamic-pituitary-adrenal axis and fear circuitry, including studies that have explicitly examined stress in an interpersonal context (Ditzen et al., 2009; Linnen et al., 2012). Similarly, other studies show that the intranasal administration of oxytocin promotes trust across different tasks (Kosfeld et al., 2005; Baumgartner et al., 2008; Cardoso et al., 2012). These findings, *in combination,* are consistent with the view by Taylor et al (2000) that oxytocin may act to promote the acquisition of social support in times of stress. However, most studies have examined the effect of oxytocin on stress *or* trust separately. Thus, the central hypothesis of Taylor et al (2000) has not been explicitly

tested using an experimental design. To do so, it would be necessary to determine if the effect of oxytocin on trust is more pronounced during interpersonal difficulties. To the best of our knowledge, this question has never been empirically addressed.

We previously reported that intranasal oxytocin lowers cortisol levels in university students during a laboratory-based social rejection challenge, and that this interpersonal stressor elicits robust negative mood change (Linnen et al., 2012). We also found that students rated themselves as more open to new experiences, more extraverted, and more trusting following oxytocin administration (Cardoso et al., 2012). We re-analyzed the data from these studies to explicitly address the question of whether oxytocin promotes increased trust following acute interpersonal distress. We predicted that, according to the tend-and-befriend theory, participants who experienced a strong and persistent negative mood response to social rejection would demonstrate the greatest increase in self-perceived trust, relative to those who reported a lesser negative mood response.

Method

Participants

One hundred 18 to 35 year old students were recruited to participate in this study through advertisements placed in local universities. Exclusion criteria included history of mental/physical illness, lifetime recreational use of illicit drugs, current medication use, current tobacco use, pregnancy, and poor English language fluency. Participants were randomized to receive intranasal oxytocin (n = 48 aged 22.4 ± 3.47 years, 24 men) or placebo (n = 52 aged 21.7 ± 3.35 years, 26 men). Data on menstrual phase and oral contraceptive use were collected in women, and these variables have not been shown to moderate effects on oxytocin administration in this data set (Cardoso et al., 2012; Linnen et al., 2012). This project was approved by the Human Research Ethics Committee at Concordia University (Montréal, Canada).

Profile of Mood States: Bipolar Form (POMS)

This 72-item inventory assesses six subjective mood states: elated-depressed, agreeable-hostile, composed-anxious, sure-unsure, energetic-tired, and clearheaded-confused. The total score (sum of all scales; internal consistency, $\alpha = .86$) was used in the present study. Lower scores indicate higher negative mood.

NEO-Personality Inventory-Revised (NEO-PI-R)

This inventory contains 240 items that measure five dimensions of personality. The *trust* facet (subscale; α = .84) of the *agreeableness* scale was used in this study. Higher scores reflect greater trust. Items from this scale include, "*I think that most of the people I deal with are honest and trustworthy*."

Yale Interpersonal Stressor (YIPS)

The YIPS consists of two staged 10-minute conversations (Stroud et al., 2000). In each conversation, the participant and two same-sex confederates (who are trained to use a standardized social exclusion protocol) discuss a topic provided by the experimenter. The paradigm is presented to the participant as a tool used to study communication behavior. The confederates gradually exclude the participant from each conversation (Stroud et al., 2000). For example, while the participant is treated cordially in the first two minutes of each conversation, he or she is often disagreed with, interrupted, and ignored in the final four minutes. The YIPS has been shown to be effective in inducing negative mood (Linnen et al., 2012).

Procedure

On arrival to the laboratory, participants provided written consent, completed the first POMS measure (POMS1), and were then administered 24IU of oxytocin or a placebo using an intranasal spray— a method that reliably increases central levels of neuropeptides in humans and animals (Born et al., 2002; Neumann et al., 2013). After a fifty minute rest period, participants were administered a second POMS (POMS2) and completed the YIPS protocol. Participants completed the POMS during (POMS3) and following (POMS4) the YIPS. The NEO-PI-R was administered to participants thereafter along with a number of other self-report measures (Cardoso et al., 2012). At the time participants completed the POMS4 and NEO-PI-R, approximately 90 minutes had elapsed since drug administration. Participants were then debriefed and compensated $50.

Statistical analyses

Three variables were entered simultaneously into a multiple regression model predicting NEO-PI-R Trust scores (sample mean= 18.97 ± 5.10): drug condition (1 = Oxytocin, 0 =

Placebo), total POMS score after the YIPS (POMS4; sample mean = 137.48 ± 34.69), and the interaction between drug condition and total POMS4 score. Statistically significant interactions were followed-up with an analysis of simple slopes (Aiken and West, 1991). All higher-order variables were regressed on lower-order terms, and POMS4 scores were centered to address issues of multicollinearity and to render the main and moderating effects in this model interpretable. To control for baseline mood, separate models were run using total POMS4 mood ratings regressed on mood measured on arrival to the laboratory (POMS1) and again regressed on mood after the baseline relaxation phase (POMS2). In other words, the effect of oxytocin and mood (POMS4) on self-perceived trust was analyzed controlling for baseline mood using two possible baseline mood measures, POMS1 and POMS2. Lastly, we ran an additional Drug X Mood X Sex interaction model to rule out the possibility that sex differences influenced our results.

Results

The simultaneous regression model predicting NEO-PI-R trust scores was statistically significant ($R^2 = .129$, $F(3,96) = 4.73$, p = .004). Both drug condition ($pr^2 = .047$, b = 2.200, $t(96) = 2.27$, $p = .025$; reported previously in Cardoso et al, 2012) and POMS4 mood ($pr^2 = .044$, b = 0.030, $t(96) = 2.14$, $p = .035$) were statistically significant predictors of self-perceived trust. More importantly, as predicted, a statistically significant interaction between drug condition and mood was found ($pr^2 = .038$, b = -0.057, $t(96) = -2.04$, $p = .044$). Simple slope analysis revealed that, following social rejection, participants reporting higher levels of negative mood (1SD below the sample mean on POMS4 mood ratings) who were administered oxytocin reported greater trust than those administered placebo (b = 4.176, $t(96) = 3.04$, $p = .003$). In contrast, oxytocin had no effect on trust among those participants reporting lower levels of negative mood (1SD above the sample mean on POMS4 mood ratings) following social rejection (b = 0.223, $t(96) = 0.16$, $p = .870$; see Figure 1). The effect remained statistically significant while controlling for mood measured on arrival to the laboratory (POMS1; $t(96) = 2.67$, $p = .009$) and mood measured following 50 minutes of relaxation (POMS2; $t(96) = 2.58$, $p = .011$), which demonstrates that oxytocin improved trust in those with higher ratings of negative mood *following social rejection specifically*, rather than effecting change in those with higher ratings of negative mood in general.

The above results were specific to trust; additional regression analyses did not predict variance in the other facets of the NEO-PI-R across all factors (data not shown).

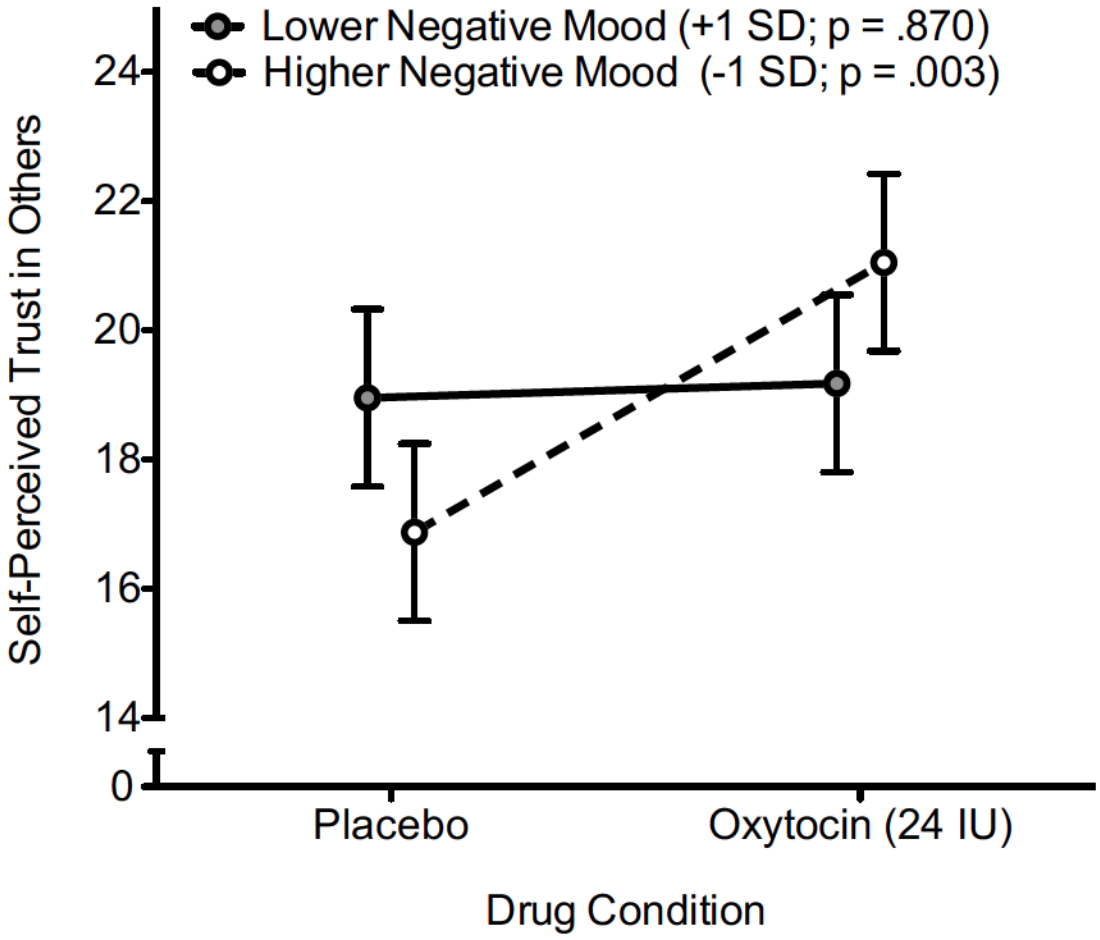

Figure 1. Following social rejection, participants reporting higher levels of negative mood (1SD below the sample mean on POMS4 mood ratings) who were administered oxytocin reported greater trust on the NEO-PI-R than those administered placebo ($b = 4.176$, $t(96) = 3.04$, $p = .003$). In contrast, oxytocin had no effect on trust among those participants reporting lower levels of negative mood (1SD above the sample mean on POMS4 mood ratings) following social rejection ($b = 0.223$, $t(96) = 0.16$, $p = .870$).

Finally, participant sex was not a statistically significant predictor of trust, nor was the Sex X Drug X mood interaction. Importantly, the Drug X Mood interaction remained statistically significant when these variables were included in the analysis (data not shown).

Discussion

While it has been previously shown that intranasal oxytocin ameliorates the negative impact of stress (Heinrichs et al., 2003; Ditzen et al., 2009; Linnen et al., 2012; Cardoso et al. 2013) and promotes trust (Kosfeld et al., 2005; Baumgartner et al., 2008; Cardoso et al., 2012), this is the first study to show that the effect of oxytocin on trust is augmented by *the experience of distress*. In fact, no relationship was observed between oxytocin and trust among those participants reporting fewer negative mood symptoms following stress. The tend-and-befriend hypothesis of oxytocin posits that oxytocin motivates individuals to affiliate in times of distress (Taylor et al., 2000), and this is the first study to provide direct experimental support of this hypothesis. Intranasal oxytocin may promote trust in part by dampening fear circuitry in the central nervous system during distress (Baumgartner et al., 2008), when activation in these circuits is greatest, and by facilitating the activation of brain circuitry important for social-approach behaviours including empathy (for a review, see Bethlehem et al., 2013). The effect documented in this study is consistent with a growing literature showing that the acute effects of oxytocin on behavior are dependent on contextual factors and individual differences (Bartz et al., 2011).

As an alternate interpretation of these data, it is possible that the response to oxytocin (i.e., increased trust) was driven by a vulnerability to social rejection, rather than negative mood per se, as individuals who did not evidence an oxytocin-related increase in trust remained euthymic following social rejection, perhaps indicating some abnormality (i.e. social detachment) in these participants. However, there was no statistical evidence that the results were driven by a small group of participants who were non-responsive to social rejection. Moreover, if there was something qualitatively different about the participants who displayed a lesser response to social rejection, one would expect that the moderation effect should be more prominent using a trait measure of social vulnerability than a state measure of mood. Additional analyses indicated that self-perceived rejection sensitivity, self-esteem and social support (reported in Cardoso et al., 2012) did not moderate the effect of oxytocin on trust in this study (data not shown). Further, lower negative mood following social rejection was associated with greater self-perceived social support and social self-esteem (data not shown), indicating that euthymic mood following social rejection was unlikely a consequence of social detachment. Taken together, the data in this study are most consistent with the tend-and-befriend hypothesis (Taylor et al., 2000). However, one

cannot rule out the possibility that participants who did not respond to social rejection with a robust negative mood response may be characterized by an abnormality in the oxytocinergic system.

An important limitation of this work is that self-perceived trust in others, as reported on a personality inventory, may not necessarily generalize to actual behavior. There is evidence in the literature that demonstrates oxytocin promotes affiliative behaviors in humans (Ditzen et al., 2009), and more work is needed to determine whether these effects are moderated by the experience of distress. Another limitation of the current study is that we report the effects of oxytocin on self-perceived trust 90 minutes following drug administration, while most studies in this area report on the acute effects of intranasal oxytocin approximately 45 minutes post-administration (e.g. Kosfeld et al., 2005). However, we have documented behavioral effects of oxytocin up to 115 minutes post administration in another study (Ellenbogen et al., 2013), and at least one study reporting on salivary oxytocin concentrations demonstrated that the molecule remains elevated up to 7 hours following intranasal administration (van IJzendoom et al., 2012).

In conclusion, the findings provide an important integration of different areas of research in the study of human oxytocinergic function. In addition to its implications for the tend-and-befriend theory, oxytocin may have important clinical benefits for those who are acutely distressed, which is consistent with a number of studies showing more pronounced effects of the neuropeptide in vulnerable populations (Bartz et al., 2011; Ellenbogen et al., 2013).

Transition paragraph

The third dissertation study indicates that the experience of distress enhances the effect of oxytocin on trust, consistent with the *tend-and-befriend* theory. So far, the results presented in this thesis demonstrate that oxytocin inhibits the physiological impetus to the *fight-or-flight* response, enhances attention to socio-emotional stimuli, and promotes trust in distressed people. The behavioral consequences of distress-motivated social support seeking, however, have not yet been evaluated in this area of research. Specifically, increasing trust, enhancing attention to emotions in faces, and inhibiting a defensive stress-response (i.e. *fight-or-flight*) during a stressful situation could theoretically have unfavorable consequences in the absence of supportive social relationships. In such a scenario, and individual would be primed for regulating the negative consequences of a stressful event with the help of a supportive ally, and the lack of such an ally would significantly reduce the utility of this response. This possibility has not yet been evaluated in experimental research, and an exploration of the context-dependent effects of oxytocin on distress-motivated social support seeking is the focus of the next and final study in this thesis. Specifically, study 4 will examine whether a 24IU dose of intranasal oxytocin increases perceived emotional support in men and women during the recall of negative emotional memories in the presence or absence of social contact. This study will also examine whether this effect is specific to the experience of distress (i.e. recalling negative memories) or whether it generalizes to to euthymic mood states (i.e. recalling neutral or positive memories). Finally, this study will also examine whether such effects are specific to women, or whether they generalize to both sexes. Throughout this thesis, evidence for sex differences in the effects of oxytocin have not been detected as would be predicted by the *tend-and-befriend* theory. To further investigate this issue, the *individual response* to oxytocin in both men and women will also be evaluated as a moderator of the effects of oxytocin on support seeking. This investigation will be critical to establishing the validity of the *tend-and-befriend* theory, and for laying the foundation for future investigations of sex differences in the stress response.

Chapter 5: Oxytocin and Social Context Moderate Social Support Seeking in Women During Negative Memory Recall

Cardoso, C., Valkanas, H., Serravalle, L., & Ellenbogen, M.A. (2016). Oxytocin and social context moderate social support seeking in women during negative memory recall. *Psychoneuroendocrinology, 70,* 63-69.

Abstract

Oxytocin is theorized to promote social support seeking under stress— an alternate biobehavioural response to challenge known as the *tend-and-befriend* response. These effects may be context dependent, however, and no study has evaluated this effect in the presence and absence of social support. The aim of this study was to evaluate the effects of oxytocin on the experience of recalling emotional autobiographical memories in two contexts— with and without social contact with an experimenter. Sixty-three individuals (32 female) participated in this randomized, double-blind, placebo-controlled, and within-subject study. During recall of negative memories in the absence of social contact, oxytocin decreased perceived emotional support relative to placebo $F(1,62)=10.75$, $p=.002$. In this same context, women who were motivated to affiliate with the experimenter following oxytocin administration showed this effect in greater magnitude $t(57)=-2.04$, $p=.047$, but showed the reverse effect (i.e. increased perceived support in response to oxytocin) when social contact with the experimenter was available $t(57)=2.78$, $p=.007$. Male participants did not evidence this pattern. These findings support the role of oxytocin in social support seeking in distressed women, and highlight the negative consequences of increasing oxytocin bioavailability in the absence of social support. Supportive relationships may be necessary to elicit the prosocial effects oxytocin.

