Oxytocin Receptor Plasticity Following a Hormone-Simulated Pregnancy in Syrian Hamsters:

Implications for Postpartum Mood Disorders

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Abstract

Despite the fact that approximately 15–20% of women develop postpartum depression and/or anxiety, and that the resulting outcomes for both the mother and her child are negative, the underlying neurobiological mechanisms of the disorders remain poorly understood. Previous research suggests that ovarian hormone fluctuations as well as changes in oxytocin signaling that occur at parturition and in the postpartum likely play a role in the etiology of these disorders. Given the increase in oxytocin-producing neurons in the paraventricular nucleus (PVN) of the hypothalamus following a hormone simulated pregnancy, Experiment 1 sought to examine oxytocin receptor levels in PVN efferents, particularly in the medial amygdala (MeA), nucleus accumbens (NAc), bed nucleus of the stria terminalis (BNST), and raphe nuclei. Results indicate an increase in oxytocin receptor density in the raphe nuclei among hormone-withdrawn animals as compared to controls, suggesting that the region could be implicated in the etiology of anxiety-like behavior during the postpartum period. Unexpectedly, behavioral results indicate reduced non-specific locomotor ability as measured by the Open Field Test and increased anxiety-like behavior as measured by the Elevated Plus Maze in hormone-withdrawn animals. Experiment 2 sought to explore whether neurodegeneration was responsible for the decreased oxytocin-producing neurons found in hormone-sustained animals. Unexpectedly, cell death is visible in hormone-withdrawn and not hormone-sustained animals, suggesting that some neuroplasticity may be taking place. Overall, these two experiments add to our understanding of the brain and behavior following hormone-simulated pregnancy in hamsters, which may inform our understanding the postpartum period in humans.
Keywords: Postpartum mood disorders, postpartum depression, postpartum anxiety, hormone-simulated pregnancy, oxytocin, paraventricular nucleus, medial amygdala, nucleus accumbens, bed nucleus of the stria terminalis, raphe nuclei, neurodegeneration
Oxytocin receptor plasticity following a hormone-simulated pregnancy in Syrian hamsters: Implications for postpartum mood disorders

The months leading up to and immediately following childbirth are often depicted as a time of great excitement and bliss as a mother welcomes her baby into the world (Latvala, 2013). However, the reality is that the postpartum period is a time of great change, both biologically and socially, in a woman’s life (Perani & Slattery, 2014). Their new realities can lead some women to feel exhausted, sad, and fearful in the first few weeks after delivery. This transitional reaction to motherhood has been termed the "baby blues" and is estimated to affect over half of all mothers (Hilt, 2015). However, these symptoms are much more severe and longer-lasting for a subset of new mothers who suffer from postpartum depression (PPD) and/or anxiety (PPA), the two most prevalent postpartum mood disorders (Perani & Slattery, 2014).

In fact, it is estimated that over 8.5% of mothers suffer from anxiety disorders in the postpartum, with generalized anxiety disorder (GAD) and obsessive compulsive disorder (OCD) being the most common (Goodman, Watson, & Stubbs, 2016). An even greater number of women, approximately 12.5%, suffer from postpartum depressive disorders (Le Strat, Dubertret, & Le Foll, 2011). Comorbidity is high between the two disorders, with approximately 2–5% of women suffering from both PPD and PPA (Fairbrother, Janssen, Antony, Tucker, & Young, 2016; Reck et al., 2008). However, it is likely that the prevalence is even higher since considerable stigma around PPD can prevent women from admitting they have a problem and are in need of help (Tam, Newton, Dern, & Parry, 2002).

