OXYTOCIN EFFECTS ON HUMAN AFFECT AND COGNITION

A Thesis

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Allison Elizabeth Gaffey

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Michelle M. Wirth, Director

Graduate Program in Psychology
Notre Dame, Indiana
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The hormone oxytocin (OT) has been associated with stress-reduction and social affiliation, and influences cognition and memory. Intranasal OT is used to manipulate OT in the brain to study the hormone’s effects. However, extant literature associating OT with cognition is inconsistent, few studies have examined OT and cognition in women, and it is unclear whether OT exerts global or targeted cognitive effects. In a double-blind design, forty-two women received 24 I.U. intranasal OT or saline before completing an N-back working memory task and viewing stimuli from an emotional memory task. Forty-eight hours later, participants completed the N-back again and were tested on memory of the emotional pictures. Saliva samples were also provided to examine OT’s effects on other hormones. OT inhibited working memory and did not affect emotional memory. Hormone analyses revealed OT increased cortisol and progesterone, and decreased testosterone.
To Marcia and Michael for my first introduction to oxytocin.
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CHAPTER 1:

INTRODUCTION

1.1 Overview

As a woman approaches you at a party, her name lingers on the tip of your tongue. Sarah? No. Megan? Kate? A flush of embarrassment creeps over you as you frantically search for her name. But what if you could take a hormone to enhance your social memory and decrease your anxiety? The peptide hormone oxytocin (OT) is hypothesized to perform these and many other functions. OT is associated with stress-reduction, increases due to affiliation/bonding, and influences social cognition and memory (Campbell, 2010). These effects have been well documented in animals. However, we do not currently understand how OT’s connection with social affiliation and stress affects human cognition and emotion regulation. Clarifying OT’s associations with affiliation and other effects will further knowledge of the hormone’s effective use.

OT is synthesized by magnocellular neurons in the paraventricular nucleus of the hypothalamus (PVN), the supranoptic nucleus (SON) and outside of the hypothalamus in the stria terminalis and medial amygdala (de Vries & Miller, 1998; de Vries & Panzica, 2006; Nelson, 2005). OT produced by PVN and SON neurons is released from the posterior pituitary into the bloodstream. Neurons elsewhere in the brain (e.g., in the amygdala) also use OT as neuromodulators. Peripheral sources of OT include the uterus,
placenta, amnion, testes, heart, and corpus luteum in the ovary (Nelson, 2005). Thus, there are distinct “blood” and “brain” hormone sources of OT.

OT’s most evolutionarily ancient purposes include salt/water balance (i.e. blood osmolarity) and smooth muscle contraction. From these earlier functions, the hormone was likely incorporated into present roles in parental and pair bonding. For example, OT is essential for forming selective partner preferences and instigating parental behavior in prairie voles (a rodent species; Carter, 1998). In mammals, OT activates uterus contractions during birth and initiates lactation (i.e., milk letdown) (Nelson, 2005). Infant suckling also triggers OT release in various brain regions in sheep and rodent mothers (Kendrick et al., 1988; Nelson & Panksepp, 1998; Neumann et al., 1993). High levels of OT are required for a mother sheep to learn to recognize and bond with her offspring (Becker & Breedlove, 2002). Below is an outline of how each of OT’s functions, informed by animal research, is also essential to humans.

1.2 Oxytocin, Stress and the HPA axis

Because OT increases during stress, it may be considered a stress hormone (Bartz & Hollander, 2006; Carter & Altemus, 1997). Consequently, OT affects several aspects of the stress response. Studies in lab animals have shown that OT administration tends to decrease physiological and behavioral aspects of stress-induced neuronal activation and hypothalamic pituitary adrenal (HPA) axis activity (Campbell, 2010). OT may exert stress-dampening effects both by preventing excessive HPA activity/glucocorticoid (GC) levels and by lowering anxiety. Numerous studies in mice
and rats have illustrated OT’s anxiolytic influence (Mantella et al., 2005; Blume et al., 2008; Windle et al., 1997). For example, OT knockout mice (i.e., mice missing the gene for OT or OT receptors) display more anxiety, higher corticosterone (i.e., the rodent form of cortisol), and higher markers of neural activity in stress and anxiety-related brain regions (e.g., the amygdala) after exposure to a stressor (Amico et al., 2004; Mantella et al., 2004). In rodents OT’s stress-reduction effects (reduced HPA activity and reduced anxiety behavior) have been localized to the central amygdala and the PVN (Neumann et al., 2000; Neumann, et al., 2002; Bale et al., 2001; Blume et al., 2008). These pathways may play an important role in determining OT’s effects on HPA axis activity and anxiety, and delineating other behaviors associated with OT.

Similar to studies in animals, research suggests that OT may also down-regulate stress and HPA axis activity in humans (Bartz & Hollander, 2006; Ditzen et al., 2007; Ditzen et al., 2009). Numerous studies have demonstrated that OT administration decreases ACTH and cortisol responses to stress (Chiodera and Coiro, 1987; Ditzen et al., 2007; Ditzen et al., 2009; Heinrichs et al., 2003; Legros et al., 1982, 1984; Suh et al., 1986). For example, Heinrichs and colleagues (2003) randomly assigned men to receive exogenous OT or placebo, and social support or no support, before undergoing acute stress. Compared to controls, men who received both OT and social support had lower salivary cortisol levels after stress. Therefore, OT and behaviors associated with OT (i.e., affiliation) may be influential in buffering against the negative effects of stress. As in the Heinrichs et al (2003) study, researchers have examined HPA activity and other OT
effects in humans by administering OT intranasally. Administration of small doses of OT (20, 24 or 40 International Units or I.U.) has proved to be a safe and effective tool for exploring the effects of OT on cognition and behavior (MacDonald et al., 2011). Notably, small peptides administered intranasally do enter the brain, and may initiate central and peripheral responses (Born et al., 2002; Illum, 2000). This method is particularly exciting as we can actually directly manipulate OT in the brain, to test effects on human cognition. Thus, intranasal OT provides causal information unavailable by merely measuring OT levels and observing how those levels correlate with stress and/or cognition (e.g., memory).

Research examining OT’s effects on stress humans was originally conducted in lactating women. During lactation, a time when circulating OT levels are high, women exhibit lower adrenocorticotropic hormone (ACTH; a hormonal index of HPA activity), and attenuated cortisol and glucose responses to physical stress compared to non-lactating controls (Altemus et al., 1995; Bartz & Hollander, 2006). In one study, lactating women were randomly assigned to either hold or breast-feed their infant for 15 minutes before undergoing psychosocial stress. While cortisol and ACTH significantly increased in both groups in response to stress, women who breast-fed before stress had significantly lower post-stress total (assessed in blood plasma) and free (assessed in saliva) cortisol compared to those who only held their infant before stress (Heinrichs et al., 2001). These results suggest that the breast-feeding group had higher OT, which reduced cortisol. However, OT levels were not tested, and we cannot be certain that OT alone
was responsible for the effects. Similar studies examining OT and stress should also be interpreted carefully. Many factors, including how an individual construes a stressful situation, can impact the magnitude of a cortisol response (Lupien et al., 2007).

OT may also affect parent-child HPA axis activity in concert. In a recent study, fathers were administered OT or placebo before interacting with their infant (Weisman et al., 2012). Fathers and infant respiratory sinus arrhythmia (RSA; a measure of parasympathetic nervous system input to the heart via the vagus nerve) was measured during their play interactions. Father and infant interactions were also coded for social engagement. Intranasal OT administration was correlated with fathers’ elevated RSA during play with their infant and an increased frequency of key parenting behaviors that support parent-infant bonding. Parallel physiological and behavioral increases were also found in infants’ RSA responses and engagement behaviors. Though replication is needed, this study importantly suggests that OT administration to one person might have parallel physiological effects on another.

What are the pathways by which OT affects HPA activity, stress, and anxiety? One hypothesis suggests that OT influences the HPA axis by decreasing activity in the sympatho-adrenal system and increasing vagal nerve activity (an important part of the generally calming parasympathetic nervous system; Higa et al., 2002; Uvnas-Moberg, 1997). Specifically, OT terminals in the brain’s solitary vagal complex may modulate reflex control of the heart to facilitate vagal activity and slow the heart (Higa et al., 2002). In animals, paraventricular (i.e., PVN) OT neurons project to the vagal nuclei
(Buijs et al., 1985), and plasma OT increases in response to vagal nerve stimulation in rats (Stock & Uvnas-Moberg, 1988). Together, this evidence suggests that OT may directly reduce HPA activity and promote positive feedback of vagal reactions.

Reducing fear and/or amygdala activation may also lower HPA activity, particularly in response to stress. Studies in humans have shown that OT decreases amygdala activation in response to fearful and angry facial expressions (Kirsch et al., 2005). A more specific model suggests that OT reduces activation in the central amygdala, a structure influential in producing physiological responses to fear. Importantly, the central amygdala contains many OT receptors (Viviani & Stroop, 2008) and projects to the hypothalamus (the origin of the HPA axis response to stress and an area of OT synthesis) and the dorsal vagal complex (influential in moderating vagal parasympathetic activity indicated above). The central amygdala also projects to the rostral ventrolateral medulla, a structure which may be essential in regulating cardiovascular responses (e.g., heart rate) to fear and anxiety triggered in the amygdala. The medulla has also been shown to deactivate in response to OT (Viviani & Stroop, 2008), possibly resulting in lower anxiety and increased calmness. However, as OT binds to receptors in many different brain areas, this model represents only one possible pathway for OT’s anxiolytic and HPA effects. Overall, structures governing the fear response may be responsible for OT’s similar effects. Pathways regulating OT’s stress/anxiety-reducing effects may also be central to OT’s influence on cognition and memory.
Growing research suggests that OT’s anxiolytic, HPA and other effects may also depend on important individual differences. For example, a study of college students revealed that individuals with “A” alleles (AA or AG) out of a known OT receptor genetic polymorphism (i.e., coding variation) had higher heart rate reactivity and affective stress reactivity during a startle anticipation task compared with those homozygous for the “G” allele (Rodrigues et al., 2009). Early life stress (e.g., child abuse) may also affect OT levels and OT’s influence on HPA activity. In healthy adult women, a history of child maltreatment, and especially emotional abuse, was associated with lower cerebrospinal fluid OT concentrations (Heim et al., 2009). These associations have also been studied in men. When given intranasal OT, compared to controls, men who had experienced early parental separation took longer to return to baseline cortisol levels after a laboratory stressor (Meinlschmidt & Heim, 2007). This evidence suggests that significant child stressors correlate with central changes in OT levels as well as OT effects on the HPA axis. Studies examining OT and stress should consider the possibility that OT effects may be mediated by these and other factors.

As previously reviewed, OT is influential in parental and social bonding in animals. Importantly, stress and anxiety reduction are both necessary preconditions for and results of social interaction and bonding (Bartz et al., 2011; Campbell, 2010). Many hormones and chemicals involved in bonding/affiliation are also anti-anxiety (e.g., progesterone; Wirth, 2011). Therefore, OT’s connection with social affiliation may explain the hormone’s anxiolytic properties. The bio-behavioral “tend and befriend”
model of stress regulation hypothesizes that increased OT during stress, particularly in 
women, may result in greater nurturing activities and affiliation thereby protecting 
against the negative effects of stress (Taylor et al., 2000). This model has instigated 
many studies examining OT, attachment, and social support’s ability to influence mental 
and physical health (Bartz et al., 2010; Light et al., 2005; Taylor, 2002). For example, 
Light and colleagues (2005) had women spend intimate time with their partner, 
concluding with a 20 second hug, before undergoing a stressor. OT and blood pressure 
were measured before the interaction, as well as during and after the stressor. Higher 
initial OT and lower blood pressure were associated with women’s report of more 
frequent hugs (and potentially greater intimacy) outside of the laboratory setting, and 
lower cardiovascular responses throughout the study. We can presuppose that OT may 
be part of the biological mechanism by which positive social contact and social support 
buffer against stress.

