

Oxytocin and Social Sensitivity: Implications for Vulnerability to Stress and  
Depressive Symptoms

by

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## Abstract

Oxytocin is a neuropeptide that has been implicated in several prosocial behaviors, such as trust, empathy and social affiliation, and might contribute to mental health disorders marked by social disturbances. Indeed, Chapter 1 indicated that the accumulated evidence points to interactions between oxytocin, and various neuroendocrine, neurotransmitter, and inflammatory processes in the emergence of depressive disorders. However, the perspective was discussed that oxytocin does not uniformly enhance prosocial behaviors, but instead increases the salience of social cues, so that positive and negative events might elicit exaggerated responses that influence affective states. The current studies examined the role of oxytocin in relation to various stressors and mental health outcomes. In Study 1 ( $N = 288$ ), it was determined that a genetic variant on the oxytocin receptor gene (OXTR) moderated the influence of early-life events in relation to later depressive symptoms. In the absence of this polymorphism individuals who experienced early-life maltreatment displayed high levels of depressive symptoms, but this was not evident in the presence of this polymorphism. In essence, in the absence of the polymorphism, prosocial behaviors are present, but so is social sensitivity, potentially rendering individuals more vulnerable to the effects of early-life adversity. Study 2 ( $N = 128$ ) revealed that individuals carrying the same ‘prosocial’ genetic variant on the OXTR were more emotionally sensitive and biologically reactive to social ostracism, further supporting the social sensitivity view. Consistent with this, in Study 3 ( $N = 243$ ), individuals carrying a genetic variant on a gene that controls for oxytocin release (CD38 gene), which had previously been viewed as a ‘protective’ variant, displayed poorer peer

and parental relationships and enhanced suicidal ideation. Finally, Study 4 ( $N = 67$ ) revealed that although oxytocin was inversely related to distrust scores and baseline cortisol levels, this hormone was unaffected by a stressor or the presence of social support. These studies highlight the role of oxytocin in stressor responses, social sensitivity and, in turn, vulnerability to mental health outcomes. At the same time, the data also suggest that interpreting the findings solely on the basis of a prosocial framework may be too narrow.

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## **Introduction**

Oxytocin has been the focus of many studies centered on understanding the mechanisms underlying prosocial behaviors. In this regard, oxytocin manipulated through nasal spray promotes prosocial moods and behaviors, such as empathy, trust and optimism (Kosfeld et al., 2005; Saphire-Bernstein et al., 2011; Shamay-Tsoory et al., 2013). However, oxytocin may also have a ‘dark side’, as this hormone may promote aggression and violence in certain contexts (DeWall et al., 2014). In fact, it is thought that rather than promoting prosocial behaviors, oxytocin may serve to increase the salience of social cues, and thus in a positive environment, elevated oxytocin may confer prosociality, but in a negative environment, enhanced sensitivity may elicit negative outcomes. In line with this view, social sensitivity might contribute to psychological illnesses, such as depression, by emphasizing the negativity associated with some situations.

A review of the relevant literature, presented in Chapter 1, discusses the implications of oxytocin to depressive disorders. It is thought that levels of oxytocin may be disturbed in depression, and that oxytocin interacts with neuroendocrine, neurotransmitter and immune processes that may contribute to this disorder. Throughout the review, the social sensitivity perspective of oxytocin is recognized. In this regard, it is proposed that although oxytocin can promote prosocial attributes, such as social support seeking, which may be protective against depression, in certain negative contexts, oxytocin may confer vulnerability to illness. The view that oxytocin increased social sensitivity was hardly explored in the human oxytocin research field, but as this appeared

to be a viable hypothesis, it was of interest to examine this perspective further. In Study 1, we examined the relationship between a genetic variant of the oxytocin receptor gene (OXTR) and depressive symptoms in the context of early-life maltreatment. The sensitivity hypothesis was further explored upon examining the OXTR single nucleotide polymorphism (SNP) in relation to an acute social ostracism stressor (Study 2). In Study 3, the OXTR SNP as well as an additional SNP on the CD38 gene, which controls for oxytocin release, was evaluated in relation to traumatic life events, depressive symptoms and suicidal ideation scores. Finally, Study 4 assessed endogenous oxytocin and cortisol levels in response to a strong psychosocial stressor in which social support from a close friend was manipulated.

## Chapter 1

### The Role of Oxytocin in Understanding Depression

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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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<sup>1</sup> This manuscript was written in collaboration with O.A. McInnis. R.J.M and O.A.M. contributed equally.

## **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; 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., 2011a; 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, 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.

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

#### ***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 et al., 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 (Grippio 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.

### ***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 towards negative stimuli (Macleod et al., 1986), and oxytocin treatment could potentially influence this disorder by affecting sensitivity to social cues (Bartz, 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

towards 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 minutes after its administration, oxytocin could be detected in CSF of humans, but was not evident when assessed 45 or 60 minutes 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 minutes 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.

#### *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 towards 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.

#### *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 minutes 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.

#### *Oxytocin and stress reactivity*

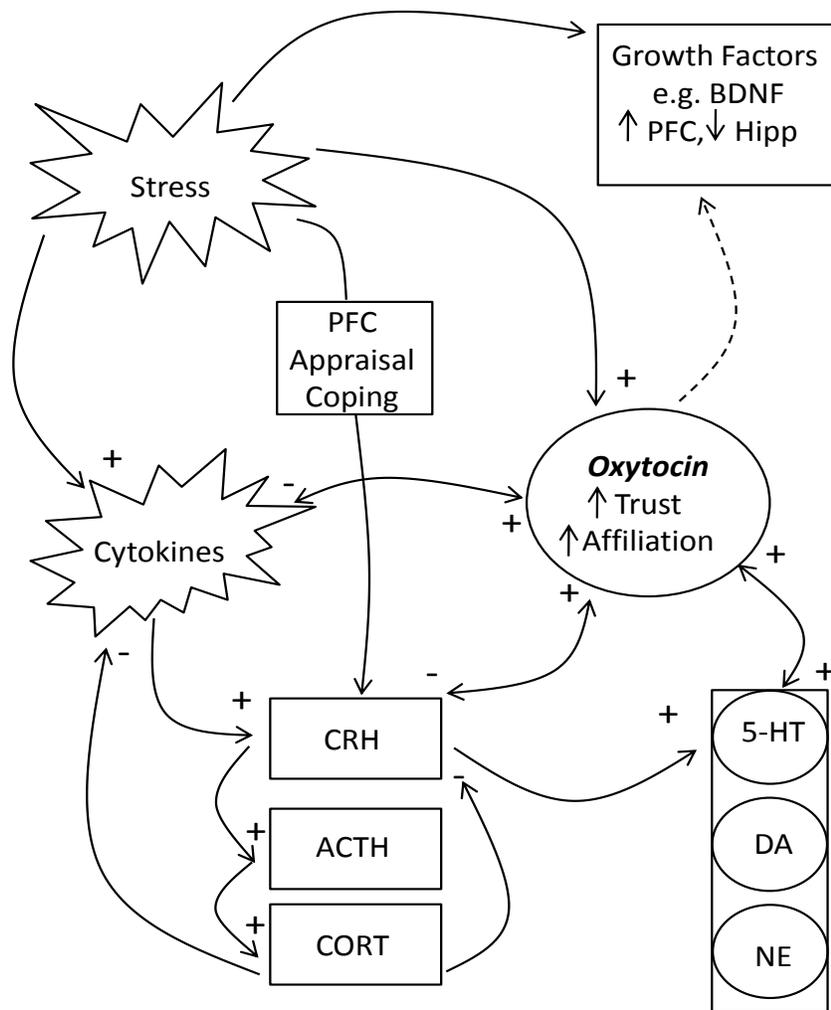
Although much of the research assessing the OXTR polymorphism focused on prosocial behaviors, there have also been reports concerning the effects of this polymorphism in relation to the impact of stressors. For instance, individuals with the AA/AG genotypes of OXTR rs53576 displayed higher heart rate 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.

## **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 Figure 1, which will be referred to repeatedly as each of the interactions is discussed.



*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.

***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 (Figure 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 CRH<sub>1</sub> 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 CRH<sub>1</sub> receptor binding is necessary for the release of oxytocin following a stressor (Keck et al., 2003), but no differences were reported between CRH<sub>1</sub> 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 CRH<sub>1</sub> 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 Figure 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<sub>A</sub> 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 (Pettersson 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, 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; 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 hours following the stressor. In contrast, chronic restraint resulted in delayed oxytocin release followed by more rapid normalization that was complete within 3 hours (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.

#### *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, 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 Oliveria 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, 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 towards ingroup members in a financial decision making game, while simultaneously increasing defensive behaviors towards 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

junction, 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.

### **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; 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 (Figure 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 Figure 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 (Mottelese 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., 2011). 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; Marazziti and Catena-Dell'Osso, 2008). Thus, modifying 5-HT processes during critical developmental periods might influence oxytocin functioning, which could engender later psychological disturbances (Eaton et al., 2011).

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).

### **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 (Figure 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).

## **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 (Kovacs 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, D<sub>1</sub> receptors, which play an inhibitory role in pair-bonding, activating D<sub>2</sub> along with oxytocin receptors in the NAc seems to be necessary for pair-bond formation among male prairie voles (Aragona et al. 2005; 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 D<sub>2</sub> receptors in the NAc to induce pair-bonding in the absence of mating, whereas blockade of the D<sub>1</sub> 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 D<sub>2</sub> 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 (Figure 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 FreundMercier, 1997). In this regard, infusion of oxytocin into the cAmyg selectively

attenuated anxiety responses, an action that could be diminished by pretreatment with a D<sub>1</sub> 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 x 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 D<sub>2</sub> 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 et al., 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., 2011a; 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 towards 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 towards 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.

### **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.

### **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 Figure 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 et al., 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 Figure 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-Pelegri 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.

## **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; 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 (Mendelwicz 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 et al., 2010).

### **Concluding comments**

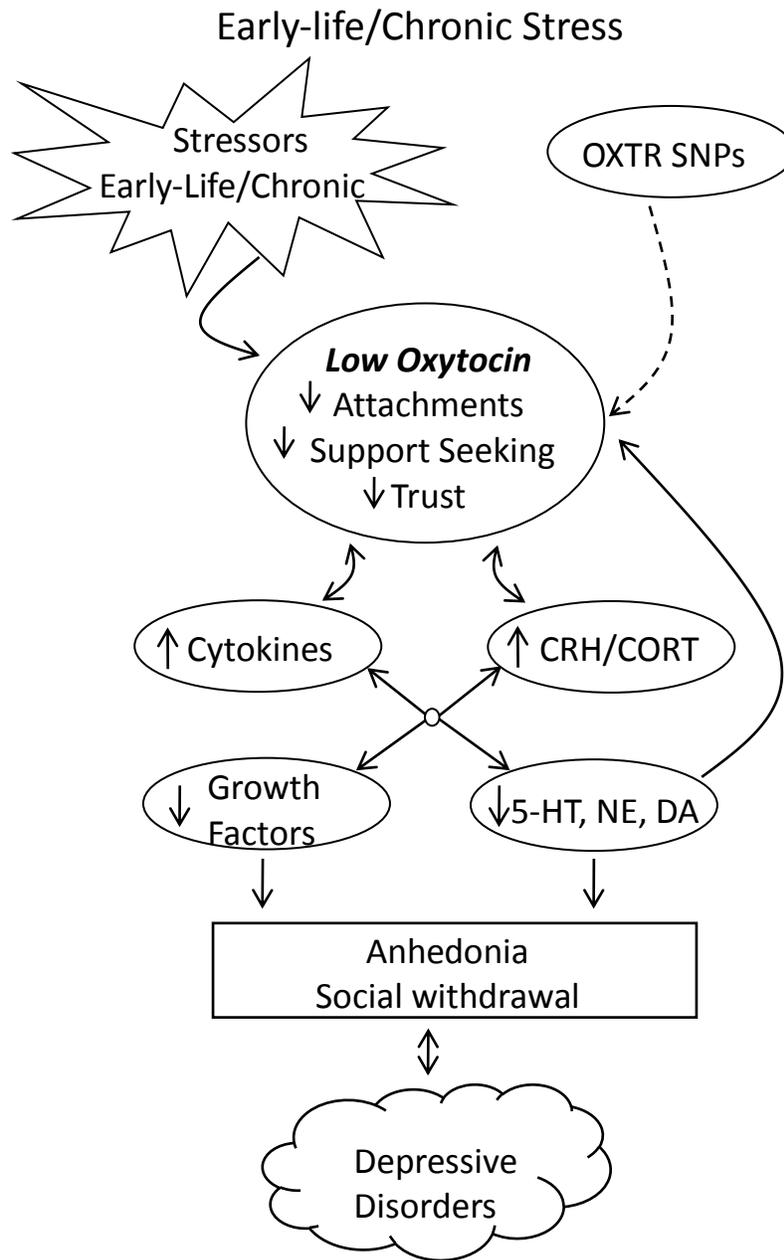
Although there is sufficient evidence linking oxytocin with the development of depression in animal-based studies, there are data that do not readily align with this perspective, particularly studies that involved human participants. Specifically, studies examining oxytocin levels in relation to depression 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 vs 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 Figure 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 Figure 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, 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

### Oxytocin and Early-life Maltreatment

McQuaid, R.J., McInnis, O.A., Stead, J.D., Matheson K., Anisman, H. (2013). A paradoxical associated of an oxytocin receptor gene polymorphism: Early-life adversity and vulnerability to depression. *Frontiers in Neuroscience*, 7: 128.

Oxytocin, as discussed in Chapter 1, is involved not only in a wide array of prosocial behaviors, but also seems to be involved in depressive disorders. This perspective is supported by evidence that oxytocin interacts with monoamine, neuroendocrine and inflammatory processes, all of which are thought to be involved in depression. In fact, oxytocin may be linked to depression through its role in prosocial behaviors, as it is likely that prosocial attributes, such as trust in others, can affect social relationships and in turn, mental health outcomes. In particular, a SNP on the OXTR, rs53576, has been tied to prosocial behaviors, showing that G carriers tend to be more trusting, optimistic and empathetic (Krueger et al., 2012; Rodrigues et al., 2009; Saphire-Bernstein et al., 2011). Yet, it was reported that African American individuals who had the GG genotype of the OXTR SNP, and experienced severe early-life maltreatment, displayed *elevated* emotional dysregulation (Bradley et al., 2011). Considering these divergent outcomes, it was of interest to examine how these ‘prosocial’ G carriers (i.e., that carry at least one G allele) would respond to a negative environment. It was thought that the G carriers might be more sensitive to social stimuli, irrespective of whether they were negative or positive events. Increased social sensitivity could result in greater prosociality in a positive environment, but in a negative environment, this might confer vulnerability to poor mood and perhaps stress-related pathological outcomes. Thus, in

Study 1 we examined the social sensitivity hypothesis of the OXTR SNP, rs53576. Specifically, it was investigated whether individuals with the G allele of the OXTR SNP, rs53576, who experienced early-life maltreatment would display elevated depressive symptomatology. Furthermore, it was thought that the AA carriers, who were presumably less sensitive to their environments, would be relatively unaffected by early-life maltreatment.

## **Abstract**

Several prosocial behaviors may be influenced by the hormone oxytocin. In line with this perspective, the oxytocin receptor gene (OXTR) 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 and 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 and 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  $\mu$ L 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 ( $p < .05$ ).

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%

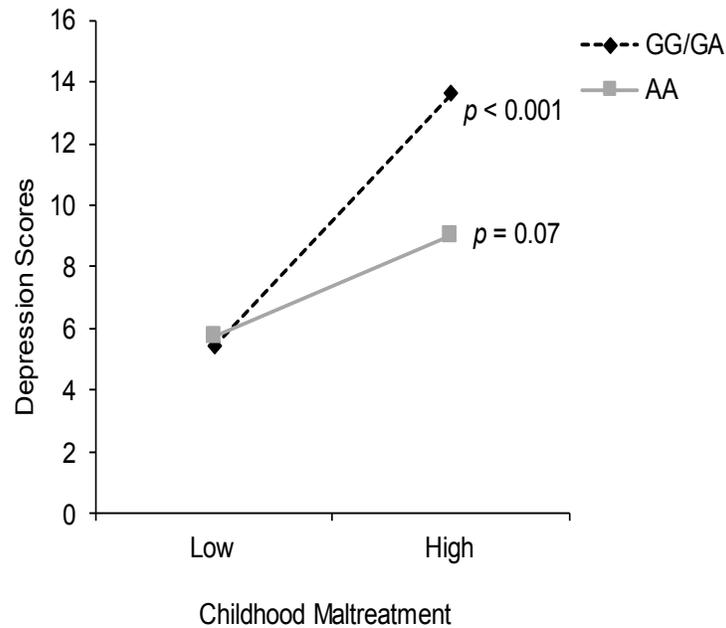
Females were found to have higher depressive scores than males,  $t(1, 163.6) = 2.60, p = .01$ , and they also reported higher levels of childhood maltreatment,  $t(1, 225.4) = 2.49, p = .01$ . However, there were no gender differences on distrust levels  $t(1, 286) = -0.15, p = .89$ , nor was the Gender 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 the 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.



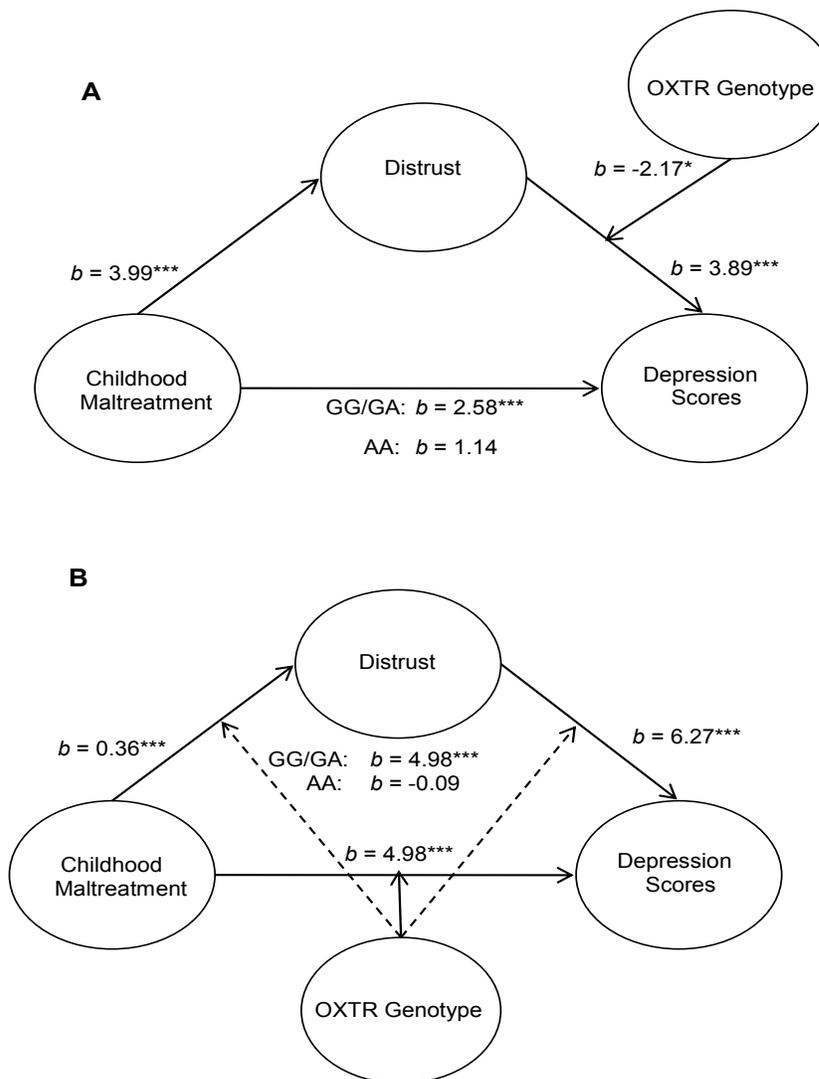
*Figure 1.* The relation between childhood maltreatment and depression scores as a function of the OXTR rs53576 genotype (GG/GA versus AA). The simple slopes analyses revealed that genotype groups did not differ at lower levels of childhood maltreatment. However, depressive symptoms increased significantly 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 and 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 behaviors are often moderated by contextual factors, thus leading to weak and/or inconsistent findings (Bartz et al., 2011a). 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., 2011a). 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., 2011a).

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.

## Chapter 3: Study 2

### Oxytocin and Social Ostracism

McQuaid, R.J., McInnis, O.A., Matheson K., Anisman, H. (2015). Distress of ostracism: Oxytocin receptor gene polymorphism confers sensitivity to social exclusion. *Social Cognitive and Affective Neuroscience*. [Epub ahead of print].

Study 1 revealed that individuals with one or two copies of the G allele of the OXTR SNP, rs53576, who experienced high levels of early-life maltreatment, displayed elevated depressive symptoms. Although most earlier studies had concluded that the G allele of this OXTR SNP facilitated beneficial prosocial attributes, the data from those studies could often be interpreted to reflect that individuals with the G allele of the OXTR SNP were more sensitive to and/or affected by positive events, and there was scant information regarding the link between the SNP and responses associated with early-life stressors. The findings of the initial study support a social sensitivity hypothesis of the OXTR SNP, although it was unclear whether these findings were specific to early-life adversity or whether they would extend beyond early-life to acute negative social interactions encountered in adulthood. Thus, in Study 2 we examined the OXTR SNP in relation to an on-line social rejection paradigm. In keeping with a social sensitivity hypothesis, we predicted that the G allele carriers of the OXTR SNP would be more affected and/or reactive to social ostracism, whereas the less sensitive AA carriers would be largely unaffected by social rejection.

## **Abstract**

A single nucleotide polymorphism (SNP) on the oxytocin receptor gene (OXTR), rs53576, 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 the 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., 2011a).

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., 2013a).

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***

The current study comprised 128 White female Carleton University undergraduate students with a mean age of 19.82 ( $SD = 3.86$ ). The OXTR genotype could be determined for 126 individuals. A homogenous ethnic sample was used in the present 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, due to 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 shown 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*

<b>Ethnicity</b>	<b>G/G</b>	<b>A/G</b>	<b>A/A</b>
White ( <i>n</i> = 126)	56	52	18
Black ( <i>n</i> = 38)	25	13	0
Asian ( <i>n</i> = 25)	3	12	10
Arab/West Asian ( <i>n</i> = 14)	8	4	2
South Asian ( <i>n</i> = 12)	3	5	4
Latin American/Hispanic ( <i>n</i> = 6)	2	4	0
South East Asian ( <i>n</i> = 3)	1	2	0
Aboriginal ( <i>n</i> = 3)	1	1	1
Other ( <i>n</i> = 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. Additionally, two participants were excluded based on previous experience playing Cyberball.

### ***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), 20 minutes after arrival to the laboratory (baseline) as well as 15 and 30 minutes following Cyberball. Immediately following the test session, saliva samples, were frozen at -80°C. Following the manufacturers protocol, a competitive radioimmunoassay (RIA), <sup>125</sup>I kit (ICN Biomedicals Inc., Irvine, CA), was used to determine, in duplicate, salivary cortisol levels. The intra- and interassay variability was less than 10%. The minimum detectable of cortisol was .02 µg/dL and the specificity was 100% cortisol. In some instances, (*n* = 8), participants did not have three valid cortisol measures and thus were appropriately removed from the repeated measures analyses.

### ***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 µL 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 were 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) (Spielberger, 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, 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 was used. Further to this a 2 (Cyberball condition) x 3 (OXTR genotype) analyses of covariance was also conducted for blood pressure, controlling for baseline 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. Additionally, an area under the curve (AUC) analysis was performed for cortisol using a formula proposed by Pruessner et al., (2003).

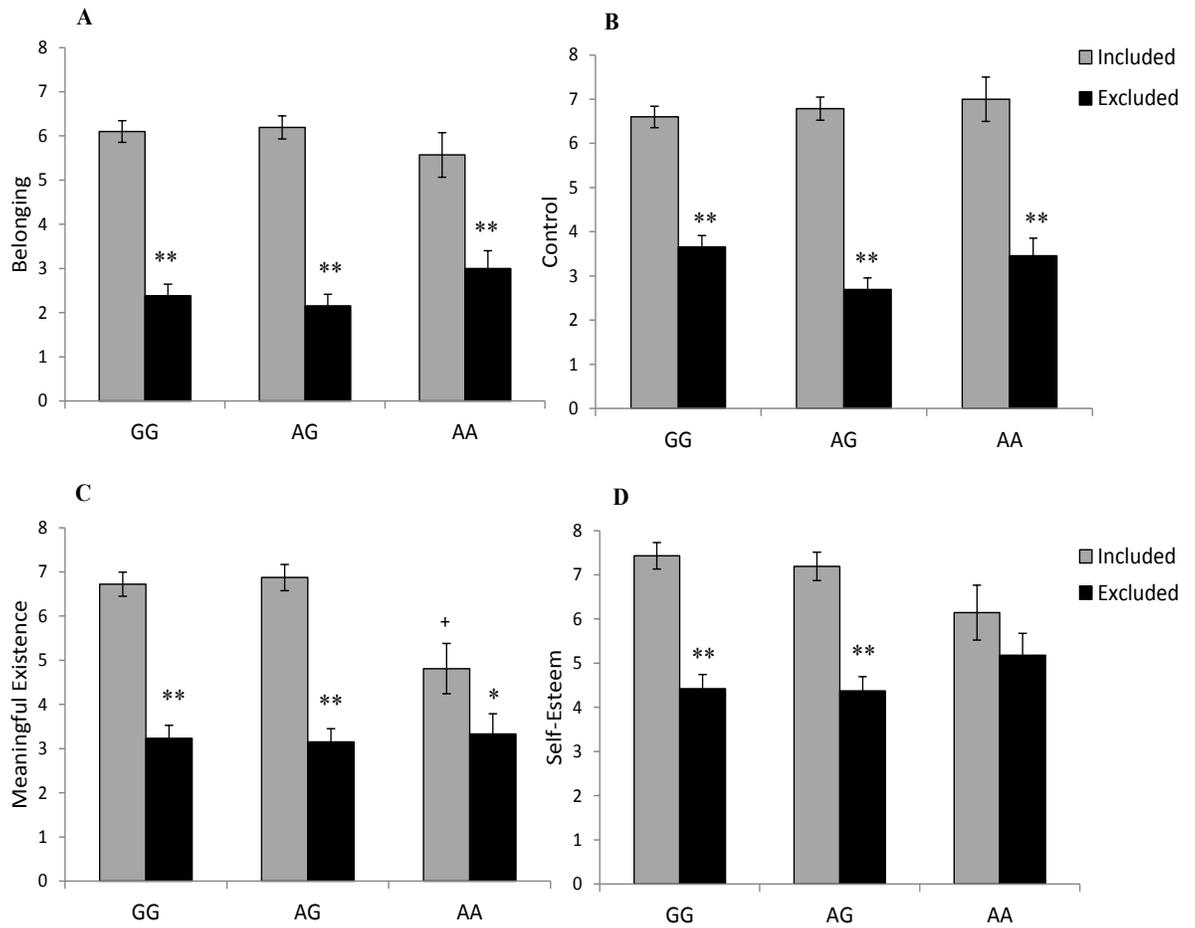
## **Results**

### ***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.



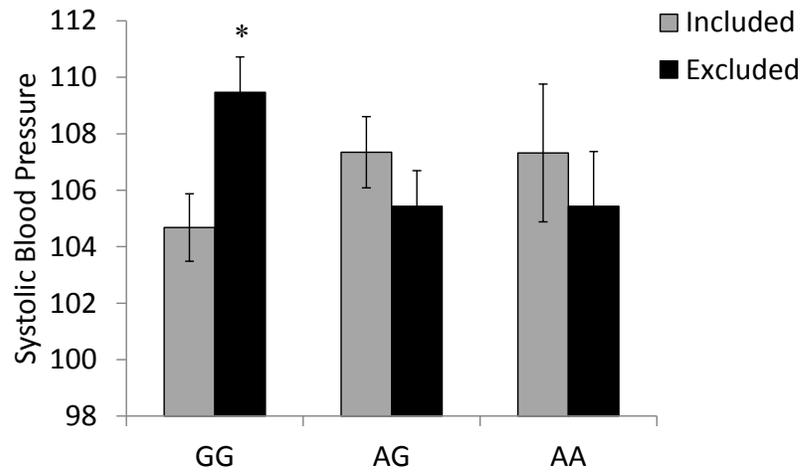
*Figure 1.* Feelings of belonging (A), control (B), meaningful existence (C) and self-esteem (D) among individuals with the GG, AG, or AA OXTR genotypes who were either included or excluded during the Cyberball game. Data represents means  $\pm$  S.E.M. \* $p < 0.05$ , \*\* $p < 0.001$  relative to included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

To further support these analyses, and considering the small group sizes, it was also important to compute one standard measure of cortisol. As such, the AUC was calculated following a method described by Pruessner et al., (2003). There are two formulas for AUC, namely, AUC with respect to the ground ( $AUC_G$ ) and AUC with respect to increase ( $AUC_I$ ). As the current data did not display an appreciable cortisol increase following Cyberball, we used the  $AUC_G$  formula. Results indicated a significant Cyberball X OXTR genotype interaction,  $F(2, 112) = 4.58, p < .05$ . The follow-up simple effects support the repeated measures findings that G/G individuals who were excluded displayed higher cortisol than their included counterparts ( $p < .05$ ), an effect not apparent among AG and AA individuals.

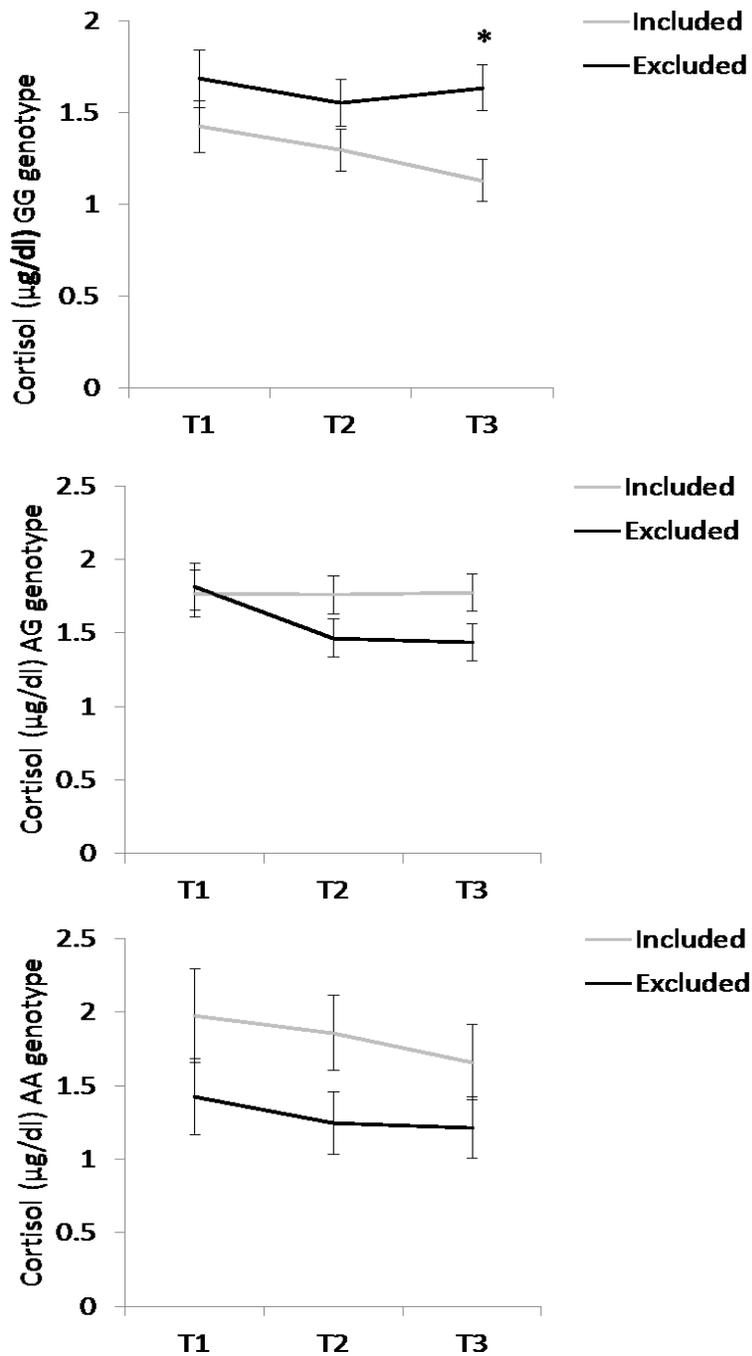


Figure 3. Cortisol levels in saliva ( $\mu\text{g}/\text{dl}$ ) collected at three time points including before Cyberball (T1), 15 minutes following Cyberball (T2) and 30 minutes following Cyberball (T3). The graph represents individuals with the GG genotype (top panel), AG genotype

(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**

#### **Oxytocin, Depression, Suicidal Ideation and Social Sensitivity: Implications of a CD38 Gene Polymorphism**

In Study 2, it was determined that G carriers of the OXTR SNP, were more affected by ostracism, displaying lower self-esteem, elevated blood pressure, and sustained cortisol levels following social rejection. Taken together, Studies 1 and 2 suggest that the G allele of the OXTR SNP, rs53576, facilitated social sensitivity to negative stressors. In Study 3 we extended this research beyond the OXTR SNP found on the oxytocin receptor gene, and examined another important component of neural oxytocin system functioning, namely the CD38 gene that controls oxytocin release. It had been reported that mice without the CD38 gene could produce oxytocin, but that it was not being released, resulting in elevated oxytocin in the hypothalamus of mice (Jin et al., 2007). A SNP found on the CD38 gene, rs3796863, has been identified as being involved in altered social processes. Specifically, the C allele, which is the common allele variant, has been suggested to be the ‘risk’ allele for disorders marked by social disturbances, such as autistic spectrum disorders. Depressive disorders may also be accompanied with disturbed social processes, and thus in Study 3 we examined whether the CD38 SNP, rs3796863, and the OXTR SNP, rs53576, were associated with altered social relationships as well as to depressive symptomatology. Furthermore, disturbed social connectedness and social support may be particularly relevant to certain features of depressive disorders, namely suicidal ideation. As suicidal behaviors are particularly relevant to young adults, being a leading cause of death in this age group, we also

examined the relation between CD38 and the OXTR SNPs to suicidal ideation among first and second year university students.