Introduction

The mammalian hormone oxytocin is known for its role in a myriad of social processes, including pair bonding, social cognition, and stress-regulation (Carter, 2014). Oxytocin is produced in the hypothalamus and acts both as a neuromodulator in the central nervous system and as a hormone in the periphery (Carter, 2014). Because estrous hormones and stages of maternity modulate endogenous oxytocin expression in humans, Taylor and colleagues (2000) posit that the roles of oxytocin in social affiliation and stress-regulation could be implicated in a sexually differentiated biobehavioural response to stress known as the *tend-and-befriend* theory. Specifically, it is theorized that because women are smaller in stature and provide more care for offspring than men, the traditional fight-or-flight response may be maladaptive under some circumstances in women. When faced with an environmental challenge, tending to offspring could increase their chances for survival and reproduction, and befriending peers in a social group could serve as protection for women against altercations with physically dominant men. Together, it is posited that increased flexibility in the biobehavioural response to stress (i.e. not just fight-or-flight, but also tend-and-befriend) confers increased evolutionary fitness (Taylor et al., 2000). The role of oxytocin in the tend-and-befriend response, however, has not received a great deal of attention in experimental research on stress to date; women were underrepresented in experimental research on stress when Taylor and colleagues developed the tend-and-befriend theory 15 years ago (17% female; Taylor et al., 2000), and women are still underrepresented in experimental research on stress and oxytocin today (32% female; Cardoso et al., 2014b).

Exogenous oxytocin administered using a nasal spray increases its levels in human cerebrospinal fluid (Striepens et al., 2013), and studies have documented increased trust, positive communication behaviours, attention to emotion in faces, and stress regulation following its application (Kosfeld et al., 2005; Ditzen at al., 2009; Cardoso et al., 2013a; Cardoso et al., 2014a), all of which are potential mechanisms by which oxytocin could promote social support seeking under stress. Correlational evidence suggests that endogenous oxytocin may function to promote support seeking under stressful circumstances: Women in distressed relationships show elevated levels of endogenous oxytocin in plasma (Taylor et al., 2006; Taylor et al., 2010), female children in distress show increased oxytocin in urine following contact with their a caregiver (Seltzer et al., 2010), and variations in the oxytocin receptor gene predict social support seeking in distressed men and women (Kim et al., 2010). Experimental evidence also suggests

that intranasal oxytocin promotes increased trust in others following social rejection, but only in those who experience high levels of distress (Cardoso et al., 2013b), which may indicate that oxytocin increases openness to social support during stressful circumstances. The direct, and sexually differentiated, effect of oxytocin on social support seeking outlined in *tend-and-befriend*, however, has never been demonstrated in experimental research. Further, it is unclear how the promotion of social support seeking in distressed people might influence them in circumstances where social support is withheld or unavailable. Indeed, a number of studies now show context-dependent effects of oxytocin, where the administration of oxytocin in a negative, competitive, or non-social context is associated with null or negative effects (Bartz et al., 2011b; Scheele et al., 2014).

To directly examine whether the effect of oxytocin on social support seeking is moderated by sex, distress, and the availability of social contact, men and women recalled positive, neutral, and negative autobiographical memories in the laboratory under two contexts: with and without social contact with an experimenter. We also examined whether participants' perceptions of the experimenter further moderated this effect. Participants received intranasal oxytocin (24IU) or a placebo on two occasions one week apart in a double-blind, placebo-controlled, randomized, and within-subject experiment. Three hypotheses were put forth. First, consistent with past studies, oxytocin administration was predicted to have context-dependent effects on behavior (Bartz et al., 2011b; Cardoso et al., 2013b). In response to oxytocin and relative to the placebo, we expected participants to experience increased emotional support when recalling negative autobiographical memories with an experimenter, and decreased emotional support when recalling such memories in social isolation. Second, we predicted that oxytocin would have the strongest effects in those persons most sensitive to the manipulation of social context (Bartz et al., 2011b, Huffmeijer et al., 2013). In response to oxytocin and relative to the placebo, we predicted that increased rapport and motivation to affiliate with the experimenter would amplify the magnitude of the effects outlined in the first hypothesis (i.e. a stronger relationship with the experimenter would increase individual receptiveness to social support). Finally, these effects would be greater in magnitude in women, relative to men, consistent with the tend-and-befriend theory and recent intranasal administration studies comparing male and female participants (Taylor et al., 2000; Ditzen et al., 2013; Fischer-Shofty et al., 2013; Yao et al., 2014).

Additional analyses were conducted to demonstrate the validity of the experimental manipulation of affect and support using autobiographical memory recall, as well as the manipulation of social context. We predicted that recalling negative autobiographical memories would induce negative mood in participants, relative to recalling positive and neutral memories. Relative to autobiographical memory recall with an experimenter, we also predicted that participants would feel less emotionally supported when recalling negative memories via a computer, without contact with the experimenter.

Method

Participants

Sixty-four participants (32 female) between the ages of 18 and 35 (M = 24.6, SD = 4.22) were recruited to participate in this study through online advertisements (e.g. Craigslist). Exclusion criteria included current use of tobacco, current use of any prescription drugs, current or past use of illicit drugs (excluding cannabis, which required one year abstinence), severe medical conditions, history of consultation with psychiatric or psychological services, and suspected pregnancy in women. Only participants who were fluent in English were included in the study. Of the 25 females not taking the oral contraceptive pill, 6 were in the follicular menstrual phase and 19 were in the luteal phase across all test sessions. The Human Research Ethics Committee at Concordia University (Montréal, Canada) provided ethical approval of the study.

Modified Autobiographical Memory Test

The modified autobiographical memory test was used in a previous study (Cardoso et al., 2014c), but with no manipulation of social context, and is based on the well-established procedures reported by Williams and Broadbent (1986). Participants were asked to recall specific episodic memories that occurred within a 24-hour period in response to positive, negative, and neutral cue words. In the *social* condition, cue words were verbally presented by a same-sexed experimenter in-person. In the *non-social* condition, cue words were presented visually on a computer to the participant, who was alone in a room that was being monitored remotely. Participants recalled memories in response to 30 unique cue words in each condition that alternated between positive, negative, and neutral valence. One hundred and twenty cue words

were randomized into four word lists that were counterbalanced across both conditions (social, non-social) and across two test sessions (placebo, oxytocin). Examples of positive cue words included "happy," "proud," and "brave." Examples of neutral cue words included "radio," "river," and "chair." Finally, examples of negative cue words included "lonely," "angry," and "afraid."

Participants rated their experience of recalling of each memory on the following dimensions: 1) how positive they felt, 2) how negative they felt, 3) how comfortable they felt, and 4) how emotionally supported they felt. Each dimension was rated on a scale from 1 *(not at all)* to 7 *(extremely)*. These ratings were completed immediately after recalling each memory. The test-retest reliability of these ratings across sessions (oxytocin, placebo) ranged from .58 (affect) to .89 (comfort, support).

Experimenter Perception Questionnaire (EPQ)

The EPQ is an 8-item questionnaire that was developed in-house to measure participants' assessment of the experimenters' social competence and desirability (see Table 1 for the list of questions). Each question was rated on a scale from 1 (*not at all*) to 7 (*extremely* or *absolutely*). Principal axis factoring revealed two factors: the first factor consisted of items related to *rapport* with the experimenter (placebo: Eigenvalue = 5.01, $R^2 = 46\%$; oxytocin: Eigenvalue = 4.69, $R^2 = 40\%$), and the second factor consisted of items relating to *motivation to affiliate* with the experimenter (placebo: Eigenvalue = 1.53, $R^2 = 30\%$; oxytocin: Eigenvalue = 1.59, $R^2 = 32\%$). Factor loadings are presented in Table 1. The test-retest reliability of ratings for the *rapport* and *motivation to affiliate* factors across sessions (oxytocin, placebo) was .80 and .81 respectively.

Table 1. Descriptive statistics and factor loadings for the Experimenter Perception Questionnaire (EPQ)

		Factor Loadings	
	Mean (SD)	Factor 1	Factor 2
Placebo			
The experimenter is…			
Trustworthy	5.73 (1.14)	.908	
Friendly	5.79 (1.26)	.825	
Sympathetic	5.32 (1.49)	.815	
Supportive	5.30 (1.50)	.852	
Competent	5.79 (1.06)	.818	
The experimenter is someone I would…			
Consider including in my social circle	3.75 (1.59)		.881
Like to get to know better	3.79 (1.64)		.947
Like to meet again	4.10 (1.70)		.800
Oxytocin			
The experimenter is…			
Trustworthy	5.65 (1.14)	.749	
Friendly	5.83 (1.09)	.792	
Sympathetic	5.38 (1.38)	.799	
Supportive	5.21 (1.35)	.838	
Competent	5.73 (1.08)	.812	
The experimenter is someone I would…			
Consider including in my social circle	3.95 (1.81)		.887
Like to get to know better	3.73 (1.56)		.940
Like to meet again	4.14 (1.68)		.885

Note: N = 63. Factor loadings < .20 are suppressed.

Procedure

Eligible participants were scheduled for two laboratory visits one week apart, keeping the time of day consistent across test sessions. Female participants were scheduled for both sessions on days that they were taking the active oral contraceptive pill to control for variations in estrous hormones. Females not taking the oral contraceptive pill were scheduled for both test sessions within 0-11 or 17-25 days of the first day of menstruation. Females contacted the laboratory on the first day of their menstruation to ensure that they were accurately scheduled within either phase. Upon arrival to their first test session, participants self-administered 24IU of intranasal oxytocin (*Syntocinon*, Novartis) or a placebo with matched inactive ingredients, with the order of administration across both test sessions counter-balanced across participants. Drug administration was conducted in accordance with recent guidelines on intranasal oxytocin administration (Guastella et al., 2013). Participants then rested in a chair and read neutral magazines for thirty minutes. Participants then completed a 60-minute eye-tracking experiment, which is not reported here. Participants were subsequently administered the social (with a research assistant) and non-social (via computer monitor) autobiographical memory tests described previously, which lasted approximately 60 minutes, with the order of test conditions (*social, non-social*) counter-balanced across participants. The effects of oxytocin on autobiographical memory recall are not presented here. Following completion of the social and non-social memory recall tests, participants completed the EPQ. At the end of the first session, participants were scheduled for the second test session a week later. In this study, male and female participants were assigned to one of two experimenters based on their availability, and these experimenters then carried out the study procedures for both laboratory visits (oxytocin and placebo). Following the completion of the second test session, participants were fully debriefed and remunerated $70 CAN.

Statistical analyses

The effect of social condition on self-reported affect (positive-negative), comfort, and emotional support was examined using a series of repeated measure ANOVAs. The effect of sex of the participant, cue word valence, drug condition, and interactions between these variables on self-reported affect, comfort, and emotional support was examined using a series of 2 (male, female) X 3 (positive, neutral, negative) X 2 (oxytocin, placebo) mixed ANOVAs. For these latter analyses, social and non-social conditions were examined separately to help simplify the

interpretation of these effects and improve statistical power (McLelland & Judd, 1993). Statistically significant omnibus effects with more than 2 levels were followed up with orthogonal contrasts (i.e. negative vs. positive + neutral; positive vs. neutral). Effect size statistics were calculated for all statistically significant ANOVAs (eta-squared), as were the mean difference scores (M_{DS}, or $M_1 - M_2$) of statistically significant contrasts with 95% confidence.

Using simultaneous multiple regression, the effects of drug condition on each of the cue valence contrasts for affect, comfort, and emotional support (6 per condition; oxytocin-placebo) were examined for moderation by sex and by changes in the *rapport* factor and the *motivation to affiliate* factor on the EPQ in response to oxytocin (oxytocin-placebo). Simple slope analyses were used to probe statistically significant interaction effects. All analyses were computed using the statistics program R.

Results

Validation of experimental manipulations: Affect, comfort, and emotional support during memory recall

Consistent with our hypothesis, cue word valence was a statistically significant predictor of self-reported affect during memory recall in the social $F(2,122) = 219.4$, $p < .001$, $\eta^2 = .78$ and non-social conditions $F(2,122) = 259.12$, $p < .001$, $\eta^2 = .80$. An analysis of orthogonal contrasts revealed that participants felt less positive and more negative when recalling memories in response to negative cue words relative to positive and neutral cue words in the social $F(1,62) = 253.39$, $p < .001$, $M_{DS} = -3.70$, 95%CI[-4.16, -3.23] and non-social conditions $F(1,62) = 282.38$, $p < .001$, $M_{DS} = -3.74$, 95%CI[-4.18, -3.29]. They also felt more positive and less negative when recalling memories in response to positive relative to neutral cue words in the social $F(1,62) = 48$, $p < .001$, $M_{DS} = 0.80$, 95%CI[0.57, 1.02] and non-social conditions $F(1,62) = 30.13$, $p < .001$, $M_{DS} = 0.59$, 95%CI[0.38, 0.80]. In addition to these findings, we found that participants also felt more emotionally supported in the social condition relative to the non-social condition $F(1,62) = 85.82$, $p < .001$, $\eta^2 = .58$.

The effects of sex, cue word valence, and social context on self-perceived affect, comfort, and emotional support during autobiographical memory recall are summarized in Fig. 1A-C.

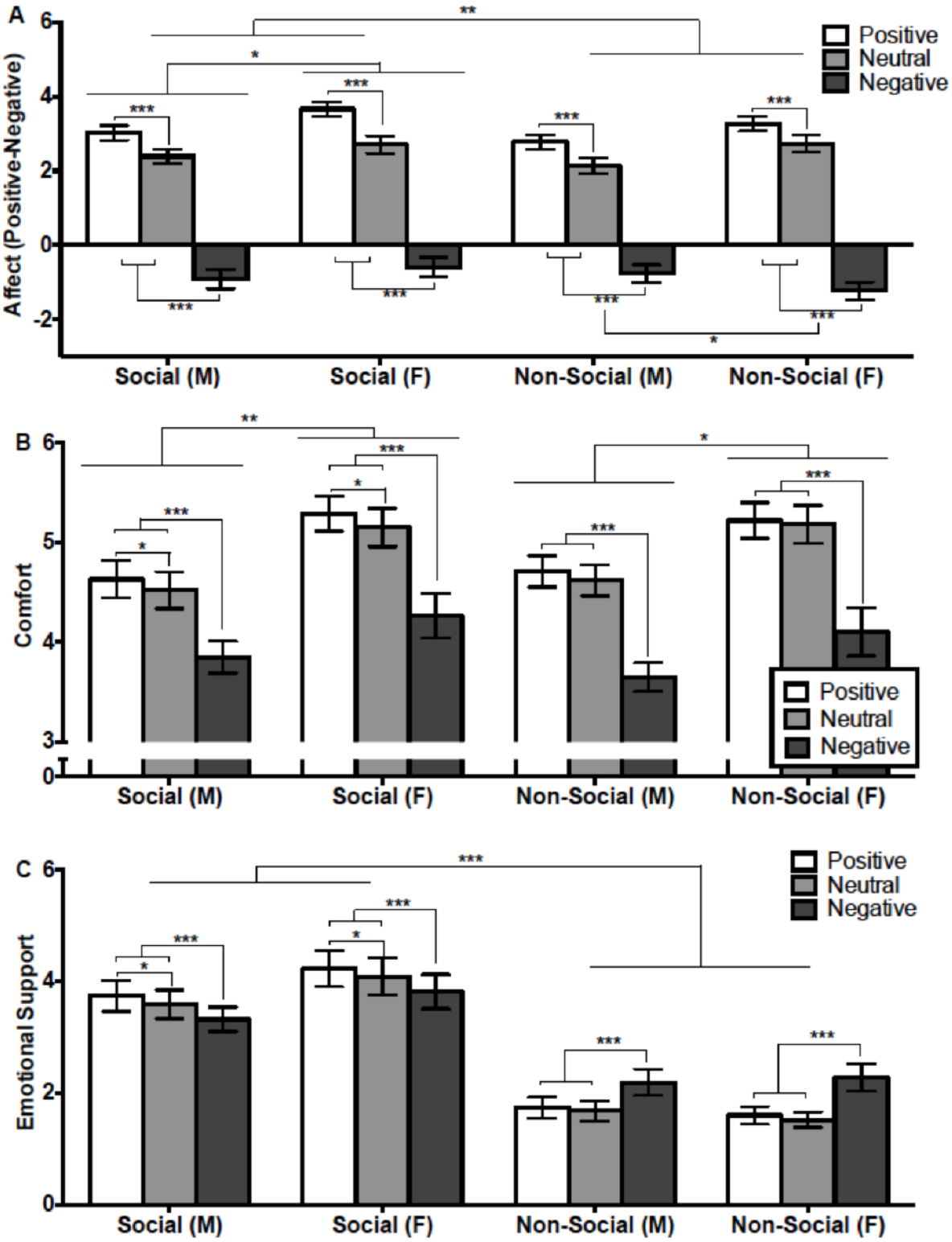

Figure 1. The main and interactive effects of sex, cue word valence (positive, neutral, and negative), and social context on the emotional experience of recalling autobiographical memories collapsed across drug condition, including **(A)** self-reported affect (positive-negative), **(B)** self-reported comfort, and **(C)** self-

reported emotional support. Error bars represent 1 SEM in either direction. *M = Male. F = Female.* * $p < .05$, ** $p < .01$, *** $p < .001$.

