Postpartum Oxytocin Receptor Plasticity And Anxiety-Like Behavior in Syrian Hamsters: The Potential Role of the Dorsal Raphe Nuclei

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Abstract

The postpartum period is a time of high vulnerability for females to develop depression. Not only does postpartum depression significantly hinder the motivation and enjoyment that a mother feels in her new role, but also the development of the offspring, doubling the impact of this unique affective disorder. Oxytocin, a hormone involved in birth, maternal behavior, depression, and anxiety may help to untangle the link between postpartum hormonal levels and the occurrence of postpartum depression. The present study examined oxytocin receptor plasticity in efferents of the paraventricular nucleus, the main source of central oxytocin, in an animal model following a hormone-simulated pregnancy. Estrogen-withdrawn females exhibited a significantly greater density of oxytocin receptors in the dorsal raphe nuclei and there were no significant differences in receptor density between hormonal treatment groups in other efferents examined. Estrogen-withdrawn females also displayed higher levels of anxiety-like behavior than estrogen-sustained animals in the Elevated Plus Maze. Ultimately, the present study aimed to understand the role of oxytocin signaling in postpartum mood disturbances and how oxytocin may meet the demand as a novel, targeted treatment for postpartum depression.

Key words: postpartum depression, oxytocin, paraventricular nucleus, and efferents
Postpartum Oxytocin Receptor Plasticity And Anxiety-Like Behavior in Syrian Hamsters: The Potential Role of the Dorsal Raphe Nuclei

Postpartum depression (PPD) is a unique and devastating affective disorder that produces severely altered mood and behavior in mother, and lasting implications for infant development. Postpartum depression is also one of the most common mental illnesses, affecting up to 15% - 20% of perinatal women (Pawluski, Lonstein & Fleming, 2017, Perani & Slattery, 2014). Despite its far-reaching implications, PPD has been considered one of the most underrepresented and least treated affective disorders (Brummelte & Galea, 2009). Unfortunately, the under treatment of PPD may result from more than just a lack of research. Many mothers feel as though the risk of current treatment methods, namely antidepressants, on infants through breast milk is too high (Marcus, 2009). Mothers may also feel ashamed of their altered emotional state during a time of expected elevated mood (Latvala, 2013). No matter the cause of postpartum depression’s underrepresentation, it is clear that the current state of treatments for PPD is inadequate for an affective disorder with such far-reaching effects.

For postpartum depression to be considered one of the most common mental illnesses, the current state of treatment options for this disorder is undoubtedly subpar. Currently, the most commonly utilized pharmacological treatment for PPD, the use of SSRIs, is insufficient at improving maternal and infant outcomes (Forman, O’Hara, Stuart, Gorman, Larsen, & Coy, 2007). Thus, it is imperative that the neurobiology of postpartum depression and its potential therapeutic targets be better understood. Due to oxytocin’s involvement in in birth, maternal behavior, depression and anxiety, and its relation to postpartum estrogen levels, individual variations in postpartum oxytocin levels may shed light on why some women experience postpartum depression, while others do not. Ultimately, it is the present study’s hypothesis that postpartum oxytocin levels may serve as a potential biomarker for postpartum depression.
Overall, by understanding the typical response to the postpartum period as it relates to oxytocin, targets for maladaptive responses to the postpartum period may be more near than ever.

**Postpartum Depression: Diagnosis**

Postpartum depression is currently diagnosed under major depressive disorder in the Diagnostic Statistical Manual of Mental Disorders (5th ed.; *DSM–5*; American Psychiatric Association, 2013), and under postpartum mood disorders in the International Statistical Classification of Diseases (10th ed.; ICD-10). In the DSM-V, postpartum depression is categorized as “major depressive disorder with peripartum onset”. “Peripartum onset” refers to the appearance of mood symptoms during pregnancy or in the 4 weeks following delivery. However, suggestions have been made for this period to be lengthened, as symptoms of PPD can appear both before parturition and beyond the 4-week period (Ross, Murray, & Steiner, 2005). Similarly, questions have been raised regarding the proposed similarities between behavioral manifestations of PPD and MDD. While the following sections detail the distinctions between the two, it is undoubtedly true that PPD and MDD share similar behavioral and neurobiological features. As well, there is limited research into the neurobiology of PPD to support their distinctiveness. Despite this lack of research to support their distinct neurobiology, the characteristic features used to diagnose PPD ultimately neglect a wide variety of the clinical symptoms that many depressed mothers may experience.

Postpartum depression can be diagnosed if a women experiences five or more of the symptoms of MDD during the peripartum period, as defined above, for longer than two weeks (APA, 2013). Symptoms used to diagnose both MDD and PPD include depressed mood, decreased interest in pleasure, changes in sleep patterns, fatigue, and thoughts of suicide. However, many symptoms of PPD are not described by its current diagnostic criteria, including
alterations in women's maternal care, alterations in empathy, stress, motivation, emotional reaction to stimuli and executive functioning are some of the (Moses-Kolko et al., 2014). Research has also shown that mothers with PPD express a delay in adapting to be a mother and a greater chance to have mechanical infant caring (maternal care in an unthinking or unfeeling manner) (Barr, 2008). These are just some examples of the behavioral symptoms that distinguish postpartum depression from major depressive disorder.

Apart from underrepresenting many of the symptoms of PPD, the current diagnostic criteria for PPD may, in fact, prevent many mothers from seeking treatment. Postpartum depression may often be under-diagnosed because its behavioral symptoms, such as changes in sleep patterns and changes in appetite, are also associated with typical perinatal adjustment (Brummelte & Galea, 2009). While an adjustment period to the demands of infant care is typical, many women are reluctant to seek treatment for longer-lasting behavioral symptoms. Thus, many women who experience symptoms of PPD may either cast these symptoms aside as normal, or be hesitant to seek treatment. This hesitation may result from the fact that treatment for postpartum depression has inevitable pharmacological effects on infants through breast milk (Marcus, 2009), or because mothers may be ultimately reluctant to admit feelings of depression during a time of expected bliss (Latvala, 2013). Overall, reluctance to admit feelings of depressed mood, the fear of the potential impact of treatment on infant development, and expectations of normal adjustment may influence both PPD’s under-diagnosis and the development of novel treatments.

Symptoms of postpartum depression may also be exacerbated by individual differences in ability to adjust to parenting, a characteristic of PPD that is also unique. The psychosocial adaptations that differentiate PPD from other affective disorders include a demand for increases in responsiveness, sensitivity, and warmth. The postpartum period also requires that mothers
rapidly gain parenting confidence, parenting competence, and the ability to cope with parenting stress (Letourneau et al., 2017). Individual differences in the severity of PPD symptoms can also have influences on each other in a self-propelling manner. Indeed, postpartum depression negatively impacts a mother’s enjoyment in her maternal role and the mother and the child’s relationship (Letourneau, Dennis, Cosic & Linder, 2017). Thus, as postpartum depressed mothers interact with their children less, some mothers may feel even more depressed mood. These necessary social adjustments and increased demands for mothers in the perinatal period relate to PPD’s unique psychosocial environment, further differentiating it from MDD.

The worldwide prevalence of postpartum depression also differs significantly from that of major depressive disorder. While many mothers imagine the postpartum window to be one of excitement and bliss (Latvala, 2013), a large percentage of postnatal women are significantly affected by negative mood. In fact, 50-70% of women who give birth experience a less debilitating form of PPD, termed the baby blues. The baby blues usually begin at day 3 or 4 following delivery and cease within 2 weeks (O’Hara et al., 1991). Yonkers and colleagues’ also suggest that 50% of women with postpartum depression experience depressed affect prior to delivery, as well (2001). A subset of the women who experience baby blues for longer than two weeks, from 15-20%, are diagnosed with postpartum depression (Pawluski, Lonstein & Fleming, 2017, Perani & Slattery, 2014). Thus, it is highly common for women to experience depressed mood during and after pregnancy. In contrast, major depressive disorder has a prevalence of approximately 7%, alerting to the fact that postpartum depression is much more common than major depressive disorder (American Psychiatric Association, 2013). Ultimately, is clear that a new framework for understanding postpartum depression is necessary.
Postpartum Depression and Infant Development

The effects of postpartum depression on infant development are far-reaching and long lasting. During infancy, offspring are in a period of development that is especially sensitive to its environment. This sensitive period of development is also characterized by the most rapid increase in neurons in any period of life (Thomas & Johnson, 2008). Neuronal development, and ultimately, neuronal pruning establishes the groundwork through which infants come to know and understand their environment. This rapid neuronal growth and pruning means that the sensitive period of development is especially responsive to negative stimuli. Research has shown that early life adversity in the form of maternal depression can affect the emotional competency, intellectual ability, and the future parenting style of the infant (Letourneau, Dennis, Cosic & Linder, 2017). One longitudinal study also found a significant correlation between maternal depression at 3 months postpartum and myriad of setbacks at 11 years old, including lower IQ, increased problems with attention, decreased mathematical reasoning, and an increased likelihood for the need for special educational (Hay et al., 2001). However, even when successfully treated, PPD has significant effects on the infant. Hay et al. (2001) found that even a decade after their mother’s recovery from PPD, children continued to be at risk for behavioral, emotional, and academic set backs. Overall, it is clear that postpartum depression can have long lasting impacts on infant development.

Not only is the intellectual development and ability of the child is significantly hindered by maternal depression, but also the emotional development (Letourneau, Dennis, Cosic & Linder, 2017). This emotional development, or lack thereof, decreases children’s ability to process their emotions on their own (Letourneau, Dennis, Cosic & Linder, 2017). Postpartum depressed mothers are also less likely to respond to their children’s needs (Michalska et al.,
Failure to respond to a child’s needs also decreases the child’s ability to regulate their emotions. The research behind the effects of maternal depression on child development is certainly robust. Multiple meta-analytic reviews have shown that maternal depression is strongly associated with their children’s negative affect and maladaptive behavioral problems, making a strong case for an understanding of maternal depression as an early life adversity (Beck, 1999; Goodman et al., 2011). Ultimately, postpartum depressed mothers are unable to meet the needs of a child during a critical period of emotional development, producing long term effects for the child’s emotional regulation and wellbeing.