Although similar in time course, the two disorders have marked differences in their characterizations. The most recent Diagnostic and Statistical Manual of Mental Disorders (DSM-V) does not classify postpartum depression as a separate diagnosis, but rather requires a
diagnosis of major depressive disorder (MDD) with a peripartum onset (American Psychiatric Association [APA], 2013). A diagnosis of major depressive disorder is made if at least five of nine possible symptoms (i.e., depressed mood, loss of interest, loss of appetite, insomnia, severe restlessness, fatigue, feelings of worthlessness, inability to concentrate, and recurrent thoughts of death or suicide) last for two or more weeks. At least one of the symptoms must be depressed mood or loss of interest or pleasure. The peripartum onset adds that the major depressive episode must occur anytime from the start of pregnancy through the first 4 weeks following delivery (APA, 2013). On the other hand, postpartum anxiety does not have any formal diagnosis in the DSM-V (Pawluski, Lonstein & Fleming, 2017). Instead, it is generally characterized by the same symptoms involved in anxiety disorders that occur outside the postpartum period. When associated with GAD, these symptoms include excessive and uncontrollable concern or worry associated with restlessness, fatigue, irritability, muscle ache, or difficulty sleeping. When associated with OCD, these symptoms include excessive, repetitive, and intrusive thoughts, impulses, or behaviors (APA, 2013). Moreover, both PPD and PPA are generally characterized by the symptoms of depression or anxiety at other times in one's life, but they occur specifically during the postpartum period.

Not all women are at equal risk for developing these symptoms. In fact, several demographic and psychosocial factors are known to predict postpartum depression and anxiety. For instance, PPD is more common in women of low socioeconomic status, low educational achievement, non-marital status (Field, 2017), young marital age (Abdollahi et al., 2014), and young maternal age (Zaidi, Nigam, Anjum, & Agarwalla, 2016). PPD is also more common in women who give birth to a female child (Zaidi et al., 2016). In addition, women with a previous diagnosis of depression or anxiety during or prior to pregnancy (Bloch, Rotenberg, Koren, &
Klein, 2006), low social support, poor relationship quality with their partner (Field, 2017), previous stressful life events, low self-esteem, and feelings of loneliness (Zaidi et al., 2016) are at an increased risk of suffering from PPD. Many of these risk factors for PPD are also predictors of postpartum anxiety including previous mental health diagnosis, particularly anxiety disorders prior to pregnancy, anxiety disorders during pregnancy, and depressive disorders during pregnancy (Martini et al., 2015). However, some risk factors that are unique to the development of postpartum anxiety include prior pregnancy loss (Giannandrea, Cerulli, Anson, & Chaudron, 2013), the baby blues (Reck, Stehle, Reinig, & Mundt, 2009), and preoccupied and fearful attachment styles (Nanni & Troisi, 2017).

These feelings of anxiety and depression during and following pregnancy are risk factors for adverse outcomes for both mothers and their offspring in the months following birth. First, postpartum depression can lead to disturbed early interactions between the mother and her baby (Field, 2010). For instance, depressed mothers are more irritable and hostile, less engaged in play and communication with their babies, and less tender towards their infants at 3 months than non-depressed mothers (Lovejoy, Graczyk, O’Hare, & Neuman, 2000). These early interactions influence and may be detrimental to the infant’s development later in life (Field, 2010). Second, several caregiving practices are jeopardized by the effects of postpartum depression. For instance, mothers with PPD are more likely to give up breastfeeding, less likely to establish a sleep routine for their infant, and less likely to bring their child to age-appropriate wellness visits and give them up-to-date vaccinations (Field, 2010). Thus, PPD symptoms lead to poor caregiving practices that can negatively impact the offspring.

Evidence further suggests that the effects of postpartum depression and anxiety influence offspring’s cognition, behavior, and socialization later in life. For instance, children of mothers
with PPD have diminished cognitive and emotional development at one year of age (Beck, 1998). Further, early school-age children of mothers who suffered from PPD have lower ego-resiliency, lower peer social competence, and lower school adjustment than their peers of mothers who were not depressed (Kersten-Alvarez et al., 2012). Postpartum anxiety shows similar consequences for offspring. For instance, children born to mothers with postpartum anxiety show emotional problems at four years of age, and boys also displayed problems of inattention and hyperactivity (O’Connor, Heron, Golding, Beveridge, & Glover, 2002). Thus, the harmful effects of postpartum depression and anxiety do not simply cause harm during the first few months following birth but rather go on to influence the children throughout their development.

