

Oxytocin, Social Interactions and Coping: Implications for Depression

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## Abstract

Oxytocin is a neuropeptide that has been implicated in a range of social behaviors, such as attachment, trust, and empathy. Additionally, it has been suggested that through its promotion of prosocial behaviors, oxytocin might contribute to mental health outcomes. Chapter 1 described research indicating that oxytocin interacts with several neuroendocrine, neurotransmitter, and inflammatory processes that are linked to depressive disorders. Moreover, it was suggested that oxytocin might not always be beneficial, but instead serves to increase the salience of social cues, such that positive or negative experiences result in greater responses, thereby influencing affective states. In Study 1 (N=225), unsupportive social interactions were related to higher depressive symptoms through greater emotion- and lower problem-focused coping, but these relations were stronger among individuals that carried a single nucleotide polymorphism (SNP) on the oxytocin receptor gene (OXTR). In essence, individuals with a genetic variant thought to be linked to lower oxytocin endorsed potentially less advantageous coping methods. Study 2 (N=476) similarly revealed that unsupportive relations were associated with negative affective states, and this association was stronger among those with the OXTR SNP, as well as those with an oxytocin-related genetic variant on a gene involved in the regulation of oxytocin release (CD38). Study 3 (N=128) revealed that these genetic variants were related to altered reactions to experimentally manipulated social ostracism. In contrast to the previous studies that linked lower oxytocin functioning to negative outcomes, in the context of social ostracism, individuals with the OXTR allele thought to be tied to greater oxytocin functioning, were more psychosocially and physiologically reactive to social rejection. This finding is consistent

with a social sensitivity perspective of oxytocin. A social sensitivity effect was not as apparent when examining relations between the CD38 gene and responses to ostracism. Finally, Study 4 (N=67) revealed that endogenous levels of oxytocin were related to coping strategies in response to a psychosocial stressor, and the strategies endorsed varied as a function of whether individuals had support present or not. These findings highlight the potential influence of oxytocin on stress-related coping processes, which could in turn affect vulnerability to mood outcomes.

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## Table of Contents

Title.....	i
Abstract.....	ii
Acknowledgements.....	iv
Table of Contents.....	v
List of Tables.....	viii
List of Figures.....	ix
List of Appendices.....	xi
Introduction.....	1
Chapter 1: The Influence of Oxytocin in Depressive Disorders.....	3
Introduction.....	4
The role of oxytocin in depression.....	7
Animal-based studies.....	7
Human-based studies.....	8
Oxytocin polymorphisms, prosociality and depression.....	13
Early-life stress and oxytocin.....	14
Oxytocin and stress reactivity.....	18
HPA axis and oxytocin.....	19
Corticotropin releasing hormone (CRH), adrenocorticotrophic hormone (ACTH) and glucocorticoids in relation to oxytocin.....	21
Interpersonal and psychosocial stressors in humans.....	25
Serotonin and oxytocin.....	30
Norepinephrine and oxytocin.....	33

Dopamine and oxytocin.....	35
Growth factors and oxytocin.....	40
Inflammation and oxytocin.....	41
Treatments based on oxytocin administration.....	47
Chapter 2: Study 1.....	55
Abstract.....	57
Introduction.....	58
Methods.....	62
Results.....	66
Discussion.....	75
Chapter 3: Study 2.....	81
Abstract.....	82
Introduction.....	83
Methods.....	87
Results.....	90
Discussion.....	95
Chapter 4: Study 3.....	101
Abstract.....	102
Introduction.....	103
Methods.....	105
Results.....	112
Discussion.....	121
Supplementary Analyses.....	126

Chapter 5: Study 4.....	130
Introduction.....	131
Methods.....	133
Results.....	138
Discussion.....	142
General Discussion.....	145
Limitations and Conclusions.....	151
References.....	153
Appendix A: Study Questionnaires.....	196
Appendix B: Experience-dependent effects of genes: Responses to stressors.....	226
Appendix C: A paradoxical association of an oxytocin receptor gene polymorphism: early-life adversity and vulnerability to depression.....	261
Appendix D: Relations between Plasma Oxytocin and Cortisol: The Stress Buffering Role of Social Support.....	286

## List of Tables

### Chapter 2- Study 1

Table 1. <i>Mean, standard deviation, and range for study variables by OXTR rs53576 genotype</i> .....	67
Table 2. <i>Mean, standard deviation, and t-test values of study variables by gender</i> .....	68
Table 3. <i>Relations between depressive symptoms, social support, unsupport and coping</i> .....	69

### Chapter 3- Study 2

Table 1. <i>Mean and standard deviations of unsupportive social interactions, negative and positive affect as a function of the OXTR rs53576 and CD38 rs3796863 polymorphisms</i> .....	92
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### Chapter 4- Study 3

Table 1. <i>Oxytocin receptor gene polymorphism distributions by ethnicity</i> .....	107
--	-----

### Chapter-5- Study 4

Table 1. <i>Pearson correlations between baseline oxytocin and coping strategies, as well as depressive symptoms</i> .....	141
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## List of Figures

### Chapter 1

*Figure 1.* A schematic depiction of the hypothesized interactions between oxytocin and other neurochemical and hormonal systems in response to an acute stressor.....20

*Figure 2.* Hypothesized relations between early-life stressful experiences and the evolution of depression.....52

### Chapter 2- Study 1

*Figure 1.* Schematic of the moderated multiple mediation analyses examining parental unsupport.....72

*Figure 2.* Schematic of the moderated mediation analyses examining peer unsupport....74

### Chapter 3- Study 2

*Figure 1.* The relationship between unsupportive social interactions and negative affect as moderated by the CD38 rs3796863 and the OXTR rs53576 polymorphisms.....94

### Chapter 4- Study 3

*Figure 1.* Feelings of belonging (A), control (B), meaningful existence (C) and self-esteem (D) among individuals with the GG, AG, or AA OXTR genotypes who were either included or excluded during the Cyberball game.....114

*Figure 2.* Systolic blood pressure levels collected 30 minutes following either inclusion or exclusion during the Cyberball game (controlling for baseline systolic blood pressure) among individuals with the GG, AG or AA OXTR genotypes.....117

*Figure 3.* Cortisol levels in saliva ( $\mu\text{g}/\text{dl}$ ) collected at three time points including before Cyberball (T1), 15 minutes following Cyberball (T2) and 30 minutes following Cyberball (T3).....120

**Chapter 5- Study 4**

*Figure 1.* The relationship between depressive symptoms and problem-focused coping as moderated by support condition..... 139

## **List of Appendices**

Appendix A. Measures.....	176
Appendix B. Experience-dependent effects of genes: Response to stressors.....	197
Appendix C. A paradoxical association of an oxytocin receptor gene polymorphism: early-life adversity and vulnerability to depression.....	232
Appendix D. Relations between plasma oxytocin and cortisol: The stress buffering role of social support. ....	257

## **Introduction**

Supportive relationships are crucial for well-being, and play an important role in how individuals' cope with stressors (Cohen and Wills, 1985; Thoits, 2011). In contrast, a lack of support or obtaining unsupportive responses when support is reasonably expected can undermine an individual's ability to cope with stressful experiences (Ingram et al., 1999; Song and Ingram, 2002). Prosocial behaviors and their influence in promoting resilience have been tied to the presence of certain neurotransmitters and hormones. Indeed, the neuropeptide oxytocin has been well characterized in its involvement in social bonding, parent-infant attachment, lactation and birth processes (Macdonald and Macdonald 2010). Beyond these behaviors, the discovery that oxytocin could be manipulated via administration of a nasal spray, has implicated this neuropeptide in several other social behaviors, such as empathy (Rodrigues et al., 2009), trust (Kosfeld et al., 2005), altruism (De Dreu et al., 2011) and optimism (Saphire-Bernstein et al., 2011). Although oxytocin has often been characterized as a promoter of prosocial behaviors, an alternative view is that it serves to increase sensitivity to social cues. In this regard, oxytocin might promote sensitivity to both positive and negative social experiences and as such in a negative environment, individuals with higher oxytocin functioning could be more vulnerable to the development of poor mood outcomes such as depression.

In Chapter 1, a review of the literature will describe the possible role of oxytocin in relation to depressive disorders. Specifically, through its interactions with neurotransmitters, neuroendocrine and immune system factors, oxytocin might be implicated in depressive symptoms. Indeed, it is recognized that many factors, including other genetic variants unrelated to the oxytocin system, are involved in the development

of depressive disorders (for a more detailed discussion see McInnis et al., 2015, Appendix B). Chapter 1 will also explore two different perspectives of oxytocin's involvement in depression. Specifically, the social sensitivity view of oxytocin, which posits that in a negative environment this neuropeptide might promote vulnerability to depressive disorders by enhancing attention to negative cues, and another perspective that suggests that through its promotion of effective social coping processes (i.e., support seeking), oxytocin might limit the development of depressive disorders.

To explore these two different, yet not necessarily competing perspectives, in Study 1, we examined the association of a genetic variant of the oxytocin receptor gene (OXTR) rs53576 on both social support and unsupportive social interactions, and how these associations were tied to coping, as well as depressive symptoms. In Study 2, the OXTR polymorphism as well as a polymorphism on an oxytocin-related gene involved in oxytocin release (CD38) were examined in relation to unsupportive social interactions and affective states. In Study 3, we aimed to assess whether these same two polymorphisms were tied to psychosocial and hormonal responses to an experimentally manipulated paradigm of social ostracism. Finally, in Study 4, the associations between endogenous levels of oxytocin and coping strategies in response to a laboratory psychosocial stressor and the buffering role of a social support manipulation were examined.

## Chapter 1

### The Influence of Oxytocin in Depressive Disorders

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#### Abstract

Depression is accompanied by an array of neurobiological variations, including altered HPA axis activity, monoamine, growth factor and inflammatory immune functioning. In addition, a recent perspective has entertained the possible role for oxytocin in depressive disorders. Given the involvement of oxytocin in prosocial behaviors such as attachment, affiliation, trust, and social support seeking, it is not surprising this neuropeptide might be involved in the development or maintenance of depressive disorders. This view is supported by evidence that oxytocin interacts with various neuroendocrine, neurotransmitter, and inflammatory processes that have previously been implicated in depression. Thus, it might be profitable to consider the contribution of oxytocin in the context of several neurobiological changes provoked by stressors. The current review examines the relation between oxytocin and depression with a specific focus on the interactions between the oxytocinergic system and stressor-provoked biological and psychosocial responses. The possibility is also considered that oxytocin might increase the salience of social cues, such that positive or negative experiences result in exaggerated responses that may influence affective states.

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## **1. Introduction**

Despite the prevalence of depression and its broad consequences, our understanding of the pathophysiology of this disorder and its treatment is still somewhat limited in several respects. To be sure, antidepressant treatments have been effective for many patients, although the efficacy of drug treatments can be further improved through the judicious use of combination therapies (Blier et al., 2010; Millan, 2006). There is room for antidepressant treatments to be improved with respect to the success rate for given agents, reducing the time lag for treatment effects to appear, diminishing side effects, and limiting the recurrence of illness. In an effort to do so, several potential mechanisms and target systems have been evaluated beyond those based on serotonin (5-HT) processes. These have included the neuroplasticity associated with growth factors, such as brain-derived neurotrophic factor (BDNF) and fibroblast growth factor (FGF-2) (Audet and Anisman, 2013; Duman and Aghajanian, 2012), variations of  $\gamma$ -aminobutyric acid (GABA) processes (Krystal et al., 2002; Northoff et al., 2007), glutamate changes (Krystal et al., 2002 and Sanacora et al., 2012), altered dopamine functioning (Nestler and Carlezon, 2006), inflammatory immune signaling molecules (i.e. pro-inflammatory cytokine expression), as well as variations of hypothalamic-pituitary adrenal (HPA) axis hyperactivity or increased corticotropin releasing hormone (CRH) functioning within mesolimbic brain regions (Dantzer et al., 2008, Maes, 2011; Miller et al., 2009).

In addition to these potential contributors to depressive disorders, a role for variations of other central neuropeptides, such as oxytocin, has been offered in the etiology of depression (Catena-Dell'Osso et al., 2013). The conventional view of oxytocin as a hormone responsible for parturition and lactation has undergone considerable expansion

over the last decade, having been identified as a key regulator in an array of prosocial behaviors (MacDonald and MacDonald, 2010). Among other things, oxytocin has been associated with social bonding, attachment, love (Carter, 1998; Insel and Hulihan, 1995), trust (Kosfeld et al., 2005), positive communication (Ditzen et al., 2009), empathy (Rodrigues et al., 2009) and altruism (De Dreu et al., 2011), all of which share characteristics, but at the same time are distinct prosocial behaviors. The view that oxytocin is a common denominator for each of these is clearly a viable perspective, but given the complexity of each of these behaviors, it is likely that they involve multiple biological processes, with oxytocin probably being one major contributing element in this regard. Indeed, crosstalk occurs between the oxytocinergic system and monoamine (5-HT, norepinephrine (NE) and dopamine (DA)) activity (Liu and Wang, 2003; Vacher et al., 2002), and there is reason to believe that these interactions, at least in part, subserve the regulation of ‘prosocial’ behaviors, especially those that involve affective components.

It also appears that oxytocin acts as a neuromodulator in brain regions, such as amygdala, hypothalamus and nucleus accumbens (NAc), that contribute to depressive disorders in which disturbed social interactions are often apparent (Duman et al., 1997; Nestler et al., 2002). While not dismissing the perspective that oxytocin influences social behaviors, to account for the divergent outcomes that have been linked to this hormone, it was suggested that oxytocin functions, among other things, to make individuals more sensitive to social cues or to influence reactions to such cues. It follows that if oxytocin makes social conditions more salient, both positive and negative events may have more dramatic consequences (Averbeck, 2010; Bartz et al., 2011; Cardoso et al., 2014). In

essence, oxytocin might influence neural plasticity in response to environmental circumstances, and ‘for better or for worse’, might affect later behavioral and psychological outcomes (Belsky et al., 2007, Belsky et al., 2009; Belsky and Pluess, 2009).

Complex illnesses are typically biochemically heterogeneous and no doubt are influenced by multiple social experiences, some of which may involve interactions with oxytocin. In this regard, the link between oxytocin and depression has not been fully deduced and inconsistent findings have been reported (Cyranowski et al., 2008; Holt-Lunstad et al., 2011; Scantamburlo et al., 2007), making it difficult to use oxytocin levels as a predictor of depressive disorders. Instead, it may be more advantageous to evaluate the contribution of oxytocin in the context of other neurochemical variations. It is our intention in the current review to examine the link between oxytocin and depression, emphasizing the interactions that exist between the oxytocinergic system and HPA axis functioning as well as with monoaminergic activity. Furthermore, given that HPA activity is closely associated with variations of growth factors and inflammatory immune system changes, which have also been implicated in depressive disorders (Anisman, 2009; Sapolsky and Plotsky, 1990), we will examine oxytocin variations in relation to these processes. It is understood that several social behaviors discussed in this review such as social support, social withdrawal, trust, bonding and attachment are only indirectly related to depressive disorders, and are also non-specific in this regard, as they have been associated with other mental health disorders. Finally, the potential for the oxytocinergic system as a target for treatments of depressive disorders will be considered.

## **2. The role of oxytocin in depression**

## ***2.1. Animal-based studies***

The first indication of oxytocin being involved in depressive behaviors came from the finding that immobility in mice in the forced swim test (a validated test for screening anti-depressants) could be diminished through intracerebroventricular (ICV) oxytocin administration (Meisenberg, 1981), just as standard antidepressants, such as selective serotonin reuptake inhibitors (SSRIs) had this effect. Systemic (intraperitoneal) oxytocin administration was subsequently reported to be as effective as imipramine in reducing immobility in this test (Arletti and Bertolini, 1987), although there has not been unanimity concerning such findings (Slattery and Neumann, 2010). A comparable antidepressant-like effect was also evident in the forced swim test in mice treated with sildenafil, a drug that diminishes sexual dysfunction by enhancing oxytocin release. As expected, the antidepressant effect of sildenafil was blocked by an oxytocin receptor antagonist (Atosiban) and was absent in mice with the oxytocin receptor gene (OXTR) deleted (Matsushita et al., 2012). In line with these findings, relative to vehicle treated animals, rats that received systemic subchronic oxytocin treatment exhibited fewer escape failures and shorter latencies to escape footshock in a learned helplessness paradigm (Arletti and Bertolini, 1987).

Interest in the role of oxytocin in depression-related behavior increased with the demonstration that this hormone plays an important role in social attraction, affiliative behavior and bonding, which might be important in relation to the development of depression (Insel and Young, 2001; Neumann, 2008). Specifically, in response to maternal isolation, prairie vole pups displayed greater distress vocalizations (i.e. calls to promote maternal retrieval) and corticosterone levels compared to the less affiliative

montane vole (Shapiro and Insel, 1990). These differences were attributed to distinct oxytocin receptor distributions in brain regions within these voles (Insel and Shapiro, 1992). Consistent with this perspective, oxytocin administration reduced the number of distress calls emitted by pups, an outcome readily promoted by anxiolytic drugs (Insel and Winslow, 1991). Such findings reinforced the view that elevated oxytocin may promote social affiliative behaviors that diminish distress (Taylor et al., 2006), and given that oxytocin has strong inhibitory effects on amygdala activation (Kirsch et al., 2005), this hormone might promote affiliative behaviors by diminishing fear and anxiety that could otherwise inhibit social interactions. As well, long term peripheral oxytocin administration among female prairie voles reversed the anhedonia elicited by persistent isolation (Grippe et al., 2009), which may be especially significant given that social isolation and withdrawal might contribute to the development, albeit indirectly, of several mental health disorders including depression.

## ***2.2. Human-based studies***

Although animal studies strongly supported a role for oxytocin in depressive-like behaviors, in clinical populations the link between oxytocin and depression seems less certain. A negative correlation was observed between severity of depressive symptoms and serum oxytocin concentrations in a clinical population (Scantamburlo et al., 2007), and a similar outcome was apparent in a healthy university student sample (Gordon et al., 2008). Moreover, circulating oxytocin levels were reduced in patients with major depression and during a depressive episode among female patients diagnosed with bipolar affective disorder (Frasch et al., 1995; Ozsoy et al., 2009). Additionally, nocturnal plasma oxytocin levels (examined to avoid the influence of daily activities on oxytocin

secretion) were lower among patients with major depression compared to healthy controls (Zetsche et al., 1996). Yet, cerebrospinal fluid (CSF) oxytocin concentrations in male patients with major depressive disorder did not differ from those in healthy controls (Sasayama et al., 2012). Moreover, among both female and male patients, depression was accompanied by elevated baseline oxytocin levels and greater oxytocin variability (Holt-Lunstad et al., 2011; Parker et al., 2010; van Londen et al., 1997). Similarly, during an affiliation-focused guided imagery task, greater variability in pulsatile oxytocin release and higher overall oxytocin concentrations were evident among depressed women compared to controls. But, a similar outcome was also apparent in a stress task, indicating that changes of oxytocin were not uniquely tied to positive social experiences (Cyranowski et al., 2008). At first blush, elevated oxytocin levels among depressed individuals may seem puzzling given that this hormone was also elevated in association with bonding and attachment that ordinarily acted against depression (Heinrichs and Domes, 2008). As such, it is conceivable that the elevated plasma oxytocin levels might reflect a compensatory change that favors enhanced affiliative behaviors that could mitigate depressive symptoms (Taylor et al., 2006).

It is uncertain why plasma oxytocin levels in depressed individuals are reduced in some studies, but elevated in others. To be sure, these studies were conducted using different procedures, making it difficult to identify the specific factors responsible for the diverse outcomes observed. Indeed some studies used only female participants, whereas others included both males and females. This may be of particular concern as depressed females display lower plasma oxytocin concentrations compared to controls, while depressed males showed a trend in the opposite direction (Yuen et al., 2014).

Additionally, in virtually all studies oxytocin was examined under baseline conditions, but infrequently assessed following a challenge (e.g., Cyranowski et al., 2008). Moreover, although some studies examined plasma oxytocin levels in patients with major depressive disorder, others correlated plasma oxytocin levels and depressive symptoms in healthy participants. As patients are often tested while undergoing cognitive or pharmacological treatment, it is also difficult to know to what extent concentrations of oxytocin were related to these treatments.

An additional source for the inconsistencies that have been reported comes from the analytic procedures used. In some studies oxytocin levels were determined once oxytocin was extracted (isolated) from the samples, whereas in other studies the measurements were made in unextracted blood samples. Measuring plasma oxytocin levels without first extracting it yields values that are two orders of magnitude higher than that obtained using extracted samples. These discrepancies in oxytocin values likely reflect the possibility that in unextracted samples, molecules in addition to oxytocin are being tagged and detected (McCullough et al., 2013). Accordingly, conclusions based on oxytocin determined from such samples should be considered cautiously when interpreting the associations to various behavioral and mood outcomes.

Beyond the peripheral oxytocin variations associated with depressive disorders, there have also been reports of central oxytocin changes that might accompany depression. A postmortem investigation revealed increased oxytocin- and vasopressin-immunoreactive neurons in the paraventricular nucleus (PVN) in depressed patients compared to controls (Purba et al., 1996). In contrast, however, there were no differences between depressed patients and controls in a postmortem analysis of oxytocin mRNA expression in the PVN

of the hypothalamus. This said, oxytocin mRNA expression in the PVN was increased among melancholic patients compared to non-melancholic patients, suggesting that oxytocin mRNA expression may differ depending upon the type or severity of depression (Meynen et al., 2007). This is not unusual, as melancholia has been found to be more closely aligned with other neurobiological variations relative to less severe forms of depression (Maes, 1995).

Depression is often associated with cognitive processing biases toward negative stimuli (MacLeod et al., 1986), and oxytocin treatment could potentially influence this disorder by affecting sensitivity to social cues (Bartz et al., 2011). Indeed, among individuals with high depression scores, oxytocin attenuated the attentional bias that otherwise existed in relation to masked angry faces (Ellenbogen et al., 2012). It is thought that the inability to inhibit the influence of negative stimuli on cognitive and emotional responses contributes to major depression, which may be modulated by oxytocin. As well, other behavioral features associated with depressive disorders, such as anxiety and a tendency toward greater emotion-focused coping, are also influenced by oxytocin (Cardoso et al., 2012).

Despite the evidence that oxytocin might diminish depressive mood, there are data suggesting that this hormone could actually exacerbate depressed affect. When presented with emotional faces after intranasal oxytocin administration, depressed patients showed enhanced neuronal activation within the superior frontal gyrus and insula, suggesting that this hormone can enhance neural representation of affective states (Pincus et al., 2010). Consistent with this view, when individuals with high depression scores received oxytocin treatment, they were less able to ignore emotionally salient (sad) faces relative

to placebo-treated individuals (Ellenbogen et al., 2013). This could be mediated by oxytocin's role in empathetic concern and that individuals with higher depressive symptoms are more sensitive to this effect (O'Connor et al., 2002). As such, it was proposed that 'enhanced empathy has a cost' in so far as greater emotional responses to others requires that the individual deal with these emotions (Hodges and Kline, 2001). While a greater empathetic response may be a positive characteristic among healthy individuals, the oxytocin-facilitated empathy may be detrimental among individuals with difficulty regulating emotions related to depressed mood.

Studies that evaluated the influence of oxytocin administration on prosocial behaviors and depressive mood are in many respects advantageous over those that simply evaluated the relations that existed between oxytocin levels and particular behaviors or for that matter the link between particular polymorphisms and specific behaviors. At the same time, however, as oxytocin is a large peptide, there had been some question concerning whether oxytocin administered by nasal spray actually reached the brain, or whether the observed effects of the nasally administered oxytocin stemmed from some other action of the hormone. However, it was reported that 75 min after its administration, oxytocin could be detected in CSF of humans, but was not evident when assessed 45 or 60 min after being administered (Striepens et al., 2013). Despite the small number of participants involved in this study, these findings are in line with the view that oxytocin delivered by nasal spray could gain access to the brain. However, most studies that assessed the behavioral consequences of oxytocin administration did so 45 min after the spray was administered. Thus, there is still a lack of clarity as to whether the inhaled oxytocin stemmed from the central effects of this peptide.

### *2.2.1. Oxytocin polymorphisms, prosociality and depression*

To assess the relationship between oxytocin and prosocial behaviors, several studies took advantage of the presence of a single nucleotide polymorphism (SNP) within the oxytocin receptor gene (OXTR) to determine whether its presence was aligned with the appearance or absence of particular behaviors. Of special interest was the rs53576 SNP that involves a guanine (G) to adenine (A) substitution within the OXTR. Specifically, individuals homozygous for the G allele compared to those with the GA or AA genotypes exhibited greater maternal sensitivity (Bakermans-Kranenburg and van Ijzendoorn, 2008), self-esteem (Saphire-Bernstein et al., 2011), empathy, and ability to detect emotions (Rodrigues et al., 2009). Furthermore, individuals with the GG/GA genotypes also displayed higher trust-related behaviors (Krueger et al., 2012). Interestingly, associations with the OXTR SNP may be dependent upon ethnic background given that Caucasian individuals with the GG/GA genotype, but not Asian participants, reported greater social support seeking compared to those with the AA genotype (Kim et al., 2010).

Consistent with a role for oxytocin in mood related disorders, higher negative affect in non-clinical populations was associated with the presence of the A allele for OXTR rs2254928 (Kawamura et al., 2010; Lucht et al., 2009) and rs53576 (Saphire-Bernstein et al., 2011). In contrast, however, a positive association with the OXTR rs2254298 and rs53576 GG carriers (i.e. typically viewed as the more favorable genotype) and unipolar depression was reported, and it seemed that there was some selectivity in this regard, as the association was not observed in bipolar depression (Costa et al., 2009). Additionally, a trend toward an association of two further oxytocin SNPs, namely the C allele of the

rs2740210 and the G allele of the rs4813627 SNP in relation to childhood depression was reported, although in neither case was this relationship statistically significant (Strauss et al., 2010). Thus, the relation between certain oxytocin receptor polymorphisms and depressive disorders is not clear, and the possibility cannot be dismissed that the contribution of a particular gene variant to depressive disorders may depend on environmental influences. For instance, an interpersonal stressor may favor a depressive phenotype among carriers of a particular OXTR allele, but only in the presence of certain stressors, possibly those that entail social challenges.

### *2.2.2. Early-life stress and oxytocin*

Adverse early-life experiences, such as abuse, neglect or loss, are associated with elevated risk of several disorders including, depression (Heim et al., 2008a). In this regard, a 4-fold increase in the risk of depression was observed among individuals who experienced multiple adverse childhood experiences (Felitti et al., 1998), and the risk of attempted suicide was increased accordingly (Dube et al., 2001). However, the mechanisms through which early-life stressors lead to depression are uncertain, although a role for serotonergic mechanisms, neuroendocrine variations, as well as that of growth factors has been suggested. This begs the question of whether early-life adversity might also induce oxytocinergic changes and hence directly or indirectly influence depressive symptoms. Indeed, oxytocin concentrations were reduced in the CSF of adult women who had a history of childhood abuse, and were particularly marked in relation to emotional abuse. Furthermore, CSF oxytocin concentrations were progressively lower with the number of different types of maltreatment experienced (Heim et al., 2008b). Consistent with these findings, men who reported higher levels of early-life adversity and

depressive scores also displayed lower plasma oxytocin concentrations (Opacka-Juffry and Mohiyeddini, 2012). Once more, early-life stressor experiences were associated with lower plasma oxytocin levels in a sample of men, and a mediation analysis indicated that this relationship occurred through emotional suppression (Mohiyeddini et al., 2014). Interestingly, women who experienced sexual abuse during childhood or adolescence displayed a marked oxytocin decrease 20 min following the onset of a psychosocial challenge comprising the Trier Social Stress Test (TSST), whereas this did not occur among women that had experienced distress in the form of childhood or adolescent cancer (Pierrehumbert et al., 2010). Evidently, diminished levels of the hormone could be elicited by later stressors provided that the initial experience comprised early-life abuse.

The impact of early-life adversity might help in accounting for the inconsistencies regarding endogenous oxytocin levels in depressed populations. In this respect, depressed individuals who had experienced early-life adversity might display relatively low oxytocin levels, whereas depressed individuals who had not experienced early-life adverse experiences might not. Indeed, early-life adversity can limit attachments and trust owing to low oxytocin levels (Olf, 2012), and thus undermine the psychosocial dynamics that might otherwise be used to reduce the likelihood of developing negative mental health outcomes. For instance, women who experienced early-life trauma were less likely to engage in behaviors that could stimulate oxytocin activity (Olf, 2012). Beyond the suggestion that low oxytocin levels might favor the development of depression by limiting effective social support coping, there are other factors that might also contribute to the complex relationships involved in the development of depression. For instance, the gene that codes for the glucocorticoid receptor has been linked to

depressive outcomes associated with early-life adversity (McGowan et al., 2009) and there has also been the view that the Val66Met BDNF polymorphism (Gatt et al., 2009) as well as a serotonin transporter 5-HTT polymorphism (Caspi et al., 2003) contribute to the relation between early-life stressful experiences and the development of depressive disorders.

These alternatives notwithstanding, as alluded to earlier, the presence of OXTR SNPs may interact with early-life adverse events to provoke the development of depression. It might be thought that as in the case of the 5-HTT polymorphism, individuals who carried the 'less favorable' allele of the OXTR SNP would also be at greater risk for depression, and that this outcome would be exacerbated by negative early-life experiences. This was not the case, however, as individuals with the GG or GA genotype of the OXTR rs53576 polymorphism, which is ordinarily associated with greater prosocial behaviors and social sensitivity, expressed greater depressive scores than those with the AA genotype if they had also experienced high childhood maltreatment (McQuaid et al., 2013). In line with these seemingly paradoxical findings, GG carriers of the OXTR rs53576 SNP that experienced severe childhood maltreatment displayed greater disorganized attachment styles and increased risk for emotional dysregulation compared to A carriers (Bradley et al., 2011). Furthermore, individuals with the GG/GA genotypes displayed higher levels of positive affect and resilient coping if they were raised in a stable or warm family environment, whereas this relationship was not found among AA carriers (Bradley et al., 2013).

Together, these findings point to the possibility that certain OXTR genotypes that facilitate individuals' sensitivity to a positive environment also influence sensitivity to a

negative environment (Bradley et al., 2011; Brüne, 2012). In fact, in genetically engineered mice with increased oxytocin receptors in the lateral septum, enhanced fear and anxiety was observed in response to a negative social interaction, possibly reflecting the intensification of negative social memories (Guzmán et al., 2013). It was proposed that certain genotypes promote greater plasticity and susceptibility to the effects of environmental events (Belsky et al., 2009; Belsky and Pluess, 2009). In essence, having the AA genotype of the OXTR SNP might render individuals less responsive or receptive to positive environmental experiences that could enhance their long-term well-being. At the same time, individuals with this genotype might also be less sensitive to negative events and experiences that would otherwise lead to increased risk for stress-related disorders.

The influence of early-life events in conjunction with the OXTR polymorphism on depressive outcomes has not been widely assessed, in part perhaps, owing to the greater focus devoted to polymorphisms related to 5-HTT and BDNF. One study that was conducted in this regard, revealed elevated depressive scores among adolescent girls (9–14 years old) who experienced high levels of early-life adversity in the form of maternal depression, and who carried the AG genotype of the OXTR SNP rs2254298 (Thompson et al., 2011). Why heterozygous individuals were more prone to depression than those who carried the GG alleles is uncertain, although it is possible that heterozygotes may reflect the ‘perfect combination’ in which individuals are particularly sensitive to both positive and negative experiences, but yet are less likely to develop close bonds that facilitate social coping.

### *2.2.3. Oxytocin and stress reactivity*

Although much of the research assessing the OXTR polymorphism focused on prosocial behaviors, there have also been reports concerning the effects of this polymorphism in relation to the impact of stressors. For instance, individuals with the AA/AG genotypes of OXTR rs53576 displayed higher heart rate responses during a startle anticipation task compared to individuals homozygous for the G allele (Rodrigues et al., 2009). Furthermore, male A carriers exhibited higher levels of resting sympathetic cardiac control compared to individuals with the GG genotype, but in response to a psychological stressor, greater sympathetic reactivity occurred in GG males (Norman et al., 2012). Thus, although the OXTR polymorphism may be accompanied by altered stress reactivity, this could depend upon the nature of the stressor experienced.

It might appear curious that although oxytocin was elevated in association with bonding, attachment and warm touch (Heinrichs and Domes, 2008; Young and Wang, 2004), it was also increased in association with relationship distress and following stressful experiences (Tabak et al., 2011; Taylor et al., 2006). How oxytocin could be related to these very different behavioral features is not known, but it may be that oxytocin ordinarily promotes affiliative behaviors that encourage effective social coping (Taylor et al., 2006). Indeed, oxytocin administration enhanced the encoding of positive social stimuli, which might increase the likelihood of social approach behaviors (Guastella et al., 2008). Consistent with this perspective, intranasal oxytocin increased perceived trust among those with negative mood symptoms who experienced social rejection (Cardoso et al., 2013a). Indeed, oxytocin may promote affiliative behaviors by diminishing fear/anxiety and these stress-buffering effects are evident in relation to variations of HPA axis activity ordinarily elicited by stressors (Neumann et al., 2000;

Parker et al., 2005). Thus, as depressive disorders, as well as pathological conditions such as posttraumatic stress disorder (Yehuda et al., 1990), are often characterized by HPA axis dysregulation, analysis of the link between oxytocin and elements of HPA functioning in response to stressors, may be particularly instructive.

### **3. HPA axis and oxytocin**

As described earlier, complex behaviors, such as those often ascribed to oxytocin are likely subserved by multiple neurochemical interactions, including several that have traditionally been linked to depressive disorders. In the sections that follow, we describe the relationship between oxytocin activation and HPA axis hormone interactions, as well as interactions with monoamine activity, growth factors, and inflammatory processes. These inter-relations are depicted in Fig. 1, which will be referred to repeatedly as each of the interactions is discussed.

# Acute Stress

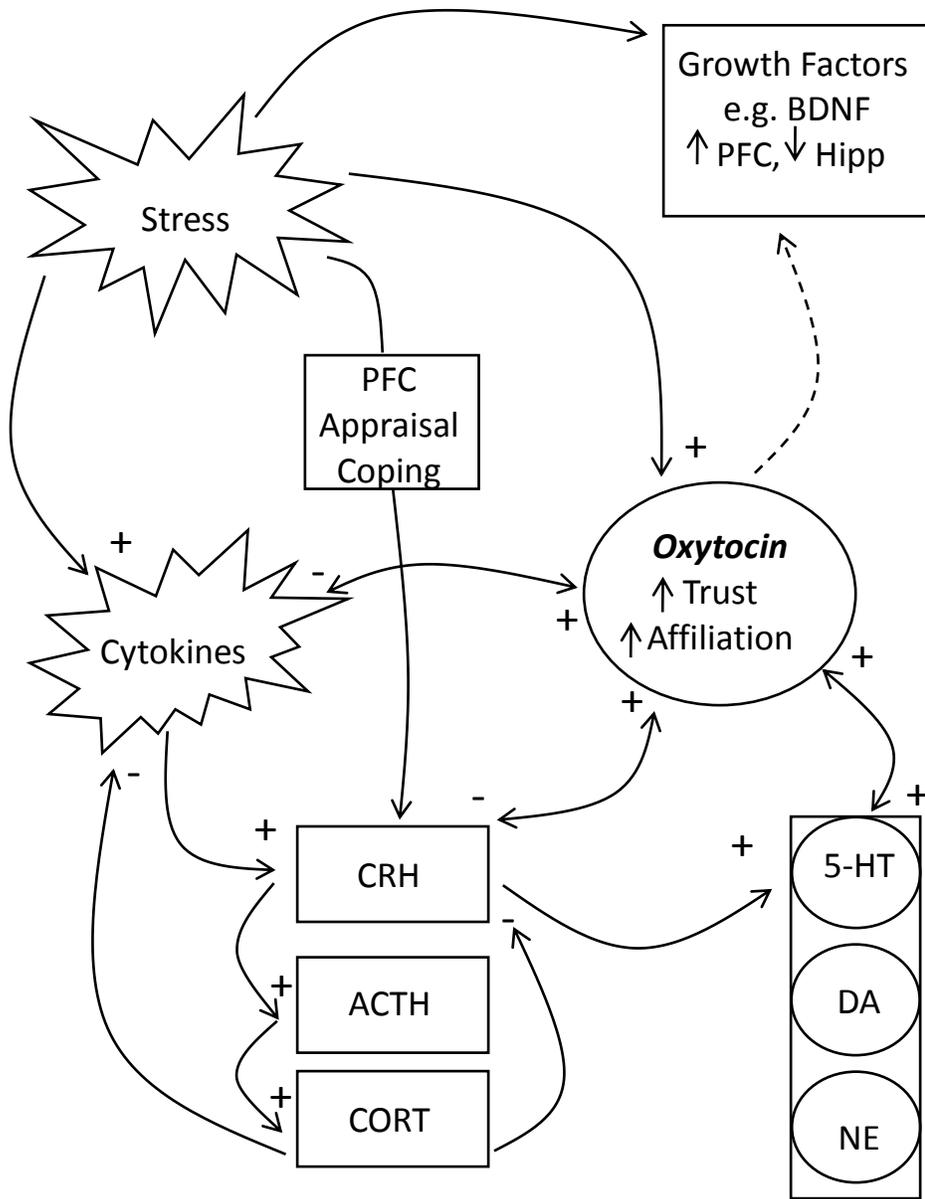


Figure 1.

A schematic depiction of the hypothesized interactions between oxytocin and other neurochemical and hormonal systems in response to an acute stressor. Stimuli appraised

as being stressful, as well as inflammatory cytokine challenges, elicit oxytocin and CRH release. In turn, oxytocin has inhibitory effects on both CRH and cytokine activity. Although stressors elicit growth factor variations, it is still unclear how oxytocin and neurotrophic factors interact (dashed line). In addition, oxytocin can elicit release of monoamines, and these neurotransmitters can have reciprocal effects to promote oxytocin release. Finally, stressor provoked oxytocin release may promote trust and affiliative behaviors as a compensatory mechanism aimed at attenuating stress responses. PFC = prefrontal cortex; Hipp = hippocampus.

### ***3.1. Corticotropin releasing hormone (CRH), adrenocorticotrophic hormone (ACTH) and glucocorticoids in relation to oxytocin***

There is reason to believe that oxytocin and CRH have reciprocal effects. In this regard, although CRH stimulates both ACTH and oxytocin release (Fig. 1), the mechanism responsible for the termination of these effects are different, with the oxytocin variations possibly occurring as a result of CRH directly or indirectly affecting magnocellular neurons (Bruhn et al., 1986). Consistent with a link between CRH and oxytocin, administration of a CRH1 receptor antagonist R121919 attenuated stressor-induced corticosterone and the oxytocin increase otherwise evident among rats bred for high anxiety-related behavior. It is tempting to conclude that CRH1 receptor binding is necessary for the release of oxytocin following a stressor (Keck et al., 2003), but no differences were reported between CRH1 mutant and wild-type mice with respect to either plasma oxytocin levels or PVN oxytocin mRNA expression elicited by a stressor (Müller et al., 2000). Thus, it remains uncertain whether and how CRH1 might contribute to oxytocin variations.

Examination of CRH mRNA expression in the PVN among oxytocin knock-out (KO) and wild-type male mice revealed no differences between the genotypes under basal conditions. A restraint stressor increased CRH mRNA expression among both genotypes, but this outcome was particularly marked in oxytocin KO mice, suggesting that oxytocin regulates CRH activity in the PVN in response to stressors, as seen in Fig. 1 (Nomura et al., 2003). Consistent with this perspective, ICV injections of oxytocin attenuated the markedly increased CRH mRNA expression in the PVN among male rats exposed to an acute restraint stressor (Bülbül et al., 2011; Zheng et al., 2010). Moreover, it appeared that the actions of oxytocin inhibition of CRH mRNA expression occurred through GABA receptors in the PVN (Bülbül et al., 2011).

Oxytocin may have long-term modulatory effects related to HPA functioning, as rats that received subcutaneous oxytocin injections over five days displayed decreased corticosterone concentrations that persisted for up to 10 days (Petersson et al., 1999). Moreover, among ovariectomized rats (to limit regulation of oxytocin by sex steroids), ICV oxytocin infusion attenuated the ACTH and corticosterone rise ordinarily observed in response to a stressor, and provoked an anxiolytic effect in the plus-maze test (Windle et al., 1997; Windle et al., 2004). Furthermore, the stressor-elicited increase of c-fos mRNA in the PVN, ventrolateral septum and dorsal hippocampus of rats was attenuated by concomitant administration of oxytocin (Windle et al., 2004), again pointing to its inhibitory influence on HPA activity.

Under natural conditions in which oxytocin levels are relatively high, as occurs in lactating female rats, stress responses were significantly diminished. For example, in

response to acute stressors lactating females displayed blunted ACTH and corticosterone levels (Walker et al., 1992; Walker et al., 2004). Interestingly, the blunted HPA responses were only present when the mom herself was challenged, but not attenuated when her pups were threatened (i.e. a stressor exposure in the presence of the pups) (Deschamps et al., 2003; Walker et al., 2004). These findings highlight the intricate interactions that exist between oxytocin and HPA functioning and raise the possibility that oxytocin might contribute to the protective “mother bear” phenomena.

Consistent with an inhibitory role for oxytocin on HPA functioning, infusion of a selective oxytocin antagonist either intraventricularly or directly into the PVN enhanced basal and stress-induced release of ACTH in male and female rats (Neumann et al., 2000). Likewise, in the absence of central oxytocin signaling, as in the case of oxytocin gene KO mice, corticosterone responses to psychogenic stressors were appreciably heightened (Amico et al., 2008; Mantella et al., 2004). Furthermore, the adaptation concerning the elevated corticosterone release that may develop in response to a chronic stressor occurred more readily among male oxytocin gene KO mice compared to wild-type animals. This enhanced adaptation might occur as a result of greater initial HPA functioning that also favors adaptation, or might reflect aspects of the HPA system being overly taxed (Bernatova et al., 2004).

Differentiating how chronic versus acute stressors affect oxytocin functioning is particularly germane to understanding stressor-related behavioral changes. In this regard, although an acute stressor readily enhanced plasma oxytocin levels, a chronic stressor did not elicit this effect, indicating a degree of stress adaptation (Hashiguchi et al., 1997). Likewise, an acute restraint stressor readily induced oxytocin release among rats, and

normalization of this response was apparent 24 h following the stressor. In contrast, chronic restraint resulted in delayed oxytocin release followed by more rapid normalization that was complete within 3 h (Danevova et al., 2013). It is possible that exposure to a chronic unpredictable, variable stressor regimen would induce a very different oxytocin profile from that provoked by a chronic predictable stressor regimen.

In addition to the variations of oxytocin levels, stressors can profoundly affect oxytocin receptor functioning. In particular, when the stressor comprised a chronic variable stressor, oxytocin mRNA expression in the PVN was reduced (Flak et al., 2011), possibly indicating that such an unpredictable and variable regimen limits the adaptation that occurs in response to chronic predictable stressors. At the same time, however, chronic social defeat in mice, despite the stressor being consistent over days, was associated with increased oxytocin receptor expression in the amygdala and lateral septum (Litvin et al., 2011). It may be that the absence of down-regulated receptor expression may be unique to these brain regions, or might be particularly germane to oxytocin given the social nature of the stressor. Whatever the case, this amounts to speculation as the experiments that have been conducted involved analyses of different stressor treatments and brain regions.

The influence of oxytocin on stressor-elicited responses may be moderated by social factors. Specifically, immobilization among socially isolated Siberian hamsters increased cortisol concentrations, but this did not occur among pair-housed animals. Similarly, the stressor-induced cortisol elevations among isolated hamsters were blunted by oxytocin treatment (Detillion et al., 2004). Isolating steers likewise induced a rapid increase of cortisol concentrations, which was attenuated by oxytocin treatment (Yayou

et al., 2008). Although the majority of studies have been consistent with these findings, there have been reports in which oxytocin treatment did not limit stressor-elicited cortisol concentrations (Lewis and Sherman, 1985; Szeto et al., 2013), or provoked enhanced corticosterone or ACTH responses (Muir and Pfister, 1988; Rault et al., 2013). The source for these between study differences is not immediately apparent given the large variations in methodologies used, including different species and stressors, as well as the different routes of oxytocin administration.

### *3.1.1. Interpersonal and psychosocial stressors in humans*

In view of the prosocial attributes of oxytocin, several reports delved into the specific prosocial behaviors associated with this hormone as well as some of the moderating variables that influence these outcomes. Early studies revealed that a low dose of oxytocin reduced plasma ACTH levels, and with higher doses ACTH and cortisol reductions were still more pronounced (Legros et al., 1984; Legros et al., 1987). It was also observed that the ACTH increase that occurs following administration of the inhibitor of cortisol release, metyrapone, could be attenuated by continuous oxytocin treatment (Chiodera and Coiro, 1987). Predictably, intranasal oxytocin attenuated the rise of salivary cortisol levels associated with an intense physical exercise stressor (Cardoso et al., 2013b; Coiro et al., 1988).

As oxytocin is thought of as a ‘social hormone’ several studies examined the effects of oxytocin on stressors of a psychosocial nature. In this regard, among males, intranasal oxytocin reduced anxiety and lowered skin conductance elicited by a public speaking task, although cortisol and ACTH levels were unchanged (de Oliveira et al., 2011). Likewise, healthy males who received intranasal oxytocin coupled with social

support during the Trier Social Stress Test (TSST; a psychosocial stressor that comprises a mock job interview and a mental arithmetic task in front of judges), exhibited greater calmness and a less pronounced rise of cortisol concentrations compared to individuals who received either oxytocin or social support or neither of these treatments (Heinrichs et al., 2003). In a related study, men with one or two copies of the prosocial G allele of the OXTR SNP rs53576 displayed less of a cortisol rise in response to the TSST when they also received social support, whereas social support did not appreciably influence cortisol levels among individuals homozygous (AA) for the polymorphism (Chen et al., 2011). Thus, it seems that the presence of the G allele of this OXTR SNP allows social support to be effective in buffering against the detrimental effects of stressors.

Most studies that evaluated the effects of intranasal oxytocin administration have involved males owing to potential complications that can arise in females due to uterine contractions and hormonal fluctuations across the menstrual cycle (Choleris et al., 2008). However, as oxytocin may be more biologically relevant to females (Taylor et al., 2010), who also experience depression more frequently (Kessler et al., 1993), examining the effects of exogenous oxytocin in females seems particularly relevant. There have been a few reports that included females in intranasal oxytocin studies in which it was shown that this treatment limited the cortisol rise elicited by the Yale Interpersonal Stressor, a social ostracism paradigm in which participants were excluded from conversations (Linnen et al., 2012). Furthermore, intranasal oxytocin increased positive communication during couple conflict, and limited the cortisol rise relative to that observed among couples that received placebo (Ditzen et al., 2009).