Along with emotional and intellectual development, offspring of mothers with PPD are thought to have difficulty with their own parenting behavior later in life. Neurobiologically, PPD has been correlated to long lasting effects in the brain of the child that negatively influences behavior. In an animal model, early life adversity as a result of postpartum depression has been shown to result in abnormal development of the oxytocin system in offspring, as well as lead to poorer quality of parenting behavior later in adulthood (Champagne & Meaney, 2001). Further research is necessary to understand the role of maternal depression on the oxytocin system in human offspring. However, it is undoubtedly clear that maternal depression can have long lasting effects on the children of depressed mothers. These effects range from behavioral to academic to emotional setbacks. The impacts of maternal depression on offspring also extend beyond the postpartum period, even if the depression was successfully treated. Overall, these data show the long lasting impacts that postpartum depression can have on infant development.
**Postpartum Depression and Maternal Care Giving**

A relatively novel concept to the study of postpartum depression has been the discovery of the maternal caregiving network. The maternal care-giving network is a group of brain regions that aids in maternal-infant relationships, motivation for the expression of maternal behaviors, and reactions to offspring, behaviors that are vital to positive child development. These maternal behaviors are supported by plasticity in the maternal care giving network, which includes the medial preoptic area, bed nucleus of the stria terminalis, amygdala, nucleus accumbens, and other sensory, limbic, and cortical systems that project to these sites (Numan, 2006). Postpartum plasticity within these regions support maternal-infant relationships, a vital aspect of child development. These relationships are maintained through maternal responses to infant needs, as well as the mothers’ ability to learn from their experiences attending to the infant (Barrett & Fleming, 2010). Sensitive caregivers respond to a complex array of infant cues using methods such as recognition and acknowledgement of infant signals, maintenance of visual contact, and appropriate empathy mirroring and vocal quality (Kim, Strathearn & Swain, 2016). Importantly, postpartum gonadal hormone levels trigger plasticity in the maternal caregiving network, which supports increased reactivity (Kim, Strathearn & Swain, 2016). Thus, the maternal care-giving network is crucially implicated in child development, and the plasticity and reactivity of these brain regions is a sign of a mother’s ability to meet her infant’s needs.

Research has shown that decreased reactivity in the maternal caregiving network is correlated with both maternal depression and less efficient caregiving (Kim, Strathearn & Swain, 2016). As previously mentioned, appropriate responses in the maternal caregiving network are dependent on the rapid hormonal changes during the postpartum period (Kim, Strathearn & Swain, 2016). The plasticity and reactivity of the maternal care-giving network is also predictive...
of the degree to which a mother can effectively generate certain emotional responses to her infant (Kim, Strathearn & Swain, 2016; Kober et al., 2008). Postpartum depressed mothers exhibit less activity in response to cries of their children compared to non-depressed mothers in the maternal care-giving network (Tail et al., 2012, Michalska et al., 2014). In one study, postpartum depressed mothers with lower levels of oxytocin also showed reduced activations in regions of the maternal caregiving network (the ventral ACC, left NAcc, inferior parietal lobule, and temporal and frontal gyri, regions) in response to videos of their own infants (Tail et al., 2012). Thus, postnatal women who lack sufficient reactivity in their maternal-caregiving network, namely those with postpartum depression, may lack the ability to meet the emotional and developmental needs of their infants through deficits in the maternal care-giving network.

**Animal Models of Postpartum Depression**

The maternal care-giving network, although only a fraction of the picture, sheds light on the widespread and complex neurobiology of postpartum depression. For a number of reasons, animal models are both advantageous and complementary to human models for understanding the neurobiology of many affective and neurological disorders. Primarily, the current state of research methods has few techniques to view cell processes as they occur, *in vivo*. Thus, the use of post-mortem brain tissue to examine the neurobiological changes associated with postpartum depression is necessary, and animal models make this tissue readily examinable. Second, although postpartum depression has one of the highest incidences of any mental illness, the use of animal models is still necessary for an adequate amount data to be available. Finally, a fact common across all animal research, animal models provide a level of experimental control and manipulation that is both impossible and unethical when using a human model. Thus, while the clinical experience of postpartum depression can be described using human research, the
supporting evidence for key neurobiological correlates of postpartum depression originates primarily from research that has utilized animal models.

Historically, two types of animal models of postpartum depression existed: stress-induced, and corticosterone-induced (Brummelte & Galea, 2009). However, in 2001, Galea, Wide, and Barr, developed a novel animal model to measure mood disturbances specifically under the hormonal conditions of the postpartum period. Galea, Wide, and Barr (2001) termed their animal model a “hormone-simulated pregnancy”, and became the first to introduce an animal model of pregnancy aimed specifically at understanding depressed mood in the postpartum period. Using this hormone-simulated pregnancy, researchers were able to induce the same gradual rise of progesterone and estradiol for the typical gestational period and, similarly, induce a sudden withdrawal of these hormones, mirroring that which occurs postpartum. To perform the hormone-simulated pregnancy (HSP), animals receive an ovariectomy to prevent natural production of estradiol and progesterone. Following the ovariectomy, estradiol and progesterone are administered daily at levels associated with the induction of maternal behavior (Galea, Wide, & Barr, 2001). Following a length of time equal to the duration of gestation, females are withdrawn from ovarian hormones, mimicking the abrupt drop in hormones that occurs following delivery.

In the study conducted by Galea et al. (2001), it was hypothesized that depressed behaviors occur following pregnancy due to a “pregnancy-induced” withdrawal from estrogen. To examine this hypothesis, Galea et al. (2001) controlled for the additional neurobiological effects of pregnancy by manipulating only the presence or absence of a postpartum period (ie. withdrawal from estrogen). This study produced shocking results, not only because it was the first study to show depressive-like behaviors in an animal model of the postpartum period, but
also because a large majority of the animals showed depressive-like behaviors. Following withdrawal from estrogen, the majority of rats displayed more depression-like behaviors compared to their still “pregnant” counterparts (animals who did not experience estrogen withdrawal). This research suggested that, following pregnancy, the drastic drop in estrogen might be related to these depression-like symptoms. Although Galea, Wide, and Barr’s research is quite extraordinary for these findings, its methods have also been called into question by the present study.

One puzzling detail of Galea, Wide, and Barr’s data is that a significant majority of the subjects that experienced estrogen-withdrawal (the typical schedule of hormones for the postpartum period) showed increased depressive-like behaviors compared to control and estrogen-sustained groups (Galea, Wide, Barr, 2001). However, these findings are drastically different from the human experience of postpartum depression, for which 80-90% of postpartum women do not experience depressed mood. Thus, although the novel animal model put forth by Galea, Wide, and Barr shows the first demonstration of ‘depressive-like’ symptoms in the postpartum period in a rodent model, the findings of this study are not directly comparable to the clinical, human prevalence of postpartum depression.

A recent study employed the hormone-simulated pregnancy model in hamsters and found a prevalence of depression much closer to that of the human population. Amaral (2017), Bodie, (2017) D’Antonio (2017), and Lee (2017) used the hormone-simulated pregnancy model employed by Galea, Wide, and Barr and became the first to measure the number of oxytocin producing neurons in the paraventricular nucleus of the hypothalamus (PVN) of female hamsters following hormone-simulated pregnancy. These studies found that levels of oxytocin immunoreactive cells correlated to anxiety-like behavior in the postpartum period. Specifically,
the group that experienced a withdrawal from estrogen showed significantly more oxytocin-producing neurons in the PVN, less anxiety-like behavior, with no difference in depression-like behaviors compared to the estrogen-sustained group. Thus, although Galea et al. (2001) was the first study to employ the hormone-simulated pregnancy model and found that the majority of their animals present with postpartum depression, Amaral (2017), Bodie, (2017), D’Antonio (2017), and Lee (2017) produced a much more typical response to “pregnancy” in their studies. Therefore, the HSP in hamsters may more accurately reflect the typical human experience of pregnancy, as opposed to being a model of postpartum depression.

While Amaral (2017), Bodie, (2017), D’Antonio (2017), and Lee (2017) did not find a significant difference between their two groups in depression, this study did find a decrease in anxiety in estrogen-withdrawn females. As estrogen-withdrawal maps onto the typical hormone schedule in pregnancy, it is hypothesized that a decrease in anxiety is the typical, adaptive response to pregnancy in humans. Aligning with the hypothesis that an HSP produces typical emotional responses to postpartum, further animal models have also shown decreases in anxiety-like behavior in the postpartum period (Macbeth, Gautreaux, & Luine, 2008). Evolutionarily, this decrease in anxiety following pregnancy may be necessary to adapt to postpartum, as the postpartum period is a time of increased stress (Fleming & Luebke, 1981). Without a natural mechanism for handling increased stress following birth, mothers in nature would be more prone to infanticide or to abandon their offspring. Thus, the current research suggests that a decrease in anxiety-like behavior with no change in depression is the natural and evolutionarily appropriate response to pregnancy and birth.

Overall, the hormone-simulated pregnancy followed by estrogen withdrawal has been shown to produce a decrease in anxiety, no significant change in depression, and an increase in
oxytocin in the PVN of female Syrian hamsters. These changes in mood and behavior may map more accurately onto the typical postpartum experience in humans. Therefore, it is believed that the HSP may model typical responses to the postpartum period, rather than producing a model of postpartum depression. However, studying the typical response to the postpartum period allows the field to better understand potential biomarkers for PPD by, eventually, disrupting the normal response to postpartum and measuring disturbances in mood. In this way, the HSP can be just as illuminating as a model of the typical response to pregnancy.

**Neurobiology of Postpartum Depression**

While largely understudied, evidence suggests that the neurobiology of postpartum depression is intricately tied to the hormonal alterations that occur during pregnancy and following parturition. Most notably, the shared role of gonadal hormones in the etiology of both PPD and MDD sheds light on the role of hormones to incidences of depression. Adolescence and the peripartum window represent the periods during which women are at the highest risk to develop depression. These two periods occur simultaneously to rapid hormonal changes, suggesting a hormonal role in mediating the onset of depression (Marcus, 2009). In support of this argument, it has been shown that females experience rates of MDD up to 1.5-3 times that of males beginning in adolescence (Kessler et al., 2003). The increase in prevalence of MDD during a time of rapid hormonal changes alerts to a similar characteristics of the postpartum period. Thus, the role of hormones in the etiology of postpartum depression warrants further attention.

As hormones may play a role in the development of PPD, an understanding of the hormonal changes associated with pregnancy and postpartum depression is necessary. The hormones that undergo the most drastic alterations during these time periods are estrogen and
progesterone. However, multiple hormones, which act on the gonads to produce internal environments that are optimal for procreation, are essential to reproduction (Christensen et al., 2012). Gonadal hormones are regulated by the hypothalamic–pituitary–gonadal axis (HPG axis), and are both necessary for reproduction and intricately tied to the neurobiology of pregnancy and PPD. Both in the periphery and centrally, the HPG axis causes gonadal alterations that have widespread effects in humans and animals. In women, the HPG axis operates both centrally and peripherally in a loop of hormones that controls ovulation. This loop begins with the gonadotropin-releasing hormone (GnRH), which acts on the anterior pituitary gland (Christensen et al., 2012). In females, the anterior pituitary gland is stimulated by GnRH to release follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH and LH then travel through the blood stream to act upon the ovaries, affecting ovarian follicle development. As the ovarian follicles develop, they release estradiol, which inhibits further release of GnRH and FSH. Finally, as estradiol levels rise, LH is triggered to release, inducing ovulation (Christensen et al., 2012). Once ovulation occurs, sperm is able to fertilize an ovum. Once an egg has been fertilized, of reproduction can occur. During the 40 weeks of pregnancy, women experience a gradual rises in estradiol and progesterone until the expulsion of the placenta with birth (Galea et al., 2001).