1.3 Oxytocin and Cognition in Animals and Humans

OT also serves a variety of cognitive functions. In non-human animals, OT 
contributes to social cognition, reinforcement and reward learning, spatial learning, and 
generally negatively affects memory (Becker & Breedlove, 2002; Bielsky & Young, 2004; 
de Wied, 1997; Engelmann et al., 1996). Compared to control rats that did not receive 
hormones, rats that received OT injections into the hippocampal dentate gyrus or the 
midbrain dorsal raphe nucleus displayed significantly attenuated passive avoidance 
behavior (indicating reduced memory) in response to receiving shocks (Kovacs et al.,
1979). Therefore, OT administration prevented some rats from learning avoidance, suggesting that OT interfered with memory consolidation. In another set of studies, OT administered peripherally to male rats reduced social recognition compared to no-OT controls (Popik, 1991). Alternately, lower central and peripheral doses facilitated social recognition in another study (Popik, 1992). These findings suggest that OT effects on social recognition may follow an inverted U-shaped dose response curve: moderate doses facilitate, and high doses attenuate social recognition (Bielsky & Young, 2004). In contrast, central OT in female rats did not facilitate social memory, but administration of OT antagonists negatively affected social memory (Engelmann et al., 1998). Therefore, OT’s effects on memory may hinge on sexually dimorphic differences.

Perhaps the most robust and well-known effect of OT on social cognition has been found in mice. Numerous studies have shown that OT- or OT receptor-knockout (OTKO) mice (i.e., missing the gene for OT or OT receptors) have deficits in social recognition, though the mice retain normal non-social learning and memory abilities (Choleris et al., 2003; Ferguson et al., 2000; Takayanagi et al., 2005). In turn, OT administration in OT-deficient mice restores their social recognition capacities (Winslow & Insel, 2002). OTKO mice also display more anxiety, higher corticosterone, and higher markers of neural activity in anxiety-related brain regions (e.g., the medial and central amygdala) after exposure to a stressor (Mantella et al., 2004). Unlike research in rats, associations between OT and memory in mouse models are not sex-dependent. Such interspecies differences limit the translation of these effects to humans. Connections
between this research and other animal or human studies of social cognition must be drawn carefully.

Other animal research indicates that OT enhances memory. For example, a ewe needs OT to help recognize and bond with her lamb (Kendrick et al., 1988). These bonds are powerful sources of both positive emotions and reward/reinforcement (when the bonded pair is together) and negative emotions and stress (when separated). Emotion and reward are intrinsically involved with cognition and learning because emotions are primary sources of reinforcement - animals are more likely to repeat, remember and pursue gratifying experiences. Mechanistically, the amygdala has been implicated in OT’s effects on social cognition (Campbell, 2010). Other evidence suggests that OT’s cognitive effects are not exclusively mediated by the amygdala, but may result from the hormone’s influence on the ventral striatum (Keverne & Curley, 2004). This is particularly interesting as that region is also associated with reinforcement (O’Doherty et al., 2004). Finally, OT may also affect social recognition by activating the neuroendocrine system (Bielsky & Young, 2004).

OT also exerts important effects on human learning and cognition (MacDonald, 2010). Intranasal OT is purported to enhance interpretation and memory of social stimuli by facilitating social cognition and increasing recognition of positive emotions (Campbell, 2010). OT has been shown to improve interpretation of facial expressions (Domes et al., 2007), increase gaze to the eye region of pictures of faces (Guastella et al., 2008), and increase pupil dilation and sensitivity to subtle differences in emotional
expressions (Leknes et al., 2012). These effects are thought to drive OT’s ability to increase pro-social behavior (Declerck et al., 2010; Evans et al., 2010; Furl et al., 2012) including in-group trust (Van Ijzendoorn et al., 2012). Other results suggest that OT’s social cognitive effects are more selective. For example, a single dose of intranasal OT slowed reaction time to correctly identify fearful facial expressions and reduced the misclassification of positive emotions as negative ones (DiSimplicio, 2009).

As indicated above, OT may impair or enhance some types of memory and learning. The relevance of this literature to the present investigation requires a careful review of related studies. One body of research suggests that OT administered before a memory test enhances interpretation and encoding of social stimuli (generally faces; Domes et al., 2007; Guastella et al., 2008; Rimmer et al., 2009; Savaskan, et al., 2008). For example, compared to placebo, Domes et al (2007) found a positive effect of OT on correct evaluation of facial expressions as more or less emotionally arousing (regardless of valence) in men. Another study examining males determined that OT increased gaze to the eye region of faces (Guastella et al., 2008), and a third study showed that OT improved recognition memory for faces over non-social stimuli (Rimmer et al., 2009). Importantly, the Rimmer et al. (2009) result suggests that OT may specifically enhance memory for social stimuli. All studies used recognition memory tests where participants identified whether they had seen test stimuli previously. Whether this methodological correspondence is tied to the similar study results is unclear.
Compared to the previously reviewed research, another study examining men and women’s memory for faces found that OT exerts more selective effects on memory. Results showed that, independent of participants’ gender, OT enhanced recognition memory of faces with neutral and angry expressions but not positive faces (Savaskan et al., 2008). These effects remained when the recognition test was repeated 24 hours after initial stimuli exposure, instead of the same day. However, the investigation used 20 I.U. instead of 24 I.U. as in previous designs, and the study was single-blind instead of double-blind. Researchers could have inadvertently influenced the results. Memory may also depend on the time of testing. In studies where memory was tested the same day, OT may still have been elevated at the time of retrieval and could have posed a problematic methodological confound. Savaskan et al (2008) is the only investigation that has tested memory beyond the day of OT and placebo administration.

Other memory studies have reported no effect or a small memory-impairing effect of OT on neutral (Bruins et al., 1992; Ferrier et al., 1980; Guastella et al., 2008) or happy faces (Savaskan et al., 2008), or did not find any effect on emotional expressions (Guastella et al., 2009; Rimmelle et al., 2009). Marsh et al. (2010) found that in men and women, OT specifically improved identification of happy facial expressions though no significant differences emerged in recognition of other expressions (anger, disgust, fear, sadness, and surprise). These effects were not affected by participants’ sex. Results have also been mixed, with improvement on one measure and impairment on another: OT slowed reaction time to correctly identify fearful facial expressions and reduced the
misclassification of positive emotions as negative ones (DiSimplicio et al., 2009). Like Rimmel et al (2009), Herzmann et al. (2012) used a similarly well-controlled design by including words, as well as social and non-social visual objects. Results indicated a memory-impairing effect on both social and non-social visual objects.

Studies that used non-social stimuli (i.e., words) found memory enhancing effects for words associated with sexuality, bonding, and social relationships (Unkelbach, Guastella, & Forgas, 2008); selective effects: no OT/placebo group difference on a priming test, while the OT group remembered fewer reproduction-related words than neutral words in free recall (Heinrichs et al., 2004); decreased memory for unpleasant words specifically (Tops et al., 2012); general memory-impairment (Fehm-Wolfsdorf et al., 1991; Ferrier et al., 1980; Geenan et al., 1988; Kennett et al., 1982; Kovacs & de Wied, 1994) or no effect (Bruins et al., 1992; Fehm-Wolfsdorf, et al., 1984; 1988). Based on this complex picture of OT's effects on human memory, it has been suggested that OT might facilitate memory for social but not for non-social stimuli (Lee et al., 2009; Rimmel et al., 2009; Savaskan et al., 2008). However, the only two studies comparing faces and non-social, visual objects found a selective memory-enhancing effect for faces (Rimmel et al., 2009) and overall impairment (Herzmann et al., 2010). Together this evidence suggests that OT might enhance some but not other encoding/consolidation processes.

While there has been burgeoning interest in OT and memory, researchers have failed to answer questions about OT’s effects on memory, and many studies were poorly
designed. Further, OT memory studies have primarily focused on declarative memory (consciously remembered facts and events), completely disregarding potential OT effects on working memory. It is remarkable that no one has ever tested OT’s influence on working memory. OT effects are possible since there are OT receptors in the prefrontal cortex (PFC) in animals (Phelps et al., 2010) and working memory is primarily dependent on the PFC (Wolf, 2009). Therefore, part of my master’s project was designed to evaluate OT’s effects on working memory to better understand OT’s effect overall.

Given the minimal information about the duration of OT’s effects in the body, differences in OT’s effects on memory and learning may be attributable to methodological (e.g., timing of the dose pre- or post-stimuli, time between OT treatment and testing) or individual differences (e.g., gender, sensitization of the individual receiving the OT due to OT receptors or genetic differences). For example, inconsistent memory results may be attributable to the valence of a facial expression as well as the type of memory test used (e.g., for social and non-social words: recognition, free recall, or cued recall, though recognition is most common). When testing OT’s influence on social memory, adequate sample sizes as well as appropriate controls and stimuli comparison groups are also important. Of all reviewed OT and memory studies in humans, only one - Guastella et al. (2009) – had a significant sample size (104 men and women). That study administered 24 I.U. of OT or placebo and used angry, happy or
neutral discrepant (one face was different in a field of several) facial displays in a visual search task. Intriguingly, effects found were not relevant to memory/cognition.

The Guastella et al. (2009) investigation is not only unique in its results but also in its methodology by including women (n = 33). Few studies have examined OT and memory in women. Previous investigations that have assessed women had extremely low sample sizes (less than 10 per group) and/or failed to control menstrual cycle phase or exclude those taking oral contraceptives (including Guastella et al., 2009). Due to little information about OT in women, examining memory in women in the present study poses an important contribution to OT research.

Addressing inconsistencies in OT research is important for other reasons. Connections between OT and memory may have crucial implications for psychological disorders characterized by social dysfunction. In previous investigations OT seems to improve emotion recognition and promote prosocial behavior in patients diagnosed with Aspergers syndrome, autistic disorder, frontotemporal dementia and schizophrenia (Andaria et al., 2010; Averbeck et al., 2012; Guastella et al., 2010; Hollander et al., 2007; Jesso, 2011). Whether OT could be part of treatments for such disorders warrants further investigation. Regardless of its effects on (social) memory, evidence is accumulating that OT generally increases attention to social stimuli.

1.4 Neurological Associations with Oxytocin

Neuroimaging studies have also supported OT’s connection with human cognition, although results are again variable. For example, in a within subjects clinical
investigation, patients with social anxiety disorder were administered OT or placebo. Compared to controls, anxiety patients showed increased mPFC and ACC activity in response to pictures of sad faces. However, OT administration significantly reduced patients’ activation to comparable levels in healthy, non-anxious controls (Labuschagne, 2011). Therefore, OT may moderate processes governing assessment of negative stimuli in those with disordered anxiety, and reduction in the ACC and mPFC (at least in certain contexts).

In healthy men, OT has been shown to reduce amygdala activity to unpleasant social stimuli and increase amygdala activation to pleasant stimuli (Gamer et al., 2010). OT also increases amygdala activity in response to scenes depicting social and non-social threat (Lischke et al., 2012). Another study involving administration of OT to women exposed them to fearful, angry, happy and neutral facial expressions (Domes et al., 2009). Results revealed greater activation in the left amygdala, the fusiform gyrus and the superior temporal gyrus in response to fear faces and in the inferior frontal gyrus in response to angry and happy faces following OT treatment compared to placebo.

Other studies have specifically associated OT with attenuated amygdala activation. Examples include OT administration resulting in reduced amygdala activity (and greater trust) in men during a trust game compared to placebo controls (Baumgartner, 2008) and in women after hearing a crying child (Riem et al., 2011). The latter study was based on research correlating higher OT during and after pregnancy with greater parental sensitivity after birth (Feldman et al., 2007). Riem and colleagues
(2011) used this paradigm to examine mothers’ neural activation in response to a crying infant after intranasal OT or placebo. Compared to controls, women given OT showed reduced amygdala activation and increased activation in the insula and the inferior frontal gyrus pars triangularis in response to the cries (Riem et al., 2011). These results suggest that OT may selectively reduce networks associated with anxiety (i.e., the amygdala) and increase those influential in empathy (i.e., the insula). As with all previously discussed OT administration studies, an important caveat of this study is that OT was assumed to increase in reaction to the manipulation but was not actually measured in participants. Beyond focusing on specific areas of activation, this body of research suggests that OT may shift cognitive processing toward positive social information (Gamer, 2011). However, like research on OT’s memory effects, the OT fMRI literature is mixed.