Just as administration of oxytocin influences cortisol stress responses, endogenous oxytocin levels might also moderate the actions of stressors. Specifically, oxytocin levels were higher and cortisol levels normalized more readily following a social stressor among children who were able to see or hear their mothers in comparison to children that had no-contact with their mothers (Seltzer et al., 2010). In contrast to these findings, analyses of the effects of couple interactions revealed that women who had positive contact with their partners before the TSST exhibited lower cortisol and heart rate responses to the stressor, but plasma oxytocin levels were unaffected (Ditzen et al., 2007). Similarly, plasma oxytocin levels among postmenopausal women were not altered by the TSST, although cortisol levels were increased. Curiously, higher plasma oxytocin levels were associated with less positive relationships with primary partners. It might, at first blush, appear paradoxical that elevated oxytocin levels were accompanied by poor positive relationships. However, as discussed earlier, elevated oxytocin levels might signal relationship distress, consequently promoting social support seeking in an effort to attenuate such feelings (Taylor et al., 2006).

Not surprisingly, individual differences related to previous early-life trauma experiences and attachment styles, may influence subsequent stress reactivity. In this regard, although intranasal oxytocin administration ordinarily reduced cortisol levels, this attenuation was less evident in men who previously experienced early parental separation (Meinlschmidt and Heim, 2007). Furthermore, among male and female participants (some of whom had experienced early-life traumatic events), those with autonomous/secure attachment reported low subjective distress in relation to the TSST, moderate cortisol and ACTH levels, and high oxytocin concentrations. In contrast, those

with preoccupied attachments displayed moderate subjective stress and HPA axis responses and low oxytocin concentrations (Pierrehumbert et al., 2012).

The rise of cortisol ordinarily elicited by a stressor occurred more readily in males with low emotional regulatory abilities, and indeed, hardly increased among those with high capabilities in this regard. Importantly, the stressor-provoked cortisol rise could be attenuated by intranasal oxytocin pretreatment in males with low emotional regulatory abilities (Quirin et al., 2011). Furthermore, among individuals with borderline personality disorder, oxytocin administration attenuated the stress-induced cortisol rise and dysphoria (Simeon et al., 2011). These findings essentially point to the possibility that oxytocin may be especially effective in buffering stress responses in particular individuals or in the context of specific psychological conditions. Further to this, oxytocin might be useful in particular disorders, such as autism (Guastella et al., 2010; Hollander et al., 2003; Hollander et al., 2007) and schizophrenia (Averbeck et al., 2011; Feifel et al., 2012; Pedersen et al., 2011), which involve disturbances of social processing or social connectedness. However, among healthy individuals, who presumably do not display social processing deficits, oxytocin administration could actually have adverse consequences as it might make them too sensitive to social cues associated with facial expressions, and it was suggested that this might render individuals inappropriately sensitive within social situations (Cardoso et al., 2014).

It has been suggested that among females, higher levels of oxytocin in times of distress may promote greater 'tend and befriend' characteristics, whereas elevated vasopressin serves a similar function among males (Taylor et al., 2010). In line with this perspective, oxytocin responses to cortisol administration led to very different outcomes

in males and females. Specifically, cortisol treatment reduced oxytocin levels and increased anxiety in males, whereas in females this treatment increased oxytocin levels and reduced anxiety (Tops et al., 2006). Moreover, cortisol administration increased oxytocin levels and decreased immediate free recall of unpleasant words among healthy females, suggesting that oxytocin might diminish negative stressful memories and, in turn, negative affect (Tops et al., 2012).

Beyond oxytocin's role in 'tend and befriend' responses, it may also promote a 'tend and defend' response. Indeed, intranasal oxytocin facilitated parochial altruism, referring to an individual self-sacrificing in order to benefit one's ingroup and protect the ingroup from outgroup threat. In this regard, oxytocin treatment promoted increased cooperation and trust toward ingroup members in a financial decision making game, while simultaneously increasing defensive behaviors toward competing outgroup members (De Dreu et al., 2010). This enhancement of parochial altruism may strengthen social ties or social identity in relation to one's ingroup, which reduces the risk of developing depressive symptomatology (Cruwys et al., 2013). Indeed, as alluded to earlier, the stress buffering effects of oxytocin may be particularly relevant to stress-related disorders, such as depression. Of course, depression is a complex illness that likely encompasses multiple processes, such as alterations of monoaminergic systems, inflammatory processes, and growth factors, and interactions between oxytocin and these systems may contribute to certain aspects or symptoms of depressive disorders. At this juncture, however, it should also be recognized that although variations in these systems have been associated with depression they have also been implicated in several other mental health disorders indicating non-specificity regarding their actions.

#### **4. Serotonin and oxytocin**

Aspects of mood disorders, such as social withdrawal, anxiety and depressive symptoms, have been associated with changes of 5-HT activity and receptor variations, which to some extent can be managed with SSRIs (Maes and Meltzer, 1995; Ressler and Nemeroff, 2000). However, the view has been expressed that the effectiveness of SSRIs are not as reliable as one would like (Pigott et al., 2010), and attention has increasingly turned to still other processes, including neurotrophic (growth) factors, such as BDNF (Duman et al., 1997). In this regard, it is possible that the positive effects of SSRIs stem, from their actions on BDNF, which might require several weeks to take effect (Mahar et al., 2014).

Serotonin contributes to the modulation of social behaviors elicited by social stressors and aggressive behaviors (Cools et al., 2008; Nelson and Trainor, 2007). The finding that 5-HT and oxytocin both have effects on social processes begs the question as to whether and how these two systems interact with one another. In fact, repeated citalopram treatment produced an increase of plasma oxytocin levels in rats and it was suggested that SSRIs might invoke an antidepressant effect through their actions on oxytocin (de Jong et al., 2007 and Uvnäs-Moberg et al., 1999). Likewise, activation of 5-HT<sub>2A/C</sub> receptors (Bagdy, 1996), and treatment with the 5-HT<sub>1A</sub> agonists, ipsapirone and buspirone also increased plasma oxytocin levels (Bagdy and Kalogeras, 1993; Saydoff et al., 1991). Paralleling these findings, the central administration of selective 5-HT agonists increased the expression of oxytocin mRNA in hypothalamic nuclei, including both the supraoptic nucleus (SON) and the PVN (Jørgensen et al., 2003), which is consistent with reports that 5-HT and 5-HT fibers influence brain regions rich in

oxytocin (Emiliano et al., 2007; Ho et al., 2007; Sawchenko et al., 1983). As well, in adult rats, tryptophan and 5-HT agonists (Van de Kar et al., 1995) can regulate oxytocin, and chronic treatment with the SSRI fluoxetine inhibits oxytocin responses to a 5-HT<sub>1A</sub> autoreceptor agonist (8-OH-DPAT) (Li et al., 1993).

Consistent with the animal studies demonstrating that 5-HT influences oxytocin release (Fig. 1), in healthy adult male subjects, administration of fenfluramine, a 5-HT agonist, stimulated the release of both plasma oxytocin and prolactin (Lee et al., 2003). Further, a positive correlation between plasma oxytocin and platelet 5-HT transporter (SERT) levels (assessed by specific [<sup>3</sup>H] Par binding parameters) was observed (Marazziti et al., 2012). Although limited, these findings are consistent with the perspective that some of the antidepressant effects of 5-HT enhancing drugs might involve actions on brain oxytocin functioning. This said, however, in a small sample of depressed patients, changes of plasma oxytocin concentrations were not apparent following SSRI treatment (Keating et al., 2013), making it possible that the positive effects of SSRIs develop as a result of processes independent of oxytocin.

As shown in Fig. 1, the relationship between 5-HT and oxytocin appears to be a reciprocal one. In particular, PET analyses revealed that among healthy males, intranasal oxytocin administration increased 5-HT<sub>1A</sub> receptor binding potential in the dorsal raphe nucleus, amygdala/hippocampal complex, insula, and orbitofrontal cortex (Mottolese et al., 2014). This finding may be relevant in pointing to therapeutic targets of depressive disorders as these illnesses are often characterized by decreased 5-HT<sub>1A</sub> receptor binding potential (Drevets et al., 2007). Beyond the 5-HT<sub>1A</sub> receptor variations, in rodents, oxytocin infusion facilitated 5-HT release within the median raphe nucleus and reduced

anxiety-related behaviors, an outcome that was blocked by infusion of a 5-HT<sub>2A/2C</sub> receptor antagonist (Yoshida et al., 2009).

Oxytocin variations that occur during neonatal development can have marked organizational effects on 5-HT functioning. For example, an oxytocin manipulation on the first postnatal day in male prairie voles resulted in long-term 5-HT alterations that included greater axonal densities within the hypothalamus and amygdala. It has been suggested that interactions that occur between oxytocin and 5-HT could potentially be involved in the regulation of anxiety-related behavior, and may also contribute to other behavioral variations (Eaton et al., 2012). In this regard, neonatal oxytocin has been postulated to be involved in social behavioral disturbances observed in disorders, such as schizophrenia, autism and depression (Carter, 2007 and Marazziti; Catena-DelloSso, 2008). Thus, modifying 5-HT processes during critical developmental periods might influence oxytocin functioning, which could engender later psychological disturbances (Eaton et al., 2012).

The developmental trajectories related to 5-HT and oxytocin could also be affected by genetic factors, thereby influencing depressive disorders. One of the more promising gene candidates for depression has been the serotonin-transporter-linked-promoter-region polymorphism (5-HTTLPR) on the gene SLC6A4 (located on chromosome 17q11.1-q12), which codes for the 5-HT transporter, 5-HTT. This polymorphism involves a deletion wherein the short (s) allele has been linked to lower mRNA and protein expression of 5-HTT compared to that evident in the presence of the long (l) allele (Lesch et al., 1996). As alluded to earlier, the presence of the short allele was not itself aligned with depressive disorders, but the psychopathology was more likely

to develop if individuals with this polymorphism had also encountered early-life or adult stressor experiences (Caspi et al., 2003). Interactions have also been investigated between this polymorphism and variations of the OXTR on depression-related behavioral phenotypes. Although no interaction was observed between 5-HTTLPR and OXTR rs53576 polymorphisms in relation to maternal depression (Bakermans-Kranenburg and van Ijzendoorn, 2008), an interactive effect was reported between OXTR rs2268498 and 5-HTTLPR on negative emotionality (Montag et al., 2011). Individuals homozygous for the L allele of the 5-HTTLPR (the allele associated with lower risk for depressive disorders) and homozygous for the T allele of rs2268498 (an allele which is presumed to be associated with more efficient oxytocin signaling), had the lowest negative emotionality scores. In effect, the combination of these two genetic variants may represent a resilience factor relevant to negative mood outcomes, such as depressive disorders (Montag et al., 2011).

## **5. Norepinephrine and oxytocin**

In addition to 5-HT, norepinephrine (NE) has long been considered to be a possible player regarding the underlying processes associated with depression and anxiety (Ressler and Nemeroff, 2000). However, with the initial enthusiasm regarding the presumed effectiveness of 5-HT-acting antidepressant treatments, the interest in NE was supplanted by a focus on 5-HT. In the past decade, there has again been an interest in multiple neurotransmitters in depressive disorders, leading to the introduction of the serotonin-norepinephrine reuptake inhibitors (SNRIs).

There is good reason to suspect that NE may be involved in the regulation of oxytocin secretion. The release of NE within the SON is facilitated by parturition (Herbison et al., 1997) and lactation (Crowley and Armstrong, 1992), and disruption of hypothalamic NE transmission can inhibit oxytocin release during lactation (Bealer and Crowley, 1998). More than this, however, NE fibers originating from the locus coeruleus, which might be important for vigilance associated with stressors, innervate oxytocin neurons in the PVN and SON (Cunningham and Sawchenko, 1988), and NE facilitates oxytocin release from the magnocellular neurons of the hypothalamus (Fig. 1) (Bealer and Crowley, 2000). Likewise, when oxytocin receptors are blocked, NE release is attenuated in response to a stressor (Onaka et al., 2003), and abolishing NE projections to the SON diminished subsequent oxytocin responses ordinarily elicited by fear stimuli (Zhu and Onaka, 2002). Further, injection of NE in combination with corticosterone increased hippocampal oxytocin receptor mRNA expression and plasma oxytocin levels, just as exposure of mice to a predator scent had such effects. Corticosterone alone did not seem to be responsible for the stressor-provoked increase of oxytocin receptor expression, as the added presence of NE was necessary to promote this outcome. Interestingly, animals that experienced a predator scent and then received hippocampal oxytocin infusion, displayed reduced anxiety upon re-exposure to stressor-related cues 7 days later, raising the possibility that endogenous hippocampal oxytocin may alter the consolidation of traumatic memories, leading to attenuated anxiety responses (Cohen et al., 2010).

The development of depression is thought to be linked to appraisals of stressful experiences and how individuals cope with potential threats (Lazarus, 1996; Matheson and Anisman, 2003). In this regard, social support is thought to be a particularly important method of dealing with stressors. Thus, oxytocin's action in promoting social affiliation and attachment could be a contributing factor in effective coping and indirectly limiting the evolution of mental health disturbances. In rodents, olfactory processes are essential for social affiliation, and as oxytocin is present in the olfactory bulb and enhances NE release at this site, the interaction between these factors might be particularly relevant to social behaviors (Lévy et al., 1993). In male rats, 6-hydroxydopamine induced depletion of NE in the olfactory bulb, which disturbs social recognition responses, was not attenuated by bilateral infusion of oxytocin. In effect, NE release within the neocortex might be necessary in order to promote social affect as well as social learning (Kraemer, 1992), and oxytocin's role in preserving social recognition requires a functional NE olfactory bulb pathway (Dluzen et al., 1998; Nelson and Panksepp, 1998). Indeed, the role of oxytocin and NE in attachment formation is particularly germane to psychological disorders; especially as insecure attachment styles have been associated with elevated depressive symptomatology (Roberts et al., 1996).

## **6. Dopamine and oxytocin**

Dopamine in conjunction with oxytocin has emerged as a potential mediator of mother-infant bonding (Shahrokh et al., 2010), pair bonding (Insel and Shapiro, 1992; Liu and Wang, 2003), social cognition (Ross and Young, 2009), sexual behavior (Baskerville and Douglas, 2008), and drug reward (Kovács et al., 1998). In this regard, for instance, oxytocin receptor density was particularly high in the mesocorticolimbic DA

pathway, including the prefrontal cortex (PFC) and nucleus accumbens (NAc) (Insel and Shapiro, 1992) and that the monogamous behavior of prairie voles was, in part, subserved by oxytocin-provoked activation of DA reward processes (Gingrich et al., 2000; Insel, 2003; Insel and Shapiro, 1992; Melis et al., 2007; Melis et al., 2009; Wang et al., 1999; Young et al., 2001). The DA-oxytocin interactions are not limited to mesolimbic circuits, as injecting oxytocin into the amygdala increased mesolimbic DA functioning (Melis et al., 2009), and conversely, DA influenced oxytocin receptor expression in the central nucleus of the amygdala (cAmyg) through activation of protein kinase A (Bale et al., 2001). As will be discussed shortly, the latter findings are consistent with the view that oxytocin-DA interactions might also contribute to emotional states, such as fear/anxiety that are mediated by amygdala nuclei.

Unlike, D1 receptors, which play an inhibitory role in pair-bonding, activating D2 along with oxytocin receptors in the NAc seems to be necessary for pair-bond formation among male prairie voles (Aragona et al., 2005 and Gingrich et al., 2000). It was likewise observed that among female prairie voles, activation of oxytocin receptors in the NAc and PFC was necessary for pair bonding (Young et al., 2001). Indeed, oxytocin can interact with D2 receptors in the NAc to induce pair-bonding in the absence of mating, whereas blockade of the D1 receptors does not affect bond formation (Liu and Wang, 2003). Given that pair-bonding and sexual behaviors, as well as self-administration of addictive drugs, involve activation of the mesolimbic DA pathway, it was suggested that they may share underlying biological processes (Insel, 2003; MacLean, 1990; Nelson and Panksepp, 1998). Parenthetically, the demonstration that D2 and oxytocin receptor heteromers are present in the NAc raised the interesting possibility that pharmaceuticals

designed to target these cells may be influential in modifying social deficits associated with behavioral disorders (Romero-Fernandez et al., 2013).

It appears that strong reciprocal interactions exist between oxytocin and DA that might contribute to social connectedness (Fig. 1). Oxytocin might promote affiliative behaviors, but in the absence of DA and activation of rewards pathways, these social approach behaviors may not be perceived as rewarding and thus might not be adopted (Depue and Morrone-Strupinsky, 2005; Taylor, 2006). In this regard, depressive disorders are often characterized by marked social withdrawal, sexual dysfunction and anhedonia that could potentially involve disruptions of DA and oxytocin networks (Baskerville and Douglas, 2010).

There is also reason to suppose that beyond its other functions, oxytocin might serve to diminish fear and/or anxiety, possibly through actions on DA neural circuits. Dopaminergic fibers originating from the ventral tegmental area project to the amygdala, and might influence emotional states through the involvement of oxytocin (Veinante and Freund-Mercier, 1997). In this regard, infusion of oxytocin into the cAmyg selectively attenuated anxiety responses, an action that could be diminished by pretreatment with a D1 antagonist. Paralleling these findings, intranasal oxytocin administration to humans attenuated amygdala activation elicited by anxiety-provoking social stimuli (Kirsch et al., 2005; Labuschagne et al., 2010; Petrovic et al., 2008). As oxytocin, like DA, is released in response to stressors, they may act cooperatively to attenuate anxiety and stress responses (Bale et al., 2001).

Studies examining certain oxytocin and DA genetic variants have provided insights into how these two systems might cooperate to affect responses to social stimuli.

A functional oxytocin SNP identified on the CD38 gene rs3796863, which influences central oxytocin release in mice and in humans, was associated with reduced parental touch and lower plasma oxytocin levels (Feldman et al., 2012; Jin et al., 2007). Analyses that included imaging and gene techniques, revealed interactions between the CD38 gene and a DA genetic variant for the degrading enzyme catechol-O-methyltransferase (COMT; val158met rs4860) while participants were presented with various social stimuli. Among A carriers of the CD38 gene (those who presumably had elevated oxytocin) attenuated amygdala responses to social stimuli occurred if they were also homozygous for the met allele of the COMT rs4860 (the genotype associated with more DA availability). Thus, the influence of the CD38 genetic variant on amygdala activation may be dependent on the presence of the COMT polymorphism (Sauer et al., 2013). Other Oxytocin  $\times$  DA gene interactions have been investigated in relation to psychosocial factors that may confer negative mood states. A longitudinal study revealed that girls homozygous for the G allele of the OXTR SNP rs53576 (i.e., who might be more socially sensitive) showed increasing loneliness over time, but their mood was stable if they also carried at least one A1 allele for the DA gene DRD2, a variant associated with reduced D2 receptor binding in the ventral striatum (van Roekel et al., 2013).

It seems that oxytocin and DA systems might also interact to influence stressor reactivity. Among female carriers of the C allele for OXT rs4813625 (a SNP whose immediate behavioral effects are not well delineated), elevated DA responses (reflected by reductions in receptor availability determined through PET analyses) were elicited by a stressor challenge in comparison to those who were homozygous for the G allele, an effect not observed among males. Additionally, the C allele carriers reported lower

emotional well-being, higher attachment anxiety and trait anxiety, and these measures were accompanied by elevated striatal DA responses to the stressor (Love et al., 2012). The elevated DA stress response was particularly pronounced in the right ventromedial caudate, which receives inputs from the amygdala, hippocampus, anterior cingulate and orbitofrontal cortex and is thought to integrate emotional, cognitive and motivational information (Haber and Knutson, 2010; Mogenson and Yang, 1991). It was suggested that among C-allele carriers of this oxytocin gene, the DA responses to stressors was particularly elevated, possibly influencing the salience of a stressor (Love et al., 2012).

In line with such a perspective, it has been proposed that the oxytocinergic system also serves to increase the salience of social cues so that positive or negative events may have more profound consequences (Averbeck, 2010; Bartz et al., 2011; Burkett and Young, 2012). In her review, Love (2014) outlined how DA is influential in mediating salience attribution and oxytocin is capable of affecting this process. It was suggested that oxytocin could influence attributions and motivations toward social stimuli so as to affect whether they are appraised positively or negatively. For example, administration of oxytocin increases the effort animals are willing to exert to gain access to social stimuli, even at the cost of not attaining other rewards (e.g., food or drugs). Thus, it is possible that through its interactions with the DA system, oxytocin may enhance both motivational salience toward social cues and influence the motivational value assigned to certain stimuli (Love, 2014). From this perspective, low levels of DA activity might favor anhedonia and depression, and this relationship would be strengthened if the anhedonia were particularly salient, as in the case when oxytocin levels were high. By the same token, it could be argued that in the presence of low oxytocin, the salience of the

rewarding effects associated with high DA might not be manifested, and this too could favor the development of depressive symptoms. In effect, the dance between oxytocin and DA must be well coordinated.

## **7. Growth factors and oxytocin**

Growth factors, such as BDNF and fibroblast growth factor (FGF-2) as well as vascular endothelial growth factor (VEGF) and neurotrophin-3 and-4 have been receiving increasing attention in relation to psychiatric disorders. Although these growth factors have most often been related to depression, they are also associated with schizophrenia, bipolar disorder (Gaughran et al., 2006) and neurodegenerative disorders (Siegel and Chauhan, 2000), reflecting non-specificity regarding their actions. In this respect, variation of these growth factors may set the stage for the emergence of pathology, but the nature of the pathology that emerges is determined by the specific neuronal and neurochemical variations that are promoted.

There have been several reports indicating that depression in humans was accompanied by diminished size of the hippocampus, likely stemming from reductions of BDNF and FGF-2 (Evans et al., 2004; Kempermann and Kronenberg, 2003). Presumably, disturbances of the structure and function of the hippocampus could contribute to disturbed cognition, anhedonia and depressed mood (McEwen, 1999), and treatments that increased growth factors had the effect of ameliorating depression (Chen et al., 2001). It was suggested that increased neurogenesis, plasticity, and neural survival through the activation of a mitogen-activated protein (MAP) kinase cascade and subsequent enhanced phosphorylation of cAMP response element-binding protein (CREB) in the hippocampus served as mediators of antidepressant efficacy (D'Sa and Duman, 2002). As oxytocin

induces phosphorylation of CREB through activation of MAP kinase signaling, and hence hippocampal neural plasticity (Matsushita et al., 2012; Tomizawa et al., 2003), oxytocin could facilitate antidepressant effects through activation of this pathway (Matsushita et al., 2012). Interestingly, administration of sildenafil citrate (a widely used medication to treat erectile dysfunction) to mice led to activation of MAP kinase signaling and phosphorylation of CREB, which was associated with an antidepressant-like effect in a forced swim test. This anti-depressant action was inhibited by blocking the oxytocin receptor and was absent in receptor knockout mice (Matsushita et al., 2012). Further, in rats, oxytocin but not arginine vasopressin, stimulated neuronal growth and attenuated glucocorticoid- or stress-induced suppression of hippocampal neurogenesis (Leuner et al., 2012). Thus, it was postulated that oxytocin may encourage antidepressant-like actions through the activation of a MAP kinase cascade and the induction of BDNF expression (Matsuzaki et al., 2012). To date, however, human research concerning oxytocin's involvement with growth factors in depressive disorders have been largely unexamined, although oxytocin treatment was reported to increase BDNF and nerve growth factor mRNA expression in human glioma cell lines (Bakos et al., 2013). As such, it is still premature to conclude that an interaction between oxytocin and growth factors contribute to the evolution or maintenance of depressive disorders.

## **8. Inflammation and oxytocin**

A role for inflammatory factors, particularly cytokines, such as interleukin (IL)-1 $\beta$ , IL-6, and tumor necrosis factor (TNF)- $\alpha$ , in depressive disorders has received increasing support. Among other things, circulating pro-inflammatory cytokine levels were elevated in depressed patients (Maes, 1995), and administration of pro-

inflammatory cytokines (e.g., interferon- $\alpha$  administered in the treatment for hepatitis C and for some types of cancer) induced symptoms of depression, an effect that could be attenuated by antidepressant medications (Capuron and Miller, 2004; Harrison et al., 2009). There have been varied suggestions as to how these cytokine effects on depression occur. One view is that cytokines reduce 5-HT activity by activating indoleamine-2,3-dioxygenase (IDO), thereby reducing the availability of the 5-HT precursor tryptophan, or because IDO metabolites have neurotoxic effects that result in the loss of 5-HT neurons (Dantzer et al., 2011; Maes et al., 2011). It is also possible that cytokines directly affect neurotransmitters or growth factors, which then influence depression (Audet and Anisman, 2013). Importantly, pro-inflammatory cytokines are not simply markers of depression, and seem to be centrally involved in the development of this illness (Dantzer et al., 2008; Maes, 2011; Miller et al., 2009).

Initial formulations of cytokine involvement in depression focused on the perspective that pro-inflammatory factors, despite having limited access under normal conditions, found their way into the CNS, thus eliciting depression (Maier and Watkins, 1998). While not dismissing this possibility, it was considered that these cytokines were also released from microglia (Nadeau and Rivest, 1999) and served in a macrophage-like capacity in brain to eliminate debris. As it turns out, cytokine levels are elevated in brain in response to any of several brain insults, including concussion and stroke and can also be elicited by peripheral administration of inflammatory agents and by stressful events (Kamm et al., 2006; Ledebuer et al., 2002; Miyahara et al., 2000; Nguyen et al., 1998, Sriram et al., 2006; Zhu et al., 2006). It was assumed that at low levels these cytokines

might act in a neuroprotective capacity, but at higher levels they might be neurodestructive and thus encourage cognitive disturbances, including depressive illness.

There have been indications that oxytocin may interact with inflammatory factors to promote depressive behaviors. Specifically, pro-inflammatory cytokines ordinarily promote the release of CRH and hence ACTH and corticosterone, and elicit monoamine variations in limbic brain regions (Anisman and Merali, 1999), thereby promoting features of depressive disorders. As already indicated, oxytocin can attenuate HPA axis responses to stressors, and it seems that it can directly or indirectly attenuate pro-inflammatory cytokine responses, as shown in Fig. 1 (Clodi et al., 2008; Oliveira-Pelegrin et al., 2013) and could thereby limit depressive symptoms. Conversely, plasma oxytocin levels were markedly increased in response to endotoxin administration (Kasting, 1986) and intravenous IL-1 $\beta$  injections (Naito et al., 1991). Further, intra-arterial administration of IL-1 $\beta$  elicited a large increase of c-fos expression in magnocellular neurosecretory oxytocin cells in the PVN and SON (Buller et al., 1998), whereas ICV infusion of IL-1 $\beta$  only increased release of oxytocin in the SON (Landgraf et al., 1995). Unlike the effects of IL-1 $\beta$ , it appeared that IL-6 did not enhance plasma oxytocin levels (Naito et al., 1991), indicating a degree of specificity regarding the link between cytokine treatment and oxytocin changes in brain.

Pregnancy and parturition are periods during which complex interactions occur between oxytocin and inflammatory processes (Brunton and Russell, 2008). Ordinarily, oxytocin is released in high amounts during parturition and breastfeeding and it was suggested that this may protect females against adverse consequences of inflammatory factors (Kimura, 1995). In fact, IL-1 $\beta$  administration enhanced plasma oxytocin among

female rats, but this effect was much less notable when rats were in the late stages of pregnancy. Furthermore, the opioid antagonist naloxone induced an oxytocin secretory response to IL-1 $\beta$  that was much greater in pregnant than in virgin rats. The opioid inhibition of the oxytocin secretion elicited by systemic IL-1 $\beta$  that typically occurs during late pregnancy, might reflect an effort to conserve oxytocin stores necessary for parturition, and could also serve to prevent pre-term labor induced by oxytocin release (Brunton et al., 2006).

As shown in Fig. 1, pro-inflammatory cytokines can enhance oxytocin, and it seems that oxytocin can attenuate cytokine responses, which could potentially protect against inflammatory-related conditions. In view of the relation between oxytocin and inflammatory processes, as well as between oxytocin and social behaviors, it is possible that social interactions and social support may be important components of the anti-inflammatory properties of oxytocin. In this regard, stressor exposure impaired wound healing, a process that requires high levels of inflammatory factors, however, this outcome was evident in isolated, but not socially housed Siberian hamsters. Predictably, oxytocin treatment facilitated wound healing among isolated hamsters, and administration of an oxytocin antagonist delayed healing among socially housed animals (Detillion et al., 2004). Consistent with the view that oxytocin together with social interactions or social support has anti-inflammatory implications, central oxytocin treatment reduced frontal cortex IL-1 $\beta$  expression and attenuated the depressive-like behavior in mice housed in isolation following induced nerve injury. However, in mice that were housed in pairs, depressive-like behavior as well as increased IL-1 $\beta$  expression in the frontal cortex only developed following treatment with an oxytocin receptor

antagonist (Norman et al., 2010). It also appeared that in humans assessed during a couple social support interaction test, blister wound healing occurred more readily among individuals who were in the upper oxytocin quartile compared to those in the lower quartile (Gouin et al., 2010). Provisionally, these data suggest that the well-established relationship between social isolation and poor health and well-being might involve oxytocin functioning.

In line with the anti-inflammatory effects of oxytocin, subcutaneous injection of this peptide abolished the sepsis-induced increase of TNF- $\alpha$  expression in female rats (İşeri et al., 2005). Moreover, upon applying the bacterial endotoxin lipopolysaccharide (LPS) to peritoneal macrophage cultures that had been obtained in the early-phase of sepsis, TNF- $\alpha$ , IL-1 $\beta$  and nitrite (an indicator of oxidative stress) were elevated, but with the exception of IL-10 these outcomes were attenuated by oxytocin (Oliveira-Pelegrin et al., 2013). Likewise, oxytocin treatment among healthy male humans limited the LPS-induced changes that were ordinarily observed, including increases of IL-6, TNF- $\alpha$ , IL-4, IL-1ra, macrophage inflammatory protein-1 $\alpha$  and-1 $\beta$  as well as VEGF (Clodi et al., 2008).

Parenthetically, the anti-inflammatory effects of oxytocin are not limited to brain-related variations or those that involve cytokine functioning that affect the brain. For instance, oxytocin functions to maintain cardiovascular homeostasis (Gutkowska et al., 1997; Gutkowska and Jankowski, 2008). Specifically, following a myocardial infarction, oxytocin infusion in male rats attenuated the TNF- $\alpha$  and IL-6 mRNA expression otherwise evident in heart tissue and enhanced the promotion of transforming growth factor- $\beta$ , a cytokine involved in healing processes and tissue fibrosis (Jankowski et al.,

2010). Oxytocin may also slow the progression of atherosclerosis, as this peptide attenuated IL-6 secretion from LPS-stimulated THP-1 macrophages, a commonly used cell line to assess monocyte and macrophage activity related to cardiovascular functioning (Szeto et al., 2008). These findings are in keeping with reports that chronic oxytocin treatment resulted in less atherosclerosis in the thoracic aorta relative to that evident in vehicle-treated animals (Nation et al., 2010; Szeto et al., 2013). Heart disease and depressive disorders are comorbid conditions, and cytokines may be responsible for this concordance (Frasure-Smith et al., 2009). Given the link between cytokines and oxytocin, it is possible that this hormone might act in a moderating capacity in the relation between cytokines and both depressive illness and heart disease.

Together, these findings suggest that oxytocin is released in response to an acute immune challenge, and might be particularly responsive to treatments that increase endogenous IL-1 $\beta$  and TNF- $\alpha$ . Furthermore, oxytocin can aid in wound healing, attenuate chronic pain associated with nerve injury, reduce pro-inflammatory cytokine expression in response to an acute endotoxin challenge and whole body inflammatory states. In effect, oxytocin is activated in response to immunogenic challenges, and the elevated oxytocin then serves to limit further cytokine responses. Considering the strong actions of pro-inflammatory cytokines in the development of depression, coupled with the inhibitory actions of oxytocin on cytokine functioning, low levels of oxytocin could reflect one path through which this hormone might be involved in the development of depression. As indicated earlier, postpartum depression is characterized by reduced plasma oxytocin levels (Skrundz et al., 2011), and the development of postpartum depression may be linked to the increased pro-inflammatory and reduced anti-

inflammatory state that occurs during labor and delivery (Corwin et al., 2008). Thus, women with lower oxytocin levels might not have the proper inhibitory signals in place to re-instate the pro-inflammatory/anti-inflammatory balance, which might culminate in a postpartum depressive state.

## **9. Treatments based on oxytocin administration**

The first indication of oxytocin being effective as an adjunct in treating depression was based on a single case report which revealed improved mood when oxytocin was administered for two weeks in conjunction with escitalopram treatment (Scantamburlo et al., 2011). Findings such as these were likely not a reflection of the antidepressant further increasing oxytocin availability. Specifically, when patients with major depression received SSRI treatment for 12 weeks, plasma oxytocin levels were unchanged despite a 50% reduction in depressive symptoms (Keating et al., 2013). Similarly, following a range of treatments, including SSRIs, tricyclic antidepressants, or electroconvulsive therapy (ECT), serum oxytocin levels were unaffected among patients even though depressive scores were reduced (Ozsoy et al., 2009). In fact, when ECT enhanced plasma oxytocin levels among patients with major depression this was not necessarily accompanied by improved clinical outcome (Devanand et al., 1998). Yet, among depressed patients, the clinical response to ECT was positively correlated to plasma neurophysin, a carrier protein for oxytocin (Scott et al., 1986 and Scott et al., 1989). Beyond endogenous oxytocin levels, antidepressant treatment responses have also been examined in relation to various OXTR SNPs, and neither OXTR rs2254928 nor rs53576 were associated with treatment response, resistance to treatment, or remission following successful treatment (Mendlewicz et al., 2012).

Despite the fact that oxytocin manipulations do not influence depression, and antidepressants hardly affect the hormone's level, this does not imply that oxytocin is not involved in the development of depression or that oxytocin is ineffective prophylactically. In this regard, treatments effective in diminishing symptoms of an existing disorder might be ineffective in preventing the development of the illness, and conversely, those effective in an intervention capacity, might not be useful in the treatment of an existing illness. In the case of elevated oxytocin levels, enhanced trust and social support seeking could promote appropriate coping methods to preclude the development of depressive illness in response to stressors. Indeed, oxytocin did facilitate the individual's willingness to share emotions related to a painful memory (Lane et al., 2013). To be sure, oxytocin in itself might not diminish symptoms in clinically depressed individuals, but could conceivably serve as an adjunct to antidepressant treatments, or in treating particular aspects of depressive disorders (Baskerville and Douglas, 2010).

## **10. Concluding comments**

Although there is sufficient evidence linking oxytocin with the development of depression in animal-based studies, there are data that do not readily align with this perspective, particularly studies that involved human participants. Specifically, studies examining oxytocin levels in relation to depression in humans have yielded results that are inconsistent across studies that are likely attributable to methodological differences, measurement-related issues, and several contextual factors. Furthermore, given the diversity of symptoms and presumed mechanisms that are associated with depressive disorders, there is no a priori reason to believe that oxytocin, any more than any other hormone or neurotransmitter, would be associated with all instances or subtypes of

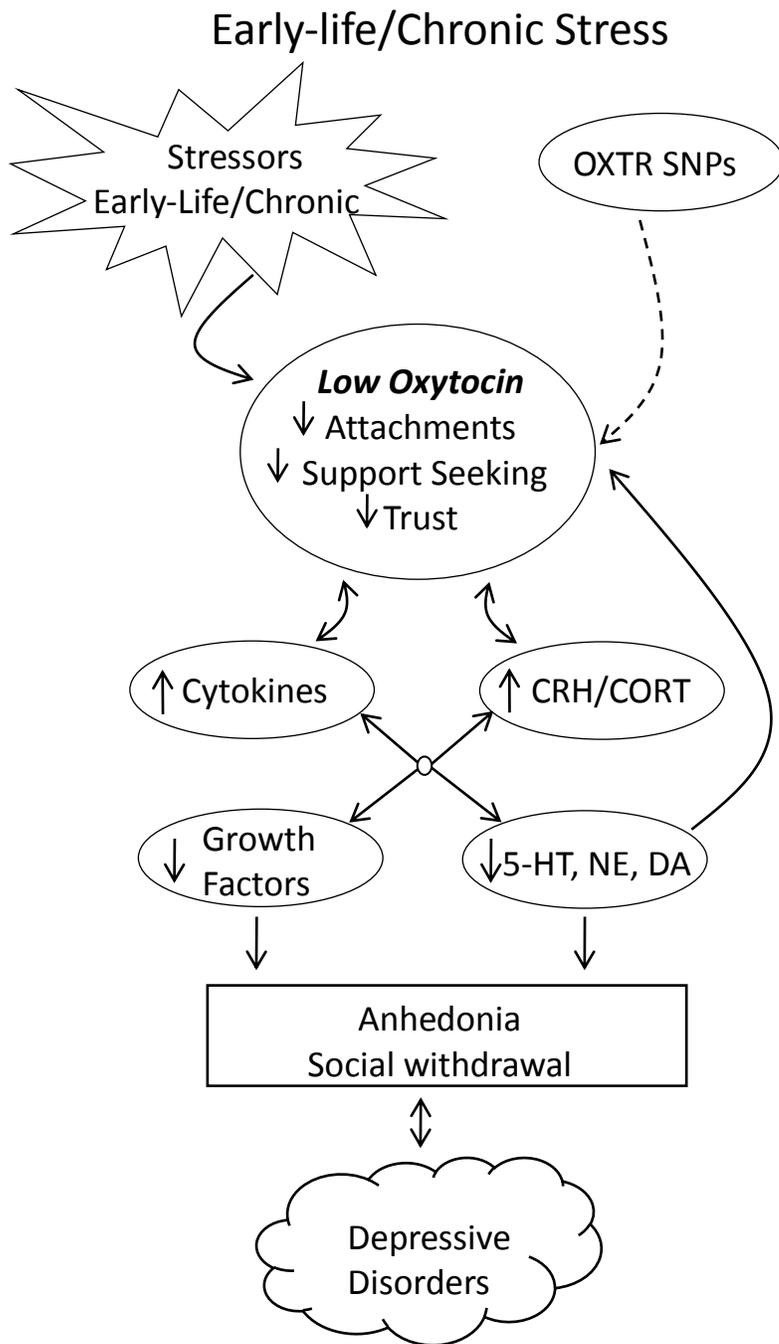
depressive illness. Subtypes of depression (e.g., typical versus atypical depression; dysthymia versus acute depression) that present with different features, may be differentially responsive to pharmacotherapy, and might involve different biological substrates (e.g., Ravindran et al., 1995). In this regard, oxytocin may be particularly pertinent to atypical depression, which is marked by social rejection sensitivity (Tops et al., 2008). Moreover, lower plasma oxytocin concentrations were associated with increased risk for later postpartum depression, and women experiencing this form of depression displayed low levels of oxytocin (Skrundz et al., 2011).

With these caveats in mind, we have offered the view that oxytocin could directly or indirectly favor the development of depression, but its involvement in this regard might be greatest when the antecedent stressor is one that involves negative social interactions (e.g., in response to social rejection, or among individuals with high rejection sensitivity), or where feelings of social isolation are prominent features of the illness. In this regard, diminished oxytocin functioning might lead to a reduction of trust, attachment and seeking social support, any of which could encourage social withdrawal, a common feature of depressive disorders. Alternatively, as oxytocin enhances prosocial behaviors and social support seeking, its administration may well encourage those behaviors that act against the development and/or maintenance of depression, and as such, might serve as an adjunctive treatment to diminish depressive illnesses in a subset of individuals.

Stressful events, particularly those experienced in early-life or encountered chronically may engender several neurochemical changes that could be aligned with the later development of depressive symptoms. In this regard, variations of monoamine or

CRH functioning in mesocorticolimbic regions that occur as a direct result of stressors or indirectly through activation of pro-inflammatory cytokines, could encourage depressive symptoms. Thus, it might be profitable to consider the contribution of oxytocin in the context of these other neurotransmitter changes that are provoked by stressors. As can be seen in Fig. 1, oxytocin ordinarily has inhibitory effects on HPA and cytokine activity, and the increase of oxytocin associated with acute stressors would limit their prolonged actions. In effect, cortisol and pro-inflammatory cytokines would still be released so that their positive effects would be realized, but the risk for the damaging effects of excessively high levels would be mitigated. At the same time, the oxytocin itself might act to increase social behaviors that could be used to act against the adverse effects of stressors. Indeed, among those with the highest levels of oxytocin, or when oxytocin is exogenously administered, the positive effects of social support are most prominent (Heinrichs et al., 2003). However, as shown in Fig. 2, under conditions where oxytocin is reduced, as observed among individuals who had encountered early-life stressors, the relaxation of the inhibitory effects of this peptide could favor elevated cortisol and pro-inflammatory cytokine functioning. This, in turn, might result in excessive utilization of biogenic amines, culminating in a decline of their levels, and increased propensity toward depression. In this regard, a core feature of depression, anhedonia, may be subserved, in part, by low oxytocin levels in combination with reduced dopamine processes so that the perceived rewarding value gained by social support is diminished. As well, the stressor and cytokine variations would lend themselves to reduced levels of neurotrophic factors that also would permit the development of depression. In effect, the confluence of several

neurobiological changes exerted by stressors might provide the (im)perfect ingredients that foster depressive disorders.



*Figure 2.*

Hypothesized relations between early-life stressful experiences and the evolution of depression. Early-life stressful experiences (and perhaps chronic stressors) may lead to

inadequate or low oxytocin functioning that might favor poor psychosocial processes, such as reduced attachments, social support seeking and trust. The diminished inhibitory actions of oxytocin on stressor elicited pro-inflammatory cytokine and HPA axis functioning may result in enhanced cytokine and HPA axis responses. This, in turn might lead to excessive utilization of monoamines, resulting in a decline in their levels. Reduced monoamines coupled with low oxytocin functioning may contribute to the development of depressive symptoms, such as anhedonia and social withdrawal. These alterations in conjunction with reduced neurotrophic factor expression, causing impaired neuroplasticity, might culminate in the development of depressive disorders.

Finally, there are considerable data indicating that early experiences may result in developmental trajectories that either encourage depression or promote the sensitization of neurochemical processes so that later challenges are more apt to promote increased vulnerability to depressive disorders. In line with these perspectives, it has been suggested that negative early-life experiences, particularly if they involve neglect or abuse, may undermine trust and social support coping. If oxytocin makes social conditions more salient, irrespective of whether these experiences are positive or negative, it would be expected that in the presence of effective oxytocin functioning, positive early-life events might favor the development of effective social coping so that individuals would become resilient in relation to stressors. However, the knife cuts both ways, and a functioning oxytocin system might also allow for negative experiences to have the adverse consequences that they so often do. It follows that if oxytocin functioning is impaired, for whatever reason (e.g., in the presence of a polymorphisms that could potentially limit oxytocin receptor sensitivity), the benefits that could be

accrued from positive events might not be realized, but by the same token, the negative consequences related to stressful experiences might likewise not occur (McQuaid et al., 2013). This perspective, like that expressed by Belsky (e.g., Belsky et al., 2007; Belsky et al., 2009; Belsky and Pluess, 2009) in relation to the impact of the 5-HTTLPR polymorphism, suggests that oxytocin might, in a sense, influence plasticity to environmental circumstances, thereby ‘for better or for worse’ affecting later behavioral and psychological outcomes.

Moving forward, to establish oxytocin's role in depressive disorders, several considerations might be profitable. For instance taking an endophenotypic approach linking symptoms of depression to oxytocin variations may facilitate individualized treatments. In this regard, evidence demonstrating that specific oxytocin genotypes are more plastic, might inform which individuals may be most responsive to cognitive behavioral therapies versus those that are more responsive to drug treatments. Individualized treatments focused on specific symptoms and biomarkers, may also be fruitful if considered together with culture and gender, particularly as oxytocin or its receptors might differ with these variables. Thus, alone or in combination with other systems, oxytocin may have effects on social processes that influence psychopathology. Finally, given that early-life and even prenatal stressors are known to influence the developmental trajectory of various neuroendocrine, growth factor and behavioral/cognitive processes, it may be productive to consider such moderating factors in experimental analyses of oxytocin's influence on depressive disorders.

## Chapter 2-Study 1

### The Moderating Influence of OXTR on Social Interactions, Coping, and Depressive Symptoms

Mcinnis, O. A., McQuaid, R. J., Matheson, K., & Anisman, H. (2015). The moderating role of an oxytocin receptor gene polymorphism in the relation between unsupportive social interactions and coping profiles: Implications for depression. *Frontiers in Psychology*, 6, 1133.

As described in Chapter 1, oxytocin promotes prosocial behaviors which might serve to buffer against the negative impact of stressors, and in turn, limit the development of depressive disorders. Alternatively, oxytocin might enhance sensitivity to social cues, so that for better or for worse, this hormone can promote both positive and negative mood outcomes depending on environmental context. The interaction between oxytocin and environment has been examined through a SNP on the OXTR rs53576. This SNP has been associated with decreased prosocial characteristics, such that compared to those with the SNP absent (i.e., those with the G allele), A carriers tend to be less trusting (Kosfeld et al., 2005), empathetic (Rodrigues et al., 2009) and optimistic (Saphire-Bernstein et al., 2011). However, we also observed that in the context of a negative early life environment, in the form of childhood maltreatment, individuals with the G allele, reported greater depressive symptoms than those with the AA genotype, possibly suggesting that oxytocin promotes social sensitivity (McQuaid et al., 2013, in Appendix C). Considering these two views, in Study 1 we explored whether the OXTR rs53576 was associated with perceptions of social support and unsupportive relations and whether these associations were tied to coping methods and depressive symptoms. It was of interest to explore whether the greater social sensitivity of those with the GG genotype could be accompanied by emotion-focused coping in response to unsupportive social

interactions, and more effective coping skills in the presence of social support. However, it was also recognized that, A carriers, who tend to have greater negative mood (and may be less sensitive to social interactions), might be more likely to adopt unfavorable coping methods that involve more emotion- rather than problem-focused coping styles.

## Abstract

Oxytocin is a hormone that is thought to influence prosocial behaviors and may be important in modulating responses to both positive and negative social interactions. Indeed, a single nucleotide polymorphism (SNP) of the oxytocin receptor gene (OXTR) has been associated with decreased trust, empathy, optimism and social support seeking, which are important components of coping with stressors. In the current study, conducted among undergraduate students ( $N=225$ ), it was shown that parental and peer social support was related to fewer depressive symptoms through elevated problem-focused coping and lower emotion-focused coping, and these effects were independent of the OXTR polymorphism. Unsupportive social interactions from parents were associated with more severe depressive symptoms through the greater use of emotion-focused coping, and this relation was moderated by the OXTR genotype. Specifically, individuals who carried the polymorphism on one or both of their alleles demonstrated increased emotion-focused coping following unsupportive responses compared to those without the polymorphism. Likewise, lower problem-focused coping mediated the relation between parental and peer unsupportive responses to depressive symptoms, but this mediated relation was only evident among carriers of the polymorphism. These findings suggest that carrying this OXTR polymorphism might favor disadvantageous coping styles in the face of negative social interactions, which in turn are linked to poor mood. Regardless of genotype, parental and peer social support are fundamental in determining stress-related coping and well-being.