## **Abstract**

Although the neuropeptide oxytocin has been associated with enhanced prosocial behaviors, it has also been linked to aggression and mental health disorders. Thus, it was suggested that oxytocin might operate by increasing the salience of social stimuli, irrespective of whether these are positive or negative. Indeed, in the context of a negative environment, such as early-life adversity, oxytocin may confer vulnerability to depressive symptomatology or related disorders, whereas these negative outcomes did not occur in the presence of an oxytocin receptor gene (OXTR) single nucleotide polymorphism (SNP). The current study, ( $N = 243$ ), conducted among White university students, examined the relation of trauma, depressive symptoms and suicidal ideation to both an OXTR SNP, rs53576, and a SNP on the CD38 gene that controls oxytocin release, rs3796863. Individuals with the polymorphism on both alleles (AA genotype) of the CD38 SNP, rs3796863, have displayed elevated plasma oxytocin levels and may be considered more socially inclined. However, consistent with the social sensitivity perspective, in the current study, individuals carrying the AA genotype actually displayed elevated feelings of alienation from parents and peers as well as increased levels of suicidal ideation compared to CC homozygotes. Suicidal ideation scores were further elevated among individuals with the AA genotype who also experienced high levels of trauma. In contrast, there was no relationship between the OXTR SNP, rs53576 and suicidal ideation. These findings support a social sensitivity hypothesis of oxytocin, such that the AA genotype of the CD38 SNP, which has been considered the ‘protective allele’

was, in this instance, associated with *increased* sensitivity and susceptibility to disturbed social relations and suicidal ideation.

## **Introduction**

Although there are many risk factors of suicidal behavior, the presence of a psychiatric disorder, such as depressive illness or posttraumatic stress disorder (PTSD) accounts for a large number of suicide attempts and/or completions (Mann et al., 2003). Traumatic life events, encountered in childhood or as adults, are also strongly linked to depression (Heim et al., 2008a) and suicidal behaviors (Dube et al., 2001; Seedat et al., 2005). Often, suicide attempts are preceded by suicidal thoughts and intentions (Han et al., 2015), and although only some individuals who engage in suicidal ideation proceed to an attempt, it is a significant indicator of suicide risk (McAuliffe, 2002).

Several biological correlates of suicide have previously been identified, including altered serotonin and GABA receptor variations (Anisman et al., 2012) as well as that of the neurotrophin FGF-2 (Evans et al., 2004). In addition, suicidal ideation has been associated with single nucleotide polymorphisms (SNPs) on the serotonin transporter and receptor genes (Kim et al., 2014; Wang et al., 2009), in addition to methylation of the brain derived neurotrophic factor (BDNF) gene (Kim et al., 2015). Oxytocin has been linked to depression (McQuaid et al., 2014), and there has been evidence indicating disturbed oxytocin functioning in association with suicidal intent (Jokinen et al., 2012).

Oxytocin, a neuropeptide that has widespread central and peripheral effects, has frequently been examined in relation to prosocial behaviors. Individuals who receive intranasal oxytocin administration display enhanced generosity (Zak et al., 2007), trust (Kosfeld et al., 2005), empathy (Domes et al., 2007; Shamay-Tsoory et al., 2013), and helping behavior (Riem et al., 2013). However, oxytocin does not consistently enhance

prosociality, having been shown to increase envy and gloating (Shamay-Tsoory et al., 2009), lying to benefit one's in-group (Shalvi and De Dreu, 2014), defensive aggressive behaviors towards competing outgroup members (De Dreu et al., 2010), and aggression and violent behaviors towards a romantic partner among individuals high in trait aggression (DeWall et al., 2014). To explain the divergent outcomes associated with oxytocin, it was suggested that oxytocin increases sensitivity to social cues such that both positive and negative events will become more salient and have more dramatic consequences (Averbeck, 2010; Bartz et al., 2011a; Cardoso et al., 2014).

Social sensitivity associated with oxytocin is also apparent in analyses involving a genetic variant of the oxytocin receptor gene (OXTR). Specifically, a SNP within the OXTR, rs53576, which involves a guanine (G) to adenine (A) substitution, has been associated with many prosocial behaviors. In particular, individuals who have two copies of the G allele of this OXTR SNP display beneficial attributes including high levels of trust (Krueger et al., 2012), empathy (Rodrigues et al., 2009; Smith et al., 2014), self-esteem, and lower negative affect (Saphire-Bernstein et al., 2011). However, it was also found that individuals with the GG genotype who experienced severe childhood maltreatment displayed *greater* disorganized attachments and *increased* emotional dysregulation compared to A carriers (Bradley et al., 2011). Similarly, G carriers who experienced early-life maltreatment reported higher depressive scores compared to individuals with the AA genotype (McQuaid et al., 2013), as well as increased reactivity to social ostracism (McQuaid et al., 2015). These findings suggest, similar to the effects resulting from oxytocin administration, that context is important, such that G carriers may

be more prosocial in certain environments, but in the face of adversity, the more socially sensitive G allele carriers may be more vulnerable to deleterious outcomes.

The important role of the gene, CD38, in oxytocin neural transmission has recently been identified (Jin et al., 2007). This gene is required for oxytocin secretion, as CD38 knockout mice display greatly reduced CSF and plasma oxytocin, and these mice also exhibited deficits in social memory and recognition (Jin et al., 2007). In human studies it was found that peripheral CD38 gene expression was positively correlated with oxytocin levels (Kiss et al., 2011). Others have found that the common variant of a SNP involving a cytosine (C) to adenine (A) switch on the CD38 gene, rs3796863, was also related to autism spectrum disorder (ASD) (Lerer et al., 2010; Munesue et al., 2010). Based on this finding, the C-allele, although more common, has been determined the 'risk' allele and was associated with lower CD38 expression in lymphoblastoid cells obtained from ASD patients (Lerer et al., 2010). Although the literature examining the CD38 rs3796863 SNP is limited, and has been largely in relation to ASD, it was found that the C allele homozygotes display reduced plasma oxytocin levels and diminished parental touch among a sample of healthy individuals (Feldman et al., 2012). It was recently revealed, however, that upon experiencing chronic stress, A carriers of the CD38 SNP displayed elevated social anxiety compared to CC homozygotes, an effect in-line with a social sensitivity perspective (Tabak et al., 2015).

The association of oxytocin with disorders comprising disturbed social functioning is not limited to autism (Guastella, et al., 2010), but also schizophrenia (Feifel et al., 2012) depressive disorders (McQuaid et al., 2014; Scantamburlo et al.,

2007) and suicidal behaviors (Jokinen et al., 2012; Lee et al., 2009). In this regard, individuals who had previously attempted suicide displayed lower cerebrospinal fluid (CSF) oxytocin concentrations compared to healthy controls (Lee et al., 2009).

Furthermore, among the male suicide attempters, low CSF oxytocin concentrations were associated with suicide intent, although there were no differences in CSF or plasma oxytocin concentrations between individuals who had completed suicide and those who attempted but survived (Jokinen et al., 2012).

Given the relationship between oxytocin and suicidal intent, in the current investigation we examined whether genetic variants of the OXTR SNP rs53576 and the CD38 gene SNP rs3796863 would be related to suicidal ideation among young adults. Furthermore, as experiencing traumatic events is a risk factor for suicidal behaviors (Dube et al., 2001; Seedat et al., 2005) and individuals who have experienced trauma displayed lower CSF oxytocin concentrations (Heim et al., 2008b), we also examined the potential interaction between traumatic life events and the OXTR and CD38 genotypes in relation to suicidal ideation. As oxytocin has been implicated in social bonding and social support, we explored whether the OXTR and CD38 genotypes were associated with altered relationships to parents and peers through feelings of alienation. These relations were examined among University students enrolled in a first year psychology course, as the transition to university is a particularly stressful period (Compas et al., 1986) associated with high levels of distress (Kidder et al., 2000). Furthermore, suicide is one of the leading causes of death among young adults in Canada (Statistics Canada, 2008).

## **Methods**

### *Participants*

Participants were recruited through the university's online computerized system and comprised 243 Carleton University students enrolled in a first year psychology course (154 females and 89 males), with a mean age of 19.17 ( $SD = 2.04$ ). Due to population stratification effects, the current study examined a homogeneous White sample.

### *Procedure*

Upon signing informed consent, participants completed a series of demographic questions, including current psychological disorders, as well as measures of current depressive symptoms including suicidal ideation, traumatic life events and feelings of alienation from parents and peers. Following completion of the questionnaires, saliva samples were obtained for later genotyping. Upon completion of the study, which took up to 1.5 hours to complete, participants were debriefed and compensated with course credit. The current study was approved by the Carleton University Ethics Committee for Psychological Research.

### *Genotyping*

Samples for genotyping were collected using Norgen collection kits (Norgen Biotek Corp., Thorold, Ontario Canada). Genomic DNA was extracted from the sample collection kit according to the manufacturer's instructions and diluted to approximately equal concentration (10 ng/ $\mu$ L). Samples were sent for genotyping to McGill University and Génome Québec Innovation Centre (Montreal, Canada). Using polymerase chain

reaction (PCR) the DNA was amplified, and QIAxcel was used to determine amplification status. Shrimp alkaline phosphatase was used to remove all unincorporated dNTPs. One probe per marker was used to do a single base extension and the product was desalted using 6mg of resin. The product was spotted on a Sequenom 384-well chip using a Samsung Nanodispenser and the chip read by a Mass Spectrometer. A manual analysis was done for each marker. Primer sequences were as follows: CD38 forward:

ACGTTGGATGGTTGCTGCTCCTGCTGTTTT, CD38 reverse:

ACGTTGGATGAAGGTGCACAGACCACTTAG, CD38 probe:

TCCTGCTGTTTTTTTGACCA, OXTR forward:

ACGTTGGATGTCCCCATCTGTAGAATGAGC, OXTR reverse:

ACGTTGGATGGCACAGCATTCATGGAAAGG, OXTR probe:

CTCTGTGGGACTGAGGA

The allele distribution of the CD38 rs3796863 SNP was 106 CC individuals (33 males, 73 females) and 105 CA individuals (50 males, 55 females) and 26 AA individuals (4 males, 22 females). These genotype distributions met Hardy-Weinberg Equilibrium expectations,  $\chi^2(1) = 0, p > 0.05$ . Six individuals were excluded from analyses that included genotype as a genotype could not be determined from the samples collected. For the OXTR SNP, rs53576, the genotype distributions were; 109 GG individuals (35 males, 74 females), 106 AG individuals (43 males, 63 females), and 26 AA individuals (10 males, 16 females). Two individuals could not be genotyped for this OXTR SNP and thus were excluded from any analyses including genotype. These genotype distributions also met Hardy-Weinberg Equilibrium expectations,  $\chi^2(1) = 0, p > 0.05$ .

## *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 ( $\alpha = .90$ ). To be sure that findings pertaining to depressive symptoms were not influenced by feelings of suicidal ideation, analyses were also carried out excluding the suicidal ideation question from the total BDI score, and both types of analyses yielded the same results. Suicidal ideation was determined through the use of a question on the 21-item BDI that assesses suicidal thoughts and behaviors. This question includes the following response options: 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), and 3 (I would kill myself if I could).

*Number of different types of traumas experienced.* The Traumatic Life Events Questionnaire TLEQ (Kubany et al., 2000) was used to measure the number of different types of traumatic events experienced. This measure includes 23 types of traumatic events experienced (e.g. natural disasters, assaults, death of a loved one). We also added four questions that included experiences of physical and psychological bullying, as well as divorce of parents and emotional abuse in the home. These were added as it was thought that they were important events that could impact mental health and as such, should be included when examining total traumatic experiences. We scored responses as

either a 0 (not having experienced the event) or 1 (having experienced this event either once or multiple times). Then we formed categories that included 0 (having experienced 0-2 different types of traumas), 1 (having experienced 3-7 different types of traumas) and 2 (having experienced 8 or more different types of traumas). Additionally, we calculated the trauma frequency score by summing across all responses, and this correlated strongly with our categories of different traumas  $r = .69, p = .000$ .

*Alienation from parents and peers.* A 15 item subscale from the Inventory of Parent and Peer Attachment (IPPA) (Armsden and Greenberg, 1987) was used to assess the quality of attachment to parents and peers in one domain, namely alienation. A mean score was determined for parental ( $\alpha = .88$ ) and peer ( $\alpha = .81$ ) alienation.

### **Statistical Analyses**

Statistical analyses were performed using SPSS for Windows 18.0 (SPSS Science, Chicago, Illinois, USA). Analyses assessing genotype differences on parental and peer alienation, depression scores and suicidal ideation were performed using a one-way analysis of variance (ANOVA). Analyses examining interactive effects used a 3 (traumatic life events; 0-2 traumas, 3-7 traumas, or 8+ traumas) x 3 (OXTR genotype: GG, AG, or AA; or CD38 genotype: CC, AC, AA) ANOVA. Follow-up comparisons comprised t-tests with a Bonferroni correction to maintain the alpha level at 0.05.

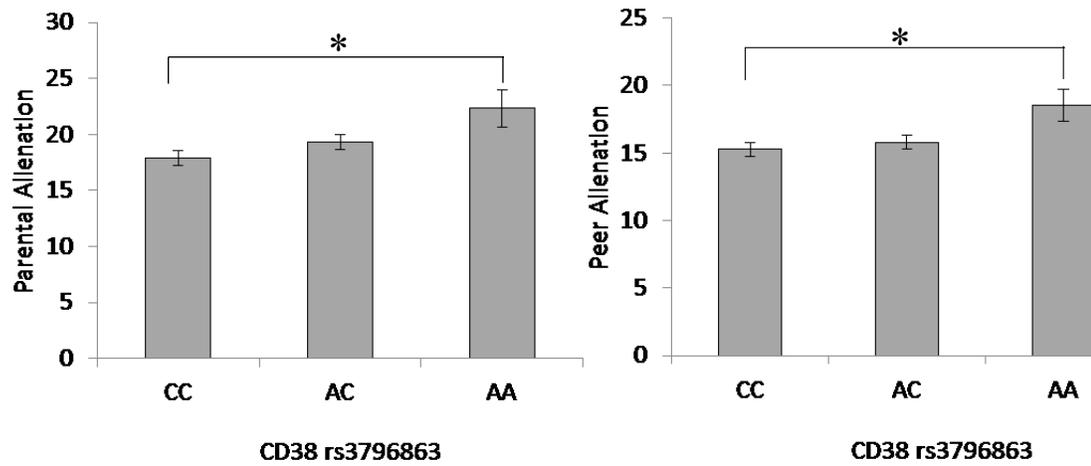
### **Results**

#### *CD38 SNP rs3796863*

There were no differences found between genotype groups and the incidence of a current psychological disorder  $\chi^2 (2) = 1.46, p = .48$ . However, genotype distributions

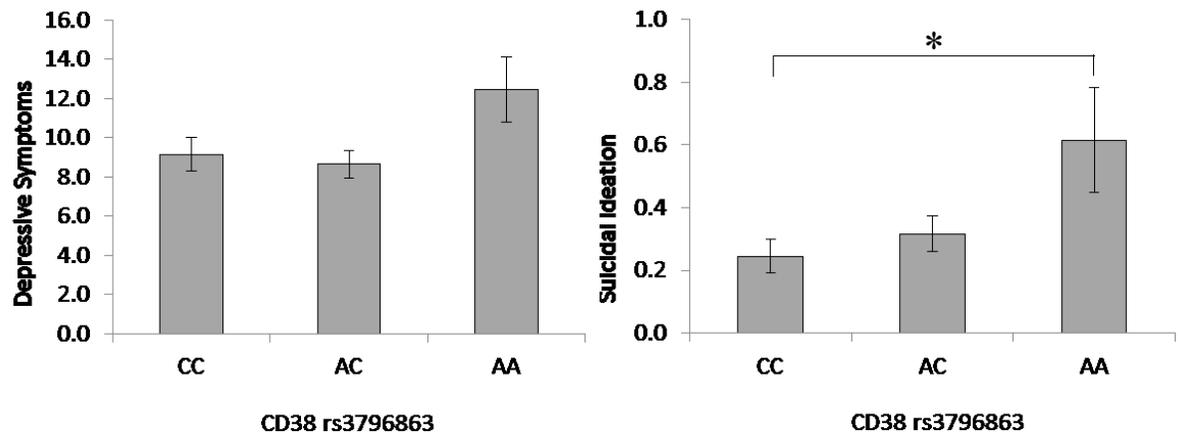
differed based on sex  $\chi^2 (2) = 11.89, p = .003$ , such that there were significantly more females than males with CC and AA genotypes,  $\chi^2 (1) = 15.09$  and  $12.46, p's = .000$ , respectively. Although the genotype frequencies differed according to sex, there were no sex differences, or Sex x CD38/OXTR genotype interactions on peer or parental alienation, suicidal ideation or depression scores.

Approximately 15% of individuals reported experiencing 0-2 traumas, 57% reported 3-7 traumas, and 27% indicated they experienced 8+ traumas. As expected, the number of reported traumatic life events did not differ across genotypes,  $\chi^2 (4) = 1.84, p = .77$ . It was determined that there were differences between CD38 genotypes on feelings of alienation from parents and peers,  $F (2, 234) = 4.30, 4.12, p's = .02, \eta^2 = .04$  and  $.03$ , respectively. As shown in Figure 1, follow-up tests revealed that compared to individuals homozygous for the C allele, those with the AA genotype reported greater feelings of alienation from parents and peers  $p's = .01$ . Individuals with the AC genotype did not differ in levels of alienation from the other genotypes.



*Figure 1.* Alienation from parents (left) and peers (right) among individuals with the CC, AC, or AA CD38 genotypes. Data represent means  $\pm$  SEM.  $*p = .01$  compared to individuals with the CC genotype.

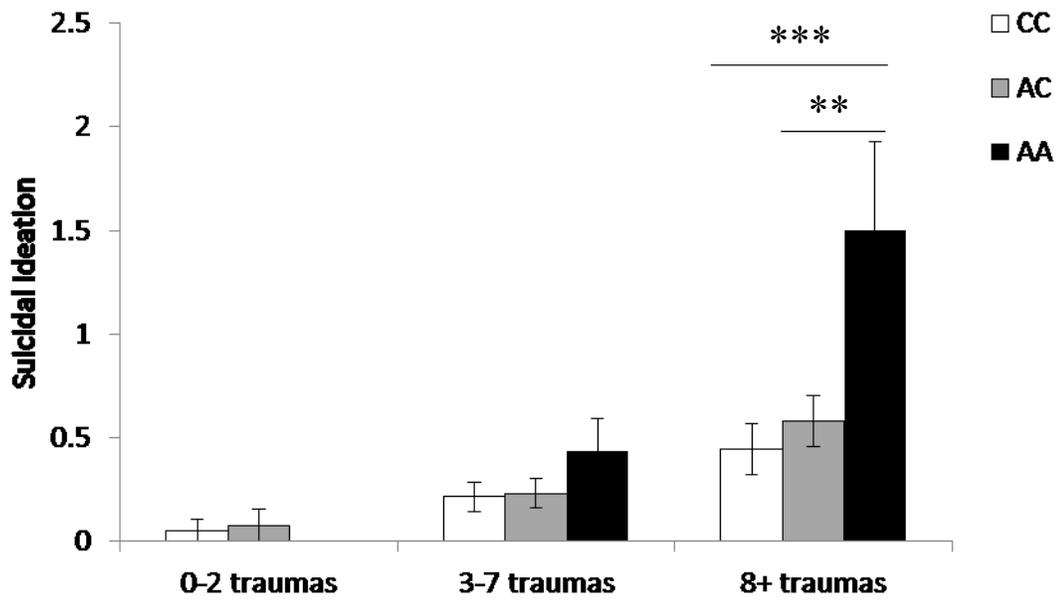
Depression scores among individuals with the CC and AC genotype were very similar ( $M = 9.16$ ;  $SE = 0.86$  and  $M = 8.66$ ;  $SE = 0.70$ , respectively) and tended to be lower relative to that of the AA genotype ( $M = 12.46$ ;  $SE = 1.68$ ), although these groups did not significantly differ from one another,  $F(2, 234) = 2.32$ ,  $p = .10$ . Suicidal ideation scores differed between CD38 genotypes,  $F(2, 233) = 3.95$ ,  $p = .02$ ,  $\eta^2 = .03$ . As displayed in Figure 2, individuals with the CC genotype ( $M = 0.25$ ;  $SE = 0.05$ ) displayed lower suicidal ideation scores compared to AA carriers ( $M = .62$ ;  $SE = 0.17$ ), ( $p = .02$ ), while levels did not significantly differ between the AC ( $M = .32$ ;  $SE = 0.06$ ) and AA individuals ( $p = .07$ ).



*Figure 2.* Depressive symptoms (left) and suicidal ideation scores (right) among individuals with the CC, AC, or AA CD38 genotypes. Data represent means  $\pm$  SEM. \* $p < .05$  compared to CC homozygotes.

We examined the potential interactive effects of the CD38 genotype and trauma to predict both depression and suicidal ideation. There was a main effect of trauma on depression levels,  $F(2, 228) = 12.19, p = .00, \eta^2 = .10$ , such that with an increasing number of traumas experienced, depression scores were elevated. However, neither CD38 genotype nor a CD38 genotype x Trauma interaction predicted depression scores,  $F(2, 228) = 1.12, p = .33$ , and  $F(4, 228) = 0.82, p = .51$ , respectively.

In contrast, suicidal ideation varied as a function of the CD38 genotype x Trauma interaction,  $F(4, 227) = 2.52, p = .04, \eta^2 = .04$ . The simple effects comprising this interaction can be seen in Figure 3, which revealed that for lower levels of trauma (0-2 traumas) and moderate levels of trauma (3-7 traumas), suicidal ideation did not differ across genotypes. However, upon experiencing higher levels of trauma (8+ traumas), individuals with the AA genotype displayed greater levels of suicidal ideation compared to CC individuals ( $p = .000$ ) and AC individuals ( $p = .001$ ). This analysis was also conducted controlling for depression scores as a covariate and the CD38 genotype x Trauma interaction remained significant,  $F(4, 226) = 2.89, p = .02, \eta^2 = .05$ .



*Figure 3.* Suicidal ideation as a function of the number of traumas experienced among individuals with the CC, AC, or AA CD38 genotypes. Data represent means  $\pm$  SEM. \*\* $p = .001$ , \*\*\* $p = .000$ .

*OXTR SNP rs53576*

There were no genotype differences based on sex  $\chi^2 (2) = 1.71, p = .43$ .

Furthermore, there were no differences across genotypes regarding currently having a psychological disorder,  $\chi^2 (2) = 0.66, p = .72$

As expected, the number of reported traumatic life events did not differ across genotypes  $\chi^2 (4) = 6.22, p = .18$ . Unlike that found for the CD38 gene, feelings of alienation to parents and peers were similar among OXTR genotype groups,  $F (2, 238) = 0.03, p = .97$ , and  $F (2, 238) = .56, p = .57$  respectively. Depression scores were very similar for the GG ( $M = 9.25; SE = 0.78$ ), AG ( $M = 9.13; SE = 0.79$ ), and AA ( $M = 9.92; SE = 1.60$ ) OXTR genotypes,  $F (2, 238) = 0.09, p = .91$ . Likewise, suicidal ideation scores did not differ across OXTR genotypes,  $F (2, 237) = 0.006, p = .99$ , GG ( $M = 0.32; SE = 0.56$ ), AG ( $M = 0.32; SE = 0.66$ ), and AA ( $M = 0.31; SE = 0.62$ ).

Similar to CD38, it was of interest to examine whether there would be a possible OXTR genotype x Trauma interaction to predict depression and suicidal ideation scores. Once more, there was an effect of trauma on depression levels,  $F (2, 232) = 5.36, p = .005, \eta^2 = .04$ , such that depression scores were elevated with a greater number of traumas experienced. However, no interactive effect was found,  $F (4, 232) = 0.52, p = .72$ . Upon examining a possible OXTR genotype x Trauma interaction to predict suicidal ideation levels, a main effect of trauma was found,  $F (2, 231) = 6.50, p = .002, \eta^2 = .05$ . Specifically, an increasing number of traumas reported was predictive of higher suicidal ideation scores. However, an interaction between the OXTR genotype x Trauma did not

predict suicidal ideation  $F(4, 231) = 0.63, p = .64$ . This analysis was repeated controlling for depression scores as a covariate, and the results remained identical.

## **Discussion**

In the current study, individuals with the AA genotype for the CD38 SNP reported greater suicidal ideation compared to C carriers and this was especially exaggerated when high levels of trauma were experienced. At first blush this might seem counterintuitive, as A carriers of this particular SNP have been associated with greater oxytocin levels (Feldman et al., 2012), and thus have been viewed as being protective in relation to social disturbances, including ASD (Lerer et al., 2010; Munosue et al., 2010). In line with the view that A carriers might be more socially inclined, individuals with the AC or AA genotype for the CD38 SNP exhibit more sensitive parenting methods (Feldman et al., 2012), and have faster reaction times to social stimuli such as emotional faces (Sauer et al., 2012). These findings support the notion that the less socially reactive CC homozygotes, who may have lower plasma oxytocin levels, are at risk for ASD, whereas A carriers of the CD38 SNP may be more sensitive to social stimuli. Thus, our finding that compared to CC homozygotes, A carriers showed elevated suicidal ideation that was still more pronounced in the context of high levels of trauma, is consistent with a social sensitivity perspective. This perspective was supported by a recent report showing that in the context of chronic stress, the A carriers of CD38 SNP displayed elevated social anxiety (Tabak et al., 2015).

Individuals with the AA genotype for the CD38 SNP also reported greater feelings of alienation from both parents and peers. This is particularly relevant as low

parental expressive support was related to elevated suicidal ideation (Winfrey Jr and Jiang, 2010) and low levels of social connectedness and social support have been associated with increased rates of suicide attempts (Compton et al., 2005). Indeed, having strong ties to social networks and social identities can reduce depressive symptoms (Cruwys et al., 2013), and thus the perceptions of disturbed parental and peer relationships among the individuals with the AA genotype would be expected to be related to suicidal ideation. Although speculative, this may also be indicative of the AA carriers being more sensitive, and therefore perceiving greater levels of alienation. This might be particularly relevant as individuals with the AA genotype did not experience greater levels of trauma compared to those with other genotypes. Given the involvement of oxytocin in social bonding and attachment (Carter, 1998; Insel and Hulihan, 1995), it is possible that the link between oxytocin and mental health disturbances occur through altered social processes (McQuaid et al., 2014).

The perception that oxytocin largely promotes prosocial/beneficial behaviors has been challenged by the suggestion that the effects of oxytocin depend on contextual and person factors (Bartz et al., 2011a). In this regard, oxytocin may promote antisocial effects among individuals with borderline personality disorder (Bartz et al., 2011b). Moreover, in voles intranasal oxytocin promotes maternal aggression in response to an intruder (Jia et al., 2008), and mice with increased oxytocin receptors displayed elevated fear and anxiety in response to a negative social stressor (Guzmán et al., 2013). Although oxytocin administration in humans may promote prosocial behaviors, it could instigate adverse outcomes if taken among individuals who do not display social processing

deficits, possibly because oxytocin treatment renders these individuals inappropriately sensitive within social situations (Cardoso et al., 2014).

In the current investigation, individuals with the G allele for the OXTR SNP, rs53576, and who are often thought to be more socially sensitive, did not display elevated suicidal ideation or depression scores even having experienced high levels of trauma. Although it was not predicted that this SNP would be related to suicidal ideation, given our earlier finding that G carriers displayed elevated depressive symptoms upon experiencing early-life maltreatment (McQuaid et al., 2013), we had expected that G carriers who had experienced high levels of trauma, would display elevated depression scores. In line with this, it has been reported that African American GG homozygotes of this OXTR SNP who experienced severe childhood maltreatment displayed elevated emotional dysregulation and disorganized attachment (Bradley et al., 2011). However, the current investigation assessed traumatic life events that comprised non-interpersonal events, such as a natural disaster, as well as interpersonal events, such as the death of a loved one. General trauma in the present investigation differed appreciably from maltreatment that we previously assessed, as the latter was perpetrated by a parental figure or guardian. Indeed, maltreatment was accompanied by feelings of distrust among the sensitive G carriers, which mediated the relation between maltreatment and depressive symptomatology (McQuaid et al., 2013). In the current study, an analysis was also performed with the OXTR SNP in relation to depression scores and trauma exclusively comprising interpersonal events. The results remained identical and non-significant, likely suggesting that it is not only the interpersonal nature of a trauma, but

also the aspect of maltreatment that is particularly aversive among G carriers of the OXTR SNP.

Although it has been reported that the CD38 gene regulates oxytocin release (Jin et al., 2007), the functionality of the rs3796863 SNP is not fully understood. It was shown that plasma oxytocin levels were elevated in A carriers compared to CC homozygotes (Feldman et al., 2012). Based on this, our current findings could potentially reflect elevated plasma oxytocin (AA carriers) among individuals high in suicidal ideation. However, the association between AA carriers and elevated oxytocin concentrations were only demonstrated in a single study, and in order to make this link, it will need to be replicated. The influence of oxytocin on suicide-related behaviors is made more complicated by the fact that some studies assessed oxytocin in blood, whereas others evaluated CSF oxytocin, and studies that assessed both indicated that oxytocin concentrations in CSF may not be reflective of plasma oxytocin levels (Jokinen et al., 2012).

It was reported that individuals who previously attempted suicide (as opposed to exhibiting suicidal ideation) displayed low levels of CSF oxytocin concentrations, although this was not found with respect to levels of plasma oxytocin (Jokinen et al., 2012; Lee et al., 2009). Moreover, plasma oxytocin was not related to overall suicidal intent, although it was negatively associated with a planning subscale in men (Jokinen et al., 2012). In contrast to these studies, in the present investigation we assessed suicidal ideation, which is not synonymous with intent, as intent is a central factor linking suicidal ideation and actions (McAuliffe, 2002). Thus, the associations reported between suicidal

intent and the CD38 SNP may not be directly relevant to the relation between suicidal ideation and this SNP. Moreover, it is uncertain whether the relation between CD38 SNP and suicidal ideation is linked to altered levels of plasma oxytocin.