Since the amygdala is crucial for emotional enhancement of memory (Abercrombie et al., 2005; Buchanan & Lovallo, 2001; LaBar & Cabeza, 2006; Payne et al., 2007), if increased OT reduces amygdala activation, then OT may impair emotional memory. OT could also exert opposite effects on the amygdala depending on whether people are at rest or under stress. During stress, OT could decrease amygdala activity and generally suppress emotional memory effects unless stimuli are socially meaningful (when memory would be enhanced). At rest, OT could enhance amygdala activity for social memory only and suppress all other memory (e.g., working). This pattern could help explain mixed findings in both the memory and fMRI literatures on OT.
1.5 Proposed Study

Overall, animal research shows that OT exerts myriad effects on social behavior, stress and cognition. Other evidence suggests similar connections in humans. Although human cognitive processes are very different from those in animals, OT may influence social behavior similarly via three proposed mechanisms: by enhancing attachment, by decreasing stress and anxiety, and/or by enhancing attention to social cues (Campbell, 2010). OT receptors have also been identified in brain areas pertaining to reward, reinforcement, and spatial knowledge, which may support the hormone’s role in other cognitive functions (Skuse & Gallagher, 2008; Young & Wang, 2004). Thus, how OT acts in the human brain remains unclear. Human (and animal) memory, particularly emotional memory, is heightened by physiological arousal, so OT’s stress-reducing properties may actually impair emotional memory. Therefore, it is unclear whether OT’s influence on memory and social behavior is due to global anti-stress effects, or more targeted cognitive effects (like increasing attention to social stimuli). These competing theories must be tested to understand OT’s neuroendocrine impact on humans.

As earlier studies have not used cognitive tasks targeted at explaining OT’s association with memory (e.g., evaluating memory with and without stress, comparing more than one type of memory, or measuring social and non-social memory over different periods of time), processes connecting those effects with stress and social behavior are unknown. Overall, there are four important limitations in the existing literature: 1) With two exceptions (Herzmann et al., 2010; Rimmele et al., 2009), recent
studies have only assessed social memory, and have not examined broader cognitive effects or other types of memory. 2) Previous research assessing OT’s impact on memory has yielded very mixed results. 3) No previous research has tested OT’s effects on working memory despite significant biological connections. 4) Although all humans have OT, the hormone is especially relevant in women, as OT synthesis and OT receptors are regulated by estrogen (Brett & Baxendale, 2001). However, most studies examining cognition have only assessed OT’s effects in men (four studies have examined men and men, two studies with women only – Domes et al., 2010 and Tops et al., 2012); little is known about relations between OT and cognition in women. As OT is particularly associated with female pair bonding in animal models (Carter et al., 2009), some researchers have suggested sex-specific roles for OT in humans as well (Donaldson et al., 2008). Examining women in the present study will begin to address questions about OT effects in women.

To clarify these problems, this proposal seeks to answer the question: How does intranasal OT affect human memory? For example, does OT down-regulate anxiety and memory overall, or does OT improve memory for social material at the expense of other material? Finally, are OT’s effects exclusive to declarative or short-term memory, or do they include working and long-term memory? As these are broad questions it was necessary to refine the present investigation to two types of memory – working and emotional.
To clarify OT’s effects on memory, we tested the main hypothesis that intranasal OT will exert differential effects on several aspects of cognition. Specifically, due to OT’s anxiolytic effects, OT will impair working memory and suppress the enhancement of emotional memory. This study also aimed to test possible mechanisms by which OT responses to stress affect emotional and working memory. Thus, we hypothesized that cortisol will moderate relations between OT and memory. Because emotional memory affects the amygdala and OT down-regulates amygdala function, emotional memory is the ideal test to determine whether OT exerts global or targeted effects on memory. Even if these hypotheses prove to be inaccurate, results will inform further research significantly by helping to elucidate mechanisms by which stress and hormones may affect human memory and cognition.
2.1 Participants

Women (N = 42, average age = 20.26, S.D. ± 2.46) were recruited via email advertisements and flyers posted on campus to complete the study for cash compensation. Potential participants completed an extensive screening interview by phone to identify psychological or health disorders before being enrolled in the study. During the interview, participants were checked for the following inclusion criteria: 1) being 18-30 years of age; 2) being a native English speaker; 3) normal or corrected to normal vision; 4) being in the early follicular phase (i.e., days 1-6) of the menstrual cycle to control hormone levels; and 5) agreeing to take an over-the-counter urine pregnancy test (First Response Pregnancy Test, Church & Dwight Co. Inc., Princeton, N.J.) upon arrival for Session 1. Exclusion criteria participants were screened for included: 1) currently taking any medication including oral or other contraceptives; 2) pregnant, nursing or may be pregnant; 3) having a history of or a current psychological disorder (this was self-reported rather than through a diagnostic interview); 3) having any current physical disorder (e.g., hypothyroidism, high blood pressure); 4) significant alcohol use (greater than 10 drinks per week); and 5) refusal to take an over-the-counter
pregnancy test or to share the results after taking a test upon arriving at the lab. An M.D. collaborator (George Knowles of IUSB Medical School) was consulted on what kinds of medications of health conditions we should exclude. Dr. Knowles was also on-call during each session in case we wanted to ask him about any side effects of the OT.

2.2 Procedures

Study sessions occurred between 16:00 and 19:00 to control for diurnal changes in hormones. Participants were asked to avoid eating, vigorous exercise, caffeine, or brushing their teeth for at least two hours prior to their study time. Additionally, due to uncertainty about the possible pharmacological interaction with the OT, participants were asked to refrain from drinking alcohol or taking recreational drugs from 48 hours before their first session until study completion.

During Session 1, following consent, participants were directed to the restroom to complete the pregnancy test. After showing a negative result on the test (no potential participant refused to share the test outcome or revealed a positive test result), participants completed a baseline saliva sample (more details below), a questionnaire to capture initial affect and state anxiety, and a symptom checklist to evaluate health status prior to beginning the study and compare against any potential side effects reported about experimental treatment. Participants were then escorted to a private room where they self-administered three puffs per nostril (24 I.U.) of synthetic OT (Syntocinon, Novartis, Basel, Switzerland) or a placebo saline solution (Salinex, Muro Pharmaceutical Inc., Tewksbury, MA), following instructions that had been placed in an
opaque box with the bottle. Any labeling identifying the solution inside the bottle had been removed prior to the experiment. Participants were also provided with a stopwatch and a box of tissues. Before administering the spray, preparatory directions stated that participants should blow their nose to clear their nasal passages and pump the container until spray is released. For spray administration, directions instructed individuals to lean slightly forward and administer one puff of spray per nostril three times, for three sets of sprays per nostril and six sprays overall. Between each set of sprays participants timed one minute on the stopwatch. After all sprays were administered, participants resealed the box with the treatment bottle and returned to their previous computer. Though spray directions indicated that participants should check with the experimenter if they had questions about spray administration procedures, study records indicate that no participant required assistance. Also, experimenters never viewed the contents of the box to help ensure that the administration was double-blind. Therefore, we can assume that all experimenters were blind to treatment during administration. While waiting for the OT to reach the bloodstream and the brain, participants completed additional questionnaires to evaluate affect and other information detailed below.

In this study, consistent with other intranasal OT studies (Ellenbogen et al., 2012), participants did not complete cognitive tasks until forty-five minutes after spray administration. This methodology is also supported by evidence from a previous study by Born et al (2002) when healthy humans were intranasally administered 40 or 80 I.U.
of peptide hormones. Hormone levels were measured in participants’ cerebrospinal fluid (CSF) and blood serum before and after administration. Results showed that arginine vasopressin, a peptide hormone virtually identical to OT, had significant serum and CSF increases after about 30 minutes and CSF levels did not decrease up to the final concentrations at 80 minutes. Unfortunately, we do not know how long OT actually increases in CSF, or how long it takes for OT levels to return to normal after administration.

Forty-five minutes after administration, participants completed two cognitive tasks: an N-back task and the encoding portion of the Emotional Picture Memory Task (details below), in counterbalanced order. Since little is known about how OT impacts physiological indices of stress, participants provided 4 saliva samples between and after the tasks (for 5 samples total during session 1) to assess changes in hormones (i.e., cortisol, progesterone, and testosterone) and completed affect and anxiety questionnaires. Participants also completed the symptom checklist after two post-treatment questionnaires (Q1-2 and Q1-5) to assess emergence of any side effects of OT. Participants returned to the lab for Session 2, 48 hours after Session 1, when we presumed that OT levels had fallen back down to normal (based on the Born et al., 2002 results). However, we actually do not know how long levels stay elevated. At that time participants completed the N-back task a second time, as well as a test of their recognition memory of the Session 1 EPMT stimuli. The order of the Session 2 tasks was not counter-balanced. All participants completed the EPMT task first. During Session 2,
participants again completed the symptom checklist, the affect and anxiety assessments and provided saliva samples at the beginning and end of the session. Examples of session timelines are shown in Figures 2.1 and 2.2 below. Though all efforts were made to maintain the double-blinding, study experimenters were asked to report which condition they thought participants were in and why. During Session 1 immediately following OT or saline treatment, at the end of Session 1, and during Session 2, adjacent to other questionnaires (Q1-1, Q1-5, and Q2-1), participants reported which condition they thought they were in. Participants could respond OT, Control, or Not Sure.
Figure 2.1. Example of Session 1 study timeline. Note that the order of N-back and EPMT tasks was counterbalanced.

Figure 2.2. Study Session 2 timeline.
2.3 Measures

2.3.1 Demographics

A professional online survey distribution tool, the Qualtrics Survey Research Suite (Qualtrics, Provo, Utah), was used to capture self-report data. A demographic questionnaire assessed participants’ age, race/ethnicity and recent health behavior patterns (e.g., caffeine and alcohol intake, recent exercise, sleep) as those factors could influence hormones or cognitive performance.

During the forty-five minutes post-administration, participants also completed questionnaires measuring depressive symptoms (Beck Depression Inventory, Beck et al., 1979), trait anxiety (Beck Anxiety Inventory, Beck et al., 1988), reassurance seeking (Depressive Interpersonal Relationships Inventory-Reassurance Seeking, Joiner et al., 1992), social anxiety and phobia (Social Interactions Scales 1 and 2, Mattick & Clarke, 1998), attachment style (Inventory of Parent and Peer Attachment, Armsden & Greenberg, 1987), and styles of empathy (Interpersonal Reactivity Index, Davis, 1980). The Barrett and Russell Affect Questionnaire (BRAQ) and the Positive and Negative Affective Scale (PANAS) were used to evaluate change in affect (Q1-1, Q1-2, Q1-3, Q1-4, Q1-5, Q2-1, Q2-2). The questionnaires of interest to the present report are explained in greater detail below.

2.3.2 Barrett and Russell Affect Questions (BRAQ)

This measure consisted of affect-related adjectives and statements based on a model developed by Feldman Barrett & Russell (1998). The BRAQ assess pleasant (e.g.,
happy, pleased), unpleasant (e.g., miserable, troubled), pleasant activated (e.g., interested, excited), pleasant-deactivated (e.g., relaxed, calm), unpleasant-activated (e.g., distressed, upset), unpleasant deactivated (e.g., bored, droopy), activated (e.g., alert, aroused), deactivated (e.g., still, quiet) affective feelings. This assessment uses two formats: (1) an adjective list, and (2) a list of statements. In both formats, participants are instructed to “stop for a moment and think about how you are feeling.” They then indicate the degree to which an affective adjective (e.g., “distressed”) or statement (e.g., “I feel at ease.”) describes them using a 5-point Likert scale (1 = not at all to 5 = extremely). The questionnaires are thought to differ from Watson et al.’s (1988) PANAS items in that their positive and negative affect dimensions are intended to describe overall arousal or activation as well as affective valence. For example, unpleasant affect, low in activation, would relate to feelings of boredom. Capturing respondents’ activation is particularly important in order to potentially associate affective states with physiological states. However, as the questionnaire merely captures subjective reports of activation, the affective effects may have no direct causal connection with any physiological outcomes (Barrett & Russell, 1998).