Despite these staggering consequences to both the mother and her baby, the treatment options available for postpartum depression and anxiety are lacking. The most common methods of treatment for depression are antidepressants, a pharmacological intervention, and cognitive behavioral therapy (CBT), a psychotherapeutic treatment (McGrath et al., 2013). However, both options are insufficient at mitigating the consequences for women and their offspring. First, infants exposed to the class of antidepressant drugs known as selective serotonin reuptake inhibitors (SSRIs) in utero are at risk for disrupted motor and language development, developing attention deficit hyperactivity disorder, and developing congenital heart defects and pulmonary hypertension (Brummelte & Galea, 2016). Similar developmental disturbances are found in the children of women taking SSRIs while breastfeeding (Wisner, Perel, & Findling, 1996). In response, many doctors have advised their patients taking antidepressants to refrain from breastfeeding their infants (Bellissima, Ververs, Visser, & Gazzolo, 2012). Thus, many women may be reluctant to take existing antidepressants during pregnancy or postpartum, leaving
psychotherapy as the next likely treatment option. However, research shows that even though mothers treated with psychotherapy for PPD report less parental stress than untreated mothers, they still rate their children lower in attachment security, higher in behavior problems, and more negative in temperament at 18 months of age than nondepressed mothers (Forman, O'Hara, Stuart, Gorman, Larsen, & Coy, 2007). Thus, despite the decrease in depressive symptoms for the mother, both antidepressants and psychotherapy appear insufficient at enhancing maternal functions and improving child outcomes.

The lack of effective treatment options is in part due to the current limited understanding of the etiology and neurobiology of postpartum depression and anxiety (Pawluski et al., 2017). Given the high incidence of postpartum depression and anxiety, as well as the severe consequences for both mothers and their infants, it is important that research aimed at uncovering the underlying mechanisms of the illness is conducted. Then, more effective treatments can be developed.

**Pregnancy, the Postpartum Period, and Other Periods of Hormonal Fluctuation**

Given that women are most likely to develop depression in times of hormonal fluctuation such as during pregnancy, the postpartum, and menopause (Brummelte & Galea, 2010; Woods et al., 2008), it is likely that alterations in hormones and the brain play a role in the etiology of depression during these times in a woman’s life. Thus, in order to begin investigating potential causes and mechanisms for postpartum depression and anxiety, one must first understand the standard changes in the behavior, brain, and hormones of women during typical pregnancy and the postpartum.

**Behavioral changes.** There are normally marked changes in behavior during pregnancy and the postpartum period. Specifically, during the first trimester of pregnancy, most women's
feelings are related to the physiological changes (e.g., nausea, vomiting, dizziness) that are prevalent during that time. By the last trimester, anxiety and emotional distress become the most significant symptoms (Rofé, Littner, & Lewin, 1993). However, most women experience increased positive mood and reduced anxiety after giving birth. This leads to the mother’s typical expression of caretaking activities to protect and nurture her child, and in many species, to the development of a close bond between mother and infant (Agrati & Lonstein, 2016).

**Hormonal changes.** Pregnancy and the postpartum period are also times of heightened and dramatic changes in hormone levels. In fact, there is an established literature on the changes in the hormones progesterone, estrogen, prolactin, cortisol, oxytocin, thyroid, and vasopressin during these times (Hendrick, Altshuler, & Suri, 1998). The key hormones for this review are described in subsequent sections.

**Ovarian hormones.** Estrogen and progesterone are two of the main hormones involved in pregnancy. Estrogen helps to stimulate hormone production and growth in the adrenal gland of the developing fetus and to enhance the mother's uterus, enabling it to respond to oxytocin, another hormone involved in pregnancy. Progesterone helps relax smooth muscle, including the muscle wall of the uterus, and plays a role in the immune system, helping the body tolerate the foreign DNA of the fetus (Bowen, 2000). The levels of estrogens (i.e., estradiol, estriol, and estrone) and progesterone rise steadily throughout pregnancy (Hendrick et al., 1998). Both hormones are produced by the corpus luteum until around the end of the first trimester when their production is taken over by the placenta (Kumar & Magon, 2012). Following delivery, there is an abrupt decrease in the levels of estrogens and progesterone with the removal of the placenta. These levels reach their pregravid amount by the fifth day postpartum (Hendrick et al., 1998).
Cortisol. Another hormone that changes throughout pregnancy and the postpartum is cortisol. Cortisol plays an important role in mobilizing the mother’s reserves for her offspring during gestation, primarily through the activation of her hypothalamic-pituitary-adrenal (HPA) axis (Michel & Bonnet, 2014). The HPA axis is a complex feedback loop involved in the stress response and homeostatic balance and thus regulates the release of cortisol (Pecoraro & Dallman, 2009; Varghese & Sherwood Brown, 2001). When activated, the hypothalamus releases corticotropin-releasing hormone (CRH) to the pituitary gland, causing the release of adrenocorticotropic hormone (ACTH) to the adrenal glands, which then produces cortisol (Webster et al., 1996). During pregnancy, the mother’s HPA axis is progressively activated in response to increasing nutritional demand of her developing offspring (Michel & Bonnet, 2014). Thus, just as with estrogen and progesterone levels, cortisol levels also rise throughout pregnancy (Fleming, Ruble, Krieger, & Wong, 1997), peaking in the third trimester due to placental production of CRH (Hendrick et al., 1998). In fact, maternal cortisol levels reach approximately three times that of nonpregnant levels during this time (Skalkidou, Hellgren, Comasco, Sylvén, & Sundström Poromaa, 2012). These high levels fall at delivery (Hendrick et al., 1998) and return to normal baseline levels within a couple of days after birth (Skalkidou et al., 2012).