Effects of oxytocin on affect, comfort, and emotional support during memory recall

When examining self-reported emotional support in the non-social condition, a statistically significant interaction between drug condition and cue word valence was detected $F(2,122) = 8.134$, $p < .001$, $\eta^2 = .12$. Orthogonal contrasts revealed that, in the non-social condition, participants felt *less* emotionally supported in the oxytocin condition, relative to placebo, when recalling memories in response to negative cue words relative to positive and neutral cue words, $F(1,62) = 10.75$, $p = .002$, $M_{DS} = -0.29$, 95%CI[-0.47, -0.11] (see Fig. 2). No statistically significant differences were observed across drug condition when participants recalled memories in response to positive relative to neutral cue words in the non-social condition $F(1,62) = 2.07$, $p = .156$. There were no other statistically significant main effects of drug condition, or interaction effects between drug condition, sex, and cue word valence on any other variable (affect, comfort, support) in the social and non-social conditions $F(1\text{-}2,61\text{-}122) = 0.04$—1.85, all $p > .05$.

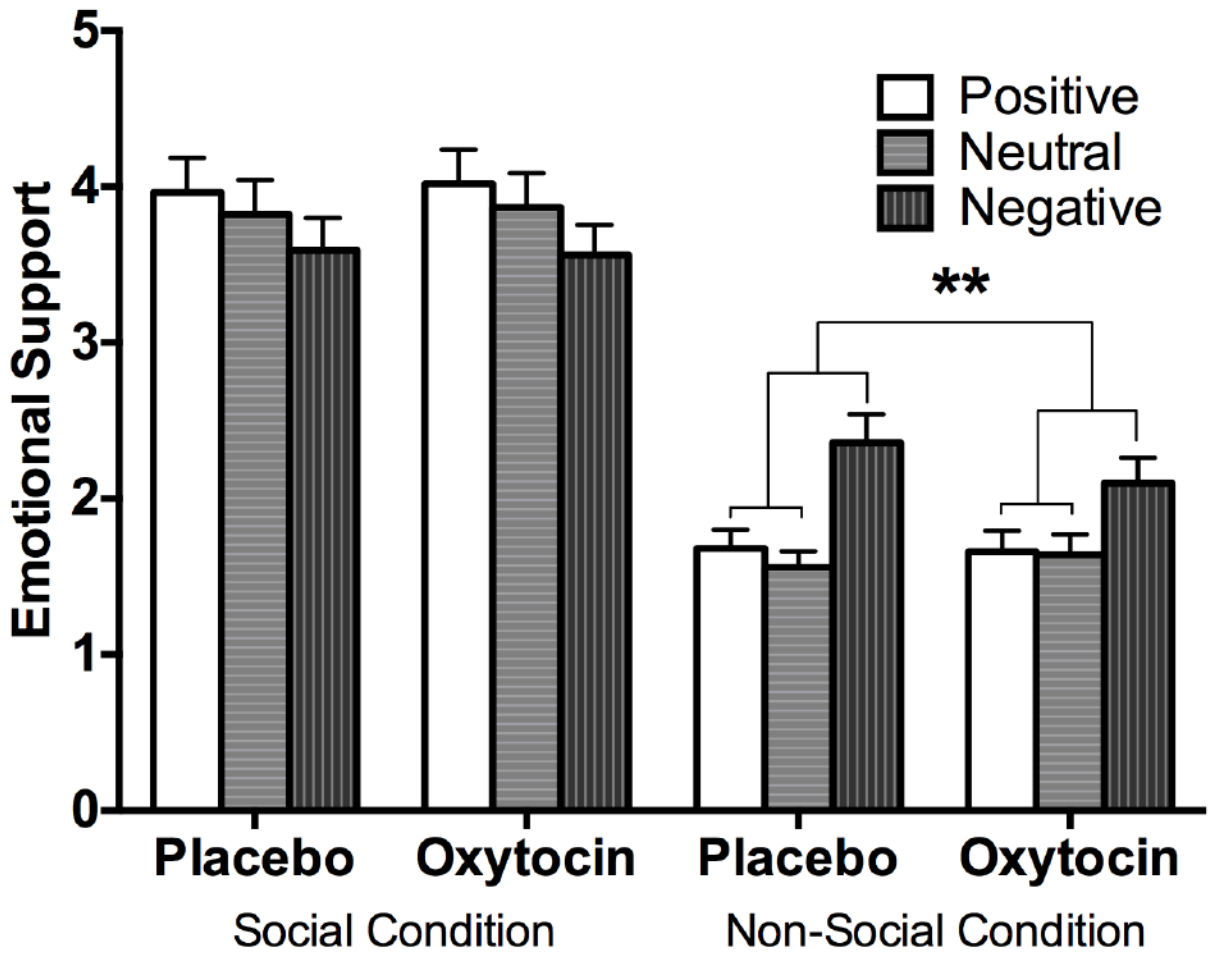

Figure 2. Emotional support during the recall of autobiographical memories in response to positive, neutral, and negative cue words. Participants felt less emotionally supported while recalling negative memories in the non-social condition under the influence of oxytocin, relative to the placebo. Error bars represent 1 SEM in either direction. ** $p < .01$.

Are the effects of oxytocin moderated by rapport and affiliation with the experimenter?

A paired-samples t-test revealed that oxytocin had no statistically significant main effect on the combined scores of either the rapport $t(62) = -0.15$, $p = .883$ or the motivation to affiliate $t(62) = 0.23$, $p = .819$ factors of the EPQ. The individual responses to oxytocin on these constructs, relative to the placebo, were examined as moderators of the effect of oxytocin on emotion and support while recalling autobiographical memories. These variables were created by subtracting the placebo score on each principal axis factor (rapport, motivation to affiliate) from

the score in the oxytocin condition (oxytocin minus placebo). The means, standard deviations, and inter-correlations of all variables in this section are presented in Table 2 and Table 3.

Table 2. Descriptive statistics for contrast scores across sex and drug condition

	Male[a]				Female[b]			
	Oxytocin		Placebo		Oxytocin		Placebo	
Variable	M	SD	M	SD	M	SD	M	SD
Non-social affect contrast 1	-3.25	1.60	-3.21	1.86	-4.17	2.00	-4.29	2.20
Non-social affect contrast 2	0.59	0.99	0.70	0.97	0.52	1.30	0.56	1.06
Non-social comfort contrast 1	-1.04	0.92	-0.99	0.89	-1.04	0.98	-1.16	1.12
Non-social comfort contrast 2	0.10	0.35	0.08	0.35	0.08	0.58	0.02	0.37
Non-social support contrast 1	0.31	0.73	0.63	0.80	0.58	0.74	0.85	1.03
Non-social support contrast 2	0.04	0.31	0.08	0.34	0.01	0.42	0.15	0.59
Social affect contrast 1	-3.56	1.85	-3.68	1.85	-3.81	2.25	-3.72	2.00
Social affect contrast 2	0.79	1.32	0.47	1.20	1.03	1.05	0.88	1.25
Social comfort contrast 1	-1.02	0.91	-0.97	0.98	-0.90	0.91	-1.02	1.02
Social comfort contrast 2	0.15	0.49	0.06	0.54	0.14	0.40	0.15	0.55
Social support contrast 1	-0.33	0.64	-0.36	0.85	-0.43	0.99	-0.24	0.90
Social support contrast 2	0.12	0.36	0.19	0.53	0.18	0.50	0.09	0.40
Factor contrast 1: rapport	-0.34	0.97	-0.24	0.99	0.33	0.93	0.23	0.97
Factor contrast 2: affiliation	-0.10	1.04	-0.06	1.08	0.10	0.97	0.06	0.93

Note: [a]$n = 31$. [b]$n = 32$. Contrast 1 = negative – (neutral + positive)/2. Contrast 2 = positive – neutral.

Table 3. Correlation statistics for the contrast scores in (1) the placebo condition and in (2) the oxytocin condition (relative to placebo).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Sex	--	.05	.02	.11	.04	.04	-.09	-.07	-.05	.11	-.09	-.15	.15	.15	.06
2. Non-social affect contrast 1	-.26*	--	-.18	.55*	-.15	-.43*	.18	.47*	-.07	.43*	-.04	.16	-.17	-.01	.19
3. Non-social affect contrast 2	-.07	-.11	--	-.28*	47*	.13	-.35*	.03	.26*	-.13	.10	.04	.22	-.12	.02
4. Non-social comfort contrast 1	-.08	.80*	-.03	--	-.29*	-.27*	.11	.24	-.06	.47*	.09	.27*	-.01	.13	.11
5. Non-social comfort contrast 2	-.08	-.21	.46	-.26*	--	-.04	-.03	-.03	.04	-.22	.10	.04	.08	-.21	-.1
6. Non-social support contrast 1	.12	-.57*	.01	-.59*	.18	--	-.11	-.23	.01	-.27*	-.01	-.08	.31*	-.33*	-.1
7. Non-social support contrast 2	.07	-.03	-.32*	-.15	.02	.15	--	0.10	.01	-.02	.00	-.05	-.17	.00	-.0
8. Social affect contrast 1	-.01	.79*	-.19	.68*	-.18	-.40*	-.06	--	.17	.61*	.26*	.46*	-.20	-.11	.16
9. Social affect contrast 2	.17	-.14	.46*	.02	.25*	.10	-.01	-.13	--	.23	.60*	.12	.21	.02	.16
10. Social comfort contrast 1	-.03	.67*	-.04	.78*	-.24	-.48	-.20	.72*	.03	--	.22	.54*	-.21	.01	.23
11. Social comfort contrast 2	.08	-.09	.15	-.06	.34*	.08	.19	.00	.58*	-.04	--	.16	.29*	-.02	.18
12. Social support contrast 1	.07	.09	.04	.20	-.04	-.35*	-.08	.21	.05	.40*	.10	--	-.26*	-.03	.23
13. Social support contrast 2	-.11	-.15	.25*	-.30*	.31*	.31*	-.02	-.12	.15	-.38*	.38*	-.17	--	-.13	-.0
14. Factor contrast 1: rapport	.24	-.51*	.07	-.34*	.07	.18	.00	-.50*	.20	-.40*	.23	.01	.32*	--	.14
15. Factor contrast 2: affiliation	.06	-.24	.23	-.13	.11	.13	.00	-.24	.08	0.12	.07	.15	.14	.52	--

Note: $N = 63$. Upper right quadrant = Oxytocin – placebo. Lower left quadrant = Placebo alone. Contrast 1 = negative – (neutral + positive)/2. Contrast 2 = positive – neutral. *$p < .05$,

Moderation effects in the non-social condition

We conducted a regression model predicting oxytocin-induced changes in perceived emotional support while recalling negative, relative to neutral and positive memories, in the non-social condition. Independent variables were oxytocin-related change in rapport with the experimenter, oxytocin-related change in motivation to affiliate with the experimenter, sex, and the interaction between sex and these variables. The overall model was statistically significant $F(5,57) = 2.655$, $p = .032$, $R^2 = .19$. Participants who experienced increased rapport with the experimenter in response to oxytocin felt less emotionally supported while recalling negative memories in the non-social condition in response to oxytocin, relative to the placebo $t(57) = -2.33$, $p = .023$, $b = -0.39$, 95%CI[-0.73, -0.06], $sr^2 = .08$. The interaction between sex and oxytocin-related changes in affiliation with the experimenter also approached statistical significance $t(57) = -1.92$, $p = .059$, $sr^2 = .05$. A follow-up analysis of simple slopes revealed that females who experienced an oxytocin-induced increase in motivation to affiliate with the experimenter (+1 SD) also experienced a further decrease in emotional support while recalling negative memories in the non-social condition under the influence of oxytocin, relative to the placebo $t(57) = -2.04$, $p = .047$, $b = -0.35$, 95%CI[-0.69, -0.01]. In contrast, no such additional effect was observed in males $t(57) = 0.85$, $p = .40$ (see Fig. 3A).

Moderation effects in the social condition

We repeated the above analyses predicting oxytocin-induced change in perceived emotional support while recalling negative, relative to neutral and positive memories in the social condition. A statistical trend was observed for the overall model $F(5,57) = 2.12$, $p = .077$, $R^2 = .16$, and an analysis of the individual predictors revealed a statistical trend for the interaction between sex and oxytocin-related changes in affiliation with the experimenter $t(57) = 1.90$, $p = .063$, $sr^2 = .05$. A follow-up analysis of simple slopes revealed that females who experienced an oxytocin-induced increase in motivation to affiliate with the experimenter (+1 SD) in the social condition felt more supported while recalling negative memories under the influence of oxytocin, relative to the placebo $t(57) = 2.78$, $p = .007$, $b = 0.50$, 95%CI[0.14, 0.87]. In contrast, no such effect was observed in males $t(57) = -0.24$, $p = .811$ (see Fig. 3B).

We repeated the above analysis for memory recall elicited with positive and neutral cue words, and found no statistically significant effects, and regressions analyses predicting self-

reported affect and comfort, using the same predictors as above, yielded no statistically significant findings $F(5,57) = 0.48—1.61$, all $p > .05$.

To demonstrate that the described statistically significant effects were specific to the individual response to oxytocin, rather than simple changes in rapport and motivation to affiliate with the experimenter across test sessions (independent of drug condition), we repeated the regression analyses described above using changes in affiliation and rapport across sessions (time effect) rather than drug condition as model predictors and none of these regression effects reached statistical significance (data not shown).

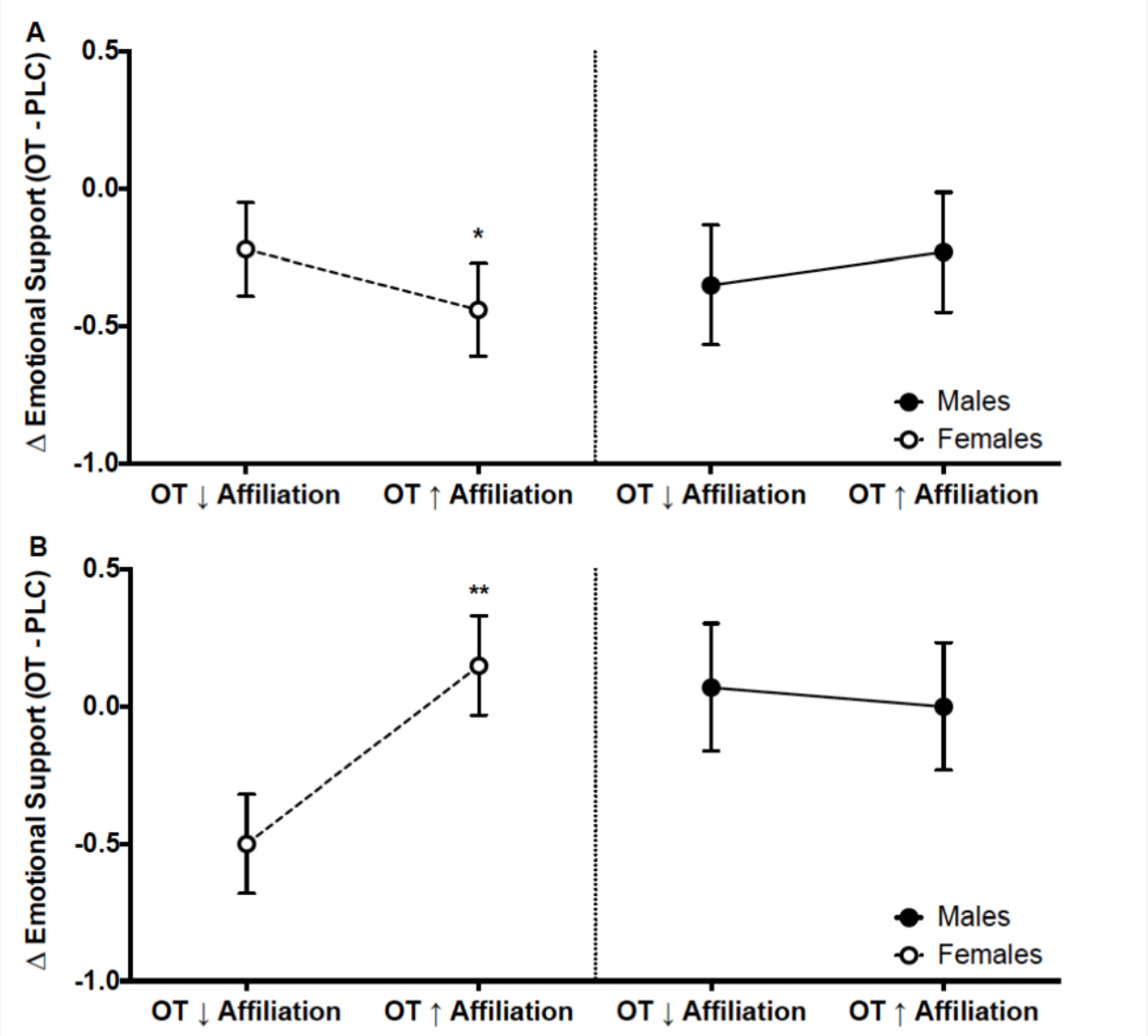

Figure 3. Simple slope analysis of the interactive effects sex of the participant and oxytocin-induced changes in motivation to affiliate with the experimenter (-1SD, +1SD) predicting

oxytocin-induced changes in self-reported feelings of emotional support in the **(A)** non-social and **(B)** social negative memory recall conditions. Among females (left side of the figure), increased motivation to affiliate with the experimenter following oxytocin was associated with *decreased* emotional support when recalling negative memories in the non-social context, but it was associated with *increased* emotional support when recalling negative memories during in the social context. These effects were absent in male participants (right side of the figure). Error bars represent 1 SE in either direction. *OT = Oxytocin. PLC = Placebo.* * $p < .05$, ** $p < .01$.