Interestingly, the relationship between the AA genotype of the CD38 SNP and experiences of trauma on suicidal ideation was not recapitulated in relation to depression. Although suicidal ideation is strongly linked to depression, it is also associated with other psychiatric disorders as well as non-psychiatric conditions (e.g., in association with chronic illness), and conversely, not all instances of depression are accompanied by suicidal ideation (Mann et al., 2003). Diverse neurobiological mechanisms might similarly be linked to suicidal ideation, varying with the context in which this occurs. Oxytocin seems to be involved in depressive disorders (Matsushita et al., 2012), and it interacts with many biological systems, such as monoaminergic, HPA, and immune responses (McQuaid et al., 2014), however, the contribution of oxytocin in relation to suicidal ideation would not be expected to fully map onto depressive state.

There were several limitations concerning the conclusions that could be drawn from the present findings. The functionality of the OXTR SNP, rs53576, and the CD38 SNP, rs3769863, are not fully understood. It has been suggested that the OXTR SNP, located on intron 3, might be involved in transcriptional suppression (Mizumoto et al., 1997), but it is also possible that the effects are associated with linkage(s) to other functional OXTR SNPs (Lin et al., 2007). Furthermore, although elevated plasma oxytocin occurs in A carriers of the CD38 SNP (Feldman et al., 2012), replication of this effect is necessary in which the oxytocin is extracted from plasma before the assay is

conducted. Unextracted plasma oxytocin levels can be more than 100 times higher compared to the same extracted sample (McCullough et al., 2013; Szeto et al., 2011). A second limitation of this study concerned the moderate sample size used, and it would have been advantageous to have a larger number of minor allele carriers for the CD38 and OXTR SNPs, particularly when examining incidences of suicidal ideation which are relatively infrequent in a university student sample. Finally, even though suicidal ideation in those with the AA genotype of the CD38 SNP was exacerbated in the context of high levels of trauma, this does not necessarily translate into elevated intent, nor is it necessarily linked to later suicidal efforts.

## Chapter 5: Study 4

### The Role of Social Support and Oxytocin in Stressor Responses

Study 3 determined that the SNP, rs3796863, on the CD38 gene was associated with feelings of alienation from parents and peers as well as greater levels of suicidal ideation, particularly in the context of traumatic life events. The previous studies have each focused on examining genetic variants of the oxytocin system in relation to various stressors, such as early-life maltreatment, an acute social rejection stressor, or traumatic life events. In keeping with the context of stress, it was of interest to examine the influence of an intense but acute psychosocial stressor, the Trier Social Stress Test (TSST), on endogenous oxytocin levels. Furthermore, given the notion that the influence of oxytocin on stressor reactions might be moderated by social factors, we also examined whether the presence of social support would buffer cortisol responses to the TSST. Certainly there have been many studies that examined the influence of intranasal oxytocin on cortisol stress responses. However, there have been few human reports demonstrating the influence of an acute stressor on endogenous oxytocin levels. It should be added, that there is some question concerning the validity of several reports that assessed oxytocin levels in response to stressor or other challenges. Specifically, in some studies oxytocin was analyzed without first being extracted from blood samples, and thus cross-reactivity may have occurred with other substrates so that oxytocin was measured together with these, leading to unrealistically high indications of this hormone (McCullough et al., 2013; Szeto et al., 2011). In the present study we examined the influence of the TSST on cortisol and oxytocin levels that had been extracted prior to the

assay, among female participants who either had a close friend present for support or did not receive social support.

## **Abstract**

Stress responses can be attenuated by exogenous oxytocin administration, and these stress-buffering properties may be moderated by social factors. Yet, the influence of acute stressors, alone or in combination with social support, on endogenous oxytocin levels is not well understood. In the current investigation, ( $N = 67$ ), undergraduate women were assessed in the Trier Social Stress Test (TSST) with either social support available from a close female friend, no social support, or they were not stressed. The TSST elicited marked elevations of state anxiety and negative emotions, which were attenuated among women who received social support. Furthermore, baseline oxytocin levels were inversely related to women's distrust scores and basal cortisol levels in plasma. Despite these associations, oxytocin levels were unaffected by the TSST, irrespective of oral contraceptive use or estrogen levels. In contrast, cortisol elevations were elicited by the psychosocial stressor, but this was only apparent among women taking oral contraceptives, an effect that was prevented when social support was available. From these findings, it appears that although oxytocin may be linked to prosocial behaviors, it does not seem that a stressor that produces anxiety and increases cortisol necessarily alters oxytocin levels, nor does it seem as if changes in plasma oxytocin accompany the stress attenuating effects of social support on cortisol levels. This said, plasma oxytocin levels might not map on well to variations of oxytocin in stress-relevant brain regions, and studies in rodents have indeed demonstrated that brain oxytocin changes are fundamental to the stress buffering effects of social support.

## **Introduction**

Oxytocin, a neuropeptide produced in the hypothalamus, has gained considerable attention given its presumed role in underlying prosocial behaviors (Donaldson and Young, 2008). In this regard, higher endogenous oxytocin levels have been associated with greater trust behaviors (Zak et al., 2005; Keri et al., 2011), enhanced 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; Shamay-Tsoory et al., 2013), positive communication (Ditzen et al., 2009), helping behavior (Riem et al., 2013), and parochial altruism (De Dreu et al., 2010).

Through interactions with other biological systems, oxytocin is involved in a wide range of physiological and behavioral processes, some of which appear to 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 levels associated with a physical stressor (Cardoso, et al., 2013b; Coiro et al., 1988), limited the cortisol rise elicited by social ostracism (Linnen et al., 2012) and that associated with a 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 levels was significant in those studies that involved a task that elicited a robust HPA-axis response as well as in studies involving clinical samples (Cardoso et al., 2014).

Considering the role of oxytocin in social affiliation and bonding, 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 (Marmot and Wilkinson, 2005; Uchino, 2006) and mental health (Cruwys et al., 2013), and provides an important method of dealing with stressors, thus attenuating the propensity to illness (Holt-Lunstad et al., 2008; Luo and Wang, 2009). In this regard, following a psychosocial stressor, social support diminished cortisol as well as activation of the dorsal anterior cingulate cortex, a brain region previously associated with social distress (Eisenberger et al., 2007). In fact, males who received social support coupled with intranasal oxytocin prior to the TSST, displayed a less pronounced cortisol rise compared to individuals who received either social support, oxytocin or neither of these treatments (Heinrichs et al., 2003). Likewise, among children who were able to see or hear their mothers following a social stressor, oxytocin levels were higher and cortisol levels were attenuated compared to children that had no-contact with their mothers (Seltzer et al., 2010).

The majority of studies examining the effects of intranasal oxytocin administration were restricted to males owing to potential complications that can arise in females, such as uterine contractions and/or fluctuations of hormone levels across the menstrual cycle (Choleris et al., 2008). Yet, as estrogen influences transcription of oxytocin as well as its receptor (Choleris et al., 2008), oxytocin is thought to be particularly relevant to females (Taylor et al., 2010). Furthermore, although there are many studies that assessed the influence of intranasal oxytocin on stress responses, there

have been few reports that examined the effect of an acute stressor on endogenous oxytocin levels in humans. Moreover, of these reports, the data concerning oxytocin release in response to acute psychosocial stressors have been inconsistent (Ditzen et al., 2007; Pierrehumbert et al., 2012; Seltzer et al., 2010; Taylor et al., 2006).

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. It was hypothesized that having social support from a close female friend would attenuate the cortisol stress response. It was less certain whether endogenous oxytocin levels among females would rise in response to the TSST. Oxytocin has frequently been measured in blood samples, over the past 10 years, many studies conducted these analyses in plasma samples which had not been extracted, and thus tagged molecules other than oxytocin, yielding apparently high levels of this hormone that may have been, in fact, confounded by the presence of other hormones (McCullough et al., 2013; Szeto et al., 2011). Thus, in the current study it was important to determine the effects of a stressor on circulating oxytocin in properly extracted samples, particularly in females. It was predicted that oxytocin would increase in response to a stressor, more so in the presence of social support, and that this would be accompanied by a diminished cortisol response.

## **Methods**

### ***Participants***

This study included female undergraduate students ( $N = 67$ ) from Carleton University ( $M_{age} = 19.37$ ,  $SD = 2.08$ ) 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 had 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), stress-no support (participants arrived alone to the laboratory session) or controls (no stress, no friend).

### *Laboratory Session*

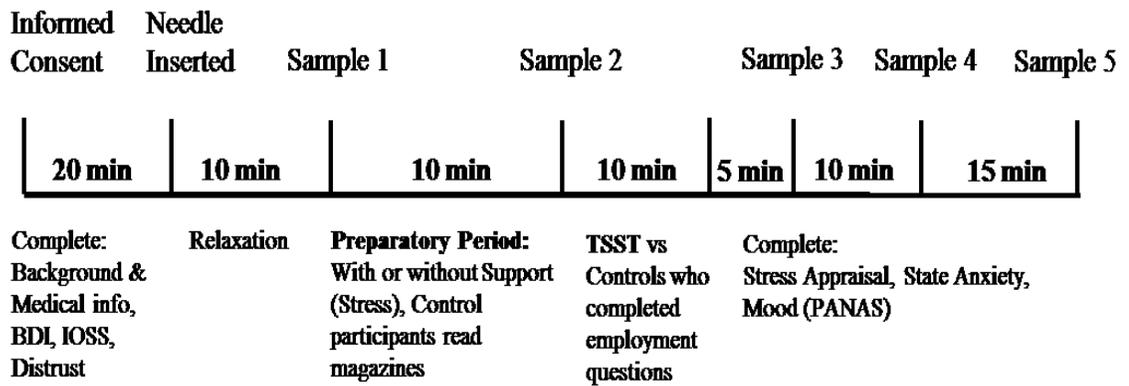
All procedures in this study were approved by the Carleton University Ethics Committee for Psychological Research. Laboratory sessions were conducted between 1300 and 1730 hr, and women were asked not to eat, drink (with the exception of water) or smoke for at least an hour before arriving to the session. Figure 1 provides a visual depiction of the laboratory session procedures. Once informed consent was signed, participants filled out a number of questionnaires assessing demographic information, 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. In the stress conditions, participants underwent the TSST in which they 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. This TSST procedure has consistently been shown to induce activation of the hypothalamic-pituitary-adrenocortical (HPA) axis response (Kudielka et al., 2004).

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. In the control condition, participants 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 stress 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 the 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.



*Figure 1.* Timeline of procedures for the laboratory session

## *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. Participants responded to one of four responses from 1 (not at all) to 4 (very much), where higher scores indicate greater state anxiety. Total score 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 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 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%. 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 ± 2pgmL.

## **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) (TSST 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 (TSST 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 TSST 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, as well as stress appraisal and state anxiety scores. Pearson's correlation coefficients were also determined between the friend's psychosocial scores and the participant's 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 depression,  $F(2, 64) = .21, p = .81$ . Following the TSST, stress appraisals varied as a function of condition,  $F(2, 64) = 37.08, p = .000, \eta^2 = .54$ . As shown in Figure 2A, both the stress-support and the stress-no support conditions reported elevated stressfulness compared to

controls,  $p = .000$  and  $p = .000$ , respectively. Upon examining state anxiety, it was determined that levels also differed according to condition,  $F(2, 64) = 9.94, p = .000, \eta^2 = .24$ . Specifically, individuals who were stressed but did not receive support exhibited elevated state anxiety compared to controls,  $p = .000$ , an effect not apparent among the stressed individuals who received social support  $p = .15$  (Figure 2B).

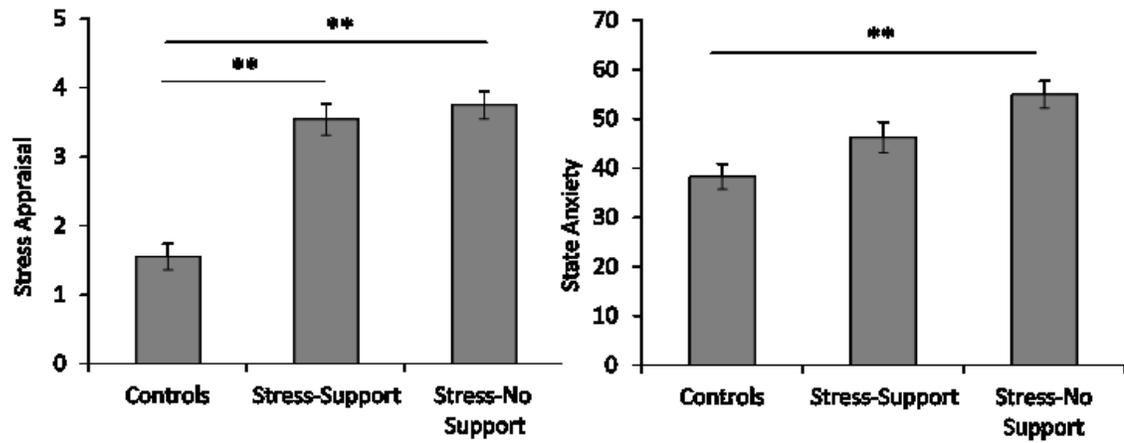


Figure 2. Stress appraisal scores (A) and State anxiety levels (B) among the controls, stress-support and stress-no support conditions following the TSST. Data represents means  $\pm$  S.E.M.  $**p < 0.001$ .

A significant MANOVA revealed differences in affective states following the TSST as a function of stressor condition,  $F(10, 122) = 4.77, p = .000, \eta^2 = .28$ . As shown in Figure 3, there was an overall effect of stress for feelings of shame,  $F(2, 64) = 13.88, p = .000, \eta^2 = .30$ , such that higher levels of shame occurred among both the stress-no support and stress-support conditions compared to controls,  $p = .000$  and  $p = .001$  respectively. Levels of sadness also differed between conditions,  $F(2, 64) = 6.10, p = .004, \eta^2 = .16$ . Specifically, the stressed-no support group displayed elevated sadness compared to controls,  $p = .000$ . In contrast, this was not apparent among individuals who were stressed but received support,  $p = .32$ , although their sadness did not differ from that of individuals who were stressed but had not received support,  $p = .35$ . Feelings of anger differed between conditions,  $F(2, 64) = 7.90, p = .001, \eta^2 = .20$ , such that individuals in the stress-no support condition had more anger compared to controls,  $p = .001$ , an effect not apparent among individuals in the stress-support condition. Further, as shown in Figure 3, anger tended to be higher in the stress-no support condition compared to the stress-support condition, although this only approached significance,  $p = .06$ . Fear and negative affect followed a similar trend to anger,  $F(2, 64) = 10.21, p = .000, \eta^2 = .24$  and  $F(2, 64) = 10.54, p = .000, \eta^2 = .25$  respectively. In this instance, individuals who were stressed and had no support displayed elevated fear and negative affect compared to controls,  $p$ 's = .000 and stress-support counterparts,  $p$ 's = .02.

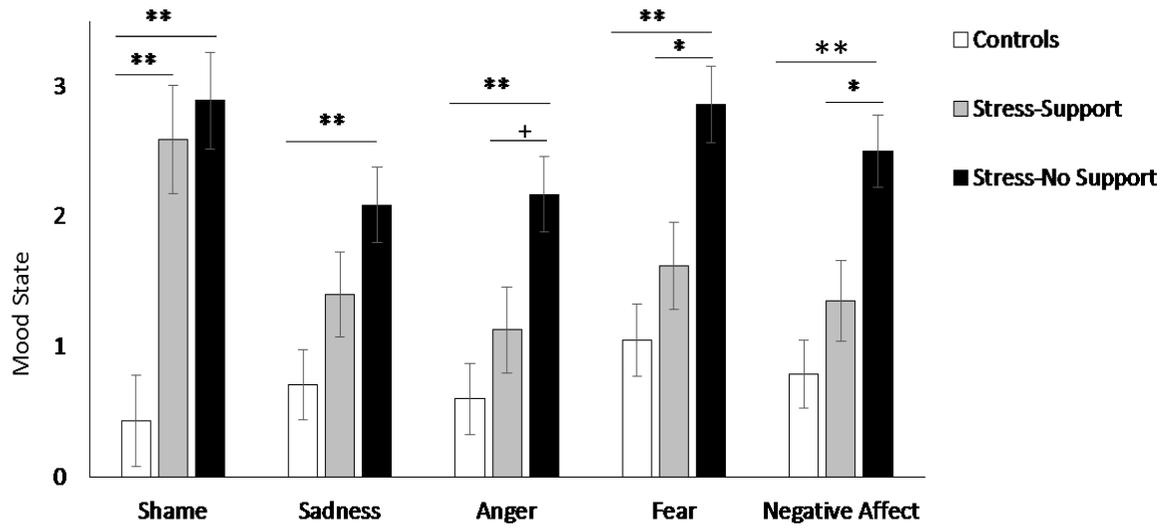


Figure 3. Affective states including sadness, anger, fear, shame and overall negative affect among the controls, stress-support and stress-no support conditions following the TSST. Data represents means  $\pm$  S.E.M. +  $p = 0.06$ , \* $p < 0.05$ , \*\* $p \leq 0.001$ .

### *Friends' responses*

Upon examining the degree of closeness between the friend and the participant, it appeared that there was no relationship. Specifically, friends' perceptions of closeness and participants' perceptions of closeness with each other were unrelated,  $r = .02, p = .93$ . Furthermore, friends' perceptions of closeness was not related to participants' psychosocial responses to the TSST, including state anxiety  $r = 1.5, p = .56$ , stress appraisals,  $r = .17, p = .50$ , 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. Similarly, the friends' level of empathy did not relate to the participants' state anxiety  $r = -.05, p = .86$ , or stress appraisal,  $r = -.02, p = .93$  scores.

Interestingly, empathy scores of the friend were inversely related to the participants' cortisol levels at baseline,  $r = -.60, p = .01$ , and following the TSST,  $r = -.50, p = .04$ . 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,  $r = .56, p = .02$ . A similar association was not observed in relation to oxytocin scores following the TSST.

### ***Biological Responses***

It was first of interest to examine 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 relationship was found

among distrust and oxytocin,  $r = -.28, p = .03$ . Specifically, lower baseline oxytocin levels were associated with greater levels of distrust. As well, as intranasal oxytocin may attenuate cortisol levels (Cardoso, et al., 2013; Coiro et al., 1988), we examined the relationship between oxytocin and cortisol and found that higher oxytocin at baseline was indeed associated with lower baseline cortisol levels,  $r = -.28, p = .04$ .

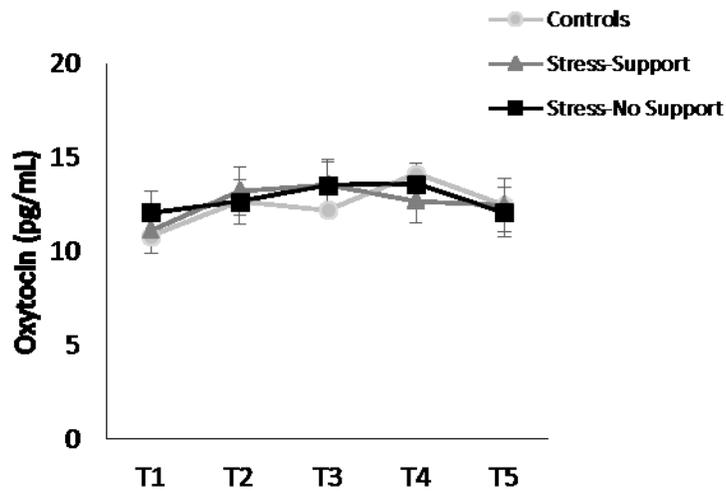
Table 1.

*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. 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 levels of oxytocin baseline,  $F(2, 59) = 0.65, p = .53$ . Furthermore, over the course of the TSST or 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 4,

oxytocin levels in response to the TSST did not vary by condition,  $F(8, 180) = 0.40$ ,  $p = .92$ .



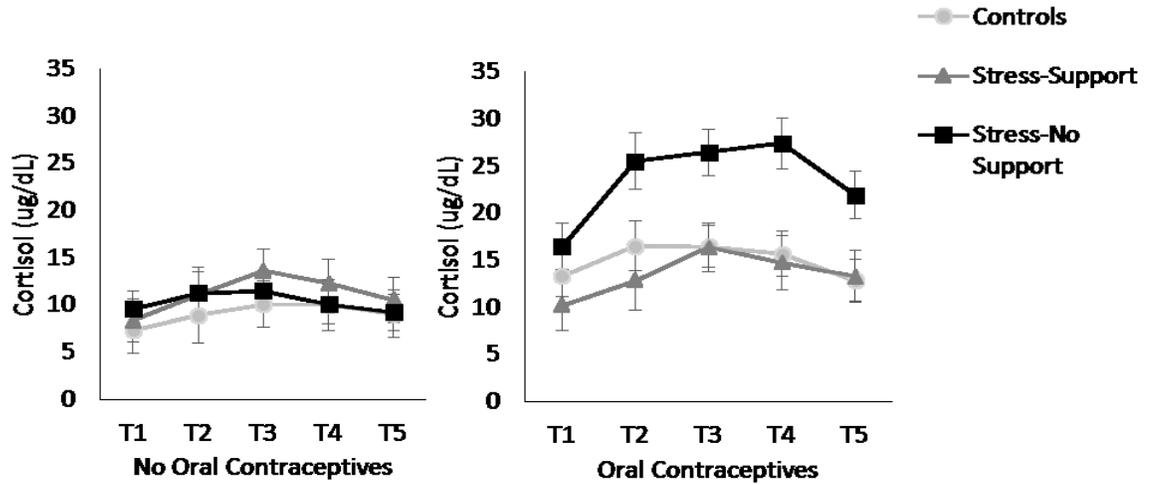
*Figure 4.* Plasma oxytocin levels (pg/mL) collected at five time points including 15 minutes prior to the TSST (T1), immediately before the TSST began (T2) and five minutes (T3), 15 minutes (T4) and 30 minutes (T5) following the TSST. The graph represents individuals in the control, stress-support and stress-no support conditions. Data represents means  $\pm$  S.E.M.

Following the TSST, correlations were examined between psychosocial responses (stress appraisals, state anxiety scores) and cortisol levels as a function of condition.

Among individuals in the stress-no support condition, stress appraisals were correlated with cortisol levels following the TSST (i.e. at time-points T-3, T-4 and T-5),  $r = .50, .56$  and  $.45$ , respectively,  $p$ 's  $< .05$ . In this same group of individuals, levels of state anxiety and cortisol were significantly correlated at each of the five time-points (T1-T5 respectively),  $r$ 's ranged from  $= .45$  to  $.63$ ,  $p$ 's  $< .05$ . Interestingly, no such relationships were found among the controls or the individuals who were tested in the TSST, but received social support.

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 area under the curve (AUC) analyses indicated that although there was no main effect of condition on cortisol AUC,  $F(2, 50) = 2.22$ ,  $p = .12$ ,  $\eta^2 = .08$ , there was a main effect of oral contraceptives,  $F(2, 64) = 6.50$ ,  $p = .014$ ,  $\eta^2 = .12$ , with individuals who were taking oral contraceptives having a larger AUC. Additionally, cortisol varied as a function of the TSST Condition x Oral contraceptives interaction,  $F(2, 50) = 5.44$ ,  $p = .007$ ,  $\eta^2 = .18$ . As shown in Figure 5, and confirmed by 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,  $p = .004$ . Further, while there were no differences between controls and individuals who were stressed with support, AUC was somewhat greater in the stress-no support group compared to the stress-support group,  $p = .07$ .



*Figure 5.* 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 in the control, stress-support and stress-no support conditions represented separately by oral contraceptive use. Data represents means  $\pm$  S.E.M.

## **Discussion**

In the current study the TSST increased stressor appraisals irrespective of social support, whereas state anxiety scores following the TSST were significantly elevated only among individuals who were stressed and did not receive support. In essence, it appeared that although participants in both conditions were equally cognizant of the stressor, social support was able to buffer some of the anxiety associated with the TSST. Similarly, individuals who were stressed and did not receive support displayed marked elevations of anger, fear, and sadness as well as overall negative affect relative to non-stressed to controls, whereas individuals with support who were stressed displayed considerably lower levels of these emotions following the TSST. Again these findings point to the stress-buffering effects of social support (Cohen and Willis, 1985).

Interestingly, although the support of a close friend acted against some of the negative emotions promoted by the TSST, feelings of shame were not diminished by support. Why shame specifically was not attenuated by social support is not immediately apparent; however, feelings of shame may be a unique emotion (relative to the others measured) being a threat to the ‘social self’, and has indeed been related to cortisol changes associated with the TSST (Dickerson et al., 2004; Gruenewald et al., 2004). In effect, the presence of a close friend might not have been sufficiently potent to attenuate intense feelings, such as a threatened social self.

It was interesting that the independent assessments made by participants and friends of their relational closeness were not consistent with one another. Furthermore, friends who reported a high degree of closeness and/or empathy was not related to low

levels of anxiety or negative affect elicited by the TSST among participants. Likewise, participants' closeness to the friend was unrelated to 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, although admittedly this might not reflect the perceived effectiveness of the support.

### *Basal oxytocin*

Given the presumed relationship between oxytocin and prosocial behaviors, and that oxytocin was also implicated in inferring the mental states of others (Domes et al., 2007), it is interesting that having a friend present with higher 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 data are simply correlational and thus preclude this conclusion. This said, in line with this suggestion, empathy is related to endogenous oxytocin release, particularly among females (Barraza and Zak et al., 2009), and intranasal oxytocin promotes empathetic behaviors (Domes et al., 2007; Shamay-Tsoory et al., 2013). However, it has yet to be shown whether empathy of one individual can affect oxytocin release in a close other.

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 involving secret sharing (Kéri et al., 2009, Kéri and Kiss, 2011). As well, intranasal

oxytocin was reported to increase trust related behaviors in a monetary game (Kosfeld et al., 2005), and in relation to trust with confidential information (Mikolajczak et al., 2010). Unlike the studies showing a relationship between endogenous oxytocin and trust (Keri et al., 2009; Zak et al., 2005), it was reported that endogenous oxytocin levels and trust were not related in a prisoner's dilemma paradigm (Christensen et al., 2014). It was suggested that these discrepant results may be due to the procedure in the former studies not extracting oxytocin from samples prior to the assay (Christensen et al., 2014). In the current study, the relation between oxytocin and distrust was found among samples that were extracted prior to the assay, although scores of distrust on a self-report measure might not map on well to the behavioral assessment of trust.

Oxytocin levels at baseline were inversely related to cortisol levels, which is consistent with reports that oxytocin administration reduces cortisol levels (Cardoso, et al., 2013b; Ditzen, et al., 2009; Linnen et al., 2012). Although oxytocin is thought to contribute to affiliative/social approach behaviors by diminishing fear/anxiety (Taylor et al., 2006), it is uncertain whether the oxytocin link to cortisol, or the combined actions of oxytocin and cortisol variations were tied to prosocial behaviors.

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

Although studies in animals have indicated oxytocin elevations following acute stressors (Danevova et al., 2013; Hashiguchi et al., 1997), in humans such effects are less apparent. Cortisol treatment in humans is known to increase oxytocin levels (Tops et al., 2006; 2012), and thus it might have been expected that a stressor would elicit a similar outcome. However, as previously observed (Ditzen et al., 2007; Taylor et al., 2006),

oxytocin levels were unaffected by the TSST. This was the case regardless of their circulating estradiol levels or whether women were using oral contraceptives. As will be discussed shortly, the TSST was effective in promoting cortisol changes, indicating that the procedure itself was stressful and thus the lack of oxytocin change could not be attributed to the use of an ineffective stressor protocol.

Cortisol profiles among women taking oral contraceptives, as in earlier reports (Kirschbaum et al., 1999, Nielsen et al., 2013), were distinct from non-oral contraceptive users. Females taking oral contraceptives in the current study displayed elevated baseline plasma cortisol levels as well as greater plasma cortisol responses to the TSST compared to females not taking oral contraceptives. Indeed, it was only the former condition that the TSST effectively increased cortisol levels. It was previously reported that oral contraceptive users typically display a blunted cortisol response measured in saliva, whereas an appreciable increase (55%) was apparent in blood (Kirschbaum et al., 1995; 1996, 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 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; Kirschbaum et al.,

1992) 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, 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 (Smith and Wang, 2014). In line with this view, 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 was 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. Our findings are more in-line with the report that women who had positive physical interactions with their partners (i.e. shoulder message) before the TSST and who displayed attenuated cortisol levels, did not show elevated oxytocin concentrations (Ditzen et al., 2007). Given that plasma oxytocin levels do not necessary reflect changes in brain oxytocin (Landgraf and Neumann, 2004), it is possible that central oxytocin release, in fact, buffers HPA axis activity, but plasma levels of this hormone might not reflect the 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. Finally, in the present study a group was not included 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.

These caveats notwithstanding, the current study addressed several important issues concerning endogenous oxytocin functioning in relation to stress. The number of studies that included females in research pertaining to stress and oxytocin have been limited, particularly those in which intranasal oxytocin was administered (owing to complications relating to hormonal fluctuations across the menstrual cycle). For example, it is known that intranasal oxytocin and social support interact to attenuate cortisol responses elicited by stressors among men (Heinrichs et al., 2003), but whether this same relation occurs among women is uncertain. This is of particular importance as the oxytocin system may be especially relevant among females (Insel and Hulihan, 1995; Taylor et al., 2010; Young and Wang 2004) and is regulated by estrogen (Choleris et al., 2008). In fact, increasing evidence has pointed to differential actions (or correlations) of endogenous and exogenous oxytocin on various behaviors among men and women (Ditzen et al., 2013; Hoge et al., 2014; Taylor et al., 2010; Tops et al., 2006). Beyond questions related to gender differences, there is a need for studies examining endogenous

oxytocin levels in response to stressors as a function of whether oxytocin was extracted from blood samples prior to assay, especially given the uncertainty concerning what is actually being measured in unextracted samples (see Szeto et al., 2011, regarding a discussion of this topic).

Summarizing, the present investigation suggested that basal oxytocin levels are associated with attitudes, such as distrust as well as with basal levels of cortisol. A psychosocial stressor in the form of the TSST, however, did not affect plasma oxytocin levels, although the stressor was effective in eliciting anxiety and among women using oral contraceptives the stressor increased cortisol levels. Moreover, social support was found to attenuate anxiety and cortisol response to the TSST. Thus, it is tempting to conclude that social support acts independent of oxytocin in determining HPA responses, but it is premature to do so given that plasma oxytocin might not be an adequate reflection of stress-related oxytocin functioning within stress-relevant brain regions.

## General Discussion

The perspective that certain genotypes are adaptive while others are ‘risk’ or vulnerability factors is an overly simplistic view. Instead, it has been suggested that genetic variants are neither positive nor negative, but might ‘for better or for worse’ influence plasticity that permits adaptation to the environment or affects sensitivity to environmental triggers (Belsky et al., 2007, 2009; Belsky and Pluess, 2009). This perspective can be extended beyond the 5-HTTLPR polymorphism discussed by Belsky, being applicable to oxytocin genetic variants. In this regard, the data presented in Study 1, 2 and 3 were consistent with the view that oxytocin genetic variants were related to sensitivity to social cues and experiences. In particular, Study 1 revealed that relative to those with the AA genotype, G carriers of the OXTR SNP, who had been considered more prosocial (and conceivably the more advantageous genotype), were, in fact, more negatively affected by early-life adversity in the form of maltreatment. Likewise, in Study 2, acute social ostracism provoked greater behavioral and physiological variations among individuals with the GG genotype. In Study 3, in which the CD38 gene was assessed, the A carriers who had previously been thought to have the ‘protective’ allele displayed disturbed peer and parental relationships and relatively high suicidal ideation.