## **Introduction**

Supportive relationships and social connectedness are important predictors of health and well-being that serve as a buffer against several negative consequences of stressors (Cohen and Wills, 1985; Thoits, 2011). In contrast, a lack of social support has been associated with increased risk of chronic health conditions, such as heart disease and diabetes (House et al., 1988; Holt-Lunstad et al., 2010). Thus, enhancing social connectedness and social identity may attenuate depressive symptomatology (Cruwys et al., 2014, 2015). The experience of unsupportive social relationships, comprise negative or ineffective social interactions, when help or advice is sought during a challenging or stressful time (Ingram, et al., 1999; Ingram, et al., 2001). These unsupportive responses from others include the minimization of problems, blaming the individual, distancing themselves from an individual and their problems, and bungling attempts to provide support. Importantly, the experience of unsupportive social interactions predicts depressive symptoms above and beyond the contribution of social support (Ingram et al., 1999; Song and Ingram, 2002). Despite the established beneficial effects of social support and the profound impact of unsupportive social interactions on well-being, the biological mechanisms underlying their influence remain largely unknown and under-investigated.

Oxytocin is a hormone that may contribute to a constellation of social behaviors, ranging from trust (Kosfeld et al., 2005) and attachment (Buchheim et al., 2009) to positive communication (Ditzen et al., 2009) and intergroup cooperation (De Dreu et al., 2010). The involvement of oxytocin in these prosocial behaviors in humans has been demonstrated following its administration through a nasal spray (Bakermans-Kranenburg

and van Ijzendoorn, 2013). As well, support for the involvement of oxytocin in mediating social behavior has come from genetic studies. Specifically, variations in the gene coding for the oxytocin receptor OXTR, in which a single nucleotide polymorphism (SNP) rs53576, which involves a guanine (G) to adenine (A) substitution, has been associated with diminished prosocial behaviors (Kumsta and Heinrichs, 2013). In this regard, compared to individuals who were homozygous for the G allele (i.e., the SNP was not present), A carriers tended to be less empathetic (Rodrigues et al., 2009), displayed lower parental sensitivity (Bakermans-Kranenburg and van Ijzendoorn, 2008), and lower trust-related behaviors (Krueger et al., 2012). This SNP has also been associated with lower positive affect (Lucht et al., 2009), and self-esteem as well as greater depressive symptoms (Saphire-Bernstein et al., 2011). In effect, individuals who carry this SNP on one or both alleles (AG or AA genotype) appear to be less socially inclined and potentially at a greater risk for mental health disturbances.

Although coping strategies are not intrinsically negative or positive, depression is frequently associated with the endorsement of lower levels of problem-focused coping and higher levels of emotion-focused coping (Matheson and Anisman, 2003). For instance, depressive disorders have been tied to greater levels of rumination (Aldao et al., 2010) and emotional containment (Ravindran et al., 2002), as well as decreased social support seeking (Matheson and Anisman, 2003) and reduced use of cognitive restructuring (Ravindran et al., 2002). Given that A carriers are less apt to use social support as a means of coping, and benefit less from this coping method, it is possible that the presence of the OXTR SNP might favor the adoption of a relatively narrow range of effective coping strategies (i.e., those that do not rely on social support resources). As a

result, the A allele might be associated with greater vulnerability to the negative impacts of stressors relative to those with the G allele.

There have been several reports, however, that do not comfortably align with the perspective that the A allele of the OXTR rs53576 gene is associated with vulnerability to disturbed social and emotional functioning. Indeed, the G allele of the OXTR was associated with greater social sensitivity (Bradley et al., 2011; Hostinar et al., 2014; McQuaid et al., 2013), which in the context of negative early life experiences, may be accompanied by greater emotional dysregulation (Bradley et al., 2011) and elevated depressive symptoms among adults (McQuaid et al., 2013). As well, maltreated adolescents who were homozygous for the G allele were more likely to perceive lower social support and reported greater internalizing of symptoms compared to maltreated A allele carriers (Hostinar et al., 2014). The social sensitivity perspective is in line with the suggestion that certain genetic variants may promote behavioral and emotional plasticity, so that environmental and experiential factors, irrespective of whether they are positive or negative, have greater effects on later outcomes (Belsky et al., 2009; Belsky and Pluess, 2009). In essence, the presence of the GG alleles might be accompanied by elevated sensitivity to social cues, irrespective of whether these involved a positive and nurturing early-life environment or one that was more negative, and as a result influence social inclinations and mood in adulthood (Bradley et al., 2011; Hostinar et al., 2014; McQuaid et al., 2013).

The elevated sensitivity to environmental factors and the heightened neuroplasticity associated with increased oxytocin functioning (Lin, Huang and Hsu, 2012) and with the G allele, could promote the adoption or development of social coping

methods (McQuaid et al., 2014a). Indeed, within a stable or warm family environment, G carriers reported greater positive affect and ‘resilient’ coping, an association that was not observed among those with the AA genotype (Bradley et al., 2013). Conversely, those with the AA genotype sought less emotional social support during distress compared to G carriers (Kim et al., 2010), and also appeared to be less able to benefit from social support (Chen et al., 2011). Among adolescents who carried an A allele, but not among GG homozygotes, experiences of maternal depression predicted lower social functioning, which, in turn, was associated with elevated depressive symptoms (Thompson et al., 2014).

Although unsupportive relationships can have profound effects on mood states, it is uncertain whether the effects of such relationships vary as a function of oxytocin levels or the presence of the OXTR polymorphism. As well, coping methods (e.g., emotion-, avoidant- and problem-focused coping) which are also important predictors of well-being have not been investigated in association with the genetic variants of the OXTR. In the present investigation we assessed experiences of social support and unsupport from both parents and peers in relation to depressive symptoms and whether these relations were mediated by coping styles. It was of particular interest to determine whether the OXTR rs53576 genotype moderated these mediated relationships. It is possible that the greater social sensitivity of those with the GG genotype would be accompanied by emotion-focused coping in response to unsupportive social interactions, and more effective coping skills in the presence of social support. In contrast, A carriers, who tend to have a more negative affect (and may be less sensitive to social interactions), might be more likely to adopt disadvantageous coping methods that involve emotion- more than problem-

focused coping styles, irrespective of perceiving support or experiencing unsupportive interactions.

## **Methods**

### **Participants**

Participants included 232 White/Euro-Caucasian female ( $n = 189$ ) and male ( $n = 43$ ) undergraduate students. Participants were recruited through a university online-recruitment system as well as through campus advertisements. Ages ranged between 17-35 years of age ( $M=19.75$ ,  $SD=2.78$ ). Current living arrangements varied, with the majority of participants living with either friends/roommates (52.16%), or with parents (31.47%), and the remaining participants reporting living alone (5.60%), with a significant other (4.74%), or other arrangements (6.03%; e.g., living with children).

### **Procedure**

Following the provision of informed consent, participants were provided with a series of questionnaires that assessed demographic information, current symptoms of depression, coping styles, as well as levels of perceived support and unsupportive interactions from parents and peers. Following completion of questionnaires, a single saliva sample was collected from participants for DNA analyses. All participants were provided with a written debriefing explaining the purpose and objectives of the study, as well as researcher contact information. All procedures for the present study were approved by the Carleton University Ethics Committee for Psychological Research.

### **Genotyping**

Saliva samples for DNA analyses were collected using an Oragene OG-500 saliva sample collection kit purchased from DNA Genotek (Ottawa, ON). Manufacturer's

instructions were followed for the extraction of genomic DNA and following extraction samples were diluted to approximately equal concentrations (20ng/  $\mu\text{L}$ ). DNA samples were genotyped using quantitative polymerase chain reaction (qPCR). The amplification reactions were performed using approximately 1  $\mu\text{L}$  (20ng) of genomic template, 0.6  $\mu\text{L}$  of each primer (with a concentration of 10  $\mu\text{M}$ ), 1.2  $\mu\text{L}$  of dNTP, 1.5  $\mu\text{L}$  of 10X buffer, 1.5  $\mu\text{L}$  of  $\text{MgCl}_2$ , 0.3  $\mu\text{L}$  of Salmon Sperm DNA, 0.15  $\mu\text{L}$  of Taq polymerase, 0.015 of SYBR green, 8.135  $\mu\text{L}$  of water. The total volume of the resulting solution was 15  $\mu\text{L}$ . Solutions were plated in duplicate and qPCR products were run on 2% agarose gel electrophoresis to visualize and confirm qPCR results. The primer sequences used for qPCR were the following:

OXTR F1 forward: TCCCTGTTTCTGTGGGACTGAGGAC

OXTR F2 forward: TCCCTGTTTCTGTGGGACTGAGGAT

OXTR reverse: TCCCTGTTTCTGTGGGACTGAGGAT

Allele distribution for the OXTR polymorphism comprised 104 individuals with the homozygote GG genotype, (87 female, 17 male), 89 individuals with the heterozygote AG genotype (71 female, 18 male), and 32 individuals with the homozygote AA genotype (25 female, 7 male). Genotype distributions did not differ as a function of gender  $\chi^2_{(1)} = 0.73, p = .70$ . Additionally, genotype distributions for males,  $\chi^2_{(1)} = 0.35, p = .55$ , and females,  $\chi^2_{(1)} = 2.79, p = .09$ , met Hardy-Weinberg Equilibrium expectations. The initial sample size was 232 but there were seven individuals for whom the genotype could not be determined and hence they were excluded from any subsequent analyses making the overall  $N = 225$ . Further, due to the infrequency of the AA genotype, a

dominant model was used wherein all A carriers (AA and AG were pooled) were compared to individuals with the GG genotype.

### **Measures**

*Depressive symptoms.* Depressive symptoms were assessed using the Beck Depression Inventory (BDI) (Beck et al., 1961). This is a 21-item questionnaire in which participants respond to each item by selecting one of four options that range from low to high depression symptomology. The scores were calculated as the total sum across all items (Cronbach's  $\alpha = .90$ ).

*Unsupportive Social Interactions.* Levels of unsupportive social interactions from parents and peers were assessed using the Unsupportive Social Interactions Inventory (USII) (Ingram et al., 2001). This 24-item scale was administered twice (once for parents, and once for peers) and assessed the degree of perceived unsupport individuals received from their parents or peers when turning to them during a recent stressful or challenging time. Participants responded to each item ranging from none (0) to a lot (4). The unsupport scale comprised four subscales that included distancing (behavioral or emotional disengagement; e.g., "Would not seem to want to hear about it"), bumbling (behaviors that are awkward, or uncomfortable; e.g., "Would try to cheer me up when I was not ready to"), minimizing (attempts to minimize the individual's concerns; e.g., "Would feel that I was overreacting") and blaming (finding fault or criticism; e.g., "Would make "I told you so" or similar comments"). The four subscales were highly correlated with one another (ranging from  $r = .47$  to  $.65$  (Parents) and  $r = .42$  to  $.58$  (Peers)), and so total mean scores of unsupport were used (Peers: Cronbach's  $\alpha = 0.92$ ; Parents: Cronbach's  $\alpha = 0.93$ ).

*Social Support.* Perceived social support from parents and peers was assessed using the Social Provisions Scale (Cutrona and Russell, 1987). Participants were asked to respond to this shortened 12-item scale twice (once for parents, and once for peers) by rating the degree to which their parents or peers are currently providing them with different forms of support including, guidance, reassurance of worth, reliable alliance, social integration, opportunity to provide nurturance and attachment. This shortened version has been shown to demonstrate good construct validity (Russell et al., 1984). Total mean scores of social support were used and demonstrated good reliability (Peers: Cronbach's  $\alpha = 0.87$ ; Parents: Cronbach's  $\alpha = 0.81$ ).

*Coping Styles.* The Survey of Coping Profile Endorsement (Matheson and Anisman, 2003) is a 50-item scale that assesses the means individuals use to cope. Participants indicated on a scale of never (1) to almost always (5), the extent to which they would use the behavior as a way of dealing with problems or stressors in recent weeks. The underlying factor structure was determined using a principal component analysis (PCA) with a varimax rotation. Three factors emerged which encompassed emotion-, avoidant- and problem-focused coping. The factor loadings were similar to that of previous findings (McQuaid et al., 2014 and Raspopow et al., 2013) and Cronbach's alphas for the three factors confirmed that they were well-constructed. Emotion-focused coping comprised ruminations, emotional expression, blaming others, self-blame and wishful thinking (Cronbach's  $\alpha = .90$ ). Avoidant coping comprised, cognitive distraction, passive resignation and emotional containment (Cronbach's  $\alpha = .82$ ). Problem-focused coping comprised problem solving, cognitive restructuring, active distraction, humor and social support seeking (Cronbach's  $\alpha = .85$ ).

## **Statistical Analyses**

The statistical analyses were performed using IBM SPSS Statistics 20 for Windows (Armonk, NY: IBM Corp.). Independent samples t-tests were performed to assess differences of OXTR and gender on scores of depression, coping and experiences of unsupportive social interactions as well as, social support. Pearson correlation scores were calculated to assess the relations between self-reported scores for depression, unsupportive social interactions, social support, and coping. Moderated mediation analyses were conducted using bootstrapping procedures and confidence intervals based on 5000 resamples (Preacher et al., 2007). Unstandardized scores were used for all regression analyses. In the moderated mediation analyses OXTR genotype was treated as the moderator, unsupport or social support were used as independent variables, coping styles as mediator variables and depressive symptoms as the outcome.

## **Results**

There were no differences as a function of individuals' genotype on depression ( $t(1, 223) = -0.04, p = .97$ ), perceived social support from parents ( $t(1, 223) = 1.14, p = .26$ ) or peers ( $t(1, 223) = -0.38, p = .70$ ), or unsupport from parents ( $t(1, 223) = -0.06, p = .95$ ) or peers ( $t(1, 223) = -0.54, p = .59$ ). Likewise, differences were not observed across genotypes with respect to emotion-focused ( $t(1, 223) = 0.37, p = .71$ ), avoidant-focused ( $t(1, 223) = 0.77, p = .44$ ), or problem-focused coping ( $t(1, 223) = -0.38, p = .70$ ; see Table 1 for descriptives). Analyses were also conducted to determine if any of the variables of interest varied as a function of gender. In this regard, reported depressive symptoms were higher among females,  $t(1, 91) = 4.56, p < .001$ , as were reports of emotion- and avoidant-

focused coping,  $t(1, 85) = 4.24, p < .001$ , and  $t(1, 230) = 2.23, p < .05$ , respectively (see Table 2 for all descriptives and t-test values).

*Table 1. Mean, standard deviation, and range for study variables by OXTR rs53576 genotype.*

	GG	AG	AA	Overall
Beck Depression Inventory	$M = 9.18 \pm 7.55$ Range: 0-31.00	$M = 9.08 \pm 8.31$ Range: 0-35.50	$M = 9.61 \pm 9.18$ Range: 0-33.00	$M = 9.15 \pm 8.01$ Range: 0-35.50
Social Support (Parents)	$M = 3.22 \pm 0.49$ Range: 1.29-4.00	$M = 3.30 \pm 0.59$ Range: 1.29-4.00	$M = 3.11 \pm 0.75$ Range: 1.42-4.00	$M = 3.24 \pm 0.57$ Range: 1.29-4.00
Social Support (Peers)	$M = 3.46 \pm 0.35$ Range: 2.00-4.00	$M = 3.43 \pm 0.47$ Range: 1.85-3.92	$M = 3.30 \pm 0.58$ Range: 1.87-4.00	$M = 3.42 \pm 0.44$ Range: 1.85-4.00
Unsupport (Parents)	$M = 1.40 \pm 0.76$ Range: 0-3.50	$M = 1.40 \pm 0.82$ Range: 0.08-3.50	$M = 1.43 \pm 0.94$ Range: 0.38-3.50	$M = 1.40 \pm 0.80$ Range: 0-3.50
Unsupport (Peers)	$M = 1.20 \pm 0.59$ Range: 0.17-3.25	$M = 1.24 \pm 0.63$ Range: 0.08-2.88	$M = 1.27 \pm 0.74$ Range: 0.17-3.13	$M = 1.23 \pm 0.63$ Range: 0.08-3.25
Emotion-Focused Coping	$M = 1.92 \pm 0.80$ Range = 0-3.80	$M = 1.86 \pm 0.76$ Range: 0.15-3.47	$M = 1.94 \pm 0.80$ Range: 0.57-3.25	$M = 1.90 \pm 0.77$ Range: 0-3.80
Avoidance-Focused Coping	$M = 2.09 \pm 0.65$ Range: 0.64-3.50	$M = 2.01 \pm 0.67$ Range: 0.33-3.39	$M = 2.07 \pm 0.64$ Range: 0.67-3.28	$M = 2.06 \pm 0.65$ Range: 0.33-3.50
Problem-Focused Coping	$M = 2.55 \pm 0.53$ Range: 1.02-3.69	$M = 2.56 \pm 0.58$ Range: 0.80-3.72	$M = 2.51 \pm 0.65$ Range: 1.34-3.61	$M = 2.56 \pm 0.65$ Range: 0.80-3.90

Table 2. Mean, standard deviation, and t-test values of study variables by gender.

	Males	Females	t-test values
Beck Depression Inventory	$M = 5.33 \pm 5.47$	$M = 10.02 \pm 8.23$	$t(1, 92) = 4.56, p < .001$
Social Support (Parents)	$M = 3.37 \pm 0.43$	$M = 3.21 \pm 0.60$	$t(1, 89) = -2.02, p < .05$
Social Support (Peers)	$M = 3.45 \pm 0.42$	$M = 3.42 \pm 0.44$	$t(1, 230) = -0.47, p = .64$
Unsupport (Parents)	$M = 1.07 \pm 0.54$	$M = 1.47 \pm 0.84$	$t(1, 94) = 3.98, p < .001$
Unsupport (Peers)	$M = 1.23 \pm 0.47$	$M = 1.22 \pm 0.66$	$t(1, 84) = -0.17, p = .86$
Emotion-Focused Coping	$M = 1.54 \pm 0.56$	$M = 1.98 \pm 0.79$	$t(1, 85) = 4.24, p < .001$
Avoidance-Focused Coping	$M = 1.86 \pm 0.62$	$M = 2.10 \pm 0.65$	$t(1, 230) = 2.23, p < .05$
Problem-Focused Coping	$M = 2.59 \pm 0.50$	$M = 2.56 \pm 0.59$	$t(1, 230) = -0.28, p = .78$

Note: When Levene's Test of Equality of Variances was violated equal variances not assumed are reported.

As expected, depression scores were positively correlated with unsupportive relations from parents ( $r = .59, p < .001$ ) and peers ( $r = .44, p < .001$ ), and negatively related to social support from parents ( $r = -.62, p < .001$ ) and peers ( $r = -.47, p < .001$ ). As predicted as well, depressive symptoms were positively related to emotion-focused coping ( $r = .62, p < .001$ ) and avoidant-focused coping ( $r = .41, p < .001$ ), whereas problem-focused coping was negatively associated with depression scores ( $r = -.43, p < .001$ ) (Table 3).

Table 3. Relations between depressive symptoms, social support, unsupport and coping.

	1.	2.	3.	4.	5.	6.	7.
1. Social Support (Parents)							
2. Social Support (Peers)	.39***						
3. Unsupport (Parents)	-.65***	-.31***					
4. Unsupport (Peers)	-.30***	-.48***	.61***				
5. Emotion-Focused Coping	-.37***	-.34***	.46***	.45***			
6. Avoidant-Focused Coping	-.25***	-.25***	.35***	.32***	.51***		
7. Problem-Focused Coping	.40***	.51***	-.26***	-.21**	-.16*	-.10	
8. Depressive Symptoms	-.62***	-.47***	.59***	.44***	.62***	.41***	-.43***

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

*Parental support and unsupport.* It was of interest to examine the influence of OXTR genotype on the mediated relations between parental social support, unsupport and depressive symptoms through coping styles. Preliminary analyses revealed that avoidant-focused coping was not an important mediator of these relations (95% CI {-0.12, .64}), and was thus excluded from subsequent analyses examining the moderating role of OXTR genotype. Moderated multiple mediation analyses were performed using bootstrapping techniques and confidence intervals based on 5000 iterations (Preacher et al., 2007), in which we assessed whether the association between parental social support and depressive symptoms mediated by problem- as well as emotion-focused coping was moderated by the OXTR genotype. In particular, it was tested whether the OXTR genotype moderated the path between social support and coping styles.

These analyses revealed that the OXTR genotype did not moderate the mediating role of problem-focused coping ( $b = 0.03, t = 0.20, p = .83$ ) or emotion-focused coping ( $b = -0.12, t = -0.68, p = .50$ ) on the relations between levels of social support from parents and depressive symptoms. In effect, regardless of the genotype, social support was related to depressive affect and this was mediated by greater problem- and lower emotion-focused coping (95% CI  $\{-1.91, -0.58\}$ , 95% CI  $\{-2.98, -1.39\}$ , respectively). Alternative models assessing whether OXTR moderated the association between both problem- and emotion-focused coping on depressive symptoms were found not to be significant.

Although OXTR genotype did not influence the mediating role of coping between parental social support and depressive symptoms, it was of interest to examine the moderating role of OXTR genotype in the context of unsupportive social interactions. Analyses were performed to determine the moderating influence of OXTR on the association between unsupport from parents and problem-focused coping to predict depressive symptoms. These analyses revealed that the OXTR genotype moderated the mediating role of problem-focused coping on the relation between levels of unsupport from parents and depressive symptoms  $b = -0.18, t = -1.96, p = .05$ . Specifically, unsupportive interactions with parents were associated with higher depressive symptoms and this was mediated through lower problem-focused coping. However, this mediated relationship was only present among individuals who carried an A allele (95% CI  $\{0.42, 1.70\}$ ) and, as expected, was absent among those with the GG genotype (95% CI  $\{-0.25, 0.80\}$ ) for the OXTR gene (Figure 1). Moreover, the OXTR genotype moderated the mediating role of emotion-focused coping in the relation between unsupport from parents

and depressive symptoms,  $b = 0.23$ ,  $t = 1.94$ ,  $p = .05$ . Perceptions of unsupportive relations were associated with higher emotion-focused coping, which, in turn was related to higher depressive symptoms. Unlike problem-focused coping, this mediated relationship was observed irrespective of the OXTR genotype, but was stronger among A allele carriers (95% CI {1.73, 3.22}) compared to individuals with the GG genotype (95% CI {0.54, 2.35}) (Figure 1). It should be noted that the moderated effect of the OXTR polymorphism was small, and thus at this juncture the results should be interpreted cautiously. Once again, alternative models assessing whether OXTR moderated the path between both problem- and emotion-focused coping on depressive symptoms were not significant.

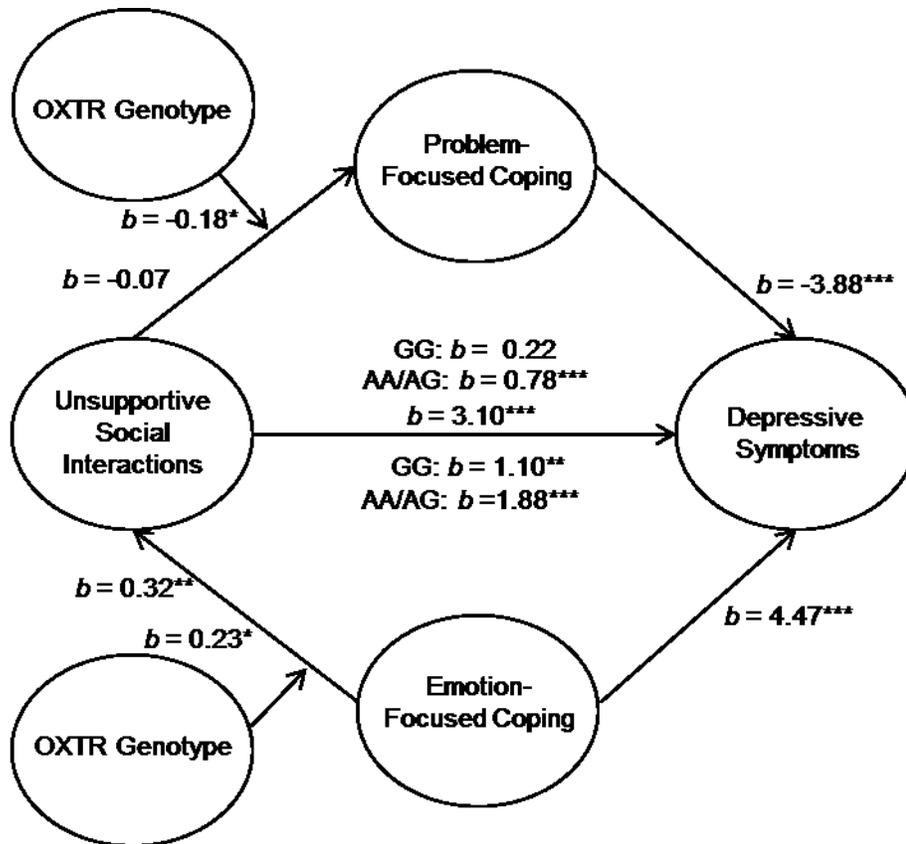


Figure 1. Schematic of the moderated multiple mediation analyses examining parental unupport. The relation between unsupportive social interactions from parents and depressive symptoms through problem-focused coping was moderated by OXTR rs53576 genotype, such that it was only significant among A carriers. As well, the relation between unsupportive responses from parents and depressive symptoms through emotion-focused coping as moderated by OXTR genotype. This mediated model was significant irrespective of genotype, but was stronger among A carriers.  $*p \leq .05$ ,  $**p < .001$ ,  $***p < .001$ .

*Peer support and unsupport.* In addition to assessing the associations between OXTR and unsupportive responses from parents, we examined the relation between unsupport and coping styles as well as between social support from peers and coping styles. As observed with social support from parents, peer support in relation to depressive symptoms through coping styles was not moderated by the OXTR genotype. Indeed, peer support was important regardless of genotype such that greater levels of perceived peer social support were associated with greater problem- and lower emotion-focused coping and this was related to lower depressive symptoms (problem-focused: 95% CI {-3.93, -1.29}; emotion-focused: 95% CI {-4.82, -2.02}). Furthermore, the OXTR genotype did not moderate the mediated relation between unsupport from peers and depressive symptoms through emotion-focused coping,  $b = 0.07$ ,  $t = 0.49$ ,  $p = .63$ . In contrast, the OXTR genotype moderated this relation when problem-focused coping was considered as a mediator,  $b = -0.27$ ,  $t = -2.20$ ,  $p < .05$ . This mediated relation was observed among A allele carriers (Figure 2), but was entirely absent among those with the GG genotype.<sup>2</sup>

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<sup>2</sup> As the BDI scores were positively skewed (skewness  $z = 6.48$ ), additional analyses were undertaken of the square root transformed BDI scores. The results of this analysis fully mapped on to that using the non-transformed data.

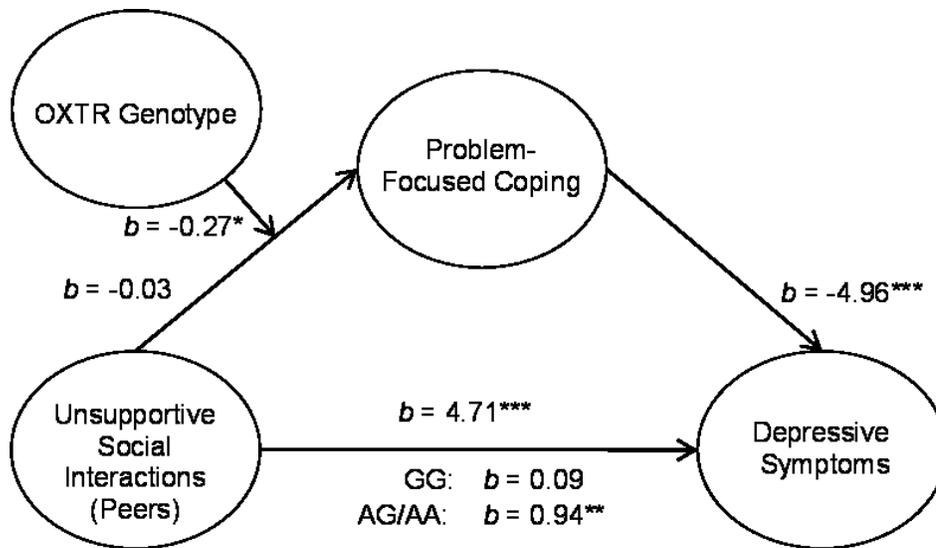


Figure 2. Schematic of the moderated mediation analyses examining peer unsupport. The relation between unsupportive social interactions from peers and depressive symptoms through problem-focused coping was moderated by OXTR rs53576 genotype, only being significant among A carriers.  $*p < .05$ , and  $***p < .001$ .

Due to the potential influence of gender on some of the factors assessed in the moderated mediation analyses (i.e., emotion-focused coping and depressive symptoms) the data were re-analyzed using gender as a covariate. These analyses revealed similar results, in that OXTR remained a non-significant moderator of models in which social support was used as the independent variable. As well, the moderated effect of the OXTR genotype on the relation between peer unsupport and problem-focused coping and depressive symptoms remained unchanged. When examining the moderated effect of OXTR in models where parental unsupport was used as the independent variable, the  $p$ -value for problem-focused coping was reduced. However, the moderated effect of OXTR on emotion-focused coping changed marginally from  $p = .05$  to  $p = .07$ . The overall direction of relationships remained unchanged.

### **Discussion**

The current findings revealed that the OXTR polymorphism rs53576 moderated the association between unsupportive social interactions from parents and peers and problem-focused coping responses in their relation to depressive scores. Specifically, this mediated relation was evident in A carriers, but absent among those with the GG genotype. It seems that in the presence of the A allele it was less likely that individuals would adopt problem-focused strategies in the face of unsupportive interactions, which could potentially contribute to depressive disorders. The current findings also indicated that the adoption of emotion-focused coping in association with perceived unsupportive parental responses was tied to greater depressive symptoms, and this was particularly notable among A carriers. It is uncertain why this heightened relation existed. It is possible that diminished reliance on social support seeking among A carriers was

accompanied by exaggerated emotion-focused coping efforts under conditions of unsupportive responses. In line with these findings, adolescents who carried the A allele for the OXTR rs53576 reported greater levels of loneliness if they also perceived their social network more negatively (van Roekel et al., 2013). The present findings are consistent with those indicating that depressive mood is accompanied by elevated emotion-focused coping at the expense of problem-focused coping (Matheson and Anisman, 2003). Whether this reflects actions of coping on depression, altered coping secondary to depression, or variations in the sensitivity to social cues, it is uncertain given the correlational nature of the present data.

It is somewhat puzzling that the relation between peer unsupport and emotion-focused coping was present irrespective of genotype, whereas this relationship was moderated by the OXTR genotype in the context of parental unsupport. However, for individuals in this age group, responses from peers may be especially significant (Wilkinson, 2004) and hence regardless of genotype, peer unsupport may be highly linked to emotion-focused coping. This speaks to the fact that the effects of social interactions on coping and well-being are not all similarly influenced by genetic predispositions.

The current findings indicated that perceptions of both parental and peer *social support* were associated with depressive symptoms through emotion- and problem-focused coping. Moreover, these relations were not influenced by the oxytocin genotype, which contrasts with the pattern observed with respect to unsupportive social interactions. Social support is fundamental to well-being and it is possible that in relation to coping styles, differences related to genotype are less marked. This said, there have been reports

of social support interacting with the OXTR genotype, indicating that in comparison to individuals with the AA genotype, G carriers of the OXTR rs53576 exhibited diminished stress responses (i.e., decreased cortisol) when social support was available (Chen et al., 2011). In the present investigation, however, the interaction with the OXTR polymorphism was limited to unsupportive relations and was not apparent with respect to social support. Follow-up statistical analyses indicated that the lack of an association of the OXTR polymorphism with social support and coping was apparent irrespective of whether or not AG carriers were pooled with the AA or GG genotypes. However, the small number of AA individuals in the analyses makes it necessary for further replication to determine the relation (or lack of it) between the OXTR polymorphism, social support and coping styles.

Finally, the current data are consistent with previous studies that linked both unsupport and coping styles with depressive symptoms (Ingram et al., 1999; McQuaid et al., 2014; Raspopow et al., 2013), and these relations were more apparent among A carriers. Although these data are in line with the view that the A allele is a vulnerability factor in relation to depressive symptoms, they are not consistent with the social sensitivity hypothesis that G allele carriers are more sensitive, rendering them more susceptible to the consequences of a negative environment (Bradley et al., 2011; Hostinar et al., 2014; McQuaid et al. 2013). It is possible, however, that the relationship between particular genotypes and negative events might vary developmentally. In particular, the heightened social sensitivity associated with the G allele of the OXTR rs53576 was more closely aligned with mood symptoms when the negative social interactions were experienced early in life, as in the case of childhood abuse or neglect (Bradley et al.,

2011; Hostinar et al., 2014; McQuaid et al., 2013). It should be added that the nature of unsupportive social interactions experienced among adults differs appreciably from that of childhood maltreatment, and thus a comparison of these stressful experiences may be inappropriate. Furthermore, it is possible that the link between oxytocin functioning and social sensitivity may vary with specific contextual conditions. For instance, oxytocin might have prosocial effects in a test involving positive social behaviors, but might have very different actions in situations involving social exclusion or ostracism. We observed that G carriers were more sensitive to the effects of an acute experience of social ostracism, although it is uncertain whether these same individuals would be more likely to adopt social support seeking as a primary coping strategy (McQuaid et al., 2014).

Although the present study indicated an association of the A allele with seemingly less productive coping processes, there are several limitations that should be considered. The modest sample size and the number of variables examined may be problematic in a gene-association study (Ohashi and Tokunaga, 2001), and thus the present findings ought to be considered as being provisional, pending a replication of this study. Also, due to the limited number of participants, we were unable to examine the relative risk for negative mood outcomes across the three OXTR genotypes. Examination of the genotypes separately can be particularly informative and the choice to collapse and use a dominant model may not always be appropriate. For example, following a social stressor that comprised social ostracism, when assessing psychosocial measures we observed that responses of participants with the heterozygote AG genotype for the OXTR rs53576 aligned more closely to those with the AA genotype, whereas on physiological measures (cortisol and blood pressure) the heterozygotes displayed profiles that were more similar

to individuals with the GG genotype (McQuaid et al., 2014). In the present investigation, the choice to combine individuals carrying the AA and AG alleles was predicated on earlier studies examining this OXTR SNP (Bakermans-Kranenburg and van IJzendoorn, 2008; Krueger et al., 2012; Rodrigues et al., 2009; Saphire-Bernstein et al., 2011), although a meta-analysis failed to detect a significant combined effect of the OXTR rs53576 polymorphism on social behaviors (Bakermans-Kranenburg and van IJzendoorn, 2014). However, this does not imply that alternative analytic approaches are inappropriate. Ultimately, evaluating the three genotypes independently, despite the low incidence of the AA genotype (approximately 15% in Euro-Caucasians), would be ideal.

Males and females differed on several dimensions (e.g., depressive symptoms, emotion-and avoidance-focused coping, parental unsupport and support), but these differences did not vary as a function of the OXTR genotype. As the sample largely comprised females (approximately 80%) and only a modest number of males were assessed, the contribution of the OXTR genotype to these gender differences warrants further research. This is especially the case as oxytocin may interact with estrogen and with menstrual cycle (Choleris et al., 2003), and it is possible that relations between behavior and the OXTR genotype might also vary with menstrual cycle. However, when gender was treated as a covariate the moderated effect of the OXTR genotype became less significant when examining parental unsupport to depressive symptoms through emotion-focused coping. Further, due to the cross-sectional nature of the study the directionality of the variables of interest is not known. This greatly limits the interpretation of the mediation analyses, and as such, inferences about temporal relations between the variables cannot be inferred. The possibility remains that participants'

current depressive symptoms could have biased their perceptions of unsupportive social interactions and social support. Finally, although there have been several studies linking the OXTR rs53576 gene polymorphism to prosocial behaviors, the functionality of this polymorphism is uncertain (i.e., whether this SNP actually disturbs the receptors responsivity) (Inoue et al., 1994). Nevertheless, it has been suggested that this polymorphism may contribute to the suppression of the protein making up these receptors (i.e., transcription suppression) and hence the presence of these receptors themselves (Mizumoto et al., 1997).

Despite the limitations, the present findings are consistent with the view that A carriers may be more susceptible to negative mood outcomes through the use of less effective coping methods. Yet, the link to psychological disorders, such as depression, is exceedingly complex, especially as genetic factors that are beneficial in certain environments, particularly those that involve social interactions, may be unfavorable in others.

## Chapter 3-Study 2

### Oxytocin, Unsupportive Social Interactions and Negative Affect

McInnis, O.A., McQuaid, R.J., Matheson, K., Anisman, H. Unsupportive social interactions and affective states: The moderating role of two oxytocin-related polymorphisms. *Psychoneuroendocrinology* (in revision).

Study 1 indicated that individuals with the A allele of the OXTR rs53576 polymorphism reported more emotion- and less problem-focused coping in relation to unsupportive social interactions and this was tied to greater depressive symptoms. Although, the SNP OXTR rs53576 has been frequently studied in association with prosocial behaviors, another less investigated oxytocin-related polymorphism has been associated with alterations in both oxytocin levels and social behaviors. The SNP rs3796863 is located on the CD38 gene and is thought to be involved in controlling the release of oxytocin (Higashida et al., 2012). The C allele of the CD38 polymorphism has been associated with lower oxytocin (Feldman et al., 2012) and decreased social functioning (Sauer et al., 2012). The purpose of study 2 was to extend the findings of study 1, to determine if a different oxytocin-related gene was also associated with unsupportive social interactions and mood outcomes. We examined both the CD38 and OXTR polymorphism in association with unsupportive social relations and affective states. In this regard, it was thought that individuals who carried alleles that are thought to be associated with lower oxytocin functioning (i.e., the A allele of the OXTR polymorphism and the C allele of the CD38 polymorphism) would report greater negative affect in association with unsupportive social interactions.

## Abstract

Two single nucleotide polymorphisms (SNPs) on oxytocin-related genes, specifically the oxytocin receptor (OXTR) rs53576 variant and the CD38 rs3796863 variant (involved in the regulation of central oxytocin release), have been associated with alterations in prosocial behaviors. In the current study, conducted among undergraduate students ( $N=476$ ), no association was detected between perceived levels of unsupportive social interactions (unsupport) and A carriers of the OXTR polymorphism SNP. In contrast, individuals with the CD38 SNP on both alleles (AA genotype), a variant previously associated with elevated oxytocin, reported greater perceptions of unsupport from peers compared to those with the CC genotype. As expected, perceived unsupport from peers was associated with greater negative affect, which was moderated by both genetic variants. Specifically, A carriers of the OXTR polymorphism and CC carriers of the CD38 polymorphism (both of which are thought to be linked to lower oxytocin functioning) reported greater negative affect that was associated with perceived unsupport. In effect, the findings are consistent with the perspective that genes that are thought to be tied to lower oxytocin functioning are related to increased vulnerability to poor mood outcomes which might be tied to negative social interactions. At the same time, having a genetic constitution associated with higher oxytocin was accompanied by increased perceptions of unsupport. These seemingly paradoxical findings may be related to previous observations that those with genes associated with increased prosocial behaviors are also more likely to exhibit relatively effective coping styles to deal with challenges.

## **Introduction**

Oxytocin, a hormone produced in the supraoptic and paraventricular nuclei of the hypothalamus, is thought to influence a wide range of prosocial behaviors, emotions and attitudes (Bartz, 2011; Feldman, 2012). In this regard, administering oxytocin through an intranasal spray promotes prosocial behaviors, such as generosity (Zak et al., 2007), trust (Kosfeld et al., 2005), attention to positive cues (Domes et al., 2013), and more positive behaviors during couple conflict (Ditzen et al., 2009), and may have implications for mental health disorders (McQuaid et al., 2014). In line with the actions of oxytocin administration, prosocial behaviors vary with genetic variants of the oxytocin receptor (OXTR). Specifically, the single nucleotide OXTR polymorphism (SNP) rs53576, which occurs on the third intron of the OXTR gene and involves a guanine (G) to adenine (A) substitution, was associated with lower positive affect and self-esteem (Lucht et al., 2009; Saphire-Bernstein et al., 2011), social support seeking (Kim et al., 2010) and prosocial behaviors (Krueger et al., 2012; Rodrigues et al., 2009; Tost et al., 2010). Moreover, those carrying the SNP reported greater depressive symptoms (Saphire-Bernstein et al., 2011) and were at elevated risk for autism spectrum disorder (ASD) (Wu et al., 2005). Despite studies linking the A allele for the rs53576 SNP with lower social functioning and negative affect, a meta-analysis suggested that the evidence for this association was weak (Bakersmans-Kranenburg & van IJzendoorn, 2014).

An alternative view of the contribution of oxytocin to behavioral change has been that rather than encouraging prosocial behaviors, this hormone serves to increase sensitivity to social cues and experiences (Bradley et al., 2011; Hostinar et al., 2014; McQuaid et al., 2013). For instance, among individuals G carriers, negative early life

experiences might promote negative mood outcomes in subsequent adulthood, which are limited among A carriers (Bradley et al., 2011; Hostinar et al., 2014; McQuaid et al., 2013). As well, the G allele has been tied to heightened sensitivity in the form of greater feelings of social rejection following social ostracism in a laboratory setting (McQuaid et al., 2015). Commensurate with this social sensitivity view, it was proposed that certain genetic variants could promote neuronal plasticity, so that environmental experiences, regardless of whether they were positive or negative, would have a greater influence on later behavioral and emotional outcomes (Belsky et al., 2009; Belsky and Pluess, 2009). Clearly, conflicting perspectives exist as to whether the A or G allele of the OXTR polymorphism represents a vulnerability or protective factor. Yet, the influence of this SNP may be dependent on the specific situation in which individuals are assessed. As well, the OXTR polymorphism represents only one element that determines the influence of oxytocin's effects on behaviors. Moreover, this OXTR rs53576 SNP resides on a noncoding region of the OXTR gene, and as such its functionality is uncertain (Inoue et al., 1994).

Beyond examining OXTR variants, there have been efforts to assess polymorphisms in the gene which codes for CD38, a transmembrane glycoprotein widely expressed on immune cells, such as macrophages and lymphocytes, and is also involved in the regulation of central oxytocin release (Higashida et al., 2012; Jin et al., 2007). In mice with this gene deleted, peripheral and central levels of oxytocin are reduced and impairments of social processes are elicited (Jin et al., 2007), and it was suggested that CD38 is critical for social recognition in mice via its regulation of oxytocin secretion (Higashida et al., 2012). Higher circulating levels of CD38 gene

expression in humans were related to higher plasma oxytocin levels (Kiss et al., 2011). The rs3796863 SNP located in intron 7 on the CD38 gene involves a cytosine (C) to adenine (A) substitution (Malavasi et al., 2008). Healthy individuals homozygous for the C allele displayed lower plasma oxytocin levels (Feldman et al., 2012), and among males, the CC genotype was associated with deficits in the processing of social stimuli (Sauer et al., 2012). In fact, it may be significant that war-exposed children who were classified as having a high risk genetic profile which included carriers of the CC genotype for CD38 rs3796863, as well as other risk alleles on the oxytocin receptor and vasopressin gene, were more likely to develop a psychiatric illness by mid-childhood (Feldman et al., 2014).

There have been reports that C carriers of this polymorphism might be at a greater risk for ASD (Munesue et al., 2010), and among those with severe forms of ASD, the C allele was associated with decreased CD38 expression (Lerer et al., 2010). However, as with the OXTR rs53576 polymorphism, not all studies supported the perspective that the C allele of the CD38 polymorphism represents a vulnerability factor to negative behavioral and emotional outcomes or to ASD. Examination of autistic-like traits among a general population sample failed to find an association of the CD38 rs3796863 SNP (Hovey et al., 2014). Moreover, among adolescents who carried the A allele, the experience of chronic interpersonal stress was associated with greater social anxiety and depressive symptoms compared to adolescents with the CC genotype, a finding which is in line with the potential role of higher oxytocin being accompanied by greater sensitivity to negative events (Tabak et al., 2015). In an entirely different context, however, individuals with the

CC genotype were more likely to perceive their romantic partner as responsive following an expression of gratitude (Algoe and Way, 2014). Clearly, given the still limited number of studies examining the relation between the CD38 gene and behavior, it is difficult to discern the link between these gene and specific prosocial behaviors or pathological conditions.

Although oxytocin has been examined in response to various social stressors as well as in relation to social support and mood outcomes (Heinrichs et al., 2003; Olf et al., 2013; McQuaid et al., 2014; Taylor et al., 2000), its relation to negative social responses, such as those of a unsupportive nature, have not been investigated. Unsupportive social interactions refer to experiencing negative or upsetting responses from another individual when support was reasonable expected (Ingram, et al., 1999; Ingram, et al., 2001). Instances of unsupportive responses have been linked to depressive symptoms even when accounting for the contribution of social support (Ingram et al., 1999; Song and Ingram, 2002). The purpose of the present study was to examine the relation between both the OXTR rs53576 and the CD38 polymorphisms and unsupportive social interactions, by examining whether they moderated the relation between perceived unsupportive responses from parents and peers and current positive and negative affect. It was predicted that perceived unsupport from parents and peers would be associated with higher reports of negative- and lower positive current mood states and that these associations would be stronger among individuals with the CC genotype for the CD38 polymorphism and the A carriers of the OXTR (those who presumably have lower oxytocin functioning).

## **Methods**

### *Participants*

A total of 476 White/Euro-Caucasian male ( $n = 131$ ) and female ( $n = 345$ ) undergraduate students were recruited through campus postings and a university online-recruitment system. Participants' ages ranged from 17-35 years ( $M=19.44$ ,  $SD=2.45$ ).

### *Procedure*

All participants provided written informed consent, and were presented with several questionnaires that measured demographic information, current mood, and unsupportive social interactions from parents and peers. Following this, a saliva sample was obtained from participants for subsequent DNA extraction. Upon study completion, all participants were given a written debriefing form, as well as researcher contact information. All procedures for the current study were approved by the Carleton University Ethics Committee for Psychological Research.