We repeated the above analysis for memory recall elicited with positive and neutral cue words, and found no statistically significant effects, and regressions analyses predicting self-reported affect and comfort, using the same predictors as above, yielded no statistically significant findings $F(5,57) = 0.48—1.61$, all $p > .05$.

To demonstrate that the described statistically significant effects were specific to the individual response to oxytocin, rather than simple changes in rapport and motivation to affiliate with the experimenter across test sessions (independent of drug condition), we repeated the regression analyses described above using changes in affiliation and rapport across sessions (time effect) rather than drug condition as model predictors and none of these regression effects reached statistical significance (data not shown).

Discussion

Two key findings emerged from these data. Women who experienced an oxytocin-induced increase in motivation to affiliate with the experimenter also felt more emotionally supported when disclosing negative memories to them. This effect was not observed in the absence of distress (i.e. when recalling positive or neutral memories), nor in male participants, and was dependent on the actual presence of experimenter in the testing room (i.e. the social condition). Previous research has shown that endogenous oxytocin is higher in distressed women and young girls (Taylor et al., 2006; Taylor et al., 2010; Seltzer et al., 2010), and that oxytocin increases dispositional trust in distressed people (Cardoso et al., 2013a). The results of this study support the role of oxytocin in the sexually differentiated biobehavioural response to challenge known as *tend-and-befriend* (Taylor et al., 2000), and suggests that in the presence of desired social relationships, oxytocin promotes receptiveness to social support in distressed women. The present findings lend further support of sex-specific effects of exogenous oxytocin administration on neural and behavioral responses (Rilling et al., 2014). Intranasal administration of

oxytocin, for example, improves the perception of kinship relations and reduces sympathetic arousal during couple conflict in women, but not in men (Ditzen et al., 2013; Fischer-Shofty et al., 2013).

The second finding highlights the crucial role of context in understanding the effects of oxytocin on social behavior. In the absence of close proximal social support, oxytocin had a *negative* effect on perceived emotional support. In the non-social condition (test administered via computer), oxytocin decreased perceived emotional support in men and women during the disclosure of negative autobiographical memories. This effect was pronounced in women who reported an oxytocin-induced increase in motivation to affiliate with the experimenter, and in those who reported more rapport with the experimenter. This effect was not observed when participants disclosed neutral or positive memories. Thus, while theoretical models (i.e. tend-and-befriend) and empirical evidence supports the role of oxytocin in social support seeking in distressed people (Taylor et al., 2000; Taylor et al., 2006; Taylor et al., 2010; Seltzer et al., 2010; Cardoso et al., 2013a), it appears that this increased support seeking may have negative consequences in contexts where social support is not readily available and, by extension, in certain at-risk populations (Bartz et al., 2011b). Consistent with this contention, while oxytocin is shown to increase self-disclosure and recall of positive social memories with a receptive experimenter (Lane et al., 2013; Cardoso et al. 2014c), one study found that oxytocin increased anxiety in depressed participants undergoing a psychotherapy session with an unresponsive therapist (MacDonald et al., 2013). Women may be highly sensitive to these context-dependent effects. Recently, the administration of oxytocin, relative to placebo, decreased efforts to repair trust following betrayal in an economic trust game in women, but not men, and this effect was greatest among female participants with high trait forgiveness, similar to the present study (Yao et al., 2014). Taken together, the effect of oxytocin on social support seeking may be maladaptive in instances where social relationships are either untrustworthy or not available, particularly among women. These findings may have important implications for understanding sex differences in the prevalence of depression, which has been hypothesized to be related to sex-specific interpersonal factors (Kendler & Gardner, 2014).

A limitation of the current study is that participants disclosed autobiographical memories in response to cue words with a stranger. Therefore, this task may not be an ecologically valid test of social support seeking in the natural environment, where distress is not cued in a structured way and support is less frequently sought from strangers. This study, however, lays the foundation for investigations on the relation between exogenous oxytocin and social support seeking in more naturalistic settings. The direction of effect between participants' ratings of emotional support and motivation to affiliate is not fully known, as both measures were collected following drug administration. However, drug administration had no main effect on ratings of motivation to affiliate or rapport with the experimenter, indicating that these measures were likely assessing trait differences among participants. Another

limitation of this study is that the pathway by which intranasal oxytocin influences social behavior is currently unknown, as this methodology increases bioavailability not only in the brain, but also in the periphery (Churchland and Winkielman, 2012; Guastella et al., 2013). Clearly, more research is needed at this juncture to definitively substantiate the pathway by which oxytocin promotes social support seeking. Finally, the results of the current study should be interpreted cautiously. The reported findings necessitated the decomposition of complex interactions to evaluate the proposed hypotheses, and some of the interactions (participant sex and oxytocin-related changes in affiliation, for example) fell short of conventional levels of significance. However, simple slope analyses in such cases revealed robust sex differences (i.e. significant effects in female participants, but not in male participants). Still, further replication in larger samples, to test complex interactions, will be required to substantiate these preliminary results.

This is the first study to provide experimental support for the direct involvement of oxytocin in the sexually differentiated biobehavioral response to challenge known as *tend-and-befriend*. Considering that women have been greatly underrepresented in this field of research (Taylor et al., 2000; Cardoso et al., 2014b), the inclusion of both men and women in studies on stress, particularly those emphasizing the role of oxytocin in stress and social functioning, is an important future direction. A number of factors could have been responsible for the observed sex-related differences under the influence of oxytocin, including altered levels of ovarian hormones in women including endogenous oxytocin (Taylor et al., 2000). It will be important moving forward to study interactions among different hormones under naturalistic conditions and in response to challenge. The present study has important clinical implications. There are a number of clinical trials underway that aim to investigate the therapeutic applications of oxytocin (McQuaid et al., 2014; Young and Barrett, 2015), and the results of this study suggest that the availability of desired social supports and social feedback is necessary to elicit the prosocial effects of exogenous oxytocin. Using oxytocin therapeutically in social-isolated depressed populations, for example, may be contraindicated as a stand-alone intervention. Going forward, the tend-and-befriend theory will be an important area of investigation, as it has the potential to shed light sex differences in stress, social support seeking, and psychiatric illnesses, particularly with respect to the affective disorders (Taylor et al., 2000; McQuaid et al., 2014).

Chapter 6: General Discussion

The principal objective of this thesis was to study the role of oxytocin in regulating different aspects of stress and social behaviour in humans. Specifically, these areas of investigation included the role of oxytocin in (1) regulating the physiological response to stress, (2) increasing the perception of emotion in social stimuli, and (3) promoting distress-dependent social support seeking. These interconnected effects are theorized to drive the *tend-and-befriend* response to stress, whereby individuals are motivated to affiliate under stress, which is an alternate coping strategy to the traditional *fight-or-flight* response to stress. This response is also posited to be greater in women because attending to offspring and building social support networks may be more adaptive than fighting or fleeing as a means of mitigating the negative consequences of a stressful from an evolutionary perspective (Taylor et al., 2000). While biopsychosocial research on sex differences in the stress response has often produced equivocal results (Linnen et al., 2012; Stroud et al., 2002), women are under-represented in stress research and their inclusion represents an important direction in this field (Taylor et al., 2000). It is argued that the *tend-and-befriend* stress response is driven by the hormone and neuropeptide oxytocin for two reasons. First, oxytocin has been shown to be germane to stress-regulation and social cognition in non-human animal models. Second, the oxytocinergic system is up-regulated by estrogen and undergoes greater activity during the perinatal period, making it a sexually dimorphic hormone that could differentially influence coping behaviour in men and women across different contexts (Taylor et al., 2000).

Across all four chapters in this thesis, the role of oxytocin in stress-regulation, emotion perception, and distress-dependent social support seeking was explored using pharmacological manipulations of oxytocin. This methodology was used for a number of reasons. The first reason is that the evidence base used to establish the *tend-and-befriend* theory is largely based on the central effects of oxytocin in non-human animals (Taylor et al., 2000). A number of studies have shown that oxytocin and related neuropeptides increase in concentration in the CSF following nasal administration (Born et al., 2002; Striepens et al., 2013), which partly supports, but does not confirm, the nose-to-brain pathway (Guastella et al., 2013). The second reason is that the methodology used to quantify endogenous concentrations of oxytocin in the periphery is unreliable in humans (Churchland, & Winkielman, 2002; McCullough, Churchland, & Mendez, 2013). Thus, even if the nose-to-brain pathway cannot be confirmed, pharmacologically

increasing the bioavailability of oxytocin in humans is a more reliable method of investigating this system irrespective of whether concentrations increase the CNS or in the periphery (Guastella et al., 2013; Legros, 2001; Neuman et al., 2013). This methodology has some notable limitations with respect to interpreting the role of oxytocin in the *tend-and-befriend* response in humans. One such limitation is that exogenous administration of a 24IU dose of nasal oxytocin increases endogenous bioavailability of oxytocin to supra physiological levels (i.e. 2-4 times natural levels), which may lack ecological validity for how endogenous oxytocin effects change in behavior in the natural environment. Another such limitation is that pharmacologically manipulating oxytocin does not inform our understanding of how it is released endogenously and under what circumstances it is released. Keeping these limitations in mind, the roles of oxytocin in stress-regulation, emotion-perception, and distress-dependent social support seeking, as they were empirically examined in this thesis using intranasal oxytocin administration methodology, are summarized next.

Chapter two confirmed that a 24IU dose of intranasal oxytocin, relative to a 48 IU dose or a placebo, inhibits cortisol rise during physical stress. This effect demonstrates that oxytocin inhibits the cortisol response to stress irrespective of social context as long as the HPA-axis is stimulated (i.e. cortisol increases), and this effect is independent of changes in mood. This finding is further confirmed by a meta-analytic review that shows a larger inhibitory effect of intranasal oxytocin on the cortisol response to stress in studies that showed the greatest stimulation of the HPA-axis irrespective of social context and changes in mood (Cardoso, Kingdon, & Ellenbogen, 2014). This study included an analysis of 675 participants across 18 studies that examined the effect of intranasal oxytocin on the cortisol response to various laboratory tasks. Thus, given that oxytocin directly regulates cortisol output and not mood, this effect may instead promote behaviours aimed at regulating the negative consequences of a stressful event, namely stress-coping strategies. One example of this includes a study that demonstrated an anxiolytic effect of oxytocin in people with poor coping skills who experienced social rejection, whereas those with relatively stronger coping skills did not show this effect (Cardoso et al., 2012). In summary, the results of chapter two support the role of oxytocin in the inhibition of the cortisol response to stress and helps to create methodological guidelines for administration doses of intranasal oxytocin in humans, which varies widely in the extant literature (Cardoso, Kingdon, & Ellenbogen, 2014). The results of this chapter improve our

understanding of one possible component mechanism of the *tend-and-befriend response.*

Chapter three confirmed that a 24IU dose of intranasal oxytocin, relative to a placebo, increased the perception of emotion in human faces. This effect generalized to all emotional expressions and was greater in magnitude for emotional expressions that were displayed at lower intensities. Such expressions included disgust, anger, and surprise in the presented study. This effect is consistent with other studies showing that oxytocin increases the perceived emotional intensity of facial expressions irrespective of valence, particularly low-intensity emotions (Leknes, 2013). This effect had a paradoxical influence on emotion identification accuracy; healthy participants were more likely to be oversensitive to the displayed emotions, and they were consequently less accurate in characterizing these stimuli (i.e. identifying the subtle and complex emotional distinctions in natural human facial expressions). This finding is consistent with the social saliency hypothesis, which posits that oxytocin improves social cognition by enhancing the salience of socio-emotional information in the environment (Bartz et al., 2011). This effect has important implications for predicting the consequences of increasing the bioavailability of oxytocin in humans, which may result in positive or negative outcomes depending on the social context. For example, oxytocin could lead individuals to feel more threatened during an interaction with an irritable social partner, or alternatively, it could promote feelings of warmth when bonding with a gregarious peer. This effect could also influence humans differently depending on individual differences. For example, some studies have shown that in individuals that are oversensitive to the emotions of other at baseline show decreased trust and cooperation following oxytocin administration (Bartz et al., 2011b), while those with deficits in this area show improved empathy (Bartz et al., 2010). These context- and person-dependent effects could have implications for the consequences of oxytocin-induced distress-motivated social support seeking. Specifically, while oxytocin could theoretically enhance attention to supportive peers in times of distress, it could also enhance feelings of rejection if such peers are not available. This possibility is supported by the extant literature on oxytocin, which encompasses both salubrious and deleterious effects of the hormone on social functioning depending on individual and contextual factors (Bartz et al., 2011). The role of oxytocin and context in modulating the consequences of the *tend-and-befriend* response is described later on this section. The results of chapter three support the role of oxytocin in enhancing social awareness, which is one of the posited mechanisms by which the *tend-and-befriend* response

promotes social support seeking in times of distress.

Chapters four and five explicitly examined the distress-dependent effects of oxytocin on social bonding and social-support seeking in humans. Chapter four showed that intranasal oxytocin, relative to a placebo, specifically enhanced dispositional trust in distressed people following social rejection relative to those whose mood was euthymic. This result represents preliminary evidence that the effect of oxytocin on dispositional trust is dependent, in part, on the experience of distress in humans, which is consistent with the *tend-and-befriend* theory. This finding is particularly important given that trust is one of the most widely studied topics among scientists interested in the effects of oxytocin on social bonding (Carter, 2014). Chapter five showed that oxytocin selectively decreased perceived social support in participants who recalled negative autobiographical memories in the absence of social feedback; this effect was not present during the recall of positive or neutral memories. In other words, the effect of oxytocin on feeling unsupported was dependent on (1) the experience of distress and (2) the absence of a social partner. We also showed that women who reported increased motivation to affiliate with the experimenter following oxytocin administration showed this effect in greater magnitude (i.e. felt less supported when no support was available), consistent with the sexually differentiated effects outlined in the *tend-and-befriend* theory. This theory was further bolstered by that fact that distressed women who reported increased motivation to affiliate with the experimenter following oxytocin administration, relative to placebo, reported feeling *more* supported in the presence of an experimenter. These distress-dependent effects of oxytocin on perceived support, which were in part dependent on showing willingness to affiliate with the experimenter, were not observed in men. The results of these two chapters provide the first pieces of evidence to suggest that oxytocin selectively alters social bonding and perceived social support in distressed humans, particularly in women, depending on the social context. These findings are in keeping with the *tend-and-befriend* theory, and suggest that the distress-dependent effect of oxytocin on increasing social support seeking may be adaptive in some circumstances. However, it should be noted that sex differences in the effects of oxytocin are equivocal in the extant literature and the studies presented in this thesis (Cardoso, Kingdon, & Ellenbogen, 2014). Specifically, we did not find support for sex differences in the effects of oxytocin in chapter three and four, and we only examined male subjects in chapter two. Thus, continued research in this area to understand how oxytocin might be implicated in sex differences in the human stress response is essential at this

juncture. In summary, the results of these chapters and those reviewed previously support the role of oxytocin in promoting a number of effects that are consistent with the *tend-and-befriend* theory, including the role of oxytocin in (1) inhibition of the cortisol rise in response to stress, (2) enhancement of the salience of emotion in social stimuli, and (3) distress-dependent enhancement of trust and social support seeking. The implications of these findings are described in the next section.