Oxytocin. Oxytocin is another hormone involved in pregnancy and the postpartum. Primarily synthesized in the paraventricular nucleus (PVN) of the hypothalamus, oxytocin is almost entirely transported to the posterior pituitary where it is secreted into the bloodstream (Meddle, Bishop, Gkoumassi, van Leeuwen, & Douglas, 2007). Peripherally, oxytocin acts to regulate childbirth and lactation in the postpartum (Lee, Macbeth, Pagani, & Young, 2009). Specifically, oxytocin levels rise sharply at delivery to stimulate uterine muscle contractions that
forcefully expel the fetus and also to induce milk ejection for breastfeeding (Gimpl & Fahrenholz, 2001; Hendrick et al., 1998; Russell, Leng, & Douglas, 2003).

Oxytocin also acts centrally as a neurotransmitter where it plays an important role in parturition and lactation as well as in the onset of maternal behavior (Insel, Young, & Wang, 1997; Meddle et al., 2007; Russell & Brunton, 2009). During childbirth, an increase in oxytocin is known to target specific brain regions including the supraoptic nucleus (SON) of the hypothalamus, medial preoptic area (mPOA) of the hypothalamus, bed nucleus of the stria terminalis (BNST), olfactory bulbs, and medial amygdala. It is believed that this increased expression of oxytocin facilitates the onset and maintenance of maternal behavior (Lee et al., 2009).

**Brain changes.** In addition to the hormone fluctuations that occur throughout pregnancy and the postpartum, there are changes in the brain that may result from these shifts (Barha & Galea, 2017). First, there is an overall reduction in healthy women’s brain sizes during pregnancy that is smallest at term. This reduction is only temporary, as pre-pregnancy brain size is recovered by about six months after delivery (Oatridge et al., 2002). However, even though overall brain size recovers, there is evidence that pregnancy results in long-lasting changes to particular regions within the brain (Barha & Galea, 2017; Hoekzema et al., 2017). More specifically, researchers have identified extensive gray matter volume reductions in the anterior and posterior cortical midline and specific sections of the bilateral lateral prefrontal and temporal cortex of postpartum women in comparison to non-pregnant women that were still present during a two-year follow up. Additionally, these changes in gray matter volumes significantly predict quality of attachment between the mother and her newborn as well as absence of hostility toward the infant in the postpartum period (Hoekzema et al., 2017). Thus, although it appears that
overall brain size recovers from reductions during pregnancy, there is sophisticated evidence that suggests that individual brain regions endure lasting alterations due to pregnancy, which has been correlated with changes observed in behavior.

**Possible Mechanisms for Postpartum Depression and Anxiety in Humans**

Based on the changes in behavior and hormones seen during normal pregnancy and the postpartum, researchers have proposed several hypotheses for possible mechanisms of postpartum depression and anxiety. These include alterations and dysregulation of ovarian hormones, cortisol secretion, and oxytocin from what would typically be expected throughout pregnancy and the postpartum. A description and evidence for each will be described.