**Estrogen-withdrawal hypothesis.** In the third trimester, endogenous estrogen, synthesized primarily from the placenta, rises to levels 100-1000 times above baseline levels (approximately 15,000 pg/ml) (Brummelte & Galea, 2010). During birth, the placenta is expelled, and postnatal women experience a drastic drop in estradiol levels. Since estrogen levels rise throughout the gestational period and drop dramatically with the expulsion of the placenta, numerous studies have proposed an “estrogen withdrawal” hypothesis for the induction of PPD.
(Galea et al., 2001). The “estrogen withdrawal” hypothesis posits that postpartum mood disturbances are due to a drastic and sudden decrease in endogenous estrogen levels. Therefore, estrogen has become a key variable for investigation related to the etiology of postpartum depression.

**Oxytocin and postpartum depression.** As previously mentioned, the central hypothesis of this paper posits that the estrogen withdrawal following parturition is a precursor to postpartum depression. However, based on previous research, the present research also hypothesizes that individual differences in experiences of postpartum depression is based on estrogen withdrawal-induced plasticity of oxytocin signaling. The following sections will detail oxytocin’s role in maternal mood, behavior, and implications for the postpartum depression.

Importantly, oxytocin’s involvement in regulating the necessary responses to pregnancy is well documented in the literature. In the periphery, oxytocin is the hormone that galvanizes both uterine contractions during labor and lactation. During labor, oxytocin is released into the bloodstream and binds to oxytocin receptors in the uterine wall to facilitate contraction of the smooth muscle (Rinaman, Sherman, & Stricker, 2000). Similarly, oxytocin receptors concentrated around the milk ducts and alveoli in the mammary glands are activated as result of suckling (Rinaman, Sherman, & Stricker, 2000). While peripheral oxytocin, or oxytocin in the body, regulates key functions of the peripartum period, central oxytocin, or oxytocin in the brain, is crucial to understanding the characteristic neurobiology of postpartum depression.

Central oxytocin is intricately tied to maternal mood. The oxytocin system is relevant for regulating stress, depression, anxiety, and sociality (Gimpl & Fahrenholz, 2001; Szymanska, Schneider, Chateau-Smith, Nezelof, & Vulliez-Coady, 2017). Administration of oxytocin antagonists increase anxiety-like behavior in pregnant and postpartum animals (Neumann,
Torner, & Wigger, 2000) animals, while central administration of oxytocin at a dose of 10 µg produces anxiolytic effects outside of the postpartum period (Ring et al., 2006). Similarly, increased levels of central oxytocin have been correlated with lower levels of anxiety following a rapid decrease in estrogen (Amaral, 2017; Bodie, 2017; D’Antonio, 2017; & Lee, 2017).

Research has also alluded to the role of oxytocin in postpartum depression, such that oxytocin levels 2 days after delivery correlate to postpartum depressive symptoms in women (Jobst et al., 2016). In a meta-analysis on oxytocin as a potential biomarker for depression in the perinatal period, Moura, Canavarro, & Figueiredo-Braga (2015) also found that lower depressive symptoms are associated with higher levels of oxytocin. Thus, it is clear that oxytocin is a key neurotransmitter in regulating maternal depression and anxiety (Gimpl & Fahrenholz, 2001), aspects of maternal mood which postpartum depression severely affects.

Oxytocin also has a well-documented influence on mother-infant relationships. Oxytocin plays a large role in sociality, specifically pair bonding (Williams et al., 1994), paternal care (Parker et al., 2001), maternal behavior (Pedersen et al., 1982), and parental sensitivity (Szymanska, Schneider, Chateau-Smith, Nezelof, & Vulliez-Coady, 2017). Oxytocin is also implicated in maternal-infant relationships through its effects on maternal mood. As oxytocin released at parturition and from tactile inputs (Lonstein, 2007; Jobst et al., 2016), and has anxiolytic effects (Russell & Brunton, 2017), the presence of oxytocin may affect individual differences in readiness to adapt to motherhood through its anxiolytic effects. These far reaching effects show that increased levels of oxytocin are correlated with increased social and care giving behaviors, and detail the importance of appropriate levels of oxytocin on improving maternal-infant relationships and maternal self-efficacy, perhaps through positive alterations in mood. Ultimately, oxytocin is not only a key neurotransmitter in regulating maternal anxiety and
depression, but also maternal care-giving behavior. Thus, oxytocin is a promising avenue for finding targeted therapeutic treatments for postpartum depression.

Optimal postnatal oxytocin levels are also dependent on the appropriate levels of estrogen following parturition. The signals induced by peripartum gonadal hormone levels are known to kick-start the normal maternal response to pregnancy and postpartum, which involves increased oxytocin (Kim, Statehearn, & Swain, 2016). While these gonadal hormones, namely estradiol and progesterone, are crucial in preparing the female brain and body for postpartum, the oxytocin system may explain individual differences in readiness to adapt to motherhood. Moreover, animal models have informed the literature that oxytocin and estradiol are elaborately related to each other, and that optimal maternal behavior is dependent on low levels of estradiol and high levels of oxytocin (Numan, 2007; Rosenblatt, Olufowobi, & Siegel, 1998). Overall, these data suggest that neuroplastic differences in postpartum oxytocin levels, as influenced by peripartum changes in gonadal hormones, could illuminate the differences between mothers with and without postpartum depression.

**Efferents of the paraventricular nucleus.** Centrally, two brain regions produce the vast majority of oxytocin: the paraventricular nucleus of the hypothalamus (PVN) and the supraoptic nucleus (SON) (Russell & Brunton, 2017). While the SON regulates peripheral levels of oxytocin, the PVN regulates central oxytocin and projects it widely throughout the brain. The locations of these projections, or efferents of the PVN, are brain regions where the presence of oxytocin receptors has been confirmed. Neuroanatomical tract tracing studies have also confirmed that the PVN projects to certain efferents (Geerling, Shin, Chimenti, & Loewy, 2010; Luiten, Ter Horst, Karst, & Steffens, 1985). Efferents of the PVN include the brainstem (nucleus tractus solitarii), the spinal cord (dorsal horns), the limbic brain (amygdala and hippocampus),
the nucleus accumbens, and the ventromedial hypothalamic nucleus. Functionally, oxytocin has a wide range of effects in these widespread brain regions, including appetite control, anxiolysis, autonomic regulation, and social behaviors (Russell & Brunton, 2017). For the purpose of understanding oxytocin plasticity as it relates to postpartum depression, four efferents of the PVN have been selected. The bed nucleus of the stria terminalis (BNST), medial amygdala (MA), dorsal raphe nuclei (RN), and the nucleus accumbens (NAcc) are efferents of the PVN that are well documented as containing oxytocin receptors (Adan et al., 1995; Boccia, Petrusz, Suzuki, Marson, & Pedersen, 2013; Tribollet, Dubois-Dauphin, Dreifuss, Barberis, & Jard, 1992) being sensitive to gonadal hormones (Alves, Weiland, Hayashi, & McEwen, 1998) and having implications for maternal behavior. As such, these efferents are excellent candidates for examining postpartum oxytocin receptor plasticity as it relates to postpartum mood disturbances.

The presence of projections from the PVN to the four efferents previously mentioned is well documented (Geerling, Shin, Chimenti, & Loewy, 2010; Yoshida et al., 2009). Having established that these locations are responsive to oxytocin, evidence for the locations’ hormonal sensitivity is the next step in determining their aptitude for the present study. Importantly, research suggests that oxytocin levels are related to gonadal hormone fluctuation, such that estradiol has the ability to increase oxytocin levels (Kim, Strathearn & Swain, 2016). Several studies have also shown that the dorsal raphe nuclei (Alves, Weiland, Hayashi, & McEwen, 1998) among other regions in the present research have receptors for estrogen, progesterone, and oxytocin. In general, it is accepted that estrogen up-regulates oxytocin receptor gene expression while progesterone inhibits expression (Russell & Brunton, 2017). Interestingly, progesterone decreases prior to delivery (Christensen et al., 2012) suggesting that there is an endogenous mechanism for oxytocin receptor up-regulation prior to the postnatal period, which increases
sensitivity to oxytocin. This sensitivity is extremely important for maternal behavior, as oxytocin is deeply implicated in the necessary response to motherhood. As well, in the RN and NAcc, estrogen and progesterone regulate dopamine and serotonin release, which are intricately implicated in depression and anxiety (Alves, Weiland, Hayashi, & McEwen, 1998; Thompson & Moss, 1994). Finally, the medial amygdala and the BSNT are regions in the maternal care-giving network whose plasticity is dependent on postnatal levels of estrogen (Numan, 2006). Thus, it has been established that PVN projections are present in particular brain regions, and that postnatal levels of both estrogen and progesterone regulate some of these efferents.

Supporting the role of oxytocin action in the BNST, MA, RN, and NAcc in postpartum depression is research that suggests that these regions are not only sensitive to estrogen and progesterone, but that their sensitivity is related to both oxytocin and maternal behavior. For example, postpartum depressed mothers with lower levels of oxytocin also showed reduced activations in the maternal caregiving network, which includes all four of these efferents, in response to videos of their own infants (Tail et al., 2012). Also in the nucleus accumbens, higher oxytocin receptor levels are correlated with increased consolidation of maternal memory, or the onset of maternal responsiveness (D'Cunha, King, Fleming, & Levy, 2011). Research has also shown that parturition induces increased expression of oxytocin receptor mRNA in the bed nucleus of the stria terminal (BNST), among other PVN efferents (Broad, Lévy, Evans, Kimura, Kevern, & Kendrick, 1999). Third, oxytocin infusion into the raphe nuclei has been shown to reduce anxiety-related behaviors (Yoshida et al., 2009), while lesions to the median raphe nuclei in rats produced extremely low incidences of maternal behavior when compared to sham-operated groups (Yurino, Tsukahara, Korányi, & Yamanouchi, 2001). Ultimately, the four PVN efferents being assessed for oxytocin receptor plasticity in the present study have been chosen
based on a depth of knowledge on their hormone sensitivity and implications in maternal mood and behavior.