Neuroimaging data have supported the social sensitivity view, revealing that G carriers of OXTR SNP, rs53576, displayed enhanced amygdala activation in response to emotional faces (Tost et al., 2010). As well, A carriers of the CD38 SNP, rs3796863, were shown to process social stimuli faster and display enhanced amygdala activity compared to CC homozygotes, an effect that was more apparent upon receiving

intranasal oxytocin treatment (Sauer et al., 2012). Typically oxytocin treatment attenuates amygdala activity (Kirsch et al., 2005; Labuschagne et al., 2010; Petrovic et al., 2008), which is thought to be a result of increased activation of GABAergic neurons in the amygdala (Huber et al., 2005). In essence, the increased social behaviors associated with high oxytocin or oxytocin administration could be a result of decreased amygdala activity and reduced social fear (Kirsch et al., 2005; Taylor et al., 2006). However, as the oxytocin genetic variants associated with social sensitivity have been related to increased amygdala activity (Sauer et al., 2012; Tost et al., 2010), it should be considered that other factors ultimately contribute to the emergence of social behaviors. For instance, GABA variations in the amygdala may be determined by the opposing actions of oxytocin and vasopressin functioning (Huber et al., 2005). Accordingly, it may be necessary to consider functioning of both these systems in assessing such sensitivity in relation to social behaviors. In fact, there have been several reports in line with this perspective, although it was argued that the contribution of vasopressin may be more relevant for males than for females (Dumais and Veenema, 2015; Taylor et al., 2010;), given that vasopressin functioning is influenced by testosterone (Delville et al., 1996; Viau et al., 1999).

Although the effects found in Studies 1-3 were consistent with a social sensitivity perspective, it is not the intention of this collection of research to imply that G carriers of the OXTR SNP are more sensitive across all social situations, contexts and/or stressors. In fact, we have also obtained data that do not support this perspective (McInnis et al., 2015). In this regard, upon experiencing feelings of unsupport from parents and peers, A

carriers of the OXTR SNP displayed higher emotion-focused coping and lower problem-focused coping, which were associated with greater depressive scores (see McInnis et al., 2015b manuscript in Appendix B). These findings suggest that GG homozygotes might be able to cope more effectively in certain circumstances. In fact, GG homozygotes were more likely to seek social support, although this was a culturally dependent effect (Kim et al., 2010), and social support was more effective at attenuating stressor responses among G carriers of the OXTR SNP (Chen et al., 2011). Thus, it seems the presence of the G allele, allows for the use of social support as a coping mechanism. The views that G carriers cope more effectively, and are also more socially sensitive, are not mutually exclusive. For instance, given the results of Study 2 in which G carriers were more reactive to social ostracism in the laboratory, it is conceivable that these individuals would also have been more likely to seek social support following the session, resulting in attenuated stressor effects.

The relationship between a particular genotype and a behavior might vary with the context in which this was assessed. By example, it has been demonstrated that the presence of a 5-HTT mutation was accompanied by increased depression provided that individuals had experienced either early-life distress or more current stressors (Caspi et al., 2003). It might similarly be the case that the relationship between oxytocin SNPs and behavioral functioning is dependent on a constellation of experiences and situations in which individuals were assessed (see McInnis et al., 2015a in Appendix C). In line with this perspective, studies in animals have indicated that diverse stressors may have very different brain neurochemical consequences, and even when similar outcomes are

observed, they may occur through different pathways (Merali et al., 2008; Morrow et al., 2000; Mountney et al., 2011). In this regard, even subtle differences between stressors could promote different outcomes. A stressor in the form of social ostracism is not akin to general feelings of an unsupportive relationship, just as trust is not analogous with empathy. Many complex behaviors have been ascribed to oxytocin, and are likely subserved by multiple neurochemical interactions. As we indicated previously, oxytocin interacts with CRH, 5-HT, NE and DA, each of which might have differing effects depending upon the individual, and may also vary with the nature or course of the stressor (McQuaid et al., 2014).

It might have been expected that a potent stressor, such as the TSST would influence oxytocin levels, but this outcome, in Study 4, was not observed. Certainly, animal studies suggested that oxytocin release occurs in response to various stressors (Danevova et al., 2013; Hashiguchi et al., 1997), although it is conceivable that oxytocin changes might be most evident following stressors of a chronic nature or early-life stressors that could influence the developmental trajectory. Indeed, CSF and plasma oxytocin levels were reduced in adults who had a history of early-life stress (Heim et al., 2008b; Opacka-Juffry and Mohiyeddini, 2012), although such individuals might also be more likely to have been depressed. Oxytocin changes might also vary with the specific nature of the stressor. In this regard, considering that males tend to show greater cortisol responses to achievement-oriented stressors (e.g., the TSST) (Kajantie and Phillips, 2006; Kirschbaum et al., 1992), whereas women display greater cortisol responses to rejection stressors (Stroud et al., 2002), this might similarly be evident in relation to oxytocin.

Albeit speculative, it is possible that in Study 4, women might have shown elevated plasma oxytocin responses to a social rejection stressor rather than the TSST. Given that oxytocin is thought to be involved in prosocial behaviors, it would follow that social rejection might be particularly pertinent in relation to oxytocin functioning.

It might also be the case that oxytocin changes in response to a stressor might uniquely occur among individuals with a particular oxytocin genotype. In fact, there is evidence suggesting that G carriers of the OXTR SNP display greater physiological responses (heart rate, blood pressure and cortisol) to social stressors (Norman et al., 2012; McQuaid et al., 2015). Ideally, in Study 4, it would have been of interest to examine genotypes for the OXTR and CD38 SNPs, however, this was not practical owing to the small number of participants, and the mixed ethnic sample (which will be discussed shortly in relation to gene association studies).

The unique and complex relations between the OXTR genotypes were highlighted in Study 2, in which individuals with the heterozygote (AG) genotype responded differently depending upon the variable in question. In this regard, following ostracism, individuals with the AG genotype showed psychosocial responses that were similar to GG homozygotes, but physiological reactivity more comparable with that of the AA homozygotes. These findings underscore the importance of examining all genotypes separately, if at all possible. The majority of studies involving oxytocin SNPs have chosen to collapse across the genotypes (e.g., combining the AG individuals with either the GG or AA homozygous individuals). This decision may have been based on previous reports or on the fit of the data, which is likely the more appropriate consideration (albeit

subject to bias). However, as shown in Study 2, collapsing across the genotypes could have caused an effect to be incorrectly interpreted or missed entirely. In fact, one report speaks to this issue as it was found that adolescent girls with the heterozygote (AG) OXTR genotype, who experienced early-life adversity in the form of maternal depression, displayed the *highest* depression scores (Thompson et al., 2011). Thus, in Study 3, all genotypes were analyzed separately. Although a clearly favorable approach, it is hampered by the statistical tests available that can be used to assess moderations and moderated mediations that don't allow for inclusion of trichotomous moderators.

Upon examining the OXTR genotype distributions, it was apparent that the genotype frequencies varied across ethnic groups, an effect this has been well documented regarding other polymorphisms (Gelernter et al., 1997). As shown in Table 1, among White and Black participants, the G allele was the most common allele variant, and the AA genotype was relatively rare. However, the opposite distribution was found among Asian participants, in which the most common allele variant was the A allele and, in fact, no Asian individuals with the GG genotype existed in this sample. What was equally interesting was that the distributions and trends across ethnic groups differed depending upon the specific SNP being assessed. As shown in Table 2, upon examining the allele frequencies for the CD38 SNP, it was the White and Asian individuals that displayed similar genotype distributions such that the C allele was the more common variant and the AA genotype was less frequent. However, Black individuals for this SNP displayed an opposite distribution in which the A allele was the common variant and the CC genotype was relatively rare.

Table 1

*Study 3 OXTR Polymorphism Distributions for Selected Ethnicities*

	<b>Ethnicity</b>	<b>G/G</b>	<b>A/G</b>	<b>A/A</b>
OXTR	White ( <i>n</i> = 241)	109	106	26
	Black ( <i>n</i> = 61)	39	20	2
	Asian ( <i>n</i> = 33)	0	17	16

Table 2

*Study 3 CD38 Polymorphism Distributions for Selected Ethnicities*

	<b>Ethnicity</b>	<b>C/C</b>	<b>A/C</b>	<b>A/A</b>
CD38	White ( <i>n</i> = 237)	106	105	26
	Black ( <i>n</i> = 61)	6	30	25
	Asian ( <i>n</i> = 33)	14	16	3

The different distributions of genotypes across ethnicities create a problem for mixed ethnic samples in gene association studies. This is particularly the case as associations with the SNPs may be dependent upon ethnic background. For instance, under distress G carriers who were American were more likely to seek social support than AA carriers, an effect that was not found among Korean G carriers, possibly owing to cultural differences in social norms (Kim et al., 2010). For example, although American norms favor social support seeking, it is often discouraged in the Korean culture. Considering the issues surrounding a mixed ethnic sample for examining gene-associations, this highlights an important limitation in Study 1. Thus, in Study 2 and 3, data from a large number of individuals with diverse backgrounds were collected, but only data from Euro-Caucasian (White) individuals were included in the analyses.

The effects of oxytocin are not just related to ethnicity, but as alluded to earlier have been shown to be person and context specific (Bartz et al., 2011a). In this regard, males who received intranasal oxytocin and had a secure attachment remembered their mother as more caring and close compared to placebo-treated individuals. In contrast, males who were anxiously attached reported their mother to be less caring and close relative to individuals that received placebo (Bartz et al., 2010). An important moderating factor that ought to be considered more often in research involving intranasal oxytocin, concerns sex differences that may exist. Females are often excluded from intranasal oxytocin research because of potential complications related to the menstrual cycle and uterine contractions (Choleris et al., 2008). Indeed, it seems that the effects associated with oxytocin are not comparable between males and females. For instance, females who received oxytocin treatment rated faces more positively compared to controls, whereas males rated them more negatively upon receiving oxytocin (Hoge et al., 2014). As well, although endogenous oxytocin levels were associated with relational distress among females, vasopressin served this function among males (Taylor et al., 2010). What might be most important, particularly in the context of the current research, is a recent finding that depressed females displayed lower plasma oxytocin concentrations compared to controls, but depressed males tended to display elevated oxytocin concentrations (Yuen et al., 2014).

Taken together, what does this suggest for oxytocin treatment as a viable option for individuals with mental health disorders? A social sensitivity perspective might predict that in certain individuals, oxytocin treatment would be beneficial in treating

depressive affect, but in others it might be detrimental (Cardoso et al., 2014). Indeed, there is reason to believe that individuals with certain oxytocin genotypes are more affected by environmental triggers, and these individuals could potentially become too sensitive with oxytocin treatment. It might similarly be profitable to consider endogenous oxytocin levels to determine the potential efficacy of oxytocin treatment. In line with this perspective, individuals who have pervasive social deficits, such as in autism spectrum disorders, and who display reduced plasma oxytocin levels (Modahl et al., 1998), show improved social cognition following intranasal oxytocin treatment (Guastella et al., 2010; Hollander, 2007). However, unlike autism spectrum disorders, it is not clear whether depressed individuals have reduced or elevated plasma oxytocin levels (McQuaid et al., 2014). As depression is a heterogeneous disorder, it might be that certain subtypes or symptoms of depressive disorders are more likely to be accompanied by disturbed oxytocin functioning. In this regard, it would be expected that oxytocin treatment would be beneficial among depressed individuals with low endogenous oxytocin levels, but this same treatment among depressed individuals who have normal or elevated endogenous oxytocin levels might be counter-productive. This suggestion is in line with the view that if healthy individuals use oxytocin treatment (i.e. with a presumably normal functioning oxytocin system), they may become overly sensitive to social cues, leading to behavioral disturbances (Cardoso et al., 2014).

### **Limitations and conclusions**

As indicated in each of the chapters, there are limitations to the conclusions that can be drawn from the current investigations. With regard to gene association studies, the

use of mixed ethnic samples are problematic, however, it is also problematic to examine only the most dominant ethnic group. In this regard, only White individuals were considered in Studies 2 and 3. However, we have since obtained data, as parts of Studies 1-3, from a large number of individuals comprising diverse ethnicities. Thus, going forward, these data will be combined across ethnic groups so that the relations between oxytocin genetic variants and mental health outcomes can be examined within other ethnic groups of interest, such as Black and Asian individuals. Beyond limitations related to ethnicity, the functionality of the OXTR and CD38 SNPs have not been well documented (e.g., are these SNPs related to endogenous oxytocin levels, or alternatively, are they having their effects through linkage with other functional SNPs?). Finally, although examining individual SNPs on the OXTR and CD38 gene separately has been very informative, it might be more profitable to consider a haplotype approach. This approach would identify a collection of specific genetic variants across the OXTR/CD38 genes associated with depression. The OXTR and CD38 gene have numerous polymorphic sites, thus, examining whether certain haplotypes across these genes are associated with depressive mood will ultimately give a more comprehensive depiction of the role of oxytocin SNPs in depression.

## References

- Amico, J.A., Cai, H.M., Vollmer, R.R., 2008. Corticosterone release in oxytocin gene deletion mice following exposure to psychogenic versus non-psychogenic stress. *Neurosci. Lett.* 442, 262–266.
- Anisman, H., 2009. Cascading effects of stressors and inflammatory immune system activation: implications for major depressive disorder. *J. Psychiatry. Neurosci.* 34, 4-20.
- Anisman, H., Merali, Z., 1999. Anhedonia and anxiogenic effects of cytokine exposure. *Adv. Exp. Med. Biol.* 461, 199-233.
- Anisman, H., Merali, Z., Poulter, M.O. Gamma-Aminobutyric Acid Involvement in Depressive Illness Interactions with Corticotropin-Releasing Hormone and Serotonin. In: Dwivedi Y, editor. *The Neurobiological Basis of Suicide*. Boca Raton (FL): CRC Press; 2012.
- Apicella, C.L., Cesarini, D., Johannesson, M., Dawes, C.T., Lichtenstein, P., Wallace, B., et al., 2010. No Association between Oxytocin Receptor (OXTR) Gene polymorphisms and experimentally elicited social preferences. *PLOS ONE* 5, e111153.
- Aragona, B.J., Liu, Y., Yu, Y.J., Curtis, J.T., Detwiler, J.M., Insel, T.R., et al., 2005. Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. *Nat. Neurosci.* 9, 133-139.
- Arletti, R., Bertolini, A., 1987. Oxytocin acts as an antidepressant in two animals models of depression. *Life Sci.* 41, 1725-1730.

- Armsden, G. C., Greenberg, M. T., 1987. The inventory of parent and peer attachment: Individual differences and their relationship to psychological well-being in adolescence. *J. Youth Adolesc.* 16(5), 427-454.
- Aron, A., Aron E. N., Smollan, D., 1992. Inclusion of other in the self scale and the structure of interpersonal closeness. *J. Pers. Soc. Psychol.* 63 (4), 596-612.
- Audet, M.C., Anisman, H., 2013. Interplay between pro-inflammatory cytokines and growth factors in depressive illness. *Front. Cell. Neurosci.* 7, 68.
- Averbeck, B.B., Bobin, T., Evans, S., Shergill, S.S., 2011. Emotion recognition and oxytocin in patients with schizophrenia. *Psychol. Med.* 42, 259-266.
- Averbeck, B.B., 2010. Oxytocin and the salience of social cues. *Proc. Natl. Acad. Sci. U.S.A.* 107, 9033-9034.
- Bagdy, G., 1996. Role of the hypothalamic paraventricular nucleus in 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, and 5-HT<sub>2C</sub> receptor-mediated oxytocin, prolactin and ACTH/corticosterone responses. *Behav. Brain Res.* 73, 277-280.
- Bagdy, G., Kalogeras, K.T., 1993. Stimulation of 5-HT<sub>1A</sub> and 5-HT<sub>2</sub>/5-HT<sub>1C</sub> receptors induce oxytocin release in the male rat. *Brain Res.* 611, 330-332.
- Bakermans-Kranenburg, M.J., van Ijzendoorn, M.H., 2008. Oxytocin receptor (OXTR) and serotonin transporter (5-HTT) genes associated with observed parenting. *Soc. Cogn. Affect. Neurosci.* 3, 128-134.
- Bakos, J., Strbak, V., Paulikova, H., Krajnakova, L., Lestanova, Z., Bacova, Z., 2013. Oxytocin receptor ligands induce changes in cytoskeleton in neuroblastoma cells. *J. Mol. Neurosci.* 50, 462-468.

- Bale, T.L., Davis, A.M., Auger, A.P., Dorsa, D.M., McCarthy, M.M., 2001. CNS region-specific oxytocin receptor expression: importance in regulation of anxiety and sex behavior. *J. Neurosci.* 21, 2546-2552.
- Barraza, J.A., Zak, P.J., 2009. Empathy toward strangers triggers oxytocin release and subsequent generosity. *Ann. N.Y. Acad. Sci.* 1167(1), 182-189.
- Bartz, J., Simeon, D., Hamilton, H., Kim, S., Crystal, S., Braun, A., et al., 2011b. Oxytocin can hinder trust and cooperation in borderline personality disorder. *Soc. Cogn. Affect. Neurosci.* 6, 556-563.
- Bartz, J.A., Zaki, J., Bolger, N., Ochsner, K.N., 2011a. Social effects of oxytocin in humans: context and person matter. *Trends Cogn. Sci.* 15, 301-309.
- Bartz, J.A., Zaki, J., Ochsner, K.N., Bolger, N., Kolevzon, A., Ludwig, N., et al., 2010. Effects of oxytocin on recollections of maternal care and closeness. *Proc. Natl. Acad. Sci. U.S.A.* 107(50), 21371-21375.
- Baskerville, T.A., Douglas, A.J., 2010. Dopamine and oxytocin interactions underlying potential contributions to behavioral disorders. *CNS Neurosci. Ther.* 16, e92-e123.
- Baskerville, T.A., Douglas, A.J., 2008. Interactions between dopamine and oxytocin in the control of sexual behaviour. *Prog. Brain Res.* 170, 277-290.
- Bealer, S.L., Crowley, W.R., 2000. Neurotransmitter interaction in release of intranuclear oxytocin in magnocellular nuclei of the hypothalamus. *Ann. N.Y. Acad. Sci.* 897, 182-191.

- Bealer, S.L., Crowley, W.R., 1998. Noradrenergic control of central oxytocin release during lactation in rats. *Am. J. Physiol.* 274, E453-458.
- Beck, A.T., Ward, C.H., Mendelson, M., Mock, J., Erbaugh, J., 1961. An inventory for measuring depression. *Arch. Gen. Psychiatry* 4, 561-571.
- Belsky, J., Jonassaint, C., Pluess, M., Stanton, M., Brummett, B., Williams, R., 2009. Vulnerability genes or plasticity genes? *Mol. Psychiatry* 14, 746-754.
- Belsky, J., Bakermans-Kranenburg, M.J., van IJzendoorn, M.H., 2007. For better and for worse differential susceptibility to environmental influences. *Curr. Dir. Psychol. Sci.* 16(6), 300-304.
- Belsky, J., Pluess, M., 2009. Beyond diathesis stress: Differential susceptibility to environmental influences. *Psychol. Bull.* 135, 885-908.
- Bernatova, I., Rigatto, K.V., Key, M.P., Morris, M., 2004. Stress-induced pressor and corticosterone responses in oxytocin-deficient mice. *Exp. Physiol.* 85, 549-557.
- Blier, P., Ward, H.E., Tremblay, P., Laberge, L., Hébert, C., Bergeron, R., 2010. Combination of antidepressant medications from treatment initiation for major depressive disorder: A double-blind randomized study. *Am. J. Psychiatry* 167, 281-288.
- Bradley, B., Davis, T.A., Wingo, A.P., Mercer, K.B., Ressler, K.J., 2013. Family environment and adult resilience: contributions of positive parenting and the oxytocin receptor gene. *Eur. J. Psychotraumatol.* 4, 21659.
- Bradley, B., Westen, D., Mercer, K.B., Binder, E.B., Jovanovic, T., Crain, D., et al.,

2011. Association between childhood maltreatment and adult emotional dysregulation in a low-income, urban, African American sample: moderation by oxytocin receptor gene. *Dev. Psychopathol.* 23, 439-452.
- Brodsky, B. S., Stanley, B., 2008. Adverse childhood experiences and suicidal behavior. *Psychiatr. Clin. North Am.* 31(2), 223-235.
- Bruhn, T.O., Sutton, S.W., Plotsky, P.M., Vale, W.W., 1986. Central administration of corticotropin-releasing factor modulates oxytocin secretion in the rat. *Endocrinology.* 119, 1558-1563.
- Brüne, M., 2012. Does the oxytocin receptor (OXTR) polymorphism (rs2254298) confer ‘vulnerability’ for psychopathology or ‘differential susceptibility’? Insights from evolution. *BMC Med.* 10, 38.
- Brunton, P.J., Russell, J.A., 2008. Keeping oxytocin neurons under control during stress in pregnancy. *Prog. Brain Res.* 170, 365-377.
- Brunton, P.J., Sabatier, N., Leng, G., Russell, J.A., 2006. Suppressed oxytocin neuron responses to immune challenge in late pregnant rats: a role for endogenous opioids. *Eur. J. Neurosci.* 23, 1241–1247.
- Bülbül, M., Babygirija, R., Cerjak, D., Yoshimoto, S., Ludwig, K., Takahashi, T., 2011. Hypothalamic oxytocin attenuates CRF expression via GABA<sub>(A)</sub> receptors in rats. *Brain Res.* 1387, 39-45.
- Buller, K.M., Xu, Y., Day, T.A., 1998. Indomethacin attenuates oxytocin and hypothalamic-pituitary-adrenal axis responses to systemic interleukin-1 $\beta$ . *J. Neuroendocrinol.* 10, 519-528.

- Burkett, J.P., Young, L.J., 2012. The behavioral, anatomical and pharmacological parallels between social attachment, love and addiction. *Psychopharmacology* 224, 1-26.
- Caldwell, W., McInnis, O.A., McQuaid, R.J., Liu, G., Stead, J.D., Anisman, A., et al., 2013. The role of the val66met polymorphism of the brain derived neurotrophic factor gene in coping strategies relevant to depressive symptoms. *PLOS One* 8, e65547.
- Capuron, L., Miller, A.H., 2004. Cytokines and psychopathology: lessons from interferon alpha. *Biol. Psychiatry* 56, 819-824.
- Cardoso, C., Ellenbogen, M.A., Linnen, A.M., 2014. The effect of intranasal oxytocin on perceiving and understanding emotion on the Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT). *Emotion* 14, 43-50.
- Cardoso, C., Linnen, A.M., Jooper, R., Ellenbogen, M.A., 2012. Coping style moderates the effect of intranasal oxytocin on the mood response to interpersonal stress. *Exp. Clin. Psychopharmacol.* 20, 84-91.
- Cardoso, C., Ellenbogen, M.A., Orlando, M.A., Bacon, S.L., Jooper, R., 2013b. Intranasal oxytocin attenuates the cortisol response to physical stress: A dose-response study. *Psychoneuroendocrinology* 38, 399-407.
- Cardoso, C., Ellenbogen, M.A., Serravalle, L., Linnen, A.M., 2013a. Stress-induced negative mood moderates the relation between oxytocin administration and trust: Evidence for the tend-and-befriend response to stress? *Psychoneuroendocrinology* 38, 2800-2804.

- Carter, C.S., 1998. Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology* 23, 779-818.
- Carter, C.S., 2007. Sex differences in oxytocin and vasopressin: implications for autism spectrum disorders? *Behav. Brain Res.* 176, 170-186.
- Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., et al., 2003. Influence of life-stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 301, 386-389.
- Catena-Dell'Osso, M., Fagiolini, A., Marazziti, D., Baroni, S., Bellantuono, C., 2013. Non-monoaminergic targets for the development of antidepressants: focus on neuropeptides. *Mini Rev. Med. Chem.* 13, 2-10.
- Chen, B., Dowlatshahi, D., MacQueen, G.M., Wang, J.F., Young, L.T., 2001. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol. Psychiatry* 50, 260-265.
- Chen, F.S., Kumsta, R., von Dawans, B., Monakhov, M., Ebstein, R.P., Heinrichs, M., 2011. Common oxytocin receptor gene (OXTR) polymorphism and social support interact to reduce stress in humans. *Proc. Natl. Acad. Sci. U.S.A.* 108, 19937-19942.
- Chiodera, P., Coiro, V., 1987. Oxytocin reduces metyrapone-induced ACTH secretion in human subjects. *Brain Res.* 420, 178-181.
- Choleris, E., Devidze, N., Kavaliers, M., Pfaff, D.W., 2008. Steroidal/neuropeptide interactions in hypothalamus and amygdala related to social anxiety. *Prog. Brain Res.* 170, 291-303.

- Christensen, J.C., Shiyanov, P.A., Estep, J.R., Schlager, J.J., 2014. Lack of association between human plasma oxytocin and interpersonal trust in a prisoner's dilemma paradigm. *PLOS One* 9(12), e116172.
- Clodi, M., Vila, G., Geyeregger, R., Riedl, M., Stulnig, T.M., Struck, J., et al., 2008. Oxytocin alleviates the neuroendocrine and cytokine response to bacterial endotoxin in healthy men. *Am. J. Physiol. Endocrinol. Metab.* 295, E686–E691.
- Cohen, H., Kaplan, Z., Kozlovsky, N., Gidron, Y., Matar, M.A., Zohar, J., 2010. Hippocampal microfusion of oxytocin attenuates the behavioural response to stress by means of dynamic interplay with the glucocorticoid-catecholamine responses. *J. Neuroendocrinol.* 22, 889-904.
- Cohen, S., Wills, T.A., 1985. Stress, social support, and the buffering hypothesis. *Psychol. Bull.*, 98(2), 310.
- Coiro, V., Passeri, M., Davoli, C., Bacchi-Modena, A., Bianconi, L., Volpi, R., et al., 1988. Oxytocin reduces exercise-induced ACTH and cortisol rise in man. *Acta. Endocrinol. (Copenh)*, 119, 405-412.
- Compas, B.E., Wagner, B.M., Slavlin, L.A., Vannatta, K., 1986. A prospective study of life events, social support, and psychological symptomatology during the transition from high school to college. *Am. J. Community Psychol.* 14, 241–257.
- Compton, M.T., Thompson, N.J., Kaslow, N.J., 2005. Social environment factors associated with suicide attempt among low-income African Americans: The protective role of family relationships and social support. *Soc. Psychiatry Psychiatr. Epidemiol.* 40(3), 175-185.

- Cools, R., Roberts, A.C., Robbins, T.W., 2008. Serotonergic regulation of emotional and behavioural control processes. *Trends Cogn. Sci.* 12, 31-40.
- Cornelis, M.C., Glymour, M.M., Chang, S-C., Tchetgen, E.J.T., Liang, L., Koenen, K.C., et al., 2012. Oxytocin receptor (OXTR) is not associated with optimism in the Nurses' Health Study. *Mol. Psychiatry* 17, 1157-1159.
- Corwin, E.J., Johnston, N., Pugh, L., 2008. Symptoms of postpartum depression associated with elevated levels of interleukin-1 beta during the first month postpartum. *Biol. Res. Nurs.* 10, 128-133.
- Costa, B., Pini, S., Gabelloni, P., Abelli, M., Lari, L., Cardini, A., et al., 2009. Oxytocin receptor polymorphisms and adult attachment style in patients with depression. *Psychoneuroendocrinology* 34, 1506-1514.
- Crowley W.R., Armstrong W.E.. 1992. Neurochemical regulation of oxytocin secretion in lactation. *Endocr. Rev.* 13, 33–65.
- Cruwys, T., Dingle, G.A., Haslam, C., Haslam, A.S., Jetten, J., Morton, T.A., 2013. Social group memberships protect against future depression, alleviate depression symptoms and prevent depression relapse. *Soc. Sci. Med.* 98, 179-186.
- Cunningham, E.T., Sawchenko, P.E., 1988. Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. *J. Comp. Neurol.* 274, 60-76.
- Cyranowski, J.M., Hofkens, T.L., Frank, E., Seltman, H., Cai, H.M., Amico, J.A., 2008. Evidence of dysregulated peripheral oxytocin release among depressed women. *Psychosom. Med.* 70, 967–975.

- Danevova, V., Kvetnansky, R., Jezova, D., 2013. Kinetics of oxytocin response to repeated restraint stress and/or chronic cold exposure. *Horm. Metab. Res.* 45, 845-848.
- Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W., Kelley, K.W., 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 9, 46-56.
- Dantzer, R., O'Conner, J.C., Lawson, M.A., Kelley, K.W., 2011. Inflammation-associated depression: from serotonin to kynurenine. *Psychoneuroendocrinology* 36, 426-436.
- Davis, M.H., 1983. Measuring individual differences in empathy: Evidence for a multidimensional approach. *J. Pers. Soc. Psychol.* 44, 113-126.
- De Dreu, C.K., Greer, L.L., Handgraaf, M.J., Shalvi, S., Van Kleef, G.A, Baas, M., et al., 2010. The Neuropeptide oxytocin regulates parochial altruism in intergroup conflict among humans. *Science* 328, 1408-1411.
- De Dreu, C.K., Greer, L.L., Van Kleef, G.A., Shalvi, S., Handgraaf, M.J., 2011. Oxytocin promotes human ethnocentrism. *Proc. Natl. Acad. Sci. U.S.A.* 108, 1262-1266.
- de Jong, T.R., Veening, J.G., Olivier, B. Waldinger, M.D., 2007. Oxytocin involvement in SSRI-induced delayed ejaculation: a review of animal studies. *J. Sex. Med.* 4, 14-28.
- Delville, Y., Mansour, K.M., Ferris, C.F., 1996. Testosterone facilitates aggression by modulating vasopressin receptors in the hypothalamus. *Physiol. Behav.* 60(1), 25-29.

- Demare, D., 1996 The childhood maltreatment questionnaire: examining long-term correlates of childhood maltreatment. Paper presented at APA, Toronto, Canada.
- de Oliveira, D.C., Zuardi, A.W., Graeff, F.G., Queiroz, R.H., Crippa, J.A., 2011. Anxiolytic-like effect of oxytocin in the simulated public speaking test. *J. Psychopharmacol.* 26, 497-504.
- Depue, R.A., Morrone-Strupinsky, J.V., 2005. A neurobehavioral model of affiliative bonding: implications for conceptualizing a human trait of affiliation. *Behav. Brain Sci.* 28, 313-350.
- Deschamps, S., Woodside, B., Walker, C.D., 2003. Pups presence eliminates the stress hyporesponsiveness of early lactating females to a psychological stress representing a threat to the pups. *J. Neuroendocrinol.* 15, 486-497.
- Detillion, C.E., Craft, T.K., Glasper, E.R., Prendergast, B.J., DeVries, A.C., 2004. Social facilitation of wound healing. *Psychoneuroendocrinology* 29, 1004-1011.
- Devanand, D.P., Lisanby, S., Lo, E.S., Fitzsimons, L., Cooper, T.B., Halbreich, U., et al., 1998. Effects of electroconvulsive therapy on plasma vasopressin and oxytocin. *Biol. Psychiatry* 44, 610-616.
- DeWall, C.N., Gillath, O., Pressman, S.D., Black, L.L., Bartz, J.A., Moskowitz, J., et al., 2014. When the love hormone leads to violence: oxytocin increases intimate partner violence inclinations among high trait aggressive people. *Soc. Psychol. Personal. Sci.* 1948550613516876.
- Dickerson, S.S., Gruenewald, T.L., Kemeny, M.E., 2004. When the social self is threatened: Shame, physiology, and health. *J. Pers.* 72(6), 1191-1216.

- Ditzen, B., Nater, U.M., Schaer, M., La Marca, R., Bodenmann, G., Ehlert, U., et al., 2013. Sex-specific effects of intranasal oxytocin on autonomic nervous system and emotional responses to couple conflict. *Soc. Cogn. Affect. Neurosci.* 8, 897-902.
- Ditzen, B., Neumann, I.D., Bodenmann, G., von Dawans, B., Turner, R.A., Ehlert, U., et al., 2007. Effects of different kinds of couple interaction on cortisol and heart rate responses to stress in women. *Psychoneuroendocrinology* 32, 565-574.
- Ditzen, B., Schaer, M., Gabriel, B., Bodenmann, G., Ehlert, U., Heinrichs, M. 2009. Intranasal oxytocin increases positive communication and reduces cortisol levels during couple conflict. *Biol. Psychiatry* 65, 728-731.
- Dluzen, D.E., Muraoka, S., Landgraf, R., 1998. Olfactory bulb norepinephrine depletion abolishes vasopressin and oxytocin preservation of social recognition responses. *Neurosci. Lett.* 254, 161-164.
- Domes, G., Heinrichs, M., Glascher, J., Buchel, C., Braus, D.F., Herpertz, S.C., 2007. Oxytocin attenuates amygdala response to emotional faces regardless of valence. *Biol. Psychiatry* 62, 1187-1190.
- Domes, G., Heinrichs, M., Michel, A., Berger, C., Herpertz, S.C., 2007. Oxytocin improves “mind-reading” in humans. *Biol. Psychiatry* 61(6), 731-733.
- Donaldson, Z.R., Young, L.J., 2008. Oxytocin, vasopressin, and the neurogenetics of sociality. *Science* 322, 900-904.
- Doyle, C., 2001. Surviving and coping with emotional abuse in childhood. *Clin. Child Psychol. Psychiatry* 6, 387-402.