**Ovarian-steroid-withdrawal hypothesis.** Given that estradiol and progesterone levels steadily rise throughout pregnancy and then sharply decline following childbirth, these two hormones have attracted much attention in research on postpartum depression (Schiller, Meltzer-Brody, & Rubinow, 2015). As such, one hypothesis for the cause of postpartum depression is the ‘ovarian-steroid-withdrawal hypothesis’ (Bloch et al., 2000; Galea, Wide, & Barr, 2001; Hendrick et al., 1998), a theory that postpartum depression develops due to the dramatic drop of ovarian hormones in the brain following delivery (Arpels, 1996). One of the first studies to investigate the possible role of changes in ovarian hormones in PPD in humans did so by inducing hypogonadism in women with and without a history of postpartum depression. Specifically, the women were given injections to mimic the rise of estrogen and progesterone during pregnancy, and then withdrawn from both hormones. The results showed that over 60% of the women with a previous episode of PPD developed mood symptoms during the withdrawal period while the women without a history of PPD displayed no changes in mood (Bloch et al., 2000). This suggests both that ovarian steroid hormone withdrawal may contribute to the
etiology of PPD, and that women with a history of postpartum depression may be particularly sensitive to changing levels of estradiol and progesterone. Furthermore, in a separate study, increased levels of androgens and estrogens measured 4 weeks before birth or from mixed umbilical cord blood at birth were associated with depressive symptoms in the postpartum period (Parizek et al., 2014). This suggests that women who develop PPD have atypical hormone levels in pregnancy which may contribute to their sensitivity to the withdrawal period following birth.

**Hypothalamic pituitary adrenal (HPA) axis dysregulation.** Given that cortisol levels are also fluctuating during pregnancy and the postpartum, researchers have proposed that cortisol and the HPA axis may be involved in the mechanism of postpartum depression (Glynn, Davis, & Sandman, 2013). Indeed, abnormal levels of cortisol or CRH throughout pregnancy, the early postpartum period, and late postpartum period have been observed in women with postpartum mood disorders. First, several studies have shown that women with elevated CRH levels in mid-pregnancy are more likely to develop postpartum depressive symptoms (Glynn & Sandman, 2014; Iliadis et al., 2016). Further, an accelerated increase in CRH levels during pregnancy is also associated with symptoms of PPD (Glynn & Sandman, 2014). Second, plasma cortisol is greater in women suffering from PPD than in healthy controls in the early postpartum (Maes et al., 1992). Another comprehensive study by Bloch et al. (2005) found that women who have previously suffered from an episode of PPD report more depressive symptoms and show greater cortisol responses during exposure to a hormone simulated pregnancy. This suggests that the HPA axis and mood are altered in response to pregnancy hormones in vulnerable women. Finally, compared to non-depressed postpartum women and non-postpartum healthy women, women diagnosed with a current major depressive disorder in the late postpartum period surprisingly have reduced cortisol levels at 3 and 6 months postpartum (de Rezende et al., 2016;
Jolley, Elmore, Barnard, & Carr, 2007). All of these abnormal cortisol levels throughout pregnancy and the postpartum are indicative of impairment in HPA axis regulation (Ising et al., 2007), which may contribute to PPD or PPA.

**Oxytocin hypothesis.** Alterations in oxytocin have also been implicated in the etiology of postpartum depression and anxiety (Kim et al., 2014). Central, but not peripheral, oxytocin release is thought to counteract the HPA axis (Heinrichs et al., 2001), thereby diminishing anxiety and stress. If oxytocin levels are altered, the HPA axis may be altered as well, possibly leading to depressive or anxious symptoms. As a matter of fact, there is evidence to suggest that atypical oxytocin levels in pregnancy predict depressive symptoms in the postpartum. Specifically, women with lower plasma oxytocin concentrations in mid-pregnancy report greater PPD symptoms at 2 weeks postpartum than women without these symptoms (Skrundz, Bolten, Nast, Hellhammer, & Meinlschmidt, 2011). Another comprehensive study found that while oxytocin plasma levels significantly increase from the 35th week of pregnancy until 6 months postpartum in healthy women, these plasma levels decrease from the 38th week of pregnancy until 2 days after delivery in women who develop postpartum depressive symptoms (Jobst et al., 2016). Together, these studies suggest that atypical oxytocin levels during pregnancy may contribute to postpartum depression.