2.3.3 Positive and Negative Affect Scale

The Positive and Negative Affect Scale (PANAS) assesses positive and negative affect at the time of questionnaire administration (Watson, Tellegen, & Clark, 1988). These items form two scales: positive affect and negative affect. In this study, the PANAS items were combined into a single “affect” questionnaire with items from the
University of Wales Mood Adjective Checklist (MACL; Matthews et al., 1990) to examine three bipolar dimensions: Hedonic Tone (pleasant vs. unpleasant mood), Tense Activation (nervous vs. relaxed) and Energetic Activation (vigorous vs. tired). All items were answered on a 5-point scale (1 = very slightly or not at all to 5 = extremely).

Notably, the PANAS was used in addition to the BRAQ to evaluate affect because the PANAS is more commonly used in research and is possibly more valid and reliable. However, the BRAQ may be a better measure for capturing activation connected to affective states (Barrett & Russell, 1998), central to the present investigation. As this exploratory study uses a physiological manipulation, including BRAQ activation subscales may capture related OT-induced affective changes better than the PANAS.

2.3.4 Beck Depression Inventory and Beck Anxiety Inventory

The Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) are well-validated measures of individuals’ symptoms of depression and anxiety (Beck et al., 1979; Beck et al., 1988). Individuals respond on a scale of 0, indicating the symptom was not present, to 3, indicating the highest severity of that symptom.

Although participants also completed other questionnaires (mentioned above) following administration, data from these questionnaires will not be considered in these analyses.

2.3.5 N-back Working Memory Task

The n-back task is commonly used to train or investigate working memory resources (Gevins & Cutillo, 1993; Kirchner, 1958). Participants are asked to monitor a
series of stimuli and respond whenever a stimulus presented is the same as the stimulus presented $n$ trials previously, where $n$ is a pre-specified integer, usually 1, 2, or 3. However, not every stimuli presented during the $n$-back requires a response. Therefore, individuals’ must pay close attention to identify patterns warranting a response, and respond accordingly. The task requires constant monitoring and manipulation of remembered information and is therefore thought to test many key working memory processes (Gevins & Cutillo, 1993; Owen et al., 2005). The $n$-back version used in this study was a dual $n$-back (Jaeggi et al., 2003). Therefore participants had to monitor two different types of stimuli at once – stimuli shape and stimuli location. The task consisted of 12 stimulus blocks (4 total practice blocks: two blocks of 1-back practice and two blocks of 2-back practice) with feedback about correct and incorrect responses, and 8 experimental blocks (four blocks of 1-back and four blocks of 2-back without feedback). In both study sessions participants first completed 6 blocks of the 1-back version (2 practice and 4 experimental), before completing 6 blocks of the 2-back version (2 practice and 4 experimental). Participants completed two different $n$-backs because the 2-back version is very challenging without first learning the 1-back. It was also important to compare performance across different levels of difficulty to evaluate potential effects of the OT.

Each block consisted of 30 stimulus trials. Stimuli (shapes) were displayed in different locations on a 9-section grid (though the middle section was not used so stimuli could only appear in 8 sections) for 500 ms with an inter-stimulus interval of
Although there are many different versions of the \( n \)-back task, this version used the type of shape (11 possible shapes, created in Microsoft PowerPoint: star, cross, circle, square, heart, star, square, curved arrow, heart, moon, triangle) and the shape location on the grid as the two similarity/match conditions. See Figure 2.3 for an example of several of task frames. Measures of participants’ performance on the \( n \)-back included percentage of: 1) hits (making a correct response to a shape), 2) misses (not responding on a trial when there was in fact a match), 3) false alarms (FA, a response to a shape or location when an individual should not respond), 4) \( Pr \) (accuracy, e.g., hits minus false alarms), 5) response time (RT; latency until making a response to a shape that required a response), and 6) correct rejections (when participants correctly do not respond – i.e., there was not a match). These six measures were calculated for each of: i) shape type, ii) picture location, and iii) overall. Data analyses will focus on \( Pr \) to address the hypotheses posed in this study. \( Pr \) is a measure of participants’ \( n \)-back accuracy and is therefore the best measure of working memory.
2.3.6 Emotional Picture Memory Task

Images used in the EPMT were created using elements of pictures selected from the International Affective Picture Set (IAPS), a well-validated set of emotional and non-emotional stimuli, which have been used in over 50 published studies on emotional memory and emotional responses (e.g. Bradley et al., 2001; Abercrombie et al., 2006). The EPMT pictures allow separate testing of memory for objects (negative or neutral; i.e., a gun or a fork) and backgrounds (always neutral; i.e., a street scene, a forest, a living room). The EPMT scenes have been used in many studies with results consistently
reporting that the negative objects are remembered better than neutral objects (e.g. Kensinger et al., 2007; Payne et al, 2007).

During Emotional Picture Memory Task (EMPT) “encoding” (Session 1), participants viewed composite images varying in emotional and neutral content on a computer screen for 5 seconds each (Kensinger et al., 2007; Payne et al., 2008). The central object or focus of each image was either emotional (negative) or neutral in content, and the central objects were juxtaposed on neutral backgrounds (Figure 2.4). After each picture presentation, participants were instructed to use the keyboard responses “1,” “4” and “7” to indicate if they would approach the image, stay the same distance or back away. A total of 64 images were shown to the participants: 64 neutral backgrounds, 32 negative objects, and 32 neutral objects.

In Session 2 (48 hours later), participants completed “retrieval.” Unlike in the encoding task when the images were a composite of objects juxtaposed on backgrounds, in the retrieval task either the object or the background was displayed. Participants were presented with each object and background they had viewed during encoding, in addition to 32 new objects (16 negative, 16 neutral), 32 similar objects (whereas 16 of the objects similar to were previously shown with a negative object and 16 objects were shown previously with a neutral object), 32 similar backgrounds (16 negative, 16 neutral), and 32 new backgrounds (Payne et al., 2008). Participants used the keyboard response “1,” “5” and “0” to indicate if the images were the same, similar or new compared to those seen during encoding.
2.3.7 Salivary Hormone Assessment

Saliva samples were collected to measure cortisol, progesterone and testosterone levels. This method is a non-invasive way to assess free (i.e. unbound to proteins) steroid hormone levels (Liening et al., 2010). Participants provided seven, 6 mL saliva samples throughout the course of the experiment (five in Session 1 and two in Session 2). Participants used a straw only (i.e., no gum, cotton, or other saliva flow stimulants) to deposit saliva into a test tube, and were offered water following each sample. Vials were capped and frozen after each data collection session. Following collection, saliva samples underwent three freeze-thaw cycles and centrifugation; the supernatant was used in analysis. Hormone levels were determined by twelve (four per
hormone) solid-phase 125I radioimmunoassays (Coat-A-Count, Siemens Healthcare Diagnostics, Duluth, GA) using the protocol described in previous literature (Wirth & Schultheiss, 2006). Samples were assayed in duplicate along with water-diluted standards. The intra-assay coefficients of variation (CVs), averaged across the two assays per hormone, were 12.27% for cortisol, 13.78% for progesterone and 18.93% for testosterone. The lower limits of detection ($B_0 - 3 \text{ SD}$ method) averaged across assays for each hormone were 0.07 ng/ml for cortisol, 4.66 pg/ml for progesterone and 2.22 pg/ml for testosterone.
CHAPTER 3:  

RESULTS

3.1 Statistical Methods

Data from the initial phase of the study was first analyzed by calculating means and standard deviations for demographic and control variables (age, perceived general stress), independent variables (condition: OT or Control), moderators (hormone levels, anxiety, engagement), and dependent variables (cognitive performance measures [e.g., percentage of each type of emotional picture stimuli recalled, n-back PR], affect, hormone levels). Notably, as hormone levels may have been influenced by experimental condition as well as exerted effects in concert with experimental condition, hormone levels were examined as both moderator and dependent variables in analyses. Univariate tests (ANOVA and regression) were conducted to determine group differences in cognitive performance and hormones. Significant results were followed by post-hoc Pearson correlations and t-tests. Two-tailed tests were performed for all analyses, \( p < 0.05 \). Confidence intervals are only included for significant results. As this paper describes an exploratory study, all statistics of marginal significance \( (p < 0.10) \) are reported to thoroughly describe relevant results. SYSTAT and SPSS statistical software
packages were used to complete analyses. Cortisol concentrations are reported as ng/ml. Progesterone and testosterone concentrations are reported as pg/ml.

3.2 Effectiveness of Blinding

There were no significant differences in demographic characteristics (age, race/ethnicity) and health behaviors (weekly alcohol consumption, recent caffeine intake, stress levels, sleep and wake patterns) between the two Conditions. Chi-square tests compared participants’ guesses, during each study session, of which treatment they had received with their actual treatment. Those analyses yielded non-significant results (Session 1: $\chi^2(2, N = 38) = 1.06, p = .59$; Session 2: $\chi^2(2, N = 41) = 0.29, p = .87$). In each session, many more participants thought that they received placebo than OT. At the end of Session 1, 1 of 21 OT participants and 2 of 21 Controls guessed they had received OT, while 7 of 21 OT participants and 9 of 21 Control participants thought that they had received placebo. Most women who thought that they had received placebo attributed their opinion to “not feeling any different after administering the spray.” A third group of participants reported that they “didn’t know” which substance they received ($n = 19$). Two OT and two Control participants left the study before finishing the last Session 1 questionnaire (due to other time commitments) and did not answer these questions. Forty-eight hours later, at the beginning of Session 2, 3 of 21 OT participants and 2 of 21 Controls thought they had received OT in Session 1. Further, 11 of 21 OT participants and 10 of 20 Control participants believed that they had received
placebo (Table 3.1). Based on these results we can assume that participants were indeed blind to their experimental condition.

TABLE 3.1

SELF REPORT OF TREATMENT PARTICIPANTS THOUGHT THEY RECEIVED

<table>
<thead>
<tr>
<th>Participants’ Treatment Guess</th>
<th>Session 1 Actual Treatment Conditions</th>
<th>Session 2 Actual Treatment Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxytocin</td>
<td>Placebo</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Placebo</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Not Sure</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>No Data</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>21</td>
</tr>
</tbody>
</table>

Tests were also conducted to examine study experimenter’s estimate of participants’ conditions to ensure that experimenters were blind to condition. Results showed that the experimenters’ guesses were not significantly better than chance ($\chi^2(1, N = 42) = 0.89, p = .35$). We can assume that study experimenters were blind to individuals’ treatment groups.

Previous literature has indicated a variety of mild symptoms commonly associated with receiving OT. A recent review of intranasal OT side effects across 38 randomized, controlled studies found that the most frequent side effects include increased calmness/euphoria, feeling more comfortable or having more energy, light headedness, drowsiness and/or headache reported and nasal irritation, and dry
mouth/throat (MacDonald et al., 2011). The incidence of these side effects has not been more than placebo in previously published research.

In this study, participants completed a symptom questionnaire to evaluate potential side effects resulting from OT or placebo. The symptom questionnaire was completed three times during Session 1 (Q1-1: pre-OT/placebo administration, Q1-3: 45-minutes post-administration, Q1-6: end of Session 1) and at the beginning of Session 2 (Q2-1). On the pre-administration symptom questionnaire (i.e., Q1-1), OT participants reported significantly higher worry ($\chi^2(1, N = 42) = 6.93, p < 0.01$) and marginally higher anxiety ($\chi^2(1, N = 42) = 5.54, p = 0.06$). However, on validated measures of anxiety and negative affect (assessed by the PANAS and BRAQ scales), the groups were not initially different in self reported affect. The significant Pre-Q results may have been attributable to relying on a previously used, but psychometrically flawed, checklist method to capture data on treatment side effects.