- Dube, S.R., Anda, R.F., Felitti, V.J., Chapman, D.P., Williamson, D.F., Giles, W.H., 2001. Childhood abuse, household dysfunction, and the risk of attempted suicide throughout the life span: findings from the Adverse Childhood Experiences Study. *JAMA*. 286, 3089-3096.
- Dumais, K.M., Veenema, A.H., 2015. Vasopressin and oxytocin receptor systems in the brain: Sex differences and sex-specific regulation of social behavior. *Front. Neuroendocrinol.* [Epub ahead of print].
- Duman, R.S., Aghajanian, G.K., 2012. Synaptic dysfunction in depression: potential therapeutic targets. *Science* 338, 68-72.
- Duman, R.S., Heninger, G.R., Nestler, E.J., 1997. A molecular and cellular theory of depression. *Arch. Gen. Psychiatry* 54, 597-606.
- Drevets, W.C., Thase, M.E., Moses-Kolko, E.L., Price, J., Frank, E., Kupfer, D.J., et al., 2007. Serotonin-1A receptor imaging in recurrent depression: replication and literature review. *Nucl. Med. Biol.* 34, 865-877.
- D'Sa, C., Duman, R.S., 2002. Antidepressants and neuroplasticity. *Bipolar. Disord.* 4, 183-194.
- Eaton, J.L., Roache, L., Nguyen, K.N., Cushing, B.S., Troyer, E., Papademetriou, E., et al., 2012. Organizational effects of oxytocin on serotonin innervation. *Dev. Psychobiol.* 54, 92-97.
- Eisenberger, N.I., 2012. The pain of social disconnection: examining the shared neural underpinnings of physical and social pain. *Nat. Rev. Neurosci.* 13, 421-434.
- Eisenberger, N.I., Lieberman, M.D., Williams, K.D., 2003. Does rejection hurt? An

- FMRI study of social exclusion. *Science* 302, 290-292.
- Eisenberger, N.I., Jarcho, J.M., Lieberman, M.D., Naliboff, B.D., 2006. An experimental study of shared sensitivity to physical pain and social rejection. *Pain* 126, 132-138.
- Eisenberger, N.I., Taylor, S.E., Gable, S.L., Hilmert, C.J., Lieberman, M.D., 2007. Neural pathways link social support to attenuated neuroendocrine stress responses. *Neuroimage* 35(4), 1601-1612.
- Eisenberger, N.I., Way, B.M., Taylor, S.E., Welch, W.T., Lieberman, M.D., 2007. Understanding genetic risk for aggression: clues from the brain's response to social exclusion. *Biol. Psychiatry* 61, 1100-1108.
- Ellenbogen, M.A., Linnen, A-M., Cardoso, C., Joober, R., 2013. Intranasal oxytocin impedes the ability to ignore task-irrelevant facial expressions of sadness in students with depressive symptoms. *Psychoneuroendocrinology* 38, 387-398.
- Ellenbogen, M.A., Linnen, A-M., Grumet, R., Cardoso, C., Joober, R., 2012. The acute effects of intranasal oxytocin on automatic and effortful attention shifting to emotional faces. *Psychophysiology* 49, 128-137.
- Emiliano, A.B.F., Cruz, T., Pannoni, V., Fudge, J.L., 2007. The interface of oxytocin-labeled cells and serotonin transporter-containing fibers in the primate hypothalamus a substrate for SSRIs therapeutic effects? *Neuropsychopharmacology* 32, 977-988.
- Evans, S.J., Choudary, P.V., Neal, C.R., Li, J.Z., Vawter, M.P., Tomita, H., et al., 2004.

- Dysregulation of the fibroblast growth factor system in major depression. *Proc. Natl. Acad. Sci. U.S.A.* 101, 15506-15511.
- Feifel, D., Macdonald, K., Cobb, P., Minassian, A., 2012. Adjunctive intranasal oxytocin improves verbal memory in people with schizophrenia. *Schizophr. Res.* 139, 207–210.
- Feldman, R., Zagoory-Sharon, O., Weisman, O., Schneiderman, I., Gordon, I., Maoz, R., et al., 2012. Sensitive parenting is associated with plasma oxytocin and polymorphisms in the OXTR and CD38 Genes. *Biol. Psychiatry* 72, 175-181.
- Felitti, V.J., Anda, R.F., Nordenberg, D., Williamson, D.F., Spitz, A.M., Edwards, V., et al., 1998. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study. *Am. J. Prev. Med.*,14, 245-258.
- Flak, J.N., Jankord, R., Solomon, M.B., Krause, E.G., Herman, J.P., 2011. Opposing effects of chronic stress and weight restriction on cardiovascular, neuroendocrine and metabolic function. *Physiol. Behav.* 104, 228-234.
- Frasch, A., Zetsche, T., Steiger, A., Jirikowski, G.F., 1995. Reduction of plasma oxytocin levels in patients suffering from major depression. *Adv. Exp. Med. Biol.* 395, 257-258.
- Friedman, E.M., Hayney, M.S., Love, G.D., Urry, H.L., Rosenkranz, M.A., Davidson, R.J., et al., 2005. Social relationships, sleep quality, and interleukin-6 in aging women. *Proc. Natl. Acad. Sci. U.S.A.* 102(51), 18757-18762.
- Frasure-Smith, N., Lespérance, F., Irwin, M.R., Talajic, M., Pollock, B.G., 2009. The

- relationships among heart rate variability, inflammatory markers and depression in coronary heart disease patients. *Brain Behav. Immun.* 23, 1140-1147.
- Gatt, J.M, Nemeroff, C.B., Dobson-Stone, C., Paul, R.H., Bryant, R.A., Schofield, P.R., et al., 2009. Interactions between BDNF Val66Met polymorphism and early life stress predict brain and arousal pathways to syndromal depression and anxiety. *Mol. Psychiatry* 14, 681-695.
- Gaughran, F., Payne, J., Sedgwick, P.M., Cotter, D., Berry, M., 2006. Hippocampal FGF-2 and FGFR1 mRNA expression in major depression, schizophrenia and bipolar disorder. *Brain Res. Bull.* 70, 221-227.
- Gelernter, J., Kranzler, H., Cubells, J.F., 1997. Serotonin transporter protein (SLC6A4) allele and haplotype frequencies and linkage disequilibria in African- and European-American and Japanese populations and in alcohol-dependent subjects. *Hum. Genet.* 101(2), 243-246.
- Gimpl, G., Fahrenholz, F., 2001. The oxytocin receptor system: structure, function and regulation. *Physiol. Rev.* 81, 629-683.
- Gingrich, B., Liu, Y., Cascio, C., Wang, Z., Insel, T.R., 2000. Dopamine D2 receptors in the nucleus accumbens are important for social attachment in female prairie voles (*Microtus ochrogaster*). *Behav. Neurosci.* 114, 173-183.
- Gordon, I., Zagoory-Sharon, O., Schneiderman, I., Leckman, J.F., Weller, A., Feldman, R., 2008. Oxytocin and cortisol in romantically unattached young adults: Associations with bonding and psychological distress. *Psychophysiology* 45, 349–352.

- Gouin, J.P., Carter, S.C., Pournajafi-Nazarloo, H., Glaser, R., Marlarkey, W.B., Loving, T.J., et al., 2010. Marital behavior, oxytocin, vasopressin, and wound healing. *Psychoneuroendocrinology* 35, 1082-1090.
- Greenglass, E.R., Julkunen, J., 1989. Construct validity and sex differences in Cook-Medley hostility. *Pers. Individ. Dif.* 10, 209-218.
- Greenglass, E.R., Julkunen, J., 1991. Cook-Medley hostility, anger, and the Type A behaviour pattern in Finland. *Psychol. Rep.* 68, 1059- 1066.
- Grippe, A.J., Trahanas, D.M., Zimmerman, R.R., Porges, S.W., Carter, C.S., 2009. Oxytocin protects against negative behavioral and autonomic consequences of long-term social isolation. *Psychoneuroendocrinology* 34, 1542-1553.
- Gruenewald, T.L., Kemeny, M.E., Aziz, N., Fahey, J.L., 2004. Acute threat to the social self: Shame, social self-esteem, and cortisol activity. *Psychosom. Med.* 66(6), 915-924.
- Guastella, A.J., Einfeld, S.L., Gray, K.M., Rinehart, N.J., Tonge, B.J., Lambert, T.J., et al., 2010. Intranasal oxytocin improves emotion recognition for youth with autism spectrum disorders. *Biol. Psychiatry* 67, 692–694
- Guastella, A.J., Mitchell, P.B., Mathews, F., 2008. Oxytocin enhances the encoding of positive social memories in humans. *Biol. Psychiatry* 64, 256-258.
- Gutkowska, J., Jankowski, M., 2008. Oxytocin revisited: It is also a cardiovascular hormone. *J. Am. Soc. Hypertens.* 2, 318-325.
- Gutkowska, J., Jankowski, M., Lambert, C., Mukaddam-Daher, S., Zingg, H.H., McCann, S.M., 1997. Oxytocin releases atrial natriuretic peptide by combining

- with oxytocin receptors in the heart. *Proc. Natl. Acad. Sci. U.S.A.* 94, 11704–11709.
- Guzmán, Y.F., Tronson, N.C., Jovasevic, V., Sato, K., Guedea, A.L., Mizukami, H., et al., 2013. Fear-enhancing effects of septal oxytocin receptors, *Nat. Neurosci.* 16, 1185-1187.
- Haber, S.N., Knutson, B., 2010. The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology* 35, 4-26.
- Han, B., Compton, W.M., Gfroerer, J., McKeon, R., 2015. Prevalence and correlates of past 12-month suicide attempt among adults with past-year suicidal ideation in the United States. *J. Clin. Psychiatry* 76(3), 295-302.
- Harrison, N.A., Brydon, L., Walker, C., Gray, M.A., Steptoe, A., Critchley, H.D., 2009. Inflammation causes mood changes through alterations in subgenual cingulated activity and mesolimbic connectivity. *Biol. Psychiatry* 66, 407-414.
- Hashiguchi, H., Ye, S.H., Morris, M., Alexander, N., 1997 Single and repeated environmental stress: effect on plasma oxytocin, corticosterone, catecholamines and behavior. *Physiol. Behav.* 61, 731-736.
- Heim, C., Newport, J.D., Mletzko, T., Miller, A.H., Nemeroff, C.B., 2008a. The link between childhood trauma and depression: insights from HPA axis studies in humans. *Psychoneuroendocrinology* 33, 693-710.
- Heim, C., Young, L.J., Newport, D.J., Mletzko, T., Miller, A.H. Nemeroff, C.B., 2008b. Lower CSF oxytocin concentrations in women with a history of childhood abuse. *Mol. Psychiatry* 14, 954-958.

- Heinrichs, M., Baumgartner, T., Kirschbaum, C., Ehlert, U., 2003. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biol. Psychiatry* 54, 1389-1398.
- Heinrichs, M. Domes, G., 2008. Neuropeptides and social behavior: effects of oxytocin and vasopressin in humans. *Prog. Brain Res.* 170, 337-350.
- Herbison, A.E., Voisin, D.L., Douglas, A.J., Chapman, C., 1997. Profile of monoamine and excitatory amino acid release in rat supraoptic nucleus over parturition. *Endocrinology* 138, 33-40.
- Ho, S.S., Chow, B.K., Yung, W.H., 2007. Serotonin increases the excitability of the hypothalamic paraventricular nucleus magnocellular neurons. *Eur J. Neurosci.* 25, 2991-3000.
- Hodges, S.D., Kline, K., 2001. Regulating the costs of empathy: the price of being human. *J. Socio-Econ.* 30, 437-452.
- Hoge, E.A., Anderson, E., Lawson, E.A., Bui, E., Fischer, L.E., Khadge, S.D., et al., 2014. Gender moderates the effect of oxytocin on social judgments. *Hum. Psychopharm. Clin.* 29(3), 299-304.
- Hollander, E., Bartz, J., Chaplin, W., Phillips, A., Sumner, J., Soorya, L., et al., 2007. Oxytocin increases retention of social cognition in autism. *Biol Psychiatry* 61, 498–503.
- Hollander, E., Novotny, S., Hanratty, M., Yaffe, R., DeCaria, C.M., Aronowitz, B.R., et al., 2003. Oxytocin infusion reduces repetitive behaviours in adults with autistic and Asperger's disorders. *Neuropsychopharmacology* 28, 193–198.

- Holt-Lunstad, J., Birmingham, W., Jones, B. Q., 2008. Is there something unique about marriage? The relative impact of marital status, relationship quality, and network social support on ambulatory blood pressure and mental health. *Ann. Behav. Med.* 35(2), 239-244.
- Holt-Lunstad, J., Birmingham, W., Light, K.C., 2011. The influence of depressive symptomatology and perceived stress on plasma and salivary oxytocin before, during and after a support enhancement intervention. *Psychoneuroendocrinology* 36, 1249-1256.
- Holt-Lunstad, J., Uchino, B.N., Smith, T.W., Hicks, A., 2007. On the importance of relationship quality: The impact of ambivalence in friendships on cardiovascular functioning. *Ann. Behav. Med.* 33(3), 278-290.
- Huber, D., Veinante, P., Stoop, R., 2005. Vasopressin and oxytocin excite distinct neuronal populations in the central amygdala. *Science* 308, 245–248.
- Inoue, T., Kimura, T., Azuma, C., Inazawa, J., Takemura, M., Kikuchi, T., et al. 1994. Structural organization of the human oxytocin receptor gene. *J. Biol. Chem.* 269, 32451-32456.
- Insel, T.R., Hulihan, T.J., 1995. A gender-specific mechanism for pair bonding: oxytocin and partner preference formation in monogamous voles. *Behav. Neurosci.* 109, 782-789.
- Insel, T. R., 2003. Is social attachment an addictive disorder? *Physiol. Behav.*, 79, 351-357.
- Insel, T.R., Shapiro, L.E., 1992. Oxytocin receptor distribution reflects social

- organization in monogamous and polygamous voles. *Proc. Natl. Acad. Sci. U.S.A.* 89, 5981-5985.
- Insel, T.R., Winslow, J.T., 1991 Central oxytocin administration modulates rat pup ultrasonic isolation call. *Eur. J. Pharmacol.* 203, 149-152.
- Insel, T.R., Young, L.S., 2001. The neurobiology of attachment. *Nat. Rev. Neurosci.* 2, 129-136.
- İşeri, S.O., Şener, G., Sağlam, B., Gedik, N., Ercan, F., Yeğen, B.C., 2005. Oxytocin protects against sepsis-induced multiple organ damage: role of neutrophils. *J. Surg. Res.* 126, 73–81.
- Jankowski, M., Bissonauth, V., Gao, L., Gangal, M., Wang, D., Danalache, B., et al., 2010. Anti-inflammatory effect of oxytocin in rat myocardial infarction. *Basic Res. Cardiol.* 105, 205-218.
- Jia, R., Tai, F.D., An, S.C., Broders, H., Ding, X.L., Kong, Q., et al., 2008. Effects of neonatal oxytocin treatment on aggression and neural activities in mandarin voles. *Physiol. Behav.* 95, 56–62.
- Jin, D., Liu, H.X., Hirai, H., Torashima, T., Nagai, T., Lopatina, O., et al., (2007. CD38 is critical for social behaviour by regulating oxytocin secretion. *Nature* 446, 41-45.
- Jokinen, J., Chatzittofis, A., Hellström, C., Nordström, P., Uvnäs-Moberg, K., Åsberg, M., 2012. Low CSF oxytocin reflects high intent in suicide attempters. *Psychoneuroendocrinology* 37(4), 482-490.
- Jørgensen, H., Kjaer, A., Knigge, U., Møller, M., Warberg, J., 2003. Serotonin

- stimulates hypothalamic mRNA expression and local release of neurohypophysial peptides. *J. Neuroendocrinol.*, 15, 564-71.
- Kajantie, E., 2008. Physiological stress response, estrogen, and the male–female mortality gap. *Curr. Dir. Psychol. Science*, 17(5), 348-352.
- Kajantie, E., Phillips, D.I., 2006. The effects of sex and hormonal status on the physiological response to acute psychosocial stress. *Psychoneuroendocrinology* 31(2), 151-178.
- Kamm, K., Vanderkolk, W., Lawrence, C., Jonker, M., Davis, A.T., 2006. The effect of traumatic brain injury upon the concentration and expression of interleukin-1beta and interleukin-10 in the rat. *J. Trauma*. 60, 152-157.
- Kasting N.W., 1986. Indomethacin, an antipyretic drug, prevents the endotoxin-induced and potentiates the hemorrhage-induced oxytocin release into the plasma of the male rat. *Neuroendocrinology* 42, 285–288.
- Kawamura, Y., Liu, X., Akiyama, T., Shimada, T., Otowa, T., Sakai, Y., et al., 2010. The association between oxytocin receptor gene (OXTR) polymorphisms and affective temperaments, as measured by TEMPS-A. *J. Affect. Disord.* 127, 31-37.
- Keating, C., Dawood, T., Barton, D.A., Lambert, G.W., Tilbrook, A.J., 2013. Effects of selective serotonin reuptake inhibitor treatment on plasma oxytocin and cortisol in major depressive disorder. *BMC Psychiatry* 13,124.
- Keck, M.E., Welt, T., Müller, M.B., Landgraf, R., Holsboer, F., 2003. The high-affinity

- non-peptide CRH1 receptor antagonist R121919 attenuates stress-induced alterations in plasma oxytocin, prolactin, and testosterone secretion in rats. *Pharmacopsychiatry* 36, 27-31.
- Kempermann, G., Kronenberg, G., 2003. Depressed new neurons--adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. *Biol. Psychiatry* 54, 499-503.
- Kéri, S., Kiss, I., 2011. Oxytocin response in a trust game and habituation of arousal. *Physiol. Behav.* 102(2), 221-224.
- Kessler, R.C., McGonagle, K.A., Swartz, M., Blazer, D.G., Nelson, C.B., 1993. Sex and depression in the National Comorbidity Survey. I: lifetime prevalence, chronicity and recurrence. *J. Affect. Disord.* 29, 85–96.
- Kidder, K., Stein, J., Fraser, J., 2000. The Mental Health of Children & Youth. In (Eds) *The Health of Canada's Children: A CICH Profile. Third Edition* (pp. 199-226). Ottawa: Canadian Institute of Child Health.
- Kim, H.S., Sherman, D.K., Mojaverian, T., Sasaki, J.Y., Park, J., Suh, E.M., et al., 2011. Gene-culture interaction: oxytocin receptor polymorphism (OXTR) and emotion regulation. *Soc. Psychol. Personal. Sci.* 2, 665-672.
- Kim, H.S., Sherman, D.K., Sasaki, J.Y., Xu, J., Chu, T.Q., Ryu, C., et al., 2010. Culture, Distress and oxytocin receptor polymorphism (OXTR) interact to influence emotional support seeking. *Proc. Natl. Acad. Sci. U.S.A.* 107, 15717-15721.
- Kim, J.M., Kang, H.J., Kim, S.Y., Kim, S.W., Shin, I.S., Kim, H.R., et al., 2015.

BDNF Promoter Methylation Associated with Suicidal Ideation in Patients with Breast Cancer. *Int. J. Psychiatry Med.* 49(1), 75-94.

Kim, J.M., Kim, S.W., Kang, H.J., Bae, K.Y., Shin, I.S., Kim, J.T., et al., 2014.

Serotonergic genes and suicidal ideation 2 weeks and 1 year after stroke in Korea. *Am. J. Geriatr. Psychiatry* 22(10), 980-988.

Kimura, T. Investigation of the oxytocin receptor at the molecular level. In R. Ivell and J.A. Russell, Eds., *Oxytocin: Cellular and Molecular Approaches in Medicine and Research*. New York: Plenum Press, 1995, Pp. 259.

Kirsch, P., Esslinger, C., Chen, Q., Mier, D., Lis, S., Siddhanti, S., et al., 2005.

Oxytocin modulates neural circuitry for social cognition and fear in humans. *J. Neurosci.* 7, 11489-11493.

Kirschbaum, C., Kudielka, B.M., Gaab, J., Schommer, N.C., Hellhammer, D.H.

(1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosom. Med.* 61(2), 154-162.

Kirschbaum, C., Pirke, K.M., Hellhammer, D.H., 1993. The 'Trier Social Stress Test' – a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 28, 76-81.

Kirschbaum, C., Pirke, K.M., Hellhammer, D.H., 1995. Preliminary evidence for reduced cortisol responsivity to psychological stress in women using oral contraceptive medication. *Psychoneuroendocrinology* 20(5), 509-514.

Kirschbaum, C., Platte, P., Pirke, K.M., Hellhammer, D., 1996. Adrenocortical

activation following stressful exercise: further evidence for attenuated free cortisol responses in women using oral contraceptives. *Stress Med.* 12(3), 137-143.

Kirschbaum, C., Wüst, S., Hellhammer, D., 1992. Consistent sex differences in cortisol responses to psychological stress. *Psychosom. Med.* 54(6), 648-657.

Kiss, I., Levy-Gigi, E., Kéri, S., 2011. CD 38 expression, attachment style and habituation of arousal in relation to trust-related oxytocin release. *Biol. Psychology* 88(2), 223-226.

Kosfeld, M., Heinrichs, M., Zak, P.J., Fischbacher, U., Fehr, E., 2005. Oxytocin increases trust in humans. *Nature* 435, 673-676.

Kovács, G.L., Sarnyai, Z., Szabó, G., 1998. Oxytocin and addiction: a review. *Psychoneuroendocrinology* 23, 945-962.

Kraemer, G.W., 1992. A psychobiological theory of attachment. *Behav. Brain Sci.* 15, 493-511.

Krueger, F., Parasuraman, R., Iyengar, V., Thornburg, M., Weel, J., Lin, M., et al., 2012. Oxytocin receptor genetic variation promotes human trust behavior. *Front. Hum. Neurosci.* 6, 4.

Krystal, J.H., Sanacora, G., Blumberg, H., Anand, A., Charney, D.S., Marek, G., et al., 2002. Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments. *Mol. Psychiatry* 1, S71-S80.

Kubany, E.S., Haynes, S.N., Leisen, M.B., Owens, J.A., Kaplan, A.S., Watson, S.B.,

- et al., 2000. Development and preliminary validation of a brief broad-spectrum measure of trauma exposure: The Traumatic Life Events Questionnaire. *Psychol. Assess.* 12, 210-224
- Kudielka, B.M., Schommer, N.C., Hellhammer, D.H., Kirschbaum, C., 2004. Acute HPA axis responses, heart rate, and mood changes to psychosocial stress (TSST) in humans at different times of day. *Psychoneuroendocrinology* 29(8), 983-992.
- Labuschagne, I., Phan, K.L., Wood, A., Angstadt, M., Chua, P., Heinrichs, M., et al., 2010. Oxytocin attenuates amygdala reactivity to fear in generalized social anxiety disorder. *Neuropsychopharmacology* 35, 2403-2413.
- Landgraf, R., Neumann, I. D., 2004. Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication. *Front. Neuroendocrinol.* 25(3), 150-176.
- Landgraf, R., Neumann, I., Holsboer, F., Pittman, Q.J., 1995. Interleukin-1 $\beta$  stimulates both central and peripheral release of vasopressin and oxytocin in the rat. *Eur. J. Neurosci.* 7, 592-598.
- Lane, A., Luminet, O., Rimé, B., Gross, J.J., de Timary, P., Mikolajczak, M., 2013. Oxytocin increases willingness to socially share one's emotions. *Int. J. Psychol.*, 48, 676-681.
- Lazarus, R.S., 1996. The role of coping in the emotions and how coping changes over the life course. *Handbook of emotion, adult development, and aging*, 289-306.
- Ledeboer, A., Binnekade, R., Brevé, J.J., Bol, J.G., Tilders, F.J., Van Dam A.M.,

2002. Site-specific modulation of LPS-induced fever and interleukin-1 beta expression in rats by interleukin-10. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 282, R1762-1772.
- Lee, R., Ferris, C., Van de Kar, L. D., Coccaro, E. F., 2009. Cerebrospinal fluid oxytocin, life history of aggression, and personality disorder. *Psychoneuroendocrinology* 34(10), 1567-1573.
- Lee, R., Garcia, F., van de Kar, L.D., Hauger, R.D., Coccaro, E.F., 2003. Plasma oxytocin response to pharmacological challenge to D-fenfluramine and placebo in healthy men. *Psychiatry Res.* 118, 129-136.
- Legros, J.J., Chiodera, P., Geenen, V., Smits, S., von Frenckell, R., 1984. Dose-response relationship between plasma oxytocin and cortisol and adrenocorticotropic concentrations during oxytocin infusion in normal men. *J. Clin. Endocrinol. Metab.* 58, 105-109.
- Legros, J.J., Chiodera, P., Geenen, V., von Frenckell, R., 1987. Confirmation of the inhibitory influence of exogenous oxytocin on cortisol and ACTH in man: evidence of reproducibility. *Acta Endocrinol (Copenh).* 114, 345-349.
- Lerer, E., Levi, S., Israel, S., Yaari, M., Nemanov, L., Mankuta, D., et al., 2010. Low CD38 expression in lymphoblastoid cells and haplotypes are both associated with autism in a family-based study. *Autism Res.* 3(6), 293-302.
- Lesch, K.P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., et al., 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, 274, 1527-1531.

- Leuner, B., Caponiti, J.M., Gould, E., 2012. Oxytocin stimulates adult neurogenesis even under conditions of stress and elevated glucocorticoids. *Hippocampus* 22, 861-868.
- Lévy, F., Guevara-Guzman, R., Hinton, M.R., Kendrick, K.M., Keverne, E.B., 1993. Effects of parturition and maternal experience on noradrenaline and acetylcholine release in the olfactory bulb of sheep. *Behav. Neurosci.* 107, 662-668.
- Lewis, D.A., Sherman, B.M., 1985. Oxytocin does not influence adrenocorticotropin secretion in man. *J. Endocrinol. Metab.* 60, 53-56.
- Li, Q., Levy, A.D., Cabrera, T.M., Brownfield, M.S., Battaglia, G., Van de Kar, L.D., 1993. Long-term fluoxetine, but not desipramine, inhibits the ACTH oxytocin responses to the 5-HT<sub>1A</sub> agonist, 8-OH-DPAT, in male rats. *Brain Res.* 630, 148-156.
- Lin, P.I., Vance, J.M., Pericak-Vance, M.A., Martin, E.R., 2007. No gene is an island: the flip-flop phenomenon. *Am. J. Hum. Genet.* 80, 531-538.
- Linnen, A.-M., Ellenbogen, M.A., Cardoso, C., Jooper, R., 2012. Intranasal oxytocin and salivary cortisol concentrations during social rejection in university students. *Stress* 15, 393-402.
- Litvin, Y., Murakami, G., Pfaff, D.W., 2011. Effects of chronic social defeat on behavioral and neural correlated on sociality: Vasopressin, oxytocin and the vasopressinergic V1b receptor. *Physiol. Behav.* 103, 393-403.
- Liu, Y., Wang, X., 2003. Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles. *Neuroscience* 121, 537-544.

- Love, T.M., Enoch, M-A., Hodgkinson, C.A., Pecina, M., Mickey, B., Koeppe, R.A.,  
2012. Oxytocin gene polymorphisms influence human dopaminergic function in a  
sex-dependent manner. *Biol. Psychiatry* 72, 198-206.
- Love, T.M., 2014. Oxytocin, Motivation and the Role of Dopamine. *Pharmacol.  
Biochem. Behav.* 119C, 49-60.
- Lucht, M.J., Barnow, S., Sonnenfeld, C., Rosenberger, A., Grabe, H.J., Schroeder, W., et  
al., 2009. Associations between the oxytocin receptor gene (OXTR) and affect,  
loneliness and intelligence in normal subjects. *Prog. Neuropsychopharmacol.  
Biol. Psychiatry* 33, 860-866.
- Luo, Y., Wang, H., 2009. Correlation research on psychological health impact on  
nursing students against stress, coping way and social support. *Nurse Educ. Today*  
29(1), 5-8.
- Lynch, M., Cicchetti, D., 1998. An ecological-transactional analysis of children and  
contexts: The longitudinal interplay among child maltreatment, community  
violence and children's symptomatology. *Dev. Psychopathol.* 10, 235-257.
- MacDonald, K., MacDonald, T.M., 2010. The peptide that binds: A systematic review  
of oxytocin and its prosocial effects in humans. *Harv. Rev. Psychiatry* 18, 1-21.
- Maes, M., 1995. Evidence for an immune response in major depression: a review and  
hypothesis. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 19, 11-38.
- Maes, M., 2011. Depression is an inflammatory disease, but cell-mediated immune  
activation is the key component of depression. *Prog. Neuropsychopharmacol.  
Biol. Psychiatry* 35, 664-675.

- Maes, M., Leonard, B.E., Myint, A.M., Kubera, M., Verkerk, R., 2011. The new '5-HT' hypothesis of depression: cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 35, 702-721.
- Maes, M., Meltzer, H.Y., 1995. The serotonin hypothesis of major depression. *Psychopharmacology: The fourth generation of progress*, 933-934. New York: Raven Press.
- Mahar, I., Bambico, F.R., Mechawar, N., Nobrega, J.N., 2014. Stress, serotonin, and hippocampal neurogenesis in relation to depression and antidepressant effects. *Neurosci. Biobehav. Rev.* 38, 173-82.
- Maier, S.F., Watkins, L.R., 1998. Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. *Psychol. Rev.* 105, 83-107.
- MacLean, P.D., 1990. *The triune brain in evolution: role in paleocerebral functions*. New York: Plenum Press.
- MacLeod, C., Mathews, A., Tata, P., 1986. Attentional bias in emotional disorders. *J. Abnorm. Psychol.* 95, 15-20.
- Mann, J. J., 2003. Neurobiology of suicidal behaviour. *Nat. Rev. Neurosci.* 4(10), 819-828.
- Mantella, R.C., Vollmer, R.R., Rinaman, L., Li, X., Amico, J.A., 2004. Enhanced

- corticosterone concentrations and attenuated Fos expression in the medial amygdala of female oxytocin knockout mice exposed to psychogenic stress. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 287, R1494-R1504.
- Marazziti, D., Baroni, S., Giannaccini, G., Betti, L., Massimetti, G., Carmassi, C., et al., 2012. A link between oxytocin and serotonin in humans: Supporting evidence from peripheral markers. *Eur. Neuropsychopharmacol.* 22, 578-583.
- Marazziti, D., Catena-DelloSso, C.M., 2008. The role of oxytocin in neuropsychiatric disorders. *Curr. Med. Chem.* 15, 698-704.
- Marmot, M., Wilkinson, R. (Eds.). 2005. *Social determinants of health*. Oxford University Press.
- Matheson, K., Anisman, H., 2003. Systems of coping associated with dysphoria, anxiety and depressive illness: a multivariate profile perspective. *Stress* 6, 223-234.
- Matshushita, H., Matsuzaki, M., Han, X.-J., Nishiki, T.-I., Ohmori, I., Michiue, H., et al., 2012. Anitidepressant-like effect of sildenafil through oxytocin-dependent cyclic amp response element-binding protein phosphorylation. *Neuroscience* 200, 13-18.
- Matsuzaki, M., Matsushita, H., Tomizawa, K., Matsui, H., 2012. Oxytocin: a therapeutic target for mental disorders. *J. Physiol. Sci.* 62, 441-444.
- McAuliffe, C.M., 2002. Suicidal ideation as an articulation of intent: A focus for suicide prevention? *Arch. Suicide Res.* 6, 325-338.
- McCullough, M.E., Churchland, P.S., Mendez, A.J., 2013. Problems with measuring peripheral oxytocin: Can the data on oxytocin and human behavior be trusted? *Neurosci. Biobehav. Rev.* 37, 1485-1492.