Oxytocin and sex differences in the stress response: A biopsychosocial framework

The introduction to this thesis suggested that the *tend-and-befriend* theory offers a framework for understanding sex differences in the stress response, which could help shed light on why people are at a greater risk for suffering from stress-related mental disorders beginning in adolescence, including major depressive disorder (MDD; Nolen-Hoeksema, 2001; Taylor et al., 2000). Such sex differences include the use of different coping strategies in response to stress that confer risk for, or resilience against, MDD depending on individual and contextual factors. This difference begins to emerge during the transition through puberty when ovarian hormones including estrogen and oxytocin increase in circulation (Cyranowski, Frank, Young, & Shear, 2000). The importance of interpersonal stress in predicting depression is magnified during this period in females, particularly those who are socialized to develop an inter-dependent self-concept from a young age (Cross & Madson, 1997). This inter-dependent self-concept has been shown to be important for the way that females try to mitigate the negative impact of stress. For example, it has been consistently shown that females tend to rely on peers for emotional support more often than males, which is a protective factor against stress in the presence of supportive social relationships (Cyranowski et al., 2000). In the absence of such supportive relationships, however, females tend to be more prone to rumination and negative self-talk (Cyranowski et al., 2000; Nolen-Hoeksema, 2001; Tamres et al., 2000), which confers risk for increased distress and consequent depressive symptoms (Nolen-Hoeksema, 2001). In addition to the role of oxytocin in social-support seeking outlined in this thesis, we have previously shown that oxytocin enhances the vividness and negative valence of negative autobiographical memories in those who are prone to rumination when such memories are recalled in social isolation (Cardoso, Orlando, Brown, Joober, & Ellenbogen, unpublished observations), which may provide a role for oxytocin in all of the aforementioned sex differences in stress-coping and its consequences.

The effect of oxytocin on magnifying feelings of support or the lack thereof depending on the availability of a desired social partner may explain why our ability as humans to regulate the negative impact of stress is dependent on the availability of supportive social relationships. This is particularly true of women because they demonstrate increased activity of the oxytocinergic system following puberty and during the perinatal period (Gimpl & Farenholz, 2001). This greater activity of the oxytocinergic system and its effects on stress and social behavior is synergistically increased by estrogen (Farbach, Morrell, & Pfaff, 1985; Pederson, Ascher, Monroe, & Prange, 1982; Pederson & Prange, 1979), which is higher in women and central to the sexually differentiated *tend-and-befriend* response (Taylor et al., 2000). While the inferences that can be drawn about sex differences in endogenous oxytocin activity are limited in this thesis because we exclusively manipulated oxytocin using exogenous administration, the results of the present thesis led to speculation about the cognitive and behavioral effects of the sexually dimorphic endogenous oxytocin system during stress. Specifically, effect of oxytocin on social support-seeking and rumination during stress may provide common biological pathway that promotes both of these outcomes in distressed people depending on the social context, particularly in women. This pathway may be further modulated by early socialization experiences toward inter-dependence (increased dependence on relationships) as well as early life stress (i.e. insecure attachment and decreased trust), both of which are known to influence the expression of oxytocin (Bhandari et al., 2014; Cyranowski et al., 2000; Kim et al., 2010; Heim et al., 2009). For a visual representation of the proposed pathway by which oxytocin influences coping strategies aimed at mitigating the negative impact of stress, see **Figure 1**.

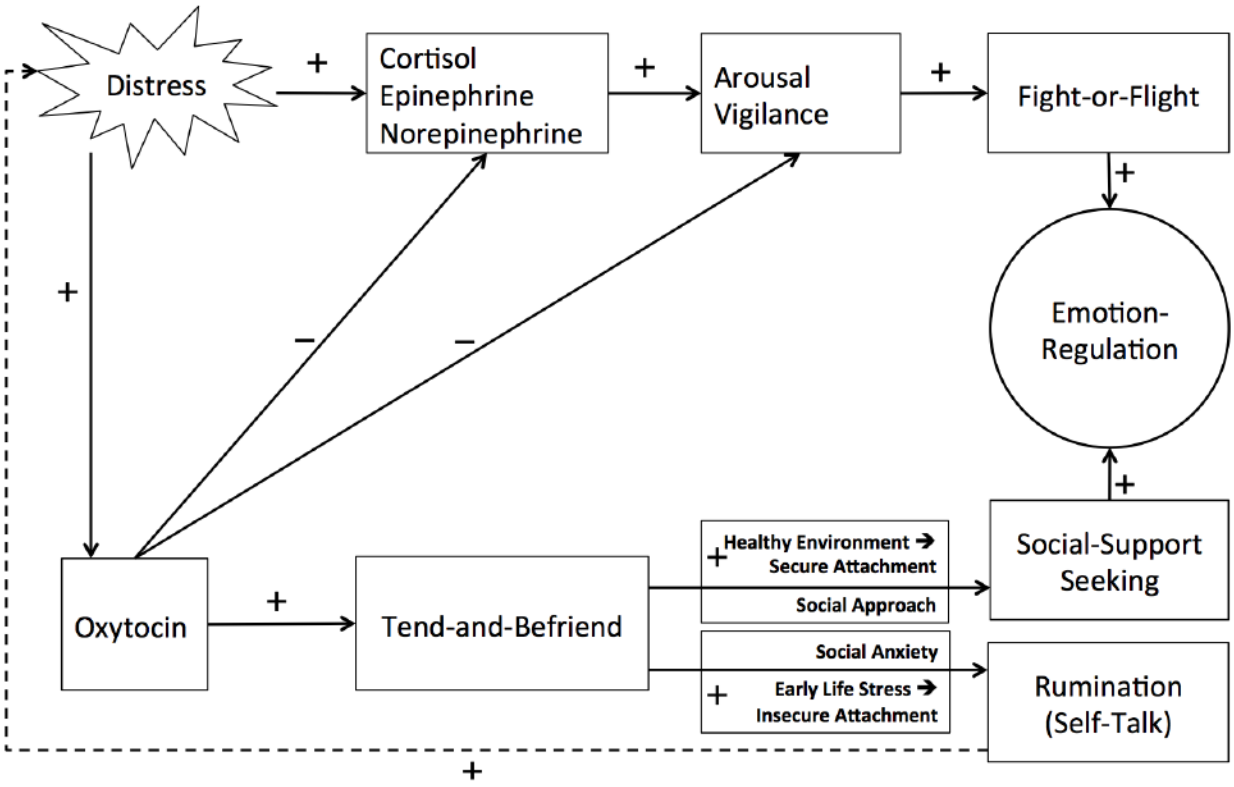

Figure 1. This figure illustrates a common pathway by which oxytocin might endogenously influence coping strategies, particularly in women. During distress, oxytocin might down-regulate the *fight-or-flight* response in favour of promoting affiliation in feminine women, namely the *tend-and-befriend* response. In individuals with a secure social attachment, this latter response might promote social-support seeking, the acquisition of support, and consequent emotion-regulation. In those without a secure attachment, the effect of oxytocin on social-support seeking might be suppressed and internalized, resulting in self-talk, rumination, and consequent increases in perceived distress if the individual remains overwhelmed.

Taken together, the role of oxytocin in the *tend-and-befriend* response, namely motivating the acquisition of social support during distress, is partly supported by the evidence presented in this thesis and may have positive or negative consequences on emotion-regulation depending on individual difference (i.e. inter-dependent self-concept and attachment security) and contextual factors (i.e. the availability of desired supportive social relationships). This theory is consistent with the extant literature on the effects of exogenous oxytocin in humans, which are shown to vary greatly depending on moderating individual and contextual factors (Bartz et al., 2011). Oxytocin has been shown to be critical to promoting the acquisition of in-group social networks

(DeDrue et al., 2010), and the results of this thesis suggests that stress may be a critical modulator of this effect. This role of oxytocin in the *tend-and-befriend* response in humans and its conditional outcomes provide a possible framework for understanding sex differences in stress, coping strategies, and related mental illnesses (i.e. MDD). Importantly, research in this area is poised to capitalize on the conditional effects of oxytocin on social-support seeking in humans (Cardoso & Ellenbogen, 2013), and a related clinical trial on the use of oxytocin as an adjunct to psychotherapy for the treatment of MDD in a supportive therapeutic context is underway (Ellenbogen & Cardoso, 2015; Protocol Registration Number *NCT02405715*). While the results of this thesis are equivocal with respect to sex differences in the exogenous effects of oxytocin, it may be the case that nasal spray methodology is not adequately suited to substantiate such effects. For example, in Chapter 5, the *individual* response to oxytocin predicted social support seeking in women (i.e., only a subset of women who were sensitive to oxytocin administration showed this effect), and without such an analysis these sex differences would not have been detected. Thus, future research will be essential to further substantiate the *tend-and-befriend* theory, particularly studies that are well suited to quantify activity of the endogenous oxytocin system (i.e. gene methylation studies, CSF studies) and that have sample sizes suitable for more complex analyses.

Limitations

One important limitation of the methodology used across all the studies in this thesis includes confusion over the pathway by which intranasal oxytocin effects change in physiology, social cognition, and behavior in humans. A number of studies have shown that intranasal oxytocin increases its concentration in the CSF of human and non-human primates (Chang, Barter, Ebitz, Watson, & Platt, 2012; Striepens et al., 2013). Similar studies have shown comparable effects in rodents (Neuman et al., 2013), and intranasal administration of related neuropeptides increases their concentrations in human CSF (Born et al., 2002). This does not provide direct evidence that exogenous oxytocin directly reaches the CNS, however; it is possible that increased CSF levels could be related to a positive feedback loop wherein exogenous oxytocin effects change in peripheral targets that then upregulate its concentration in the CNS and CSF (Guastella et al., 2013). In spite of the fact that oxytocin might directly penetrate the CNS via the olfactory bulb and the trigeminal nerve pathway (Guastella et al., 2013; Quintana,

Alvares, Hickie, & Guastella, 2015), it is important to note that the methodology required to definitively prove this pathway does not currently exist because no ligands have been developed that specifically bind to oxytocin for use in Positron Emission Tomography (PET) imaging studies. In the absence of such evidence, some authors have refuted the proposed pathway and argued that less than one percent of exogenous oxytocin can transverse the BBB (Walum, Hasse, Irwin, & Young, 2015). While these authors have casted doubt on whether the results of intranasal oxytocin studies are meaningful in the absence of such a pathway, other authors have argued that oxytocin has consistently produced meaningful, replicable, albeit small, effects across a number of indices of social cognition, physiology, and behavior (Carson, Yuan, & Labuschagne, 2015; Quintana & Woolley, 2015). These effect sizes have been substantiated in recent meta-analytic reviews (Hedges g = .20—.30; Bakermans-Kranenburg and van IJzendoorn, 2013; Cardoso, Kingdon, & Ellenbogen, 2014; Cardoso, Payne, & Ellenbogen, unpublished observations; Shahrestani et al., 2013). The effect sizes reported in this thesis are in keeping with those documented in the literature (Cardoso et al., 2014; Walum et al., 2015), and they are in keeping with *a priori* hypotheses based on a popular theoretical framework (i.e. *tend-and-befriend*) and effects documented in non-human animals. Thus, even though the pathways by which intranasal oxytocin effects change in social cognition, physiology, and behavior are not well understood, this does not detract from the validity of the effects documented in this thesis.

The second limitation of the methodology used across the studies in this thesis is that exogenous oxytocin administration increases its bioavailability to supra physiological levels (Guastella et al., 2013; Quintana, Alvares, Hickie, & Guastella, 2015). Such concentrations of oxytocin may not adequately represent the natural endogenous effects of oxytocin on social cognition, physiology, and behavior. Further, exogenous administration of oxytocin does not inform how the hormone is released in the natural environment in response to various contextual factors, thus our understanding of how such environmental factors motivate oxytocin-mediated behavior is still unclear. It is important to note that current methodologies used to investigate the endogenous release of oxytocin during various procedures is rife with measurement error (McCullough, Churchland, & Mendez, 2013), and the relation between peripheral concentrations of oxytocin and its effects in the CNS is not well understood (McCullough, Churchland, & Mendez, 2013). Thus, despite concerns over a lack of ecological validity in studies employing intranasal oxytocin methodology, alternatives to such methodology do not currently fare any

better. Importantly, there is a long history of using exogenous molecules to challenges CNS neurohormonal systems, which has yielded a great deal of knowledge about human psychophysiology (Putman & Roelofs, 2011).

Conclusion

The *tend-and-befriend* theory was developed on the premise that women and men may respond differently to stress, and that this sex difference may have a biological underpinning (Taylor et al., 2002). This idea was put forth because prior to 1995, 17% of participants in stress research were female, and this body of research was not representative of both genders. This gender gap has not improved greatly in current studies of oxytocin and stress (29%; Cardoso et al., 2014), which is why this area of research is so important. The results of this thesis show that intranasal oxytocin is a useful methodology for investigating the effects of oxytocin in humans. Specifically, this thesis has shown that oxytocin inhibits cortisol rise during stress, increases attention to emotion in human faces, enhances trust in distressed people, and produces feelings of support in distressed individuals, particularly in women, in the presence of a desired social partner (and produces the inverse effect in the absence of such a partner). These results provide the first pieces of evidence to suggest that oxytocin may motivate a sexually differentiated biobehavioral response to stress consistent with the *tend-and-befriend* theory. Specifically, oxytocin may induce distress-motivated social support seeking in women to a greater degree than it does in men, which might help explain why women are more likely to seek out social support in times of distress (Cyranowski et al., 2000), conferring both risk for and resilience against stress-related mental illnesses depending on the availability of supportive social relationships. As has been previously noted, the studies in this thesis produced equivocal results that don't definitively support this claim, as only Chapter 5 evidenced a sexually differentiated effect of oxytocin. However, it is important to note that theory in this research area strongly suggests a sexually differentiated effect (Cyranowski et al., 2000; Taylor et al., 2000), and more varied methodology (i.e. use of gene methylation studies and CSF oxytocin studies) may be essential to clarifying this issue. It is clear that this line of investigation has the potential to further our understanding of how sex influences stress and mental health, and this area of research has the potential to ameliorate important gender gaps in stress-related mental illnesses such as MDD.

References

Adolphs, R., Baron-Cohen, S., & Tranel, D. (2002). Impaired recognition of social emotions following amygdala damage. *Journal of Cognitive Neuroscience, 14,* 1264–74

Aragona, B. J., Liu, Y., Curtis, J. T., Stephan, F. K., & Wang, Z. (2003). A critical role for nucleus accumbens dopamine in partner-preference formation in male prairie voles. *The Journal of Neuroscience, 23,* 3483-90

Adolphs, R., Tranel, D., Damasio, H., & Damasio, A. (1994). Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. *Nature, 372,* 669-72

Aiken, L.S., West, S.G., 1991. Multiple regression: testing and interpreting interactions. CA, Thousand Oaks: Sage

Andari, E., Duhamel, J. R., Zalla, T., Herbrecht, E., Leboyer, M., & Sirigu, A. (2010). Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. *Proceedings of the National Academy of Sciences, 107*, 4389-94

Aviezer, H., Hassin, R. R., Ryan, J., Grady, C., Susskind, J., Anderson, A., Moscovitch, M., et al. (2008). Angry, Disgusted, or Afraid? Studies on the Malleability of Emotion Perception. *Psychological Science*, *19*, 724–32

Averbeck, B. B. (2010). Oxytocin and the salience of social cues. *Proceedings of the National Academy of the United States of America, 107,* 9033-4.