In addition to pre-treatment self-report, side effect data was also collected at three other time points: 1) 45 minutes after treatment, 2) at the end of Session 1, 3) and at the beginning of Session 2. Forty-five minutes after spray administration (Q1-3), the OT group reported marginally higher energy ($\chi^2(1, N = 42) = 3.11, p = 0.08$) and significantly greater worry ($\chi^2(1, N = 42) = 4.42, p < 0.05$) than Controls. On the final Session 1 symptom questionnaire (Q1-6), the OT group reported marginally higher worry than Controls ($\chi^2(1, N = 38) = 3.64, p = 0.06$). Once again, on the PANAS and BRAQ there were no significant differences at this time point. On the Session 2 symptom
questionnaire, the OT group reported significantly greater energy compared to Controls $(\chi^2(1, N = 42) = 4.02, p < 0.05)$. Overall, there were no symptoms created by OT other than greater energy.

In addition to comparing symptom self-report at discrete time points, it is also essential to examine symptom changes between pre- and post-treatment. Chi-square analyses were conducted to compare all participants’ Session 1 symptom report change from pre- to post-treatment. However, there were no significant differences in symptom change by group.

Overall, the OT group reported a number of side effects including relaxation, calmness, increased energy, drowsiness, dry mouth/throat, discomfort, abdominal/stomach discomfort, blurred vision, worry, headache, and sore throat. Side effects most often reported by Controls included drowsiness, dry mouth, anxiety, and feeling relaxed. See Table 3.2 for further details about the prevalence of each side effect by study condition. Together, these results show that OT may have only marginally increased energy. Group differences in worry were already present before the OT/placebo treatment.
## TABLE 3.2

PERCENTAGE OF TREATMENT SIDE EFFECTS REPORTED BY PARTICIPANTS ACROSS SESSIONS 1 AND 2

<table>
<thead>
<tr>
<th>Questionnaire</th>
<th>Q1-1</th>
<th>Q1-3</th>
<th>Q1-6</th>
<th>Q2-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OT</td>
<td>Control</td>
<td>OT</td>
<td>Control</td>
</tr>
<tr>
<td>Nausea</td>
<td>-</td>
<td>4.8</td>
<td>4.8</td>
<td>-</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>38.1</td>
<td>3.3</td>
<td>28.6</td>
<td>23.8</td>
</tr>
<tr>
<td>Energized</td>
<td>23.8</td>
<td>19.0</td>
<td>23.5*</td>
<td>4.8</td>
</tr>
<tr>
<td>Cramping</td>
<td>9.5</td>
<td>9.5</td>
<td>9.5</td>
<td>9.5</td>
</tr>
<tr>
<td>Uncomfortable</td>
<td>9.5</td>
<td>9.5</td>
<td>14.3</td>
<td>14.3</td>
</tr>
<tr>
<td>Stomach</td>
<td>9.5</td>
<td>9.5</td>
<td>-</td>
<td>9.5</td>
</tr>
<tr>
<td>Worried</td>
<td>38.1**</td>
<td>4.8</td>
<td>19.0*</td>
<td>-</td>
</tr>
<tr>
<td>Nasal</td>
<td>4.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dizziness</td>
<td>4.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dry</td>
<td>9.5</td>
<td>4.8</td>
<td>14.3</td>
<td>33.3</td>
</tr>
<tr>
<td>Anxious</td>
<td>33.3*</td>
<td>9.5</td>
<td>19.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Calmness</td>
<td>66.7</td>
<td>66.7</td>
<td>42.9</td>
<td>47.6</td>
</tr>
<tr>
<td>Lightheaded</td>
<td>9.5</td>
<td>14.3</td>
<td>4.8</td>
<td>9.5</td>
</tr>
<tr>
<td>Headache</td>
<td>9.5</td>
<td>4.8</td>
<td>14.3</td>
<td>4.8</td>
</tr>
<tr>
<td>Blurred vision</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Relaxed</td>
<td>76.2</td>
<td>81.0</td>
<td>57.1</td>
<td>66.7</td>
</tr>
<tr>
<td>Runny or itchy</td>
<td>9.5</td>
<td>4.8</td>
<td>9.5</td>
<td>-</td>
</tr>
<tr>
<td>Sore throat</td>
<td>-</td>
<td>4.8</td>
<td>4.8</td>
<td>-</td>
</tr>
</tbody>
</table>

*Note. Q1-1 was completed before OT or placebo administration, Q1-3 was completed 45 minutes post- administration, Q1-6 was completed 2 hours post- administration, and Q2-1 was completed at the beginning of Session 2. If no symptoms were reported the field was left blank in the table; **p < 0.01, *p < 0.05, ºp < 0.10, All tests were two-tailed.*
3.3 Affect Analyses

Changes in affect were evaluated six times during Session 1 (Q1-1, Q1-2, Q1-3, Q1-4, Q1-5, Q1-6) and twice during Session 2 (Q2-1 and Q2-2). ANOVAs were conducted with Condition as the independent variable and self-reported affect within each session as the repeated measures dependent variables. T-tests were used to evaluate differences by Condition at discrete times, as well as to compare pre- to post- treatment change by Condition. Significant findings on affect scales are summarized in Table 3.3.

3.3.1 Session 1 and Session 2: PANAS and MACL

T-tests determined that there were no significant differences in groups’ affect (positive affect, negative affect, hedonic tone, tense activation, energetic activation) when beginning Session 1 (Q1-1). Q1-1 affect t-tests were followed by mixed ANOVAs to assess change in affect over time. For PANAS Positive Affect, ANOVA revealed a marginal Time x Condition interaction ($F(5,175) = 2.13, p = 0.06$). To follow up on this effect, a positive affect change score was calculated (Q1-6 Positive Affect minus Q1-1 Positive Affect). However, the t-test examining Positive Affect change was not significant. Analyses replicating this model examining Session 2 PANAS Positive Affect were not significant.

ANOVA examining Session 1 MACL Energetic Activation showed a significant main effect of Condition ($F(1,39) = 6.05, p < 0.05$). Specifically, there was higher Energetic Activation in the OT condition on Q1-6 ($t(39) = -2.77, p < 0.01$). As with Positive
Affect, to assess whether the Session 1 Energetic Activation result was attributable to OT, pre- to post- treatment change scores were calculated and a t-test compared change scores by Condition. Results were not significant. Importantly, the difference in Session 1 Energetic Activation self-report effect matches initial differences found in the symptom checklist – the OT group had higher initial energy. Session 2 Energetic Activation ANOVAs were not significant. However, a t-test using a change score between Session 2 reports (Q2-1 and Q2-2) was marginal ($t(28) = -2.04$, CI: -3.15 to 0.01, $p = 0.05$). The OT condition had greater Energetic Activation than Controls in Session 1 and Session 2.

3.3.2 Session 1 and Session 2: Barrett and Russell Affect Questions

Affect was also assessed using the BRAQ (Barrett & Russell, 1998). A mixed ANOVA of Session 1 Deactivated Affect (i.e., droopy, drowsy, sluggish), examining change across all Session 1 questionnaires (with Time and Condition as between-subjects independent variables), revealed a marginal main effect of Condition ($F(1,35) = 4.09$, $p = 0.05$). Post-hoc tests revealed that compared to OT, Controls reported significantly greater Deactivated affect on Q1-6 ($t(36) = 2.04$, CI: 0.11 to 5.15, $p < 0.05$). A change score calculated from Q1-1 to Q1-6 showed a greater increase in Deactivated affect in Controls than in OT ($t(35) = 2.12$, CI: 0.11 to 5.15, $p < 0.05$). The effect matches the increase in Energetic Activation reported by the OT group in Session 1.
For Session 1 Pleasant Activation, there was a Time x Condition interaction \((F(4,140) = 3.11, p < 0.05)\). Post-hoc t-tests comparing Pleasant Activation by Condition at individual time points, as well as change from pre- (Q1-1) to post- (Q1-6) administration, were not significant. OT showed a significantly greater change in Pleasant Activation from Q1-2 (self-reported affect immediately post-administration) to Q1-6 compared to Controls \((t(34) = 2.14, CI: 0.19 \text{ to } 7.60, p < 0.05)\). However, since there was no significant group effect on change from Q1-1 to Q1-6, the Q1-2 to Q1-6 difference could be explained by group differences in pre-treatment Pleasant Activation.

Finally, for Unpleasant Deactivated affect, there was a significant main effect of Condition \((F(1,35) = 4.41, p < 0.05)\). Follow-up t-tests examining individual affect time points and change over time were not significant. Differences in Unpleasant Deactivated affect by Condition may have been present from Q1-1 but were not captured by the symptom questionnaire due to differences between the symptom checklist and BRAQ item content.

Like Session 1, Session 2 revealed significant differences in Deactivated Affect. A global, repeated-measures ANOVA showed a significant main effect of Condition \((F(1,39) = 9.30, p < 0.01)\) and a marginal Time x Condition interaction \((F(1,39) = 2.81, p = 0.10)\). During Session 2, Controls reported significantly higher Deactivated Affect than did the OT group on Q2-1 \((t(37) = 2.14, CI: 0.09 \text{ to } 3.46, p < 0.05)\) and Q2-2 \((t(34) = 3.03, p < 0.01)\). The change score post-hoc t-test from Q2-1 to Q2-2 was not significant. As observed with Session 1 Unpleasant Deactivated affect, there was a marginal Session 2
main effect of Condition ($F(1,39) = 3.50, p = 0.07$). In addition to replicating the Session 1 Condition result, Session 2 analyses revealed a marginal Time x Condition interaction ($F(1,39) = 3.81, p = 0.06$). Post-hoc tests indicated higher Unpleasant Deactivated affect in Controls than in the OT group during Q2-2 ($t(32) = 2.14$, CI: 0.15 to 6.11, $p < 0.05$). The change score post-hoc t-test was not significant. Overall, results suggested that there were minimal OT effects on affect. OT and Controls differed on Pleasant Activation, but those differences fit with our results from the symptom checklist as the OT group may have felt a little more engaged. Non-significant PANAS and MACL results suggest that OT may actually affect cognition more directly (rather than via affecting emotion). Therefore in this study, any OT-induced cognitive changes are not attributable to affect.
### TABLE 3.3

**SIGNIFICANT AFFECT MEASURES BY CONDITION GROUP AND SESSION**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Result</th>
<th>Post-Hoc</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Session 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MACL: Energetic Activation</td>
<td>Q1-6**</td>
<td>Higher in OT</td>
</tr>
<tr>
<td>BRAQ: Deactivated</td>
<td>Q1-6*</td>
<td>Higher in Controls</td>
</tr>
<tr>
<td>BRAQ: Pleasant Activation</td>
<td>Overall*</td>
<td>NS</td>
</tr>
<tr>
<td>BRAQ: Unpleasant Deactivated</td>
<td>Overall*</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Session 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRAQ: Deactivated</td>
<td>Q2-1*</td>
<td>Higher in Controls</td>
</tr>
<tr>
<td>BRAQ: Deactivated</td>
<td>Q2-2**</td>
<td>Higher in Controls</td>
</tr>
<tr>
<td>BRAQ: Unpleasant Deactivated</td>
<td>Q2-2*</td>
<td>Higher in Controls</td>
</tr>
</tbody>
</table>

*Note. *p < 0.05, **p < 0.01, two-tailed*

3.4 Effects on Salivary Cortisol, Progesterone and Testosterone

OT’s influence on other hormones (cortisol, progesterone and testosterone) was initially examined using two sets (for Session 1 and Session 2) of mixed ANOVAs. In these analyses, Condition was the independent, between-subjects variable, time was the independent within-subjects variable, with pre- (S1-1) and post-administration hormone samples (See sample study timeline: S1-2, S1-3, S1-4, S1-5) as dependent repeated measures. Area under the curve increase (AUC), a measure of change in hormone levels from baseline, was also calculated for each hormone (Pruessner et al., 2003).