- McEwen, B.S., 1999. Stress and hippocampal plasticity. *Annu. Rev. Neurosci.* 22, 105-122.
- McGowan, P.O., Sasaki, A., D'Alessio, A.C., Dymov, S., Labonté, B., Szyf, M., et al., 2009. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat. Neurosci.* 12, 342-348.
- McInnis, O.A., McQuaid, R.J., Matheson, K., Anisman, H., 2015a. Experience-dependent effects of genes: Responses to stressors. In: *Psychology of Change: Life Contexts, Experiences, and Identities*. K.J. Reynolds and N. R. Branscombe, eds. Psychology Press, New York.
- McInnis, O.A. McQuaid R.J., Matheson, K., Anisman, H., 2015b. The moderating role of an oxytocin receptor gene polymorphism in the relation between unsupportive social interactions and coping profiles: Implications for depression. *Front. Psychol.* [Accepted].
- McQuaid, R.J., McInnis, O.A., Abizaid, A., Anisman, H., 2014. Making room for oxytocin in understanding depression. *Neurosci. Biobehav. Rev.* 45, 305-322.
- McQuaid, R.J., McInnis, O.A., Matheson, K., Anisman, H., 2015. Distress of ostracism: oxytocin receptor gene polymorphism confers sensitivity to social exclusion. *Soc. Cogn. Affect. Neurosci.* nsu166.
- McQuaid, R.J., McInnis, O.A., Stead, J.D., Matheson, K., Anisman, H., 2013. The paradoxical association of the oxytocin receptor gene polymorphism: Early-life adversity and vulnerability to depression. *Front. Neurosci.* 7: 128.
- Meisenberg, G., 1981. Short-term behavioral effects of neurohypophyseal peptides in

- mice. *Peptides* 2, 1-8.
- Meinlschmidt, G., Heim, C., 2007. Sensitivity to intranasal oxytocin in adult men with early parental separation. *Biol. Psychiatry* 61, 1109-1111.
- Melis, M.R., Melis, T., Cocco, C., Succu, S., Sanna, F., Pillolla, G., et al., 2007. Oxytocin injected into the ventral tegmental area induces penile erection and increases extracellular dopamine in the nucleus accumbens and paraventricular nucleus of the hypothalamus of male rats. *Eur. J. Neurosci.* 26, 1026-1035.
- Melis, M.R., Succu, S., Sanna, F., Boi, A., Argiolas, A., 2009. Oxytocin injected into the ventral subiculum or the posteromedial cortical nucleus of the amygdala induces penile erection and increases extracellular dopamine levels in the nucleus accumbens of male rats. *Eur. J. Neurosci.* 30, 1349-1357.
- Mendlewicz, J., Crisafulli, C., Calati, R., Kocabas, N.A., Massat, I., Linotte, S., et al., 2012. Influence of COX-3 and OXTR polymorphisms on treatment outcome in treatment resistant depression. *Neurosci. Lett.* 516, 85-88.
- Merali, Z., Anisman, H., James, J.S., Kent, P., Schulkin, J., 2008. Effects of corticosterone on corticotrophin-releasing hormone and gastrin-releasing peptide release in response to an aversive stimulus in two regions of the forebrain (central nucleus of the amygdala and prefrontal cortex). *Eur. J. Neurosci.* 28(1), 165-172.
- Meynen, G., Unmehopal, U.A., Hofman, M.A., Swaab, D.F., Hoogendijk, W.J.G., 2007. Hypothalamic oxytocin mRNA expression and melancholic depression. *Mol. Psychiatry* 12, 118–119.
- Mikolajczak, M., Pinon, N., Lane, A., de Timary, P., Luminet, O., 2010. Oxytocin not

- only increases trust when money is at stake, but also when confidential information is in the balance. *Biol. Psychol.* 85(1), 182-184.
- Millan, M.J., 2006. Multi-target strategies for the improved treatment of depressive states: Conceptual foundations and neuronal substrates, drug discovery and therapeutic application. *Pharmacol. Ther.* 110, 135-370.
- Miller, A.H., Maletic, V., Raison, C.L., 2009. Inflammation and its discontents; the role of cytokines in the pathophysiology of major depression. *Biol. Psychiatry* 65, 732-741.
- Miyahara, S., Komori, T., Fujiwara, R., Shizuya, K., Yamamoto, M., Ohmori, M., et al., 2000. Effects of repeated stress on expression of interleukin-6 (IL-6) and IL-6 receptor mRNAs in rat hypothalamus and midbrain. *Life Sci.* 66, PL93-8.
- Mizumoto, Y., Kimura, T., Ivell, R., 1997. A genomic element within the third intron of the human oxytocin receptor gene may be involved in transcriptional suppression. *Mol. Cell. Endocrinol.* 135, 129-138.
- Modahl, C., Green, L.A., Fein, D., Morris, M., Waterhouse, L., Feinstein, C., et al., 1998. Plasma oxytocin levels in autistic children. *Biol. Psychiatry* 43(4), 270-277.
- Mogenson, G.J. Yang, C.R., 1991. The contribution of basal forebrain to limbic-motor integration and the mediation of motivation to action. *Adv. Exp. Med. Biol.* 295, 267-290.
- Mohiyeddini, C., Opacka-Juffry, J., Gross, J.J., 2014. Emotional suppression explains the link between early life stress and plasma oxytocin. *Anxiety Stress Coping.* [Epub Ahead of Print].

- Montag, C., Flebach, C.J., Kirsch, P., Reuter, M., 2011. Interactions of 5-HTTLPR and a variation on the oxytocin receptor gene influences negative emotionality. *Biol. Psychiatry* 69, 601-603.
- Morrow, B.A., Redmond, A.J., Roth, R.H., Elsworth, J.D., 2000. The predator odor, TMT, displays a unique, stress-like pattern of dopaminergic and endocrinological activation in the rat. *Brain Res.* 864(1), 146-151.
- Mottolose, R., Redouté, J., Costes, N., Le Bars, D., Sirigu, A., 2014. Switching brain serotonin with oxytocin. *Proc. Natl. Acad. Sci. U.S.A.* 201319810.
- Mountney, C., Anisman, H., Merali, Z., 2011. In vivo levels of corticotropin-releasing hormone and gastrin-releasing peptide at the basolateral amygdala and medial prefrontal cortex in response to conditioned fear in the rat. *Neuropharmacology* 60(2), 410-417.
- Muir, J.L., Pfister, H.P., 1988. Influence of exogenously administered oxytocin on the corticosterone and prolactin responses to psychological stress. *Pharmacol. Biochem. Behav.* 29, 699-703.
- Müller, M.B., Landgraf, R., Preil, J., Sillaber, I., Kresse, A.E., Keck, M.E., et al., 2000. Selective activation of the hypothalamic vasopressinergic system in mice deficient for the corticotropin-releasing hormone receptor 1 is dependent on glucocorticoids. *Endocrinology* 141, 4262- 4269.
- Munesue, T., Yokoyama, S., Nakamura, K., Anitha, A., Yamada, K., Hayashi, K., et al., 2010. Two genetic variants of CD38 in subjects with autism spectrum disorder and controls. *Neurosci. Res.* 67(2), 181-191.

- Nadeau, S., Rivest, S., 1999. Effects of circulating tumor necrosis factor on the neuronal activity and expression of the genes encoding the tumor necrosis factor receptors (p55 and p75) in the rat brain: a view from the blood-brain barrier. *Neuroscience* 93, 1449-64.
- Naito, Y., Fukata, J., Shindo, K., Ebisui, O., Murakami, N., Tominaga, T., et al., 1991. Effects of interleukins on plasma arginine vasopressin and oxytocin levels in conscious, freely moving rats. *Biochem. Biophys. Res. Commun.* 174, 1189-1195.
- Nation, D.A., Szeto, A., Mendez, A.J., Brooks, L.G., Zaias, J., Herderick, E.E., et al., 2010. Oxytocin attenuates atherosclerosis and adipose tissue inflammation in socially isolated ApoE<sup>-/-</sup> mice. *Psychosom. Med.* 72, 376-382.
- Nelson, E.E., Panksepp, J., 1998. Brain substrates of infant-mother attachment: contributions of opioids, oxytocin, and norepinephrine. *Neurosci. Biobehav. Rev.* 22, 437-452.
- Nelson, R.J., Trainor, B.C., 2007. Neural mechanisms of aggression. *Nat. Rev. Neurosci.* 8, 536-546.
- Nestler, E.J., Barrot, M., DiLeone, R.J., Eisch, A.J., Gold, S.J., Monteggia, L.M., 2002. Neurobiology of depression. *Neuron* 34, 13-25.
- Nestler, E.J., Carlezon, W.A. Jr., 2006. The mesolimbic dopamine reward circuit in depression. *Biol. Psychiatry* 59, 1151-1159.
- Neumann, I.D., 2008. Brain oxytocin: a key regulator of emotional and social behaviours in both females and males. *J. Neuroendocrinol.* 20, 858-865.

- Neumann, I.D., Torner, L, Wigger, A., 2000. Brain oxytocin: differential inhibition of neuroendocrine stress responses and anxiety-related behavior in virgin, pregnant and lactating rats. *Neuroscience* 95, 567-575.
- Nguyen K.T., Deak T., Owens, S.M., Kohno, T., Fleshner, M., Watkins, L.R., et al., 1998. Exposure to acute stress induces brain interleukin-1beta protein in the rat. *J. Neurosci.* 18, 2239-2246.
- Nielsen, S.E., Segal, S.K., Worden, I.V., Yim, I.S., Cahill, L.. 2013. Hormonal contraception use alters stress responses and emotional memory. *Biol. Psychol.* 92(2), 257-266.
- Nomura, M., Saito, J., Ueta, Y., Muglia, L.J., Pfaff, D.W., Ogawa, S., 2003. Enhanced up-regulation of corticotropin-releasing hormone gene expression in response to restraint stress in the hypothalamic paraventricular nucleus of oxytocin gene-deficient male mice. *J. Neuroendocrinol.* 15, 1054-1061.
- Norman, G.J., Hawkley, L., Luhmann, M., Ball, A.B., Cole, S.W., Berntson, G.C., et al., 2012. Variation in the oxytocin receptor gene influences neurocardiac reactivity to social stress and HPA function: A population based study. *Horm. Behav.* 61, 134-139.
- Norman, G.J., Karelina, K., Morris, J.S., Zhang, N., Cochran, M., DeVries, C.A., 2010. Social interaction prevents the development of depressive-like behavior post nerve injury in mice: a potential role for oxytocin. *Psychosom. Med.* 72, 519–526.
- Northoff, G., Walter, M., Schulte, R.F., Beck, J., Dydak, U., Henning, A., et al., 2007.

- GABA concentrations in the human anterior cingulate cortex predict negative BOLD responses in fMRI. *Nat. Neurosci.* 10, 1515-1517.
- O'Connor, L.E., Berry, J.W., Weiss, J., Gilbert, P., 2002. Guilt, fear, submission, and empathy in depression. *J. Affect. Disord.* 71, 19-27.
- Olf, M., 2012. Bonding after trauma: on the role of social support and the oxytocin system in traumatic stress. *Eur. J. Psychotraumatol.* 3, 18597.
- Oliveira-Pelegrin, G.R., Saia, R.S., Cárnio, E.C., Rocha, M.J., 2013. Oxytocin affects nitric oxide and cytokine production by sepsis-sensitized macrophages. *Neuroimmunomodulation* 20, 65–71.
- Onaka, T., Ikeda, K., Yamashita, T., Honda, K., 2003. Facilitative role of endogenous oxytocin in noradrenaline release in the rat supraoptic nucleus. *Eur. J. Neurosci.* 18, 3018-3026.
- Opacka-Juffry, J., Mohiyeddini, C., 2012. Experience of stress in childhood negatively correlates with plasma oxytocin concentration in adult men. *Stress* 15, 1-10.
- Ozsoy, S., Esel, E., Kula, M., 2009. Serum oxytocin levels in patients with depression and the effects of gender and antidepressant treatment. *Psychiatry Res.* 169, 249–252.
- Parker, K.J., Buckmaster, C.L., Schatzberg, A.F., Lyons, D.M., 2005. Intranasal oxytocin administration attenuates the ACTH stress response in monkeys. *Psychoneuroendocrinology* 30, 924-929.
- Parker, K.J., Kenna, H.A., Zeitzer, J.M., Keller, J., Blasey, C.M., Amico, J.A., et al.,

2010. Preliminary evidence that plasma oxytocin levels are elevated in major depression. *Psychiatry Res.* 178 359–362.
- Peacock, E.J. Wong, P.T., 1990. The Stress Appraisal Measure (SAM): A multidimensional approach to cognitive appraisal. *Stress Med.* 6, 227-236.
- Pedersen, C.A., Gibson, C.M., Rau, S.W., Salimi, K., Smedley, K.L., Casey, R.L., et al., 2011. Intranasal oxytocin reduces psychotic symptoms and improves Theory of Mind and social perception in schizophrenia. *Schizophr. Res.* 132, 50–53.
- Petersson, M., Hulting, A.-L., Uvnäs-Moberg, K., 1999. Oxytocin causes a sustained decrease in plasma levels of corticosterone in rats. *Neurosci. Lett.* 264, 41-44.
- Petrovic, P., Kalisch, R., Singer, T., Dolan, R.J., 2008. Oxytocin attenuates affective evaluations of conditioned faces and amygdala activity. *J. Neurosci.* 28, 6607-6615.
- Pierrehumbert, B., Torrisi, R., Ansermet, F., Borghini, A., Halfon, O., 2012. Adult attachment representations predict cortisol and oxytocin responses to stress. *Attach. Hum. Dev.* 14, 453-576.
- Pierrehumbert, B., Torrisi, R., Laufer, D., Halfon, O., Ansermet, F., Popovic, B.M. 2010. Oxytocin response to an experimental psychosocial challenge in adults exposed to traumatic experiences during childhood or adolescence. *Neuroscience* 166, 168-177.
- Pigott, H.E., Leventhal, A.M., Alter, G.S., Boren, J.J., 2010. Efficacy and effectiveness of antidepressants: current status of research. *Psychother. Psychosom.* 79, 267-279.

- Pincus, D., Kose, S., Arana, A., Johnson, K., Morgan, P.S., Borckardt, J., et al., 2010. Inverse effects of oxytocin on attributing mental activity to others in depressed and healthy subjects: a double-blind placebo controlled fMRI study. *Front. Psychiatry* 1, 134.
- Preacher, K.J., Curran, P.J., Bauer, D.J., 2006. Computational tools for probing interaction effects in multiple linear regression, multilevel modeling, and latent curve analysis. *J. Educ. Behav. Stat.* 31, 437-448.
- Preacher, K.J., Hayes, A.F., 2004. SPSS and SAS procedures for estimating indirect effects in simple mediation models. *Behav. Res. Methods. Instrum. Comput.* 36, 717-731.
- Preacher, K.J., Rucker, D.D., Hayes, A.F., 2007. Assessing moderated mediation hypotheses: Theory, methods, and prescriptions. *Multivariate Behav. Res.* 42, 185-227.
- Preti, A., Melis, M., Siddi, S., Vellante, M., Doneddu, G., Fadda, R., 2014. Oxytocin and autism: a systematic review of randomized controlled trials. *J. Child Adolesc. Psychopharmacol.* 24(2), 54-68.
- Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* 28(7), 916-931.
- Purba, J.S., Hoogendijk, W.J., Hofman, M.A., Swaab, D.F., 1996. Increased number of

- vasopressin- and oxytocin-expressing neurons in the paraventricular nucleus of the hypothalamus in depression. *Arch. Gen. Psychiatry* 53, 137-143.
- Quirin, M., Kuhl, J., Düsing, R., 2011. Oxytocin buffers cortisol responses to stress in individuals with impaired emotion regulation abilities. *Psychoneuroendocrinology* 36, 898-904.
- Rault, J.-L., Carter, C.S., Garner, J.P., Marchant-Forde, J.N., Richert, B.T., Lay, D.C. Jr., 2013. Repeated intranasal oxytocin administration in early life dysregulates the HPA axis and alters social behavior. *Physiol. Behav.* 112-113, 40-48.
- Ravindran, A.V., Griffiths, J., Waddell, C., Anisman, H., 1995. Stressful life events and coping styles in relation to dysthymia and major depressive disorder: Variations associated with alleviation of symptoms following pharmacotherapy. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 19, 637-653.
- Ressler, K.J., Nemeroff, C.B., 2000. Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. *Depress. Anxiety* 12, 2-19.
- Riem, M.M., Bakermans-Kranenburg, M.J., Huffmeijer, R., van IJzendoorn, M.H., 2013. Does intranasal oxytocin promote prosocial behavior to an excluded fellow player? A randomized-controlled trial with Cyberball. *Psychoneuroendocrinology* 38(8), 1418-1425.
- Roberts, J.E., Gotlib, I.H., Kassel, J.D., 1996. Adult attachment security and symptoms of depression: The mediating roles of dysfunctional attitudes and low self-esteem. *J. Pers. Soc. Psychol.* 70, 310-320.

- Rodrigues, S.M., Saslow, L.R., Garcia, N., John, O.P., Keltner, D., 2009. Oxytocin receptor genetic variation related to empathy and stress reactivity in humans. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21437-21441.
- Romero-Fernandez, W., Borroto-Escuela, D.O., Agnati, L.F., Fuxe, K., 2013. Evidence for the existence of dopamine d2-oxytocin receptor heteromers in the ventral and dorsal striatum with facilitatory receptor-receptor interactions. *Mol. Psychiatry* 18, 849-850.
- Ross, H.E., Young, L.J., 2009. Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Front. Neuroendocrinol.* 30, 534-547.
- Sanacora, G., Treccani, G., Popoli, M., 2012. Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology* 62, 63-77.
- Saphire-Bernstein, S., Way, B.M., Kim, H.S., Sherman, D.K., Taylor, S.E., 2011. Oxytocin receptor gene (OXTR) is related to psychological resources. *Proc. Natl. Acad. Sci. U.S.A.* 108, 15118-15122.
- Sapolsky, R.M., Plotsky, P.M., 1990. Hypercortisolism and its possible neural bases. *Biol. Psychiatry* 27, 937-952.
- Sasayama, D., Hattori, K., Teraishi, T., Hori, H., Ota, M., Yoshida, S., et al., 2012. Negative correlation between cerebrospinal fluid oxytocin and negative symptoms of male patients with schizophrenia. *Schizophr. Res.* 139, 201-206.
- Sauer, C., Montag, C., Wörner, C., Kirsch, P., Reuter, M., 2012. Effects of a common

- variant in the CD38 gene on social processing in an oxytocin challenge study: possible links to autism. *Neuropsychopharmacology* 37(6), 1474-1482.
- Sauer, C., Montag, C., Reuter, M., Kirsch, P., 2013. Imaging oxytocin x dopamine interactions: an epistasis effect of CD38 and COMT gene variants influences the impact of oxytocin on amygdala activation to social stimuli. *Front. Neurosci.* 7, 45.
- Sawchenko, P.E., Swanson, L.W., Steinbusch, H.W.M., Verhofstad, A.A.J., 1983. The distribution and cells of origin of serotonergic inputs to the paraventricular and supraoptic nuclei of the rat. *Brain Res.* 277, 355-360.
- Scantamburlo, G., Ansseau, M., Geenen, V., Legros, J.-J., 2011. Intranasal oxytocin as an adjunct to escitalopram in major depression. *J. Neuropsychiatry Clin. Neurosci.* 23, E5.
- Scantamburlo, G., Hansenne, M., Fuchs, S., Pitchot, W., Maréchal, P., Pequeux, C., et al., 2007. Plasma oxytocin levels and anxiety in patients with major depression. *Psychoneuroendocrinology* 32, 407-410.
- Scott, A.I., Whalley, L.J., Bennie, J., Bowler, G., 1986. Oestrogen stimulated neurophysin and outcome after electroconvulsive therapy. *Lancet* 1, 1411–1414.
- Scott, A.I., Whalley, L.J., Legros, J.J., 1989. Treatment outcome, seizure duration, and the neurophysin response to ECT. *Biol. Psychiatry* 25, 585–597.
- Seedat, S., Stein, M.B., Forde, D.R., 2005. Association between physical partner violence, posttraumatic stress, childhood trauma, and suicide attempts in a community sample of women. *Violence Vict.* 20(1), 87-98.

- Seidel, E.M., Silani, G., Metzler, H., Thaler, H., Lamm, C., Gur, R.C., et al., 2013. The impact of social exclusion vs. inclusion on subjective and hormonal reactions in females and males. *Psychoneuroendocrinology* 38, 2925-2932.
- Smith, A.S., Wang, Z., 2014. Hypothalamic oxytocin mediates social buffering of the stress response. *Biol. Psychiatry* 76(4), 281-288.
- Smith, K.E., Porges, E.C., Norman, G.J., Connelly, J.J., Decety, J., 2014. Oxytocin receptor gene variation predicts empathetic concern and autonomic arousal while perceiving harm to others. *Soc. Neurosci.* 9, 1-9.
- Seltzer, L.J., Ziegler, T.E., Pollak, S.D., 2010. Social vocalizations can release oxytocin in humans. *Proc. Biol. Sci.* 277, 2661-2666.
- Shahrokh, D.K., Zhang, T.Y., Diorio, J., Gratton, A., Meaney, M.J., 2010. Oxytocin-dopamine interactions mediate variations in maternal behavior in the rat. *Endocrinology* 151, 2276-2286.
- Shalvi, S., De Dreu, C.K., 2014. Oxytocin promotes group-serving dishonesty. *Proc. Natl. Acad. Sci. U.S.A.* 111(15), 5503-5507.
- Shamay-Tsoory, S.G., Abu-Akel, A., Palgi, S., Sulieman, R., Fischer-Shofty, M., Levkovitz, Y., et al., 2013. Giving peace a chance: oxytocin increases empathy to pain in the context of the Israeli–Palestinian conflict. *Psychoneuroendocrinology* 38(12), 3139-3144.
- Shamay-Tsoory, S.G., Fischer, M., Dvash, J., Harari, H., Perach-Bloom, N., Levkovitz, Y., 2009. Intranasal administration of oxytocin increases envy and schadenfreude (gloating). *Biol. Psychiatry* 66(9), 864-870.

- Shapiro, L.E., Insel, T.R., 1990. Infant's response to social separation reflects adult differences in affiliative behavior: a comparative developmental study in prairie and montane voles. *Dev. Psychobiol.* 23, 375-394.
- Siegel, G.J., Chauhan, N.B., 2000. Neurotrophic factors in Alzheimer's and Parkinson's disease brain. *Brain Res. Brain Res. Rev.* 33, 119-227.
- Simeon, D., Bartz, J., Hamilton, H., Crystal, S., Braun, A., Ketay, S., et al., 2011. Oxytocin administration attenuates stress reactivity in borderline personality disorder: a pilot study. *Psychoneuroendocrinology* 36, 1418-1421.
- Skrundz, M., Bolten, M., Nast, I., Hellhammer, D.H., Meinlschmidt, G., 2011. Plasma oxytocin concentration during pregnancy is associated with development of postpartum depression. *Neuropsychopharmacology* 36, 1886-1893.
- Slattery, D.A., Neumann, I.D., 2010. Chronic icv oxytocin attenuates the pathological high anxiety state of selectively bred Wistar rats. *Neuropharmacology* 58, 56-61.
- Smith, K.E., Porges, E.C., Norman, G.J., Connelly, J.J., Decety, J., 2014. Oxytocin receptor gene variation predicts empathetic concern and autonomic arousal while perceiving harm to others. *Soc. Neurosci.* 9, 1-9.
- Spielberger, C.D., 1983. *Manual for the State-Trait Anxiety Inventory (Form Y)*. Palo Alto, CA: Consulting Psychologists Press.
- Sriram, K., Matheson, J.M., Benkovic, S.A., Miller, D.B., Luster, M.I., O'Callaghan, J.P., 2006. Deficiency of TNF receptors suppresses microglial activation and alters the susceptibility of brain regions to MPTP-induced neurotoxicity: role of TNF-alpha. *FASEB J.* 20, 670-682.

- Statistics Canada. CANSIM Table 102-0561. Leading causes of death, total population, by age group and sex, Canada, annual.
- Strauss, J.S. Freeman, N.L., Shaikh, S.A., Vetro, A., Kiss, E., Kapornai, K., et al., 2010. No association between oxytocin or prolactin gene variants and childhood-onset mood disorders. *Psychoneuroendocrinology* 35, 1422-1428.
- Striepens, N., Kendrick, K.M., Hanking, V., Landgraf, R., Wüllner, U., Maier, W., et al., 2013. Elevated cerebrospinal fluid and blood concentrations of oxytocin following its intranasal administration in humans. *Sci. Rep.* 3, 3440.
- Stroud, L.R., Salovey, P., Epel, E.S., 2002. Sex differences in stress responses: social rejection versus achievement stress. *Biol. Psychiatry* 52(4), 318-327.
- Szeto, A., McCabe, P.M., Nation, D.A., Tabak, B.A., Rossetti, M.A., McCullough, M. E., et al., 2011. Evaluation of enzyme immunoassay and radioimmunoassay methods for the measurement of plasma oxytocin. *Psychosom. Med.* 73(5), 393.
- Szeto, A., Nation, D.A., Mendez, A.J., Dominguez-Bendala, J., Brooks, L.G., Schneidermann, N., et al., 2008. Oxytocin attenuates NADPH-dependent superoxide activity and IL-6 secretion in macrophages and vascular cells. *Am. J. Physiol. Endocrinol. Metab.* 295, E1495–E1501.
- Szeto, A., Rossetti, M.A. Mendez, A.J., Noller, C.M., Herderick, E.E., Gonzales, J.A., et al., 2013. Oxytocin administration attenuates atherosclerosis and inflammation in Watanabe Heritable Hyperlipidemic rabbits. *Psychoneuroendocrinology* 38, 685-693.
- Tabak, B.A., McCullough, M.E., Carver, C.S., Pedersen, E.J., Cuccaro, M.L., 2013.

Variation in oxytocin receptor gene (OXTR) polymorphisms is associated with emotional and behavioral reactions to betrayal. *Soc. Cogn. Affect. Neurosci.* 9, 810-816.

Tabak, B.A., McCullough, M.E., Szeto, A., Mendez, A.J., McCabe, P.M., 2011.

Oxytocin indexes relational distress following interpersonal harms in women. *Psychoneuroendocrinology* 36, 115-122.

Tabak, B.A., Vrshek-Schallhorn, S., Zinbarg, R.E., Prenoveau, J.M., Mineka, S., Redei, E.E. et al., 2015. Interaction of CD38 Variant and Chronic Interpersonal Stress Prospectively Predicts Social Anxiety and Depression Symptoms Over 6 Years. *Clin. Psychol. Sci.* [Epub Ahead of Print].

Taylor, S.E., Gonzaga, G.C., Klein, L.C., Hu, P., Greendale, G.A., Seeman, T.E., 2006.

Relation of oxytocin to psychological stress responses and hypothalamic-pituitary-adrenocortical axis activity in older women. *Psychosom. Med.* 68, 238–245.

Taylor, S.E., Klein, L.C., Lewis, B.P., Gruenewald, T.L., Gurung, R.A., Updegraff, J.A.,

2000. Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. *Psychol. Rev.* 107, 411-429.

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

and plasma vasopressin in men biomarkers of distressed pair-bond relationships? *Psychol. Sci.* 21, 3-7.

Taylor, S. E., 2006. Tend and Befriend Biobehavioral Bases of Affiliation Under Stress.

*Curr. Dir. Psychol. Sci.* 15, 273-277.

- Thompson, S.M., Hammen, C., Starr, L.R., Najman, J.M., 2014. Oxytocin receptor gene polymorphism (rs53576) moderates the intergenerational transmission of depression. *Psychoneuroendocrinology* 43, 11-19.
- Thompson, R.J., Parker, K.J., Hallmayer, J.F., Waugh, C.E., Gotlib, I.H., 2011. Oxytocin receptor gene polymorphism (rs2254298) interacts with familial risk for psychopathology to predict symptoms of depression and anxiety in adolescent girls. *Psychoneuroendocrinology* 36, 144-147.
- Tomizawa, K., Iga, N., Lu, Y.F., Moriwaki, A., Matsushita, M., Li, S.T., et al., 2003. Oxytocin improves long-lasting spatial memory during motherhood through MAP kinase cascade. *Nat. Neurosci.* 6, 384-390.
- Tops, M., Buisman-Pijlman, F.T., Boksem, M.A., Wijers, A.A., Korf, J., 2012. Cortisol-induced increases of plasma oxytocin levels predict decreased immediate free recall of unpleasant words. *Front. Psychiatry* 3, 43.
- Tops, M., Riese, H., Oldehinkel, A.J. Rijdsdijk, F.V., Ormel, J., 2008. Rejection sensitivity relations to hypocortisolism and depressed mood state in young women. *Psychoneuroendocrinology* 33, 551-559.
- Tops, M., van Peer, J.M., Wester, A.E., Wijers, A.A., Korf, J., 2006. State-dependent regulation of cortical activity by cortisol: An EEG study. *Neurosci. Lett.* 404, 39-43.
- Tost, H., Kolachana, B., Hakimi, S., Lemaitre, H., Verchinski, B.A., Mattay, V.S., et al.,

2010. A common allele in the oxytocin receptor gene (OXTR) impacts prosocial temperament and human hypothalamic-limbic structure and function. *Proc. Natl. Acad. Sci. U.S.A.* 107(31), 13936-13941.
- Uchino, B. N., 2006. Social support and health: a review of physiological processes potentially underlying links to disease outcomes. *J. Behav. Med.* 29(4), 377-387.
- Uvnäs-Moberg, K., Bjökstrand, E., Hillegaard, V., Ahlenius, S., 1999. Oxytocin as a possible mediator of SSRI-induced antidepressant effects. *Psychopharmacology (Berl)*. 142, 95-101.
- Vacher, C.M., Fretier, P., Creminon, C., Calas, A., Hardin-Pouzet, H., 2002. Activation by serotonin and noradrenaline of vasopressin and oxytocin expression in the mouse paraventricular and supraoptic nuclei. *J. Neurosci.* 22, 1513-1522.
- Van de Kar, L.D., Rittenhouse, P.A., Li, Q., Levy, A.D., Brownfield, M.S., 1995. Hypothalamic paraventricular, but not supraoptic neurons, mediate the serotonergic stimulation of oxytocin secretion. *Brain Res. Bull.* 36, 45-50.
- van Londen, L., Goekoop, J.G., van Kempen, G.M., Frankhuijzen-Sierevogel, A.C., Wiegant, V.M., van der Velde, E.A., et al., 1997. Plasma levels of arginine vasopressin elevated in patients with major depression. *Neuropsychopharmacology* 17, 284-292.
- van Roekel, E., Verhagen, M., Engels, R.C., Goossens, L., Scholte, R.H., 2013. Oxytocin receptor gene (OXTR) in relation to loneliness in adolescence: interactions with sex, parental support and DRD2 and 5-HTTLPR genotypes. *Psychiatr. Genet.* 23, 204-213.

- Veinante, P., Freund-Mercier, M. J., 1997. Distribution of oxytocin- and vasopressin-binding sites in the rat extended amygdala: a histoautoradiographic study. *J. Comp. Neurol.* 383, 305-325.
- Viau, V., Chu, A., Soriano, L., Dallman, M.F., 1999. Independent and overlapping effects of corticosterone and testosterone on corticotropin-releasing hormone and arginine vasopressin mRNA expression in the paraventricular nucleus of the hypothalamus and stress-induced adrenocorticotrophic hormone release. *J. Neurosci.* 19(15), 6684-6693.
- Walker, C.D., Deschamps, S., Proulx, K., Tu, M., Salzman, C., Woodside, B., et al., 2004. Mother to infant or infant mother? Reciprocal regulation of responsiveness to stress in rodents and the implications for humans. *J. Psychiatry Neurosci.* 29, 364-382.
- Walker, C.-D., Lightman, S.L., Steele, M.K., Dallman, M.F., 1992. Suckling is a persistent stimulus to the adrenocortical system of the rat. *Endocrinology* 130, 115-125.
- Wang, S., Zhang, K., Xu, Y., Sun, N., Shen, Y., Xu, Q., 2009. An association study of the serotonin transporter and receptor genes with the suicidal ideation of major depression in a Chinese Han population. *Psychiatry Res.* 170(2), 204-207.
- Wang, Z., Yu, G., Cascio, C., Liu, Y., Gingrich, B., Insel, T.R., 1999. Dopamine D2 receptor-mediated regulation of partner preferences in female prairie voles (*Microtus ochrogaster*): a mechanism for pair bonding? *Behav. Neurosci.* 113, 602-611.