Bakermans-Kranenburg, M.J., & van IJzendoorn, M.H. (2013). Sniffing around oxytocin: review and meta-analyses of trials in healthy and clinical groups with implications for pharmacotherapy. *Translational Psychiatry, 3,* e258

Bales, K. L., Kim, A. J., Lewis-Reese, A. D., & Carter, C. S. (2004). Both oxytocin and vasopressin may influence alloparental behavior in male prairie voles. *Hormones and Behavior, 45,* 354-61

Bales, K. L., Pfeifer, L. A., & Carter, C. S. (2004). Sex differences and developmental effects of manipulations of oxytocin on alloparenting and anxiety in prairie voles. *Developmental Psychobiology, 44,* 123-31

Baron-Cohen, S., Wheelwright, S., Hill, J., Raste, Y., & Plumb, I. (2001). The "reading the mind in the eyes" test revised version: A study with normal adults, and adults with asperger

syndrome or high-functioning autism. *Journal of Child Psychology and Psychiatry, 42,* 241-51

Bartz, J., Simeon, D., Hamilton, H., Kim, S., Crystal, S., Braun, A., Vicens, V., et al. (2011b). Oxytocin can hinder trust and cooperation in borderline personality disorder. *Social Cognitive and Affective Neuroscience*, *6*, 556–63

Bartz, J. A., & Hollander, E. (2006). The neuroscience of affiliation: Forging links between basic and clinical research on neuropeptides and social behavior. *Hormones and Behavior, 50,* 518-28

Bartz, J. A., Zaki, J., Bolger, N., Hollander, E., Ludwig, N. N., Kolevzon, A., & Ochsner, K. N. (2010). Oxytocin selectively improves empathic accuracy. *Psychological Science, 21,* 1426-28

Bartz, J. A., Zaki, J., Bolger, N., & Ochsner, K. N. (2011a). Social effects of oxytocin in humans: context and person matter. *Trends In Cognitive Sciences, 1*5, 301-9

Batson, C. D. (2009). These things called empathy: Eight related but distinct phenomena. In J. Decety (Ed). *The social neuroscience of empathy* (pp. 3-15). London: MIT Press

Baumgartner, T., Heinrichs, M., Vonlanthen, A., Fischbacher, U., & Fehr, E. (2008). Oxytocin shapes the neural circuitry of trust and trust adaptation in humans. *Neuron, 58,* 639-50

Bethlehem, R. A. I., van Honk, J., Auyeung, B., & Baron-Cohen, S. (2013). Oxytocin, brain physiology, and functional connectivity: A review of intranasal oxytocin fMRI studies. *Psychoneuroendocrinology, 38,* 962-74

Bhandari, R., Bakermans-Kranenburg, M. J., van der Veen, R., Parsons, C. E., Young, K. S., Grewen, K. M., ... & van IJzendoorn, M. H. (2014). Salivary oxytocin mediates the association between emotional maltreatment and responses to emotional infant faces. *Physiology & Behavior, 131,* 123-128.

Blair, R. J. R., Colledge, E., Murray, L., & Mitchell, D. G. V. (2001). A selective impairment in the processing of sad and fearful expressions in children with psychopathic tendencies. *Journal of Abnormal Child Psychology, 29,* 491–8

Born, J., Lange, T., Kern, W., McGregor, G. P., Bickel, U., & Fehm, H. L. (2002). Sniffing neuropeptides: A transnasal approach to the human brain. *Nature Neuroscience, 5,* 514–16

Bosch, O. J., Krömer, S. A., Brunton, P. J., & Neumann, I. D. (2004). Release of oxytocin in the hypothalamic paraventricular nucleus, but not central amygdala or lateral septum in lactating residents and virgin intruders during maternal defence. *Neuroscience, 124,* 439-48

Brackett, M. A., Mayer, J. D., & Warner, R. M. (2004). Emotional intelligence and the prediction of behavior. *Personality and Individual Differences, 36,* 1387–1402

Brackett, M. A., Rivers, S. E., & Salovey, P. (2011). Emotional intelligence: implications for personal, social, academic, and workplace success. *Social and Personality Psychology Compass, 5,* 88-103

Brannick, M. T., Wahi, M. M., & Goldin, S. B. (2011). Psychometrics of Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT) scores. *Psychological Reports*, *109*, 327–37

Bruins, J., Hijman, R., & Van Ree, J. M. (1992). Effect of a single dose of des-glycinamide-[arg^{8}]vasopressin or oxytocin on cognitive processes in young healthy subjects. *Peptides, 13,* 461-8

Buchheim, A., Heinrichs, M., George, C., Pokorny, D., Koops, E., Henningsen, P., ... & Gündel, H. (2009) Oxytocin enhances the experience of attachment security. *Psychoneuroendocrinology, 34,* 1417-22

Buckley, J., & Eston, R. (2007). Ratings of perceived exertion. In: Winter, E.M., Jones, A.M., Davison, R.C.R., Bromley, P.D., Mercer, T.H. (Eds.), Sport and Exercise Physiology Testing— The British Association of Sport and Exercise Sciences Guide Volume II: Exercise and Clinical Testing. Routledge, New York, pp. 120-129

Campbell, A. (2010). Oxytocin and human social behavior. *Personality and Social Psychology Review, 14,* 281-95

Cardoso, C., & Ellenbogen, M.A. (2013). Oxytocin and psychotherapy: Keeping context and person in mind. *Psychoneuroendocrinology, 38,* 3172-3173

Cardoso, C., & Ellenbogen, M.A. (2014). Tend-and-befriend is a beacon for change in stress research: a reply to Tops. *Psychoneuroendocrinology, 45,* 212-3

Cardoso, C., Ellenbogen, M. A., & Linnen, A-M, 2012. Acute intranasal oxytocin improves positive self-perceptions of personality. *Psychopharmacology, 220,* 741-49

Cardoso, C., Ellenbogen, M.A., & Linnen, A.M. (2014). The effect of intranasal oxytocin on perceiving and understanding emotion on the Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT). *Emotion, 14,* 43-50

Cardoso, C., Ellenbogen, M. A., Orlando, M. A., Bacon, S. L., & Joober, R. (2013). Intranasal oxytocin attenuates the cortisol response to physical stress: A dose-response study. *Psychoneuroendocrinology. 38,* 399-407

Cardoso C, Ellenbogen MA, Serravalle L, & Linnen A-M. (2013). Stress-induced negative mood moderates the relation between oxytocin administration and trust: Evidence for the tend-and-befriend response to stress? *Psychoneuroendocrinology, 38,* 2800-4

Cardoso, C., Kingdon, D., & Ellenbogen, M.A. (2014). A meta-analytic review of the impact of intranasal oxytocin administration on cortisol concentrations during laboratory tasks: Moderation by method and mental health. *Psychoneuroendocrinology, 49,* 161-70

Cardoso, C., Linnen, A-M., Joober, R., & Ellenbogen, M. A. (2012). Coping style moderates the effect of intranasal oxytocin on the mood response to interpersonal stress. *Experimental and Clininical Psychopharmacology, 20,* 84-91

Cardoso, C., Orlando, M.A., Brown, C.A., & Ellenbogen, M.A. (2014). Oxytocin and enhancement of the positive valence of social affiliation memories: an autobiographical memory study. *Social Neuroscience, 9,* 186-95

Cardoso, C., Payne, J. W., & Ellenbogen, M.A. (unpublished observations). A meta-analysis of the inhibitory effect of intranasal oxytocin on amygdala activation in humans: a neuroimaging perspective. *Unpublished manuscript*

Carson, D. S., Yuan, H., & Labuschagne, I. (2015). Improving Research Standards to Restore Trust in Intranasal Oxytocin. *Biological Psychiatry*.

Carter, C. S. (1998). Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology, 23,* 779-818

Carter, C.S. (2014). Oxytocin pathways and the evolution of human behavior. *Annual Reviews of Psychology, 65,* 17-39

Cavanaugh, J., Mustoe, A.C., Taylor, J.H., & French, J.A. (2014) Oxytocin facilitates fidelity in well-established marmoset pairs by reducing sociosexual behavior toward opposite-sex strangers. *Psychoneuroendocrinology, 49,* 1-10

Champagne, F. A., Chretien, P., Stevenson, C. W., Zhang, T. Y., Gratton, A., & Meaney, M. J. (2004). Variations in nucleus accumbens dopamine associated with individual differences in maternal behavior in the rat. *The Journal of Neuroscience, 24,* 4113-23

Champagne, F., Diorio, J., Sharma, S., & Meaney, M. J. (2001). Naturally occurring variations in

maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proceedings of the National Academy of Sciences, 98,* 12736-41

Chang, S. W., Barter, J. W., Ebitz, R. B., Watson, K. K., & Platt, M. L. (2012). Inhaled oxytocin amplifies both vicarious reinforcement and self reinforcement in rhesus macaques (Macaca mulatta). *Proceedings of the National Academy of Sciences, 109,* 959-964.

Cho, M. M., DeVries, A. C., Williams, J. R., & Carter, C. S. (1999). The effects of oxytocin and vasopressin on partner preferences in male and female prairie voles (Microtus ochrogaster). *Behavioral Neuroscience, 113,* 1071-9

Churchland, P.S., & Winkielman, P. (2012). Modulating social behavior with oxytocin: How does it work? What does it mean? *Hormones and Behavior, 61*, 392–399.

Cohen, H., Kaplan, Z., Kozlovsky, N., Gidron, Y., Matar, M. A., & Zohar, J. (2010). Hippocampal microinfusion of oxytocin attenuates the behavioural response to stress by means of dynamic interplay with the glucocorticoid-catecholamine responses. *Journal of Neuroendocrinology, 22,* 889-904

Coiro, V., Passeri, M., Davoli, C., Bacchi-Modena, A., Bianconi, L., Volip, R., & Choidera, P. (1988). Oxytocin reduces exercise-induced acth and cortisol rise in man. *Acta Endocrinology, 119,* 405-12

Colonnello, V., Chen, F. S., Panksepp, J., & Heinrichs, M. (2013). Oxytocin sharpens self-other perceptual boundary. *Psychoneuroendocrinology, 38,* 2996-3002

Cross, S. E., & Madson, L. (1997). Models of the self: self-construals and gender. *Psychological Bulletin, 122,* 5.

Cyranowski, J. M., Frank, E., Young, E., & Shear, M. K. (2000). Adolescent onset of the gender difference in lifetime rates of major depression: a theoretical model. *Archives of General Psychiatry, 57,* 21-27

Dabrowska, J., Hazra, R., Ahern, T. H., Guo, J. D., McDonald, A. J., Mascagni, F., ... & Rainnie, D. G. (2011). Neuroanatomical evidence for reciprocal regulation of the corticotrophin-releasing factor and oxytocin systems in the hypothalamus and the bed nucleus of the stria terminalis of the rat: Implications for balancing stress and affect. *Psychoneuroendocrinology, 36,* 1312-26

Davies, C.T. & Few, J.D. (1973). Effects of exercise on adrenocortical function. *Journal of Applied Physiology, 35,* 887-91

Decety, J. (2011). Dissecting the Neural Mechanisms Mediating Empathy. *Emotion Review, 3,* 92–108

Decety, J., & Jackson, P. L. (2004). The functional architecture of human empathy. *Behavioral and Cognitive Neuroscience Reviews, 3,* 71–100

de Dreu, C. K., Greer, L. L., Handgraaf, M. J., Shalvi, S., Van Kleef, G. A., Baas, M., ... & Feith, S. W. (2010). The neuropeptide oxytocin regulates parochial altruism in intergroup conflict among humans. *Science, 328,* 1408-1411.

de Kloet, R.R., Joels, M., & Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nature Reviews Neuroscience, 6,* 463-75

DeVries, A.C., DeVries, M.B., Taymans, S., & Carter, C.S. (1995). Modulation of pair bonding in female prairie voles (Microtus ochrogaster) by corticosterone. *Proceedings of the National Academy of Sciences, 92,* 7744-7748

DeVries, A.C., DeVries, M.B., Taymans, S.E., & Carter, C.S. (1996). The effects of stress on social preferences are sexually dimorphic in prairie voles. *Proceedings of the National Academy of Sciences, 93*, 11980-11984

DeVries, A.C., Guptaa, T., Cardillo, S., Cho, M., & Carter, C.S. (2002). Corticotropin-releasing factor induces social preferences in male prairie voles. *Psychoneuroendocrinology, 27,* 705-714.

DeVries, A.C., Craft, T.K., Glasper, E.R., Neigh, G.N., & Alexander, J.K. (2007). 2006 Curt P. Richter award winner: Social influences on stress responses and health. *Psychoneuroendocrinology. 32,* 587-603.

Domes, G., Heinrichs, M., Michel, A., Berger, C., & Herpertz, S. C. (2007). Oxytocin improves "mind-reading" in humans. *Biological Psychiatry*, *61*, 731–3

Ditzen, B., Schaer, M., Gabriel, B., Bodenmann, G., Ehlert, U., & Heinrichs, M. (2009). Intranasal oxytocin increases positive communication and reduces cortisol levels during couple conflict. *Biological Psychiatry, 65,* 728-31

Dziobek, I., Rogers, K., Fleck, S., Bahnemann, M., Heekeren, H. R., Wolf, O. T., & Convit, A. (2008). Dissociation of cognitive and emotional empathy in adults with Asperger syndrome using the Multifaceted Empathy Test (MET). *Journal of Autism and Developmental Disorders, 38*, 464-73

Ebert, A., Kolb, M., Heller, J., Edel, M.A., Roser, P., & Brüne, M. (2013). Modulation of

interpersonal trust in borderline personality disorder by intranasal oxytocin and childhood trauma. *Social Neuroscience, 8,* 305-13

Ebstein, R. P., Israel, S., Lerer, E., Uzefovsky, F., Shalev, I., Gritsenko, I… & Yirmiya, N. (2009). Arginine vasopressin and oxytocin modulate human social behavior. *Annals of the New York Academy of Sciences, 1167,* 87-102

Ebner, K., Bosch, O. J., Kromer, S. A., Singewald, N., & Neumann, I. D. (2005). Release of oxytocin in the rat central amygdala modulates stress-coping behavior and the release of excitatory amino acids. *Neuropsychopharmacology, 30,* 223-30

Ellenbogen, M.A. & Cardoso, C. (2015). Therapy with an oxytocin adjunct for major depression (TOAD2015). Protocol Registration Number NCT02405715 http://clinicaltrials.gov/

Ellenbogen, M.A., Linnen, A-M., Cardoso, C., & Joober, R. (2013). Intranasal oxytocin impedes the ability to ignore task-irrelevant facial expressions of sadness in students with depressive symptoms. *Psychoneuroendocrinology, 38,* 387-98

Ellenbogen, M. A., Linnen, A. M., Cardoso, C., & Joober, R. (2014). Intranasal oxytocin attenuates the human acoustic startle response independent of emotional modulation. *Psychophysiology*, *51*, 1169-1177.

Ellenbogen, M.A., Linnen, A-M., Grumet, R., Cardoso, C., & Joober, R. (2012). The acute effects of intranasal oxytocin on automatic and effortful attentional shifting. *Psychophysiology, 49,* 127-37

Engelmann, M., Landgraf, R., & Wotjak, C. T. (2004). The hypothalamic-neurohypophysial system regulates the hypothalamic-pituitary-adrenal axis under stress: an old concept revisited. *Frontiers of Neuroendocrinology, 25,* 132-49

Fahrbach, S. E., Morrell, J. I., & Pfaff, D. W. (1985). Possible role for endogenous oxytocin in estrogen-facilitated maternal behavior in rats. *Neuroendocrinology, 40,* 526-532.

Fehm-Wolfsdorf, G., Bachholz, G., Born, J., Voigt, K., & Fehm, H.L. (1988). Vasopressin but not oxytocin enhances cortical arousal: an integrative hypothesis on behavioral effects of neurohypophyseal hormones. *Psychopharmacology, 94,* 496-500

Feifel, D., Macdonald, K., Nguyen, A., Cobb, P., Warlan, H., Galangue, B., Minassian, A… & Hadley, A. (2010). Adjunctive intranasal oxytocin reduces symptoms in schizophrenia patients. *Biological Psychiatry*, *68*, 678–80

Feldman, R., Gordon, I., Schneiderman, I., Weisman, O., & Zagoory-Sharon, O. (2010). Natural

variations in maternal and paternal care are associated with systematic changes in oxytocin following parent–infant contact. *Psychoneuroendocrinology, 35,* 1133-41

Feldman, R., Gordon, I., & Zagoory-Sharon, O. (2011). Maternal and paternal plasma, salivary, and urinary oxytocin and parent–infant synchrony: considering stress and affiliation components of human bonding. *Developmental Science, 14,* 752-61

Feldman, R., Zagoory-Sharon, O., Weisman, O., Schneiderman, I., Gordon, I., Maoz, R., ... & Ebstein, R. P. (2012). Sensitive parenting is associated with plasma oxytocin and polymorphisms in the OXTR and CD38 genes. *Biological Psychiatry, 72,* 175-81

Ferguson, J. N., Aldag, J. M., Insel, T. R., & Young, L. J. (2001). Oxytocin in the medial amygdala is essential for social recognition in the mouse. *The Journal of Neuroscience*, *21,* 8278-85

Ferguson, J. N., Young, L. J., Hearn, E. F., Matzuk, M. M., Insel, T. R., & Winslow, J. T. (2000). Social amnesia in mice lacking the oxytocin gene. *Nature Genetics, 25,* 284-8

Fertuck, E. A., Jekal, A., Song, I., Wyman, B., Morris, M. C., Wilson, S. T… & Stanley, B. (2009). Enhanced 'reading the mind in the eyes' in borderline personality disorder compared to healthy controls. *Psychological Medicine, 39,* 1979-88

Few, J.D. (1974). Effect of exercise on the secretion and metabolism of cortisol in man. *Journal of Endocrinology, 62,* 341-353.

Foley, P., & Kirschbaum, C. (2010). Human hypothalamus-pituitary-adrenal axis responses to acute psychosocial stress in laboratory settings. *Neuroscience and Biobehavioral Reviews, 35,* 91-96.