**Research Hypotheses**

The hypotheses of the current study are based upon a deep knowledge of the hormonal, behavioral, and affective changes associated with pregnancy in hamsters, combined with the findings of two previous research studies in similar lines of research. Galea, Wide, and Barr (2001) provide the present research with its experimental manipulations, specifically, the model of a hormone-simulated pregnancy. The present research will also follow in the line of Amaral (2017), Bodie, (2017) D’Antonio (2017), and (Lee, 2017). Based on the findings of the 2017 studies, the present studies are interested in differences in oxytocin receptor levels between estrogen-withdrawn, estrogen-sustained, and control groups in a hormone-simulated pregnancy. More specifically, the present studies are interested in the density of oxytocin receptors in efferents of the paraventricular nucleus of the hypothalamus following a hormone-simulated pregnancy. Oxytocin receptor plasticity is, to the best of our knowledge, a completely novel target for understanding the neurobiological substrates of postpartum depression. The first experiment in this research study will explore four separate PVN efferents, with the expectation that the main hypotheses could be confirmed or rejected in any of the efferents of the PVN.

First, the present research proposes that any oxytocin-receptor plasticity will indicate changes in the overall functional levels of oxytocin in that brain region in manner that regulates homeostasis. Thus, we hypothesize that finding increased oxytocin receptor levels would indicate a decrease in functional levels of oxytocin in that brain region. Oppositely, finding a decrease in oxytocin receptor levels would indicate an increase in functional oxytocin levels in that brain region. However, through the use of immunohistochemical staining and
autoradiography, recent studies have found discrepancies between binding sites for oxytocin and oxytocin-immunoreactive axons, such that some brain regions show abundant oxytocin binding sites with little to no oxytocin-immunoreactive axons (Rinaman, Sherman, & Stricker, 2000). Thus, a consensus has not been reached on whether or not receptor levels always map onto functional levels (Rinaman, Sherman, & Stricker, 2000). However, for the sake of the first study in the present research, it will be assumed that receptor levels and function levels of oxytocin have an inverse correlation.

The present study hypothesizes that there will be two areas of divergence between the estrogen-withdrawn and the estrogen-sustained and control groups. The estrogen-withdrawn group, who are the only animals to receive the typical schedule of hormones associated with pregnancy and postpartum, will experience a decrease in anxiety-like behavior and show a decrease in oxytocin receptor levels (i.e. an increase in functional oxytocin levels). As the only difference between the estrogen-withdrawn and the estrogen-sustained group is that the former will experience a “postpartum period”, it can be assumed that these changes are both a result of and occur in the simulated postpartum period. As Amaral (2017), Bodie, (2017) D’Antonio (2017), and (Lee, 2017) found no differences between their estrogen-withdrawn and control groups in depression-like behavior, the present studies will not measure changes in depression-like behavior.

Following Experiment 1, which will examine the variables described above, one consecutive study will also be performed to confirm the directionality of results seen in previous research (Bodie, 2017). More specifically, Experiment 2 will examine the presence of Flouro-Jade C positive neurons, a marker of neurodegeneration, in the PVN. These data will provide a clearer picture as to the differing levels of oxytocin producing neurons between experimental
conditions. Using these staining methods, it will be possible to examine whether HSP is correlated to neurodegeneration in the estrogen-sustained group.

As previous research found that females who experience an estrogen-withdrawal followed by a hormone simulated pregnancy exhibited significantly higher levels of oxytocin producing neurons than female who did not experience this estrogen drop (Bodie, 2017), Experiment 2 was used to examine possible neurodegeneration in the PVN of females who are estrogen-sustained. Thus, Experiment 2 compared the number of Fluoro-Jade C positive neurons in the PVN of both estrogen-sustained and estrogen-withdrawn females. Fluoro-Jade C (FJC) is a fluorescent anionic dye with greater ability to view morphological detail of degenerating cells than its staining predecessors (FJ and FJB). FJC is favorable because it is compatible with different histological processing and staining protocols, allowing for double-labeling (Ehara & Ueda, 2009). Third, FJC is useful in detecting neuronal cell death that occurs 24 hours to 72 hours before the tissue was extracted. Due to this characteristic, FJC has become a widely popular method for detecting the onset of neurodegeneration, allowing for a greater understanding of the temporal characteristics of degeneration. This is crucially important to the present research, as neurodegeneration is hypothesized to occur during postpartum. Thus, the purpose of FJC for the present research was to view neurodegeneration that may occur following a hormone-simulated pregnancy.

Method

Subjects: Experiment 1

The subjects of Experiment 1 were 24 adult, female Syrian hamsters (*Mesocricetus auratus*) who were purchased from Charles River Laboratories (Wilmington, Massachusetts, USA) at approximately 60 days of age. Eight hamsters were designated to the estrogen-sustained
group, eight hamsters in the estrogen-withdrawn group, and eight hamsters in a true control condition (receiving oil injections throughout). Hamsters were the animal model of choice in this research for a multitude of reasons. Primarily, hamsters were chosen because their neuroendocrine function, a key variable to the present study, is well documented. Hamsters are also solitary animals, allowing them to be housed alone to limit the confound of social interaction on oxytocin receptor levels. Thirdly, the localization of oxytocin-binding sites has also been well documented in literature of Syrian hamsters (Dubois-Dauphin et al., 1992). Syrian hamsters, in particular, are also the most commonly used type of hamster in research, accounting for 90% of the 1,000,000 hamsters used in research annually in the United States (“Hamsters: biology, care, diseases & models,” n.d.). A PubMed search for studies that have utilized Syrian hamsters also revealed over 23,000 results. Thus, the popularity for the use of Syrian hamsters combined with the wealth of knowledge into the systems of interest reveal Syrian hamster as a prime model organism for the current study.

The present study housed hamsters individually in a temperature-controlled room held at approximately 22 degrees Celsius and maintained at a reversed light cycle of 14 hours light to 10 hours dark. Lights were turned off at 10am and on at 12am. All behavioral testing was performed during the dark phase. Each animal was housed in a plastic, solid bottom cage with aspen bedding and two supplementary objects (a wooden block and a plastic tunnel). The cage dimensions were 46 x 24.5 x 20 cm. The animals had access to food and water ad libitum. All animal procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and the Haverford College Institutional Animal Care and Use Committee. All efforts were made to minimize the number of animals used and their suffering.
Procedure: Experiment 1

The 24 hamsters were divided into three groups (N=8). All 24 animals were ovariectomized, subjected to a hormone-simulated pregnancy, and underwent behavioral testing. Each animal received daily injections for 17 days during the HSP. The estrogen-withdrawal group received the typical hormonal schedule (see below) followed by estrogen-withdrawal, the estrogen-sustained group received the typical hormonal schedule followed by maintenance of estrogen levels, and the control group received cottonseed oil injections throughout. On the first and third day of behavioral testing, even numbered hamsters underwent the Open Field Test and Elevated Plus Maze, respectively. On the second and fourth day, odd numbered hamsters underwent the Elevated Plus Maze and Open Field Test, respectively. In this way, the order in which animals were subjected to behavioral testing was pseudo-randomized and counterbalanced across groups. On the fifth day following postpartum, or the 21st day of the experiment, all hamsters were anesthetized using 5% isoflurane in oxygen and euthanized.

Hormone-simulated pregnancy. To begin the hormone-simulated pregnancy (HSP), animals were subjected to an ovariectomy to ensure that endogenous fluctuations of ovarian hormones were eliminated. To preform ovariectomies, all 24 Syrian hamsters were anesthetized to reach surgical plane at 5% isoflurane aerosolized in oxygen and maintained under anesthesia at 2.5-3.5%. Subjects’ flanks were shaved and then disinfected with three alternating scrubs of ethanol and betadine to prepare the surgical site. Next, animals were subcutaneously injected with butorphanol (10mg/kg) and Baytril (10mg/kg) for pre-emptive antibiotic and analgesic treatment. An incision was then made through the skin and muscle under subjects’ rib cage, directly over the ovary. The ovary was pulled through the incision by finding the fat pad associated with it. A hemostat that was heated in a hot bead sterilizer was then placed between
the ovary and the uterine horn and a scalpel was run across the hemostat to remove the cauterized ovary. The muscle was closed with absorbable sutures and the skin was closed with wound clips. This procedure was then repeated for the opposite side. Animals were monitored for one-hour post-operatively and given subcutaneous injections of butorphanol (10mg/kg) for three subsequent days.

After a week of recovery, the hamsters were subcutaneously injected for a period of 17 days, the typical time frame for pregnancy in hamsters (Galea et al., 2001). For days 1-12, the estrogen-sustained and estrogen-withdrawn animals (N=16) were subcutaneously injected with high doses (4 mg) of progesterone and low doses (2.5 µg) of estradiol benzoate dissolved in a cottonseed oil vehicle to mimic hormone levels in early pregnancy. During days 13-16, this same group of hamsters (N=16) received only high doses of estradiol benzoate (50 µg) dissolved in the vehicle during to mimic hormone levels in late pregnancy. On day 17, the estrogen-sustained group (N=8) continued to receive high levels of estradiol benzoate for five days, while the estrogen-withdrawn group (N=8) ceased to receive estradiol and instead received a cottonseed oil vehicle. Our estrogen sustained group did not experience a drop in estradiol on day 17 and thus served as an experimental control to ensure that any neurobiological changes seen in the estrogen-withdrawn group are based on the rapid decrease in estrogen rather than the hormone-simulated pregnancy itself. Throughout these 21 days, a third group of hamsters (N=8) received a cottonseed oil injection (0.1ml) everyday, and served as a true control.

**Euthanization.** On the fifth day postpartum, the hamsters were sacrificed via anesthetized rapid decapitation. Following decapitation, the brains were immediately extracted and flash frozen on dry ice. Later, the tissues were sent to Amy Ross’ laboratory at Georgia State University to be sectioned using a cryostat. The sections were collected as 4 separate sets per
brain region (40 μm thickness) and fixed to slides (See Ehlen, Novak, Karom, Gamble, & Albers, 2008 for methods). At GSU, Dr. Amy Ross preformed autoradiography to localize radioactive ligand bound to oxytocin receptors (Stumpf, 1971).

**Measures: Experiment 1**

**Receptor autoradiography.** The neurobiological measure used to assess differences in mean cell density of oxytocin receptors was autoradiography. Autoradiography involves incubating a section of brain tissue in radioactive ligand. Oxytocin receptor binding was determined with the I 125 -labeled ornithine vasotocin analog Vasotocin, d(CH 2 ) 5 [Tyr(Me) 2, Thr 4, Orn 8, [125 I] Tyr9-NH2] (Perkin-Elmer). The tissue was allowed to thaw and dry, and was then fixed in 0.1% paraformaldehyde for 2 minutes. Following the paraformaldehyde fixative, tissue was mounted onto slides and then rinsed two times for 10 min each in (50 mM Tris, pH 7.4). Following two, ten-minute washes; the slides were then incubated in tracer buffer (0.35 mM bacitracin, Sigma-Aldrich, St. Louis, MO; 0.015mM bovine serum albumin, Sigma-Aldrich, St. Louis, MO; 100 nM I 125 vasotocin analog) for 1 hour. Next, slides were rinsed with agitation in buffer (50mM Tris, 21 mM MgCl) twice for 5 minutes each, then once for 35 minutes. All incubations and washes were performed at room temperature. Finally, the slides were dipped in 4°C deionized water and allowed to dry.