- Watson, D., Clark, L.A., Tellegen, A., 1988. Development and validation of brief measures of positive and negative affect: The PANAS scales. *J. Pers. Soc. Psychol.* 54, 1063-1070.
- Williams, K.D., 1997. Social ostracism. In R. M. Kowalski (Ed.), *Aversive interpersonal behaviors* (pp. 133–170). New York: Plenum.
- Williams, K.D., 2001. *Ostracism: The power of silence*. New York: Guilford Press.
- Williams, K.D., Cheung, C.K.T. Choi, W., 2000. CyberOstracism: Effects of being ignored over the internet. *J. Pers. Soc. Psychol.* 79, 748-762.
- Windle, R.J., Shanks, N., Lightman, S.L., Ingram, C.D., 1997. Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. *Endocrinology* 138, 2829-2834.
- Windle, R.J., Kershaw, Y.M., Shanks, N., Wood, S.A., Lightman, S.L., Ingram, C.D., 2004. Oxytocin attenuates stress-induced c-fos mRNA expression in specific forebrain regions associated with modulation of hypothalamo-pituitary-adrenal activity. *J. Neurosci.* 24, 2974-2982.
- Winfrey, L.T., Jiang, S., 2010. Youthful suicide and social support exploring the social dynamics of suicide-related behavior and attitudes within a national sample of US adolescents. *Youth Violence Juv. Justice* 8(1), 19-37.
- Yayou, K., Ito, S., Kasuya, E., Sutoh, M., Ohkura, S., Okamura, H., 2008. Intracerebroventricularly administered oxytocin attenuated cortisol secretion, but not behavioral responses, during isolation in Holstein steers. *J. Vet. Med. Sci.* 70, 665–671.

- Yehuda, R., Southwick, S.M., Nussbaum, G., Wahby, V., Giller, E.L.Jr., Mason, J.W., 1990. Low urinary cortisol excretion in patients with posttraumatic stress disorder. *J. Nerv Ment. Dis.* 178, 366-369.
- Yoshida, M., Takayanagi, Y., Inoue, K., Kimura, T., Young, L.J., Onaka, T., et al., 2009. Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice. *J. Neurosci.* 29, 2259-2271.
- Young, L.J., Lim, M.M., Gingrich, B., Insel, T.R., 2001. Cellular mechanisms of social attachment. *Horm. Behav.* 40, 133-138.
- Young, L.J., Wang, Z., 2004. The neurobiology of pair bonding. *Nat. Neurosci.* 7, 1048-1054.
- Yuen, K.W., Garner, J.P., Carson, D.S., Keller, J., Lembke, A., Hyde, S.A., et al., 2014. Plasma oxytocin concentrations are lower in depressed vs. healthy control women and are independent of cortisol. *J. Psychiatr. Res.* 51, 30-36.
- Zadro, L., Williams, K.D., Richardson, R., 2004. How low can you go? Ostracism by a computer is sufficient to lower self-reported levels of belonging, control, self-esteem, and meaningful existence. *J. Exp. Psychol.* 40, 560-567.
- Zak, P.J., Kurzban, R., Matzner, W.T., 2005. Oxytocin is associated with human trustworthiness. *Horm. Behav.* 48(5), 522-527.
- Zak, P.J., Stanton, A.A., Ahmadi, S., 2007. Oxytocin increases generosity in humans. *PLOS One* 2(11), e1128.
- Zetsche, T., Frasch, A., Jirikowski, G., Murck, H., Steiger, A., 1996. Nocturnal oxytocin secretion is reduced in major depression. *Biol. Psychiatry* 39, 584.

- Zheng, J., Babygirija, R., Bülbül, M., Cerjak, D., Ludwig, K., Takahashi, T., 2010. Hypothalamic oxytocin mediates adaptation mechanism against chronic stress in rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* 299, G946–G953.
- Zhu, L., Onaka, T., 2002. Involvement of medullary A2 noradrenergic neurons in the activation of oxytocin neurons after conditioned fear stimuli. *Eur. J. Neurosci.* 16, 2186-2198.
- Zhu, Y., Saito, K., Murakami, Y., Asano, M., Iwakura, Y., Seishima, M., 2006. Early increase in mRNA levels of pro-inflammatory cytokines and their interactions in the mouse hippocampus after transient global ischemia. *Neurosci. Lett.* 393, 122-126.
- Zöller, C., Maroof, P., Weik, U., Deinzer, R., 2010. No effect of social exclusion on salivary cortisol secretion in women in a randomized controlled study. *Psychoneuroendocrinology* 35, 1294-1298.
- Zwolinski, J., 2012. Psychological and neuroendocrine reactivity to ostracism. *Aggress. Behav.* 38, 108-125.

## Appendix A

### Study Questionnaires

#### Beck Depression Inventory

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 ever 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 all 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
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 anymore 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

### Childhood Maltreatment Questionnaire

	1	2	3	4	5
	Never	Rarely	Sometimes	Often	Very Often
1. Act emotionally “cold” toward you.	1	2	3	4	5
2. Fail to praise you when you deserved it.	1	2	3	4	5
3. Fail to listen to you or to comfort you when they knew you were sad and upset.	1	2	3	4	5
4. Seem to be emotionally detached or unexpressive with you.	1	2	3	4	5
5. Appear to be disinterested in you and your life.	1	2	3	4	5
6. Ignore you.	1	2	3	4	5
7. Fail to take you to the doctor or give you medicine when you were ill and medical attention seemed to have been needed.	1	2	3	4	5
8. Leave you alone or unattended for periods of time when, looking back now, you believe it was unsafe or inappropriate for them to have done so.	1	2	3	4	5
9. Fail to provide adequate food or clothing for you even though they had the means to do so.	1	2	3	4	5
10. Fail to care for your injuries when you were physically hurt.	1	2	3	4	5
11. Have many unpredictable moods or frequently changing moods that affected their ability to provide reliable care for you.	1	2	3	4	5
12. Leave you with certain people (e.g., strangers, other children, casual acquaintances) for periods of time when, looking back now, you believe it was unsafe inappropriate for them to have done so.	1	2	3	4	5
13. Speak to you in a way that frightened you.	1	2	3	4	5
14. Act in a way that implied they did not like or value you.	1	2	3	4	5
15. Totally disregard your input into decisions that affected you.	1	2	3	4	5
16. Put you down or treat you in a degrading manner.	1	2	3	4	5
17. Touch or handle you in a rough way that frightened you.	1	2	3	4	5
18. Make you cater to their desires or whims with little concern for your own comfort or welfare.	1	2	3	4	5

19. Spank you hard enough to cause you bruising, swelling or Bleeding. 1 2 3 4 5
20. Hit or punch you with a closed fist. 1 2 3 4 5
21. Kick you with their foot or strike you hard with a knee or elbow. 1 2 3 4 5
22. Hit you with an object such as belt, cord, kitchen utensil, board or stick. 1 2 3 4 5
23. Twist, yank or bend your leg, arm or finger in a painful manner. 1 2 3 4 5
24. Push, throw or knock you down or into an object such as a wall or a piece of furniture. 1 2 3 4 5
25. Make you show them a sexual part of your body (i.e., genitals, breasts, or buttocks). 1 2 3 4 5
26. Get you to touch their genitals, breasts or anus with your mouth or tongue. 1 2 3 4 5
27. Rub or fondle a sexual part of your body (i.e., genitals, breasts, or buttocks). 1 2 3 4 5
28. Engage in vaginal or anal intercourse with you. 1 2 3 4 5
29. Get you to do something sexual with them. 1 2 3 4 5
30. Get you to touch them in a sexual way or fondle a sexual part of their or another person`s body (i.e., genitals, breasts, or buttocks). 1 2 3 4 5
31. Who were you thinking of when you answered the 30 questions above. Please list as many apply (e.g., mother stepfather, older sister etc.) \_\_\_\_\_

### Distrust & Cynicism

	Completely Disagree	Somewhat Disagree	Somewhat Agree	Completely Agree
1. No one cares much what happens to you	0	1	2	3
2. It is safer to trust nobody	0	1	2	3
3. I think most people would lie to get ahead	0	1	2	3
4. Most people inwardly dislike putting themselves out to help other people	0	1	2	3
5. Most people will use somewhat unfair means to gain profit or an advantage rather than lose it	0	1	2	3
6. Most people are honest chiefly through fear of being caught	0	1	2	3
7. I commonly wonder what hidden reason another person may have for doing something nice for me	0	1	2	3
8. Most people make friends because friends are likely to be useful to them	0	1	2	3

## Social Ostracism and Mood Scale

### Section 1: General Questions

1. Is this your first time playing Cyberball?
  - a. YES
  - b. NO
  
2. If NO, how many times have you played Cyberball in the past?
  - a. 1 time
  - b. 2-5 times
  - c. 5 times or more
  
3. Were you able to mentally visualize yourself playing the game?

1	2	3	4	5
not at all able				very much able
  
4. How able were you to visualize the other players?

1	2	3	4	5
not at all able				very much able

Describe how you tried to visualize the other players (e.g., where were you, what sort of people were they? Was it cloudy or sunny?). What was your mental picture?

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5. Assuming that the ball should be thrown to each person equally (33% if three people), what percentage of throws was directed to you?

I received \_\_\_\_\_ % of the throws.
  
6. Please describe the amount you participated (i.e, the amount you got to throw the ball).
  - a. About the same as the other players
  - b. More than the other players
  - c. Less than the other players









Co-player 2?

1 2 3 4 5 6 7 8 9

6. How tolerant is...

Co-player 1?

1 2 3 4 5 6 7 8 9

Co-player 2?

1 2 3 4 5 6 7 8 9

7. How arrogant is...

Co-player 1?

1 2 3 4 5 6 7 8 9

Co-player 2?

1 2 3 4 5 6 7 8 9

8. How friendly is...

Co-player 1?

1 2 3 4 5 6 7 8 9

Co-player 2?

1 2 3 4 5 6 7 8 9

9. How manipulative is...

Co-player 1?

1 2 3 4 5 6 7 8 9

Co-player 1?

1 2 3 4 5 6 7 8 9

10. How fair is...

Co-player 1?

1 2 3 4 5 6 7 8 9

Co-player 2?

1 2 3 4 5 6 7 8 9

11. How loyal is...

Co-player 1?

1 2 3 4 5 6 7 8 9

Co-player 2?

1 2 3 4 5 6 7 8 9

12. How hypocritical is...

Co-player 1?

1 2 3 4 5 6 7 8 9

Co-player 2?

1 2 3 4 5 6 7 8 9

13. To what degree would you describe...

Co-player 1 as a sell out?

1 2 3 4 5 6 7 8 9

Co-player 2 as a sell out?

1 2 3 4 5 6 7 8 9

### Spielberger Trait Anxiety

	Almost 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 |

## Inventory of Parental and Peer Alienation

Almost Never or Never 1	Seldom 2	Sometimes 3	Often 4	Almost Always or Always 5
----------------------------	-------------	----------------	------------	------------------------------

### Section I

- |   |   |   |   |   |   |
|---|---|---|---|---|---|
| 1. Talking over my problems with my parents makes me feel ashamed or foolish. | 1 | 2 | 3 | 4 | 5 |
| 2. I get upset easily at home.  | 1 | 2 | 3 | 4 | 5 |
| 3. I get upset a lot more than my parents know about.                         | 1 | 2 | 3 | 4 | 5 |
| 4. I feel angry with my parents.  | 1 | 2 | 3 | 4 | 5 |
| 5. I don't get much attention at home.  | 1 | 2 | 3 | 4 | 5 |
| 6. I don't know whom I can depend on these days.                              | 1 | 2 | 3 | 4 | 5 |
| 7. My parents don't understand what I'm going through these days.             | 1 | 2 | 3 | 4 | 5 |
| 8. I feel that no one understands me.   | 1 | 2 | 3 | 4 | 5 |

### Section II

- |   |   |   |   |   |   |
|---|---|---|---|---|---|
| 1. Talking over my problems with my friends makes me feel ashamed or foolish. | 1 | 2 | 3 | 4 | 5 |
| 2. I feel the need to be in touch with my friends more often.                 | 1 | 2 | 3 | 4 | 5 |
| 3. My friends don't understand what I'm going through these days.             | 1 | 2 | 3 | 4 | 5 |
| 4. I feel alone or apart when I am with my friends.                           | 1 | 2 | 3 | 4 | 5 |
| 5. I feel angry with my friends.  | 1 | 2 | 3 | 4 | 5 |
| 6. I get upset a lot more than my friends know about.                         | 1 | 2 | 3 | 4 | 5 |
| 7. It seems as if my friends are irritated with me for no reason.             | 1 | 2 | 3 | 4 | 5 |

## Traumatic Life Events Questionnaire

1. Have you ever experienced a natural disaster (a flood, hurricane, earthquake, etc.)? (If never, go to question 2)

never once twice 3 times 4 times 5 times more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1	2	3
Age 0-12	Age 13-17	Age 18+

Did you experience fear, helplessness, or horror at what happened? yes / no

2. Were you involved in a motor vehicle accident for which you received medical attention or that badly injured or killed someone? (If never, go to question 3)

never once twice 3 times 4 times 5 times more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1	2	3
Age 0-12	Age 13-17	Age 18+

Did you experience fear, helplessness, or horror at what happened? yes / no

3. Have you been involved in any other kind of accident where you or someone else was badly hurt? (examples: a plane crash, a drowning or near drowning, an electrical or machinery accident, an explosion, home fire, chemical leak, or overexposure to radiation or toxic chemicals) (If never, go to question 4)

never once twice 3 times 4 times 5 times more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1	2	3
Age 0-12	Age 13-17	Age 18+

Did you experience fear, helplessness, or horror at what happened? yes / no

4. Have you lived, worked, or had military service in a war zone? yes / no (If no, go to question 5)

If yes, were you ever exposed to warfare or combat? (for example: in the vicinity of a rocket attack or people being fired upon; seeing someone getting wounded or killed)

never once twice 3 times 4 times 5 times more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1	2	3
Age 0-12	Age 13-17	Age 18+

Did you experience fear, helplessness, or horror at what happened? yes / no

5. Have you experienced the unexpected and sudden death of a close friend or loved one? (If never, go to question 6)

never once twice 3 times 4 times 5 times more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1	2	3
Age 0-12	Age 13-17	Age 18+

Did you experience fear, helplessness, or horror at what happened? yes / no

6. Has a loved one (who is living) ever experienced a life threatening or permanently disabling accident, assault, or illness? (examples: spinal cord injury, rape, life threatening virus) (If never, go to question 7)

never once twice 3 times 4 times 5 times more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1	2	3
Age 0-12	Age 13-17	Age 18+

Did you experience fear, helplessness, or horror at what happened? yes / no

7. Have you ever had a life threatening illness? (If never, go to question 8)

never once twice 3 times 4 times 5 times more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1

2

3

Age 0-12

Age 13-17

Age 18+

Did you experience fear, helplessness, or horror at what happened? yes / no

8. Have you been robbed or been present during a robbery – where the robber(s) used or displayed a weapon? (If never, go to question 9)

never once twice 3 times 4 times 5 times more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1

2

3

Age 0-12

Age 13-17

Age 18+

Did you experience fear, helplessness, or horror at what happened? yes / no

9. Have you ever been hit or beaten up and badly hurt by a stranger or someone you didn't know very well? (If never, go to question 10)

never once twice 3 times 4 times 5 times more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1

2

3

Age 0-12

Age 13-17

Age 18+

Did you experience fear, helplessness, or horror at what happened? yes / no

10. Have you seen a stranger (or someone you didn't know very well) attack or beat up another someone and seriously injure or kill them? (If never, go to question 11)

never once twice 3 times 4 times 5 times more than 5 times



14. Have you ever been slapped, punched, kicked, beaten up, or otherwise physically hurt by your spouse (or former spouse), a boyfriend/girlfriend, or some other intimate partner? (If never, go to question 15)

never once twice 3 times 4 times 5 times more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1	2	3
Age 0-12	Age 13-17	Age 18+

Did you experience fear, helplessness, or horror at what happened? yes / no

15. Before your 13<sup>th</sup> birthday: Did anyone – who was at least 5 years older than you – touch or fondle your body in a sexual way or make you touch or fondle their body in a sexual way? (If never, go to question 16)

never once twice 3 times 4 times 5 times more than 5 times

Did you experience fear, helplessness, or horror at what happened? yes / no

16. Before your 13<sup>th</sup> birthday: Did anyone close to your age touch sexual parts of your body or make you touch sexual parts of their body –against your will or without your consent? (If never, go to question 17)

never once twice 3 times 4 times 5 times more than 5 times

Did you experience fear, helplessness, or horror at what happened? yes / no

17. After your 13<sup>th</sup> birthday and before your 18<sup>th</sup> birthday: Did anyone touch sexual parts of your body or made you touch sexual parts of their body – against your will or without your consent? (If never, go to question 18)

never once twice 3 times 4 times 5 times more than 5 times

Did you experience fear, helplessness, or horror at what happened? yes / no

18. After your 18<sup>th</sup> birthday: Did anyone touch sexual parts of your body or made you touch sexual parts of their body – against your will or without your consent? (If never, go to question 19)

never once twice 3 times 4 times 5 times more than 5 times

Did you experience fear, helplessness, or horror at what happened? yes / no

19. Has anyone stalked you – in other words: followed you or kept track of your activities – causing you to feel intimidated or concerned for your safety? (If never, go to question 20)

never once twice 3 times 4 times 5 times more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1	2	3
Age 0-12	Age 13-17	Age 18+

Did you experience fear, helplessness, or horror at what happened? yes / no

20. Have you ever had a miscarriage? (If never, go to question 21)

never once twice 3 times 4 times 5 times more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1	2	3
Age 0-12	Age 13-17	Age 18+

Did you experience fear, helplessness, or horror at what happened? yes / no

21. Have you ever had an abortion? (If never, go to question 22)

never once twice 3 times 4 times 5 times more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1	2	3
Age 0-12	Age 13-17	Age 18+

Did you experience fear, helplessness, or horror at what happened? yes / no

22. Have you ever had something happened to you that you believe represented an experience of discrimination (e.g., religious, racial, sex)? (If never, go to question 23)
- never   once   twice   3 times   4 times   5 times   more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1	2	3
Age 0-12	Age 13-17	Age 18+

Did you experience fear, helplessness, or horror at what happened?   yes /   no

23. Have you experienced (or seen) any other events that were life threatening, caused serious injury, or were highly disturbing and distressing? (examples: lost in the wilderness; a serious animal bite; violent death of a pet; being kidnapped and held hostage; seeing a mutilated body or parts) (If never, go to question 24)
- never   once   twice   3 times   4 times   5 times   more than 5 times

Please describe: \_\_\_\_\_  
\_\_\_\_\_

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1	2	3
Age 0-12	Age 13-17	Age 18+

Did you experience fear, helplessness, or horror at what happened?   yes /   no

24. If any of the events (listed above) happened to you, check ONE event that currently causes you the most distress?

- |   |  |
|---|--|
| <input type="checkbox"/> 1: Natural disaster                | <input type="checkbox"/> 13: Family violence growing up                          |
| <input type="checkbox"/> 2: Motor Vehicle Accident          | <input type="checkbox"/> 14: Assault by partner                                  |
| <input type="checkbox"/> 3: Other kind of accident          | <input type="checkbox"/> 15: Sexual assault before 13 by someone 5 years older   |
| <input type="checkbox"/> 4: Exposure to Warfare/Combat      | <input type="checkbox"/> 16: Sexual assault before 13 by someone of the same age |
| <input type="checkbox"/> 5: Death of loved one              | <input type="checkbox"/> 17: Sexual assault from 13-18                           |
| <input type="checkbox"/> 6: Illness/accident of loved one   | <input type="checkbox"/> 18: Sexual assault after 18                             |
| <input type="checkbox"/> 7: Personal illness                | <input type="checkbox"/> 19: Experience of being stalked                         |
| <input type="checkbox"/> 8: Robbery with weapon             | <input type="checkbox"/> 20: Miscarriage   |
| <input type="checkbox"/> 9: Personally hurt by stranger     | <input type="checkbox"/> 21: Abortion  |
| <input type="checkbox"/> 10: Witnessed attack by stranger   | <input type="checkbox"/> 22: Discrimination experience                           |
| <input type="checkbox"/> 11: Threatened with injury/death   | <input type="checkbox"/> 23: Other distressing event                             |
| <input type="checkbox"/> 12: Physically punished growing up |  |

How much distress (anxiety, worry, sadness, or grief) does this event cause you?

None Happened	No Distress	Slight Distress	Moderate Distress	Considerable Distress	Extreme Distress
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25. While growing up, were you ever psychologically bullied? (If never, go to question 26)

never   once   twice   3 times   4 times   5 times   more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1	2
Age 0-12	Age 13-17

Did you experience fear, helplessness, or horror at what happened?   yes /   no

26. While growing up, were you ever physically bullied? (If never, go to question 27)

never   once   twice   3 times   4 times   5 times   more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1                      2  
Age 0-12              Age 13-17

Did you experience fear, helplessness, or horror at what happened? yes / no

27. While growing up, was your father or mother emotionally abusive towards one another (such as name calling, degrading and yelling at one another)? (If never, go to question 28)

never   once   twice   3 times   4 times   5 times   more than 5 times

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1                      2  
Age 0-12              Age 13-17

Did you experience fear, helplessness, or horror at what happened? yes / no

28. While growing up did your parents get divorced?

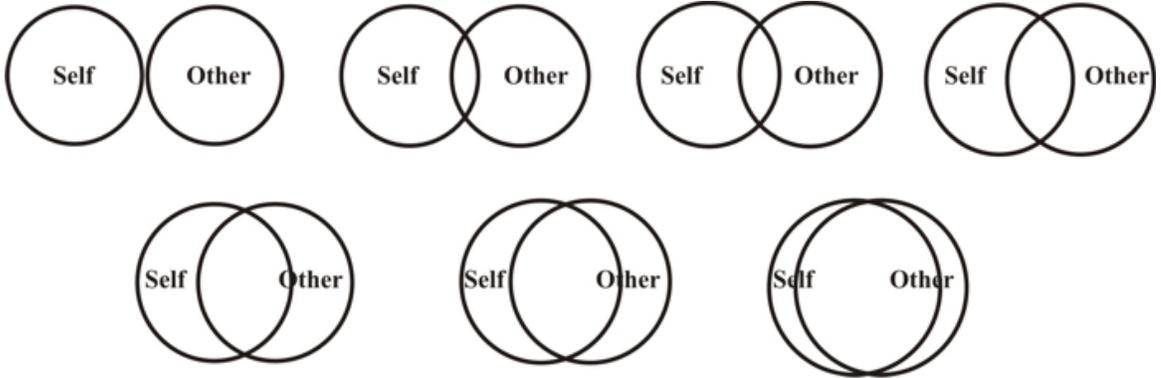
Yes \_\_\_\_\_              No \_\_\_\_\_

How old were you when this happened (you may have experienced these at multiple age groups so please circle all that apply):

1                      2  
Age 0-12              Age 13-17

Did you experience fear, helplessness, or horror at what happened? yes / no

Inclusion of the Other in the Self



### Interpersonal Reactivity Index (Empathy)

*Strongly Disagree*      *Disagree*      *Neutral*      *Agree*      *Strongly Agree*  
1                      2                      3                      4                      5

1. I daydream and fantasize, with some regularity, about things that might happen to me.  
1 2 3 4 5
2. I often have tender, concerned feelings for people less fortunate than me.  
1 2 3 4 5
3. I sometimes find it difficult to see things from the "other guy's" point of view.  
1 2 3 4 5
4. Sometimes I don't feel sorry for other people when they are having problems.  
1 2 3 4 5
5. I really get involved with the feelings of the characters in a novel.  
1 2 3 4 5
6. In emergency situations, I feel apprehensive and ill-at-ease.  
1 2 3 4 5
7. I am usually objective when I watch a movie or play, and I don't often get completely caught up in it.  
1 2 3 4 5
8. I try to look at everybody's side of a disagreement before I make a decision.  
1 2 3 4 5
9. When I see someone being taken advantage of, I feel kind of protective toward them.  
1 2 3 4 5
10. I sometimes feel helpless when I am in the middle of a very emotional situation.  
1 2 3 4 5
11. I sometimes try to understand my friends better by imagining how things look from their perspective.  
1 2 3 4 5
12. Becoming extremely involved in a good book or movie is somewhat rare for me.  
1 2 3 4 5

13. When I see someone get hurt, I tend to remain calm.  
1 2 3 4 5
14. Other people's misfortunes do not usually disturb me a great deal.  
1 2 3 4 5
15. If I'm sure I'm right about something, I don't waste much time listening to other people's arguments.  
1 2 3 4 5
16. After seeing a play or movie, I have felt as though I were one of the characters.  
1 2 3 4 5
17. Being in a tense emotional situation scares me.  
1 2 3 4 5
18. When I see someone being treated unfairly, I sometimes don't feel very much pity for them.  
1 2 3 4 5
19. I am usually pretty effective in dealing with emergencies.  
1 2 3 4 5
20. I am often quite touched by things that I see happen.  
1 2 3 4 5
21. I believe that there are two sides to every question and try to look at them both.  
1 2 3 4 5
22. I would describe myself as a pretty soft-hearted person.  
1 2 3 4 5
23. When I watch a good movie, I can very easily put myself in the place of a leading character.  
1 2 3 4 5
24. I tend to lose control during emergencies  
1 2 3 4 5
25. When I'm upset at someone, I usually try to "put myself in his shoes" for a while.  
1 2 3 4 5

26. When I am reading an interesting story or novel, I imagine how I would feel if the events in the story were happening to me.

1 2 3 4 5

27. When I see someone who badly needs help in an emergency, I go to pieces.

1 2 3 4 5

28. Before criticizing somebody, I try to imagine how I would feel if I were in their place.

1 2 3 4 5

### The Stress Appraisal Measure

	1 Not at all	2 Slightly	3 Moderately	4 Considerably	5 Extremely
1. Does this situation create tension in me?				1 2 3 4 5	
2. Does this situation tax or exceed my coping resources?				1 2 3 4 5	
3. To what extent do I perceive this situation as stressful?				1 2 3 4 5	
4. To what extent does this event require coping efforts on my part?				1 2 3 4 5	

### Spielberger State Anxiety

	Almost Never	Sometimes	Often	Almost Always
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

### Positive and Negative Affect Schedule

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
Hapy	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

## Appendix B

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*. (Accepted).

### 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 & 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), so that enhancing social connectedness and social identity may attenuate depressive symptomatology (Cruwys et al., 2014, 2015). The experience of unsupportive social relationships, comprising negative or ineffective interactions, may have deleterious consequences for well-being, even when accounting for the positive effects of social support (Ingram et al., 1999; Song & Ingram, 2002). Although the beneficial effects of social support and the profound impact of unsupportive social interactions on well-being have consistently been reported, the biological mechanisms underlying their impact 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 assessed following its administration through a nasal spray (Bakermans-Kranenburg & van Ijzendoorn, 2013) as well as through analyses of such behaviors among individuals carrying a polymorphism (i.e., a common mutation in the sequence of nucleotides that make up a DNA strand) of a gene related to the oxytocin receptor (OXTR) (Kumsta &

Heinrichs, 2013). Variations in the gene coding for the OXTR, in particular, the single nucleotide polymorphism (SNP) rs53576, which involves a guanine (G) to adenine (A) substitution, has been associated with diminished prosocial behaviors. In essence, individuals with the GG genotype do not have the SNP present, whereas individuals with the AG or AA genotype have the SNP present on one or both alleles, respectively. In this regard, compared to individuals who were homozygous for the G allele, A carriers tended to be less empathetic (Rodrigues et al., 2009), displayed lower parental sensitivity (Bakermans-Kranenburg & van Ijzendoorn, 2008), and lower trust-related behaviors (Krueger et al., 2012). As well, this SNP has been associated with lower positive affect (Lucht et al., 2009), self-esteem and 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. This said, there have been several reports indicating that the heterozygotes (i.e., carrying the AG alleles) in some instances fell in line with the GG carriers (e.g. support seeking (Kim et al., 2010), and reporting positive affect, (Lucht et al., 2009)), but in relation to other phenotypes (e.g., cortisol responses associated with ostracism) fell in line with the AA carriers (McQuaid et al., 2015).

There have been several reports, however, that do not comfortably align with the perspective that the A allele of the OXTR rs53576 gene acts as a vulnerability factor related 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). Thus, it may be too simplistic to categorize OXTR alleles as ‘risk’ factors, and it was suggested 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 & 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 & Hsu, 2012) and presumably with the G allele (McQuaid et al., 2013, 2015), could promote the adoption or development of social coping methods (McQuaid et al., 2014b). 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).

Additionally, 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 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 & 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 & 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), and hence would be associated with greater vulnerability to the negative impacts of stressors relative to those with the G allele.

The positive role of social support in dealing with stressors and in relation to depression has been well established (Heinrichs et al., 2003; Taylor et al., 2000; Thoits, 2013). However, the impact of unsupportive relationships in this regard has received limited attention. Unsupportive interactions do not simply reflect the absence of social support, but instead refer to the receipt of a negative, upsetting, or ineffective social interaction with another individual when they are approached for help or advice during a

challenging or stressful time (Ingram, et al., 1999; Ingram, et al., 2001). Unsupportive responses from others include the minimization of problems, blaming the individual, distancing from an individual and their problems, and bumbling 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 & Ingram, 2002). 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. 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. In this regard, 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 have a more negative affect (and may be less sensitive to social interactions), were expected to 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  $\mu\text{L}$  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 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 & 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. Total mean scores of social support were used (Peers: Cronbach's  $\alpha = 0.87$ ; Parents: Cronbach's  $\alpha = 0.81$ ).

*Coping Styles.* The Survey of Coping Profile Endorsement (Matheson & 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. A principal component analysis (PCA) with a varimax rotation was conducted to determine the underlying factor structure of this scale. The PCA was performed on 13 subscales based on earlier studies (Matheson & Anisman, 2003) and were included on a factor when loadings were greater than .40. Three factors emerged which encompassed emotion-, avoidant- and problem-focused coping. The factor loadings were similar to that of previous findings (McQuaid et al., 2014a; Raspopow et al., 2013) and Cronbach's alphas for the three factors confirmed that they were well-constructed. Emotion-focused coping comprised rumination, 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 the OXTR and sex 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). Standardized scores were used for all regression analyses.

### **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 ( $t(1, 223) = -0.38, p = .70$ ). Analyses were also conducted to determine if any of the variables of interest varied as a function of sex. 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.

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 ( $r = -.43, p < .001$ ).

*Parental support and unsupport.* It was of interest to examine the influence of the 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 the 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 the OXTR moderated the association between both problem- and emotion-focused coping on depressive symptoms were found not to be significant.

Although the 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 the OXTR genotype in the context of unsupportive social interactions. Analyses were performed to determine the moderating influence of the 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,  $b = 0.23$ ,  $t = 1.94$ ,  $p = .05$ . In particular, 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.