For Session 1 data, global ANOVAs examining potential Condition differences in pre- to post-administration change in individual hormone samples, and t-tests
examining AUCi were not significant for any hormone (See Figure 3.1). For cortisol (Figures 3.2 and 3.3), post-hoc t-tests comparing hormone concentrations by Condition revealed that the OT group produced significantly higher cortisol than Controls during Session 1, Time 2 (post-treatment; \( t(40) = -2.09, \text{CI: -0.73 to -0.01, } p < 0.05 \)). A similar marginal effect was also exhibited in Session 2, Time 1 (\( t(39) = -1.79, p < 0.08 \)). For progesterone, though the Session 1 ANOVA was not significant, the Session 2 ANOVA indicated a Time x Condition interaction during Session 2 (\( F(1,31) = 6.18, p < 0.01 \)). Post-hoc tests were not significant, and this result was likely produced by a greater increase in progesterone from Session 2, Sample 1 to Session 2, Sample 2 in the placebo group vs. the OT group (See Figures 3.4 and 3.5). Finally, testosterone follow-up tests revealed that Controls had significantly higher testosterone at Session 1, Time 4 compared to the OT group (Figures 3.6 and 3.7; \( t(39) = 2.18, \text{CI: 0.50 to 12.55, } p < 0.05 \)). Therefore, OT administration resulted in higher salivary cortisol and lower salivary testosterone.
Figure 3.1. Session 1 change in salivary hormones by, according to area under the curve increase (AUCi), by treatment condition.
Figures 3.2 and 3.3. Session 1 and Session 2 change in salivary cortisol over time; *p < 0.05
Figures 3.4 and 3.5. Session 1 and Session 2 change in salivary progesterone over time; *p < 0.05
Figures 3.6 and 3.7: Session 1 and Session 2 change in salivary testosterone over time; *$p < 0.05$. 

Testosterone pg/ml

Time points

Nasal Spray

Testosterone pg/ml

Time points

OT

Placebo

*
3.5 N-back Task Analyses

3.5.1 Working Memory Data Analysis Strategy

The n-back task was used to determine whether OT affects women’s working memory. Performance outcome variables were calculated for the two test stimuli (i.e., shape and location), the two different n-backs (1-back or 2 back), and within each of the two study sessions. To capture potential differences between working memory during practice and experimental blocks, variables were computed separately for practice blocks and experimental blocks respectively. The n-back produces four types of response – hits, misses, correct rejections and false alarms. It is important to correct for false alarms because otherwise people could press the keys all the time and get a perfect score. Therefore, we use Pr (hits – false alarms) to gage people’s accuracy at the working memory task. Pr variables used in n-back analyses included: 1-back Practice Pr Overall, 1-back Practice Shape Pr, 1-back Practice Location Pr, 1-back Experimental Overall Pr, 1-back Experimental Shape Pr, 1-back Experimental Location Pr, 2-back Practice Pr Overall, 2-back Practice Shape Pr, 2-back Practice Location Pr, 2-back Experimental Overall Pr, 2-back Experimental Shape Pr, and 2-back Experimental Location Pr. All Pr variables were also evaluated on reaction time. Results of those analyses are not described in this manuscript.

As the stress hormone cortisol is known to exert important effects on memory (Schoofs et al., 2008; Wolf et al., 2008; Young et al., 1999), additional n-back analyses
included both Cortisol (pre-task, Cortisol 1-3) and experimental Condition as independent variables.

On several occasions research assistant error aborted the n-back program at the end of the task and participants’ data was not saved. In Session 1, this occurred for six participants (OT: 4, Control: 2). For Session 2, this occurred for seven participants (OT: 4, Control: 3). Data from both sessions was not deleted for any participant. During both sessions, one participant’s n-back responses were more than five standard deviations outside of other participants’ response rates. Those results suggest that the individual was potentially not engaging in the task or did not understand how to perform the task and her data was excluded from all n-back analyses. Finally, one person (a Control participant) did not return for Session 2. Thus, for Session 1, N = 36 (OT: 17, Control: 19) and for Session 2, N = 33 (OT: 17, Control: 16).

3.5.2 Session 1 and Session 2 Practice Pr

To examine Overall (i.e., collapsed over shape and location) Practice Pr across the 1-back and 2-back practice blocks, we first collapsed over the two blocks of 1-back practice and the two blocks of 2-back practice to create four variables: 1) Session 1 Overall Practice 1-back Pr; 2) Session 1 Overall Practice 2-back Pr; 3) Session 2 Overall Practice 1-back Pr; 4) Session 2 Overall Practice 2-back Pr. Within each session, a global mixed ANOVA was conducted with Condition as the between-subjects independent variable and 1-back and 2-back average Pr practice variables as the two dependent
variables. These analyses were conducted for each of the two study sessions. All analyses were non-significant in Session 1 and Session 2 for Overall, Shape and Location.

An additional test combined all Overall Practice Pr (1-back and 2-back) from each session together to create Session 1 Pr Overall Practice Total and Session 2 Pr Overall Practice Total. T-tests were not significant in Session 1 or Session 2. These results suggest that Controls and the OT group performed similarly on working memory for both practice trials for 1-back and 2-back.

3.5.3 Session 1 and Session 2 Experimental Pr

In Session 1 and Session 2 mixed ANOVAs examining effects of Condition on 1- and 2-back Pr variables (Overall, Shape, Location) were not significant. Together with non-significant results from the practice blocks, the experimental n-back block analyses support the conclusion that the two groups performed similarly Overall, and on Pr, Shape, and Location; therefore OT did not appear to affect working memory.

3.5.4 Pr in Individual Blocks

Although the previous analyses examined Pr differences across Session 1 and Session 2, separate tests were also conducted to examine 1-back vs. 2-back variables on individual blocks. These tests were necessary because collapsing across blocks could conceal key Condition differences in participants’ n-back performance. Examining individual blocks could help reveal those details. Therefore, t-tests were conducted to compare Pr performance by Condition on individual blocks (1-12).
For Session 1, t-tests revealed that Controls had significantly higher Overall Pr than OT on 1-back Practice Block 1 (Block 1; \( t(28) = 2.25, CI: 0.02 \) to 0.54, \( p < 0.05 \)), 2-back Practice Block 1 (Block 7; \( t(30) = 2.10, CI: 0.01 \) to 0.54, \( p < 0.05 \)), and marginally higher performance on 2-back Experimental Block 1 (Block 9; \( t(28) = 1.71, p = 0.09 \); Figure 3.8). For 1-back Shape PR (Figure 3.9), Controls had higher Pr on 2-back Practice Block 1 (Block 7; \( t(30) = 1.75, p = 0.09 \)), 2-back Experimental Block 1 (Block 9; \( t(28) = 2.18, CI: 0.01 \) to 0.31, \( p < 0.05 \)), and 2-back Experimental Block 3 (Block 11; \( t(28) = 1.85, p = 0.08 \)). For Pr Location (Figure 3.10), Controls had higher Pr on 1-back Practice Block 1 \((t(28) = 3.09, CI: 0.13 \) to 0.64, \( p < 0.01 \)), 1-back Practice Block 2 \((t(19) = 1.77, p = 0.094)\), and 2-back Practice Block 1 \((t(31) = 1.85, p = 0.07)\). During Session 1 Controls performed similarly or better than the OT group on all individual \( n \)-back blocks. We can conclude that OT impaired working memory, at least initially.

Individual Pr variables were also examined in Session 2 by Condition. In contrast with Session 1, participants did not differ on individual blocks of Session 2 Overall Pr (Figure 3.11). There was a significant Session 2 difference on 1-back Shape Pr Practice Block 2 whereas Controls had better Shape Pr compared to OT \((t(17) = 3.33, CI: 0.03 \) to 0.14, \( p < 0.01 \); Figure 3.12). There were no Session 2 Condition differences according to Location (Figure 3.13). Thus, with the exception of one block, both conditions performed similarly across Session 2. As observed in Figures 3.8-3.13, in contrast with Session 1 Pr (which levels off), Session 2 Pr decreases sharply in both conditions. This effect is likely due to participants’ fatigue.
Together, the $n$-back results indicate that Controls performed better than OT on working memory over both sessions, both types of N-back and for Pr Overall, Pr Shape and Pr Location. As these associations were not apparent in the global ANOVAs or aggregate Pr measures, examining individual blocks revealed important differences between Conditions. Many significant variations between Conditions occurred during the practice blocks and became non-significant as OT participants improved. These results may provide evidence that OT slows or interferes with working memory. However, it is unlikely that OT exerts substantial long-term effects on working memory, as evidenced by mostly non-significant Condition relations during Session 2.
Figure 3.8. Session 1 change in Overall Pr (collapsing across shape and location) by Condition, Type of Block and Type of n-back. Pr was calculated by hits (correct recognition of an object) minus FA (pressing a key when it was not a match). There was also significance between Conditions on Blocks 1 and 7 ($p < 0.05$).
Figure 3.9. Session 1 change in Shape Pr by Condition, Type of Block and Type of n-back. Pr was calculated by hits (correct recognition of an object) minus FA (pressing a key when it was not a match). There was also significance between Conditions on Block 9 ($p < 0.05$).
Figure 3.10. Session 1 change in Location Pr by Condition, Type of Block and Type of n-back. Pr was calculated as an index of recognition accuracy. Pr was calculated by hits (correct recognition of an object) minus FA (pressing a key when it was not a match). There was also significance between Conditions on Block 1 ($p < 0.01$).
Figure 3.11. Session 2 change in Overall Pr (collapsing across shape and location) by Condition, Type of Block and Type of n-back. Pr was calculated by hits (correct recognition of an object) minus FA (pressing a key when it was not a match). There was also significance between Conditions Block 2 ($p < 0.01$).
Figure 3.12. Session 2 change in Shape Pr by Condition, Type of Block and Type of n-back. Pr was calculated by hits (correct recognition of an object) minus FA (pressing a key when it was not a match). There was also significance between Conditions on Block 2 ($p < 0.01$).
Figure 3.13. Session 2 change in Shape Pr by Condition, Type of Block and Type of n-back. Pr was calculated by hits (correct recognition of an object) minus FA (pressing a key when it was not a match). All tests examining individual blocks were ns.
3.5.5 Cortisol and Pr

As mentioned earlier, significant research suggests that cortisol impairs working memory (Schoofs et al., 2008; Wolf et al., 2008; Young et al., 1999). Therefore, additional n-back analyses included both Cortisol and Condition as independent variables. Although there are many ways to assess cortisol, these analyses examined the hormone using Session 1 pre-n-back task cortisol levels (S1-3). We can assume that those levels are more reflective of OT’s effects on cortisol and working memory since hormone levels were measured closest to the first n-back, and on the same day as OT administration.

Six global ANOVAs testing these associations included Condition and Cortisol as independent variables and one of the six Practice and Experimental Pr measures (1-back Overall Pr, 1-back Shape Pr, 1-back Location Pr, 2-back Overall Pr, 2-back Shape Pr, and 2-back Location Pr) as a dependent variable. For Session 1 there was a significant result for 1-back Location Practice Pr ($F(3,31) = 6.47, p < 0.01$). Follow-up tests showed that there was a significant negative association between Location Practice Pr and Cortisol in OT ($t(17) = -2.53, p < 0.05$) and a trend in Controls ($t(18) = -1.69, p = 0.11$). These effects were not found for 1-back Location Practice Pr in Session 2.