Once dry, the slides and a C 14 standard calibration strip (American Radiolabeled Chemicals, St. Louis, MO) were loaded into autoradiography cassettes and exposed to film (Kodak, Rochester, NY) for seven days at room temperature. Densitometry analysis was performed using Scion Image software (NIH, Bethesda, MD) and a lightbox (Imaging Research, Inc., Ontario, Canada) attached to a camera (Panasonic, Newark, NJ). Standard curves were created using the C 14 microscales on the standard calibration strip. For each brain area of
interest, three tissue sections located 60 μm apart were analyzed on the right and left sides of the brain, except for raphe nuclei sections, which were analyzed along the midline of the brain. With the exception of the MeA, a 0.35 mm² box was placed over the center of each brain area, and the optical density was recorded. A 0.35 mm x 0.75 mm box was used to analyze the MeA in order to measure dorsal and ventral MeA simultaneously. Background binding was subtracted from this measurement. Optical densities were calculated as disintegrating units per min per mg tissue (dpm/mg) (A. Ross, personal communication, April 12, 2018).

**Elevated plus maze.** The Elevated Plus Maze (EPM) is a validated behavioral measure used to assess anxiety-like behavior in rodents (Kaluzeff & Pentti Tuohimaa, 2004). It consists of a raised structure with four arms in a plus-shape. The EPM for the present experiment was 73 cm off of the ground and each arm was 51 cm x 11.5 cm. Two of the arms on the EPM were enclosed with black walls that were 39.5 cm tall, while two arms were open with only a 1 cm lip. The intersecting middle square of the EPM was 10 cm x 11 cm and was not considered a part of any of the four arms when measuring behavior. Each behavioral testing session was recorded and later analyzed using behavior tracking software. Consistent with Kaluzeff and Pentti Tuohimaa (2004), anxiety-like behavior can be assessed in the EPM by a number of variables, including time spent in the enclosed arms, self-grooming duration and frequency, and latency to leave the center square. The primary measure of anxiety-like behavior in the present experiment was the amount of time spent in the open and closed arms.

**Open field test.** The Open Field Test (OFT) is another validated behavioral measure that the present study used to assess anxiety-like behavior. Postpartum anxiety-like behavior in animals has been well established using the OFT (Macbeth, Gautreaux, & Luine, 2008). The OFT in the present experiment was placed on the ground in a behavioral testing room separate
from the housing room. The OFT dimensions were 40.5 cm x 40.5 cm x 30 cm. The camera was mounted 127.5 cm above the bottom of the OFT using a Selfie Stick. Each behavioral testing session was also recorded and later analyzed using tracking software. Consistent with Kalueff and Pentti Tuohimaa (2004), anxiety-like behavior can be assessed in the OFT by a number of variables, including self-grooming duration and frequency, latency to leave the central area, and stretch postures. The primary measure for anxiety-like behavior in the OFT was the amount of time spent in the center and time spent in the periphery of the open field. Nonspecific locomotor activity was also measured (distance traveled, velocity, amount of time spent moving vs. being stationary).

**Statistical Analysis: Experiment 1**

For behavioral measures (EPM and OFT) mean group differences in anxiety-like behavior were analyzed with a between conditions, one-way ANOVA followed by Tukey’s HSD tests for post hoc analyses. In the OFT, anxiety-like behavior was quantified by ambulatory velocity, distance traveled, time in the edge of the arena, and time in the center of the arena. For the EPM, anxiety-like behavior was quantified by time spent in the open arms and time spent in the closed arms. The statistical analyses for autoradiography were conducted using individual ANOVAs for each PVN efferent of interest, followed by Tukey’s HSD tests for post hoc analyses of mean group differences. Despite there being multiple factors in multiple levels for our neurobiological measures, a factorial ANOVA was not used because the interaction effect between OTR levels in each PVN efferent was not a variable of interest to the present study. For receptor autoradiography, the slides were labeled and densitometry analysis occurred. The data was then sent back to Haverford College where they were analyzed for mean group differences.
in oxytocin receptor density within PVN efferents, between conditions, using a one-way ANOVA, followed by Tukey’s HSD tests for post hoc analyses.

**Subjects: Experiment 2**

The procedure (including animal care, ovariectomies, and hormone-simulated pregnancy) for the subjects of Experiment 2 can be found in Bodie (2017). Subjects were euthanized using an intracardial perfusion with 25mM phosphate buffered saline (PBS) (pH=7.2) for three minutes. Brains were then removed and post fixed for one hour in 4% paraformaldehyde in 25mM PBS for 20 minutes. The tissue was then placed in a 10% sucrose solution overnight at 4 degrees Celsius, and finally stored in cryoprotectant at -20 degrees Celsius.

**Procedure: Experiment 2**

Sections containing the PVN were extracted from cryoprotectant storage (stored at -20 degrees Celsius) and washed in phosphate-buffered saline (PBS) (25mM, pH=7.2) five times for six minutes each. Following PBS washes, tissue was mounted from 100ml ddH₂O and FJC staining protocol was performed.

**Measures: Experiment 2**

**Fluoro-Jade C.** Fluoro-Jade C (FJC) is a fluorescent marker of neurodegeneration (Ehara & Ueda, 2009). To localize degenerating neurons, 40 μm thick sections were mounted on VWR Super Frost Plus microslides from 100ml ddH₂O and allowed to air dry for 24 hours. After air-drying, the slides were subjected to FJC staining protocol provided by Histo-Chem, Inc. The slides were first immersed in a 1% NaOH in 80% ethanol for 5 minutes. They were then immersed in a 70% ethanol solution for 2 minutes followed by ddH₂O for 2 minutes. Following the water rinse, slides were transferred to a 0.06% potassium permanganate solution for 10 minutes. The slides were then subjected to another 1.5 minutes ddH₂O water rinse, and then
transferred to the FJC staining solution for 10 minutes. The FJC staining solution was created from a 1ml 0.01% stock solution of FJC dye in ddH$_2$O, added to 99ml of a 0.1% acetic acid solution. After 10 minutes, slides were washed three times each for 1 minute in ddH$_2$O then air-dried in the dark for 24 hours. After the slides had been dried, they were cleared in xylene and coverslipped with DPX mountant for histology. The slides were then examined under the Nikon Eclipse 80i confocal microscope and the EZ C1 for Nikon system was used to visualize FJC positive neurons. Images were captured for demonstration and later analysis.

**Qualitative Analysis: Experiment 2**

Qualitative analyses were conducted to determine themes in Flouro-Jade C positive neurons in the PVN. Nine tissue sections (five estrogen-withdrawn, four estrogen-sustained) previously subjected to FJC staining were visualized at 20x and framed to allow visualization of the bilateral PVNs. Images were categorized into low (0-2), intermediate (3-9), and high (10+) levels of FJC-positive neurons. Experimenters who were naïve to subjects’ experimental conditions performed all qualitative analyses.

**Results**

**Study 1**

**Receptor autoradiography.** A between conditions, one-way analysis of variance (ANOVA) determined a significant difference in the density of oxytocin-immunoreactive cells between hormonal treatments in the dorsal raphe nuclei ($F(2, 21)=4.351, p =.026$) (see Tables 1 and 2). Post Hoc Tukey’s-HSD tests determined that the estrogen-withdrawn group ($M = 275.77, SD = 105.59$) had significantly more oxytocin-immunoreactive receptors than the control group ($M = 156.25, SD = 87.35$) ($p=.033$) (see Table 3). However, the estrogen-withdrawn group did not have significantly more or less oxytocin-immunoreactive receptors than the estrogen-
sustained group (see Table 2). There were no significant effects on the density of oxytocin-immunoreactive cells between hormone treatments within the medial amygdala ($F(2, 21) = 1.872, p = .179$), the BNST ($F(2, 21) = 1.746, p = .199$), or the nucleus accumbens core ($F(2, 21) = 1.133, p = .341$) or shell ($F(2, 21) = 1.736, p = .201$) (4-11). See Figures 1a through 4a for representative images of receptor autoradiography in the dorsal raphe nuclei (between all three hormone treatment groups) and in the remaining brain regions in the estrogen-withdrawn group.

**Elevated plus maze.** A between conditions, one-way analysis of variance (ANOVA) revealed a significant effect of hormone treatment on time spent in open arms ($F(2,21) = 6.082, p = .008$; Table 12). Post hoc Tukey’s HSD tests revealed that both the control ($p = .034$) and the sustained group ($p = .010$) spent significantly less time in the closed arms of the elevated plus maze than the estrogen-withdrawn group (See Table 13). A difference score (a measure of the difference between time spent in the closed arms minus time spent in the open arms) was calculated to control for individual differences in time spent in both open and closed arms. The ANOVA revealed a significant effect of hormone treatment on the difference score ($F(2,21)=3.706, p < .05$; Table 14). There was a significantly larger difference score in the estrogen-withdrawn group ($M = 107.24, SD = 47.76$) compared to the estrogen-sustained ($M = 21.12, SD = 79.63$) and control groups ($M = 29.91, SD = 76.93$). However, post hoc Tukey’s HSD tests revealed that the variance in difference score was only marginally significant ($p = .055$; Table 15) between the control group and withdrawn group and trending toward significance between the withdrawn and sustained group ($p = .09$). There was no significant effect of hormone treatment on time spent in the closed arms of the EPM ($F(2,21) = 1.456, p = .256$). See Figure 5 for a representation of time spent in closed and open arms between hormone treatment groups.
Open field test. A between conditions, one-way analysis of variance (ANOVA) revealed a significant effect of hormone treatment on subjects’ velocity in the open field test ($F(2,21) = 3.639, p = .04$; Table 16). Post hoc Tukey’s HSD test showed that the estrogen-withdrawn group displayed significantly lower velocities than the estrogen-sustained group ($p = .035$; Table 17 and Figure 6). There was no significant difference in velocity between estrogen-withdrawn and control groups ($p = .37$). The ANOVA also revealed a marginally significant effect of hormone treatment on distance moved in the open field test ($F(2,21) = 3.366, p = .054$; Table 18 and Figure 7). The post hoc Tukey’s HSD tests showed that the estrogen-withdrawn group traveled significantly lower distances in the OFT than the estrogen-sustained group ($p = .043$; Table 19). There was no significant difference in distance traveled between the estrogen-withdrawn and control groups ($p = .40$). There was also no significant effect of hormone treatment on time spent in the edge ($F(2,21) = .348, p = .710$), time spent in the center ($F(2,21) = 3.366, p = .054$), or the difference score of time spent in the edge minus center ($F(2,21) = .294, p = .748$) between hormone treatment groups.