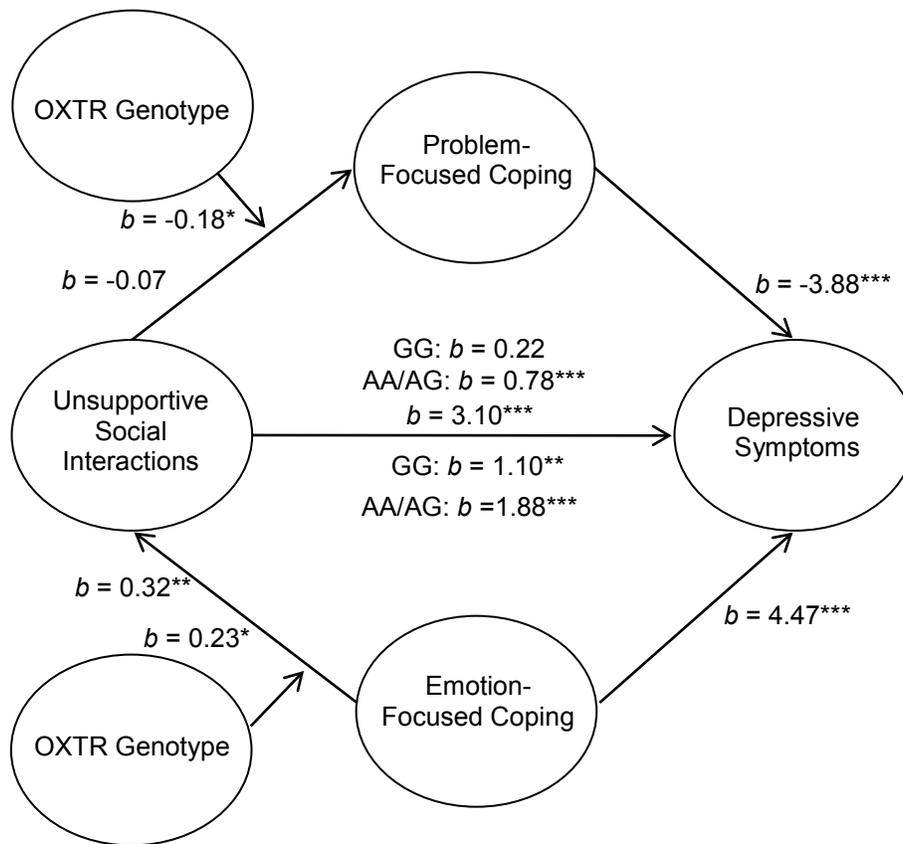
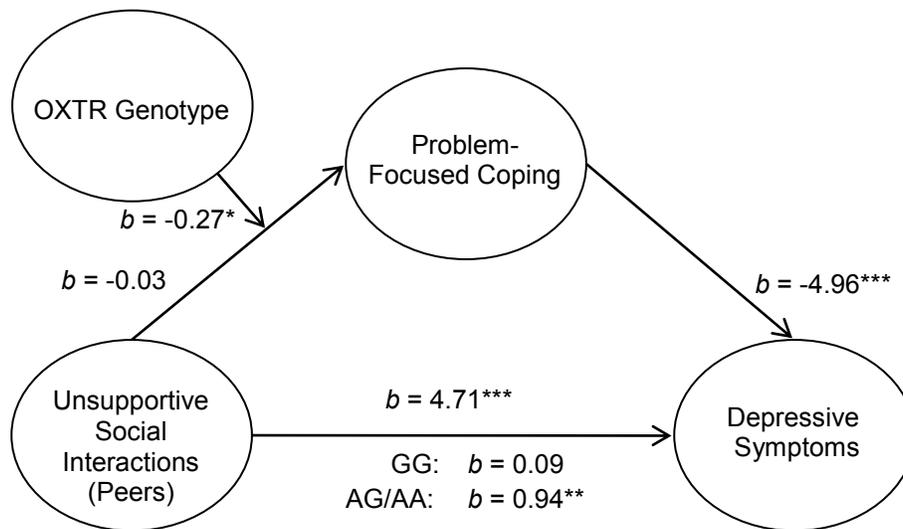


Figure 1. Schematic of the moderated multiple mediation analyses examining parental unsupport. The relation between unsupportive social interactions from parents and depressive symptoms through problem-focused coping was moderated by OXTR rs53576 genotype, such that it was only significant among A carriers. 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.  $*p \leq .05$ ,  $**p < .01$ ,  $***p < .001$ .

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

## **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. In addition to the contribution of problem-focused coping, the current findings indicated that the adoption of emotion-focused coping in association with perceived unsupportive responses from parents was tied to greater depressive symptoms, and this relation 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 & 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 analyses indicated that this was the case irrespective of whether or not AG carriers were pooled with the AA genotype for statistical analyses, although

the small number of AA individuals makes it necessary to have further validation in relation to the link (or lack of it) to social support.

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., 2014a; Raspopow et al., 2013), and it was further apparent that this relationship was still more pronounced among individuals carrying an A allele. 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. For example, we observed that G

carriers were more sensitive to the effects of an acute experience of social ostracism (McQuaid et al., 2015), although it is uncertain whether these same individuals would be more likely to adopt social support seeking as a primary coping strategy.

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 & 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., 2015). This said, 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 & 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 & 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.

It should also be noted that the sample largely comprised females (approximately 80%) and as such, the generalizability of these findings to males is uncertain. To be sure, 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. 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. For instance, 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), although 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.

## References

- Aldao, A., Nolen-Hoeksema, S., and Schweizer, S. (2010). Emotion-regulation strategies across psychopathology: A meta-analytic review. *Clin. Psychol. Rev.* 30, 217-237.
- Bakermans-Kranenburg, M. J., and Van IJzendoorn, M. H. (2014). A sociability gene? Meta-analysis of oxytocin receptor genotype effects in humans. *Psychiatr. Genet.* 24, 45-51.
- Bakermans-Kranenburg, M. J., and Van IJzendoorn, M. H. (2013). Sniffing around oxytocin: review and meta-analyses of trials in healthy and clinical groups with implications for pharmacotherapy. *Transl. Psychiatry.*, 3, e258.
- Bakermans-Kranenburg, M. J., and van IJzendoorn, M. H. (2008). Oxytocin receptor (OXTR) and serotonin transporter (5-HTT) genes associated with observed parenting. *Soc. Cogn. Affect. Neurosci.*, 3, 128-134.
- Beck, A.T., Ward, C.H., Mendelson, M., Mock, J., and Erbaugh, J. (1961). An inventory for measuring depression. *Arch. Gen. Psychiatry.*, 4, 561-571.
- Belsky, J., Jonassaint, C., Pluess, M., Stanton, M., Brummett, B., and Williams, R. (2009). Vulnerability genes or plasticity genes? *Mol. Psychiatry*, 14, 746-754.
- Belsky, J., and Pluess, M. (2009). Beyond diathesis stress: Differential susceptibility to environmental influences. *Psychol Bull.*, 135, 885-908.
- Bradley, B., Westen, D., Mercer, K., Binder, E.B., Jovanovic, T., Crain, D., Wingo, A., and Heim, C. (2011). Association between childhood maltreatment and adult emotional dysregulation in a low-income, urban, African American sample: moderation by oxytocin receptor gene. *Dev. Psychopathol.*, 23, 439-452.

- Bradley, B., Davis, T.A., Wingo, A.P., Mercer, K.B., and Ressler, K.J. (2013). Family environment and adult resilience: contributions of positive parenting and the oxytocin receptor gene. *Eur J Psychotraumatol.*, 4, 21659.
- Buchheim, A., Heinrichs, M., George, C., Pokorny, D., Koops, E., Henningsen, P., O'Connor, M.F. and Gündel, H. (2009). Oxytocin enhances the experience of attachment security. *Psychoneuroendocrinology*, 34, 1417-1422.
- Chen, F.S., Kumsta, R., von Dawans, B., Monakhov, M., Ebstein, R.P. and Heinrichs, M. (2011). Common oxytocin receptor gene (OXTR) polymorphism and social support interact to reduce stress in humans. *Proc. Natl. Acad. Sci. U S A.*, 108, 19937-19942.
- Cohen S, and Willis TA (1985). Stress, social support, and the buffering hypothesis. *Psychol Bull.*, 98, 310-357.
- Cruwys, T., Haslam, S. A., Dingle, G. A., Haslam, C., and Jetten, J. (2015). Depression and social identity an integrative review. *Pers. Soc. Psychol. Rev*, 1088868314523839.
- Cruwys, T., Haslam, S. A, Dingle, G. A., Jetten, J., Hornsey, M. J., Chong, D. E. M., & Oei, T. P. (2014). Feeling connected again: Interventions that increase social identification reduce depression symptoms in community and clinical settings. *J. Affect. Disord.*, 159, 139-146.
- Cutrona, C. E., Russell, D. W., (1987). The provisions of social relationships and

- adaptation to stress. *Advances in personal relationships*, 1, pp. 37-67. Greenwich, Conn: JAI Press.
- De Dreu, C.K., Greer, L.L., Handgraaf, M.J., Shalvi, S., Van Kleef, G.A., Baas, M., Ten Velden, F.S., Van Dijk, E. and Feith, S.W. (2010). The neuropeptide oxytocin regulates parochial altruism in intergroup conflict among humans. *Science*, 328, 1408-1411.
- Ditzen, B., Schaer, M., Gabriel, B., Bodenmann, G., Ehlert, U., and Heinrichs, M. (2009). Intranasal oxytocin increases positive communication and reduces cortisol levels during couple conflict. *Biol. Psychiatry*, 65, 728-731.
- Heinrichs, M., Baumgartner, T., Kirschbaum, C., and Ehlert, U., (2003). Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biol. Psychiatry.*, 54, 1389-1398.
- Holt-Lunstad, J., Smith, T. B., and Layton, J. B. (2010). Social relationships and mortality risk: a meta-analytic review. *PLoS Medicine*, 7, e1000316.
- Hostinar, C. E., Cicchetti, D., and Rogosch, F. A., (2014). Oxytocin receptor gene polymorphism, perceived social support, and psychological symptoms in maltreated adolescents. *Dev. Psychopathol.*, 26, 465-477.
- House, J. S., Landis, K. R., and Umberson, D. (1988). Social relationships and health. *Science*, 241, 540-545.
- Ingram, K.M., Betz, N.E., Mindes, E.J., Schmitt, M.M., and Smith, N.G. (2001). Unsupportive responses from others concerning a stressful life event: Development of the unsupportive social interactions inventory. *J. Soc. Clin.*

*Psychol.*, 20, 173-207.

- Ingram, K. M., Jones, D. A., Fass, R. J., Neidig, J. L., and Song, Y. S. (1999). Social support and unsupportive interactions: their association with depression among people living with HIV. *AIDS Care*, 11, 313-329.
- Inoue, T., Kimura, T., Azuma, C., Inazawa, J., Takemura, M., Kikuchi, T., Kubota, Y., Ogita, K., and Saji, F., (1994). Structural organization of the human oxytocin receptor gene. *J. Bio. Chem.* 269, 32451-32456.
- Kim, H.S., Sherman, D.K., Sasaki, J.Y., Xu, J., Chu, T.Q., Ryu, C., Suh, E.M., Graham, K., and Taylor, S.E. (2010). Culture, Distress and oxytocin receptor polymorphism (OXTR) interact to influence emotional support seeking. *Proc. Natl. Acad. Sci. U S A.*, 107, 15717-15721.
- Kosfeld, M., Heinrichs, M., Zak, P.J., Fischbacher, U., and Fehr, E. (2005). Oxytocin increases trust in humans. *Nature*, 435, 673-676.
- Krueger, F., Parasuraman, R., Iyengar, V., Thornburg, M., Weel., J., Lin, M., Clarke, E., McCabe, K., and Lipsky, R.H. (2012). Oxytocin receptor genetic variation promotes human trust behavior. *Front Hum Neurosci*, 6.
- Kumsta, R., and Heinrichs, M. (2013). Oxytocin, stress and social behavior: neurogenetics of the human oxytocin system. *Curr. Opin. Neurobiol.*, 23, 11-16.
- Lucht, M. J., Barnow, S., Sonnenfeld, C., Rosenberger, A., Grabe, H. J., Schroeder, W., Völzke, H., Freyberger, H.J., Herrmann, F.H., Kroemer, H., and Roszkopf, D. (2009). Associations between the oxytocin receptor gene (OXTR) and affect,

- loneliness and intelligence in normal subjects. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 33, 860-866.
- Matheson, K., Anisman, H., 2003. Systems of coping associated with dysphoria, anxiety and depressive illness: a multivariate profile perspective. *Stress*. 6, 223-234.
- McQuaid, R. J., Bombay, A., McInnis, O. A., Matheson, K., and Anisman, H., (2014a). Childhood adversity, perceived discrimination, and coping strategies in relation to depressive symptoms among First Nations adults in Canada: The moderating role of unsupportive social interactions from ingroup and outgroup members. *Cultur. Divers. Ethnic. Minor. Psychol.*, E-pub ahead of print.
- McQuaid, R.J., McInnis, O.A., Abizaid, A., and Anisman, H., (2014b). Making room for oxytocin in understanding depression. *Neurosci. Biobehav. Rev.*, 45, 305-322.
- McQuaid, R.J., McInnis, O.A., Stead, J.D., Matheson, K., and Anisman, H. (2013). A paradoxical association of an oxytocin receptor gene polymorphism: early-life adversity and vulnerability to depression. *Front Neurosci*, 7.
- McQuaid, R.J., McInnis, O.A., Matheson, K., and Anisman, H. (2015). Distress of ostracism: oxytocin receptor gene polymorphism confers sensitivity to social exclusion. *Soc. Cogn. Affect. Neurosci.*, E-pub ahead of print.
- Mizumoto, Y., Kimura, T., and Iveil, R. (1997). A genomic element within the third intron of the human oxytocin receptor gene may be involved in transcriptional suppression. *Mol. Cell. Endocrinol.* 135, 129-138.
- Ohashi, J., and Tokunaga, K. (2001). The power of genome-wide association studies of

- complex disease genes: statistical limitations of indirect approaches using SNP markers. *J. Hum. Genet.*, 46, 478-482.
- Preacher, K.J., Rucker, D.D., and Hayes, A.F. (2007). Addressing moderated mediation hypotheses: theory, methods, and prescriptions. *Multivar. Behav. Res.*, 42, 185-227.
- Raspopow, K., Matheson, K., Abizaid, A., and Anisman, H. (2013). Unsupportive social interactions influence emotional eating behaviors. The role of coping styles as mediators. *Appetite*, 62, 143-149.
- Ravindran, A. V., Matheson, K., Griffiths, J., Merali, Z., and Anisman, H. (2002). Stress, coping, uplifts, and quality of life in subtypes of depression: a conceptual frame and emerging data. *J. Affect. Dis.*, 71, 121-130.
- Rodrigues, S. M., Saslow, L. R., Garcia, N., John, O. P., and Keltner, D. (2009). Oxytocin receptor genetic variation relates to empathy and stress reactivity in humans. *Proc. Natl. Acad. Sci. U S A*, 106, 21437-21441.
- Saphire-Bernstein, S., Way, B.M., Kim, H.S., Sherman, D.K., and Taylor, S.E. (2011). Oxytocin receptor gene (OXTR) is related to psychological resources. *Proc. Natl. Acad. Sci. U S A*, 108, 15118-15122
- Song, Y.S., and Ingram, K.M. (2002). Unsupportive social interactions, availability of social support, and coping: Their relationship to mood disturbance among African Americans living with HIV. *J. Soc. Pers. Relat.*, 19, 67-85.
- Taylor, S. E., Klein, L. C., Lewis, B. P., Gruenewald, T. L., Gurung, R. A., and

- Updegraff, J. A. (2000). Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. *Psychol. Rev.*, 107, 411-429.
- Thoits, P.A. (2011). Mechanisms linking social ties and support to physical and mental health. *J. Health Soc. Behav.*, 52, 145-161.
- Thoits, P. A. (2013). Self, identity, stress, and mental health. In Handbook of the sociology of mental health (pp. 357-377). Springer Netherlands.
- Thompson, S. M., Hammen, C., Starr, L. R., and Najman, J. M. (2014). Oxytocin receptor gene polymorphism (rs53576) moderates the intergenerational transmission of depression. *Psychoneuroendocrinology*, 43, 11-19.
- van Roekel, E., Verhagen, M., Scholte, R. H., Kleinjan, M., Goossens, L., and Engels, R. C., (2013). The oxytocin receptor gene (OXTR) in relation to state levels of loneliness in adolescence: evidence for micro-level gene-environment interactions. *PloS One*, 8, e77689.
- Wilkinson, R. B. (2004). The role of parental and peer attachment in the psychological health and self-esteem of adolescents. *J. Youth Adolesc.*, 33, 479-493.

## Appendix C

McInnis, O.A., McQuaid, R.J., Matheson, K., & Anisman, H. (2015). Experience-dependent effects of genes: Responses 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, Torrissi, 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.

## References

- Abizaid, A., Luheshi, G., & Woodside, B.C. (2013). Interaction between immune and energy balance signals in the regulation of feeding and metabolism. In A. Kusnecov & H. Anisman (Eds.), *Handbook of Psychoneuroimmunology* (pp. 488-503). London: Wiley-Blackwell.
- Aguilera, M., Arias, B., Wichers, M., Barrantes-Vidal, N., Moya, J., Villa, H., ... Fañanás, L. (2009). Early adversity and 5-HTT/BDNF genes: New evidence of gene-environment interactions on depressive symptoms in a general population. *Psychological Medicine*, *39*, 1425-1432.
- Anisman, H., & Matheson, K. (2005). Stress, anhedonia and depression: Caveats concerning animal models. *Neuroscience & Biobehavioral Reviews*, *29*, 525-546.
- Anisman, H., Hayley, S., & Merali, Z. (2003). Cytokines and stress: Sensitization and cross-sensitization. *Brain, Behavior, and Immunity*, *17*, 86-93.
- Anisman, H., Merali, Z., & Hayley, S. (2008). Neurotransmitter, peptide and cytokine processes in relation to depressive disorder: Comorbidity of depression with neurodegenerative disorders. *Progress in Neurobiology*, *85*, 1-74.
- Arango, V., Huang, Y.-y., Underwood, M.D., & Mann, J.J. (2003). Genetics of the serotonergic system in suicidal behavior. *Journal of Psychiatric Research*, *37*, 375-386.
- Belsky, J., Jonassaint, C., Pluess, M., Stanton, M., Brummett, B., & Williams, R. (2009). Vulnerability genes or plasticity genes? *Molecular Psychiatry*, *14*, 746-754.

- Bergman, K., Sarkar, P., O'Connor, T.G., Modi, N. & Glover, V. (2007). Maternal stress during pregnancy predicts cognitive ability and fearfulness in infancy. *Journal of the American Academy of Childhood and Adolescent Psychiatry*, *46*, 1454-1463.
- Beydoun, H., & Saftlas, A.F. (2008). Physical and mental health outcomes of prenatal maternal stress in human and animal studies: A review of recent evidence. *Paediatric and Perinatal Epidemiology*, *22*, 438-466.
- Bland, S.T., Tamlyn, J.P., Barrientos, R.M., Greenwood, B.N., Watkins, L.R., Campeau, S., & Maier, S.F. (2007). Expression of fibroblast growth factor-2 and brain-derived neurotrophic factor mRNA in the medial prefrontal cortex and hippocampus after uncontrollable or controllable stress. *Neuroscience*, *144*, 1219-1228.
- Bradley, B., Westen, D., Mercer, K.B., Binder, E.B., Jovanovic, T., Crain, D.,... Heim, C. (2011). Association between childhood maltreatment and adult emotional dysregulation in a low-income, urban, African American sample: Moderation by oxytocin receptor gene. *Development and Psychopathology*, *23*, 439-452.
- Caldwell, W., McInnis, O.A., McQuaid, R.J., Liu, G., Stead, J.D., Anisman, H., & Hayley, S. (2013). The role of the Val66Met polymorphism of the brain derived neurotrophic factor gene in coping strategies relevant to depressive symptoms. *PLoS One*, *8*, e65547.
- Carver, C.S., Johnson, S.L., Joormann, J., Lemoult, J., & Cuccaro, M.L. (2011). Childhood adversity interacts separately with 5-HTTLPR and BDNF to predict lifetime depression diagnosis. *Journal of Affective Disorders*, *132*, 89-93.
- Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., ... Poulton,

- R. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science*, *301*, 386-389.
- Champagne, F.A. (2010). Epigenetic influence of social experiences across the lifespan. *Developmental Psychobiology*, *52*, 299-311.
- Chen, F.S., Kumsta, R., von Dawans, B., Monakhov, M., Ebstein, R.P., & Heinrichs, M. (2011). Common oxytocin receptor gene (OXTR) polymorphism and social support interact to reduce stress in humans. *Proceedings of the National Academy of Sciences*, *108*, 19937-19942.
- Cirulli, F., Francia, N., Berry, A., Aloe, L., Alleva, E., & Suomi, S.J. (2009). Early life stress as a risk factor for mental health: Role of neurotrophins from rodents to non-human primates. *Neuroscience and Biobehavioral Reviews*, *33*, 573-585
- Clasen, P.C., Wells, T.T., Knopik, V.S., McGeary, J.E., & Beevers, C.G. (2011). 5-HTTLPR and BDNF Val66Met polymorphisms moderate effects of stress on rumination. *Genes, Brain and Behavior*, *10*, 740-746.
- Covington, H.E., III, Maze, I., LaPlant, Q.C., Vialou, V.F., Ohnishi, Y.N., Berton, O., ... Nestler, E.J. (2009). Antidepressant actions of histone deacetylase inhibitors. *Journal of Neuroscience*, *29*, 11451-11460.
- Davis, E.P., Waffarn, F., & Sandman, C.A. (2011). Prenatal treatment with glucocorticoids sensitizes the HPA axis response to stress among full-term infants. *Developmental Psychobiology*, *53*, 175-183.

- Dolinoy, D.C., Huang, D., & Jirtle, R.L. (2007). Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. *Proceedings of the National Academy of Sciences, 104*, 13056–13061.
- Dube, S.R., Felitti, V.J., Dong, M., Giles, W.H., & Anda, R.F. (2003). The impact of adverse childhood experiences on health problems: Evidence from four birth cohorts dating back to 1900. *Preventative Medicine, 37*, 268–277.
- Duman, R.S., & Monteggia, L.M. (2006). A neurotrophic model for stress-related mood disorders. *Biological Psychiatry, 59*, 1116-1127.
- Egan, M., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., ...Weinberger, D.R. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell, 112*, 257-269.
- Entringer, S., Kumsta, R., Nelson, E.L., Hellhammer, D.H., Wadhwa, P.D., & Wüst, S. (2008). Influence of prenatal psychosocial stress on cytokine production in adult women. *Developmental Psychobiology, 50*, 579-587.
- Glover, V. (2011). Prenatal stress and the origins of psychopathology: An evolutionary perspective. *Journal of Child Psychology and Psychiatry, 52*, 356-367.
- Guzmán, Y.F., Tronson, N.C., Jovasevic, V., Sato, K., Guedea, A.L., Mizukami, H., ... Radulovic, J. (2013). Fear-enhancing effects of septal oxytocin receptors, *Nature Neuroscience, 16*, 1185-1187.

- Hajek, T., Kopecek, M., & Höschl, C. (2012). Reduced hippocampal volumes in healthy carriers of brain-derived neurotrophic factor Val66Met polymorphism: Meta-analysis. *World Journal of Biological Psychiatry, 13*, 178-187.
- Heim, C., Young, L.J., Newport, D.J., Mletzko, T., Miller, A.H., & Nemeroff, C.B. (2008). Lower CSF oxytocin concentrations in women with a history of childhood abuse. *Molecular Psychiatry, 14*, 954-958.
- Heinrichs, M., Baumgartner, T., Kirschbaum, C., & Ehlert, U. (2003). Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biological Psychiatry, 54*, 1389-1398.
- Huizink, A.C., Mulder, E. J.H., & Buitelaar, J.K. (2004). Prenatal stress and risk for psychopathology: Specific effects or induction of general susceptibility? *Psychological Bulletin, 130*, 115-142.
- Jetten, J., Haslam, C., & Haslam, S.A. (Eds.). (2012). *The social cure: Identity, health and well-being*. New York: Psychology Press.
- Karg, K., Burmeister, M., Shedden, K., & Sen, S. (2011). The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: Evidence of genetic moderation. *Archives of General Psychiatry, 68*, 444-454.
- Kinnally, E.L., Capitanio, J.P., Leibel, R., Deng, L., LeDuc, C., Haghghi, F., & Mann, J.J. (2010). Epigenetic regulation of serotonin transporter expression and behavior in infant rhesus macaques. *Genes, Brain, and Behavior, 9*, 575-582.
- Kinney, D.K., Munir, K.M., Crowley, D.J., & Miller, A.M. (2008). Prenatal stress and risk for autism. *Neuroscience and Biobehavioral Reviews, 32*, 1519-1532.

- Kirsch, P., Esslinger, C., Chen, Q., Mier, D., Lis, S., Siddhanti, S.,... Meyer-Lindenberg, A. (2005). Oxytocin modulates neural circuitry for social cognition and fear in humans. *Journal of Neuroscience*, 7, 11489-11493.
- Labonté, B., Suderman, M., Maussion, G., Lopez, J.P., Navarro-Sánchez, L., Yerko, V., Mechawar, N., Szyf, M., Meaney, M.J., Turecki, G. (2013). Genome-wide methylation changes in the brains of suicide completers. *American Journal of Psychiatry*, 170, 511-520.
- Leshem, M., & Schulkin, J. (2011). Transgenerational effects of infantile adversity and enrichment in male and female rats. *Developmental Psychobiology*, 54, 169-186.
- Luby, J.L., Barch, D.M., Belden, A., Gaffrey, M.S., Tillman, R., Babb C, .... & Botteron, K.N. (2012). Maternal support in early childhood predicts larger hippocampal volumes at school age. *Proceedings of the National Academy of Sciences*, 109, 1027-1041.
- Matthews, S.G., Owen, D., Kalabis, G., Banjanin, S., Setiawan, E.B., Dunn, E.A. & Andrews, M.H. (2004). Fetal glucocorticoid exposure and hypothalamo-pituitary-adrenal (HPA) function after birth. *Endocrine Research*, 30, 827-836.
- McEwen, B. S. (2000). Allostasis and allostatic load: Implications for neuropsychopharmacology. *Neuropsychopharmacology*, 22, 108-124.
- McGowan, P.O., Sasaki, A., D'Alessio, A.C., Dymov, S., Labonté, B., Szyf, M., ... Meaney, M.J. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nature Neuroscience*, 12, 342-348.

- McQuaid, R.J., McInnis, O.A., Stead, J.D., Matheson, K., & Anisman, H. (2013). A paradoxical association of the oxytocin receptor gene polymorphism: Early-life adversity and vulnerability to depression. *Frontiers in Neuroscience*, 7, 128.
- Meinlschmidt, G., & Heim, C. (2007). Sensitivity to intranasal oxytocin in adult men with early parental separation. *Biological Psychiatry*, 61, 1109-1111.
- Meyer-Lindenberg, A., Domes, G., Kirsch, P., & Heinrichs, M. (2011). Oxytocin and vasopressin in the human brain: Social neuropeptides for translational medicine. *Nature Reviews Neuroscience*, 12, 524-538.
- Morgan, C.P., & Bale, T.L. (2011). Early prenatal stress epigenetically programs dysmasculinization in second-generation offspring via the paternal lineage. *Journal of Neuroscience*, 31, 11748-11755.
- Mueller, B.R., & Bale, T.L. (2008). Sex-specific programming of offspring emotionality after stress early in pregnancy. *Journal of Neuroscience*, 28, 9055-9065.
- Nielsen, N.M., Hansen, A.V., Simonsen, J., & Hviid, A. (2011). Prenatal stress and risk of infectious diseases in offspring. *American Journal of Epidemiology*, 173, 990-997.
- Oberlander, T.F., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., & Devlin, A.M. (2008). Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics*, 3, 97-106.
- Perea, C.S., Paternina, A.C., Gomez, Y., & Lattig, M.C. (2012). Negative affectivity moderated by BDNF and stress response. *Journal of Affective Disorders*, 136, 767-774.

- Petronis, A. (2010). Epigenetics as a unifying principle in the aetiology of complex traits and diseases. *Nature*, *465*, 721-727.
- Pierrehumbert, B., Torrisi, R., Laufer, D., Halfon, O., Ansermet, F., & Popovic, B.M. (2011). Oxytocin response to an experimental psychosocial challenge in adults exposed to traumatic experiences during childhood or adolescence. *Neuroscience*, *166*, 168-177.
- Pierrehumbert, B., Torrisi, R., Ansermet, F., Borghini, A., & Halfon, O. (2012). Adult attachment representations predict cortisol and oxytocin responses to stress. *Attachment & Human Development*, *14*, 453-576.
- Poulter, M.O., Du, L., Weaver, I.C., Palkovits, M., Faludi, G., Merali, Z., ... Anisman, H. (2008). GABAA receptor promoter hypermethylation in suicide brain: Implications for the involvement of epigenetic processes. *Biological Psychiatry*, *64*, 645-652.
- Rice, F., Harold, G.T., Boivin, J., van den Bree, M., Hay, D.F., & Thapar, A. (2010). The links between prenatal stress and offspring development and psychopathology: Disentangling environmental and inherited influences. *Psychological Medicine*, *40*, 335-345.
- Rodrigues, A.J., Leão, P., Carvalho, M., Almeida, O.F., & Sousa, N. (2011). Potential programming of dopaminergic circuits by early life stress. *Psychopharmacology*, *214*, 107-120.
- Roth, T.L., Lubin, F.D., Funk, A. J., & Sweatt, J.D. (2009). Lasting epigenetic influence of early-life adversity on the BDNF gene. *Biological Psychiatry*, *65*, 760-769.
- Sánchez, M.M, Ladd, C.O., Plotsky, P.M. (2001). Early adverse experience as a

- developmental risk factor for later psychopathology: Evidence from rodent and primate models. *Developmental Psychopathology*, *13*, 419-449.
- Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, *21*, 55-89.
- Shalev, I., Lerer, E., Israel, S., Uzefovsky, F., Gritsenko, I., Mankuta, D., Ebstein, R.P., & Kaitz, M. (2009). BDNF Val66Met polymorphism is associated with HPA axis reactivity to psychological stress characterized by genotype and gender interactions. *Psychoneuroendocrinology*, *34*, 382-388.
- Shonkoff, J.P., Boyce, W.T., & McEwen, B.S. (2009). Neuroscience, molecular biology, and the childhood roots of health disparities: Building a new framework for health promotion and disease prevention. *Journal of the American Medical Association*, *301*, 2252-2259.
- Skilbeck KJ, Johnston GA, Hinton T. (2010). Stress and GABA receptors. *Journal of Neurochemistry*, *112*, 1115-1130.
- Talge, N. M., Neal, C., & Glover, V. (2007). Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? *Journal of Child Psychology and Psychiatry*, *48*, 245-261.
- Taylor, S.E. (2006). Tend and befriend: Biobehavioral bases of affiliation under stress. *Psychological Science*, *15*, 273- 277.
- Taylor, S.E., Saphire-Bernstein, S., & Seeman, T.E. (2010). Are plasma oxytocin in women and plasma vasopressin in men biomarkers of distressed pair-bond

- relationships? *Psychological Science*, 21, 3-7.
- Taylor, S.E. & Stanton, A.L. (2007). Coping resources, coping processes, and mental health. *Annual Review of Clinical Psychology*, 3, 377-401.
- Tops, M., van Peer, J.M., Wester, A.E., Wijers, A.A., & Korf, J. (2006). State-dependent regulation of cortical activity by cortisol: An EEG study. *Neuroscience Letters*, 404, 39-43.
- Wankerl, M., Wüst, S., & Otte, C. (2010). Current developments and controversies: Does the serotonin transporter gene-linked polymorphic region (5-HTTLPR) modulate the association between stress and depression? *Current Opinion in Psychiatry*, 23, 582-587.
- Weinstock, M. (2005). The potential influence of maternal stress hormones on development and mental health of the offspring. *Brain Behavior & Immunity*, 19, 296-308.
- Yoshimura, R., Kishi, T., Suzuki, A., Umene-Nakano, W., Ikenouchi-Sugita, A., Hori, H., ... Nakamura, J. (2011). The brain-derived neurotrophic factor (BDNF) polymorphism Val66Met is associated with neither serum BDNF level nor response to selective serotonin reuptake inhibitors in depressed Japanese patients. *Progress in Neuro-psychopharmacology & Biological Psychiatry*, 35, 1022-1025.
- Zou, Y.F., Ye, D.Q., Feng, X.L., Su, H., Pan, F.M., & Liao, F.F. (2010). Meta-analysis of BDNF Val66Met polymorphism association with treatment responses in patients with major depressive disorder. *European Neuropsychopharmacology*, 20, 535-544.