### *Genotyping*

Saliva samples for DNA were collected using Norgen collection kits (Norgen Biotek Corp., Thorold, Ontario Canada). Genomic DNA was extracted according to the manufacturer's instructions and samples were diluted to approximately equal concentration (10 ng/ $\mu$ L). DNA samples were sent for genotyping to McGill University and Génome Québec Innovation Centre (Montreal, Canada). Polymerase chain reaction (PCR) was used to amplify the DNA, and QIAxcel was used to determine amplification status. Unincorporated dNTPs were removed using shrimp alkaline phosphatase. One probe per marker was used to perform a single base extension and the product was desalted using 6mg of resin. Using a Samsung Nanodispenser, the product was spotted on

a Sequenom 384-well chip and the chip was read using a Mass Spectrometer. Manual analyses were performed for each marker. Primer sequences were as follows:

OXTR:

rs53576 forward: ACGTTGGATGTCCCCATCTGTAGAATGAGC

rs53576 reverse: ACGTTGGATGGCACAGCATTTCATGGAAAGG

rs53576 probe: CTCTGTGGGACTGAGGA

CD38:

rs3796863 forward: ACGTTGGATGGTTGCTGCTCCTGCTGTTTT

rs3796863 reverse: ACGTTGGATGAAGGTGCACAGACCACTTAG

rs3796863 probe: TCCTGCTGTTTTTTTGACCA

Allele frequency for the CD38 polymorphism included 210 individuals homozygous for the C allele, (156 females, 54 males), 199 CA heterozygotes (134 females, 65 males), and 53 homozygous for the A allele (43 females, 10 males). The distribution of genotypes for the OXTR polymorphism comprised: 213 individuals with the GG genotype (161 females, 52 males), 193 with the AG genotype (132 females, 61 males), and 57 with the AA genotype (41 females, and 16 males). Genotype distributions met Hardy-Weinberg Equilibrium expectations for both the CD38 polymorphism  $\chi^2_{(1)} = 0.31, p = .58$ , as well as the OXTR polymorphism  $\chi^2_{(1)} = 1.64, p = .55$ . The original sample size was 476 but there were 13 individuals for the OXTR polymorphism and 14 individuals for the CD38 polymorphism for whom the genotype could not be determined. As such, subsequent analyses involving the OXTR and CD38 polymorphisms will comprise 463 and 462 individuals, respectively.

*Measures*

*Mood.* The Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988) is a 41-item scale that examines the current mood states of participants. This scale assesses the levels of positive (e.g., “enthusiastic”, “happy”, “inspired”) and negative affective states (e.g., “distain”, “enraged”, “hostile”). Scores indicate degree of positive affect, negative affect, or may be divided into subcategories of negative affect which include sadness, anger, fear, shame contempt, and guilt. Participants reported the extent to which they were currently experiencing a given emotion on a six-point scale that ranged from not at all (0) to extremely (6). Mean scores across items were calculated for both negative affect (Cronbach’s  $\alpha = .90$ ) and positive affect (Cronbach’s  $\alpha = .91$ ).

*Unsupport.* Perceptions of unsupport were assessed using The Unsupportive Social Interactions Inventory (Ingram et al., 2001). This 24-item inventory was administered twice (once for parents and once for peers) to assess how often (from “none” (0) to “a lot” (4)) in recent weeks the participants' received negative or upsetting responses when talking to peers or parents about events in their life. The scale contains four subscales which include minimizing (efforts to force optimism, or to minimize the individual's problems), distancing (behavioral or emotional disengagement), bumbling (uncomfortable or awkward behaviors), and blaming (criticism or finding fault). The subscales were highly correlated and as such, total mean scores of unsupport were used (Peers: Cronbach’s  $\alpha = 0.92$ ; Parents: Cronbach’s  $\alpha = 0.93$ ).

### *Statistics*

The statistical analyses were performed using IBM SPSS Statistics 20 for Windows software (Armonk, NY: IBM Corp.). Statistical significance was determined at  $p < 0.05$  (two-tailed). Analyses assessing CD38 and OXTR genotype differences on perceptions of

unsupport from parents and peers, as well as positive and negative affect were conducted using multivariate analyses of variance (MANOVAs) with Bonferonni post-hoc follow-up. Hierarchical regression analyses were performed to determine whether associations between unsupport from parents and peers were moderated by CD38 and OXTR genotypes. Significant moderations were followed up using a web utility for simple slopes (Preacher et al., 2006). Standardized scores were used in all regression analyses.

## Results

A MANOVA revealed that negative and positive affect did not vary as a function of either the CD38 gene, *Pillai's Trace*,  $F(4, 914) = 0.79, p = .53$ , or the OXTR polymorphism, *Pillai's Trace*,  $F(4, 916) = 0.54, p = .70$ . In contrast, perceptions of unsupportive social interactions varied by CD38 genotype, *Pillai's Trace*,  $F(4, 914) = 3.05, p = .02, \eta^2 = 0.013$ . Specifically, whereas perceptions of parental unsupport did not differ as a function of the CD38 genotype,  $F(2, 455) = 2.16, p = .12$ , unsupport from friends differed significantly differ by genotype,  $F(2, 455) = 3.58, p = .03$ , such that individuals with the AA genotype perceived higher levels of unsupport from friends than those with the CC genotype ( $p = .05$ ) (See Table 1 for descriptives). The OXTR polymorphism was not associated with levels of perceived parental or peer unsupport *Pillai's Trace*,  $F(4, 916) = 0.05, p = .99$ .

*Table 1. Mean and standard deviations of perceived unsupportive social interactions, negative and positive affect as a function of the OXTR rs53576 and CD38 rs3796863 polymorphisms.*

	CD38 CC	CD38 CA	CD38 AA	OXTR GG	OXTR AG	OXTR AA
Unsupport Peers	1.18 ± 0.63	1.30 ± 0.69	1.42 ± 0.80*	1.26 ± 0.68	1.26 ± 0.68	1.25 ± 0.74
Unsupport Parents	1.26 ± 0.77	1.41 ± 0.78	1.28 ± 0.66	1.32 ± 0.75	1.34 ± 0.77	1.33 ± 0.85
Negative Affect	1.02 ± 1.10	0.98 ± 1.07	1.02 ± 1.17	0.93 ± 0.97	1.07 ± 1.22	0.99 ± 1.12
Positive Affect	3.12 ± 1.36	2.94 ± 1.35	2.82 ± 1.25	2.97 ± 1.38	3.03 ± 1.33	2.87 ± 1.22

\*  $p \leq .05$ , AA individuals greater than those with the CC genotype.

To determine whether gender differences existed on the outcomes assessed multivariate analyses were conducted. No gender differences were observed between males and females on current negative affect, although males reported more positive affect  $F(1, 455) = 19.83, p < .001$ . Males and females did not differ on perceptions of peer unsupportive social interactions, but there was a significant gender difference on perceived levels of parental unsupport, wherein females reported greater levels than males  $F(1, 455) = 4.68, p < .05$ .

#### *Moderating Role of the CD38 and the OXTR polymorphism*

In examining the moderating role of the CD38 polymorphism in the relation between perceived unsupport from parents and peers to positive affect no significant effects were observed  $\Delta R^2 = 0.00, \Delta F(1, 455) = 0.02, p = .90$ , and  $\Delta R^2 = 0.00, \Delta F(1, 455) = 0.00, p = .95$ , respectively. However, when negative affect was assessed as the outcome, a significant moderated effect for the CD38 polymorphism was found  $\Delta R^2 = 0.01, \Delta F(1, 455) = 6.82, p < 0.01$ . The regression analysis revealed that perceived unsupport from friends was related to higher negative affect,  $b = 0.56, t = 7.41, p < 0.001$ , but this was moderated by the CD38 genotype,  $b = -0.25, t = -2.61, p < 0.01$ . Simple slopes revealed that the relation between higher perceptions of unsupport and negative affect was stronger among individuals who carried the CC genotype compared to A carriers, although the relation between perceived unsupport and negative mood was still present among these individuals (Figure 1A). No moderating effect of CD38 was observed when examining the association between unsupportive relations from parents and negative affect  $\Delta R^2 = 0.00, \Delta F(1, 455) = 0.91, p = .34$ .

Very similar relationships were found upon examining the OXTR polymorphism.

In this regard, the association between perceived unsupport from friends and parents and positive mood was not moderated by the OXTR polymorphism  $\Delta R^2 = 0.00$ ,  $\Delta F(1, 455) = 0.70$ ,  $p = .40$ , and  $\Delta R^2 = 0.01$ ,  $\Delta F(1, 455) = 3.36$ ,  $p = .07$ , respectively. However, the OXTR polymorphism moderated the relation between perceived unsupport from friends and negative affective state,  $\Delta R^2 = 0.01$ ,  $\Delta F(1, 455) = 4.41$ ,  $p < .05$ ,  $b = 0.20$ ,  $t = 2.10$ ,  $p < .05$ . In particular, a stronger relationship between perceived unsupport from friends and negative affect was observed among individuals who carried the A polymorphism, compared to those with the GG genotype, although this relation was also apparent in those with the GG genotype (Figure 1B). The relationship between perceptions of unsupport from parents and negative affect was not moderated by OXTR genotype  $\Delta R^2 = 0.00$ ,  $\Delta F(1, 455) = 0.74$ ,  $p = .39$ . Additional analyses were conducted to determine the moderating effects of the OXTR or CD38 polymorphisms on the relations between perceived unsupport from peers and negative affect to determine whether each of the polymorphisms had unique effects. A hierarchical regression analysis revealed that when the OXTR and CD38 polymorphisms were in the regression model together, the interaction terms remained significant, although the effect for the OXTR polymorphism decreased marginally. In essence, these two polymorphisms had their own unique relations with negative affect, and had additive effects in this regard.

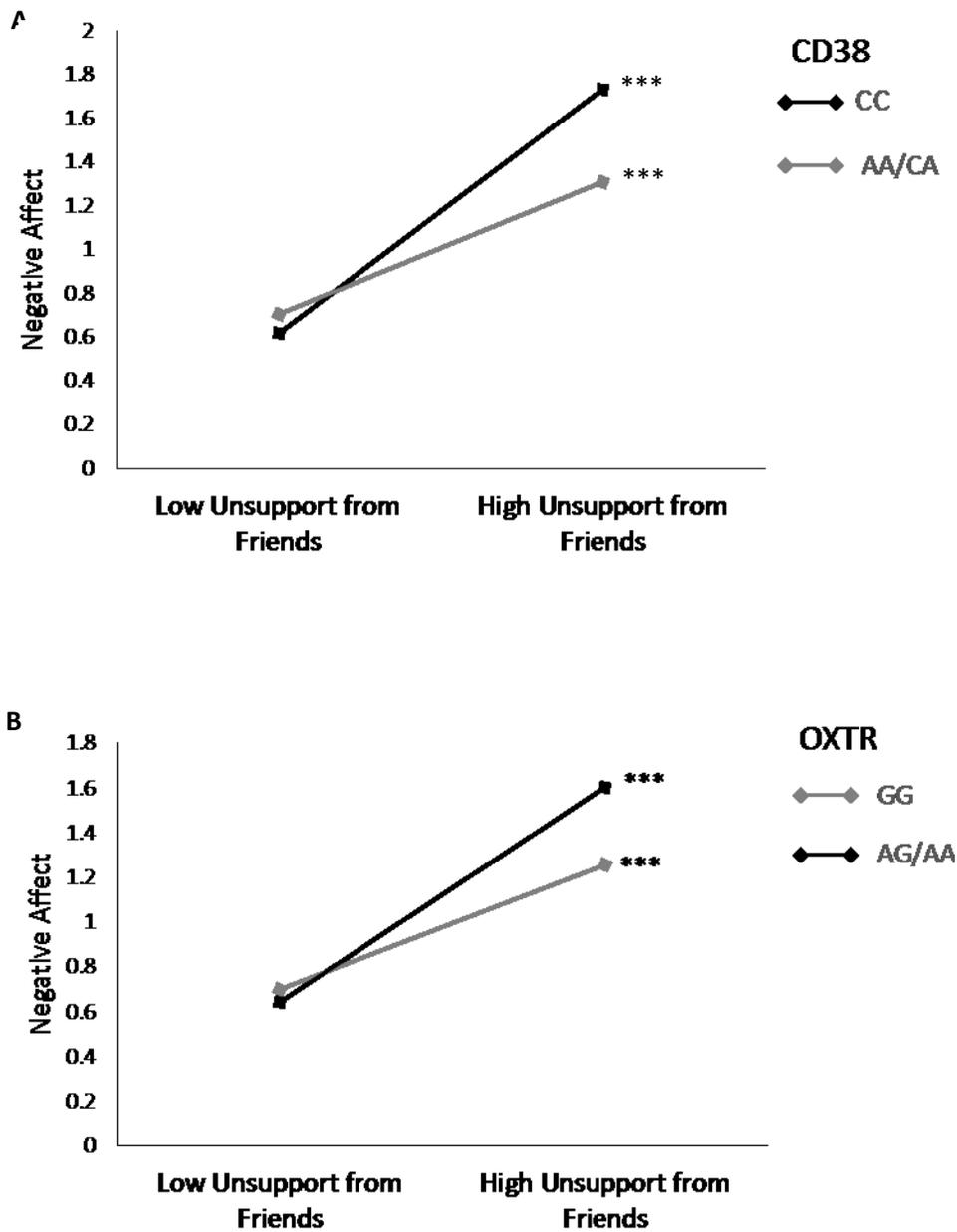


Figure 1. The relationship between unsupportive social interactions and negative affect as moderated by the CD38 rs3796863 and the OXTR rs53576 polymorphisms.

Perceptions of unsupport from peers were associated with greater reports of negative affect, and this association was stronger among those with the CC genotype of the CD38

polymorphism compared to A carriers (Panel A). Similarly, the relation between peer unsupport and negative mood was stronger among A carriers of the OXTR polymorphism compared to those with the GG genotype (Panel B),  $***p < .001$ .

## **Discussion**

The current findings indicated a modest association between the AA genotype of the CD38 polymorphism rs3796863 and greater perceptions of peer unsupport compared to those homozygous for the CC genotype. The AA genotype has been related to higher peripheral levels of plasma oxytocin (Kiss et al., 2011). As such, the observation that individuals with the AA genotype perceived more negative social interactions is in line with the view that oxytocin might promote elevated sensitivity to social cues. However, this effect was small and replication is required to determine the robustness of this association. Nonetheless, this finding is consistent with reports that the AA genotype of the CD38 polymorphism promotes greater social sensitivity to chronic interpersonal stress (Tabak et al., 2015). The OXTR rs53576 polymorphism was not associated with perceptions of unsupport, a finding consistent with previous reports that this polymorphism is not directly related to perceived levels of unsupport (McInnis et al., 2015).

Despite the observation that the AA genotype of the CD38 polymorphism was associated with higher perceptions of peer unsupportive responses, our findings did not suggest a stronger association between perceptions of unsupport and current negative affective state among A carriers compared to those with the CC genotype. Instead, it appeared that the association between perceived unsupport from peers and current negative affective state was stronger among individuals with the CC

genotype (those who have been shown to have lower peripheral oxytocin) compared to A carriers. The observation that individuals with the CC genotype reported negative mood in association with higher perceptions of unsupport is consistent with studies that have suggested it might represent a vulnerability factor to lower social functioning (Feldman et al., 2012b; Sauer et al., 2012). Despite this, the current findings are entirely correlational and it is unclear whether negative mood was a result of experiences of unsupport or whether mood states influenced participants' perceptions of their social interactions.

The current findings regarding the CD38 polymorphism are somewhat paradoxical, but nonetheless speak to the challenges in delineating its role as a 'risk' versus a 'protective' allele. On the one hand, the AA genotype was associated with higher perceptions of peer unsupport, which supports the perspective that this genotype is accompanied by elevated social sensitivity. On the other hand, the finding that higher negative affect was associated with perceived unsupport among individuals with the CC genotype compared to A carriers is in line with the view that low oxytocin would be related to negative mood outcomes. Together, these findings demonstrate the complex nature of gene associations with environment and well-being. In this regard, the effects of certain genetic variants might vary depending on the context of the stressor experience, as well as in relation to the specific outcome measured.

Consistent with the moderating role of CD38 in the relation between perceived unsupport from peers and negative mood, we also observed that the OXTR polymorphism rs53576 moderated this association. Specifically, A carriers of the

OXTR polymorphism perceptions of peer unsupport was associated with higher current negative affect, and this association was weaker among individuals with the GG genotype. Additionally, the moderating effect of each of the two polymorphisms on the relation between perceived unsupport and negative affect was small and served additively in this capacity. It has been speculated that both the A allele of the OXTR polymorphism and the C allele of the CD38 polymorphism are associated with lower oxytocin functioning, whether directly or through their associations with other functional polymorphisms. As such, the pattern observed between these two polymorphisms in relation to perceived unsupportive responses and negative affect might be indicative of a link between lower oxytocin functioning and poor mood stemming from negative social interactions. However, given the current study is association-based it is too premature to draw such conclusions. Commensurate with the present findings, we previously reported that the A allele of the OXTR polymorphism rs53576 was associated with ineffective coping methods in relation to perceived unsupport, which was, in turn, linked to greater depressive symptoms compared to individuals homozygous for the G allele (McInnis et al., 2015). Similarly, compared to individuals with the GG genotype, adolescents who carried an A allele for the OXTR polymorphism demonstrated a stronger relation between negative social experiences and loneliness (van Roekel et al., 2013).

The findings of this study should be interpreted cautiously given that both the OXTR and CD38 polymorphisms are located on intronic regions, and their functionality remains unknown. It would have been ideal to examine levels of oxytocin in association with these genetic variants to determine if levels of oxytocin varied according to the

SNPs as well as with the psychosocial measures examined. Nevertheless, it has been demonstrated previously that lower endogenous oxytocin levels accompanied the CC genotype of the CD38 polymorphism, suggesting that this SNP might be linked to a functional SNP on the CD38 gene (Lin et al., 2007). The finding that the oxytocin-related variants assessed only moderated the associations between perceived unsupport from peers and negative affect and gene associations were not observed in relation to perceived parental unsupport or when positive affect was assessed as the outcome was surprising. Because of this, the current findings should be viewed as preliminary pending further replication. The correlational nature of the present study precludes conclusions about the direction between perceived unsupport and negative mood. Moreover, questionnaire used to assess mood states measured current emotions, and as such it is not possible to determine whether the emotional states were in any way the result of unsupportive social interactions. Finally, in the present investigation the number of females was markedly greater than the number of males. In our studies with university students (e.g., McQuaid et al., 2013; McQuaid et al., 2015; McInnis et al., 2015) this skewed distribution was commonly observed, possibly because of the greater number of females enrolled in an Introductory Psychology course, and/or females were more disposed to volunteering in experiments. Regardless of the source, it would be advantageous to be able to assess the relations between oxytocin polymorphisms and behavioral responses, particularly as there is reason to suppose that oxytocin might not have identical effects in the two sexes. In females, for instance, oxytocin may contribute to tend-and-befriend characteristics (Taylor et al., 2000), whereas in males, it has been observed that oxytocin promotes a

tend-and-defend behavioral response, although it is uncertain whether this is also present in females (De Dreu et al., 2010).

## **Conclusion**

Although correlational, the present findings are in line with two different, but not necessarily competing, perspectives regarding oxytocin functioning and social experiences. The observation that genetic variants thought to be linked to lower oxytocin functioning is associated with poor mood which was tied to perceived negative social interactions is consistent with the view that oxytocin is linked to prosocial behaviors that, in turn, limit negative affect. Yet, individuals who presumably have higher oxytocin (AA carriers of the CD38 polymorphism) also reported greater perceptions of negative social interactions, which is in line with the perspective that elevated oxytocin increases sensitivity to social cues, irrespective of whether these are positive (Bradley et al., 2013) or negative (Bradley et al., 2011; Hostinar et al., 2014; McQuaid et al., 2013).

One factor which could account for the differential association with perceived negative interpersonal experiences could be related to the coping methods individuals employ following such experiences. While individuals with higher oxytocin might be more socially sensitive, the possibility remains that they are also more likely to engage in advantageous coping involving social support seeking, which could limit the extent of negative mood outcomes. Moreover, the current study was based on normal conditions, and hence does not provide information concerning the influence of the polymorphisms in the presence versus absence of a challenge. For example, a genetic variant could affect oxytocin in one manner under quiescent conditions or in the presence of positive stimuli, but behave very differently in the context of negative events. Ultimately, closely

examining the context under which certain genetic variants are assessed is key for delineating if, or to what extent, they might impact well-being.

### Chapter 4-Study 3

#### The Relation of an Oxytocin Polymorphism to Psychosocial and Hormonal Responses to Social Rejection

McQuaid, R. J., McInnis, O. A.<sup>3</sup>, Matheson, K., & Anisman, H. (2015). Distress of ostracism: oxytocin receptor gene polymorphism confers sensitivity to social exclusion. *Social cognitive and affective neuroscience*, nsu166.

Studies 1 and 2 revealed that individuals with oxytocin polymorphisms that are thought to be tied to lower oxytocin functioning and decreased prosocial behaviors, also appeared to be more likely to report negative mood in association with unsupportive social interactions. However, these studies examined the relations of oxytocin polymorphisms under baseline circumstances, which could differ substantially in other contexts, such as under a challenge or early-life adversity. Indeed, we have previously observed that G carriers of the OXTR polymorphism rs53576 who reported childhood maltreatment had greater levels of depressive symptoms than those with the AA genotype, suggesting that G carriers might be more socially sensitive (McQuaid et al., 2013). Thus, in Study 3 we extended the stress-oxytocin link beyond self-report measures and examined the association of the OXTR SNP to both psychosocial and hormonal responses following a laboratory-based manipulation of social ostracism.

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<sup>3</sup> Author contributions: R.J.M, O.A.M and H.A. contributed to the inception and design of the current experiment. Testing and data collection were performed by R.J.M. and O.A.M. Data analysis and the writing of the manuscript were performed by R.J.M., O.A.M., K.M., and H.A. All authors approved the final version of the paper for submission.

### **Abstract**

A single nucleotide polymorphism (SNP) on the oxytocin receptor gene (OXTR) involving a guanine (G) to adenine (A) substitution has been associated with altered prosocial features. Specifically, individuals with the GG genotype (i.e., the absence of the polymorphism) display beneficial traits including enhanced trust, empathy and self-esteem. However, because G carriers might also be more socially sensitive, this may render them more vulnerable to the adverse effects of a negative social stressor. The current investigation, conducted among 128 White female undergraduate students, demonstrated that relative to individuals with AA genotype, G carriers were more emotionally sensitive (lower self-esteem) in response to social ostracism promoted through an on-line ball tossing game (Cyberball). Furthermore, GG individuals also exhibited altered blood pressure and cortisol levels following rejection, effects not apparent among A carriers. The data support the view that the presence of the G allele not only promotes prosocial behaviors, but also favors sensitivity to a negative social stressor.

## Introduction

Oxytocin, a neuropeptide known for its role in childbirth, breastfeeding and infant-mother bonding (Gimpl and Fahrenholz, 2001), influences social behaviors, and might thus contribute to disorders, including autism, schizophrenia, anxiety and depressive disorders, which involve social disturbances (Feifel et al., 2012, Guastella, et al., 2010; Scantamburlo et al., 2007). Several single nucleotide polymorphisms (SNPs) have been identified on the oxytocin receptor gene (OXTR), but one in particular, rs53576, which involves a guanine (G) to adenine (A) substitution, seems particularly relevant to prosocial behaviors. Compared to A allele carriers (i.e., the polymorphism is present), individuals with two G alleles exhibit a range of favorable attributes, such as high levels of trust (Krueger et al., 2012), self-esteem (Saphire-Bernstein et al., 2011), empathy (Rodrigues et al., 2009; Smith et al., 2014), maternal sensitivity (Bakermans-Kranenburg and van Ijzendoorn, 2008) and may be more attune to social cues (Rodrigues et al., 2009). Individuals homozygous for the G allele also exhibited lower depressive symptoms compared to A carriers (Saphire-Berstein et al., 2009), and G carriers displayed higher positive affect (Lucht et al., 2009).

Although it is tempting to consider the G allele of the rs53576 SNP as advantageous and the A allele as a risk/vulnerability factor for negative mood states, this may be an overly simplistic view. In fact, in an African American sample comprising individuals who had experienced severe childhood maltreatment, those with the GG genotype (i.e., in the absence of the polymorphism) displayed greater disorganized attachments and increased emotional dysregulation compared to their A carrier counterparts (Bradley et al., 2011). In line with these findings, in the context of early-life maltreatment, G carriers displayed greater depressive scores than individuals with the AA

genotype (McQuaid et al., 2013). Together, these findings suggest that although the G allele may be associated with beneficial prosocial features, in some contexts, in other contexts as in the case of early-life adversity, the social sensitivity associated with the G allele may render individuals more vulnerable to behavioral disturbances. From this perspective, oxytocin might not just serve as a prosocial hormone, but might also influence the salience of or sensitivity to social cues, irrespective of whether these are positive or negative (Averbeck, 2010; Bartz et al., 2011).

In addition to affecting behavioral and emotional responses to stressors, the OXTR polymorphism has been associated with several physiological responses to stressors. Compared to A allele carriers, individuals with the GG genotype of the OXTR SNP displayed lower awakening salivary cortisol levels (Norman et al., 2012), and lower heart rate responses to an anticipatory startle stimulus (Rodrigues et al., 2009). However, in response to a psychosocial stressor, those with the GG genotype showed *greater* sympathetic reactivity (Norman et al., 2012) as well as increased sympathetic and subjective arousal when presented with stimuli showing others in distress (Smith et al., 2014). Although some of these findings are inconsistent with one another, it is possible that carrying a G allele may confer particular sensitivity to stressors involving a social component.

Ostracism is a powerful social stressor (Eisenberger, 2012; Williams, 2001) that induces strong negative emotions even when it occurs briefly (Williams et al., 2000). For instance, being ostracized within a virtual ball-tossing game, Cyberball, is accompanied by lower feelings of belonging, self-esteem, meaningful existence, and control (Zadro et al., 2004). It is of particular interest that social rejection in this context activates the same

neural pain networks, including the dorsal anterior cingulate cortex (dACC) and the insula, that are associated with bodily injury (Eisenberger et al., 2003, 2006). Given the contribution of oxytocin to social behaviors, it is possible that this hormone contributes to the processes underlying social rejection sensitivity. Indeed, in response to social ostracism elicited by participants being excluded from conversations, intranasal oxytocin reduced cortisol levels compared to placebo (Linnen et al., 2012) and increased self-perceived trust among those reporting negative mood (Cardoso et al., 2013).

As oxytocin administration modulates responses to social rejection, it might also be expected that OXTR rs53576 genotypes would influence reactions to social ostracism. In the current study we examined the OXTR SNP in relation to ostracism elicited by exclusion in a Cyberball game among a sample of White females. It was predicted that following rejection, G carriers would report more pronounced responses to ostracism, including lower belonging, control, self-esteem, and meaningful existence, which are influenced by ostracism (Williams, 1997, 2001). Further, if G carriers are more prosocial, it would be expected that compared to their AA counterparts, G carriers would judge their Cyberball co-players less harshly following rejection. Finally, it was predicted that G carriers would be physiologically more reactive to social stressors, displaying higher blood pressure and cortisol levels upon rejection compared to individuals with two A alleles.

## **Methods**

### ***Participants***

This study comprised 128 white female Carleton University undergraduate students with a mean age of 19.82 (standard deviation=3.86). The OXTR genotype could be determined for 126 individuals. A homogenous ethnic sample was used in this study as marked

cultural differences have been found in association with this OXTR SNP (i.e. Caucasians who have at least one G allele are more likely to seek emotional social support, an effect not found among Asian G carriers; Kim et al., 2010). Thus, because of population stratification, data were collected from non-white participants ( $n = 122$ ) but were not included in any analyses. The ethnicity of these participants included Black (32.5%,  $n=38$ ), Asian (21.4%,  $n=25$ ), other, (13.7%,  $n=16$ ), Arab (12.0%,  $n=14$ ), South Asian (10.3%,  $n = 12$ ), Latin American (5.1%,  $n = 6$ ) and Aboriginal (2.6%,  $n= 3$ ). It would have been of interest to assess the influence of genotype across different ethnic groups, but this was precluded owing to the small number of participants in each of the ethnic groups. The distributions of the OXTR genotypes vary substantially across ethnic groups. As listed in Table 1, for example, Black individuals and Asian individuals display the complete opposite OXTR genotype distributions. Further to this issue, not all three OXTR genotypes could even be represented in each ethnic group.

*Table 1. Oxytocin receptor gene polymorphism distributions by ethnicity*

Ethnicity	G/G	A/G	A/A
Caucasian ( $n = 126$ )	56	52	18
Black ( $n = 38$ )	25	13	0
Asian ( $n = 25$ )	3	12	10
Arab/West Asian ( $n = 14$ )	8	4	2
South Asian ( $n = 12$ )	3	5	4
Latin American/Hispanic ( $n = 6$ )	2	4	0
South East Asian ( $n = 3$ )	1	2	0
Aboriginal ( $n = 3$ )	1	1	1
Other ( $n = 15$ )	7	5	3

Participants were recruited from an online computerized recruitment system used by the university. Eighteen percent ( $n = 23$ ) of participants reported a family income of less than \$45,000, whereas almost half of participants reported a family income between \$45,000 and \$90,000 (44.5%,  $n = 57$ ) and 35.1% ( $n = 45$ ) reported a family income greater than \$90,000. Self-reported religion included Catholic (31.3%,  $n = 40$ ), Agnostic (23.4%,  $n = 30$ ), Protestant (20.3%,  $n = 26$ ), Atheist (16.4%,  $n = 21$ ), Other (5.5%,  $n = 7$ ), Buddhist (1.6%,  $n = 2$ ) and Jewish (0.8%,  $n = 1$ ).

### ***General Procedure***

All procedures in this study were approved by the Carleton University Ethics Committee for Psychological Research. Once informed consent was signed, participants provided a saliva sample for DNA genotyping using Oragene OG-500 collection kits

(DNA Genotek, Inc., Ottawa, Ontario, Canada). Participants were informed that the purpose of the study was to assess mental visualization through playing an online ball tossing game (Cyberball). Prior to beginning Cyberball, participants relaxed over a 20 min period, and also completed demographic information and a trait anxiety questionnaire. Once participants finished playing Cyberball, they completed several questionnaires including those assessing feelings of rejection and judgments regarding their Cyberball co-players. Saliva samples for cortisol assays and blood pressure measurements were obtained at baseline (20 minutes after arrival to the laboratory), as well as 15 and 30 minutes following Cyberball. Participants were then fully debriefed. Each session took up to 1.25 hr to complete.

### ***Cyberball task***

Cyberball is a well-established computerized game used to induce feelings of social rejection (Williams et al., 2000). Participants were tested individually, but were led to believe that they were playing with two other university students from other laboratories connected to the same server. In actuality, the other players did not exist and the game was computer simulated. As previously described (Williams et al., 2000), to increase the validity of Cyberball, prior to beginning, participants' pictures were taken and they were told that their pictures were uploaded onto the on-line server so that their two co-players would be able to see them, and photographs of two virtual players were shown to the participants throughout the game. Participants were randomly assigned to one of two conditions, inclusion or exclusion. In the included condition, participants passed and received a virtual ball an equal amount of times as other players throughout the game. In contrast, excluded participants received the ball twice at the beginning and

then never again. The game lasted approximately two and a half minutes for both conditions.

### ***Salivary Cortisol***

Saliva samples were collected in Salivette<sup>R</sup> tubes, (Sarstedt, Germany). Immediately following the test session, saliva samples, were frozen at -80°C. A commercial radioimmunoassay (RIA) kit (ICN Biomedicals Inc., Irvine, CA) was used to determine, in duplicate, salivary cortisol levels. The intra- and interassay variability was less than 10%.

### ***Genotyping***

Genomic DNA was extracted from the Oragene OG-500 collection kits according to the manufacturer's protocol and diluted to equal concentration of 20 ng/μL.

Quantitative polymerase chain reaction (qPCR) was used for genotyping. A total volume of 15 μl was used to perform the amplification reactions which contained approximately 1 μL (20 ng) of genomic template, 0.6 μL of each primer (concentration 10 μM), 1.2 μL of dNTP, 1.5 μL 10X Buffer, 1.5 μL of MgCl<sub>2</sub>, 0.3 μL of Salmon Sperm DNA, 0.15 μL of Taq polymerase, 0.015 of SYBR green and 8.135 μL of water. All q-PCR plates were run in duplicate and genotypes were called blind. All qPCR products were then electrophoresed on 2% agarose gel and visualized to confirm qPCR results. The Bio-Rad Iq5 Primer sequences used for qPCR included: OXTR F1 forward:

TCCCTGTTTCTGTGGGACTGAGGAC, OXTR F2 forward:

TCCCTGTTTCTGTGGGACTGAGGAT, OXTR reverse:

ACCCAAGAGGCTGGTTTGGGGTT.

The genotype distribution for the OXTR polymorphism was 56 individuals with the GG genotype, 52 GA individuals and 18 AA individuals. These distributions met the

expectations for Hardy-Weinberg Equilibrium,  $\chi^2(1) = 1.07, p = .30$ . We were not able to confirm an OXTR genotype for two individuals who were therefore excluded from any analyses including the OXTR genotype.

### ***Measures***

*Social Ostracism.* The Social Ostracism and Mood Scale (Williams, 2001; Zadro et al., 2004) was used to assess the effectiveness of the ostracism manipulation through questions such as, “what percentage of the throws were directed to you?” and, “to what extent you currently feel accepted or rejected?”. In addition, the questionnaire contained 11 items on a 9-point scale of 1 (not at all) to 9 (very much so) that assessed participants levels of four fundamental needs proposed by Williams (1997, 2001). These comprised: *belonging* (e.g. I felt like an outsider during the Cyberball game;  $\alpha = .78$ ), *control* (e.g. I felt in control during the Cyberball game;  $\alpha = .75$ ), *self-esteem* (e.g. I felt somewhat inadequate during the Cyberball game;  $\alpha = .79$ ), and *meaningful existence* (I felt non-existent during the Cyberball game, and I felt that my performance had some effect on the direction of the game;  $\alpha = .74$ ). Mean scores for each of the four needs was calculated.

*Co-player judgments.* Participants reported judgments about both of their Cyberball co-players on a scale of 1 (not at all) to 9 (very much so) on thirteen characteristics that included how likable, good, attractive, prejudiced, trustworthy, tolerant, arrogant, friendly, manipulative, fair, loyal, hypocritical, and to what degree they believed they were sell-outs. Ratings for each co-player were calculated together to obtain a mean score on each judgment.

*Anxiety Symptoms.* Trait anxiety levels were assessed by the Spielberger State-Trait Anxiety Inventory (STAI) (Speilberger, 1983). A 20-item trait anxiety scale was used to measure general anxiety symptoms before playing Cyberball, where participants responded to statements regarding how often they *generally* felt each feeling (e.g. nervous and restless) on a scale of 1 (almost never) to 4 (almost always). Total scores were calculated by summing across all items ( $\alpha = .95$ ).

### **Statistical Analyses**

Statistical analyses were performed using SPSS for Windows 18.0 (SPSS Science, Chicago, Illinois, USA). Analyses assessing initial differences on trait anxiety scores between Cyberball conditions as well as the Cyberball manipulation checks were performed using an independent samples t-test. Analyses assessing the social ostracism outcomes (i.e. belonging, control, self-esteem and meaningful existence) and co-player judgments were analyzed using 2 (Cyberball condition: excluded versus included) x 3 (OXTR genotype: GG, AG or AA) MANOVAs. For blood pressure scores, both a 2 (Cyberball condition) x 3 (OXTR genotype) x 3 (Time: 1 to 3 time-points) mixed measures ANOVA with Time serving as the within-group factor, and a 2 (Cyberball condition) x 3 (OXTR genotype) analyses of covariance was conducted, controlling for baseline blood pressure levels. Cortisol was analyzed using a 2 (Cyberball condition) x 3 (OXTR genotype) x 3 (Time: 1 to 3 time-points) mixed measures ANOVA with Time serving as the within-group factor. Follow-up comparisons comprised t-tests with a Bonferonni correction to maintain the alpha level at 0.05.

### **Results**

#### ***Psychosocial measures***

As expected, there were no initial differences on trait anxiety between OXTR genotype groups,  $F(2, 123) = .91, p = .40$  or Cyberball conditions,  $t(1, 126) = .31, p = .76$ . Following Cyberball, analyses of two manipulation checks revealed that participants who were excluded reported receiving the ball less than included participants,  $t(1, 83.99) = 23.65, p < .001$ , and participants in the ostracism condition reported feeling more rejected relative to their included counterparts,  $t(1, 125) = -11.42, p < .001$ .

A MANOVA revealed a significant difference in the four needs as a function of the Cyberball condition, Pillai's Trace  $F(4, 117) = 49.39, p < .001, \eta^2 = .63$ . Furthermore, there was a significant Cyberball x OXTR genotype interaction for the four needs, Pillai's Trace  $F(8, 236) = 2.82, p < .01, \eta^2 = .09$ . Individual ANOVAs revealed that irrespective of OXTR genotype, Cyberball exclusion significantly reduced feelings of belonging,  $F(1, 126) = 236.56, p < .001, \eta^2 = .65$ , and control,  $F(1, 126) = 171.15, p < .001, \eta^2 = .58$  (Figure 1A and 1B). There was a significant Cyberball x OXTR genotype interaction on meaningful existence,  $F(2, 120) = 3.74, p < .05, \eta^2 = .06$ . As shown in the follow-up analyses of the simple effects, depicted in Figure 1C, under conditions where participants had been included in the Cyberball game, self-reports of meaningful existence were lower among the AA carriers compared to AG ( $p < .001$ ) and GG individuals ( $p < .001$ ). However, following exclusion in the Cyberball game, meaningful existence diminished to a greater extent in the GG ( $p < .001$ ) and AG ( $p < .001$ ) genotypes than in those with the AA genotype ( $p < .05$ ), so that similar levels of meaningful existence were self-reported across the genotypes. The self-esteem profile was very much like meaningful existence but the Cyberball x OXTR genotype interaction was shy of significance,  $F(2, 120) = 2.67, p = .07, \eta^2 = .04$ . Nonetheless, follow-up tests of the simple effects based on *a priori*

predictions revealed that self-esteem was reduced among excluded individuals with the GG or AG genotype compared to their respective counterparts in the included condition,  $p$ 's < .001 (Figure 1D). In contrast, this difference was not evident among individuals who carried two A alleles.

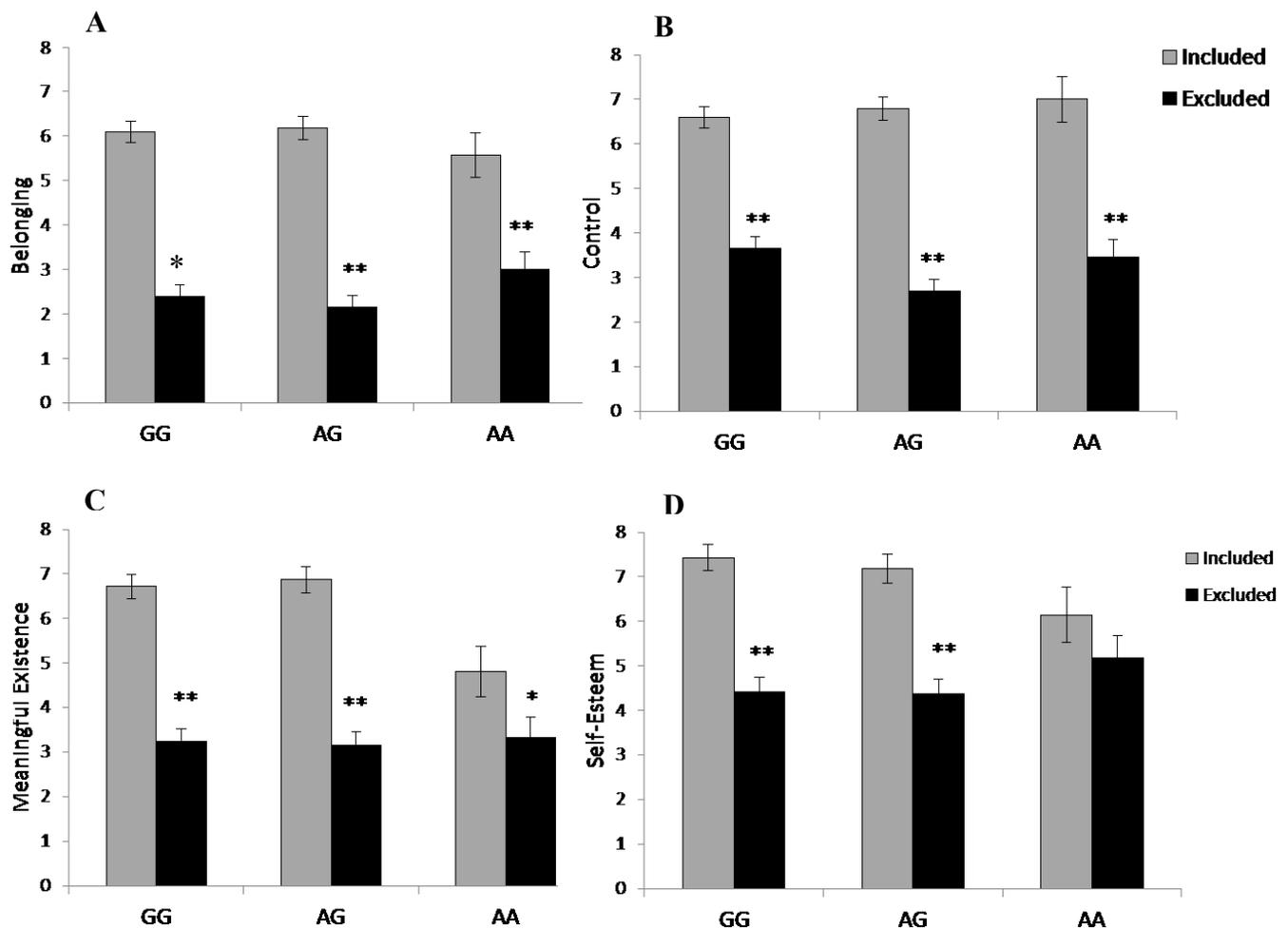


Figure 1. Feelings of belonging (A), control (B), meaningful existence (C) and self-esteem (D) among individuals with the GG, AG, or AA OXTR genotypes who were either included or excluded during the Cyberball game. Data represents means  $\pm$  S.E.M. \* $p < 0.05$ , \*\* $p < 0.001$  relative to included counterparts, and +  $p < 0.001$  relative to included GG and AG individuals.

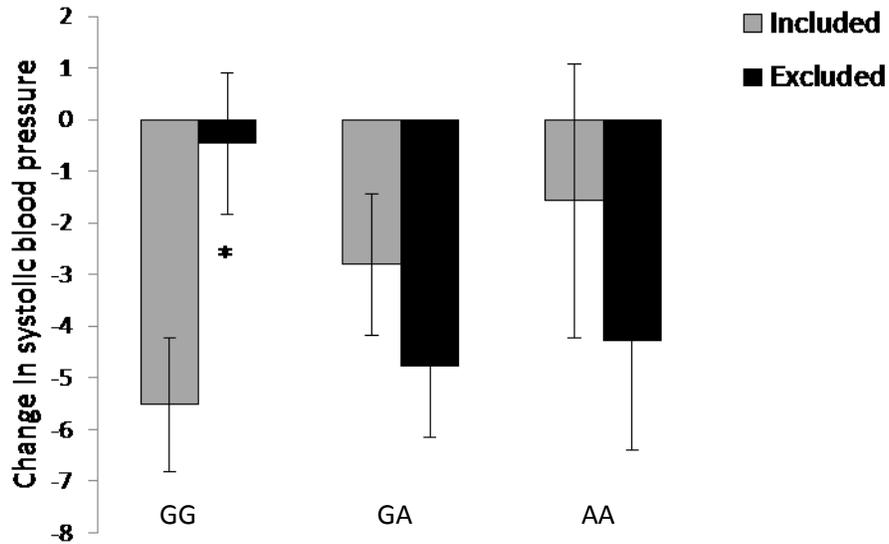
It was of interest to examine how being excluded would affect individual judgments concerning the Cyberball co-players, and to examine whether this occurred more readily in relation to a specific OXTR genotype. A MANOVA revealed a

significant difference in co-player judgments between excluded and included participants irrespective of OXTR genotype, Pillai's Trace  $F(13, 108) = 8.21, p < .001, \eta^2 = .50$ . Individual ANOVAs revealed that excluded participants viewed their co-players as less likeable,  $F(1,120) = 68.42, p < .001, \eta^2 = .36$ , good,  $F(1,120) = 35.00, p < .001, \eta^2 = .22$ , trustworthy,  $F(1,120) = 20.44, p < .001, \eta^2 = .15$ , tolerant,  $F(1,120) = 25.30, p < .001, \eta^2 = .17$ , friendly,  $F(1,120) = 53.04, p < .001, \eta^2 = .31$ , fair,  $F(1,120) = 95.38, p < .001, \eta^2 = .44$ , and loyal,  $F(1,120) = 6.12, p < .05, \eta^2 = .05$ , as well as more prejudiced,  $F(1,120) = 14.65, p < .001, \eta^2 = .11$ , arrogant,  $F(1,120) = 34.59, p < .001, \eta^2 = .22$ , manipulative,  $F(1,120) = 12.33, p < .01, \eta^2 = .09$ , hypocritical,  $F(1,120) = 8.90, p < .01, \eta^2 = .07$ , and were more likely report them as sell-outs,  $F(1,120) = 15.67, p < .001, \eta^2 = .12$  compared to included counterparts. Despite the negative opinion of their co-players, the ostracized participants were not more likely to describe them as less attractive compared to participants who were included,  $F(1, 126) = 2.90, p = .09$ . In effect, the participants' negative views were limited to personality characteristics of their co-players, but not their physical appearance.