Study 2

Fluoro-Jade C. Qualitative analyses revealed that the majority of tissue scored in the low range (0-2) for presence of FJC-positive neurons in bilateral PVNs were tissue from females in the estrogen-sustained group. See Figures 8a and 8b for representative images of FJC staining in both hormonal treatment groups.

Discussion

Receptor Autoradiography

The present research found significantly higher densities of oxytocin-immunoreactive receptors in the dorsal raphe nuclei of the estrogen-withdrawn group compared to the control
group, but not the estrogen-sustained group. There were no significant differences in the density of oxytocin-immunoreactive cells between hormone treatments in the bed nucleus of the stria terminals, medial amygdala, and the nucleus accumbens (core and shell). These results suggest, as our hypotheses stated, that the gradual rise in hormones during pregnancy is not enough to induce plasticity (as the estrogen-sustained and withdrawn groups were not significantly different) but that a rise followed by a drastic drop in estrogen is required, supporting the estrogen-withdrawal posited by previous research (Galea et al., 2001). Due to the region-specific nature of oxytocin receptor plasticity following hormone-simulated pregnancy, future studies can examine variables specific to the dorsal raphe nuclei that may be implicated in the neuroplasticity, behavioral, and affective characteristics of postpartum depression.

The raphe nuclei are well accepted as the foremost producer of centrally circulating serotonin. In particular, the dorsal raphe nuclei contain around 80% of the forebrain-projecting serotonergic neurons (Lowry et al., 2008). Serotonin and its pharmacological manipulator (selective serotonin reuptake inhibitors, or SSRI s) are also the first line of treatment for both depression and anxiety during the postpartum period (Lanza di Scalea & Wisner, 2009) and beyond (Bystritsky, Khalsa, Cameron, & Schiffman, 2013). Importantly, Yoshida et al. (2009) recently discovered that the majority of serotonergic neurons in the RN contain oxytocin receptors and that oxytocin binding to these receptors can induce serotonin release. Thus, the present research hypothesizes that oxytocin receptor increases in the dorsal raphe nuclei following estrogen-withdrawal elevate the potential for serotonin release and, concurrently, alterations in anxiety and depression.

Extensive research into postpartum neuroplastic changes in serotonin signaling in the dorsal raphe nuclei also supports the need for increased serotonin signaling postpartum.
Plasticity of the serotonergic system following pregnancy is implicated in effective postpartum caregiving behaviors as well as the motivation to provide care (Holschbach & Lonstein, 2017). The suckling-induced oxytocin surges that facilitate lactation are dependent on serotonin signaling in the dorsal raphe nuclei (Barofsky et al., 1983), while maternal behaviors such as nursing, huddling, and nest creation are significantly impaired in animals who have the transcription factor for serotonin neuron differentiation and the enzyme necessary for neuronal serotonin synthesis knocked out (Angoa-Perez et al., 2014; Lerch et al., 2008). In terms of serotonin’s relationship to postpartum mood, multiple studies have correlated serotonin levels to symptoms of postpartum depression and identified genetic risk factors related to serotonin and for postpartum depression, such as the 5-HTT genotype (Shapiro, Fraser, & Sequin, 2012; Yildiz, Senturk, Yildiz, Cakmak, Budak, & Cakar, 2017). All of these data suggest that serotonin signaling is necessary for adaptation to maternal responsibilities on both a behavioral and affective level. These data also support the hypothesis that an increase in oxytocin-receptor levels enhance serotonin release, and that postpartum increases in oxytocin receptors in the dorsal raphe nuclei have the ability to promote serotonin signaling and alter maternal mood.

Previously, we hypothesized that the level of oxytocin receptors within efferents of the PVN would be represented inversely to the synaptic levels of oxytocin in a manner that would maintain homeostasis. Maintaining neurotransmitter homeostasis is a well established principle that involves modulation of neurotransmitter release and receptor expression (Davis & Muller, 2015). Through these processes, increases in synaptic neurotransmitter levels result in decreased receptor expression. The present results do not support this hypothesis. Estrogen-withdrawn females, those who would hypothetically require the highest levels of synaptic levels of oxytocin, had significantly more oxytocin-immunoreactive receptors. It is therefore possible that
synaptic levels of oxytocin increase alongside increases in oxytocin receptors, significantly inflating oxytocin signaling from both sides. As oxytocin plays a pivotal role during the postpartum period, we interpret the increase in oxytocin receptors in the estrogen-withdrawn group as a sign that oxytocin signaling does experience a surge in both producing and receiving elements. This magnification is potentially essential for oxytocin to perform its role as an anxiolytic, to promote infant bonding, and to increase effective maternal behavior.

The findings of the present research map well onto previous research of oxytocin signaling following hormone-simulated pregnancy. Specifically, Bodie (2017) and colleagues demonstrated that oxytocin-producing neurons in the PVN increase during the postpartum period. The present research shows that there is also oxytocin receptor plasticity in the dorsal raphe nuclei, an efferent of the PVN, in the postpartum period. Thus, it can be posited that estrogen-withdrawal following pregnancy increases oxytocin producing and binding in a region-specific manner. Relevant research has examined the relationship between the PVN and its axonal projections to the brainstem, further supporting the story posited by the present research. Geerling, Shin, Chimenti, & Loewy (2010) labeled neurons in the rat PVN with an anterograde axonal tracer to demonstrate the projections of PVN to various brain regions and found that the PVN projects to the dorsal raphe nuclei. These findings demonstrate that not only do increases of oxytocin producing neurons in the PVN and oxytocin receptors in the RN occur, but also that projections between these two regions exist. Ultimately, the present research provides a promising story of oxytocin signaling between the PVN and the dorsal raphe nuclei during the postpartum period.
Elevated Plus Maze.

Analyses of behavior in the Elevated Plus Maze revealed that the estrogen-sustained group displayed significantly less anxiety-like behavior than the estrogen-withdrawn group, but not the control group. As well, a calculation of the difference score (time spent in closed arms minus time spent in open arms) allowed a more accurate representation of anxiety-like behavior through a consideration of individual differences. The analysis of difference score revealed that the estrogen-withdrawn group had the greatest difference score. However, after performing post hoc Tukey’s HSD tests, the variance in difference scores were determined to be marginally significant (when compared to the control group) and trending towards significance (when compared to the estrogen-sustained group). The trend toward significance during post hoc analyses is most likely due to the increased stringency of post hoc analyses. Analyses did not reveal a significant effect of hormone treatment on the amount of time subjects spent in the closed arms of the EPM.

The present research has thus rejected its hypothesis that the estrogen-group should exhibit the least anxiety-like behavior. Instead, results show that the estrogen-withdrawn group displays the most anxiety-like behavior. These results are opposite that of Bodie (2017), who found a marginal significance of less anxiety-like behavior in the estrogen-withdrawn group. The research with which the present paper had based its animal model on, Galea et al., 2001, also found that the estrogen-withdrawn group displayed decreased anxiety-like behavior. However, Galea et al., 2001 utilized only an Open Field Test. A third paper, Stoffel & Craft (2004) found no difference in anxiety-like behavior as measured through the EPM following hormone-simulated pregnancy. The use of the EPM following hormone-simulated pregnancy has therefore produced wildly different findings of anxiety-like behavior across the literature. The present
research hypothesizes that this may be for one of two reasons: (1) the EPM is an unreliable measure of anxiety-like behavior following hormone-simulated pregnancy or (2) the use of a hormone-simulate pregnancy to assess levels of anxiety-like behavior in the “postpartum” period does not accurately reflect the clinical experience of behavior in the postpartum period following reproduction due to variables associated with oxytocin signaling.

There are many benefits of animal models, but also many caveats to the aspect of assessing their behavior. One of these complications is that research can never be certain that the behavior of interest is indeed the behavior being measured. Due to equal amounts of data suggesting both that a hormone-simulated pregnancy increases and decreases anxiety-like behavior, it is difficult for the present paper to reconcile its findings with the existing literature. As well, the preset study raises serious concerns for the validity of the Elevated Plus Maze as a measure of anxiety-like behavior. Due to concerns about the validity of the behavioral data from the EPM, both in the context of existing literature and based on problems with data collection, the present study analyzed an additional set of behavioral data that was collected from a pilot study. The animals in the pilot study underwent the same hormone-simulated pregnancy and behavioral testing conditions, and the pilot study data was collected under more valid circumstances. The results of the pilot study revealed no significant difference in anxiety-like behavior (measured as time in open arms, closed arms, and difference score). Thus, the pilot study’s results contradicted both the present study’s findings and the findings of previous literature, like Galea et al. (2001). While it is unclear what the best measure of anxiety-like behavior currently is, the present findings call into question the efficacy of the EPM to produce consistent and reliable results of anxiety-like behavior across studies.
The present research posits that measuring this behavior following hormone-simulated is less clinically relevant and, perhaps, less accurate than measuring anxiety-like behavior following actual reproduction. For two reasons, the presence of an offspring may in fact reduce anxiety more than just estrogen-withdrawal in the hormone-simulated pregnancy. First, oxytocin signaling is thought to be higher following vaginal delivery as compared to cesarean section (Marchini, Lagerkrantz, Winberg, & Uvnas-Moberg, 1988) and second, transient levels of oxytocin are known to increase through breastfeeding and infant contact (Jobst et al., 2016). The previously discussed role of oxytocin in producing anxiolytic effects may play a significant role that the HSP simply cannot produce. For this reason, it is possible that the EPM is not an invalid measure of anxiety-like behavior, but rather, that oxytocin during the postpartum period produces anxiolytic effects only if animals experience reproduction and offspring interaction. Thus, the absence of these experiences in the present paper may explain the lack of decreased anxiety-like behavior in the estrogen-withdrawn group.

**Open Field Test.**

Analyses of anxiety-like behavior measured in the Open Field Test revealed a significant effect of hormone-treatment on subjects’ velocity and a marginally significant effect of on distance moved. However, these measures are considered non-specific locomotor activity, rather than measures of anxiety-like behavior. The estrogen-withdrawn group displayed slower average velocities and traveled less than the estrogen-sustained group, but not the control group. It is possible to argue that slower, less movement aligns with both increased and decreased anxiety-like behavior. On one hand, rodents in the wild may run faster and for longer distances when they are feeling anxious of predators. However, on the other hand, moving slowly for smaller distances might decrease the chance that a rodent is spotted by a predator, making anxious
rodents travel slower and for shorter distances. As well, the estrogen-sustained group showed the highest average velocity and distance traveled, making it possible to suggest that the estrogen-sustained group were actually hyperactive, instead of estrogen-withdrawn group being hypoactive. Overall, the results of the non-specific locomotor analyses are inconclusive in discerning the effects of hormone-treatment on these measures. No significant effects of hormone-treatment on anxiety-like behavior (time spent in the middle, time spent on the edge, and difference score) were found. The insignificance of measures of anxiety-like behavior in the OFT may be due to the previously discussed limitations of the EPM.