Flury, J. M., Ickes, W., & Schweinle, W. (2008). The borderline empathy effect: Do high BPD individuals have greater empathic ability? Or are they just more difficult to "read"? *Journal of Research in Personality, 42,* 312–32

Francis, D. D., Champagne, F. C., & Meaney, M. J. (2000). Variations in maternal behaviour are associated with differences in oxytocin receptor levels in the rat. *Journal of Neuroendocrinology, 12,* 1145-8

Francis, D. D., Young, L. J., Meaney, M. J., & Insel, T. R. (2002). Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors: gender differences. Journal of neuroendocrinology, 14(5), 349-353.

Gallese, V., Keysers, C., & Rizzolatti, G. (2004). A unifying view of the basis of social

cognition. Trends in Cognitive Sciences, 8, 396–403

Gamer, M., Zurowski, B., & Büchel, C. (2010). Different amygdala subregions mediate valence-related and attentional effects of oxytocin in humans. *Proceedings of the National Academy of the United States of America, 107,* 9400-5

Geary, D. C., & Flinn, M. V. (2002). Sex differences in behavioral and hormonal response to social threat: Commentary on Taylor et al. (2000). *Psychological Reviews, 109,* 745-50

Gimpl, G., & Fahrenholz, F. (2001). The oxytocin receptor system: structure, function and regulation. *Physiological Reviews, 81,* 629-83

Guastella, A. J., Einfeld, S. L., Gray, K. M., Rinehart, N. J., Tonge, B. J., Lambert, T. J., & Hickie, I. B. (2010). Intranasal oxytocin improves emotion recognition for youth with autism spectrum disorders. *Biological Psychiatry*, *67*, 692–4

Guastella, A.J., Hickie, I.B., McGuinness, M.W., Otis, M., Woods, E.A., Disinger, H.M… & Banati, R. B. (2013). Recommendations for the standardisation of oxytocin nasal administration and guidelines for its reporting in human research. *Psychoneuroendocrinology, 38,* 612-25

Guastella, A.J., Howard, A.L., Dadds, M.R., Mitchell, P., & Carson, D.S. (2009). A randomized controlled trial of intranasal oxytocin as an adjunct to exposure therapy for social anxiety disorder. *Psychoneuroendocrinology, 34, 917-23*

Guastella, A.J., & MacLeod, C. (2012). A critical review of the influence of oxytocin nasal spray on social cognition in humans: evidence and future directions. *Hormones and Behavior*, 61, 410-8

Guastella, A. J., Mitchell, P. B., & Dadds, M. R. (2008). Oxytocin Increases Gaze to the Eye Region of Human Faces. *Biological Psychiatry*, *63*, 3–5

Goldman, M. B., Gomes, A. M., Carter, C. S., & Lee, R. (2011). Divergent effects of two different doses of intranasal oxytocin on facial affect discrimination in schizophrenic patients with and without polydipsia. *Psychopharmacology, 216,* 101-10

Grimm, S., Pestke, K., Feeser, M., Aust, S., Weigand, A., Wang, J… & Bajbouj, M. (2014). Early life stress modulates oxytocin effects on limbic system during acute psychosocial stress. *Social Cognitive and Affective Neuroscience, 9,* 1828-35.

Hall, S.S., Lightbody, A.A., McCarthy, B.E., Parker, K.J., & Reiss, A.L. (2012). Effects of intranasal oxytocin on social anxiety in males with fragile X syndrome.

Psychoneuroendocrinology. 37, 509-18

Heim, C., Young, L. J., Newport, D. J., Mletzko, T., Miller, A. H., & Nemeroff, C. B. (2009). Lower CSF oxytocin concentrations in women with a history of childhood abuse. *Molecular Psychiatry, 14,* 954-958

Heinrichs, M., Baumgartner, T., Kirschbaum, C., & Ehlert, U. (2003). Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biological Psychiatry, 54,* 1389-98

Hodges, S. D., & Kline, K. J. K. (2001). Regulating the costs of empathy: The price of being human. *The Journal of Socio-Economics, 30,* 437–52

Huber, D., Veinante, P., & Stroop, R. (2005). Vasopressin and oxytocin excite distinct neuronal populations in the central amygdala. *Science, 308,* 245-248

Huffmeijer, R., Alink, L.R.A., Tops, M., Grewen, K.M., Light, K.C., Bakermans-Kranenburg, M.J., & Van IJzendoorn, M.H. (2012). Salivary levels of oxytocin remain elevated for more than two hours after intranasal oxytocin administration. *Neuroendocrinology Letters, 33*, 101-5

Hurlemann, R., Patin, A., Onur, O. A., Cohen, M. X., Baumgartner, T., Metzler, S… Kendrick, K.M. (2010). Oxytocin enhances amygdala-dependent, socially reinforced learning and emotional empathy in humans. *The Journal of Neuroscience, 30,* 4999-5007

Ickes, W. (2001). Measuring empathic accuracy. In J. A. Hall & F. J. Bernieri (Eds.) Interpersonal sensitivity: Theory and measurement (pp. 219–241). Lawrence Erlbaum Associates Publishers: Mahwah, NJ

Insel, T. R., & Hulihan, T. J. (1995). A gender-specific mechanism for pair bonding: oxytocin and partner preference formation in monogamous voles. *Behavioral Neuroscience, 109,* 782-9

Insel, T. R., & Young, L. J. (2001). The neurobiology of attachment. *Nature Reviews Neuroscience, 2,* 129-36

Kendrick, K. M., Keverne, E. B., & Baldwin, B. A. (1987). Intracerebroventricular oxytocin stimulates maternal behaviour in the sheep. *Neuroendocrinology, 46,* 56-61

Keri, S., & Benedek, G. (2009). Oxytocin enhances the perception of biological motion in humans. *Cognitive, Affective & Behavioral Neuroscience, 9,* 237–41

Keverne, E. B., & Kendrick, K. M. (1992). Oxytocin Facilitation of Maternal Behavior in

Sheep. *Annals of the New York Academy of Sciences, 652,* 83-101

Kim, H.S., Sherman, D.K., Sasaki, J.Y., Xu, J., Chu, T.Q., Ryu C... & Taylor, S.E. (2010). Culture, distress, and oxytocin receptor polymorphism (OXTR) interact to influence emotional support seeking. *Proceedings of the National Academy of Sciences of the United States of America, 107,* 15717-21

Kim, H., Somerville, L. H., Johnstone, T., Alexander, A. L., & Whalen, P. J. (2003). Inverse amygdala and medial prefrontal cortex responses to surprised faces. *Neuroreport*, *14*, 2317–22

Kirsch, P., Esslinger, C., Chen, Q., Mier, D., Lis, S., Siddhanti, S… Meyer-Lindernberg, A. (2005). Oxytocin modulates neural circuitry for social cognition and fear in humans. *Journal of Neuroscience, 25,* 11489-93

Klatt, J. D., & Goodson, J. L. (2013). Oxytocin-like receptors mediate pair bonding in a socially monogamous songbird. *Proceedings of the Royal Society B: Biological Sciences, 280,* 20122396

Knobloch, H. S., Charlet, A., Hoffmann, L.C., Eliava, M., Khrulev, S., Cetin, A. H… Grinevich, V. (2012). Evoked axonal oxytocin release in the central amygdala attenuates fear response. *Neuron, 73,* 553-66

Kosfeld, M., Heinrichs, M., Zak, P.J., Fischbacher, U., & Fehr, E. (2005). Oxytocin increases trust in humans. *Nature, 435,* 673-6

Kubzansky, L.D., Mendes, W.B., Appleton, A.A., Block, J., & Adler, G.K. (2012). A heartfelt response: oxytocin effects on response to social stress in men and women. *Biological Psychology, 90,* 1-9

Kudielka, B. M., Hellhammer, D. H., & Wüst, S. (2009). Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. *Psychoneuroendocrinology 34,* 2-18

Jackson, P. L., Rainville, P., & Decety, J. (2006). To what extent do we share the pain of others? Insight from the neural bases of pain empathy. *Pain*, *125*, 5–9

Jesso, S., Morlog, D., Ross, S., Pell, M. D., Pasternak, S. H., Mitchell, D. G. V… & Finger, E. C. (2011). The effects of oxytocin on social cognition and behaviour in frontotemporal dementia. *Brain: A Journal Of Neurology, 134,* 2493-501

Lane, A., Luminet, O., Rimé, B., Gross, J.J., de Timary, P., & Mikolajczak, M. (2013). Oxytocin

increases willingness to socially share one's emotions. *International Journal of Psychology, 48,* 676-81

Lang, R.E., Heil, J.W.E., Ganten, D., Hermann, K., Unger, T., & Rascher, W. (1983). Oxytocin unlike vasopressin is a stress hormone in the rat. *Neuroendocrinology, 37,* 314-16

Legros, J.J. (2001). Inhibitory effect of oxytocin on corticotrope function in humans: are vasopressin and oxytocin ying-yang neurohormones? *Psychoneuroendocrinology. 26,* 649-55

Legros, J.J., Choidera, P., & Demey-Ponsart, E. (1982). Inhibitory influence of exogenous oxytocin on adrenocorticotropin secretion in normal human subjects. *Journal of Clinical Endocrinology and Metabolism, 55,* 1035-9

Legros, J.J., Choidera, P., Geenen, C., & von Frenckell, R. (1984). Dose-response relationship between plasma oxytocin and cortisol and adrenocorticotropin concentrations during oxytocin infusion in normal men. *Journal of Clinical Endocrinology and Metabolism, 58,* 105-9

Legros, J.J., Choidera, P., Geenen, C., & von Frenckell, R. (1987). Confirmation of the inhibitory influence of exogenous oxytocin on cortisol and ACTH in man: evidence of reproducibility. *Acta Endocrinology, 114,* 345-9

Leknes, S., Wessberg, J., Ellingsen, D.-M., Chelnokova, O., Olausson, H., & Laeng, B. (2013). Oxytocin Enhances Pupil Dilation and Sensitivity to "hidden" Emotional Expressions. *Social Cognitive and Affective Neuroscience, 8,* 741-9.

Leuner, B., Caponiti, J. M., & Gould, E. (2012). Oxytocin stimulates adult neurogenesis even under conditions of stress and elevated glucocorticoids. *Hippocampus, 22,* 861-8

Lev-Ran, S., Shamay-Tsoory, S. G., Zangen, A., & Levkovitz, Y. (2012). Transcranial magnetic stimulation of the ventromedial prefrontal cortex impairs theory of mind learning. *European Psychiatry, 27,* 285–9

Liberzon, I., & Young, E. A. (1997). Effects of stress and glucocorticoids on CNS oxytocin receptor binding. *Psychoneuroendocrinology. 22,* 411-22

Lin, M.-T., Huang, K.-H., Huang, C.-L., Huang, Y.-J., Tsai, G. E., & Lane, H.-Y. (2012). MET and AKT genetic influence on facial emotion perception. *PLoS ONE*, *7*, e36143

Linnen, A-M., Ellenbogen, M. A., Cardoso, C., & Joober, R. (2012). Intranasal oxytocin and

salivary cortisol concentrations during social rejection in university students. *Stress: The International Journal on the Biology of Stress, 15,* 393-402

Lischke, A., Berger, C., Prehn, K., Heinrichs, M., Herpertz, S. C., & Domes, G. (2012). Intranasal oxytocin enhances emotion recognition from dynamic facial expressions and leaves eye-gaze unaffected. *Psychoneuroendocrinology*, *37*, 475–81

Liu, Y., & Wang, Z. X. (2003). Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles. *Neuroscience, 121,* 537-44

Lopes, P.N., Salovey, P., & Straus, R. (2003). Emotional intelligence, personality, and the perceived quality of social relationships. *Personality and Individual Differences, 35,* 641–58

Loup, F., Tribollet, E., Dubois-Dauphin, M., & Dreifuss, J. J. (1991). Localization of high-affinity binding sites for oxytocin and vasopressin in the human brain. An autoradiographic study. *Brain Research, 555,* 220-32

Lukas, M., Toth, I., Reber, S. O., Slattery, D. A., Veenema, A. H., & Neumann, I. D. (2011). The neuropeptide oxytocin facilitates pro-social behavior and prevents social avoidance in rats and mice. *Neuropsychopharmacology, 36,* 2159-68

Luminet, O., Grynberg, D., Ruzette, N., & Mikolajczak, M. (2011). Personality-dependent effects of oxytocin: Greater social benefits for high alexithymia scorers. *Biological Psychology, 87,* 401–6

Macdonald, K., & Macdonald, T. M. (2010). The peptide that binds: a systematic review of oxytocin and its prosocial effects in humans. *Harvard Review of Psychiatry, 18,* 1-21

MacDonald, K., MacDonald, T.M., Brüne, M., Lamb, K., Wilson, M.P., Golshan, S., & Feifel, D. (2013). Oxytocin and psychotherapy: a pilot study of its physiological, behavioral and subjective effects in males with depression. *Psychoneuroendocrinology 38,* 2831-43

Marsh, A. A., Yu, H. H., Pine, D. S., & Blair, R. J. (2010). Oxytocin improves specific recognition of positive facial expressions. *Psychopharmacology, 209,* 225-32

Maul, A. (2012). The validity of the Mayer–Salovey–Caruso Emotional Intelligence Test (MSCEIT) as a measure of emotional intelligence. *Emotion Review*, *4,* 394-402

Mayer, J. D., Salovey, P., & Caruso, D. R. (2000). Emotional intleligence as zeitgeist, as personality, and as a standard intelligence. In Bar-On, R. & Parker, J. D. A. (Eds.) *Handbook of Emotional Intelligence* (pp. 92-117). San Francisco, CA: Jossey-Bass.

Mayer, J. D., Salovey, P., & Caruso, D. R. (2002). *Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT) user's manual.* Toronto, ON: MHS

Mayer, J. D., Salovey, P., & Caruso, D. R. (2012). The validity of the MSCEIT: Additional analyses and evidence. *Emotion Review, 4,* 403-8

McCullough, M. E., Churchland, P. S., & Mendez, A. J. (2013). Problems with measuring peripheral oxytocin: Can the data on oxytocin and human behavior be trusted? *Neuroscience & Biobehavioral Reviews, 37,* 1485-1492

McClelland, G.H., Judd, C.M. (1993). Statistical difficulties of detecting interactions and moderator effects. *Psychological Bulletin, 114,* 376-390

McQuaid, R.J., McInnis, O.A., Abizaid, A., Anisman, H. (2014). Making room for oxytocin in understanding depression. *Neuroscience and Biobehavioural Reviews, 45,* 305-22

McRae-Clark, A.L., Baker, N.L., Maria, M.M., & Brady, K.T. (2013). Effect of oxytocin on craving and stress response in marijuana-dependent individuals: a pilot study. *Psychopharmacology, 228,* 623-31

Meinlschmidt, G., & Heim, C. (2007). Sensitivity to intranasal oxytocin in adult men with early parental separation. *Biological Psychiatry, 61,* 1109-11.