ANOVA also revealed marginal results for 1-back Overall Practice Pr ($F(3,30) = 2.68, p = 0.07$) and 1-back Shape Practice Pr ($F(3,31) = 2.53, p = 0.08$). Neither result was found in Session 2. Post-hoc tests of 1-back Overall Practice Pr showed a marginal negative effect between memory and cortisol in Controls only ($t(18) = -1.84, p = 0.08$).
Post-hoc tests for 1-back Shape Practice Pr showed a significant negative effect of Cortisol on Pr for Controls only (t(18) = -2.24, p < 0.05). Cortisol and OT together influenced Pr during Session 1 n-back practice. Cortisol impaired OT participants’ working memory for Location and impaired Controls’ working memory for Shape and (suggestively) Overall as well. These effects were found for Session 1 Practice 1-back blocks only. This indicates that even though the two groups showed similar cortisol patterns in Session 2, cortisol may not have affected n-back Pr as significantly as in Session 1, when participants were first learning the task.

Overall, Controls performed better than the OT group on the n-back. The normal negative association between Cortisol and working memory was absent in women who received OT for Shape and Overall but was robust for Location. As cortisol has been shown to effect working memory (Wolf, 2009), one could speculate that higher cortisol levels observed in the OT group during Session 1, may have contributed to the OT group’s negative correlation between Location Practice Pr and cortisol. It is unclear why this effect is only present for one of the three Pr variables. However, cortisol seems to moderate OT’s effects on working memory.
3.6 Emotional Picture Memory Task Analyses

3.6.1 EPMT Data Analysis Strategy

For the EPMT, one participant did not identify any recall pictures as same or similar and was excluded from these analyses. Notably, this was a different participant than the one who was an outlier in the n-back and was excluded from those analyses. A second participant did not return for Session 2, and therefore had no recall test, leaving an N of 40. Mixed ANOVAs and t-tests were conducted to compare the effect of Condition on EPMT recall. In these analyses, Condition was the independent variable. Dependent variables were constructed using participants’ rates of correctly categorizing an object or background viewed during Session 1 encoding as same (i.e., the original object or background viewed during encoding), similar (i.e., an object or background sharing some characteristics of an original object or background) or new (i.e., an object or background that the participant had not viewed during encoding). Participants’ responses were coded based on the emotional content of a given stimuli (i.e., negative or neutral) and the stimuli type (object and background). Therefore, during encoding, participants could have categorized a negative background, negative object, neutral background or neutral object as the same, similar or new. Participants’ responses were first evaluated for specific memory (categorizing same stimuli as same). In line with previous research, analyses also examined participants’ memory for the “gist,” Gist memory variables were constructed based on participants’ categorization of objects or
backgrounds presented during recall (same) as those presented during encoding (same or similar).

A second set of mixed ANOVAs included participants’ cortisol levels to evaluate potential joint effects of Cortisol and Condition on emotional memory. Three different cortisol variables were used individually as moderators in these analyses. Cortisol variables included measures from pre-EPMT encoding (Cortisol 1-3), pre-EPMT recall (Cortisol 2-1) and an overall measure of cortisol change (AUCiCort). AUCiCort, or area under the curve increase, represents global change in cortisol calculated from each individual’s baseline across Session 1 (Pruessner et al., 2003).

3.6.2 Emotional Trade-off Effect

Previous studies using the EPMT have found a consistent effect of a “trade-off” of better memory for negative or emotional objects vs. backgrounds, where such an object-background gap is reduced for neutral stimuli (Kensinger et al., 2006; Payne et al., 2008). To examine whether the association was present in this study, a 2 x 2 ANOVA was conducted with Emotion (Negative and Neutral) and Stimuli Type (Object and Background) as the two repeated-measures dependent variables. Results revealed that the trade-off was indeed present in this study, as indicated by an Emotion x Stimuli Type interaction \( (F(1,17) = 21.67, p < 0.001) \). To evaluate whether this trade-off effect was altered by OT, Condition was added into the previous model as an independent variable. Results revealed a marginal Stimuli x Condition interaction \( (F(1,36) = 3.44, p = 0.07) \), but
the three-way interaction of Stimuli x Condition x Emotion was not significant (see Figure 3.14). Further exploratory t-tests examining specific memory by each type of stimuli (negative and neutral objects and backgrounds individually) were not significant. Therefore, OT did not have any influence on the trade-off effect or specific memory.
Figure 3.14. Specific emotional memory by Condition. Data is displayed according to Type of Stimuli (Objects & Backgrounds) and Emotion (Negative & Neutral).
To examine gist memory, composite variables were created using participants’ identification of previously viewed objects and backgrounds as same or similar (when the stimuli actually were the same), divided by all participants’ responses to same stimuli (i.e., same + similar / same + similar + new). However, results were not significant. When examining these gist responses by each type of stimuli (negative objects and backgrounds separate from neutral objects and backgrounds), t-tests revealed a marginal effect of better gist memory for negative backgrounds in the OT group ($t(34) = -1.80, p = 0.08$) than in Controls (Figure 3.15). Therefore, gist memory was not influenced by OT.
Figure 3.15. Gist emotional memory by Condition. Data is displayed according to Type of Stimuli (Objects & Backgrounds) and Emotion (Negative & Neutral).
3.6.3 Hormones and Emotional Memory

Similar to working memory, cortisol can also affect declarative memory, especially of emotional material (Wolf, 2008). To evaluate OT and cortisol’s effects together, the three cortisol variables of interest (Cortisol 1-3, Cortisol 2-1, AUCiCort) were each separately added to all previous analyses.

All mixed ANOVAs examining cortisol’s influence on specific memory of negative stimuli were not significant. Additional ANOVAs including cortisol to examine gist memory were also not significant. Overall, specific and gist memory were not affected by an interaction of cortisol and OT, and OT did not affect emotional memory in general.
CHAPTER 4:
DISCUSSION

4.1 Working Memory

The current study provides evidence that a single 24 I.U. dose of intranasally administered OT negatively impacts working memory in women. Though these results vary in the type of working memory that is impaired - sometimes overall Pr, sometimes Pr shape or Pr location - associations are always in a negative direction. Importantly, results were unique from any clear influences of OT on affect. OT had the greatest effect on working memory during practice blocks, when participants were still learning the task. OT may initially impair working memory, but with practice, women in the OT group performed equivalently to women in the Control group. Therefore, OT’s working memory effects seem to be a minor and recoverable. Several studies have shown that OT exerts either memory-enhancing or memory-inhibiting effects in women (Marsh et al., 2010; Savaskan et al., 2008; Tops et al., 2012), but no investigation has explicitly examined working memory in either men or women. Therefore the present findings represent an important advance in our knowledge about OT’s cognitive effects, in women particularly.
Cortisol also exerted effects on women’s working memory. This is particularly important because working memory may be more sensitive to glucocorticoids (GC) like cortisol than emotional memory also tested in this study (Lupien et al., 1999). While most cortisol differences by condition did not reach significance, in line with our results, even subtle cortisol differences in the OT group seemed to negatively affect working memory. Although cortisol only seemed to significantly affect working memory in Session 1, the fact that the OT group showed generally impaired working memory and higher cortisol than Controls is unlikely coincidental.

Notably, cortisol’s connection with OT and working memory was a side finding. We did not design the study to look at these interactions, but these results should be explored in future research manipulating cortisol in order to directly examine the effects of cortisol and OT together on working memory. Perhaps if OT was administered adjacent to a stressor, therefore coupling HPA and sympathetic activation, we would observe more profound negative effects on working memory (Elzinga & Roelofs, 2005).

It was also important that OT’s effects on cortisol seemed to last into Session 2. Other Session 2 hormones also exhibited similar patterns. Does this evidence mean that OT is still affecting women’s systems two days later? Unfortunately we do not know how long OT is elevated in the brain after the nasal spray treatment. Born et al (2002) (examining the closely related peptide hormone vasopressin) previously assessed hormone increases in participants’ cerebrospinal fluid up to 80 minutes after spray administration. Notably, vasopressin levels were still significantly elevated at the 80
minute sample. Therefore it is possible that OT levels could have still been elevated in Session 2. Although long-term physiological effects seem unlikely given the reportedly short half-life (estimated at 6-7 minutes; Robinson & Verbalis, 2003) of OT, our results suggest otherwise. It is possible that OT administration sets into motion a chain of events that are self-sustaining or lasting. Most studies have not evaluated long-term physiological changes in those administered OT. In one striking example, men who received an OT infusion exhibited decreased central cognitive potential (measured by EEG) on the day of OT administration (Geenan et al., 1988). This physiological effect was still observed one week after treatment, but without other symptoms. Together with our results, we can suggest that both central and peripherally administered OT linger in the system, and exert long-term physiological effects.

4.2 Emotional Memory

The emotional memory trade-off effect was present in the EPMT overall. However, OT did not specifically affect the trade-off. OT also did not affect specific or gist memory globally. These results partially align with our hypothesis that due to anxiolytic effects, OT would suppress enhancement of emotional memory. First, there were no clear anxiolytic effects of OT to associate with EPMT effects. Second, there were no group differences on any EPMT memory variables. Therefore, the emotional enhancement of memory was not uniquely suppressed by OT. When added to those
models, cortisol did not moderate the trade-off effect, or affect global memory overall or by condition.

In contrast with the working memory, these null results suggest that OT may suppress negative effects of cortisol on emotional memory. Findings match other literature where higher cortisol is commonly negatively associated with memory in general, and positively associated with memory for emotionally salient stimuli specifically (Smeets et al., 2008; Wolf, 2009). Unlike many studies using the EPMT, cortisol’s effects on memory do not always differentially affect emotional and negative information (Abercrombie et al., 2003). One study showed increased emotional ratings of neutral stimuli in individuals administered higher levels of cortisol (Abercrombie et al., 2005). Although this evidence contrasts with our null emotional memory effects, despite higher cortisol in Controls, arousal ratings of stimuli are not the same for memory for stimuli. The Abercrombie et al. (2005) results are also similar to the affective results in our study: affect measured by the PANAS and BRAQ in both studies was not related to cortisol. In contrast with this and other studies of cortisol and memory, we did not raise cortisol endogenously or expose our participants to acute stress. Perhaps our results would align with previous research on cortisol and emotional memory, and produce condition differences in the EPMT trade-off effect, when using a stress manipulation while assessing OT. Manipulating cortisol and observing effects on OT and emotional memory is an important future research direction.
Although this study did not examine emotional stimuli with only social relevance (e.g., negative and neutral faces rather than objects and backgrounds), previous work assessing OT’s effects on emotional and declarative memory with social content has found mixed results (Bartz et al., 2011). As mentioned earlier, it is possible that cortisol and OT’s effects on non-social memory differ from memory for social stimuli. Yet, no research prior to our investigation has examined non-social emotional memory in women.

However, one study has examined cortisol, memory, and OT together in women. Using a within-subjects design, women were administered cortisol to evaluate effects on plasma OT and memory for lists of pleasant and unpleasant words (Tops et al., 2012). Memory was tested via written free recall immediately after exposure to the word lists. Collapsing across groups, results showed an inverse relationship between recall for unpleasant words and OT: better recall with lower OT and worse recall with higher OT. The authors concluded that cortisol-induced OT increases could mediate effects of stress and cortisol on memory, potentially by decreasing negative memory. An important caveat to this study is that we don’t know how closely blood OT reflects levels of OT in the brain, if at all. As we purposefully manipulated OT rather than cortisol levels it is difficult to compare these results. However, this study and our investigation provide converging suggestive evidence that OT moderates cortisol’s effects on memory. OT may buffer both cortisol’s effects to boost memory of negative stimuli, and the negative association between cortisol and memory. A further point is that in both studies we
were only manipulating one of the two hormones. The next step needed to advance this research is a study in which both hormones are manipulated.