**Fluoro-Jade C positive neurons in the PVN.**

Tissue was subjected to Fluoro-Jade C staining to elucidate the underlying processes of previous research showing significantly lower numbers of oxytocin producing neurons in the PVN of estrogen-sustained females (Bodie, 2017). It was originally hypothesized that the estrogen-sustained group would show greater amounts of FJC-positive neurons. This theory was based in the possibility that estrogen-sustained females experienced greater neurodegeneration of oxytocin-producing neurons, explaining the process by which estrogen-sustained females had significantly less numbers of oxytocin-producing neurons than estrogen-withdrawn group (Bodie, 2017). Qualitative analyses revealed more FJC-positive neurons in the PVN of females who were estrogen-withdrawn. Thus, the FJC results were in the opposite direction of the present hypothesis.

While neurodegeneration cannot explain the difference in oxytocin-producing neurons in the PVN between hormonal treatments in the present research, neurodegeneration may help elucidate the process of increases in oxytocin-producing neurons in the PVN (Bodie, 2017). Originally, the neurodegeneration hypothesis for the estrogen-sustained group, as opposed to
neurogenesis in the estrogen-withdrawn group was favored because it is logical to assume that neurodegeneration is less costly in terms of energy supplies than neurogenesis. However, neurodegeneration in the PVN of the estrogen-withdrawn group may also be energy efficient in that it leaves greater energy stores to allow the number of oxytocin-producing neurons to increase. Importantly, FJC staining in the present study is unspecific, meaning FJC-positive neurons are not labeled to a specific type of neurons. This means that FJC-positive neurons could be non-oxytocin producing neurons, supporting the theory that neurodegeneration makes room for the increase in oxytocin signaling following pregnancy. On the other hand, if the FJC-positive neurons are oxytocin-producing neurons, then the theory that neurodegeneration in the PVN supports increased oxytocin neurogenesis cannot be supported. Ultimately, due to the lack of double labeling, it is difficult to make any conclusions regarding the role of neurodegeneration in the PVN of estrogen-withdrawn animals. If future analyses using FJC double-labeled tissue are performed, it would be possible to determine the role of postpartum neurodegeneration in the PVN.

Alongside the possibility that PVN neurodegeneration supports oxytocin neurogenesis, it is also possible that the increased cell death seen in estrogen-withdrawn females is a result of neurodegeneration during pregnancy. There has been ample evidence to suggest that mechanisms of pruning occur following pregnancy, and a cautious argument has been posited that postpartum pruning is an adaptive response to create more efficient circuitry required for maternal behaviors. For example, Gregg et al. (2007) found that pregnant animals are more able to re-myelinate white matter lesions while Hoekzema et al. (2017) demonstrated a decrease in grey matter volume in regions associated with social cognition in postpartum women. Together, a decrease in grey matter and an increase in myelination suggest that pregnancy induces neuronal pruning to
make cognition faster and more efficient. Thus, the greater number of FJC-positive neurons in the estrogen-withdrawn group may reflect previous findings of pregnancy-induced pruning.

**Strengths and Limitations**

Prior to this study, there had been no previous research that examined the relationship between postpartum estrogen levels, alterations in mood, and oxytocin receptor plasticity in efferents of the paraventricular nucleus of the hypothalamus. Thus, the findings of the present research have genuine applications to the study of postpartum depression. With the understanding that oxytocin receptor plasticity increases within the dorsal raphe nuclei following pregnancy, it may be possible to produce targeted therapies for postpartum depression. Due to the effects of SSRIs on infant development through breastfeeding (Lanza di Scalea & Wisner, 2009), an all too necessary alternative for the pharmacotherapy of postpartum depression may be informed by the present research. Thus, pharmacology that targets oxytocin-induced serotonin signaling in the dorsal raphe nuclei has the potential to alleviate the current challenges of treating postpartum depression. Ultimately, the findings of the present research are both novel and significant for the fields of behavioral endocrinology and the study of postpartum depression.

While previous research using the hormone-simulated pregnancy was limited due to the lack of a true control group, the present research added a control group to the hormone-simulated pregnancy that received all the same protocol, including an ovariectomy, with daily injections of cottonseed oil instead of estrogen and progesterone. Future research should continue the practice of using a true control group. The HSP is also the best method to tightly control hormone levels to ensure proper experimental conditions. As well, no other animal model of pregnancy has been developed specifically to study the postpartum period. Finally, by using the HSP, researchers can
mimic pregnancy without the need for insemination or reproduction, reducing the cost of animal lives. These details are some of the most salient strengths of the present research.

Previous research has viewed the hormone-simulated pregnancy as a model of postpartum depression. While hypothesizing, the present research altered this perspective to view the hormone-simulated pregnancy as a model of the typical response to pregnancy. This perspective switch was based in findings that showed no significant differences in anhedonia between hormone treatment groups and a decrease in anxiety (Bodie, 2017) which are typical responses to pregnancy. While Galea, Wide, & Barr (2001) assumed that the HSP can model postpartum depression, this perspective disregards the fact that anywhere from 80% - 85% of women do not experience postpartum depression, suggesting that the majority of the animals in this model would not experience alterations in mood following estrogen-withdrawal. However, the results of the present research reject the hypothesis that estrogen-withdrawal induces decreases in anxiety. There is clearly a need for future research to examine the HSP, as it is currently unclear whether the HSP is a model of typical responses to pregnancy or a model of postpartum depression.

This study has limitations particularly due to the fact that it was not examining true biological reproduction and that it lacked double-labeling with FJC staining. There are many reasons why a model of postpartum depression must eventually be performed in animals who actually give birth. Two significant reasons are that oxytocin signaling is thought to be higher following vaginal delivery as compared to cesarean section (Marchini, Lagerkrantz, Winberg, & Uvnas-Moberg, 1988) and that transient levels of oxytocin are known to increase through breastfeeding and infant contact (Jobst et al., 2016). While the use of a hormone-simulated pregnancy is beneficial to reducing the cost to animal lives, this model may limit the extent to
which oxytocin receptors increase and produce anxiolytic effects. If oxytocin signaling is less increased in the HSP because of a lack of vaginal delivery and interactions with offspring, then it is possible that oxytocin receptor plasticity and mood alterations would also be less drastic. In terms of FJC double-labeling, it has been previously mentioned that FJC staining in the present research cannot determine the kinds of neurons undergoing degeneration. It is crucial that caution is taken when interpreting these data, and the present research does not attempt to make any strong suggestions for the role of neurodegeneration in postpartum mood disturbances due to the lack of double-labeling.

In terms of limitations regarding behavioral data, the major limitations of this study are the use of imperfect behavioral assays of anxiety-like behavior and individual differences in anxiety-like behavior prior to hormone-simulated pregnancy. Extensive research has attempted to develop assays of anxiety-like behavior that accurately assesses anxiety in rodents. While the Elevated Plus Maze and Open Field Test combine rodent’s aversion to light areas and open areas, pushing anxious animals to darker, more closed areas (Lezak, Missig, & Carlezon, 2017), the laboratory setting under which the assays are conducted (bright rooms full of experimenters) may induce feelings of anxiety due to fear of predation. As well, there is currently no way to be certain that the measures of anxiety-like behavior used in the present paper and similar research are truly measuring anxiety. Individual differences in anxiety prior to hormone-simulated pregnancy were also not accounted for in analyses, and these pre-existing states of anxiety may have affected between-group differences in behavior following the HSP. Finally, the present research used a behavior tracking software that had the tendency to incorrectly track movement which led to its inaptitude for measuring non-specific locomotor activity in the EPM. The
accuracy of behavior tracking in a few instances may have affected the results of behavioral measures.

**Future Directions**

The present study employed a hormone-simulated pregnancy to elucidate the role of oxytocin in the etiology of postpartum depression. To localize and examine the presence of oxytocin between experimental conditions, autoradiography was the primary neurobiological measure. However, there are myriad directions that future studies could take to quantify differences in oxytocin before, during, and after pregnancy. In the future, it would be possible and advantageous to perform a series of experiments in order to confirm results from this research and previous studies of its kind.

The present research suggests that a polymerase chain reaction (PCR) could be performed to establish that the mRNA expression of oxytocin in the PVN is indeed increased in the estrogen-withdrawn group. As previous research has shown an increase in oxytocin-producing neurons in the PVN of the estrogen-withdrawn group (Bodie, 2017), PCR for gene expression of oxytocin in the PVN would confirm these results, as it would be unusual for the number of oxytocin-immunoreactive cells to increase without increases in mRNA expression for oxytocin. Second, it will be necessary to confirm the direction of results shown in previous research. Bodie (2017) and the present research found significantly higher levels of oxytocin producing neurons (in the PVN) and oxytocin receptors (in the RN) in the estrogen–withdrawn group. However, it is unclear whether the significant difference between the estrogen-withdrawn group and the estrogen-sustained group is due to neurogenesis (in the withdrawn group) or neurodegeneration (in the sustained group). Thus, a second future direction for this line of research would be to
examine histology using tissue that is double-labeled for oxytocin and a marker of neurogenesis and tissue that is double-labeled for oxytocin and FJC, or a similar marker of neurodegeneration.

If the results of the present research and findings from previous research (Bodie, 2017) are confirmed using PCR, the logical next step in this research would be to perform the same behavioral testing in females who produce offspring. As previously mentioned, there are numerous variables associated with reproduction that involve oxytocin signaling, and the present paper hypothesizes that the findings here may be even more pronounced with the delivery of offspring. Following a version of the present research during which females reproduce, if findings are consistent, it would be possible to conduct pharmacological studies targeting the dorsal raphe nuclei and measure postpartum mood alterations following a hormone-simulated pregnancy. As the present study found that increases in anxiety-like behavior were correlated with increases in oxytocin receptor levels in the dorsal raphe nuclei, future studies could elucidate these relationships by administering an oxytocin receptor antagonist directly into the dorsal raphe nuclei and comparing disturbances in mood between females who reproduce and females who do not. These pharmacological studies are probably the most direct method for ascertaining the relationship between oxytocin signaling in the dorsal raphe nuclei and postpartum mood disturbances.