### ***Physiological measures***

Prior to the Cyberball session, systolic blood pressure differences were not apparent as a function of OXTR genotypes,  $F(2,120) = 0.61, p = .54$ , or the Cyberball conditions,  $F(1,120) = 0.15, p = .70$ . Systolic blood pressure varied as a function of Cyberball condition x OXTR genotype over Time,  $F(4, 238) = 2.53, p < .05$ . Upon examining the follow-up analyses comprising this effect, blood pressure levels for included GG individuals declined across the session ( $p < .001$ ), an effect not apparent among the AG ( $p = .13$ ) or AA ( $p = 1.0$ ) genotypes. Following exclusion, systolic blood

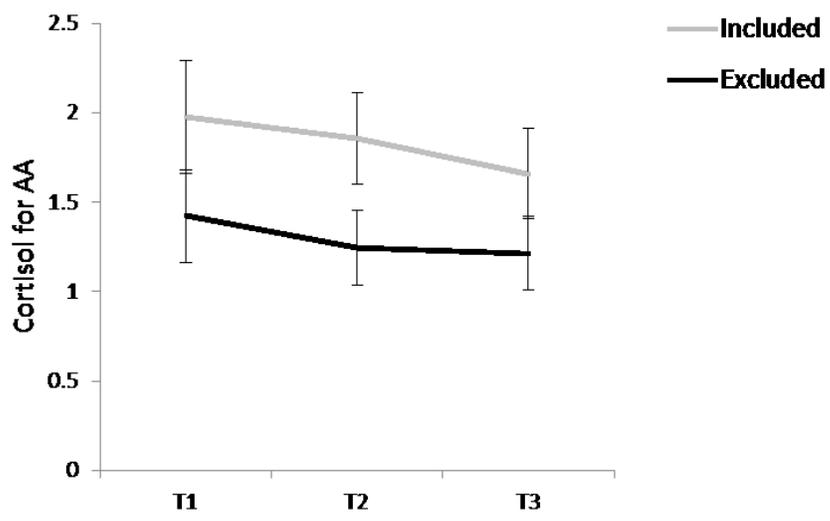
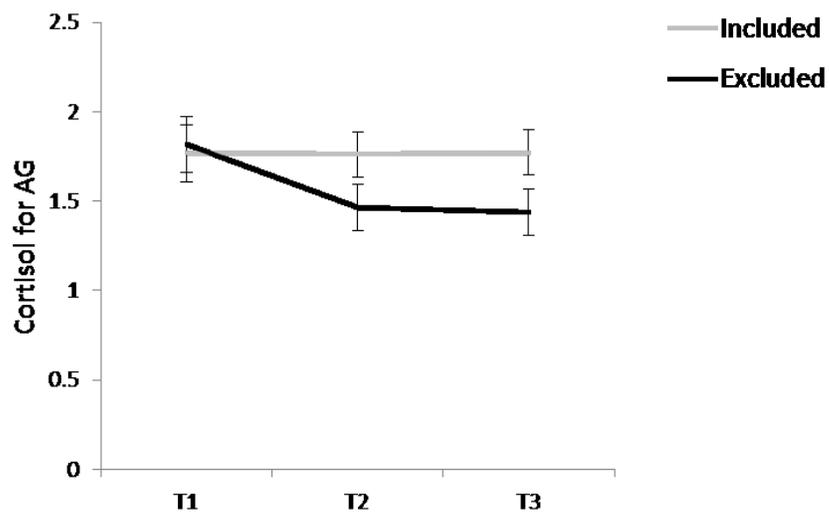
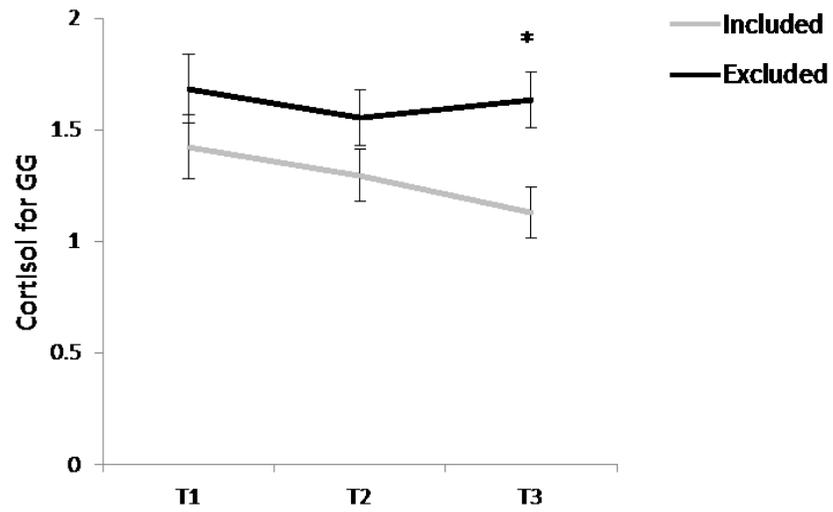
pressure among individuals with the GG genotype remained elevated and thus did not change as a function of time, ( $p = 1.0$ ). In contrast, individuals with the AG genotype had blood pressure scores that declined over the session, ( $p < .01$ ). Among individuals with the AA genotype blood pressure declined somewhat over the session, but this effect was not significant ( $p = .14$ ), likely owing to the limited power associated with the small number of AA individuals. A follow-up examining systolic blood pressure 30 minutes after Cyberball (controlling for baseline levels), varied as a function of the OXTR genotype x Cyberball interaction,  $F(2,118) = 4.14, p < .05, \eta^2 = .07$ . As depicted in Figure 2 and confirmed by the follow-up tests, among excluded individuals with the GG genotype, systolic blood pressure was elevated relative to that of individuals in the included condition during Cyberball ( $p < .01$ ). In contrast to the effect of exclusion among GG individuals, a comparable effect of exclusion was not apparent among AG ( $p = .29$ ) or AA individuals ( $p = .54$ ). This said, among those with the AA genotype a large amount of variability was evident, likely owing to the small number of individuals in this group. Unlike systolic blood pressure, diastolic blood pressure did not vary as a function of the OXTR genotype x Cyberball conditions.



*Figure 2.* Systolic blood pressure levels collected 30 minutes following either inclusion or exclusion during the Cyberball game (controlling for baseline systolic blood pressure) among individuals with the GG, AG or AA OXTR genotypes. Data represents means  $\pm$  S.E.M. \* $p < 0.01$  relative to included GG individuals.

The number of cigarettes smoked, current medications including oral contraceptives, time of day and waking time did not influence cortisol and thus these variables were not controlled for in subsequent analyses. Although cortisol levels are sensitive to some laboratory stressors, such as the Trier Social Stress Test (TSST: Kirschbaum et al., 1993), which involves public speaking and mental arithmetic in front of a small audience, the levels of cortisol typically do not increase appreciably following exclusion in the Cyberball situation (Seidel et al., 2013; Zöller et al., 2010; Zwolinski, 2012). However, in the present study it was of interest to determine whether cortisol would vary with genotype. Consistent with earlier findings, relative to baseline, cortisol levels did not vary as a function of the Cyberball condition,  $F(2, 111) = 0.53, p = .57$ , but instead declined over the course of the session,  $F(2, 111) = 4.40, p < .05, \eta^2 = .04$ . The analyses also revealed a significant Cyberball x OXTR genotype effect,  $F(2, 112) = 4.82, p = .01, \eta^2 = .08$ , such that individuals with the GG genotype that had been excluded during Cyberball displayed cortisol levels that exceeded those of included participants ( $p < .05$ ). In contrast, among those carrying an A allele, Cyberball exclusion did not significantly influence cortisol levels and, in fact, cortisol in those that were excluded were marginally lower than those in the included condition. Given the *a priori* hypothesis that the effects of the Cyberball manipulation would vary by genotype over the course of the session (i.e., baseline vs. the post-testing period), follow up tests were conducted to assess whether the effects of the Cyberball manipulation and genotype interaction further varied as a function of the time of saliva sampling. As shown in Figure 3, analyses of the simple effects revealed that among those with the GG genotype who were in the included condition within the Cyberball game, cortisol tended to decline over the course of the

session. In contrast, among the GG individuals who had been in the exclusion condition, cortisol levels did not decline over the course of the session and as a result the cortisol levels in this group significantly exceeded that in the included counterparts at T3 ( $p < .01$ ). In contrast to the effect seen in those with the GG genotype, among the AG and AA individuals, these differences between groups were not evident, and there was no indication of elevated cortisol among individuals who had been excluded in the Cyberball game relative to those individuals who were in the included condition.



*Figure 3.* Cortisol levels in saliva ( $\mu\text{g}/\text{dl}$ ) collected at three time points including before Cyberball (T1), 15 minutes following Cyberball (T2) and 30 minutes following Cyberball (T3). The graph represents individuals with the GG genotype (top panel), AG genotype (middle panel) and AA genotype (bottom panel) who were either included or excluded during the Cyberball game. Data represents means  $\pm$  S.E.M.  $*p < 0.05$  relative to included GG individuals.

## **Discussion**

As expected, individuals with one or two copies of the G allele could, in several ways, be distinguished from those with the AA genotype. In the absence of ostracism, individuals with the AA genotype tended to express low meaningful existence relative to G carriers. The idea that AA individuals generally feel that their presence matters less is in line with reports showing that they tend to have a more negative disposition comprising poor affect and low optimism (Saphire-Bernstein et al., 2011). When individuals were rejected in the Cyberball game, however, those carrying the G allele exhibited a more pronounced decline in their feeling that their presence in the game mattered (meaningful existence). This effect was less prominent among individuals with the AA genotype because they had lower levels of meaningful existence in the included condition in the absence of a manipulation.

As previously reported (Saphire-Bernstein et al., 2011), although individuals with the AA genotype tended to express low levels of self-esteem, they were not especially sensitive to rejection in the Cyberball game. In contrast, individuals carrying the G allele showed a decline of self-esteem upon being ostracized, potentially reflecting the elevated sensitivity of G carriers in response to a social stressor. The other two dimensions of

needs described by Williams (2001), feelings of belonging and control, were also affected by ostracism, irrespective of genotype, and thus all individuals perceived the rejection accurately, reflected by the lower levels of belonging and control, but the degree to which this impacted their sense of self (i.e. self-esteem) was limited in the AA individuals.

The behavioral outcomes were in line with the physiological responses, suggesting that individuals with the GG genotype were more reactive to ostracism. When individuals with the GG genotype were excluded within the Cyberball game, their systolic blood pressure was elevated relative to that of their included counterparts. This difference, however, was not apparent among AG or AA individuals who experienced ostracism, just as individuals with the GG genotype displayed greater sympathetic reactivity to a psychosocial stressor (Norman et al., 2012). However, individuals with the GG genotype also display less sympathetic reactivity in response to a non-social stressor (Rodrigues et al., 2009). Thus, it is possible that GG carriers might only be more reactive to stressors of a social nature.

As previously reported (Seidel et al., 2013, Zöller et al., 2010; Zwolinski, 2012), in the current investigation, exclusion in the Cyberball game did not elicit a cortisol rise, and in the main cortisol levels declined over the course of the session. However, among ostracized individuals with the GG genotype the decline of cortisol was not apparent so that 30 min following Cyberball cortisol levels were greater among ostracized participants than among those in the included condition. This effect, , was not apparent among ostracized AG or AA individuals, reinforcing the perspective that the GG individuals are sensitive to social insults, whereas this sensitivity may be limited in the presence of the polymorphism. These findings are very much in line with the perspective

that genetic variants associated with greater interpersonal sensitivity result in increased reactions to social exclusion in the form of enhanced neural activity in the dACC and anterior insula (Eisenberger et al., 2007).

It is interesting that individuals with the AG genotype displayed psychosocial responses similar to GG carriers, but physiological reactivity like that of AA carriers. Although this might seem surprising, oxytocin interacts with other hormones and neurotransmitter systems, and it is likely that different outcomes or behaviors (i.e. psychosocial responses versus physiological reactivity) involve these diverse interactions (McQuaid et al., 2014). For instance, oxytocin may interact with mesolimbic dopamine functioning so that the rewarding attributes of particular stimuli take on greater salience (Love, 2014), and oxytocin also influences amygdala activity (Kirsch et al., 2005; Petrovic et al., 2008), possibly through actions on  $\gamma$ -aminobutyric acid (GABA), so that fear reactions are altered (Huber et al., 2005). The divergent outcomes related to oxytocin interactions with other hormones in the context of specific behaviors among those who are heterozygous regarding the OXTR polymorphism, speaks to the importance of examining the three OXTR genotypes separately whenever possible.

Several beneficial traits have been observed among G carriers; yet, it was also proposed that individuals with this genotype might be more sensitive to their environments (Bradley et al., 2011; McQuaid et al., 2013). In this regard, individuals with one or two copies of the G allele displayed greater emotional dysregulation (Bradley et al., 2011) and depressive symptoms (McQuaid et al., 2013) in the context of high levels of early-life maltreatment. Conversely, G carriers displayed higher positive affect and resilience if they were raised in a warm family environment (Bradley et al., 2013).

These findings are congruent with the view that certain genotypes confer greater plasticity in the context of both positive and negative environmental stimuli, thereby affecting behavior ‘for better or for worse’ (Belsky et al., 2009). However, the data supporting this view have not been unanimous. For instance, youth with at least one A allele and raised with a depressed mother, experienced particularly high levels of depressive symptoms at age 15 (Thompson et al., 2014). Maternal depression certainly might offer a negative environment, although this may not necessarily be equivalent to experiencing maltreatment in the form of abuse and/or neglect, which likely constitutes a breach of trust that might have a greater impact on G carriers (McQuaid et al., 2013).

There are several limitations of the current study that should be acknowledged. Although we and others have suggested that individuals with the G allele of the OXTR rs53576 SNP may be more socially sensitive, possibly owing to the oxytocin system operating differently than in AA individuals, the functionality of this particular SNP is still unknown. It has been hypothesized that this OXTR SNP, which is located on intron 3, may be involved in transcriptional suppression (Mizumoto et al., 1997), but it may also be that the effects observed in the current investigation were due to linkage(s) with other functional OXTR SNPs (Lin et al., 2007). In addition, the sample size in the current study was modest, and it certainly would have been ideal to have greater power through a larger number of AA participants. Despite these limitations, the current findings suggested that individuals with the GG genotype, who are typically viewed as having many beneficial traits, were emotionally and biologically more affected by ostracism. At the same time, even in the face of this brief rejection from unknown co-players, ostracized participants tended to judge them harshly, irrespective of their oxytocin genotype. Evidently,

regardless of their genotype, individuals are able to recognize slights experienced, but in line with our previous suggestion (McQuaid et al., 2013), those with the GG genotype for this OXTR SNP are more adversely affected by negative social experiences. The current findings provide support for the view that oxytocin functioning, besides promoting prosocial behaviors, might also enable higher social sensitivity or reactivity to social challenges. In this regard, it has been suggested (Cardoso et al., 2014) that treatment with an oxytocin nasal spray might enhance mood state among some individuals, but others may engender excessive sensitivity, rendering individuals more vulnerable to the negative impacts of social stressors. Knowledge of an individual's genotype might be useful as a biomarker to determine vulnerability to adverse effects of social stressors and might be useful in predicting the efficacy of treatment options.

### Chapter 4-Study 3-Supplementary Analysis

#### An Examination of the Contribution of the CD38 polymorphism in Association with Responses to Social Ostracism

In addition to examining responses following social ostracism among individuals carrying the OXTR variant, it was of interest to examine the contribution of a polymorphism on the CD38 gene. This is a transmembrane glycoprotein broadly expressed on immune cells, such as macrophages and lymphocytes, and additionally serves as a regulator of central oxytocin release (Jin et al., 2007). In mice Peripheral and central levels of oxytocin are reduced and social processes are impaired when this gene is deleted (Jin et al., 2007). Likewise, higher circulating levels of CD38 gene expression in humans were related to higher plasma oxytocin levels (Kiss et al., 2011).

A SNP rs3796863 located in intron 7 on the CD38 gene involves a cytosine (C) to adenine (A) substitution (Malavasi et al., 2008). Among healthy individuals, the CC genotype was associated with lower plasma oxytocin levels (Feldman et al., 2012), and among males, those homozygous for the C allele displayed deficits in the processing of social stimuli (Sauer et al., 2012). It was reported that risk of autism spectrum disorder (ASD) was increased among C carriers of this polymorphism (Munesue et al., 2010), and among those with greater ASD severity, the C allele was associated with decreased CD38 expression (Lerer et al., 2010). However, not all reports support the view that the C allele of the CD38 polymorphism acts as a vulnerability factor to negative behavioral and emotional outcomes. In this regard, among older adolescent A carriers, chronic interpersonal stress was subsequently related to higher symptoms of social anxiety and depression compared to adolescents

with the CC genotype. This finding is in line with the perspective that higher oxytocin promotes greater sensitivity to negative events (Tabak et al., 2015). Thus, the purpose of this additional analysis was to examine whether CD38 rs3796863 would be associated with greater sensitivity to social rejection, as was observed with the OXTR rs53576 polymorphism.

### ***Psychosocial measures***

There were no initial differences on trait anxiety as a function of the CD38 genotype<sup>4</sup>,  $F(2, 102) = 0.39, p = .69$ . Upon examining the four needs in response to the Cyberball manipulation, a MANOVA revealed a significant difference in these needs as a function of the Cyberball condition, Pillai's Trace  $F(4, 93) = 42.91, p < .001, \eta^2 = .65$  and CD38 genotype, Pillai's Trace  $F(8, 188) = 2.65, p < .01, \eta^2 = .10$ . As previously described, individual ANOVAs revealed that Cyberball exclusion significantly reduced feelings of belonging,  $F(1, 102) = 153.46, p < .001, \eta^2 = .62$ , control,  $F(1, 102) = 88.98, p < .001, \eta^2 = .48$ , self-esteem  $F(1, 102) = 48.50, p < .001, \eta^2 = .34$  and meaningful existence,  $F(1, 102) = 104.23, p < .001, \eta^2 = .52$ . However, there was no Cyberball x CD38 genotype interaction on the four needs, Pillai's Trace  $F(8, 188) = 1.18, p = .31, \eta^2 = .05$ .

Irrespective of Cyberball condition, the CD38 polymorphism was associated with differences in belonging,  $F(2, 102) = 8.37, p < .001, \eta^2 = .15$ , self-esteem  $F(2, 102) = 4.43, p < .05, \eta^2 = .08$ , meaningful existence,  $F(2, 102) = 7.67, p < .01, \eta^2 = .14$ , but not control,  $F(2, 102) = 12.33, p = .09, \eta^2 = .05$ . Specifically, those with the AA genotype reported higher self-esteem compared to those with the CC ( $p < .05$ ) or CA ( $p < .05$ )

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<sup>4</sup> The distribution of CD38 genotypes were as follows: CC= 56, AC = 34, AA = 12. The  $N$  for this additional analysis was smaller 102 versus 126 in the OXTR analysis.

genotype. Likewise, those with the AA genotype reported higher feelings of belonging and meaningful existence than individuals with the CC ( $p$ 's < .01) or CA ( $p$ 's < .01) genotype. There were no differences on judgments of co-players as a function of CD38 polymorphism, Pillai's Trace  $F(24, 172) = 1.36, p = .13$ , nor was there a significant CD38 genotype x Cyberball condition interaction, Pillai's Trace  $F(24, 172) = 0.56, p = .95$ .

### ***Physiological measures***

As with the OXTR polymorphism, systolic blood pressure at baseline did not differ as a function of the CD38 genotype,  $F(2, 102) = 0.12, p = .61$ . In contrast to what was observed with the OXTR polymorphism, systolic blood pressure did not vary as a function of Cyberball condition x CD38 genotype over time,  $F(2, 196) = 0.85, p = .43$ , nor did it differ upon examining the change score (i.e., T3 minus T1)  $F(2, 101) = .07, p = .93$ . Assessment of cortisol revealed a Cyberball x CD38 genotype interaction which approached significance,  $F(2, 89) = 2.72, p = .07, \eta^2 = .06$ . As with the OXTR analysis, follow up tests were conducted to assess whether the effects of the Cyberball manipulation and genotype interaction varied as a function of the time of saliva sampling. In contrast to the difference observed at T-3 (30 minutes following Cyberball) with the OXTR polymorphism, there was a difference observed at T-2 for the CD38 polymorphism. Specifically, among those with the AC genotype, being excluded was associated with higher cortisol at 15 minutes following Cyberball, compared to their included counterparts ( $p < .05$ ). In contrast, this difference was not observed among the CC or AA genotypes.

## **Conclusion**

The results of this analysis indicated that the CD38 polymorphism may be related to higher feelings of social rejection. Curiously, this was the case irrespective of whether individuals were excluded or included in the Cyberball game. Specifically, C carriers of this polymorphism reported lower levels of self-esteem, meaningful existence and belonging. These findings are somewhat in line with previous reports which have indicated that individuals with the C allele might be at greater risk for negative emotional outcomes potentially due to lower oxytocin functioning (Feldman et al., 2012). These findings are also partially in line with what was observed when examining the OXTR polymorphism in relation to feelings of rejection. However, in contrast to what was observed with the OXTR polymorphism, the analyses were not suggestive of a social sensitivity effect for the A allele of the CD38 polymorphism. There were very few individuals with the AA genotype, and thus it is possible that with a greater sample size a sensitivity effect might have become apparent. Finally, it is curious that heightened cortisol was observed in response to rejection in the CD38 heterozygotes and not in those with the AA alleles. There have been reports of heterozygotes of other oxytocin-related polymorphisms demonstrating heightened sensitivity to adverse events and thus the present findings might not be particularly unusual (Thompson et al., 2011).

## **Chapter 5-Study 4**

### **Coping, Oxytocin and Responses to Social Support**

The preceding studies examined the relations between oxytocin-related genetic polymorphisms and factors such as support, unsupport, coping and depressive symptoms. Study 3 revealed that the G allele of the OXTR polymorphism rs53576 was associated with greater reactions to experimentally manipulated ostracism. The purpose of Study 4 was to examine how endogenous levels of plasma oxytocin relate to support, coping and depressive affect. Moreover, given the potential stress-buffering effect that oxytocin might have on stress reactivity, it was also an aim of Study 4 to determine how this hormone might influence responses to a psychosocial stressor, specifically the Trier Social Stress Test (TSST). As well, there has been some suggestion that oxytocin may have an additive effect on attenuating stressor responses when combined with social support. Thus, Study 4 also examined the influence of having a close friend present prior to undergoing the TSST on psychosocial responses to stress, and whether plasma oxytocin levels were related differently to such responses depending on the presence or absence of support.

## **Introduction**

Social support can serve as a protective factor against the negative impacts of stressors and may thereby promote well-being. In this regard, greater levels of social support reduced the risk of experiencing a relapse of a depressive episode among those with major depression (Cruwys et al., 2014). It has been suggested that social support might buffer against depressive symptoms by influencing neuroendocrine activity in response to stressors. By example, the presence of social support attenuated cortisol responses to a psychosocial stressor in the form of the Trier Social Stress Test (TSST; Ditzen et al., 2008), as well as neural activity in brain regions associated with distress (Eisenberger et al., 2007). Despite the consistent links between social support and well-being, the underlying mechanisms through which support enacts its effects remain largely unknown.

Oxytocin is a neuropeptide that has been implicated in modulating stressor responses and the effects of social support. It has been suggested, that through its interactions with neurotransmitter, immune and neuroendocrine factors, oxytocin can moderate responses to stress and as a result, might thus contribute to psychological disorders, such as depression (McQuaid et al., 2014). By example, administration of oxytocin via a nasal spray was associated with lower cortisol levels in response to a physical stressor, compared to those given placebo (Cardoso, et al., 2013). Likewise, intranasal oxytocin also attenuated the cortisol rise associated with social rejection (Linnen et al., 2012) and couple conflict (Ditzen, et al., 2009). Moreover, in the presence of social support, oxytocin may have additive effects. In this regard, men who were given oxytocin and had a close friend present prior to undergoing the TSST, had lower levels of

cortisol in response to the stressor than those given support or oxytocin alone (Heinrichs et al., 2003). Despite these findings, a meta-analysis reported that intranasal oxytocin was not significantly associated with lower cortisol levels in response to laboratory stressors, although oxytocin did attenuate cortisol among clinical populations and under conditions where the stressor provoked a strong HPA-axis response (Cardoso et al., 2014).

While social support can be an important coping resource in the face of a stressor, other approaches to coping may also influence psychological outcomes. Although coping strategies are not intrinsically adaptive or maladaptive, depressive symptoms are frequently tied to the endorsement of lower problem- and higher emotion-focused coping (Matheson and Anisman, 2003). Specifically, factors such as rumination (Aldao et al., 2010) and emotional containment (Ravindran et al., 2002) have been linked to depression. As well, lower levels of methods that often are linked to problem-focused coping, such as support seeking (Matheson and Anisman, 2003) and cognitive restructuring (Ravindran et al., 2002), have been associated with negative mood outcomes. Few studies have examined the relationship between different forms of coping and oxytocin; however, among women who were high in emotion-focused coping, intranasal oxytocin reduced anxiety in response to an interpersonal stressor (Cardoso et al., 2012). Further, a single nucleotide polymorphism (SNP) rs53576 on the oxytocin receptor gene has been tied to higher emotion- and lower problem-focused coping in response to unsupportive social interactions (McInnis et al., 2015).

In light of the already established linkages, the present investigation was conducted to examine the relations between, coping, depressive symptoms, social support and oxytocin in response to a psychosocial stressor among female undergraduate

students. It was predicted that higher plasma oxytocin levels at baseline would be associated with greater problem- and lower emotion-focused coping strategies in response to the TSST. However, these relations would be most evident among those who receive social support. Additionally, social support would moderate the relationship between depressive symptoms at baseline and coping strategies in response to the TSST. Specifically, those with high depressive symptoms would report higher problem- and lower emotion-focused strategies in the presence of social support compared to those with no social support.

## **Methods**

### ***Participants***

Female undergraduate students ( $N = 67$ ) from Carleton university were recruited using an online system (SONA). Participants ages ranged from 17-30 years ( $M_{age} = 19.37$ ,  $SD = 2.08$ ). Reported ethnicity of participants was White (50.7%,  $n = 34$ ), Black (19.4%,  $n = 13$ ), Arab/West Asian (9.0%,  $n = 6$ ) Asian (6.0%,  $n = 4$ ), Latin American/Hispanic (4.5%,  $n = 3$ ), South Asian (2.0%,  $n = 3$ ), South East Asian (1.5%,  $n = 1$ ), Aboriginal (1.5%,  $n = 1$ ), and other (1.0%,  $n = 3$ ). Demographic information was also collected from a separate group of individuals who participated as friends of those participants who were assigned to the social support group ( $n = 18$ ). The mean age of these participants was 18.78 ( $SD = 1.17$ ; range: 17-21 years) and they comprised an ethnically diverse sample the majority of whom were White (61.1%,  $n = 11$ ), with the remainder reporting various ethnicities including, Black (16.7%,  $n = 3$ ), Asian (5.6%,  $n = 1$ ), South Asian (5.6%,  $n = 1$ ), South East Asian (5.6%,  $n = 1$ ), and other (5.6%,  $n = 1$ ).

### ***General Procedure***

Ethical approval for the research was obtained from the Carleton University Ethics Committee for Psychological Research. Participant eligibility was determined using an online prescreening survey to determine whether inclusion criteria were met. Specifically, participants were excluded if they reported a history of problems related to blood draws, such as nausea, fainting, dizziness and fear. As well, participants were ineligible if they reported use of medications or a current medical condition that could influence hormone functioning. The online screening questionnaire also asked whether participants would be able to attend the laboratory session with a close female friend. Participants who met eligibility criteria were then randomly assigned to one of three conditions, control ( $n = 26$ ), stress-no support ( $n = 23$ ) and stress-social support in which participants brought a friend with them to the laboratory session ( $n = 18$ ). To help reduce selection bias, those participants who indicated they could bring a close females friend were randomly assigned to one of the three conditions, including that in which no friend was present.

### *Laboratory Session*

A more detailed description of the laboratory session can be found in (McQuaid et al., 2015, see Appendix D in this document). Briefly, prior to arriving to the laboratory, women were asked not to eat, drink or smoke for 1 hour before their scheduled session. The experimental procedures were completed between 1300 and 1730 hr. Following the receipt of written informed consent, participants were asked to complete several questionnaires including demographic information that assessed several factors, particularly those that could influence hormone levels (e.g., medications, menstrual cycle, smoking etc). Once the participant had completed these measures, a registered nurse

inserted a catheter into the participant's arm for the blood draw. This was then followed by a relaxation period of 10 minutes to allow participants to acclimate their surroundings.

Upon completion of the relaxation period, those that were assigned to the stressor conditions were informed that as part of an employment task, they would be asked to perform a five-minute speech for a panel of judges, as well as complete a five-minute mental arithmetic task for the judges and the task would be videotaped. Participants were given 10-minutes to prepare for the speech. Those that were assigned to stress-support group were able to have their friend present in the room and assisting them during the 10-minute preparatory period, whereas the stress-no support groups prepared alone. Prior to entering the experimental room, the friends were instructed to provide support in the best way they knew how for the participant. Once the preparatory period was completed, participants in both stressor conditions underwent the TSST alone. Participants assigned to the control condition completed a written employment task where they were asked to describe previous job/volunteer experience on a form. Psychosocial measures, such as coping strategies were completed following the control task or TSST.

Blood samples were continuously drawn using a Dakmed ambulatory pump. Samples were collected at five time-points were used for samples analysis, which comprised, 15 minutes prior to the TSST or control task, immediately preceding the stressor/task, and five, 15 and 30 minutes following the TSST or control task. The blood draw rate was increased at time points of interest for analysis.

### ***Measures***

*Depression.* The Beck Depression Inventory (BDI; Beck, Mendelson, Mock, & Erbaugh, 1961) consists of 21-items measuring depressive symptoms. Higher values are indicative

of greater severity of depressive symptoms. Questions are structured in a manner that assesses a particular aspect of depression such as sadness or anhedonia. Total scores were calculated by summing across all items (Cronbach's  $\alpha = .88$ ).

*Coping.* To assess strategies participants use to cope, the shortened 27-item version of the Survey of Coping Profile Endorsement (SCOPE; Matheson & Anisman, 2003) was used. This assessed emotion-focused, problem-focused, and avoidance-focused coping strategies in response to the laboratory stressor. Each question was rated on a five-point Likert scale, ranging from never (0), to almost always (4). The underlying factor structure was determined using a principal component analysis (PCA) with a varimax rotation. Three factors were determined that comprised emotion-, avoidant- and problem-focused coping. Emotion-focused coping comprised rumination, emotional expression, blaming others, and self-blame (Cronbach's  $\alpha = .84$ ). Avoidance coping comprised, cognitive distraction, passive resignation, emotional containment and wishful thinking (Cronbach's  $\alpha = .74$ ). Problem-focused coping comprised cognitive restructuring, problem solving, active distraction, humor and social support seeking (Cronbach's  $\alpha = .87$ ).

#### *Blood Collection*

Continuous blood samples were collected at a low draw rate. Chilled serum separator and EDTA coated vacutainer tubes were used to collect serum and plasma, respectively. Blood samples for oxytocin analysis were collected in separate chilled EDTA tubes in which aprotinin was added to diminish degradation. The blood draw rate was increased at time points of interest for analysis. Specifically, at 15 minutes prior to the TSST or the control task, immediately preceding the stressor or control task, and at five, 15 and 30 minutes following. Subsequently, samples were centrifuged at 4°C and

2100g for 15 minutes. Plasma and serum were aliquoted into Eppendorf tubes and stored at -80°C.

#### *Plasma Oxytocin*

All oxytocin samples were extracted following the procedures outlined by Enzo Life Science Inc., (Farmingdale, NY). Per sample, 1mL of plasma was evaporated using nitrogen gas, and the samples were then stored at -20°C prior to assay. Plasma concentrations of oxytocin were determined in accordance with manufacturer's instructions using an ELISA kit from Enzo Life Science Inc. Intra-assay variability was less than 12%.

#### **Statistical Analyses**

Statistical analyses were conducted using IBM SPSS Statistics 20 for Windows software (Armonk, NY: IBM Corp.). Statistical significance was determined at  $p < 0.05$  (two-tailed). A univariate analysis of variance (ANOVA) was conducted to determine whether baseline depressive symptoms varied across the stressor groups. Multivariate analysis of variance (MANOVA) was conducted to determine whether stressor conditions differed on coping strategies endorsed following the stressor. Hierarchical regression analyses were performed to determine whether relations between depressive symptoms and coping strategies in response to stress were moderated as a function of support individual's received. As well, hierarchical regressions were conducted to determine whether the relations between baseline oxytocin and coping strategies were moderated by support condition. Statistically significant moderations were probed further using a web utility for simple slopes (Preacher et al., 2006). Standardized scores were used in all

regression analyses. Associations between baseline oxytocin and coping strategies were analyzed using Pearson product moment correlations.

## Results

As anticipated, baseline depressive symptoms did not vary as a function of the subsequent stressor condition,  $F(2, 64) = .21, p = .81$ . Similarly, no difference between baseline oxytocin were observed across these conditions  $F(2, 64) = .64, p = .53$ .

Additionally, a MANOVA indicated no differences across the stressor conditions with respect to levels of endorsement of problem-, emotion- or avoidance-focused coping following the TSST, *Pillai's Trace*  $F(6, 126) = 1.126, p = .35$ .

The presence of social support prior to the stressor experience moderated the relation between depressive symptoms and problem-focused coping strategies,  $\Delta R^2 = 0.11, \Delta F(1, 40) = 5.17, p < 0.05$ . The analysis also revealed that perceived depressive symptoms were related to lower problem-focused strategies in response to the psychosocial stressor,  $b = -0.60, t = -3.00, p < 0.01$ , and this was moderated by the support condition,  $b = 0.61, t = 2.27, p < 0.05$ . Simple slopes analyses revealed that the relation between higher depression at baseline and lower problem-focused coping in response to the stressor was only significant among those with no social support (Figure 1). Support condition did not moderate the relation between depressive symptoms and emotion- or avoidance-focused coping strategies,  $\Delta R^2 = 0.06, \Delta F(1, 40) = 0.01, p = .97$ , and  $\Delta R^2 = 0.08, \Delta F(1, 40) = 0.73, p = .40$ , respectively.

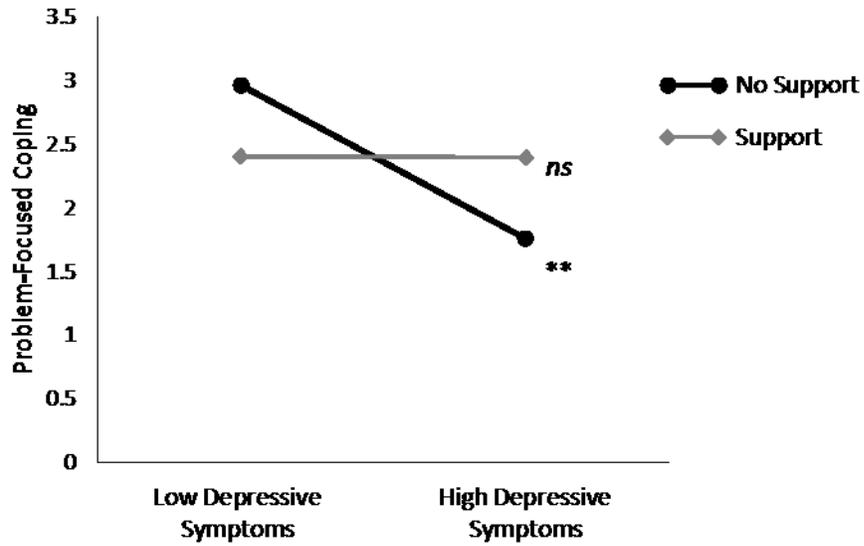


Figure 1. The relationship between depressive symptoms and problem-focused coping as moderated by support condition. Higher reported depressive symptoms prior to undergoing the TSST were associated with the endorsement of lower problem-focused strategies, but only among those without support,  $**p < .01$ .

Contrary to prediction, support condition did not moderate the relation between baseline oxytocin and problem-focused coping,  $\Delta R^2 = 0.00$ ,  $\Delta F(1, 36) = 0.10$ ,  $p = .76$ , emotion-focused coping,  $\Delta R^2 = 0.00$ ,  $\Delta F(1, 36) = 0.01$ ,  $p = .92$ , or avoidance-focused coping,  $\Delta R^2 = 0.00$ ,  $\Delta F(1, 36) = 0.00$ ,  $p = .99$ . However, as it was of interest to determine whether oxytocin was associated with specific strategies (e.g., support seeking, problem-solving, rumination etc.) correlations between baseline oxytocin and the various coping methods were analyzed across the groups separately. As seen in Table 1, among those in the stressor condition without support, higher oxytocin was related to higher self-reported cognitive distraction,  $r = .45$ ,  $p < .05$ , and humor  $r = .49$ ,  $p < .05$ , as well as a trend towards increased social support seeking  $r = .40$ ,  $p = .06$ . Individuals who had social support present, higher baseline oxytocin was associated with greater problem solving,  $r = .50$ ,  $p < .05$ , whereas in the control (no stress) condition, lower oxytocin was associated with higher self-blame  $r = .44$ ,  $p < .05$  and lower humor  $r = -.53$ ,  $p < .05$ .

*Table 1.* Pearson correlations between baseline oxytocin and coping strategies, as well as depressive symptoms

	Baseline Oxytocin		
	Control	Stress-No Support	Stress-Support
Rumination	-.17	.13	.26
Emotional expression	.03	.12	.01
Blaming others	-.05	.30	-.09
Self-blame	-.44*	-.26	-.07
Cognitive distraction	-.19	.45*	.23
Passive resignation	.19	.08	-.15
Emotional containment	-.15	.24	.07
Wishful thinking	-.08	.17	.30
Cognitive restructuring	.15	.17	.34
Problem solving	.21	.27	.50*
Active distraction	.12	.11	.10
Humor	-.53*	.49*	.24
Social support seeking	.04	.40 <sup>+</sup>	.44

\* $p < .05$ , <sup>+</sup> $p = .06$

## Discussion

As expected, in the current study, higher depressive symptoms prior to the TSST were accompanied by the endorsement of lower problem-focused coping strategies in response to the psychosocial stressor; however, in the presence of social support this relationship was absent. Previous studies reported that depressive symptoms were often accompanied by lower problem-focused strategies (Matheson and Anisman, 2003; Ravindran et al., 2002). Further, social support can act as a potent stress buffer against some of the negative impacts of stressors (Cohen and Wills, 1985) and it is possible that this might partially occur through the promotion of more effective coping methods (McInnis et al., 2015). Moreover, social connectedness can be particularly beneficial for individuals with depressive disorders (Cruwys et al. 2014; Ditzen et al., 2008), and as such, the findings of the current study are not all that surprising.

As depicted in Figure 1, at low levels of depressive symptoms, participants in the support condition endorsed fewer problem-focused coping strategies than those without support. It is possible that those with a close friend present might have relied more heavily on them as a coping resource rather than endorsing the use of other strategies. However, given the correlational nature of the current study it is uncertain whether this is the case. It was somewhat surprising that the social support condition did not impact the relation between depressive symptoms and emotion- or avoidance-focused coping strategies, although it was uncertain why this was the case. This said, it is conceivable that variations of problem-focused coping associated with social support could reduce the need for variations of other coping methods.

Support condition did not moderate the association between baseline oxytocin and problem-, emotion-, and avoidance-focused coping. However, because oxytocin genetic variants might be associated with the endorsement of specific strategies during times of distress (e.g., support seeking; Chen et al., 2011), it was of interest to further examine associations between oxytocin and the various strategies employed in response to the TSST. In fact, baseline oxytocin was associated with certain coping strategies in response to the stressor and these relations differed across the stressor conditions. Although the effects were small, it is curious that higher oxytocin was tied to the use of cognitive distraction, humor and support seeking in the absence of support but these same relations were not present among those in the support condition. Indeed, among those with support, higher oxytocin was only associated with greater problem-solving. It is not known why oxytocin was related to these particular strategies, but not others. Although entirely speculative, it is possible that factors, such as humor and support seeking, might be tied to the hormone's ability to promote prosocial behaviors, and in the presence of support, participants did not need to rely on such methods. Among those with support, higher oxytocin was only associated with greater endorsements of problem-solving. This finding is in line with the perspective that oxytocin might favor more advantageous coping when faced with social stressors (McInnis et al., 2015), as well as the view that support in combination with oxytocin could promote better responses to stress (Heinrichs et al., 2003).

There are several limitations that should be considered when interpreting the current findings. The sample size was relatively small, particularly given the number of variables assessed. Additionally, it would have been ideal to include a group in which

support was available without a stressor manipulation. This would have allowed for an understanding of how oxytocin and support influence coping strategies in the absence of a stressor. Furthermore, given the correlational nature of the study, it is uncertain to what extent the coping strategies were directly tied to oxytocin, or whether oxytocin was related to third variable that had not been assessed (e.g., appraisals).

Taken together, the social support manipulation appeared not to attenuate the commonly found relation between depressive symptoms and less advantageous coping methods. Thus, social interventions were linked to enhanced use of problem-focused coping methods when experiencing a stressful event. Although the findings are preliminary, they are consistent with view that oxytocin might be important in stress responses, potentially acting through certain coping processes.

## General Discussion

As repeatedly indicated, most investigators have taken the view that oxytocin plays a fundamental role in relation to social functioning. However, in this regard, several different perspectives have been offered concerning the actions of oxytocin, although it should be said that these might not be mutually exclusive, possibly being context-dependent. One of the earliest views was that oxytocin was integral in promoting prosocial behaviors, particularly in women in whom oxytocin was critical for bonding, and also contributed to a ‘tend and befriend’ characteristic (Taylor et al., 2010). A somewhat similar position was adopted, but oxytocin in males was seen as a basic feature of parochial altruism so that oxytocin contributed to a ‘tend and defend’ characteristic in which individuals high in oxytocin would favor their own group and defend against incursions by others (De Dreu et al., 2010). Yet another perspective, that we and others have adopted (Bartz et al., 2011; Bradley et al., 2011; McQuaid et al., 2013; McQuaid et al., 2015) is that oxytocin increases sensitivity or reactivity to social stimuli irrespective of their valence. Essentially, in the presence of high oxytocin levels or a well functioning oxytocin system, positive social events would have a greater impact and at the same time, negative social experiences would also take on greater significance. The presently reported findings provide support for each of these views, but to a considerable extent these are dependent upon the situation in which individuals are assessed.

The view that certain polymorphisms represent vulnerability factors, while others may serve as protective factors that limit negative outcomes, is likely an over generalization concerning the role of specific genetic variants in mood outcomes. In this regard, Studies 1, 2, and 3 demonstrated the difficulty of determining the specific conditions under which, for example, alleles linked to higher oxytocin would be

protective, or would be associated with enhanced sensitivity to negative environments. Specifically, Study 1 indicated that A carriers of the OXTR SNP (those with presumably low oxytocin functioning) reported greater depressive symptoms in relation to unsupportive social interactions and this appeared to be mediated through less effective coping methods. Similarly, Study 2 indicated that both the CD38 and OXTR alleles that are associated with lower oxytocin were tied to greater negative affect in relation to unsupportive relationships. However, in this same study, individuals with a CD38 genotype linked to higher oxytocin also reported greater perceptions of unsupportive interactions, possibly suggesting a social sensitivity effect. In line with the social sensitivity view of oxytocin, Study 3 demonstrated that an acute manipulation of social rejection was related to greater psychosocial and physiological responses among those with the OXTR GG genotype. Yet, when additional analyses were conducted to determine associations of the CD38 polymorphism on responses to social ostracism, a social sensitivity effect was not readily apparent. Although the findings of these studies are intriguing, the lack of simplicity in determining the associations of oxytocin-related polymorphisms to responses to social stimuli and mood outcomes in turn raises the question as to the utility of assessing these polymorphisms in determining well-being.

Although the relationships observed in Studies 1-3 are complex, it is possible that other factors might account for the differential linkages observed, which might be helpful in clarifying the contribution of particular oxytocin polymorphisms to mood outcomes. In this regard, the ways in which individuals with certain polymorphisms cope with social situations could be an explanatory factor in these varying associations. Indeed, in Study 1, the OXTR SNP was tied to higher emotion-focused and lower problem-focused coping

in association with unsupportive relationships, which were linked to greater depression scores. These findings suggest that those with the GG genotype might endorse more effective coping under certain conditions. In line with this view, it has been reported that those homozygous for the G allele tend to seek more social support during times of distress (Kim et al., 2010) and G carriers were more responsive to the stress-buffering effects of social support (Chen et al., 2011). It could be that the G allele promotes more effective social coping methods, which is not contrary to the view that they may also be more socially sensitive. By example, although it was observed in Study 2 that individuals with the G allele were more responsive to social rejection, they might also have been more likely to seek social support following the laboratory session, which could have favoured more beneficial outcomes to the experience of ostracism.

Across this collection of studies, it is apparent that context is critical in determining the outcomes associated with certain oxytocin genetic variants. It should be acknowledged, however, that this is not exclusive to polymorphisms linked to the oxytocin system; the importance of environmental and contextual factors has been demonstrated in relation to many other polymorphisms tied to various neurobiological systems (McInnis et al., 2015, in Appendix A). For instance, Caspi et al (2003) reported that individuals who carried a mutation on the serotonin transporter gene (in which the gene was short) were at a greater risk for later depression provided that they had experienced either early-life or more recent life stressors. Other studies, such as those conducted in animals have demonstrated that various stressors can confer diverse brain neurochemical alterations, and even similar responses to stressors might result from very different underlying mechanisms (Merali et al., 2008; Morrow et al., 2000; Moutney et

al., 2011). In fact, slight variations in stressor characteristics and experiences could favour very distinct outcomes. Thus, it shouldn't be surprising that a given polymorphism might be tied to more negative outcomes in the context of unsupportive relationships, but yet not in the context of ostracism or childhood adversity. Attempting to paint certain genotypes with the same wide brush will likely not be conducive to delineating their role in social and emotional processes. Moreover, as described previously, many of the social and emotional variables assessed in these studies are complex and as such likely involve multiple systems in addition to oxytocin, such as CRH, DA, 5-HT and NE (McQuaid et al., 2014), which may further contribute to the number of diverse outcomes which can emerge from various stressors.

In addition to oxytocin genetic variants, circulating levels of oxytocin might also be important in determining stress-related outcomes. Indeed, several animal studies have suggested that endogenous oxytocin may attenuate responses to stressors (Nomura et al., 2003; Bülbül et al., 2011; Zheng et al., 2010). Additionally, there is evidence suggesting that oxytocin could be important in buffering against the negative impact of stressors in humans (Cardoso et al., 2013; Ditzen et al., 2009; de Oliveria et al., 2011). However, no studies have examined whether levels of oxytocin are associated with specific stress-coping methods. In Study 4, we observed that women with higher oxytocin reported greater problem-solving if they also had social support; however, among women without support present, oxytocin was related to other coping methods, such as cognitive distraction, support seeking and humor. Such findings suggest that oxytocin may enact some of its stress-buffering effects through its relation to coping processes. It would have been ideal to understand the interplay between oxytocin levels, oxytocin genetic variants

and coping methods in response to stressors. This is particularly the case as there is evidence that individuals with the G allele of the OXTR polymorphism might be more responsive to social support (Chen et al., 2011). As well, findings in Study 1 which indicated that those with GG genotype also endorsed more effective coping methods suggests that the OXTR genotypes, as well as the CD38 polymorphism, could have been important contributors to the relationships observed in Study 4. However, to examine these polymorphisms in such a small mixed ethnic sample across the three stressor conditions would have been impractical.

One factor which varied across Studies 1-3 was whether the three genotypes were examined separately, or were collapsed such that certain allele carriers were grouped together for analysis and compared to the dominant homozygote group. This is an important issue to acknowledge, as there may be unique contributions of each genotype making a 'dominant model' approach to analysis inappropriate in certain cases. In this regard, in Study 3 it was observed that the heterozygotes of the OXTR polymorphism (AG) aligned with individuals with the GG genotype when psychosocial indices of ostracism were examined, but the heterozygotes responded physiologically to ostracism in a way that was more similar to those with the AA genotype. Moreover, in the supplementary analysis examining the CD38 gene, only the heterozygotes (CA), displayed higher cortisol in relation to being socially excluded compared to their socially included counterparts. Such findings highlight the importance of analyzing the three genotypes separately. However, many studies which have examined oxytocin and other genetic variants, have cited the collapsing across genotype groups to be premised on previous studies that chose this approach. If such a strategy had been chosen in Study 3,

relevant effects might have been missed or interpreted differently. It is recognized that in higher level statistical models, such as moderated mediations, a trichotomous variable could not have been included in this type of statistical analysis. However, in recognition of the potential differences across the three genotypes, in Studies 1 and 2 orthogonal contrasts were conducted to ensure that the heterozygotes did not differ from the homozygotes prior to collapsing.