4.3 Physiological Effects

Salivary levels of cortisol, progesterone and testosterone were generally similar between OT and placebo, with a few exceptions. At one Session 1 time point, the OT group demonstrated higher cortisol than the Control group. Session 1 progesterone results exhibited a similar pattern, though non-significant. In contrast, women who received OT tended to have lower testosterone than Controls during Session 1. Interestingly, similar patterns cortisol, progesterone and testosterone results were observed in both study sessions. This evidence suggests that in the absence of a social stressor, OT may actually enhance stress-related hormones (cortisol, progesterone) and decrease testosterone in women. Session 2 hormone patterns mirroring Session 1 results may be attributable to delayed effects. It is possible that OT administration sets into motion a chain of events that lead to HPA axis or central changes in hormone production. Environmental conditioning (returning to our laboratory space where these reactions were originally instigated) may also explain the Session 2 hormone results. These findings have important implications for clinical research using intranasal OT. In those and other instances we need to consider endocrinological side effects of OT administration-- in other words, the effects of OT on other hormone systems.
No other study has examined effects of exogenous OT administration on
testosterone or progesterone increase. Of the existing research, studies in rats suggest
that testosterone mediates OT action in the hypothalamus (Johnson et al., 1989).
Testosterone and OT have been proposed to act antagonistically in the human amygdala
in response to social cues since exogenous testosterone decreases trust and out-group
affiliative behaviors while exogenous OT exerts opposite effects (Bos et al., 2010). These
actions may partially explain attenuated fear/anxiety, increased in-group favoritism and
increased out-group derogation accompanying OT administration (De Dreu et al., 2010;
2011). While testosterone has been shown to reduce generosity in an economic game,
OT exerts the opposite effect (Zak et al., 2007; 2009).

It is noteworthy that saliva samples from six participants were mistakenly left
out of the freezer. Though the samples were assayed for all hormones, assay quality
control characteristics seemed impacted to some extent in the progesterone but not the
cortisol and testosterone assays. Because of these observations, progesterone results
should be treated with caution and may need to be replicated.

Future directions for the physiological effects observed in this study could
include repeating the same study to examine OT’s physiological effects in men and
compare with effects in women, testing OT’s effects on women’s hormones in reaction
to acute stress and social situations, and examining effects of different OT doses on
these hormones. It would also be informative to examine OT’s effect on other
hormones, like estradiol.
4.4 Proposed Mechanisms

Previous research has shown that OT affects animal and human memory, especially for social stimuli (Bartz et al., 2011; Campbell, 2010). But what is the mechanism of these lasting effects? Evidence currently points to OT triggering activity in the PFC and the amygdala (Viviani & Stoop, 2008). While OT receptors are distributed across many areas of the brain, animal models have demonstrated that receptors are particularly abundant in those two structures (Phelps et al., 2010; Young & Wang, 2004). Rodent and human research has also shown that working memory is dependent on the PFC (Wolf, 2009). Therefore, the PFC is likely a primary structure in working memory effects observed in women who received OT. In a study reviewed earlier, OT reduced ACC and mPFC activity in individuals’ with social anxiety who were shown sad faces (Labuschagne, 2011). It is possible that reduction in PFC activity might be a mechanism for our working memory effects. Declarative and emotional memory retrieval are also thought to be mediated by the PFC, hippocampus and the amygdala (Ranganath et al. 2003; Wolf, 2008; Wolf, 2009). Finally, in addition to OT, the PFC is a significant target for GCs due to large amounts of GC receptors (Lupien & Lepage, 2001; Pryce, 2001; Sanchez et al., 2000). It follows that OT’s effects on working memory are exerted by action in the PFC and that these actions are likely very sensitive to GCs such as cortisol.

The amygdala may also be largely responsible for OT’s effects on working and emotional memory (Viviani & Stroop, 2008). In rats, there are different OTR in the amygdala. One group is excited by OT and another group is inhibited by OT (Huber et al.,
Importantly, OT can also bind to vasopressin receptors, but at a lower affinity compared with oxytocin receptors (Akerlund et al., 1999). There are also vasopressin receptors in the medial amygdala and other areas of the brain, which may connect to OT’s action in the amygdala, and suggest that OT’s relations to memory may be mediated by other structures (Huber et al., 2005). In addition to effects on memory, OT has also been shown to influence affective processes, including reducing anxiety (Campbell, 2010). While those effects were not observed in this study, they are also likely attributable to amygdala activation. However, OT’s effects on the amygdala may depend on specific patterns of activation in different subregions (Ellenbogen et al., 2012). Though the basolateral amygdala is known to interact with the PFC to regulate GC effects on working memory (Roozendaal et al., 2004, 2006), in line with research reviewed earlier, the central amygdala may play a larger role in these OT’s connections with mood and anxiety (Viviani & Stroop, 2008).

The PFC has been shown to regulate amygdala activity, specifically in the basolateral amygdala (de Quervain et al., 2009). As our OT group did not show increased memory for emotional stimuli, perhaps the study experience (without testing memory of specifically social stimuli) was not arousing enough to appropriately activate the amygdala. Therefore, it is possible that OT’s unique effects on the PFC depend on the social-salience of stimuli and that the amygdala is specifically attuned to social stimuli.
What is the role of cortisol in OT’s memory effects? Why was working memory affected, while emotional memory was not? In line with previous research, it is possible cortisol could affect working memory, without affecting declarative memory (as in the EPMT) or arousal (Lupien et al., 1999). It is likely that OT affected working memory directly or through some other mechanism given that there were few effects of OT on cortisol.

Based on this literature it is likely that OT affects memory primarily via the PFC and the amygdala. Similar to other instances where the PFC “gates” the amygdala (Quirk & Beer, 2006), one could suggest that OT’s effects on memory are first mediated by the PFC. Our declarative memory task was emotional and likely did activate the amygdala but there were no effects of OT. However, our working memory task was not (presumably) emotional and probably did not engage the amygdala, but definitely engaged the PFC (based on fMRI and other research reviewed) as there were OT effects on working memory. This argues for OT affecting the PFC more than the amygdala. Studies utilizing brain imaging methods are required to further explore how OT interacts with the PFC to mediate OT’s effects on working memory.

GCs are potent elicitors of amygdala activity, when coupled with a significant sympathetic nervous system response (Sapolsky, 2003). Therefore, to make matters more complicated, OT’s effects on memory and other cognitive processes also likely contingent on the level of arousal an individual is experiencing. This is especially the
case related to cortisol and memory. Greater HPA arousal would likely make non-social stimuli less memorable, impairing memory overall.

In sum, OT likely affects memory through the PFC though further brain imaging research is needed parse these interactions.

4.5 Limitations

Though this was a carefully-controlled, double-blind study, a number of limitations should be acknowledged. One important limitation relates to the validity of the memory tasks used. While the n-back and EPMT have been used to evaluate memory in other studies, we do not know whether they were the best or most relevant tasks to assess effects of OT on memory.

Though the tasks were counterbalanced during Session 1, they were not during Session 2. This detail could have produced order effects during Session 2 on one or both tasks.

Incidental encoding (women were not told that they would be tested on their memory for EPMT stimuli) rather than intentional encoding was used for the declarative memory task. Incidental encoding has been previously shown to result in lower recall (Mandler, 1967). Perhaps intentional encoding would have produced more robust effects on memory. Intentional encoding could have also made participant’s performance on the tasks more socially relevant (potentially through demand characteristics), as OT is influential in social cognition and social reward/reinforcement.
Also, as the study did not test memory for explicitly social stimuli, we cannot directly compare these non-social results with previous examinations of OT and social stimuli.

Related to OT specifically, although menstrual phase was controlled, we did not assess women’s baseline or change in blood plasma OT levels. This information could have helped evaluate the extent to which the intranasal OT was metabolized and evaluating whether OT was still elevated during Session 2. There could have been different effects on memory in women with lower pre-administration (i.e., endogenous baseline) OT levels, or vice versa. There are many individual variations in OT sensitivity and resulting effects (Poulin et al., 2012).

Unfortunately we do not know if/how much variability there was in actual OT or placebo spray administration including the number of spray puffs that they actually gave themselves and how much got into their brains. Cognitive changes may relate to the dose of OT given. As evidenced by mixed study results with different OT doses (among other methodological variations), and commonly found U-shaped dose curves for other hormones (e.g. cortisol; Wolf 2008), participants’ given lower doses might exhibit very different effects, even stronger effects, than found in the current study.

Finally, though our OT and Control groups were larger than most studies examining cognition in men and/or women, it is possible that with a larger sample, we could have detected more robust effects. Another limitation is that since this study only examined these effects in women it is difficult to compare these results to the many studies of OT and memory in men.
4.6 Considerations for Clinical Use of OT

OT’s effects on memory have important implications for various types of psychopathology. This is particularly the case when considering OT’s interaction with cortisol on memory. Many types of psychopathology including schizophrenia show significant working memory impairment, among many other memory deficits (Van Snellenberg, 2009; Wolf, 2008). Our results linking OT with impaired working memory may have important implications for the clinical use of OT.

Researchers have been eager to examine OT’s efficacy in treating psychopathology and other conditions (e.g., autism) characterized by social dysfunction (Andaria et al., 2010; Averbeck et al., 2012; Guastella et al., 2010; Hollander et al., 2007; Jesso, 2011). In one investigation, men and women with schizophrenia were treated with intranasal OT over a three week period (Feifel et al., 2012). Though short-term verbal memory improved, working memory performance did not. Reflecting on our working memory results in healthy women, this is not surprising. Compared to a 24 I.U. dose, the clinical investigation administered 20 I.U. twice daily for one week and then 40 I.U. twice daily thereafter – well above normal physiological levels. If a linear relationship exists between OT and working memory (which is unknown at present), such large doses could be detrimental. While the Feifel et al (2012) study did not report any significant impairment, there may be unforeseen long-term effects on neuroendocrine or other physiological systems (Geenan et al., 1988).
A major issue with this type of research is that participants usually remain on anti-psychotics or other medications during clinical investigations. These drugs could interact with OT to produce different effects than would be observed in healthy, non-medicated individuals. OT may also exert unique effects in clinical populations due to recognized differences in neural architecture (e.g., reduced hippocampal volumes in PTSD patients) reported in those groups (Wolf, 2008).

Although OT seems to dampen working memory in our study, it does not do so dramatically. However, should we risk potentially handicapping those with known cognitive deficits like schizophrenia? GC treatment has been consistently shown to impair working memory more in clinical populations compared to healthy controls (Grossman et al., 2006). OT might exert similar effects to GCs by impairing clinical populations more than healthy people. Finally, what are the potential effects of giving a person an OT dose over a long period of time, days or months? It is possible that OT levels and related effects may increase over time or that individuals could build a tolerance to OT effects. All these questions advocate caution when studying OT and translating research results.

4.7 Conclusions

Of the various theories about OT's effects (Campbell, 2010), OT's previously described influence on memory seems to derive from its ability to increase attention to social stimuli (Bartz et al., 2011). To better understand this hormone, it is also critical to
evaluate OT’s effects outside of explicitly social contexts. Therefore, the present investigation used working and emotional memory tasks, as well as assessed affect and salivary hormone levels.

Together these measures tested the hypothesis that intranasal OT would suppress working and emotional memory globally. As working memory what somewhat suppressed by OT, and emotional memory was not enhanced in women administered OT, we can conclude that the proposed memory hypothesis was supported in both cases. Related to cortisol, it was hypothesized that OT effects on memory would be moderated by individuals’ cortisol levels, and that OT and cortisol would together decrease working memory. This hypothesis was supported for working memory but was not supported for the EPMT. Further, cortisol’s effects on working memory were different depending on the type of memory. These results are difficult to interpret.

Results from this study are theoretically interesting because they advance our understanding of OT’s effects on cognition. Findings imply that OT’s effects on cognition are not necessarily targeted toward social information, as the working memory task is not social. Instead, OT seems to exert more global effects on learning and memory. This idea makes sense considering that OT receptors are prevalent throughout the brain.

The present investigation furthers our understanding of OT’s broader impacts on the human brain and on neuroendocrine function. Additional research is necessary to best evaluate how stress and the stress hormone cortisol interact with OT in memory, as well as to inform pharmacological and clinical investigations using OT. Overall, this basic
research project is a crucial leap forward in understanding OT, a hormone vital to human behavior.
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