Finally, and aligning with the present study’s limitations, it is necessary that the field of behavioral endocrinology develop an animal model of postpartum depression and behavioral assays of anxiety/depression that more closely maps onto the human prototype. A better understanding of the natural progression of progesterone and estrogen before, during, and after pregnancy in each animal model will aid this effort, as the current hormone-simulated pregnancy utilizes the hormone levels associated with the induction of maternal behavior, rather than with
reproduction. It is also necessary to determine the effects of various days of the postpartum period on behavioral analysis. The current research performed behavioral analyses throughout the postpartum period, but future research will benefit from understanding the most appropriate day(s) during postpartum to ascertain behaviors of interest. Finally, as previous research found no significant difference in postpartum anhedonia between hormonal treatment groups, future research may determine if postpartum anxiety and depression require separate animal models. Novel animal models that accurately depict the human phenotype of postpartum mood disorders will augment understandings of the most appropriate treatments for postpartum mood disturbances.

**Final Remarks**

The impact of postpartum depression is double that of many other affective disorders, posing serious and long lasting concerns for the well-being of mothers and the development of infants. Postpartum depression is also accompanied by enormous social adjustments, including the need for responsiveness, sensitivity, warmth, parenting confidence, parenting competence, and the ability to cope with parenting stress (Letourneau et al., 2017). These necessary demands are accompanied by long-term consequences for the offspring if they are not fulfilled. Unfortunately, maternal depression can impact the emotional, behavioral, and cognitive development of offspring, even if PPD is effectively treated (Hay et al., 2001). Maternal depression even has the capability to affect the future parenting style of offspring, potentially posing a mutli-generational effect on both behavior and neurobiology (Champagne & Meaney, 2001; Letourneau, Dennis, Cosic & Linder, 2017). It is therefore vital that the field gain a clearer picture into the cause of postpartum depression in order to limit the effects of this devastating affective disorder.
The present research hopes to represent one step in the direction of understanding postpartum depression as a neurobiologically complex disorder with far-reaching and long-lasting effects on both maternal mood and infant development. From a public health perspective, it is vital that the study of postpartum depression actively continues to seek novel and effective treatments for women who suffer from this disorder. Despite the fact that postpartum depression currently affects between 15% and 20% of women, it is also considered the most underrepresented and least treated affective disorder (Brummelte & Galea, 2009; Pawluski, Lonstein & Fleming, 2017; Perani & Slattery, 2014). Thus, any step forward in the development of postpartum depression-specific treatments is a step in the right direction.

The present research, although preliminary, has serious implications for the development of unique and targeted therapies for postpartum depression. Future studies can confirm the efficacy of treatment using direct administration of oxytocin into the dorsal raphe nuclei, although this treatment is dependent on the success of many studies in between. Here, it is posited that this oxytocin receptor plasticity in the dorsal raphe nuclei has the potential to increase serotonin signaling and decrease postpartum mood disturbances. The implications for these findings are immense and deserve adequate attention in the manner of tightly controlled follow-up studies to determine the efficacy of oxytocin as a potential treatment for postpartum depression. Ultimately, the present studies provide great hope for the future of this field and, potentially, the outcomes for both postpartum women and their children.
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### Tables and Figures

Table 1. *One-Way Analysis of Variance of Oxytocin Receptor Density within the Dorsal Raphe nuclei*

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<tr>
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*Note. *p<.05*
Table 2. Oxytocin Receptor Density within the Dorsal Raphe nuclei Between Hormone Treatment Groups

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Table 3. Tukey’s HSD Post-Hoc Multiple Comparison Analyses of Oxytocin Receptor Density within the Dorsal Raphe nuclei Between Hormone Treatment Groups

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Note. *p<.05
Table 4. *Oxytocin Receptor Density within the Medial Amygdala Between Hormone Treatment Groups*

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Table 5. *One-Way Analysis of Variance of Oxytocin Receptor Density within the Medial Amygdala*

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Table 6. *Oxytocin Receptor Density within the Nucleus Accumbens (Shell) Between Hormone Treatment Groups*

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Table 7. Oxytocin Receptor Density within the Nucleus Accumbens (Core) Between Hormone Treatment Groups

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Table 8. *One-Way Analysis of Variance of Oxytocin Receptor Density within the Nucleus Accumbens (Shell)*

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Table 9. *One-Way Analysis of Variance of Oxytocin Receptor Density within the Nucleus Accumbens (Core)*

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Table 10. *Oxytocin Receptor Density within the Bed Nucleus of the Stria Terminalis Between Hormone Treatment Groups*

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<td>Estrogen-Withdrawn</td>
<td>23</td>
<td>357169.16</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 12. *One-Way Between-Subjects Analysis of Variance of Total Time Spent in the Open Arms of the Elevated Plus Maze*

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>2</td>
<td>15888.05</td>
<td>7944.03</td>
<td>6.08</td>
<td>.008*</td>
</tr>
<tr>
<td>Within Groups</td>
<td>21</td>
<td>27429.29</td>
<td>1306.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>43317.34</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note. *p* < .05
Table 13. Tukey’s’s HSD Post-Hoc Multiple Comparison Analyses of Total Time Spent in the Open Arms of the Elevated Plus Maze Between Hormone Treatment Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Estrogen-Sustained</td>
<td>-10.10</td>
</tr>
<tr>
<td></td>
<td>Estrogen-Withdrawn</td>
<td>48.82*</td>
</tr>
<tr>
<td>Estrogen-Sustained</td>
<td>Control</td>
<td>10.10</td>
</tr>
<tr>
<td></td>
<td>Estrogen-Withdrawn</td>
<td>58.93*</td>
</tr>
<tr>
<td>Estrogen-Withdrawn</td>
<td>Control</td>
<td>-48.82*</td>
</tr>
<tr>
<td></td>
<td>Estrogen-Sustained</td>
<td>-58.93*</td>
</tr>
</tbody>
</table>

Note. *p< .05
Table 14. One-Way ANOVA Between Time Spent in Closed Arms of the EPM and Hormone Condition

<table>
<thead>
<tr>
<th>Group</th>
<th>SS</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>4140.66</td>
<td>2</td>
<td>2070.33</td>
<td>1.456</td>
<td>0.256</td>
</tr>
<tr>
<td>Within</td>
<td>29852.18</td>
<td>21</td>
<td>1421.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>33992.84</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 15. Tukey’s HSD Post-Hoc Multiple Comparison Analyses of the Difference in Time Spent in the Closed Arms and Open Arms of the Elevated Plus Maze Between Hormone Treatment Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Estrogen-Sustained</td>
<td>8.79</td>
</tr>
<tr>
<td></td>
<td>Estrogen- Withdrawn</td>
<td>-77.31</td>
</tr>
<tr>
<td>Estrogen-Sustained</td>
<td>Control</td>
<td>-8.79</td>
</tr>
<tr>
<td></td>
<td>Estrogen- Withdrawn</td>
<td>-86.11</td>
</tr>
<tr>
<td>Estrogen-Withdrawn</td>
<td>Control</td>
<td>77.32</td>
</tr>
<tr>
<td></td>
<td>Estrogen- Sustained</td>
<td>86.11</td>
</tr>
</tbody>
</table>
Table 16. *One-Way Between-Subjects Analysis of Variance of Total Time Spent in the Open Arms of the Elevated Plus Maze*

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>2</td>
<td>15888.05</td>
<td>7944.03</td>
<td>6.08</td>
<td>.008*</td>
</tr>
<tr>
<td>Within Groups</td>
<td>21</td>
<td>27429.29</td>
<td>1306.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>43317.34</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note. *p < .05*
Table 17. Tukey’s HSD Post-Hoc Multiple Comparison Analyses of Velocity in the Open Field Test

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Difference</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogen-Sustained</td>
<td>-1.33</td>
<td>.40</td>
</tr>
<tr>
<td>Estrogen-Withdrawn</td>
<td>1.38</td>
<td>.37</td>
</tr>
<tr>
<td>Estrogen-Sustained</td>
<td>Control</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>Estrogen-Withdrawn</td>
<td>2.71*</td>
</tr>
<tr>
<td>Estrogen-Withdrawn</td>
<td>Control</td>
<td>-1.37</td>
</tr>
<tr>
<td></td>
<td>Estrogen-Sustained</td>
<td>-2.71*</td>
</tr>
</tbody>
</table>

Note. *p<.05
Table 18. *One-Way Between-Subjects Analysis of Variance of the Difference in Time Spent in the Closed Arms and Open Arms of the Elevated Plus Maze*

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>2</td>
<td>35921.60</td>
<td>17960.80</td>
<td>3.71</td>
<td>.04*</td>
</tr>
<tr>
<td>Within Groups</td>
<td>21</td>
<td>101780.61</td>
<td>4846.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>137702.21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note. *p < .05
Table 19. Tukey’s HSD *Post-Hoc Multiple Comparison Analyses of Distance Moved in the Open Field Test*

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Difference</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogen-Sustained</td>
<td>-762.96</td>
<td>.43</td>
</tr>
<tr>
<td>Estrogen-Withdrawn</td>
<td>797.08</td>
<td>.40</td>
</tr>
<tr>
<td>Estrogen-Sustained</td>
<td>Control</td>
<td>762.96</td>
</tr>
<tr>
<td></td>
<td>Estrogen-Withdrawn</td>
<td>1560.05*</td>
</tr>
<tr>
<td>Estrogen-Withdrawn</td>
<td>Control</td>
<td>-797.08</td>
</tr>
<tr>
<td></td>
<td>Estrogen-Sustained</td>
<td>-1560.05*</td>
</tr>
</tbody>
</table>

*Note.* *p*<.05
Figure 1a. Oxytocin receptors in the dorsal raphe nuclei of control females

Figure 1b. Oxytocin receptors in the dorsal raphe nuclei of estrogen-sustained females

Figure 1c. Oxytocin receptors in the dorsal raphe nuclei of estrogen-withdrawn females

Figure 1d. Stereotaxic atlas image used to identify the location of the dorsal raphe nuclei

(Morin & Wood, 2001)
Figure 2a. Oxytocin receptors in the medial amygdala of estrogen-withdrawn females

Figure 2b. Stereotaxic atlas image used to identify the the location of the medial amygdala

(Morin & Wood, 2001)
Figure 3a. Oxytocin receptors in the nucleus accumbens (shell and core) of estrogen-withdrawn females

Figure 3b. Stereotaxic atlas image used to identify the location of the nucleus accumbens (shell and core) (Morin & Wood, 2001)
Figure 4a. Oxytocin receptors in the BNST of estrogen-withdrawn females

Figure 4b. Stereotaxic atlas image used to identify the location of the BNST (Morin & Wood, 2001)
Figure 5.
Note. *p<.05
Figure 6.
Note. *p<.05
Figure 7.
Note. *p < .05
Figure 8a. Representative image of FJC-positive neurons in the PVN of estrogen-withdrawn females

Figure 8b. Representative image of FJC-positive neurons in the PVN of estrogen-sustained females

Figure 8c. Stereotaxic atlas image used to identify the location of the PVN (Morin & Wood, 2001)