In Studies 1-3 only White participants were included in the analyses conducted. Across the studies, the OXTR and CD38 genotype distributions varied wildly depending on ethnicity. This is not unusual having consistently been documented in relation to many polymorphisms (Gelernter et al., 1997). Indeed, among Black and White individuals the GG genotype is quite common (even more so in Black individuals) and the AA genotype is relatively rare (less than 15%), whereas among Asians these distributions are flipped, such that the AA genotype is common and the GG genotype is rare. These variations in genotype distributions vary yet again depending on the specific genetic variant examined. Specifically, when examining the CD38 polymorphism the CC genotype was more common among Asian and White individuals, whereas the AA genotype was more common among Black individuals. Thus, it is problematic for studies to examine genes in association with behavioral and emotional factors, as ethnicity may become a proxy for genotype and could impact the ability to distinguish the effects of the polymorphism from effects related to ethnicity. Because of this, the findings of studies which have included a mixed ethnic sample should be interpreted cautiously.

Unfortunately, across Studies 1-3 there were an insufficient number of participants in any one ethnic group other than those who identified as White to examine

other ethnicities separately. Examining the impact of ethnicity on the gene associations assessed would have been very informative. For example, it was observed that in relation to the OXTR polymorphism, American individuals who were G carriers reported greater social support seeking in response to stress in comparison to those with the AA genotype. However, among Koreans with the G allele this relationship was not apparent and it was suggested that this could have been due to cultural influences, such that support seeking is not the social norm among Koreans, as it is among Americans (Kim et al., 2010). Such findings, underscore the importance of examining cultural differences when assessing the contribution of genetic variants.

### **Limitations and conclusions**

As outlined in each of the chapters, findings should be interpreted cautiously given several limitations which exist across each of Sothe studies. In particular, the studies examining relationships between genetic variants and behavioural and emotional outcomes were often cross-sectional, and in some cases involved a relatively small sample size for gene association studies. Because of this, the results should be viewed as preliminary, pending replication, Furthermore, it would have been profitable to have examined the contribution of gender and/or sex as a moderator in these studies, particularly given that oxytocin has been shown to promote different behavioral effects in males and females (Taylor et al., 2010). To be sure, in Studies 1 and 2, Sex x Gene interactions were examined and none were evident on the factors assessed; however, males comprised a relatively small proportion of participants in each of these studies and as such firm conclusions cannot be drawn as to whether or not sex is an important contributor to the polymorphism associations observed. As well, the choice to examine

the single SNPs on the OXTR and CD38 genes were largely predicated on previous studies which had linked them to social behaviors and mood outcomes. There are many polymorphic sites on both of these genes, and would it be informative to examine multiple SNPs simultaneously to determine genetic risk profiles in relation to environment and in turn, mood outcomes, such as depression.

There remain many gaps in our understanding of the contribution of the genetic polymorphisms examined on outcomes such as negative affective states. Moreover, the implications of these findings for clinical conditions, such as major depression or other subtypes of depressive disorders are uncertain. Indeed, the social sensitivity perspective of oxytocin functioning might suggest that among certain individuals with depressed mood, oxytocin could be beneficial through promoting more positive perceptions of social networks, whereas in others it may favour greater negative interpretations of social situations (Cardoso et al., 2014). Ultimately when examining the potential efficacy of intranasal oxytocin as a primary or adjunct treatment for depressive disorders it might be advantageous to adopt an individualized approach, whereby oxytocin genetic variants are also taken into consideration when predicting clinical outcomes.

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## Appendix A (Measures)

### *Beck Depression Inventory (BDI)*

On this questionnaire are groups of statements. Please read the entire group of statements in each category. Then pick out ONE statement in that group which best describes the way you feel. Check off the number beside the statement you have chosen.

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1. \_\_\_ 0 = I do not feel sad  
\_\_\_ 1 = I feel sad or blue  
\_\_\_ 2a = I am blue or sad all of the time and I can't snap out of it  
\_\_\_ 2b = I am so sad or unhappy that it is very painful  
\_\_\_ 3 = I am so sad or unhappy that I can't stand it
  
2. \_\_\_ 0 = I am not particularly pessimistic or discouraged about the future  
\_\_\_ 1 = I feel discouraged about the future  
\_\_\_ 2a = I feel I have nothing to look forward to  
\_\_\_ 2b = I feel I won't every get over my troubles  
\_\_\_ 3 = I feel that the future is hopeless and things cannot improve
  
3. \_\_\_ 0 = I do not feel like a failure  
\_\_\_ 1 = I feel I have failed more than the average person  
\_\_\_ 2a = I feel I have accomplished very little that is worthwhile or that means anything  
\_\_\_ 2b = As I look back on my life, all I can see is a lot of failures  
\_\_\_ 3 = I feel I am a complete failure as a person

4. \_\_\_ 0 = I am not particularly dissatisfied  
\_\_\_ 1a = I feel bored most of the time  
\_\_\_ 1b = I don't enjoy things the way I used to  
\_\_\_ 2 = I don't get satisfaction out of anything anymore  
\_\_\_ 3 = I am dissatisfied with everything
5. \_\_\_ 0 = I don't feel particularly guilty  
\_\_\_ 1 = I feel bad or unworthy a good part of the time  
\_\_\_ 2a = I feel quite guilty  
\_\_\_ 2b = I feel bad or unworthy practically of the time now  
\_\_\_ 3 = I feel as though I am very bad or worthless
6. \_\_\_ 0 = I don't feel I am being punished  
\_\_\_ 1 = I have a feeling that something bad may happen to me  
\_\_\_ 2 = I feel I am being punished or will be punished  
\_\_\_ 3a = I feel I deserve to be punished  
\_\_\_ 3b = I want to be punished
7. \_\_\_ 0 = I don't feel disappointed in myself  
\_\_\_ 1a = I am disappointed in myself  
\_\_\_ 1b = I don't like myself  
\_\_\_ 2 = I am disgusted with myself  
\_\_\_ 3 = I hate myself
8. \_\_\_ 0 = I do not feel I am any worse than anybody else

\_\_\_ 1 = I am very critical of myself for my weaknesses or mistakes

\_\_\_ 2a = I blame myself for everything that goes wrong

\_\_\_ 2b = I feel I have many bad faults

9. \_\_\_ 0 = I don't have thoughts of harming myself

\_\_\_ 1 = I have thoughts of harming myself but I would not carry them out

\_\_\_ 2a = I feel I would be better off dead

\_\_\_ 2b = I have definite plans about committing suicide

\_\_\_ 2c = I feel my family would be better off if I were dead

\_\_\_ 3 = I would kill myself if I could

\*Individuals who respond 2a or greater will receive an additional debriefing.

10. \_\_\_ 0 = I don't cry anymore than usual

\_\_\_ 1 = I cry more now than I used to

\_\_\_ 2 = I cry all the time now. I can't stop it

\_\_\_ 3 = I used to be able to cry but now I can't cry at all even though I want to

11. \_\_\_ 0 = I am no more irritable than usual

\_\_\_ 1 = I am more irritable than usual

\_\_\_ 2 = I am much more irritable than usual

\_\_\_ 3 = I am irritable all the time

12. \_\_\_ 0 = I have not lost interest in other people

\_\_\_ 1 = I am less interested in other people than I used to be

\_\_\_ 2 = I have lost most of my interest in other people and I have little feeling for them

\_\_\_ 3 = I have lost all my interest in other people and don't care about them at all

13. \_\_\_ 0 = I make decisions about as well as ever  
\_\_\_ 1 = I am less sure of myself now and try to put off making decisions  
\_\_\_ 2 = I can't make decisions anymore without help  
\_\_\_ 3 = I can't make decisions at all anymore
14. \_\_\_ 0 = I don't feel I look any worse than I used to  
\_\_\_ 1 = I am worried that I am looking old or unattractive  
\_\_\_ 2 = I feel that there are permanent changes in my appearance and they make me  
look unattractive  
\_\_\_ 3 = I feel that I am ugly or repulsive looking
15. \_\_\_ 0 = I can work about as well as before  
\_\_\_ 1a = It takes extra effort to get started at doing something  
\_\_\_ 1b = I don't work as well as I used to  
\_\_\_ 2 = I have to push myself very hard to do anything  
\_\_\_ 3 = I can't do any work at all
16. \_\_\_ 0 = I can sleep as well as usual  
\_\_\_ 1 = I wake up more tired in the morning than I used to  
\_\_\_ 2 = I wake up 1-2 hours earlier than usual and find it hard to get back to sleep  
\_\_\_ 3 = I wake up early every day and can't get more than 5 hours sleep
17. \_\_\_ 0 = I don't get any more tired than usual  
\_\_\_ 1 = I get tired more easily than I used to

\_\_\_ 2 = I get tired from doing anything

\_\_\_ 3 = I get too tired to do anything

18. \_\_\_ 0 = My appetite is no worse than usual

\_\_\_ 1 = My appetite is not as good as it used to be

\_\_\_ 2 = My appetite is much worse now

\_\_\_ 3 = I have no appetite at all any more

19. \_\_\_ 0 = I haven't lost much weight, if any, lately

\_\_\_ 1 = I have lost more than 5 pounds

\_\_\_ 2 = I have lost more than 10 pounds

\_\_\_ 3 = I have lost more than 15 pounds

20. \_\_\_ 0 = I am no more concerned about my health than usual

\_\_\_ 1 = I am concerned about aches and pains or upset stomach or constipation or other unpleasant feelings in my body

\_\_\_ 2 = I am so concerned with how I feel or what I feel that it's hard to think of much else

\_\_\_ 3 = I am completely absorbed in what I feel

21. \_\_\_ 0 = I have not noticed any recent change in my interest in sex

\_\_\_ 1 = I am less interested in sex than I used to be

\_\_\_ 2 = I am much less interested in sex now

\_\_\_ 3 = I have lost interest in sex completely

***Unsupportive Social Interactions Inventory (USII)***

Think about times you have talked to **your friends** about events in your life during the past month. Please circle the appropriate answer in regards to how much of the following responses you have received from others.

	<b>None</b>				<b>A lot</b>
Would not seem to want to hear about it.	0	1	2	3	4
Would refuse to take me seriously.	0	1	2	3	4
Would change the subject before I wanted to.	0	1	2	3	4
Would refuse to provide the type of help or support I was asking for.	0	1	2	3	4
When I was talking about it, the person wouldn't give me enough time, or would make me feel like I should hurry.	0	1	2	3	4
Would discourage me from expressing feelings such as anger, hurt or sadness.	0	1	2	3	4
Would not seem to know what to say, or would seem afraid of saying or doing the "wrong" thing.	0	1	2	3	4
Would seem to be telling me what he or she thought I wanted to hear.	0	1	2	3	4
From voice tone, expression, or body language, I would get the feeling he or she was uncomfortable talking about it.	0	1	2	3	4
Would try to cheer me up when I was not ready to.	0	1	2	3	4
Would respond with uninvited physical touching	0	1	2	3	4

(e.g., hugging).

Would do things for me that I would want to do and  
could do myself. 0 1 2 3 4

Would feel that I should stop worrying about the  
event and just forget about it. 0 1 2 3 4

Would tell me to be strong, to keep my chin up,  
or that I should not let it bother me. 0 1 2 3 4

Would feel that I should focus on the present or  
the future and that I should forget about what has  
happened and get on with my life. 0 1 2 3 4

Would feel that it could have been worse or was  
not as bad as I thought. 0 1 2 3 4

Would say that I should look on the bright side. 0 1 2 3 4

Would feel that I was overreacting. 0 1 2 3 4

Would ask “why” questions about my role in the  
event. 0 1 2 3 4

Would make “Should or shouldn’t have” comments  
about my role in the event. 0 1 2 3 4

Would tell me that I had gotten myself into the  
situation in the first place, and now must deal  
with the consequences. 0 1 2 3 4

Would blame me, or try to make me feel responsible  
for the event. 0 1 2 3 4

Would make "I told you so" or similar comments.	0	1	2	3	4
Would seem to be disappointed in me.	0	1	2	3	4

Think about times you have talked to **your parents** about events in your life during the past month. Please circle the appropriate answer in regards to how much of the following responses you have received from others.

	<b>None</b>				<b>A lot</b>
Would not seem to want to hear about it.	0	1	2	3	4
Would refuse to take me seriously.	0	1	2	3	4
Would change the subject before I wanted to.	0	1	2	3	4
Would refuse to provide the type of help or support I was asking for.	0	1	2	3	4
When I was talking about it, the person wouldn't give me enough time, or would make me feel like I should hurry.	0	1	2	3	4
Would discourage me from expressing feelings such as anger, hurt or sadness.	0	1	2	3	4
Would not seem to know what to say, or would seem afraid of saying or doing the "wrong" thing.	0	1	2	3	4
Would seem to be telling me what he or she thought I wanted to hear.	0	1	2	3	4
From voice tone, expression, or body language, I would get the feeling he or she was uncomfortable talking about it.	0	1	2	3	4
Would try to cheer me up when I was not ready to.	0	1	2	3	4
Would respond with uninvited physical touching (e.g., hugging).	0	1	2	3	4

Would do things for me that I would want to do and could do myself.	0	1	2	3	4
Would feel that I should stop worrying about the event and just forget about it.	0	1	2	3	4
Would tell me to be strong, to keep my chin up, or that I should not let it bother me.	0	1	2	3	4
Would feel that I should focus on the present or the future and that I should forget about what has happened and get on with my life.	0	1	2	3	4
Would feel that it could have been worse or was not as bad as I thought.	0	1	2	3	4
Would say that I should look on the bright side.	0	1	2	3	4
Would feel that I was overreacting.	0	1	2	3	4
Would ask “why” questions about my role in the event.	0	1	2	3	4
Would make “Should or shouldn’t have” comments about my role in the event.	0	1	2	3	4
Would tell me that I had gotten myself into the situation in the first place, and now must deal with the consequences.	0	1	2	3	4
Would blame me, or try to make me feel responsible for the event.	0	1	2	3	4
Would make “I told you so” or similar comments.	0	1	2	3	4

Would seem to be disappointed in me.

0 1 2 3 4

### *Social Provisions Scale*

In answering the next set of questions, please think about your current relationships with your **friends**. Please indicate to what extent each statement describes your current relationships with other people (i.e., in the past few weeks). Use the following scale to indicate your opinion.

<u>STRONGLY DISAGREE</u>	<u>DISAGREE</u>	<u>AGREE</u>	<u>STRONGLY AGREE</u>
1	2	3	4

So, for example, if you feel a statement is very true of your current relationships with your friends, you would respond with a 4 (strongly agree). If you feel a statement clearly does not describe your relationships with your friends, you would respond with a 1 (strongly disagree).

	<b>Rating</b>
1. Are there friends you can depend on to help you, if you really need it	_____
2. Do you feel you could <u>not</u> turn to your friends for guidance in times of stress?	_____
3. Are there friends who enjoy the same social activities that you do?	_____
4. Do you feel personally responsible for the well-being of your friends?	_____
5. Do you feel your friends do <u>not</u> respect your skills and abilities?	_____
6. If something went wrong, do you feel that <u>none</u> of your friends would come to your assistance?	_____
7. Do your relationships with your friends provide you with a sense of emotional security and well being?	_____
8. Do you feel your competence and skill are recognized by your friends?	_____
9. Do you feel <u>none</u> of your friends share your interests and concerns?	_____
10. Do you feel <u>none</u> of your friends really rely on you for their well-being?	_____
11. Is there a trustworthy friend you could turn to for advise, if you were having problems?	_____
12. Do you feel you <u>lack</u> emotional closeness with your friends?	_____

In answering the next set of questions, please think about your current relationships with your **parents**.

STRONGLY DISAGREE                      DISAGREE                      AGREE                      STRONGLY AGREE

1

2

3

4

**Rating**

1. Can you depend on your parents to help you, if you really need it? \_\_\_\_\_
2. Do you feel you could not turn to your parents for guidance in times of stress? \_\_\_\_\_
3. Do your parents enjoy the same social activities that you do? \_\_\_\_\_
4. Do you feel personally responsible for the well-being of your parents? \_\_\_\_\_
5. Do you feel your parents do not respect your skills and abilities? \_\_\_\_\_
6. If something went wrong, do you feel that your parents would not come to your assistance? \_\_\_\_\_
7. Does your relationship with your parents provide you with a sense of emotional security and well-being? \_\_\_\_\_
8. Do you feel your competence and skill are recognized by your parents? \_\_\_\_\_
9. Do you feel your parents do not share your interests and concerns? \_\_\_\_\_
10. Do you feel your parents do not really rely on you for their well-being? \_\_\_\_\_
11. Could you turn to your parents for advice, if you were having problems? \_\_\_\_\_
12. Do you feel you lack emotional closeness with your parents? \_\_\_\_\_

***Survey of Coping Profiles Endorsed- Style***

The purpose of this questionnaire is to find out how people deal with their problems or the stresses in their lives. The following are activities that you may have done. After each activity, please indicate the extent to which you would use this as a way of dealing with stresses in recent weeks.

---

Ordinarily, in recent weeks have you:	<b>Never</b>	<b>Seldom</b>	<b>Sometimes</b>	<b>Often</b>	<b>Almost always</b>
1. Accepted that there was nothing you could do to change your situation?	0	1	2	3	4
2. Tried to just take whatever came your way?	0	1	2	3	4
3. Talked with friends or relatives about your problems?	0	1	2	3	4
4. Tried to do things which you typically enjoy?	0	1	2	3	4
5. Sought out information that would help you resolve your problems?	0	1	2	3	4
6. Blamed others for creating your problems or making them worse?	0	1	2	3	4
7. Sought the advice of others to resolve your problems?	0	1	2	3	4
8. Blamed yourself for your problems?	0	1	2	3	4
9. Exercised?	0	1	2	3	4
10. Fantasized or thought about unreal things (e.g., the perfect revenge, or winning a million dollars) to feel better?	0	1	2	3	4
11. Been very emotional compared to your usual self?	0	1	2	3	4
12. Gone over your problems in your mind over and over again?	0	1	2	3	4

Ordinarily, in recent weeks have you:	Never	Seldom	Sometimes	Often	Almost always
13. Asked others for help?	0	1	2	3	4
14. Thought about your problems a lot?	0	1	2	3	4
15. Became involved in recreation or pleasure activities?	0	1	2	3	4
16. Worried about your problems a lot?	0	1	2	3	4
17. Tried to keep your mind off things that are upsetting you?	0	1	2	3	4
18. Tried to distract yourself from your troubles?	0	1	2	3	4
19. Avoided thinking about your problems?	0	1	2	3	4
20. Made plans to overcome your problems?	0	1	2	3	4
21. Told jokes about your situation?	0	1	2	3	4
22. Thought a lot about who is responsible for your problems (besides yourself)?	0	1	2	3	4
23. Shared humorous stories etc. to cheer yourself and others up?	0	1	2	3	4
24. Told yourself that other people have dealt with problems such as yours?	0	1	2	3	4
25. Thought a lot about how you have brought your problems on yourself?	0	1	2	3	4
26. Decided to wait and see how things turn out?	0	1	2	3	4
27. Wished the situation would go away or be over with?	0	1	2	3	4

Ordinarily, in recent weeks have you:	Never	Seldom	Sometimes	Often	Almost always
28. Decided that your current problems are a result of your own past actions?	0	1	2	3	4
29. Gone shopping?	0	1	2	3	4
30. Asserted yourself and taken positive action on problems that are getting you down?	0	1	2	3	4
31. Sought reassurance and moral support from others?	0	1	2	3	4
32. Resigned yourself to your problems?	0	1	2	3	4
33. Thought about how your problems have been caused by other people?	0	1	2	3	4
34. Daydreamed about how things may turn out?	0	1	2	3	4
35. Been very emotional in how you react, even to little things?	0	1	2	3	4
36. Decided that you can grow and learn through your problems?	0	1	2	3	4
37. Told yourself that other people have problems like your own?	0	1	2	3	4
38. Wished I was a stronger person or better at dealing with problems?	0	1	2	3	4
39. Looked for how you can learn something out of your bad situation?	0	1	2	3	4
40. Asked for God's guidance?	0	1	2	3	4
41. Kept your feelings bottled up inside?	0	1	2	3	4
42. Found yourself crying more than usual?	0	1	2	3	4

Ordinarily, in recent weeks have you:	<b>Never</b>	<b>Seldom</b>	<b>Sometimes</b>	<b>Often</b>	<b>Almost always</b>
43. Tried to act as if you were not upset?	0	1	2	3	4
44. Prayed for help?	0	1	2	3	4
45. Gone out?	0	1	2	3	4
46. Held in your feelings?	0	1	2	3	4
47. Tried to act as if you weren't feeling bad?	0	1	2	3	4
48. Taken steps to overcome your problems?	0	1	2	3	4
49. Made humorous comments or wise cracks?	0	1	2	3	4
50. Told others that you were depressed or emotionally upset?	0	1	2	3	4

### *Survey of Coping Profile Endorsement*

The following are activities that you might do *in response to the employment task* you have just experienced. Please indicate the extent to which you would use these activities as a way of dealing with this task.

The following is a list of actions that people may use in response to a difficult situation. Please indicate the extent to which you would use these activities.

***In response to the employment task I would:***

	<i>Never</i>	<i>Seldom</i>	<i>Sometimes</i>	<i>Often</i>	<i>Almost always</i>
1. accept that there is nothing I could do to change the situation?	0	1	2	3	4
2. (if this situation is problematic for me), blame myself?	0	1	2	3	4
3. tell others that I was really upset about the task?	0	1	2	3	4
4. ask others for help or advice?	0	1	2	3	4
5. spend a lot of time thinking about the task?	0	1	2	3	4
6. take time for recreation or pleasure activities after the task?	0	1	2	3	4
7. make plans to overcome my concerns regarding the task?	0	1	2	3	4
8. avoid thinking about the task?	0	1	2	3	4
9. tell jokes about this situation?	0	1	2	3	4
10. think a lot about who was responsible for my this situation (besides me)?	0	1	2	3	4
11. worry about the task a lot?	0	1	2	3	4
12. make humorous comments or stories about this situation?	0	1	2	3	4
13. wish the situation would just go away or be over with?	0	1	2	3	4
14. think a lot about how I brought this situation on	0	1	2	3	4

myself?

15. decide to wait and see how things turned out?	0	1	2	3	4
16. try to keep my mind off things about the task that were upsetting me?	0	1	2	3	4
17. seek reassurance and emotional support from others following the task ?	0	1	2	3	4
18. think about how this situation was caused by other people?	0	1	2	3	4
19. cry, even if someone else was around?	0	1	2	3	4
20. look for how I could grow and learn following this situation?	0	1	2	3	4
21. tell myself that other people experience situations like this?	0	1	2	3	4
22. do things to keep busy or active following this task (e.g. exercised, went out)?	0	1	2	3	4
23. hold in my feelings about this task?	0	1	2	3	4
24. daydream about how the situation may have turned out?	0	1	2	3	4
25. try to act as if I wasn't feeling bad?	0	1	2	3	4
26. take steps to overcome the situation?	0	1	2	3	4
27. turn to God or my faith following the task?	0	1	2	3	4

*Positive and Negative Affective States*

Using the rating scale beside each item, please indicate how much each adjective describes **how you feel at the moment**. There are no right or wrong answers, we just want you to be as honest as possible in indicating how you're feeling right now.

Active	Not at all	0	1	2	3	4	5	6	Extremely
Afraid	Not at all	0	1	2	3	4	5	6	Extremely
Alert	Not at all	0	1	2	3	4	5	6	Extremely
Angry	Not at all	0	1	2	3	4	5	6	Extremely
Annoyed	Not at all	0	1	2	3	4	5	6	Extremely
Anxious	Not at all	0	1	2	3	4	5	6	Extremely
Ashamed	Not at all	0	1	2	3	4	5	6	Extremely
Attentive	Not at all	0	1	2	3	4	5	6	Extremely
Confused	Not at all	0	1	2	3	4	5	6	Extremely
Contempt	Not at all	0	1	2	3	4	5	6	Extremely
Depressed	Not at all	0	1	2	3	4	5	6	Extremely
Determined	Not at all	0	1	2	3	4	5	6	Extremely
Disdain	Not at all	0	1	2	3	4	5	6	Extremely
Disgust	Not at all	0	1	2	3	4	5	6	Extremely
Distressed	Not at all	0	1	2	3	4	5	6	Extremely
Embarrassed	Not at all	0	1	2	3	4	5	6	Extremely
Enraged	Not at all	0	1	2	3	4	5	6	Extremely
Enthusiastic	Not at all	0	1	2	3	4	5	6	Extremely
Excited	Not at all	0	1	2	3	4	5	6	Extremely
Frustrated	Not at all	0	1	2	3	4	5	6	Extremely
Guilty	Not at all	0	1	2	3	4	5	6	Extremely
Happy	Not at all	0	1	2	3	4	5	6	Extremely
Helpless	Not at all	0	1	2	3	4	5	6	Extremely

Hostile	Not at all	0	1	2	3	4	5	6	Extremely
Humiliated	Not at all	0	1	2	3	4	5	6	Extremely
Indifferent	Not at all	0	1	2	3	4	5	6	Extremely
Infuriated	Not at all	0	1	2	3	4	5	6	Extremely
Inspired	Not at all	0	1	2	3	4	5	6	Extremely
Interested	Not at all	0	1	2	3	4	5	6	Extremely
Irritable	Not at all	0	1	2	3	4	5	6	Extremely
Jittery	Not at all	0	1	2	3	4	5	6	Extremely
Nervous	Not at all	0	1	2	3	4	5	6	Extremely
Proud	Not at all	0	1	2	3	4	5	6	Extremely
Regretful	Not at all	0	1	2	3	4	5	6	Extremely
Responsible	Not at all	0	1	2	3	4	5	6	Extremely
Sad	Not at all	0	1	2	3	4	5	6	Extremely
Scared	Not at all	0	1	2	3	4	5	6	Extremely
Strong	Not at all	0	1	2	3	4	5	6	Extremely
Unhappy	Not at all	0	1	2	3	4	5	6	Extremely
Upset	Not at all	0	1	2	3	4	5	6	Extremely
Worried	Not at all	0	1	2	3	4	5	6	Extremely

### *Trait Anxiety*

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the answer that indicates how you *generally* feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

	Never	Sometimes	Often	Almost Always
1. I feel pleasant	1	2	3	4
2. I feel nervous and restless	1	2	3	4
3. I feel satisfied with myself	1	2	3	4
4. I wish I could be as happy as others seem to be	1	2	3	4
5. I feel like a failure	1	2	3	4
6. I feel rested	1	2	3	4
7. I am "calm, cool, and collected"	1	2	3	4
8. I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
9. I worry too much over something that really does not matter	1	2	3	4
10. I am happy	1	2	3	4
11. I have disturbing thoughts	1	2	3	4
12. I lack self-confidence	1	2	3	4
13. I feel secure	1	2	3	4
14. I make decisions easily	1	2	3	4
15. I feel inadequate	1	2	3	4
16. I am content	1	2	3	4

- |  |   |   |   |   |
|--|---|---|---|---|
| 17. Some unimportant thought runs through my mind & bothers me                 | 1 | 2 | 3 | 4 |
| 18. I take disappointments so keenly that I can't put them out of my mind      | 1 | 2 | 3 | 4 |
| 19. I am a steady person   | 1 | 2 | 3 | 4 |
| 20. I get in a state of tension or turmoil as I think over my recent concerns. | 1 | 2 | 3 | 4 |

**State Anxiety**

DIRECTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and then circle the answer to indicate how you feel **right** now, that is, **at this moment**. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	Not at All	Somewhat	Moderately	Very Much
	1	2	3	4
1. I feel calm .....	1	2	3	4
2. I feel secure .....	1	2	3	4
3. I am tense .....	1	2	3	4
4. I feel strained.....	1	2	3	4
5. I feel at ease .....	1	2	3	4
6. I feel upset .....	1	2	3	4
7. I am presently worrying over possible..... misfortunes.	1	2	3	4
8. I feel satisfied.....	1	2	3	4
9. I feel frightened.....	1	2	3	4
10. I feel comfortable .....	1	2	3	4

- |                                |   |   |   |   |
|--------------------------------|---|---|---|---|
| 11. I feel self-confident..... | 1 | 2 | 3 | 4 |
| 12. I feel nervous.....        | 1 | 2 | 3 | 4 |
| 13. I am jittery .....         | 1 | 2 | 3 | 4 |
| 14. I feel indecisive.....     | 1 | 2 | 3 | 4 |
| 15. I am relaxed.....          | 1 | 2 | 3 | 4 |
| 16. I feel content.....        | 1 | 2 | 3 | 4 |
| 17. I am worried.....          | 1 | 2 | 3 | 4 |
| 18. I feel confused.....       | 1 | 2 | 3 | 4 |
| 19. I feel steady .....        | 1 | 2 | 3 | 4 |
| 20. I feel pleasant.....       | 1 | 2 | 3 | 4 |

### *Co-player Feelings*

**Please circle the most accurate answer to reflect your general impression of your co-players based on your experience playing Cyberball.**

1      2      3      4      5      6      7      8      9

not at all

very much so

1. How much do you like these people?
2. How good are these people?
3. How attractive are these people?
4. How prejudiced are these people?
5. How trustworthy are these people?
6. How tolerant are these people?
7. How arrogant are these people?
8. How friendly are these people?
9. How manipulative are these people?
10. How fair are these people?
11. How loyal are these people?
12. How hypocritical are these people?
13. To what degree would you describe these people as sell outs?





1 2 3 4 5 6 7 8 9  
not at all very much so

12. I felt like an outsider during the Cyberball game.

1 2 3 4 5 6 7 8 9  
not at all very much so

13. Please indicate to what extent you currently feel

1 2 3 4 5 6 7 8 9  
Bad Good

14. Please indicate to what extent you currently feel

1 2 3 4 5 6 7 8 9  
Happy Sad

15. Please indicate to what extent you currently feel

1 2 3 4 5 6 7 8 9  
Tense Relaxed

16. Please indicate to what extent you currently feel

1 2 3 4 5 6 7 8 9  
Aroused Not aroused

17. Please indicate to what extent you currently feel

1	2	3	4	5	6	7	8	9
Friendly							Unfriendly	

18. I felt angry during the Cyberball game.

1	2	3	4	5	6	7	8	9
not at all							very much so	

19. I enjoyed playing the Cyberball game.

1	2	3	4	5	6	7	8	9
not at all							very much so	

## Appendix B

McInnis, O.A., McQuaid, R.J., Matheson, K., & Anisman, H. (2015). *Experience-dependent effects of genes: Response to stressors*. In: Psychology of Change: Life Contexts, Experiences, and Identities. K.J. Reynolds and N.R. Branscombe, eds. Psychology Press, New York.

Stressful experiences, irrespective of when they occur, can have marked ramifications on psychological and physical well-being. However, if such experiences are encountered prenatally, during early postnatal development, childhood or adolescence, they can have particularly profound effects on later well-being. In this regard, stressors can influence trajectories related to psychosocial development as well as hormonal, neurochemical, growth factor and immune processes, all of which may contribute to the emergence of pathological conditions.

What makes some individuals relatively vulnerable or resilient to the effects of stressors involves a combination of neurobiological influences and a constellation of psychosocial factors, including those related to appraisal processes and the coping methods used, as well as the individual's social networks and identities (Jetten, Haslam, & Haslam, 2012). Although genetic factors influence the expression of proteins essential for neural plasticity, memory formation, behavior, emotions, and motivations, their influence is frequently moderated by experiences. Indeed, gene x environment interactions have been observed in relation to phenotypic changes associated with inherited genetic mutations (polymorphisms), and stressful experiences can cause the suppression or amplification of gene expression (epigenetic changes) without altering the genetic code itself (Petronis, 2010).

In this chapter we will discuss the contribution of stressful events to the emergence of psychopathological conditions, why and how the effects of stressors during

early development can have especially profound consequences, and how experience and environmental factors can interact with genetic contributions in promoting both pathological outcomes and varied non-pathological conditions. In effect, people are not hard-wired in their vulnerability to illnesses, but rather social and developmental experiences can fundamentally change how we respond to subsequent stressors and the evolution of stress-related pathologies. Given the vast number of psychosocial and genetic factors that come together to promote vulnerability or resilience to stress-related pathology, only a few of these will be considered as illustrative examples.

### **Neurobiological responses to stressors**

Biological reactions to stressors represent adaptive responses to meet the demands placed on the organism. Among other things, they facilitate the ability to appraise and cope with stressors, blunt the negative psychological impact of such challenges, and prepare the individual to deal with ongoing or impending insults (e.g., enhance arousal, vigilance, and cognitive processes necessary for effective coping). In addition, energy substrates that may be needed for survival increase, affecting readiness to make appropriate behavioral, cognitive, or emotional responses to contend with stressful events. These adaptive responses also comprise regulatory changes to prevent or limit excessive activation of certain biological systems (e.g., immune functioning) that could potentially have negative effects on well-being (Sapolsky, Romero, & Munck, 2000).

Despite the remarkable adaptive capacity of neurobiological processes, some of the reactions elicited by stressors can instigate pathological conditions. For example, when the stressor is chronic and uncontrollable the utilization of essential neurotransmitters, such as serotonin, may exceed their production, leading to insufficient

levels necessary to deal with further stressors. In other instances, compensatory increases of a neurobiological substrate, such as cortisol may occur, seemingly facilitating effective coping, but if the stressor is sufficiently prolonged, then the wear and tear on biological systems may become excessive (i.e., allostatic overload), thereby favoring the development of pathology (McEwen, 2000). Under some stressor conditions, excessive levels of particular biochemicals or their products may promote neurotoxic actions, as in the case of extreme inflammatory events, and the resulting cell loss may be associated with psychological disturbances (Anisman, Merali & Hayley., 2008). In fact, the influence of stressors is exceptionally widespread, and there is hardly a biological system that is not affected in some fashion. Commensurately, the range of pathologies that can arise is broad, and it can be exceedingly difficult to tie specific stress-related biological changes to particular pathologies. These difficulties are further complicated by the fact that the processes that are associated with the initial appearance of some pathologies, may differ from those that sustain them over time or that are responsible for illness recurrence.

These difficulties notwithstanding, disturbances of several neurobiological systems have been linked to pathological conditions. Of the hormones influenced by stressors, the most widely known are those related to hypothalamic-pituitary-adrenal (HPA) functioning, comprising corticotropin releasing hormone (CRH), adrenocorticotrophic hormone (ACTH) and cortisol. Cortisol, being easily measured non-intrusively in saliva, has been a favorite among some social psychologists, but there are many other hormones that also contribute to stress processes. The sex hormones (estrogen, testosterone) influence behavior and interact with other hormones and neurotransmitters to modify behavioral outputs, as do hormones involved in energy

regulation and eating processes (e.g., leptin, ghrelin, neuropeptide Y, insulin; Abizaid, Luheshi & Woodside, 2013). Oxytocin has been implicated in prosocial behaviors, including trust, attachment, and bonding (Meyer-Linjenberg, Domes, Kirsch, & Heinrichs, 2011). In addition to these hormones, several neurotransmitters have been extensively examined in relation to stressors, with the monoamines (serotonin, norepinephrine, dopamine) receiving particular attention, although others, such as GABA, glutamate, acetylcholine and histamine also have important ramifications (Anisman et al., 2008). As well, stressors influence growth factors, such as brain derived neurotrophic factor (BDNF) and fibroblast growth factor-2 (FGF-2), which influence the survival of existing neurons, and serve to promote the growth and differentiation of new neurons and synapses. By virtue of their synaptic actions, these growth factors are essential for learning and memory, and they have also been implicated in stress-related pathologies such as depressive disorders (Duman & Monteggia, 2006).

The response of varied neurobiological systems is often influenced by the form of the stressor itself, with some systems being differentially sensitive to psychological versus physical stressors, whereas others are affected by both types of stressors as well as by impending or expected stressors (threats). Indeed, certain psychological attributes of a stressor (e.g., lack of controllability) markedly affect neurotransmitters, such as norepinephrine and serotonin (Anisman et al., 2008), but variations in growth factors (BDNF and FGF-2) tend to be more pronounced when the stressor is controllable, possibly reflecting the engagement of methods to contend with the stressor (Bland, Tamlyn, Barrientos, Greenwood, Watkins, Campeau, & Maier, 2007). As well, some biological systems are exquisitely sensitive to systemic stressors (e.g., immunogenic

agents), and are thought to have effects on some psychological disorders. Furthermore, varied types of stressors may engage different neural circuits (Anisman et al., 2008). For instance, a stressor that involves a psychosocial challenge might instigate biological changes that are different from those that entail chronic or sudden traumatic experiences. Consequently, the most efficacious treatments to deal with pathologies that follow these challenges might differ appreciably from one another.

The influence of stressors on neurobiological and behavioral outcomes varies with the severity of the stressor, as well as its controllability, predictability, uncertainty and ambiguity. The chronicity of a stressor may also have pronounced effects on neurobiological processes, depending on whether the stressor is one that is consistent over days (permitting behavioral and neurobiological adaptation to develop) or varies in an unpredictable manner. As well, if a stressor is a chronic, unpredictable one, then neurobiological systems may be overly taxed or may result in cell loss (referred to as allostatic overload), and pathology may ensue (Anisman & Matheson, 2005). One particularly important aspect concerning responses to stressors is that although the immediate neurobiological changes introduced are relatively brief, stressor experiences can have very long-lasting consequences. Specifically, stressors can result in the ‘sensitization’ of neurobiological processes so that when stressors are encountered later, even if they are somewhat different from the initial insult, rapid and marked neurobiological changes are apparent. Effects such as these have been observed with respect to neurotransmitters, hormones, growth factors and cytokines, and it is thought that sensitization processes contribute to the emergence of pathological conditions, as well as recurrence of illness after individuals have been successfully treated (Anisman,

Hayley, & Merali, 2003).

### **Early Postnatal Experiences**

Given that stressors may result in the sensitization of neurobiological systems, it is not surprising that stressors experienced early in life, a period thought to be especially sensitive to stressors, may have marked ramifications on physical and psychological well-being throughout life, and can even have consequences that carry across generations (intergenerational or transgenerational effects of stressors). Outcomes of this sort are not limited to psychological or physical stressor experiences, as they have also been observed in response to systemic challenges. Indeed, early life stressors in the form of immune challenges affect neurochemical and hormonal responses to later stressor challenges much as early life neglect may have such effects. Of course, it is important to distinguish between those childhood stressors that are mild or moderate, and that can actually have beneficial effects to the extent that children learn how to deal with stressful experiences, versus those stressors that are of a toxic nature, including physical, psychological or sexual abuse, neglect, or stressors stemming from poverty (Shonkoff, Boyce, & McEwen, 2009). These experiences may have especially profound effects on children as they frequently lack the social, cognitive, and tangible resources necessary to cope with stressors effectively. Thus, it is not surprising that severe early-life experiences have been associated with risk of depression and elevated suicidal ideation (Dube, Felitti, Dong, Giles, & Anda, 2003).

For decades it has been known that children who experienced neglect and poor early-life environmental conditions subsequently display greater adult anxiety, depression, chronic fatigue syndrome, autoimmune disorders, as well as the development

of diseases of aging, such as vascular disease and premature mortality (Shonkoff et al., 2009). As well, children from a poor nurturing environment have a hippocampus that is about 10% smaller than children from a good environment (Luby et al., 2012), which could have enormous repercussions for stress responses, mental health, as well as learning and memory processes.

There have been numerous studies, primarily in animals, assessing the neurobiological processes associated with stressors during early life, with the aim of deciphering how these might influence later pathological conditions. Many of these studies indicate that early life stressors, including neglect, alter the response to later stressors reflected in disturbed ways of coping as well as poor behavioral and emotional regulation (Sanchez, Ladd, & Plotsky, 2001). These behavioral outcomes were accompanied by several neurobiological changes when these animals were later introduced to a stressor. Although particular attention has been devoted to the effects on HPA related hormones, such as corticoids, early life insults also have protracted effects on GABA processes (Skilbeck, Johnston, & Hinton, 2010), dopamine, norepinephrine and serotonin activity (Rodrigues et al., 2011), and the levels of growth factors (Roth, Lubin, Funk, & Sweatt, 2009).

### **Prenatal Stressor Effects**

The influence of early life stressors, more than those experienced at other ages, has received extensive attention based on the view that experiences at this stage of life engender marked consequences that are manifested throughout life. It also seems that stressors in pregnant women can influence the physical and psychological health of their offspring, and such effects can extend into adulthood (Beydoun & Saftlas, 2008). One of

the most common findings concerning the influence of prenatal stressful experiences was that they were associated with shortened gestation periods and reduced birth weights (Talge, Neal, & Glover, 2007), which in turn, were predictive of later physical and psychological pathology. The negative consequences of prenatal stressors on the well-being of the offspring are exceptionally broad. Prenatal stressors have been linked to physical illnesses, with offspring at increased risk of metabolic syndrome and immune-related disorders, such as allergies and asthma, as well as a greater likelihood of being hospitalized with an infectious disease (Nielsen, Hansen, Simonsen, & Hviid, 2011). Children of mothers stressed during pregnancy were also more likely to experience neurodevelopmental disorders, including emotional and cognitive problems, increased risk of attention deficit hyperactivity, anxiety, and language delay, as well as schizophrenia and autism spectrum disorders. Importantly, many of these outcomes cannot be attributed to maternal postnatal depression and anxiety (Glover, 2011).

Paralleling the many psychological and physical disturbances stemming from prenatal stressors, multiple neurobiological alterations are elicited by such events. Among other changes, prenatal stressors are related to variations in immune functioning and increased production of immune messenger molecules, cytokines (Entringer, Kumsta, Nelson, Hellhammer, Wadhwa, & Wüst, 2008), variations of sex hormones, and increased corticotropin releasing hormone (CRH), probably of placental origin (Weinstock, 2005). Given the breadth of the neurobiological and behavioral processes associated with prenatal stressors, it is likely that these insults result in a ‘general susceptibility’ to pathology, rather than one that is related to particular pathological conditions (Huizink, Mulder, & Buitelaar, 2009).

This said, particular attention has focused on cortisol change in the mother being related to the well-being of the fetus. Treatments that increase endogenous glucocorticoid levels late in gestation, including treatment with the synthetic glucocorticoid, betamethasone, which is used to promote lung maturation in fetuses at risk of preterm delivery, may influence neurotransmitter systems and affect responses to later postnatal stressors (Davis, Waffarn, & Sandman, 2011). Evidently, changes that occur in HPA functioning prenatally have consequences that persist throughout the lifespan, and may even be associated with the premature development of pathologies related to aging (Matthews et al., 2004). It is particularly significant that the influence of prenatal stressors on later cognitive and neuroendocrine functioning can be modified by postnatal experiences, including infant-mother attachment. Specifically, elevated prenatal cortisol levels (measured in amniotic fluid at about 17 weeks of gestation) predicted poor cognitive abilities in the presence of subsequent insecure attachment, but not in children with secure attachment (Bergman, Sarkar, O'Connor, Modi, & Glover, 2010). As strong as the implications of cortisol elevation might be for potential developmental milestones, the realization of these effects vary with postnatal influences. Essentially, although it is often assumed, rightly so, that biological factors profoundly affect behavioral processes, their effects are not immutable, and can be altered by postnatal environmental events.

Given the consistency of the available data, it is certain that adverse prenatal experiences can have protracted effects on the well-being of the offspring. Yet, prenatal trauma may be confounded with other factors, especially as prenatally stressed mothers could differ in several ways from those that were not stressed. Likewise, genetic factors unrelated to the prenatal stressor may influence outcomes, or interact with prenatal

stressors to produce effects on the offspring (Rice, Harold, Boivin, van den Bree, Hay, & Thapar, 2010). Importantly, although genetic and environmental factors may both have effects on the offspring, their relative contributions (and their interactions) vary with the specific phenotype examined.

### **Epigenetics**

Most readers will know that genes refer to stretches of DNA that serve as a template for the formation of RNA, which is then translated into specific proteins. Each gene comprises a series of nucleotides (guanine, adenine, cytosine and thymine) that in sets of three make up amino acids (e.g., valine, methionine) that essentially spell out the protein (e.g., glucocorticoid receptors, neurotransmitters, growth factors) that a gene is responsible for forming. There is also an aspect of DNA, referred to as a promoter region, which serves to initiate or promote the transcription of a particular gene. Essentially, some genes contain the information for making particular proteins, and nearby regions of DNA serve as an instruction manual for that gene. If changes occur within the promoter region, then the instructions for the RNA transcription of that gene will be altered and so will the manufacture of the protein (for instance, features of puberty are determined by particular genes, but promoter genes provide the instruction as to when this should occur).

There are several ways through which a gene's action can be altered, including random mutations, polymorphisms, and through a process in which the gene is not actually altered, but its expression is suppressed or activated, the latter being referred to as epigenetic changes (Petronis, 2010). In effect, although genetic factors may contribute greatly to phenotypes associated with normal behaviors, as well as a great number of

physical and psychological pathologies, the phenotypic expression of gene-dependent phenotypes is modifiable. Moreover, as the actual DNA sequence is unaltered, epigenetic effects that occur within a germ line (e.g., sperm or ovum) can be transmitted across generations (Petronis, 2010). Essentially, DNA will be passed on across generations, but it will occur with the epigenetic contributions in place (e.g., suppression of the gene), thus affecting ensuing generations. In this sense, the sins of the father can be visited on the children and grandchildren.

Experiential and environmental factors (including pesticides and fungicides, dioxin, endocrine challenges, diet, and neglect) may also alter a gene's actions in producing proteins without actually altering the sequence of amino acids that makes up these genes. In effect, stressful events experienced at critical times, such as prenatally or in early life may result in changes within gene promoter regions. If these epigenetic changes occur in particular aspects of a promoter, then this could affect the proteins they usually form, including hormones, neurotransmitters and their receptors, and growth factors, and hence, could directly influence processes that lead to a particular phenotype or vulnerability to an illness. Likewise, epigenetic changes could affect emotional or cognitive processes, such as appraisal and coping mechanisms, thereby influencing vulnerability to stressor-related phenotypes. Nonetheless, the presence of a genetic change, even if it occurs within an important portion of a gene, does not necessarily mean that a psychological disturbance will occur, as the expression of such disturbances might require cofactors, such as stressor experiences.

Although it has been on the radar for many years among scientists studying cancer toxicology as well as plant biology, the finding that epigenetic processes might

contribute to behavioral phenotypes has resulted in this becoming a hot topic in neuroscience the past few years, particularly in regard to the influence of early life experience on later pathophysiological processes. The marked cellular proliferation and differentiation that occurs during fetal development makes it an especially sensitive period for genes to be turned on or off in response to environmental toxins as well as endocrine-acting drugs. In addition, early life experiences, including the behavior of a mother toward her pups (e.g., whether she exhibits good parenting or is neglectful) may cause the silencing of promoters that regulate genes associated with HPA functioning, so that as adults these pups are more likely to exhibit poor social behavior, increased stress responses, and poor parenting (Champagne, 2010). Likewise, a prenatal stressor administered during the first trimester of pregnancy in mice influences epigenetic changes related to glucocorticoid receptors (Mueller & Bale, 2008).