Molnar-Szakacs, I., Iacoboni, M., Koski, L., & Mazziotta, J. C. (2005). Functional segregation within pars opercularis of the inferior frontal gyrus: Evidence from fMRI studies of imitation and action observation. *Cerebral Cortex, 15,* 986–94

Neumann, I. D. (2009). The advantages of social living: brain neuropeptides mediate the beneficial consequences of sex and motherhood. *Frontiers in Neuroendocrinology, 30,* 483-96

Neumann, I.D., Kromer, S.A., Toschi, N., & Ebner, K. (2000). Brain oxytocin inhibits the (re)activity of the hypothalamo-pituitary-adrenal axis in male rats: involvement of hypothalamic and limbic brain regions. *Regulatory Peptides, 96,* 31-8

Neumann, I. D., & Landgraf, R. (2012). Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. *Trends in Neuroscience, 35,* 649-59

Neumann, I.D., Maloumby, R., Beiderbeck, D. I., Lukas, M., & Landgraf, R. (2013). Increased brain and plasma oxytocin after nasal and peripheral administration in rats and mice. *Psychoneuroendocrinology, 38, 1985-93*

Neumann, I.D., Tornet, L., & Wigger, A. (2000). Brain oxytocin: differential inhibition of neuroendocrine stress and anxiety-related behaviour in virgin, pregnant, and lactating rats. *Neuroscience, 95,* 567-75

Neumann, I. D., Wigger, A., Torner, L., Holsboer, F., & Landgraf, R. (2000). Brain oxytocin inhibits basal and stress-induced activity of the hypothalamo-pituitary-adrenal axis in male and female rats: partial action within the paraventricular nucleus. *Journal of Neuroendocrinology, 12,* 235-44

Nishioka, T., Anselmo-Franci, J. A., Li, P., Callahan, M. F., & Morris, M. (1998). Stress increases oxytocin release within the hypothalamic paraventricular nucleus. *Brain Research, 781,* 57-61

Nolen-Hoeksema, S. (2001). Gender differences in depression. *Current Directions in Psychological Science, 10,* 173-176

Norman, G.J., Cacioppo, J.T., Morris, J.S., Malarkey, W.B., Berntson, G.G., & DeVries, A.C. (2011). Oxytocin increases autonomic cardiac control: moderation by loneliness. *Biological Psychology, 86,* 174-80

Orchard, B., MacCann, C., Schulze, R., Matthews, G., Zeidner, M., & Roberts, R. D. (2009). New Directions and alternative approaches to the measurement of emotional intelligence. *Assessing Emotional Intelliegence: Plenum Series on Human Exceptionality, 4,* 321-44

Ott, V., Finlayson, G., Lehnert, H., Heitmann, B., Heinrichs, M., Born, J., & Hallschmid, M. (2013). Oxytocin reduces reward-driven food intake in humans. *Diabetes, 62,* 3418-25

Parker, K.J., Buckmaster, C.L., Schatzberg, A.F., & Lyons, D.M. (2005). Intranasal oxytocin administration attenuates the ACTH stress response in monkeys. *Psychoneuroendocrinology, 30,* 924-29

Parr, L. A., Modi, M., Siebert, F., & Young, L. J. (2013). Intranasal oxytocin selectively attenuates rhesus monkeys' attention to negative facial expressions. *Psychoneuroendocrinology, 38,* 1748-56

Pedersen, C. A., Ascher, J. A., Monroe, Y. L., & Prange, A. J. (1982). Oxytocin induces maternal behavior in virgin female rats. *Science, 216,* 648-50

Pedersen, C. A., Gibson, C. M., Rau, S. W., Salimi, K., Smedley, K. L., Casey, R. L… & Penn, D. L. (2011). Intranasal oxytocin reduces psychotic symptoms and improves Theory of Mind and social perception in schizophrenia. *Schizophrenia Research*, *132*, 50–3

Pedersen, C. A., & Prange, A. J. (1979). Induction of maternal behavior in virgin rats after intracerebroventricular administration of oxytocin. *Proceedings of the National Academy of Sciences, 76*, 6661-5

Perry, A., & Bentin, S. (2009). Mirror activity in the human brain while observing hand movements: A comparison between EEG desynchronization in the μ-range and previous fMRI results. *Brain Research, 1282,* 126–32

Perry, A., Bentin, S., Shalev, I., Israel, S., Uzefovsky, F., Bar-On, D., & Ebstein, R. P. (2010). Intranasal oxytocin modulates EEG mu/alpha and beta rhythms during perception of biological motion. *Psychoneuroendocrinology, 35,* 1446–53

Perry, A., Troje, N. F., & Bentin, S. (2010). Exploring motor system contributions to the perception of social information: Evidence from EEG activity in the mu/alpha frequency range. *Social Neuroscience, 5,* 272–84

Petersson, M., & Uvnäs-Moberg, K. (2003). Systemic oxytocin treatment modulates glucocorticoid and mineralocorticoid receptor mRNA in the rat hippocampus. *Neuroscience Letters, 343,* 97-100

Pfeifer, J. H., Iacoboni, M., Mazziotta, J. C., & Dapretto, M. (2008). Mirroring others' emotions relates to empathy and interpersonal competence in children. *NeuroImage, 39,* 2076–85

Pierrehumbert, B., Torrisi, R., Laufer, D., Halfon, O., Ansermet, F., & Beck Popovic, M. (2010). Oxytocin response to an experimental psychosocial challenge in adults exposed to traumatic experiences during childhood or adolescence. *Neuroscience. 166,* 168-77

Pincus, D., Kose, S., Arana, A., Johnson, K., Morgan, P. S., Borckardt, J., … Nahas, Z. (2010). Inverse effects of oxytocin on attributing mental activity to others in depressed and healthy subjects: a double-blind placebo controlled fMRI study. *Frontiers in Neuropsychiatric Imaging and Stimulation, 1,* 134

Popik, P., Vetulani, J., & Van Ree, J. M. (1992). Low doses of oxytocin facilitate social recognition in rats. *Psychopharmacology, 106,* 71-4

Prehn, K., Kazzer, P., Lischke, A., Heinrichs, M., Herpertz, S. C., & Domes, G. (2013). Effects of intranasal oxytocin on pupil dilation indicate increased salience of socioaffective stimuli. *Psychophysiology, 50,* 528-37

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas

for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology. 28,* 916-31

Putman, P., & Roelofs, K. (2011). Effects of single cortisol administrations on human affect reviewed: Coping with stress through adaptive regulation of automatic cognitive processing. *Psychoneuroendocrinology, 36,* 439-448.

Quintana, D. S., Alvares, G. A., Hickie, I. B., & Guastella, A. J. (2015). Do delivery routes of intranasally administered oxytocin account for observed effects on social cognition and behavior? A two-level model. *Neuroscience & Biobehavioral Reviews, 49,* 182-192

Quintana, D. S., & Woolley, J. D. (2015). Intranasal Oxytocin Mechanisms Can Be Better Understood, but Its Effects on Social Cognition and Behavior Are Not to Be Sniffed At. *Biological Psychiatry*.

Quirin, M., Kuhl, J., & Dusing, R. (2011). Oxytocin buffers cortisol responses to stress in individuals with impaired emotion regulation abilities. *Psychoneuroendocrinology. 36,* 898-904

Radke, S., & de Bruijn, E. R. (2015). Does oxytocin affect mind-reading? A replication study. Psychoneuroendocrinology, 60, 75-81.

Riem, M. M. E., Bakermans-Kranenburg, M. J., Pieper, S., Tops, M., Boksem, M. A. S., Vermeiren, R. R. J. M., …& Rombouts, S. A. R. B. (2011). Oxytocin modulates amygdala, insula, and inferior frontal gyrus responses to infant crying: A randomized controlled trial. *Biological Psychiatry, 70,* 291–7

Riem, M.M., van Ijzendoorn, M.H., Tops, M., Boksem, M.A., Rombouts, S.A., & Bakermans-Kranenburg, M.J. (2012). No laughing matter: intranasal oxytocin administration changes functional brain connectivity during exposure to infant laughter. *Neuropsychopharmacology. 37,* 1257-66

Rimmele, U., Hediger, K., Heinrichs, M., & Klaver, P. (2009). Oxytocin makes a face in memory familiar. *The Journal of Neuroscience, 29,* 38-42

Roberts, R. D., Schulze, R., O'Brien, K., MacCann, C., Reid, J., & Maul, A. (2006). Exploring the validity of the Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT) with established emotions measures. *Emotion, 6,* 663-9

Robinson, D. L., & Petersen, S. E. (1992). The pulvinar and visual salience. *Trends in Neuroscience, 15,* 127-32

Ross, H.E., & Young, L.J. (2009). Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Frontiers in Neuroendocrinology, 30,* 534-47

Sagi, A., & Hoffman, M. L. (1976). Empathic distress in the newborn. *Developmental Psychology, 12,* 175–6

Salovey, P. & Grewal, D. (2005). The science of emotional intelligence. *Current Directions in Psychological Science, 14,* 281-85

Schaller, M., & Cialdini, R. B. (1988). The economics of empathic helping: Support for a mood management motive. *Journal of Experimental Social Psychology, 24,* 163–81

Scheele, D., Striepens, N., Güntürkün, O., Deutschländer, S., Maier, W., Kendrick, K.M., & Hurlemann, R. (2012) Oxytocin modulates social distance between males and females. *The Journal of Neuroscience, 32,* 16074-9

Scheele, D., Wille, A., Kendrick, K. M., Stoffel-Wagner, B., Becker, B., Güntürkün, O., ... & Hurlemann, R. (2013) Oxytocin enhances brain reward system responses in men viewing the face of their female partner. *Proceedings of the National Academy of Sciences, 110,* 20308-13

Schneiderman, I., Zagoory-Sharon, O., Leckman, J. F., & Feldman, R. (2012). Oxytocin during the initial stages of romantic attachment: relations to couples' interactive reciprocity. *Psychoneuroendocrinology, 37,* 1277-85

Schulze, L., Lischke, A., Greif, J., Herpertz, S. C., Heinrichs, M., & Domes, G. (2011). Oxytocin increases recognition of masked emotional faces. *Psychoneuroendocrinology*, *36,* 1378–82

Seltzer, L.J., Zieglar, T.E., & Pollak, S.D. (2010). Social vocalizations can release oxytocin in humans. *Proceedings of the Royal Society B: Biological Sciences, 277,* 2661-6

Shahrestani, S., Kemp, A. H., & Guastella, A. J. (2013). The impact of a single administration of intranasal oxytocin on the recognition of basic emotions in humans: A meta-analysis. *Neuropsychopharmacology, 38,* 1929-36

Shahrokh, D. K., Zhang, T. Y., Diorio, J., Gratton, A., & Meaney, M. J. (2010). Oxytocin-dopamine interactions mediate variations in maternal behavior in the rat. *Endocrinology, 151,* 2276-86

Shalev, I., Israel, S., Uzefovsky, F., Gritsenko, I., Kaitz, M., & Ebstein, R. P. (2011). Vasopressin needs an audience: neuropeptide elicited stress responses are contingent upon perceived social evaluative threats. *Hormones and Behavior, 60,* 121-7

Shamay-Tsoory, S G, Lester, H., Chisin, R., Israel, O., Bar-Shalom, R., Peretz, A., … & Aharon-Peretz, J. (2005). The neural correlates of understanding the other's distress: a positron emission tomography investigation of accurate empathy. *NeuroImage, 27,* 468–72

Simeon, D., Bartz, J., Hamilton, H., Crystal, S., Braun, A., Ketay, S., & Hollander, E. (2011). Oxytocin administration attenuates stress reactivity in borderline personality disorder: a pilot study. *Psychoneuroendocrinology. 36,* 1418-21

Smith, A. S., Ågmo, A., Birnie, A. K., & French, J. A. (2010) Manipulation of the oxytocin system alters social behavior and attraction in pair-bonding primates, Callithrix penicillata. *Hormones and Behavior, 57,* 255-62

Smith, A.S., & Wang, Z. (2014). Hypothalamic oxytocin mediates social buffering of the stress response. *Biological Psychiatry, 76,* 281-8

Stone, V. E., Baron-Cohen, S., Calder, A., Keane, J., & Young, A. (2003). Acquired theory of mind impairments in individuals with bilateral amygdala lesions. *Neuropsychologia, 41,* 209–220.

Strathearn, L., Fonagy, P., Amico, J., & Montague, P. R. (2009). Adult attachment predicts maternal brain and oxytocin response to infant cues. *Neuropsychopharmacology, 34,* 2655-2666.

Striepens, N., Kendrick, K.M., Hanking, V., Landraf, R., Wullner, E., Maier, W., & Hurlemann, R. (2013). Elevated cerebrospinal fluid and blood concentrations of oxytocin following its intranasal administration in humans. *Scientific Reports, 3,* 3440

Stroud, L.R., Tanofsky-Kraff, M., Wilfley, D.E., Salovey, P. (2000). The Yale Interpersonal Stressor (YIPS): affective, physiological, and behavioural responses to a novel interpersonal rejection paradigm. *Annals of Behavioral Medicine, 22,* 204-13

Tamres, L.K., Janicki, D., & Helgeson, V.S. (2002). Sex differences in coping behavior: A meta-analytic review and an examination of relative coping. *Personality and Social Psychology Review*, *6*, 2-30.

Taylor, S.E., Klein, L.C., Lewis, B.P., Gruenewald, T.L., Gurung, R.A.R., & Updegraff, J.A. (2000). Biobehavioral responses to stress in females: Tend-and-befriend, not fight-or-flight. *Psychological Review, 107,* 411-29

Taylor, S.E., Saphire-Bernstein, S., & Seeman, T.E. (2010). Are plasma oxytocin in women and

plasma vasopressin in men biomarkers of distressed pair-bond relationships? *Psychological Science, 21,* 3-7

Thompson, S. M., Hammen, C., Starr, L. R., & Najman, J. M. (2014). Oxytocin receptor gene polymorphism (rs53576) moderates the intergenerational transmission of depression. *Psychoneuroendocrinology,* 43, 11-9

Triana-Del Rio, R., Tecamachaltzi-Silvarán, M. B., Díaz-Estrada, V. X., Herrera-Covarrubias, D., Corona-Morales, A. A., Pfaus, J. G., & Coria-Avila, G. A. (2015). Conditioned same-sex partner preference in male rats is facilitated by oxytocin and dopamine: Effect on sexually dimorphic brain nuclei. *Behavioural brain research, 283,* 69-77

Unkelbach, C., Guastella, A. J., & Forgas, J. P. (2008). Oxytocin selectively facilitates recognition of positive sex and relationship words. *Psychological Science, 19,* 1092-4

Unvas Moberg, K., & Francis, R. (2003). *The oxytocin factor: Tapping the hormone of calm, love, and healing*. Da Capo Press.

van Leengoed, E., Kerker, E., & Swanson, H. H. (1987). Inhibition of post-partum maternal behaviour in the rat by injecting an oxytocin antagonist into the cerebral ventricles. *Journal of Endocrinology, 112,* 275-82

van Ijzendoorn, M.H., & Bakermans-Kranenburg, M.J. (2012). A sniff of trust: meta-analysis of the effects of intranasal oxytocin administration on face recognition, trust to in-group, and trust to out-group. *Psychoneuroendocrinology, 37,* 438-43

van IJzendoom, M. H., Bhandari, R., van der Veen, R., Grewen, K. M., & Bakermans-Kraneburg, M. J. (2012). Elevated salivary levels of oxytocin persist more than 7h after intranasal administration. *Frontiers in Neuroscience, 6,* 1-6

van Ijzendoorn, M.H., Huffmeijer, R., Alink, L.R., Bakermans-Kranenburg, M.J., & Tops, M. (2011). The impact of oxytocin administration on charitable donating is moderated by experiences of parental love-withdrawal. *Frontiers in Psychology, 2,* 258

Viviani, D., Charlet, A., van den Burg, E., Robinet, C., Hurni, N., Abatis, M… & Stoop, R. (2011). Oxytocin selectively gates fear responses through distinct outputs from the central amygdala. *Science, 333,* 104-7

Walum, H., Lichtenstein, P., Neiderhiser, J. M., Reiss, D., Ganiban, J. M., Spotts, E. L., ... & Westberg, L. (2012) Variation in the oxytocin receptor gene is associated with pair-bonding and social behavior. *Biological Psychiatry, 71,* 419-26

Walum, H., Waldman, I. D., & Young, L. J. (2016). Statistical and methodological considerations for the interpretation of intranasal oxytocin studies. *Biological Psychiatry, 79,* 251-257

Weisman, O., Zagoory-Sharon, O., & Feldman, R. (2012a). Intranasal oxytocin administration is reflected in human saliva. *Psychoneuroendocrinology, 37,* 1582-6

Weisman, O., Zagoory-Sharon, O., & Feldman, R. (2012b). Oxytocin administration to parent enhances infant physiological and behavioral readiness for social engagement. *Biological Psychiatry, 72,* 982-9

Williams J. M., Broadbent, K. (1986). Autobiographical memory in suicide attempters. *Journal of Abnormal Psychology, 95,* 144-149.

Williams, J. M., & Scott, J. (1988). Autobiographical memory in depression. *Psychological Medicine, 18,* 689-95

Williams, J. R., Insel, T. R., Harbaugh, C. R., & Carter, C. S. (1994). Oxytocin administered centrally facilitates formation of a partner preference in female prairie voles (Microtus ochrogaster). *Journal of Neuroendocrinology,* 6, 247-50

Windle, R.J., Kershaw, Y.M., Shanks, N., Woods, S.A., Lightman, S.L., & Ingram, C.D. (2004). Oxytocin attenuates stress-induced c-fos mRNA expression in specific forebrain regions associated with modulation of the hypothalamo-pituitary-adrenal activity. *Journal of Neuroscience, 24,* 2974-82

Windle, R.J., Shanks, N., Lightman, S.L., & Ingram, C.D. (1997). Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. *Endocrinology, 138,* 2829-34

Winslow, J. T., & Insel, T. R. (2002). The social deficits of the oxytocin knockout mouse. *Neuropeptides, 36,* 221-9

Wispé, L. (1968). Sympathy and empathy. In D. L. Sills (Ed.), International encyclopedia of the social sciences (Vol. 15, pp. 441–447). New York: Free Press.

Witt, D. M., Winslow, J. T., & Insel, T. R. (1992). Enhanced social interactions in rats following chronic centrally infused oxytocin. *Pharmacology Biochemistry and Behavior, 43,* 855-61

Young, L.J., & Barrett, C.E. (2015). Can oxytocin treat autism? *Science, 347,* 825-6

Young, L.J., & Flanagan-Cato, L.M. (2012). Editorial comment: Oxytocin, vasopressin and social behavior. *Hormones and Behaviour, 61,* 227-9

Young, L. J., & Wang, Z. (2004). The neurobiology of pair bonding. *Nature Neuroscience, 7,*

1048-54

Zaki, J., Weber, J., Bolger, N., & Ochsner, K. (2009). The neural bases of empathic accuracy. *Proceedings of the National Academy of Sciences, 106,* 11382-7