In addition to the epigenetic variations of the glucocorticoid receptor, negative experiences can have similar effects on other biological processes that could affect psychological functioning. In monkeys, adverse life experiences influence genes associated with the serotonin transporter (5-HTT), which in humans has been linked to depressive disorders (Kinnally et al., 2010). Similarly, in female rodents, maternal care influences the gene promoter for estrogen receptor alpha ( $ER\alpha$ ) in the hypothalamus (Champagne, 2010), and prenatal stressors can affect the developmental trajectory by epigenetically altering genes controlling sex hormones (Morgan & Bale 2011). It also appears that epigenetic changes can occur within the gene for the growth factor BDNF. For instance, Roth et al. (2009) raised rat pups during the first postnatal week with adult caretakers that had been stressed and thus displayed abusive behaviors toward the pups.

When the abused pups were subsequently assessed in adulthood, epigenetic effects were apparent within the BDNF gene in the prefrontal cortex. When these pups, as adults, had their own litters, this epigenetic BDNF profile, accompanied by anxiety and poor maternal behaviors, was also apparent in the offspring. These data indicate that this trophic factor is susceptible to epigenetic changes in response to early life stressors, and implicate BDNF genes in the intergenerational behavioral effects of early life stressors. Importantly, although it is known that gene influences are malleable, at the same time, it appears that the epigenetic changes are sufficiently resilient to be passed on across generations, in the absence of other transformative experiences.

Although the prenatal and early postnatal periods are especially vulnerable to epigenetic effects as a result of stressors, such outcomes can also be elicited at other times. Indeed, when administered during adulthood, relatively intense stressors elicited epigenetic effects of the BDNF gene and promoted the emergence of depressive and PTSD-like features (Roth et al., 2009). In effect, these findings once again indicate that having been born with particular genes does not necessarily mean that the actions of the genes will be phenotypically expressed. Prenatal and early life social and environmental experiences, as well as those encountered in adulthood, can determine the influence of genes on behaviors within and across generations.

It may be of particular significance that although epigenetic changes can be stable, and hence their actions could persist over the course of an organism's life, these variations are modifiable (Petronis, 2010). For instance, the effects of particular toxins can be reversed by increasing the presence of folate in the mom's diet (Dolinoy, Huang, & Jirtle, 2007). Moreover, pharmacological treatments that attenuated epigenetic effects

also diminished behavioral disturbances that were otherwise present (Covington et al., 2009). Further to this point, the epigenetic changes of the gene for BDNF elicited by a stressor applied during the juvenile period, which promoted increased reactivity and anxiety into the next generation, could be attenuated if animals were maintained in an enriched environment (Leshem & Schulkin, 2011). Therefore, even though the sins of the father can be visited upon the children, at least some of these influences can be undone or redeemed by positive environmental factors.

Relatively few studies in humans have assessed epigenetic contributions to the relations between stressful events and behavioral disturbances. However, in infants at 3 months of age, maternal depressed/anxious mood during the third trimester of pregnancy was accompanied by greater epigenetic effects with respect to the genes for glucocorticoid receptors (measured in DNA from saliva) coupled with increased salivary cortisol stress responses (Oberlander, Weinberg, Papsdorf, Grunau, Misri, & Devlin, 2008). There have also been several studies showing that epigenetic changes are present in the prefrontal cortex and hippocampus obtained from depressed individuals who died by suicide (McGowan et al., 2009; Poulter et al., 2008). Significantly, the epigenetic modifications related to the hippocampal glucocorticoid receptor were particularly notable among those individuals who had a history of early childhood neglect/abuse (McGowan et al., 2009). While consistent with the view that early experiences are related to glucocorticoid receptor functioning, the studies linking genes to behavior do not speak to whether epigenetic changes are related causally to the psychological disturbances that might be detected.

Analyses of epigenetic changes related to psychological disturbances are

exceptionally difficult to conduct. Aside from the fact that human brain tissue is difficult to obtain, we often do not know which genes to examine and in which brain regions we should be looking. This is compounded by the fact that (a) thousands of epigenetic changes may exist at any given time, and (b) complex pathologies involve multiple brain areas, and there are different types of neurons within any region that might be differentially affected by environmental triggers. At the end of the day, the best we can end up with at this time are multiple correlations, and even if causal connections exist, it would be unclear whether the epigenetic change was responsible for producing an illness, or the illness itself caused the epigenetic change.

### **Gene polymorphisms**

Yet another way in which genetic alterations can influence behavioral outcomes involves polymorphisms (inherited gene mutations) that influence gene expression and hence behavioral phenotypes. Polymorphisms are fairly common, and their presence has frequently been assessed in order to link specific genes to psychopathological conditions. This entails finding a cohort of affected and nonaffected individuals, and then determining whether there is a match between the presence of certain gene polymorphisms and the appearance of a pathological condition. That ought to be simple enough, but it presupposes that diagnosis of an illness is correct, which is not always a simple matter as different illnesses have overlapping symptoms. Second, individuals might have similar symptoms, but that does not necessarily mean that these stem from the same underlying biological processes. Third, a vast number of polymorphisms can occur across the genome (multiple polymorphisms can even appear on any given gene), and most of these will be entirely unrelated to the pathology being studied. As a result, the

number of participants needed to do the relevant studies is huge. Finally, the expression of gene mutations in the form of pathological phenotypes might not be evident under ideal conditions, but instead will be most evident in the presence of particular challenges, such as life stressors. These difficulties notwithstanding, some of the most common polymorphisms that have been linked to behavioral outcomes indicate how life experiences can influence the behavioral expression of these gene actions.

### **The Serotonin Transporter (5-HTT)**

Although many aspects of the serotonergic system have been assessed in relation to depression, recent studies have devoted particular attention to the contribution of the serotonin transporter (5-HTT), which is responsible for taking serotonin back into the neuron after it has been released, thereby limiting its ability to activate receptors on the adjacent neuron. The antidepressant actions of serotonin reuptake inhibitors were thought to be a result of serotonin remaining in the synaptic cleft for longer periods. Consistent with postmortem analyses showing that depression/suicide was associated with 5-HTT disturbances, a 5-HTT gene promoter polymorphism (5-HTTLPR) was reported in relation to depression (Arango, Huang, Underwood, & Mann, 2003). Later studies indicated that depression and suicide were more frequent among individuals carrying particular alleles (i.e., one of several different forms of a gene). Specifically, depressive disorders were elevated among individuals carrying a polymorphism that comprised one or both copies of a short allele of the 5-HTT promoter, relative to individuals that were homozygous for the long allele (Caspi et al., 2003). What made these findings interesting was that the risk for depression associated with the short 5-HTT alleles was only elevated if individuals had also encountered major life stressors or early life trauma.

Several subsequent studies have confirmed these findings, and meta analyses indicate (Wankerl, Wüst, & Otte, 2010) that of the studies relying on interviews and objective measures of stressor experiences, almost all fully or partially replicated the initial finding. Moreover, when the data were stratified on the basis of the type of stressor individuals experienced (e.g., childhood maltreatment or specific medical conditions), the strength of the original findings was more impressive, with childhood stressors having stronger effects than adult stressors (Karg, Burmeister, Shedden, & Sen, 2011). At present, the consensus seems to be that as strong as the role of genes might be in determining a variety of phenotypes, their role in mediating complex psychological disorders may be determined by psychosocial and other challenges. It is not entirely certain how a 5-HTT polymorphism would come to be translated into a greater propensity toward depression upon exposure to stressors, but it might be the case that genetics dispose individuals to depression because of their greater sensitivity or reactivity to environmental stressors.

### **Brain Derived Neurotrophic Factor (BDNF)**

Given the presumed links between stressor experiences, BDNF changes and depressive symptoms, it is not surprising that polymorphisms related to BDNF have also been associated with responses to stressors. Indeed, a single nucleotide polymorphism (SNP) on the BDNF promoter in which the amino acid valine was substituted by methionine (referred to as Val66Met or the val/met polymorphism), was associated with several behavioral and physiological outcomes. This included disrupted cellular processing and secretion of BDNF, memory and hippocampal functioning, (Egan et al., 2003), as well as altered stress responses reflected by elevated HPA reactivity in response

to a public speaking challenge (Shalev et al., 2009). A meta-analysis confirmed that this SNP was accompanied by reduced hippocampal size (Hajek, Kopecek, & Höschl, 2012). As well, nondepressed individuals who carried either the BDNF polymorphism (or the short 5-HTT alleles) tended to ruminate more following life stressors than did those with other genotypes (Clasen, Wells, Knopik, McGeary, & Beevers, 2011), and thus might have been at increased risk for later depression.

The link between the BDNF polymorphism and depression has not been without controversy. A strong association was observed between the BDNF polymorphism and effective antidepressant treatment, particularly in Asian populations where the polymorphism is far more common than in Caucasian populations (Zou, Ye, Feng, Su, Pan, & Liao, 2010). However, other reports indicate that the presence of the polymorphism was not necessarily related to depressive disorders, and was not associated with the effectiveness of antidepressant treatment (Yoshimura et al., 2011). It is uncertain what factors are responsible for the diverse outcomes observed with regard to the BDNF polymorphism, but the large number of reports showing a relation between the Val/Met SNP and depression makes it likely that BDNF, possibly in combination with other biological processes and stressor experiences (as in the case of 5-HTT), contributes to depressive illness.

Consistent with this perspective, both human and animal studies have implicated BDNF as an important mediator between the effects of early life adversity and later stressor-related depressive symptoms. For instance, in humans, the adverse effects of early life sexual abuse in relation to depression was markedly greater among individuals carrying the BDNF polymorphism (Aguilera et al., 2009), as was the tendency toward

negative affectivity (Perea et al., 2012). Moreover, among university students who experienced early adversity, lifetime depression was particularly elevated in those carrying both the BDNF SNP and the short 5-HTT allele (Carver, Johnson, Joormann, Lemoult, & Cuccaro, 2011).

There is yet another perspective regarding BDNF that warrants consideration. BDNF plays a fundamental role in synaptic plasticity so that experiences remain in memory and affect subsequent behaviors. From this vantage, the presence of BDNF ‘allows’ early events, for better or worse, as Belsky et al. (2009) put it, to influence plasticity and hence the response to later stressful events. Thus, when the gene promoter for BDNF operates properly, early life positive events should enhance later psychological functioning, whereas adverse events in early life would result in negative outcomes. In contrast, the presence of a BDNF polymorphism would diminish the benefits that could be derived from positive early life events, but at the same time it might limit the adverse effects that might otherwise occur as a result of negative early experiences. This is precisely what happens among those carrying the Val/Met polymorphism (Caldwell et al., 2013), indicating that the influence of this polymorphism needs to be considered in the context of different experiential variables. It should also be noted that this SNP is not uniquely related to depression, having been detected in relation to schizophrenia and neurodegenerative disorders, and might thus represent a general risk factor for psychological illnesses rather than being exclusive to any single pathology.

### **Oxytocin**

Ordinarily, social support plays a pivotal role in individuals’ ability to cope with stressors, and conversely, loss of support or not obtaining support when it was reasonably

expected might comprise a powerful stressor in its own right. The social support that individuals receive is particularly important early in life, especially as close attachments and parental bonding have been consistently implicated in the development of self-esteem, resilience, secure adult attachments, and positive mental health (Taylor & Stanton, 2007). There is reason to believe that the development of prosocial behaviors and their contribution to resilience may be linked to the presence of specific hormones and brain neurotransmitters. In this regard, oxytocin was long known to play an important role in the birth process, lactation, and maternal bonding, but interest in this peptide hormone increased markedly with the demonstration that it plays an important role in a variety of prosocial behaviors, including trust, empathy, attachment, and altruism. Each of these behaviors entails complex emotional and motivational processes that likely involve multiple neurochemical mechanisms, so there is some question as to how this single hormone contributes to such a broad array of behaviors. This is further complicated by the finding that oxytocin is not only associated with prosocial behaviors, but is also released in response to stressors and may serve to attenuate HPA axis responses (Taylor, 2006). Furthermore, oxytocin administration in the form of nasal spray, which allows direct access of this hormone to the brain, has proven to be a potent means of buffering the stress response (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003).

It seems that SNPs for the oxytocin receptor gene (OXTR) play an important role in stress reactivity, and might do so by moderating the impact of social support on stress responses. Evidence for this comes from studies that evaluated a SNP in the oxytocin receptor gene, termed rs53576, which involves a guanine (G) to adenine (A) substitution.

Individuals who carry the A nucleotide on one (GA) or both (AA) alleles may exhibit altered social responses, although the need for both alleles (vs. one allele) being affected varies as a function of the specific behavior being examined. When men had social support available, men without the OXTR SNP displayed low cortisol levels in response to a psychosocial stressor. However, the effects of social support on cortisol levels were limited if men carried the polymorphism (Chen, Kumsta, von Dawans, Monakhov, Ebstein, & Heinrichs, 2011).

It might be thought that as in the case of the 5-HTT polymorphism, individuals who carried the OXTR polymorphism would be at greater risk for depression, and that this outcome would be exacerbated by negative early-life experiences. This was not the case, and in fact, those individuals without the OXTR polymorphism, and who would be expected to be relatively prosocial or socially sensitive, showed greater severity of depressive symptoms if they had experienced high childhood maltreatment compared to individuals with the OXTR SNP (McQuaid, McInnis, Stead, Matheson, & Anisman, 2013). Similarly, those without the OXTR polymorphism who experienced severe childhood maltreatment displayed greater disorganized attachment styles and increased risk for emotional dysregulation compared to individuals with the OXTR SNP (Bradley et al., 2011). These findings, although correlational, raise the possibility that certain OXTR genotypes that might facilitate sensitivity to a positive environment also influence sensitivity to a negative environment.

As indicated earlier, certain genotypes promote greater neural plasticity and thus they create greater susceptibility to environment influences. Thus, for better or for worse, in their presence, environment and experience might influence developmental trajectories

more profoundly and thus affect vulnerability to psychopathology (Belsky et al., 2009). This same sort of scenario might be applicable to the relations that exist between oxytocin and early experiences. Specifically, activation of the oxytocin system might intensify positive social experiences and memories of these experiences, but may equally intensify negative social experiences and memories (Guzmán et al., 2013). From this perspective, oxytocin may confer a disposition towards social sensitivity and increased salience of social cues that can be either favorable or disadvantageous depending on the environmental context. Conversely, a polymorphism of the OXTR gene might diminish prosocial behaviors, but this polymorphism might also limit the negative influence otherwise provoked by negative early life experiences.

Just as polymorphisms can influence stress responses, it seems that stressors in early life can promote pronounced oxytocinergic variations. Indeed, oxytocin concentrations were reduced in the cerebrospinal fluid (CSF) of adult women who had a history of childhood abuse, and this effect was particularly strong for those women who experienced emotional abuse. Furthermore, CSF oxytocin concentrations were progressively lower among individuals with multiple forms of maltreatment (Heim, Young, Newport, Mletzko, Miller, & Nemeroff, 2008). Interestingly, women who experienced sexual abuse displayed a marked oxytocin decrease following the onset of a psychosocial challenge in a laboratory context (Pierrehumbert, Torrisi, Laufer, Halfon, Ansermet, & Popovic, 2011), suggesting that among women with such early-life experiences, further stressors compromise the functioning of the oxytocin system, and might thus have implications for illnesses that could be buffered through social support.

Not surprisingly, individual differences, along with early-life adverse experiences

may influence stress reactivity. In this regard, although intranasal oxytocin ordinarily reduces cortisol levels, this attenuation was less apparent in men who previously experienced early parental separation (Meinlschmidt & Heim, 2007). Furthermore, individuals who reported autonomous attachment displayed moderate cortisol and ACTH levels and high oxytocin concentrations following a psychosocial stressor. In contrast, participants who reported preoccupied attachment displayed a moderate cortisol and ACTH response coupled with low oxytocin concentrations (Pierrehumbert, Torrisi, Ansermet, Borghini, & Halfon, 2012). The individual differences reported in relation to oxytocin are particularly pronounced among women, who tend to exhibit increased oxytocin levels and decreased anxiety in response to cortisol administration, whereas males display decreased oxytocin levels and increased anxiety (Tops, van Peer, Wester, Wijers, & Korf, 2006). In fact, it was suggested that among females, higher levels of oxytocin in times of distress may promote a greater 'tend and befriend' characteristic, whereas in times of distress elevated levels of vasopressin (a hormone similar in structure to oxytocin) may serve a similar function in males (Taylor, Saphire-Bernstein, & Seeman, 2010).

Despite ambiguities concerning the implications of stressor effects on oxytocin, it was suggested that this peptide may indirectly contribute to the development of depressive disorders. Oxytocin might promote social affiliative behaviors that serve to buffer against distress (Taylor, 2006), or it might be that the strong inhibitory effects of oxytocin on amygdala activation (Kirsch et al., 2005) diminish fear and/or anxiety that would otherwise limit affiliative behaviors. This said, oxytocin can influence stress responses that involve cortisol, corticotropin releasing hormone, inflammatory processes,

as well as serotonin and dopamine. Thus, the contribution of oxytocin to depressive disorders likely involves interaction with one or more of these other factors that can be modified by stressor experiences.

### **Conclusions**

A common perspective that was held for years was that genetic factors influenced the occurrence of pathological conditions, as did environmental factors, and the individuals' experiences. Gene x Environment interactions were included in this formula, but there was little understanding concerning how these interactions came about, and still less was understood regarding the possibility that the environment could actually influence gene functioning. It has long been clear that unspecified genetic factors play a large role in determining behavioral features, but in the past decade or so, specific genes have been linked to particular pathologies, although too often these have been met with failures to replicate (in part because of the small number of participants inappropriately used in these studies). It has also become clear that environment and experiences can promote epigenetic processes that influence behavioral outcomes, and these epigenetic modifications can be altered by biological and social factors. Moreover, polymorphisms can affect behavioral outcomes, and these are subject to modification by prenatal, early life and adult experiences. As a result of these discoveries, the nature versus nurture debates of some years ago have been largely muted, and the questions now being addressed concern how genes come to affect neurobiological substrates that influence behavioral processes, how experiential and environmental factors come to modify gene processes that link to these behavioral outcomes (e.g., through neuroplasticity, altered developmental trajectories of particular biochemicals), and how these variations come to

affect social and cognitive processes that affect well-being. Even at this relatively early stage of the analyses of gene x environmental interactions, it is certain that experiences, particularly those that involve psychosocial processes, have an enormous influence on later behavior and well-being, and do so, in part, by altering gene expression, and these actions can be transmitted across generations.

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## Appendix C

McQuaid, R. J., McInnis, O. A., Stead, J. D., Matheson, K., & Anisman, H. (2013). A paradoxical association of an oxytocin receptor gene polymorphism: early-life adversity and vulnerability to depression. *Frontiers in neuroscience*, 7.

### Abstract

Several prosocial behaviors may be influenced by the hormone oxytocin. In line with this perspective, the oxytocin receptor (OXTR) gene single nucleotide polymorphism (SNP), rs53576, has been associated with a broad range of social behaviors. In this regard, the G allele of the OXTR SNP has been accompanied by beneficial attributes such as increased empathy, optimism and trust. In the current study among university students ( $N = 288$ ), it was shown that early-life maltreatment was associated with depressive symptoms, and that the OXTR genotype moderated this relationship, such that under high levels of childhood maltreatment, only individuals with GG/GA genotype demonstrated increased depressive symptomatology compared to those with the AA genotype. In addition, the role of distrust in mediating the relation between childhood maltreatment and depression seemed to be more important among G allele carriers compared to individuals with the AA genotype. Thus, a breach in trust (i.e. in the case of early-life abuse or neglect) may have a more deleterious effect among G carriers, who have been characterized as more prosocial and attuned to social cues. The data suggested that G carriers of the OXTR might favor social sensitivity and thus might have been more vulnerable to the effects of early-life adversity.

## **Introduction**

Oxytocin, a neuropeptide produced in the hypothalamus, is involved in a broad range of physiological and behavioral processes. The role of oxytocin in pair-bonding and reproductive behaviors has been well established (Carter, 1998; Gimpl and Fahrenholz, 2001), and this peptidergic system has become the focus of understanding the biological processes underlying prosocial behaviors (Donaldson & Young, 2008). In this regard, intranasal oxytocin has been shown to increase trusting behavior (Kosfeld et al., 2005), positive communication (Ditzen et al., 2009) and in-group favoritism (De Dreu et al., 2011). Furthermore, intranasal oxytocin has been associated with reduced social stress reactivity (Heinrichs et al., 2003), and attenuated amygdala activity in response to emotional stimuli (Domes et al., 2007).

In addition to intranasal manipulations of oxytocin, studies examining human social behaviors have also recently focused on the oxytocin receptor gene (OXTR) located on chromosome 3p25 (Inoue et al., 1994). A single nucleotide polymorphism (SNP), rs53576, involving a guanine (G) to adenine (A) substitution located in the third intron of the OXTR has emerged as an important candidate in the understanding of human social behaviors. Individuals homozygous for the G allele compared to those with the GA or AA genotypes exhibit greater empathy and an increased ability to detect emotion from pictures of human faces in which only the eyes were shown (Rodrigues et al., 2009), greater maternal sensitivity (Bakermans-Kranenburg and van Ijzendoorn, 2008) and higher self-esteem and optimism (Saphire-Bernstein et al., 2011). However, optimism was not associated with the OXTR rs53576 in a large cohort of Caucasian women (Cornelis et al., 2012). It was also revealed that in a sample of Caucasian males,

those with the GG genotype displayed higher trust-related behaviors in comparison to A allele carriers (Krueger et al., 2012). Individuals with one or two copies of the G allele also showed lower cortisol levels compared to individuals with the AA genotype assessed in the Trier Social Stress Test after receiving social support (Chen et al., 2011). Furthermore, the GG/GA genotypes seek more emotional social support in Caucasian but not Asian participants (Kim et al., 2010) and have higher positive affect (Lucht et al., 2009) compared to AA genotypes.

Together, these findings indicate that having one or two copies of the G allele is associated with several positive features. However, the possibility exists that the seemingly positive effects of being a G carrier might be absent under conditions of adversity, especially those that involve negative early-life experiences. For instance, in an African American sample GG carriers exposed to severe childhood maltreatment displayed greater disorganized attachments styles and increased risk for emotional dysregulation compared to GA/AA carriers (Bradley et al., 2011). As well, among a Caucasian sample of depressed individuals, the GG genotype was associated with higher levels of adult separation anxiety compared to A carriers (Costa et al., 2009). It seems that in the face of adversity, the more prosocial and socially attuned individuals may be more sensitive and therefore more likely to be affected by adverse experiences such as abuse.

It might be expected that although the G allele has been associated with greater trust, a violation of that trust, as in the case of early-life abuse and neglect could be more upsetting for those individuals. Accordingly, in the present study we examined the OXTR SNP in relation to childhood maltreatment and mental health outcomes in a culturally

diverse sample. It was predicted that individuals with one or two copies of the G allele who self-reported early-life maltreatment would display elevated depression scores compared to those with two copies of the A allele. Furthermore, the betrayal associated with childhood maltreatment may culminate into a general distrust for others (Doyle, 2001) which may have important ramifications related to depression (Lynch & Cicchetti, 1998). Thus, we predicted that levels of distrust may be a mediator through which maltreatment might lead to depressive symptoms. Further, given that OXTR has been implicated in trust-related behaviors (Krueger et al., 2012), we hypothesized that feelings of distrust following maltreatment would be more detrimental for those with a G allele.

## **Methods**

### ***Participants***

Participants included 288 Carleton University first and second year students (213 females and 75 males), with a mean age of 19.99 ( $SD = 3.17$ ) who were recruited through the university's online computerized recruitment system. Self-reported ethnicity included White (58.0%,  $n = 167$ ), Black (11.8%,  $n = 34$ ), Asian (8%,  $n = 23$ ), Arab (5.7%,  $n = 17$ ), South Asian (5.6%,  $n = 16$ ), South East Asian (2.1%,  $n = 6$ ), Latin American (1.4%,  $n = 4$ ), Aboriginal (1.4%,  $n = 4$ ), and other (e.g., mixed ethnicity, 5.9%,  $n = 17$ ).

### ***Procedure***

Once signed informed consent was obtained, participants responded to a series of demographic questions as well as measures of current depressive symptoms, childhood maltreatment, and distrust and cynicism. Saliva samples for DNA genotyping were taken following the questionnaires. Upon completion of the study, which took up to 1 hour to complete, participants were debriefed and compensated with course credit. All

procedures in the current study were approved by the Carleton University Ethics Committee for Psychological Research.

### ***Genotyping***

Samples for genotyping were collected using Oragene OG-500 collection kits (DNA Genotek, Inc., Ottawa, Ontario, Canada). Genomic DNA was extracted from the sample collection kit according to the manufacturer's instructions and diluted to approximately equal concentration (20 ng/ $\mu$ L). Genotyping was conducted using quantitative polymerase chain reaction (qPCR). Amplification reactions were performed in a total volume of 15  $\mu$ L, containing approximately 1  $\mu$ L (20 ng) of genomic template, 0.6  $\mu$ L of each primer (concentration 10  $\mu$ M), 1.2  $\mu$ L of dNTP, 1.5  $\mu$ L 10X Buffer, 1.5  $\mu$ L of MgCl<sub>2</sub>, 0.3  $\mu$ L of Salmon Sperm DNA, 0.15  $\mu$ L of Taq polymerase, 0.015 of SYBR green and 8.135  $\mu$ L of water. All q-PCR plates were run in duplicate. Following this, all qPCR products were electrophoresed on 2% agarose gel and then visualized to verify qPCR results. The Bio-Rad Iq5 Primer sequences used for qPCR were as follows: OXTR F1 forward: TCCCTGTTTCTGTGGGACTGAGGAC, OXTR F2 forward: TCCCTGTTTCTGTGGGACTGAGGAT, OXTR reverse: ACCCAAGAGGCTGGTTTGGGGTT.

The allele distribution of the OXTR polymorphism was 118 GG individuals (22 male, 96 female) and 119 GA (38 male, 81 female) and 43 AA individuals (14 male, 29 female). The genotype distributions met Hardy-Weinberg Equilibrium expectations,  $\chi^2(1) = 1.99, p = .16$ . Based on earlier studies, there was reason to collapse across the GG and GA genotypes, but there was also precedent for collapsing across the GA and AA carriers. In the present study the depressive scores associated with the A allele was

somewhat lower than among G carriers (AA compared to G carriers), albeit not significantly so, and thus the AA recessive allele was considered in comparison to the pooled G carriers. As will be seen, this approach fit the data better than the alternative procedure, which did not distinguish between these conditions on any of the variables measured in the present investigation. This same procedure was used in studies showing that individuals with the GG/GA genotypes seek more emotional social support (Kim et al., 2010), have higher positive affect (Lucht et al., 2009), and showed lower cortisol responses to stress after social support compared to individuals with two copies of the A allele (Chen et al., 2011).

Eight individuals were excluded from analyses including genotype because we were unable to determine a genotype from the samples provided. No significant differences were found between the genotype groups based on sex,  $\chi^2(1) = 0.98, p = .32$ , and past or current mental disorders,  $\chi^2(1) = 0.004, p = .95$ . A trend was apparent for differences between genotypes based on self-reported ethnicity,  $\chi^2(8) = 14.13, p = .08$ . This is not surprising, as the distribution of the OXTR polymorphism differs between ethnicities. More specifically, among Asian ethnic groups the AA genotype is most prevalent, whereas it is least prevalent among Caucasians (Kim et al., 2011; Saphire-Bernstein et al., 2011). Similarly, in the current study, of the 21 Asian participants whose samples could be genotyped, eight of these were of the AA genotype, which is a much higher frequency than that found among Caucasians. Thus, as the Asian OXTR genotype distributions differed from that of Caucasian individuals, all of the main analyses were performed both with and without Asian participants. In both instances the observed results were very similar.

## ***Measures***

Depressive Symptoms. The 21-item Beck Depression inventory (BDI) (Beck et al., 1961) was used to assess depressive symptoms. For each item participants responded to one of four options which ranged from low to high depression symptomatology. Total scores were calculated by summing across all items ( $\alpha = .91$ ).

Childhood maltreatment. The 31-item Childhood Maltreatment Questionnaire (short form) (Demare, 1996) assessed levels of maltreatment comprising psychological ( $\alpha = .95$ ), physical ( $\alpha = .90$ ), and sexual abuse ( $\alpha = .89$ ) as well as neglect ( $\alpha = .85$ ). Each item can be rated from 1 (never) to 5 (very often) indicating the frequency of experiences.

Distrust and Cynicism. The 8-item Distrust and Cynicism Scale (derived from the Cook-Medley Hostility Scale) has been used as a reliable and valid measure of cynical distrust (GreenGlass and Julkunen 1989, 1991). Each item can be rated from 0 (completely disagree) to 3 (completely agree). Total scores were calculated by taking the mean across all items ( $\alpha = .82$ ). Items such as; '*It is safer to trust nobody*' can be found in this scale.

## **Statistical Analyses**

The statistical analyses were performed using SPSS for Windows 18.0 (SPSS Science, Chicago, Illinois, USA). Statistical significance was determined at  $p < .05$  (two-tailed). Analyses assessing differences on depression scores, childhood maltreatment, and distrust were assessed using independent samples t-tests. Correlational analysis was performed using Pearson product moment correlations. Moderations were analyzed using hierarchical linear regressions, and the significant moderations were followed up using a web utility for simple slopes (Preacher et al., 2006). Mediation analyses were conducted using Sobel's test for estimating indirect effects (Preacher and Hayes, 2004). Moderated

mediation analyses were conducted using bootstrapping procedures and confidence intervals based on 5000 resamples (Preacher et al., 2007). In all regression analyses standardized scores were used.

## Results

The depression scores among individuals with the GG and AG genotype were very similar to one another ( $M = 9.66$ ;  $SE = 0.75$  and  $M = 9.15$ ;  $SE = 0.74$ , respectively) and although somewhat elevated compared to that of the AA genotype ( $M = 7.58$ ;  $SE = 1.23$ ), these groups did not significantly differ from one another,  $t(1, 278) = 1.36$ ,  $p = .18$ . Childhood maltreatment scores also did not differ based on genotype for total maltreatment scores,  $t(1, 51.3) = -0.77$ ,  $p = .45$ , or on any subscales of maltreatment, including physical abuse,  $t(1, 51.3) = -0.70$ ,  $p = .49$ , psychological abuse,  $t(1, 278) = -0.67$ ,  $p = .50$ , sexual abuse,  $t(1, 42.4) = -0.93$ ,  $p = .36$ , and neglect,  $t(1, 50.1) = -0.96$ ,  $p = .34$ . There were too few participants who reported sexual abuse ( $N=2$ ) to provide meaningful results. As seen in Table 1, levels of maltreatment experienced in the current study were appreciable, with the most common form of abuse being of a psychological nature, whereas neglect and physical abuse were less common. In the present study approximately 10% of participants reported experiencing physical abuse or neglect at least sometimes and up to very often. Additionally, up to 30% reported experiences of psychological abuse to the same degree. Furthermore, there were no differences on levels of distrust,  $t(1, 278) = -0.27$ ,  $p = .79$ , between genotype groups. For the Student t-test, the  $p$ -value for equal variances not assumed was reported when Levene's test was significant ( $p < .05$ ).

Females were found to have higher depressive scores than males,  $t(1, 163.6) =$

Table 1. Percentage of childhood maltreatment experienced.

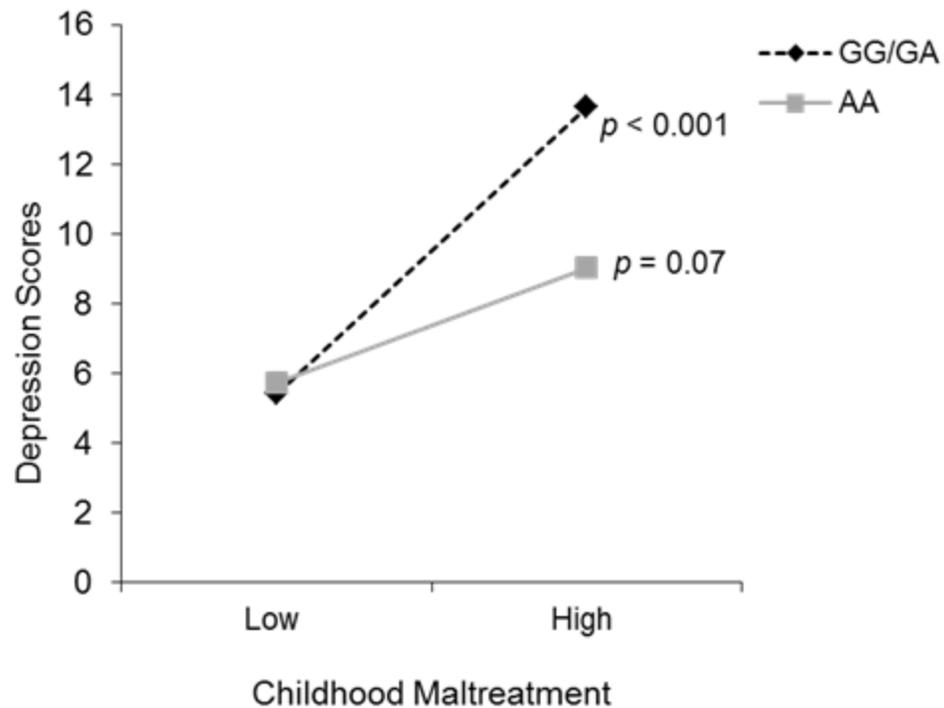
<b>Childhood Maltreatment</b>	<b>Never/Rarely</b>	<b>Sometimes</b>	<b>Often/Very Often</b>
Psychological	70.7%	15%	14.3%
Neglect	90.2%	4.9%	4.9%
Physical	89.2%	7.1%	3.7%

2.60,  $p = .01$ , and they also reported higher levels of childhood maltreatment,  $t(1, 225.4) = 2.49$ ,  $p = .01$ . However, there were no gender differences on distrust levels  $t(1, 286) = -0.15$ ,  $p = .89$ , nor was the Gender x Gene interaction significant in relation to depressive symptoms, childhood maltreatment, or distrust. Furthermore, in an effort to control for the potential influence of population stratification, analyses assessing Ethnicity x Gene interactions on depressive symptoms, distrust and childhood maltreatment were not found to be significant. In this regard, these analyses included all nine ethnicities as one of the factors, as well as a further analysis in which these ethnicities were collapsed into 5 ethnic conditions.

The relation between childhood maltreatment and depressive symptoms revealed that total scores on childhood maltreatment were related to severity of depressive symptoms  $r = .44$ ,  $p < .001$ . Likewise, psychological abuse,  $r = .46$ ,  $p < .001$ , physical abuse,  $r = .30$ ,  $p < .001$ , and neglect,  $r = .34$ ,  $p < .001$ , were positively related to depression scores. The possible moderating effect of genotype was explored regarding

the relationship between total childhood maltreatment scores, psychological abuse, physical abuse and neglect with symptoms of depression.

To examine the possible interaction between total childhood maltreatment scores and depressive symptoms with OXTR genotype, a hierarchical linear regression was conducted. Genotype and total maltreatment scores were entered on the first step, and the Genotype x maltreatment interaction term was entered on the second step. The moderating role of OXTR genotype on the relation between childhood maltreatment and depression was significant,  $\Delta R^2 = .02$ ,  $b = -2.47$ ,  $t = -2.42$ ,  $p = .02$ . Follow up simple slope analyses (Preacher et al., 2006), as shown in Figure 1, revealed that levels of depressive symptoms did not differ between genotypes at low levels of childhood maltreatment. However, at high levels of childhood maltreatment, depressive symptoms were significantly increased among those with one or two copies of the G allele ( $p < .001$ ), an effect not seen among individuals with the AA genotype ( $p = .07$ ). To further examine whether differences existed between individuals with one or more copies of the G allele, orthogonal contrasts were carried out in a hierarchical linear regression. As expected, individuals with the GG and GA genotypes displayed a similar relation between childhood maltreatment and depressive symptoms, and thus did not moderate this relationship. This moderation analysis was also conducted with ethnicity as a covariate, in an effort to control for population stratification, and the analysis remained significant.



*Figure 1.* The relation between childhood maltreatment and depression scores as a function of the OXTR rs53576 genotype (GG/GA versus AA). The simple slopes analyses revealed that genotype groups did not differ at lower levels of childhood maltreatment. However, depressive symptoms increased significantly when higher levels of childhood maltreatment were experienced, but only among those individuals with the GG/GA genotype.

We examined the potential interactive effects between the different forms of maltreatment (i.e. psychological abuse, physical abuse and neglect) and genotype in predicting depression. A multiple hierarchical linear regression was conducted between genotype and each of psychological abuse, physical abuse and neglect. Genotype and

maltreatment subscales were entered on the first step, and the Genotype x Maltreatment subscale interaction terms were entered on the second step. Neither psychological abuse, physical abuse nor neglect interacted with genotype to predict depression scores,  $\Delta R^2 = .02$ ,  $\Delta F(3, 272) = 2.07$ ,  $p = .11$ . Thus, it seems that the relation between specific forms of maltreatment and depressive symptoms were not uniquely moderated by genotype, whereas the interaction was evident when maltreatment as a whole was considered.

It was of interest to explore potential pathways through which childhood maltreatment predicted depressive symptoms. In this regard, level of distrust was examined as a possible mediator through which childhood maltreatment predicts depression scores. As expected, distrust was positively related to depression scores,  $r = .53$ ,  $p < .001$ . A mediation analyses was conducted, using bootstrapping techniques based on 5000 resamples to determine 95% confidence limits (Preacher and Hayes, 2004). The mediated effect of distrust in the relation between childhood maltreatment and severity of depressive symptoms was significant (95% CI {1.19, 2.63}), although the relation between maltreatment and depressive symptoms remained significantly evident,  $b = 1.88$ ,  $p < .001$ . An alternative model was tested in which depressive symptoms mediated the relation between childhood maltreatment and distrust, and this model was also significant, (95% CI {0.11, 0.24}).

We further examined whether this mediation relationship was moderated by OXTR genotype. Moderated mediation analyses conducted using bootstrapping procedures and confidence intervals based on 5000 resamples (Preacher et al., 2007) showed that, as expected, the OXTR genotype moderated the mediating role of distrust in the relation between childhood maltreatment and depression scores (Figure 2A).

Specifically, the relation between maltreatment and depression scores was mediated by distrust, but this mediated effect was only significant for G carriers. This moderated mediation analysis was also conducted with ethnicity as a covariate and remained significant.

An alternative moderation model, in which the OXTR genotype moderated the relation between childhood maltreatment and distrust was found not to be significant. However, when a final alternative model wherein the moderating effects of genotype on all three pathways were considered simultaneously (Figure 2B), only the moderation of the direct path between childhood maltreatment and depression scores accounted for unique variance. Thus, although distrust among those individuals with a G allele appeared to be more strongly associated with depressive symptoms than among those with AA genotype, when reports of childhood maltreatment were taken into consideration, their sensitivity to early-life maltreatment preclude effects attributable to distrust in the evolution of depressive symptoms.

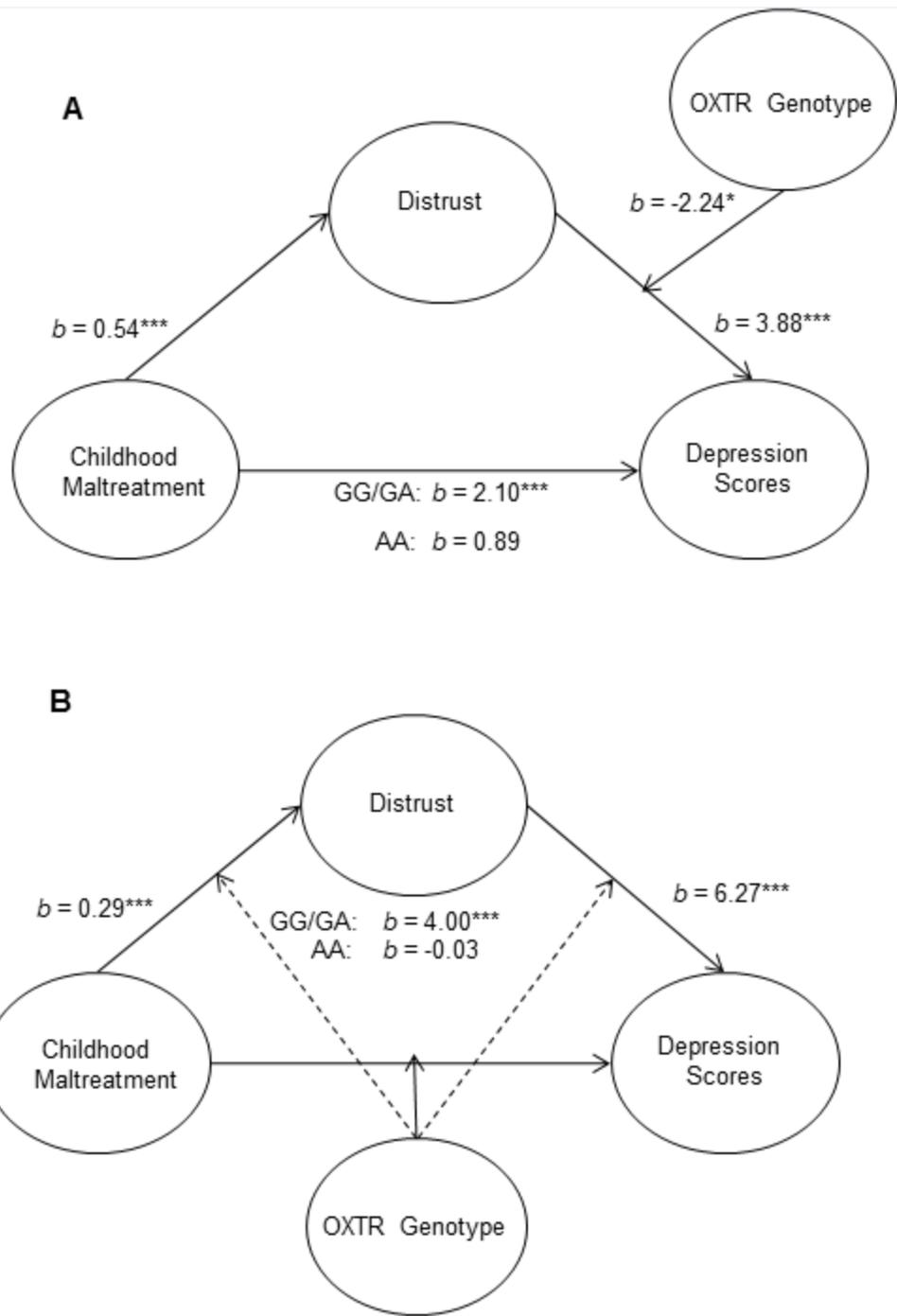


Figure 2. Schematic representations of the moderated mediation models. The relation between childhood maltreatment and depression scores through distrust was moderated by OXTR rs53576 genotype (GG/GA versus AA). This mediation model was found only to be significant among G carriers (A). An alternative model was tested, in which the

relationship between childhood maltreatment and depression scores through distrust was moderated at all three pathways. This time, only the direct path was moderated and again the effect was only observed among individuals with the GG/GA genotype (B). \* $p < .05$ , and \*\*\* $p < .001$ .

## **Discussion**

In the current investigation, there was no direct associated found between the OXTR genotypes and depression scores. This is in contrast to the previous finding that A-allele carriers displayed greater depressive symptomatology (Saphire-Bernstein et al., 2011), although these variations may be due to the fact that different measures were used to assess depressive symptoms. Furthermore in the current investigation GG and GA genotypes were collapsed together, whereas Saphire-Bernstein et al. collapsed OXTR A carriers together. Importantly, in the current study, the OXTR genotype interacted with experiences of childhood maltreatment to predict depressive symptoms. Specifically, individuals with one or two copies of the G allele reported greater severity of depressive symptoms when they reported high levels of maltreatment compared to those individuals with the AA genotype. Thus, having experienced a negative early-life environment, the more sensitive G allele carriers appeared to be at greater risk of exhibiting depressive symptoms. These findings are consistent with the report that African American individuals with the GG genotype were at increased risk for emotional dysregulation provided that they experienced three or more types of childhood maltreatment (Bradley et al., 2011). Those with the GG or GA genotype in the present study also displayed higher depressive symptoms provided that they had experienced high levels childhood maltreatment. However, it is uncertain from the available data whether this relation was

apparent with multiple different forms of maltreatment. This said, in the current investigation, the OXTR SNP interacted with total childhood maltreatment scores in predicting depressive symptoms and not with the individual subscales of maltreatment (i.e., psychological abuse, physical abuse and neglect). In effect, the different forms of maltreatment do not account for unique variance, and it is all forms of maltreatment that was sufficient to increase depression symptoms among G carriers.

These findings suggest that individuals carrying one or two copies of the G allele may be more affected by previous experiences, regardless of whether it is positive or negative. Considering the research implicating the beneficial traits associated with having the G allele of the OXTR SNP, the current findings might seem counterintuitive. However, our results are in line with the view that the same genetic factors that make individuals relatively sensitive to a negative environment also influence sensitivity to a positive environment. In this respect, it has been suggested that ‘for better or for worse’ certain genotypes are considered to promote greater plasticity and susceptible to the environment, (Belsky et al., 2009; Belsky and Pluess, 2009). Essentially, individuals with the GG or GA genotype of the OXTR SNP may thrive in a positive environment, (e.g. one that is high in social support) but, this same allele may encourage susceptibility in a negative environment. In fact, similar findings were reported with regard to the BDNF polymorphism (Val66Met) and vulnerability to childhood maltreatment. Specifically, the Val/Val genotype (which at first blush would seem to be associated with diminished vulnerability to stress-related pathology), were more negatively affected by experiences of early-life adversity compared to individuals with the Val/Met & Met/Met genotypes (Caldwell et al., 2013).

Evidently when examining the OXTR genotypes and their associations, environmental influences matter, and this extends to research focusing on other domains of the oxytocin system. In this regard, the effects of intranasal oxytocin administration on social behaviours are often moderated by contextual factors, thus leading to weak and/or inconsistent findings (Bartz et al., 2011). Furthermore, it was suggested that endogenous oxytocin levels across individuals are highly variable, and could be an indicator of sensitivity to social cues. Essentially, although high plasma oxytocin levels might be related to increased sensitivity and in turn elevated pro-social behaviors in general, the increased sensitivity among these individuals might result in elevated distress under conditions where their social needs are not met (Taylor, 2006; Bartz et al., 2011). From this perspective, oxytocin may confer a disposition towards increased sensitivity to social cues that can be either beneficial or detrimental depending on the environmental context (Bartz et al., 2011).

Beyond contextual factors, personal characteristics such as having a distrusting outlook on the world may be important when explaining the relationship between early-life adversity and depressive symptoms among specific OXTR genotypes. It has been reported that individuals with GG genotypes display greater trust in an investor-trustee money transfer game (Kruger et al., 2012). However, it has also been reported that no association existed between the OXTR SNP and human trust behaviors (Apicella et al., 2010). Thus, it was suggested that the OXTR SNP may be associated with trust-specific behaviors, but not lend itself to a general increase in trustworthy behaviors (Krueger et al., 2012). As the scale in the current investigation measured general feelings of distrust, this might account for why the OXTR genotypes did not differ in levels of distrust.

Nevertheless, distrust was found to mediate the pathway between childhood maltreatment and depressive symptoms. Although, the directionality cannot be confirmed as alternative models were also significant. Furthermore, this mediation only occurred among the G carriers, and not among individuals with the AA genotype. Thus, distrust may have greater ramifications on measures of well-being among those individuals with one or more G alleles. Given that the G allele has been associated with greater levels of trust as well as greater sensitivity, it is possible that a breach of this trust could be particularly damaging for those individuals. However, when the mediation model was tested with OXTR genotype moderating all three pathways, (as seen in Figure 2B) only the direct path was significant. This suggests that, although genotype influences the extent of the relation between distrust and depressive symptoms, it seems that the differential sensitivity to negative early-life experiences is sufficiently overwhelming that when taken into consideration, the role of distrust is obfuscated. It should also be noted that when examining emotional reactions to betrayals in trust and the OXTR rs53576, no association between these variables was reported (Tabak et al., 2013). However, given the different methodologies used and especially considering the current findings were in the context of childhood maltreatment, the different outcomes are not particularly surprising.

There are several limitations associated with the current findings. Early-life maltreatment was determined based on retrospective self-reports. The use of self-reports, although common, might be biased by the individuals' current affective state, and indeed individuals might be unaware of events that occurred years earlier. The present findings are limited in the scope of the early-life adverse events that were considered, and

generalizations beyond this population, in which maltreatment was moderate, would be inappropriate. Additionally, in the current investigation, G carriers were collapsed and compared to individuals with the AA genotype. This method provided the best fit to our data and has been used in previous studies, although there have also been reports in which A carriers were combined (GA/AA). However, additional analyses revealed that individuals with the GG versus GA genotypes did not differ from one another. Importantly, the functionality of the OXTR rs53576 remains unknown. Although it has been suggested that intron 3, in which this particular OXTR SNP is located, may contribute to transcription suppression (Mizumoto et al., 1997), it is also possible that the observed associations are largely due to linkage disequilibrium associations with other functional OXTR polymorphisms (Lin et al., 2007). Finally, the current study included a heterogeneous cultural sample. Ideally, a much larger sample size would allow for analyses to be done separately for each ethnic group as there may be important Gene x Environment differences across ethnicities. A larger sample size might also have permitted analyses to determine whether one or another form of abuse, interacting with genotype, was more closely aligned with depression. Thus, the inability to detect an interactive effect between the OXTR SNP and specific forms of maltreatment could be due to a lack of power stemming from the relatively small number of participants. A power analysis indicated that with the N in used in the current study only a small-medium effect size could be detected.

Summarizing, the results of the present study indicated that the OXTR SNP interacts with early-life adversity in the form of maltreatment to predict depressive symptoms. Specifically, depressive symptoms were most prominent among individuals

with the GG/GA genotype who experienced abuse and neglect. Being correlational, the data do not allow for causal connections to be made. Nonetheless, the present findings are consistent with the view that the OXTR genotype might favor social sensitivity so that early experiences might affect later mood states. Although the G allele has typically been characterized as being associated with beneficial attributes, it seems as if those with the G allele might be most sensitive to environmental influences, whereas individuals with the AA genotype (who are typically viewed as less socially attune or prosocial), were least affected by early-life adversity. One can imagine a breach in trust might be less detrimental to an individual who is less sensitive to their social environment, as seems to be true for the individuals with two copies of the A allele. These data provide support to the likelihood that the G allele in the OXTR SNP rs53576 is not always advantageous, and in some instances the AA genotype does not necessarily suggest an unfortunate fate.

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## Appendix D

McQuaid, R.J., McInnis, O.A., Paric, A., Al-Yawer, F., Matheson, K., Anisman, H. (2015). Relations between Plasma Oxytocin and Cortisol: The Stress Buffering Role of Social Support. *Neurobiology of Stress* (Submitted).

### Abstract

Stress responses in humans can be attenuated by exogenous oxytocin administration, and these stress-buffering properties may be moderated by social factors. Yet, the influence of acute stressors on circulating endogenous oxytocin levels have been inconsistent, and limited information is available concerning the influence of social support in moderating this relationship. In the current investigation, undergraduate women ( $N = 67$ ) were assessed in the Trier Social Stress Test (TSST) with either social support available from a close female friend, no social support being available, or women were not stressed. The TSST elicited marked elevations of state anxiety and negative emotions, which were largely attenuated among women who received social support. Furthermore, baseline oxytocin levels were inversely related to women's general feelings of distrust, as well as basal plasma cortisol levels. Despite these associations, oxytocin levels were unaffected by the TSST, and this was the case irrespective of oral contraceptive use or estrogen levels. In contrast, plasma cortisol elevations were elicited by the psychosocial stressor, varying with oral contraceptive use, an effect that was prevented when social support was available. Taken together, it does not appear that changes in plasma oxytocin accompany the stress attenuating effects of social support on cortisol levels. Furthermore, as plasma oxytocin might not reliably reflect central oxytocin levels, the linkage between oxytocin and prosocial behaviors may, for the moment, be best served by studies in which the hormone is assessed in cerebrospinal

fluid, or administered intranasally in the presence or absence of oxytocin-related polymorphisms.

### **Introduction**

Oxytocin, a neuropeptide produced in the hypothalamus, has gained considerable attention given its presumed role in prosocial behaviors (Donaldson and Young, 2008). In this regard, endogenous oxytocin levels have been associated with trust behaviors (Zak et al., 2005; Kéri et al., 2009), maternal sensitivity (Feldman et al., 2012) and empathy towards strangers (Barraza and Zak, 2009). Furthermore, administration of oxytocin by nasal spray promotes enhanced generosity (Zak et al., 2007), trust (Kosfeld et al., 2005), empathy (Domes et al., 2007), positive communication (Ditzen et al., 2009), and helping behavior (Riem et al., 2013).

Through interactions with other biological systems, oxytocin can modulate stress responses, such as cortisol and cytokine reactivity, and may thus be germane to stress-related psychological disorders (McQuaid et al., 2014). For instance, intranasal oxytocin attenuated salivary cortisol elevations elicited by a physical stressor (Cardoso, et al., 2013), limited the cortisol rise elicited by social ostracism (Linnen et al., 2012) and that associated with couple conflict (Ditzen, et al., 2009). As encouraging as these data seem, a recent meta-analysis indicated a modest non-significant effect of intranasal oxytocin in attenuating cortisol levels during stressful laboratory tasks. However, a dampening effect of oxytocin on cortisol was apparent in those studies that involved a task that elicited a robust HPA-axis response as well as in studies involving clinical populations (Cardoso et al., 2014).

It is likely that social interactions and social support are important components of the stress-attenuating effects of oxytocin. Social support and enhanced connectedness is strongly associated with improved physical (Uchino, 2006) and mental health (Cruwys et al., 2013), and provides an important method of dealing with stressors, thereby attenuating the propensity for illness development (Holt-Lunstad et al., 2008). In this regard, social support diminished cortisol and activation of the dorsal anterior cingulate cortex elicited by a psychosocial stressor (Eisenberger et al., 2007). Furthermore, in males who received social support coupled with intranasal oxytocin prior to a laboratory stressor (Trier Social Stress Test; TSST), a less pronounced cortisol rise occurred compared to individuals who received either social support, oxytocin or neither of these treatments (Heinrichs et al., 2003). Following a social stressor, children who were able to see or hear their mothers, had higher oxytocin levels and cortisol levels were diminished compared to children that had no-contact with their mothers (Seltzer et al., 2010). To be sure, the effectiveness of social support in attenuating stressor responses may vary as a function of the quality of the support provided, or the closeness and empathy of the person providing support (Holt-Lunstad et al., 2007; 2008).

There have been several studies that assessed the influence of intranasal oxytocin on stress responses, but relatively few reports examined the effect of acute stressors on endogenous oxytocin levels in humans under ordinary conditions (i.e. in the absence of lactation and mother-infant bonding). Moreover, the data that have been reported concerning oxytocin release in response to acute psychosocial stressors have been inconsistent (Taylor et al., 2006; Ditzen et al., 2007; Seltzer et al., 2010; Pierrehumbert et al., 2012), possibly being related to various procedural differences, including the way in

which oxytocin levels were determined. In particular, in some studies oxytocin was measured without first extracting the hormone from blood, and thus molecules in addition to oxytocin might have been tagged, yielding exceptionally high levels, likely reflecting oxytocin together with other factors that were present (see Szeto et al., 2011, for a discussion of this topic).

In the current investigation psychosocial responses and biological variations, including cortisol, oxytocin, and estradiol levels, were assessed in women at baseline and following the TSST. Efforts were made to eliminate factors that could confound the potential effects of stressors on oxytocin levels. For instance, in the current study, using females, the effects of a stressor were assessed on circulating oxytocin in properly extracted samples. Furthermore, as self-reported menstrual phase may be unreliable, and estrogen can regulate oxytocin functioning (i.e. transcription of oxytocin as well as its receptor; Choleris et al., 2008), estradiol levels were also assessed. As well, in the present study, the impact of support from a close female friend was assessed, considering that social support coming from a male may be less effective (Glynn et al., 1999). It was hypothesized that having social support from a close female friend would attenuate anxiety, negative mood outcomes as well as cortisol stress responses. However, it was uncertain whether or not oxytocin levels would be expected to rise in response to the stressor or social support manipulation. Conflicting findings were reported in this regard, with some indicating that oxytocin either does not increase in response to a stressor irrespective of social support (Taylor et al., 2006; Ditzen et al., 2007), increases in response to a stressor if social support is also present (Seltzer et al., 2010), or increases in response to a stressor in the absence of support (Pierrehumbert et al., 2012). Given these

disparate findings, in the present investigation we also examined whether qualities of the individual providing support, including empathy, would be related to the participants stress responses.

## **Methods**

### ***Participants***

The study included female undergraduate students ( $N = 67$ ) from Carleton University ranging in age from 17-30 years ( $M_{age} = 19.37$ ,  $SD = 2.08$ ) that were recruited using the online SONA system. Participants represented an ethnically diverse sample comprising White (50.7%,  $n = 34$ ), Black (19.4%,  $n = 13$ ) Arab/West Asian (9.0%,  $n = 6$ ) Asian (6.0%,  $n = 4$ ), Latin American/Hispanic (4.5%,  $n = 3$ ), South Asian (2.0%,  $n = 3$ ), South East Asian (1.5%,  $n = 1$ ), Aboriginal (1.5%,  $n = 1$ ), and other (1.0%,  $n = 3$ ). Living arrangements of the students varied with a large number of individuals residing with their parents (44.8%,  $n = 30$ ), or with friends/roommates (41.7%,  $n = 28$ ), while few lived alone (6.0%,  $n = 4$ ), with a significant other (3.0%,  $n = 2$ ), or other (4.5%,  $n = 3$ ).

In addition to these participants, data relevant to several psychosocial factors were collected from the friends who provided social support during the laboratory session ( $n = 18$ ). This group of individuals ranged in age from 17-21 years with a mean age of 18.78 ( $SD = 1.17$ ) and comprised an ethnically diverse sample that included White (61.1%,  $n = 11$ ), Black (16.7%,  $n = 3$ ), Asian (5.6%,  $n = 1$ ), South Asian (5.6%,  $n = 1$ ), South East Asian (5.6%,  $n = 1$ ), and other (5.6%,  $n = 1$ ).

### ***General Procedure***

The current study was conducted in two phases. Participants first completed a brief on-line pre-screening questionnaire (Part 1) that determined eligibility for the

laboratory session that comprised the TSST and a blood draw (Part 2). The on-line pre-screening questionnaire assessed the presence of a number of exclusion criteria, such as medical conditions or medications that may influence hormone functioning, as well as issues surrounding blood sampling (e.g. a fear of needles, previous history of nausea or fainting during blood collection, or difficulty with veins). Eligible participants were then randomly assigned to one of three conditions: stress-social support (participants were asked to bring a friend to a laboratory session,  $n = 18$ ), stress-no support (participants arrived alone to the laboratory session,  $n = 23$ ) or controls (no stress, no friend,  $n = 26$ ). To avoid a selection bias, all participants who stated they could bring a close female friend were randomly assigned to one of the three conditions, including that in which no friend was present.

#### *Laboratory Session*

All procedures in this study were approved by the Carleton University Ethics Committee for Psychological Research. Laboratory sessions were conducted between 1300 and 1730 hr, and women were asked not to eat, drink (with the exception of water) or smoke for at least an hour before arriving to the session. Once informed consent was signed, participants filled out a number of questionnaires assessing demographic information (including information on oral contraceptive use and menstrual cycle phase), depressive symptomatology and a relationship closeness measure (related to the friend they brought with them to the laboratory session). Upon completion of these measures, a registered nurse inserted a catheter into the participants' non-dominant arm for blood collection. Participants were then asked to relax for 10 minutes to habituate to the laboratory environment. Following the relaxation period, participants in the stress

conditions were instructed that they would be given 10 minutes to prepare for an employment task comprising a five-minute speech and five-minute mental arithmetic task in front of a panel of graduate student judges. In addition, participants were told they were being videotaped during the psychosocial stressor.

During the 10-minute preparatory period, participants in the stress-no support condition prepared for the stressor alone, whereas participants within the stress-social support condition had their friend present. The friends were instructed to provide emotional and/or instrumental support to the participant. Immediately following the 10 minute preparatory period, participants in the two stressor conditions underwent the TSST. Participants in the control condition were asked to complete an employment task, which comprised writing down their strengths and past work/volunteer experience on a form. Following the TSST or control task, participants continued completing questionnaires concerning measures of stressor appraisal, anxiety and affect scores until completion of the experiment.

Blood, which was continuously being drawn through a Dakmed ambulatory pump, was sampled at five time-points during the session, which included 15 minutes before the TSST or the written employment task (controls), immediately before the stressor/tasks began, and then at five, 15 and 30 minutes following completion of the TSST or the written employment task.

Upon arrival to the experimental session, the friend was led to a nearby room in which they signed an informed consent, and were asked to complete a questionnaire booklet assessing basic demographic information, depressive symptomatology, a closeness of relationship measure, and empathy scores. They were told about the stressful

nature of the employment task (their friend would undergo), and told that they would be assisting the participant during the 10 minute preparatory period. They were asked to provide as much support as possible to their friend. Once the preparatory period was complete, participants were asked to return to a nearby room to complete the remainder of the questionnaires. They were not present when the participant was being tested.

### ***Measures***

*Depression.* The 21-item Beck Depression Inventory (BDI) was used as a measure of depressive symptomatology (Beck et al., 1961). Each item comprises one of four options, ranging from low to high depressive symptomatology. Total scores were calculated by summing across all items, (Chronbach's  $\alpha = .88$ ).

*Closeness of Relationship.* Inclusion of other in the self scale (IOSS) (Aron et al., 1992) was used to assess closeness of the friend and the participant. This assessment provides a graphical representation of seven separate Venn-like diagrams, in which participants are asked to choose one, where increasing overlap between two circles represents a greater degree of closeness.

*Distrust and Cynicism.* An 8-item Distrust and Cynicism Scale (derived from the Cook-Medley Hostility Scale) was used to measure general feelings of cynical distrust (GreenGlass and Julkunen 1989, 1991). Each item can be rated from 0 (completely disagree) to 3 (completely agree). A total score was calculated by taking the mean across all items ( $\alpha = .82$ ). Items such as; '*It is safer to trust nobody*' can be found in this scale.

*Empathy.* The 28 item Interpersonal Reactivity Index (Davis, 1983) was used to assess total empathy scores. Each item ranges from 0 (does not describe me well) to 4 (describes me well), with higher scores reflecting greater empathy. Items such as, '*I am often quite*

*touched by things that I see happen,*' can be found in this scale. Total empathy scores were created using a mean across all items ( $\alpha = .82$ ).

*Stress appraisals.* The 28-item Stress Appraisal Measure (SAM) (Peacock and Wong, 1990) comprises multiple subscales representing appraisals of threat, challenge, centrality, stressfulness, controllability and uncontrollability. In the present study, only the four items that represent the subscale for stressfulness were used. These items were measured on a five-point scale ranging from 1 (not at all) to 5 (extremely), with higher scores indicating higher levels of perceived stress. A mean across all items created a total stress appraisal score ( $\alpha = .95$ ).

*Anxiety.* State anxiety was assessed using the Spielberger State Trait Anxiety Inventory (STAI) (Spielberger et al., 1983). The 20-item state anxiety scale was used to measure current feelings of anxiety following the TSST. Items ranged from 1 (not at all) to 4 (very much), where higher scores indicate greater state anxiety. Total scores were obtained by summing across all items ( $\alpha = .95$ ).

*Mood.* The 41-item Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988) assessed the presence and severity of positive and negative affective states following the TSST. Responses ranged on a six-point scale from 0 (not at all) to 6 (extremely). Various mood-state subscales were calculated including sadness ( $\alpha = .91$ ), anger ( $\alpha = .94$ ), fear ( $\alpha = .93$ ) and shame ( $\alpha = .96$ ). Total negative affect scores were calculated by a mean across items ( $\alpha = .94$ ).

#### *Blood Collection*

Blood samples were collected continuously (at a low draw rate) into chilled EDTA coated (for plasma) and Serum separator (for serum) vacutainer tubes. Separate

chilled EDTA tubes were used for the collection of oxytocin, which contained aprotinin. Samples were taken (at an increased draw rate) 15 minutes before the TSST or the employment task (controls), immediately before the stressor/tasks began, and then at five, 15 and 30 minutes post-task. Following collection, samples were centrifuged for 15 minutes at 4°C and 2100g, plasma and serum were immediately aliquoted into Eppendorf tubes and frozen at -80°C.

#### *Plasma Cortisol*

Plasma cortisol was determined in duplicate by a radioimmuno assay (RIA) using the <sup>125</sup>I kit obtained from ICN Biomedicals Inc., Irvine, CA. The assays were performed according to the manufacturer's instructions. The intra-assay variability was less than 8% and the minimum detectable concentration was 0.17µg/dL.

#### *Plasma Oxytocin*

Prior to the assay, oxytocin was extracted as recommended following the procedure manual from Enzo Life Science Inc., (Farmingdale, NY). For the extraction procedure, 1mL of plasma was used and samples were evaporated with nitrogen gas, following which samples were stored at -20°C until the assay. Plasma oxytocin concentrations were determined through an ELISA kit obtained from Enzo Life Science Inc., according to the manufacturer's instructions. The intra-assay variability was less than 12%. Furthermore, according to the manufacturer's kit information, cross reactivity for arginine vasopressin was less than 0.02%.

#### *Serum Estradiol*

Serum estradiol was determined through an ELISA kit obtained from Invitrogen (Camarillo, CA). This assay was performed according to the manufacturer's instructions.

The intra-assay variability was less than 5% and the minimum detectable concentration was  $5 \pm 2$ pgmL.

Of the 67 participants, there were four individuals from whom blood samples could not be obtained due to complications with small veins. As well, in some instances participants did not have five valid samples for hormone detection (e.g., failure of blood collection equipment, collapsed vein or not enough blood could be obtained), thus they were removed from any repeated measures analyses and as such the *n*'s per group will vary.

### **Statistical Analyses**

Statistical analyses were performed using SPSS for Windows 18.0 (SPSS Science, Chicago, Illinois, USA). Analyses assessing the influence of the TSST on stress appraisals and state anxiety were performed using a one-way analyses of variance (ANOVA) (Stressor Condition: controls, stress-support, stress-no support). A MANOVA was used to determine the effects of the TSST condition on mood outcomes, including sadness, anger, fear, shame and negative affect. Oxytocin was analyzed using a 3 (Stressor Condition) x 5 (Time: 5 time-points) mixed measures ANOVA with Time serving as the within-group factor. For cortisol assessment, an area under the curve (AUC) analysis with respect to increase was performed using the formula proposed by Pruessner et al., (2003) with Stressor Condition and Oral contraceptives (yes vs. no) serving as the between groups factors. Follow-up comparisons comprised t-tests with a Bonferonni correction to maintain the alpha level at 0.05. Additionally, Pearson's correlation coefficients were determined between cortisol, oxytocin, and distrust levels. Pearson's correlation coefficients were also determined between the friends' psychosocial

scores and the participants' psychosocial and biological scores.

## Results

### *Psychosocial responses*

As expected, prior to the TSST, there were no differences between individuals in the control, stress-support, or stress-no support conditions on levels of depressive symptoms,  $F(2, 64) = .21, p = .81$ . Following the TSST, stress appraisals varied as a function of the Stressor Condition,  $F(2, 64) = 37.08, p < .001, \eta^2 = .54$ . As shown in Figure 1A, individuals in both the stress-support and the stress-no support conditions reported elevated stressfulness compared to controls,  $p < .001$  and  $p < .001$ , respectively. State anxiety also differed with the Stressor Condition,  $F(2, 64) = 9.94, p < .001, \eta^2 = .24$ , such that individuals who were stressed but did not receive support exhibited elevated state anxiety compared to controls,  $p < .001$ , an effect not apparent among the stressed individuals who received social support  $p = .15$  (Figure 1B).

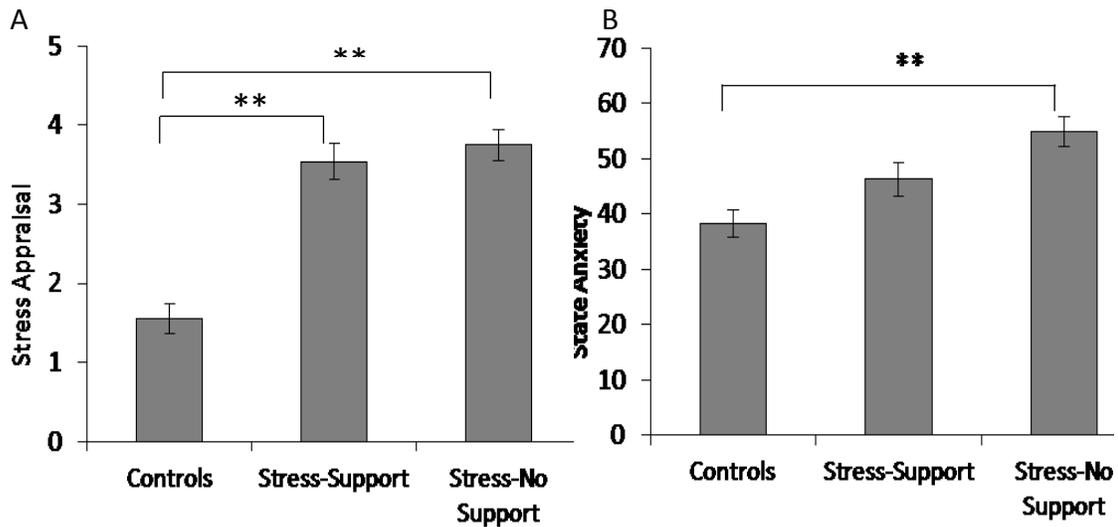


Figure 1. Stress appraisal scores (A) and State anxiety levels (B) among the controls ( $n = 26$ ), stress-support ( $n = 18$ ) and stress-no support ( $n = 23$ ) following the TSST. Data represents means  $\pm$  S.E.M.  $**p < 0.001$ .

A significant MANOVA revealed differences in affective states following the TSST as a function of the Stressor Condition,  $F(10, 122) = 4.77, p < .001, \eta^2 = .28$ . As shown in Figure 2, there was an overall effect of stress for feelings of shame,  $F(2, 64) = 13.88, p < .001, \eta^2 = .30$ , such that higher levels of shame occurred among both the stress-no support and stress-support conditions compared to controls,  $p < .001$  and  $p = .001$  respectively. Levels of sadness also differed between Stressor Conditions,  $F(2, 64) = 6.10, p = .004, \eta^2 = .16$ . Specifically, the stressed-no support group displayed elevated

sadness compared to controls,  $p < .001$ , whereas individuals who were stressed but received support did not differ from controls,  $p = .32$ . Feelings of anger differed between Stressor Conditions,  $F(2, 64) = 7.90, p = .001, \eta^2 = .20$ , such that individuals in the stress-no support condition reported more anger compared to controls,  $p = .001$ , an effect not apparent among individuals in the stress-support condition. Further, as shown in Figure 2, anger tended to be somewhat higher in the stress-no support compared to the stress-support condition, although this only approached significance,  $p = .06$ . Fear and negative affect followed a similar trend,  $F(2, 64) = 10.21, p < .001, \eta^2 = .24$  and  $F(2, 64) = 10.54, p < .001, \eta^2 = .25$  respectively. In these instances, individuals who were stressed and had no support displayed elevated fear and negative affect compared to controls,  $p$ 's = .001 and their stress-support counterparts,  $p$ 's = .02.

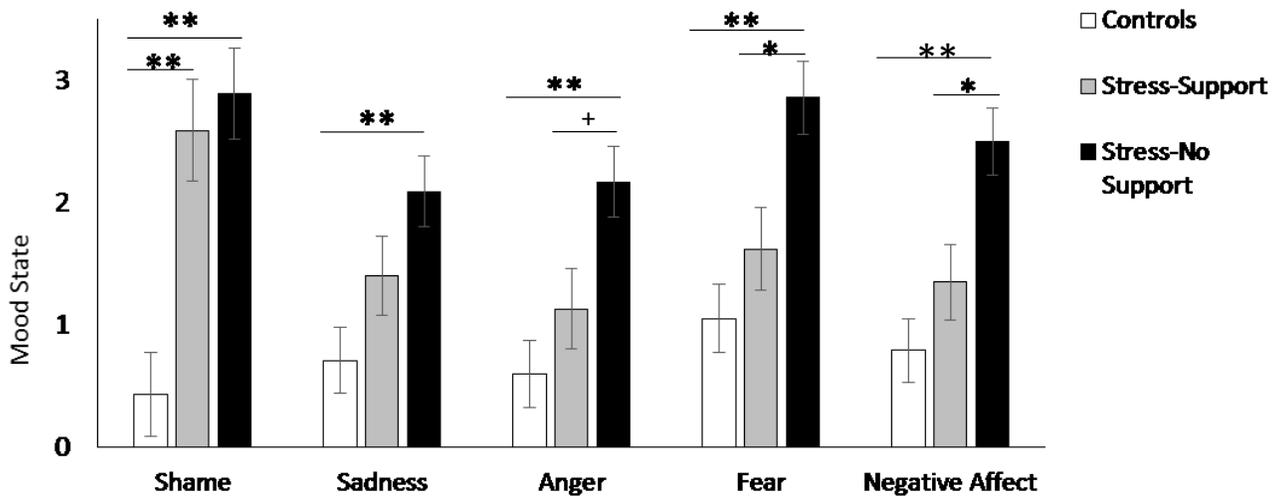


Figure 3. Plasma oxytocin levels (pg/mL) collected at five time points including 15 minutes prior to the TSST (T1), immediately before the TSST began (T2) and five minutes (T3), 15 minutes (T4) and 30 minutes (T5) following the TSST. The graph

represents individuals in the control ( $n = 15$ ), stress-support ( $n = 15$ ) and stress-no support ( $n = 18$ ) conditions. Data represents means  $\pm$  S.E.M.

### *Friends' responses*

An analysis was conducted to assess the extent to which the participants and the friends they brought to the laboratory shared the closeness they felt toward each other. Unexpectedly, the correlation of perceived closeness between the friend and the participant, indicated that friends' perceptions of closeness and participants' perceptions of closeness with each other were unrelated,  $r(15) = .02, p = .93$ . Furthermore, friends' perceptions of closeness were not related to participants' psychosocial responses to the TSST, including state anxiety  $r(15) = .15, p = .56$ , stress appraisals,  $r(15) = .17, p = .50$ , and any of the participants' biological scores at baseline or in response to the TSST. As well, the participants' perceptions of closeness also did not significantly correlate with their psychosocial or biological stress responses and similarly, the friends' level of empathy did not relate to the participants' state anxiety,  $r(16) = -.05, p = .86$ , or stress appraisal,  $r(16) = -.02, p = .93$  scores.

Interestingly, empathy scores of the friend were inversely related to the participants' cortisol levels at both baseline,  $r(15) = -.60, p = .01$ , and following the TSST,  $r(15) = -.50, p = .04$ . As well, a relationship was found between the friends' level of empathy and the participants' basal oxytocin levels, such that higher empathy from the friend was associated with elevated oxytocin levels at baseline among the participants,  $r(15) = .56, p = .02$ . A similar association was not observed in relation to oxytocin scores following the TSST.

### ***Biological Responses***

It was first of interest to examine the relationships between baseline oxytocin levels with depression and distrust scores. As shown in Table 1, depressive scores were not significantly associated with oxytocin, however, a significant inverse relationship was found between distrust and oxytocin,  $r(59) = -.28, p = .03$ . As well, oxytocin at baseline was inversely related to baseline cortisol levels,  $r(55) = .28, p = .04$ .

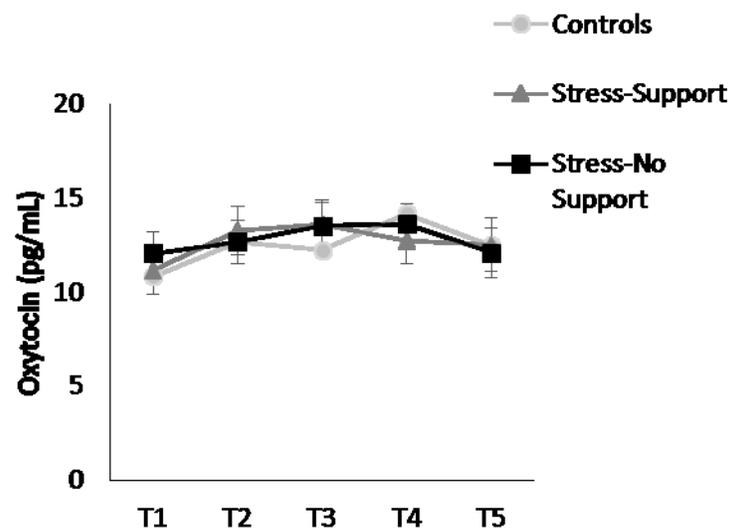
Table 1.

*Zero-order Pearson Correlations Between Depressive Symptoms, Distrust and Baseline Oxytocin, Cortisol and Estradiol*

	1	2	3	4	5
1. Depressive Symptoms	--	--	--	--	--
2. Distrust	.55**	--	--	--	--
3. Oxytocin	-.15	-.28*	--	--	--
4. Cortisol	.16	.15	-.28*	--	--
5. Estradiol	.05	-.05	.05	-.24	--

Although estrogen is known to influence oxytocin transcription, as shown in Table 1, no relationship was found between levels of oxytocin and estradiol at baseline. Almost half of the females in the current experiment reported using oral contraceptives ( $n = 30$ ), whereas 36 females were not taking oral contraceptives. As expected, females taking oral contraceptives ( $M = 40.31, SE = 8.26$ ) displayed reduced serum estradiol levels compared to females not taking oral contraceptives, ( $M = 73.84, SE = 8.47$ ),  $t(1, 52) = 2.83, p = .007$ . Similar to estradiol, oxytocin levels at baseline also did not vary based on whether females were using oral contraceptives or not,  $F(2, 59) = 0.35, p = .55$ . Therefore, for further oxytocin analyses, neither oral contraceptives nor estrogen were controlled for. There were no initial difference between controls or stressor groups on baseline levels of oxytocin,  $F(2, 59) = 0.65, p = .53$ . Furthermore, over the course of the

TSST or the written employment task (for controls), oxytocin levels did not significantly increase, although there was a trend in this direction,  $F(4, 180) = 2.14, p = .08, \eta^2 = .05$ . Additionally, as shown in Figure 3, oxytocin levels in response to the TSST did not vary by Stressor Condition,  $F(8, 180) = 0.40, p = .92$ . In addition to this analysis, AUC analyses were also conducted for oxytocin with respect to ground (full area beneath the oxytocin scores, irrespective of whether or not oxytocin rose) and with respect to increase (change of oxytocin levels). In neither instance were differences found between conditions.



*Figure 3.* Plasma oxytocin levels (pg/mL) collected at five time points including 15 minutes prior to the TSST (T1), immediately before the TSST began (T2) and five minutes (T3), 15 minutes (T4) and 30 minutes (T5) following the TSST. The graph represents individuals in the control ( $n = 15$ ), stress-support ( $n = 15$ ) and stress-no support ( $n = 18$ ) conditions. Data represents means  $\pm$  S.E.M.

Oral contraceptive users displayed elevated baseline cortisol levels ( $M = 14.19, SE = 1.61$ ) compared to individuals not taking oral contraceptives ( $M = 8.62, SE = 1.15$ ),

$t(1, 55) = -2.88, p = .006$ . However, cortisol levels did not vary as a function of menstrual phase or smoking. Thus, oral contraceptive use was included as a variable in subsequent analyses relating to cortisol. An analysis of area under the curve (AUC) indicated that although there was no main effect of Stressor Condition on cortisol,  $F(2, 50) = 2.22, p = .12, \eta^2 = .08$ , there was a main effect of Oral contraceptives,  $F(2, 64) = 6.50, p = .014, \eta^2 = .12$ , with a larger AUC being present among individuals who were taking oral contraceptives (mean difference = 154.27). Additionally, cortisol varied as a function of the Stressor Condition x Oral contraceptives interaction,  $F(2, 50) = 5.44, p = .007, \eta^2 = .18$ . As shown in Figure 4, and confirmed by the follow-up tests of the simple effects comprising this interaction, among individuals not using oral contraceptives (left hand panel), cortisol did not vary in relation to the TSST. However, for individuals using oral contraceptives, the stress-no support group displayed a larger cortisol AUC compared to controls (mean difference = 358.86),  $p = .004$ . Further, while there were no differences between controls and individuals who were stressed with support available, the AUC tended to be greater in the stress-no support group compared to the stress-support group (mean difference = 266.77),  $p = .07$ .

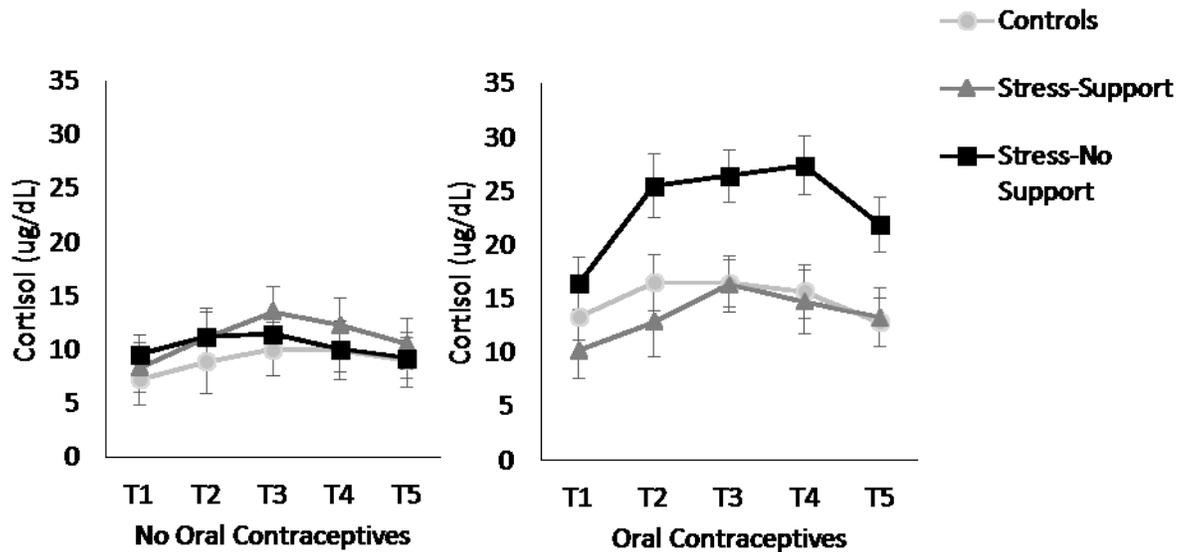


Figure 4. Cortisol levels in plasma ( $\mu\text{g/dL}$ ) collected at five time points including 15 minutes prior to the TSST (T1), immediately before the TSST began (T2) and five minutes (T3), 15 minutes (T4) and 30 minutes (T5) following the TSST. The graph represents individuals who were not taking oral contraceptives (left panel) in the control ( $n = 8$ ), stress-support ( $n = 9$ ) and stress-no support ( $n = 14$ ), conditions as well as the individuals in the control ( $n = 10$ ), stress-support ( $n = 7$ ), and stress-no support ( $n = 8$ ), conditions who were taking oral contraceptives (right panel). Data represents means  $\pm$  S.E.M.

## Discussion

In the current study, the TSST increased stressor appraisals irrespective of social support being available, whereas state anxiety scores following the TSST were only elevated among individuals who were stressed and did not receive support. In essence, although participants in both conditions were equally cognizant of the stressor, social support buffered the anxiety associated with the TSST. Similarly, relative to controls, individuals who were stressed and did not receive support displayed high levels of anger, fear, and sadness as well as overall negative affect relative to non-stressed controls. In contrast, stressed individuals who received support did not exhibit elevations of these emotions, again pointing to the stress-buffering effects of social support (Cohen and Willis, 1985). Interestingly, despite the effectiveness of social support in acting against some of the negative emotions promoted by the TSST, feelings of shame were not diminished by support. It is not immediately apparent why shame was not attenuated; however, such feelings may be a uniquely powerful emotion (relative to the others measured), being a threat to the ‘social self’, and might thus not be amenable to being appreciably diminished by social support. In this regard, it has been reported that the effects of the TSST on cortisol levels were directly related to the shame reported in this situation (Gruenewald et al., 2004).

Although the availability of social support was accompanied by reductions of several negative emotions, the independent assessments made by participants and their friends regarding their closeness were not consistent with one another (i.e., participants’ perceived closeness to the friend was not necessarily shared). Likewise, the degree of closeness and/or empathy expressed by the friend was not related to anxiety or negative affect elicited by the TSST among participants, and similarly, the participants’ perceived

closeness to the friend was unrelated to their feelings of anxiety or negative affect. It has been predicted that relationship quality is an important component of support efficacy (Holt-Lunstad et al., 2007). However, in the present investigation social support appeared to buffer against anxiety and negative emotionality irrespective of perceived closeness. In effect, simply because individuals did not share their perceived closeness was not predictive of the effectiveness of the support provided. The measure of closeness adopted (Inclusion of other in the self) is one that has been frequently used and is considered to be a valid measure of closeness (Tropp and Wright, 2001).

### *Basal oxytocin*

Given the presumed relationship between oxytocin and prosocial behaviors, and that oxytocin was also implicated in inferring the mood states of others (Domes et al., 2007), it is interesting that having a friend present with generally high levels of empathy was related to elevated oxytocin levels among participants. Although it is tempting to suggest that the higher empathy of the friend caused an oxytocin release, the correlational nature of the data preclude this conclusion. Empathy has been found to be related to endogenous oxytocin release, particularly among females (Barraza and Zak et al., 2009), and intranasal oxytocin promotes empathetic behaviors (Domes et al., 2007), but it has yet to be shown that empathy of one individual may be related to oxytocin levels in a close other. This finding, being the first of this nature to be reported, certainly needs to be replicated.

Lower baseline oxytocin levels were related to higher levels of distrust, which is consistent with the reported elevated plasma oxytocin levels associated with trust behaviors in a monetary game (Zak et al., 2005) as well as trust-related interactions

involved in sharing secrets (Kéri et al., 2009). Consistent with these reports, intranasal oxytocin administration increased trust-related behaviors in a monetary game (Kosfeld et al., 2005), and trust with confidential information (Mikolajczak et al., 2010). Unlike the studies showing a relationship between endogenous oxytocin and trust (Zak et al., 2005; Kéri et al., 2009), however, such a relationship has not always been reported (Christensen et al., 2014). It was suggested that these discrepant results may be due to the procedure in the former studies not including the extraction of oxytocin from samples prior to the assay being conducted and hence the presence of oxytocin may have been contaminated by other factors being assayed concurrently (Christensen et al., 2014). In the current study, the relation between oxytocin and basal levels of general distrust was found in samples where the hormone was extracted prior to the assay, although this does not imply that oxytocin scores would be linked to trust or distrust following an experimental manipulation.

Consistent with reports that oxytocin administration reduces cortisol levels (Ditzen, et al., 2009; Linnen et al., 2012; Cardoso, et al., 2013), baseline oxytocin was inversely related to circulating cortisol. Although oxytocin is thought to contribute to affiliative/social approach behaviors by diminishing fear/anxiety (Taylor, 2006), it is uncertain whether the oxytocin link to cortisol were tied to prosocial behaviors.

#### *Oxytocin and cortisol in relation to post-stressor responses*

Although studies in animals have indicated that oxytocin elevations are elicited by acute stressors (Danevova et al., 2013), such effects are less consistently observed in humans. Cortisol treatment in humans is known to increase oxytocin levels (Tops et al., 2012), and thus it might have been expected that a stressor would elicit a similar

outcome. However, as previously observed (Taylor et al., 2006; Ditzen et al., 2007), oxytocin levels were unaffected by the TSST. This was the case regardless of circulating estradiol levels or whether women were using oral contraceptives. It is also important to consider that oxytocin is released in a pulsatile fashion (Ludwig and Leng, 2006) and thus, it is possible that the time of collection did not capture this dynamic process. This said, blood for each sample was collected over a two minute period, and samples were taken at three post-stressor intervals, thus making this possibility less likely.

Cortisol profiles among women taking oral contraceptives, as in earlier reports (Kirschbaum et al., 1999), were distinct from non-oral contraceptive users. Compared to females not using oral contraceptives, those taking oral contraceptives in the current study displayed elevated baseline plasma cortisol levels as well as greater plasma cortisol responses to the TSST. Indeed, it was only among women using oral contraceptives that the TSST effectively increased plasma cortisol. Although it has been reported that oral contraceptive users displayed a blunted cortisol response, this only occurred when cortisol was measured in saliva, whereas an appreciable increase (55%) was apparent in blood (Kirschbaum et al., 1999). Thus, the present findings in blood are consistent with those previously reported, and reinforce the distinction between salivary ‘free’ cortisol and total cortisol levels in plasma (which mainly comprises ‘bound’ rather than free cortisol). It was suggested that among oral contraceptive users, estrogen stimulates corticosteroid-binding globulin (CBG) synthesis, resulting in reduced free bioactive cortisol (Kajantie et al., 2008) and enhanced bound cortisol. This aside, it was surprising that cortisol levels among females not taking oral contraceptives were largely unaffected by the TSST. It has been observed that women with high estrogen levels (corresponding

to the luteal phase of the cycle), display significantly increased free cortisol levels in response to stress, and also displayed blunted blood cortisol stress responses (Kirshbaum et al., 1999). The high estradiol levels among females in the current study who were not taking oral contraceptives, may have contributed to the lack of cortisol change in response to the TSST. Furthermore, it may be significant that although the TSST is a potent stressor, the effects on cortisol are typically more pronounced among males (Kajantie and Phillips, 2006) who tend to display greater responses to achievement-oriented stressors, such as mathematics and verbal tasks, whereas women show a greater reactivity to rejection related stressors (Stroud et al., 2002).

Among oral contraceptive users in the present study, social support attenuated the cortisol rise in response to the TSST that was otherwise evident among the individuals who did not receive support. It is thought that one potential mechanism by which social support may buffer cortisol stress responses is through hypothalamic oxytocin release, as treatment with an oxytocin receptor antagonist blocked the social buffering effects in rodents (Smith and Wang, 2014). Likewise, female children that received some form of support from their mothers displayed elevated oxytocin and attenuated cortisol responses to the TSST (Seltzer et al., 2010). Thus, although it might have been expected in the current investigation that the females receiving social support from a close friend would display elevated oxytocin levels following the stressor, this hormone was unaffected by the social support manipulation. Given that plasma oxytocin might not reflect changes in brain oxytocin, our findings do not necessarily suggest that plasma oxytocin levels are unimportant in the buffering effects of support (Landgraf and Neumann, 2004; Leng and Ludwig, 2015). In essence, central oxytocin release could buffer HPA axis responses to

stressors, but levels of this hormone in the periphery might not adequately reflect oxytocin changes in brain.

There were several limitations associated with the current investigation. The sample size was admittedly modest, but it is unlikely that this had any bearing on the lack of an oxytocin rise in response to the stressor or the absence of a cortisol rise among women not using oral contraceptives. In both instances, the stressor did not elicit even a hint of a hormonal change. However, as already indicated, the cortisol measure was determined in blood, and it is uncertain how the stressor and use of oral contraceptives might have interacted in affecting free cortisol in saliva. Ideally, cortisol levels should have been determined from both saliva and blood. Furthermore, a group was not included in the present study in which support was available in the absence of a stressor manipulation. Thus, it was not possible to examine the influence of social support on oxytocin levels over time in the absence of a stressor. Likewise, it would have been advantageous to include a manipulation in which a neutral party was present (as opposed to a friend) who could have acted as a distractor and thus modified the stress response. In this way it could be determined whether the buffering effects observed with respect to cortisol were linked to a close friend being present prior to the actual TSST or whether simply having another person present could have acted in this capacity, possibly by distracting participants.

These caveats notwithstanding, the current study addressed several important issues concerning endogenous oxytocin functioning in relation to stressful experiences. Only a limited number of studies have included females in research pertaining to stress and oxytocin, particularly those studies in which intranasal oxytocin was administered

(owing to complications relating to hormonal fluctuations across the menstrual cycle). Yet, increasing evidence has pointed to differential actions (or correlations) of endogenous and exogenous oxytocin on various behaviors among men and women (Taylor et al., 2010; Hoge et al., 2014), thus suggesting that this hormone may potentially serve different functions in men and women. The present investigation, in women, suggested that basal oxytocin and cortisol levels are related, and oxytocin is linked to attitudes, such as distrust and empathy. A psychosocial stressor in the form of the TSST, did not affect plasma oxytocin levels, but among women using oral contraceptives the stressor increased circulating cortisol. This outcome could be buffered by social support, and once again this occurred despite oxytocin levels in plasma being unaffected. This is not altogether surprising as plasma oxytocin might not be an accurate reflection of oxytocin functioning within stress-relevant brain regions (Landgraf and Neumann, 2004; Leng and Ludwig, 2015), and thus circulating oxytocin might not be an ideal measure to assess the linkage to the stress-attenuating properties of social support. This said, studies in animals have made it fairly clear that central oxytocin release is fundamental to the positive effects of social support on stress responses (Smith and Wang, 2014), but numerous studies in humans have relied simply on plasma oxytocin to provide relevant data to prosocial behaviors. It may be necessary to work beyond peripheral oxytocin levels in assessing complex behaviors. Efforts have been made to assess the effects of intranasal oxytocin treatment, but it is uncertain how much of the hormone actually reaches the brain. Nonetheless, short of animal studies, this approach is likely the best option at the moment, particularly when coupled with analyses of polymorphisms related to oxytocin or its receptor.

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