What’s more, these altered oxytocin levels remain and predict PPD symptoms in the late postpartum period. Specifically, women suffering from PPD at 8 weeks postpartum have lower levels of oxytocin than non-depressed mothers (Lara-Cinisomo, McKenney, Di Florio, & Meltzer-Brody, 2017). This is in line with past research looking at the association between PPD and oxytocin level in the postpartum (Moura, Canavarro, & Figueiredo-Braga, 2016).
convincing evidence for the role of oxytocin in postpartum mood disorders, further research on
the neuropeptide is of particular interest to the present study.

**Neurobiology of Postpartum Depression and Anxiety in Humans**

Relatively few studies have examined the neurobiology of postpartum depression and
anxiety in humans (Brummelte & Galea, 2016; Fiorelli et al., 2015). This is likely due to the
difficulty in recruiting both healthy and depressed and/or anxious women in the shortly defined
postpartum window as well as methodological limitations. Nonetheless, a few studies have taken
advantage of minimally invasive magnetic resonance imaging technology to study the brains of
women with PPD (Fiorelli et al., 2015), and several key regions have been implicated. First,
women with PPD have less activation in their amygdala than healthy controls to negatively
valenced stimuli (Moses-Kolko et al., 2010; Silverman et al., 2011; Silverman et al., 2007). It is
interesting to note that this finding is in contrast to a number of studies examining amygdala
activation in major depressive disorder (Fiorelli et al., 2015), which may indicate a specific
signature for PPD. Second, researchers have also studied the reaction of depressed and non-
depressed mothers to the sound of their infant’s cry in comparison to other infants’ cries and a
control sound (Laurent & Ablow, 2011; Laurent & Ablow, 2013). While non-depressed mothers
show brain activation in regions previously identified as important to parenting behavior (i.e., the
limbic subcortical regions, dorsomedial prefrontal cortex, insula, and fusiform gyrus) in response
to their infant’s cry compared to either reference stimuli, depressed mothers did not (Laurent &
Ablow, 2011). Further, this same group of depressed mothers also showed decreased activation
in the dorsal anterior cingulate cortex when exposed to distressed facial expressions and in the
orbitofrontal cortex and frontal insula when exposed to the expressions of joy in comparison to
healthy controls (Laurent & Ablow, 2013). These studies demonstrate marked differences in the neural correlates of women with PPD as opposed to healthy postpartum women.

Nonetheless, these studies only provide a broad look at the brain alterations that exist for women suffering from PPD and PPA. In order for the effective treatment and possible prevention of these disorders, an even better understanding of the neural pathophysiology of PPD and PPA is required. To do so, more invasive techniques are needed to examine the role of particular brain regions for indications of plasticity. Due to the difficulty or inability to perform such studies in humans, researchers have turned to animal models to fill this void.

**Animal Models of Postpartum Depression and Anxiety**

Despite the fact that an animal model is unlikely to share all of the behaviors, hormonal fluctuations, and brain alterations that women with complex postpartum mood disorders have, they still serve an invaluable role in deepening our understanding of the etiology of these disorders. In fact, over the past several decades, researchers have developed several behavioral tests to model depression- and anxiety-like behavior in rodents, as well as some hormonal protocols to mimic pregnancy and the postpartum in these species (Ming & Shinn-Yi, 2016; Perani & Slattery, 2014). This section will describe some of these models of postpartum mood disorders in animals.

**Quantifying depressive behaviors.** One behavioral test that measures depressive-like behavior in animals is called the Forced Swim Test (FST; Porsolt, Le Pichon, & Jalfre, 1977). The test involves putting a rodent in a chamber filled with water from which they cannot escape for a given period of time. The amount of time that the animal spends swimming or climbing the walls of the chamber is indicative of escape behaviors, while the amount of time spent floating immobile is considered a measure of learned helplessness (Slattery & Cryan, 2012), a common
symptom in both major depressive disorder and PPD (Melges, 1968). The Forced Swim Test is a valid tool to assess depressive-like behavior in animal models because other animal models of depression also exhibit immobility in the FST (Velazquez-Moctezuma & Diaz Ruiz, 1992), animals given antidepressants see reduced depressive-like behavior in the FST (Lucki, 1997), and animals given drugs that induce depression in humans also demonstrated reduced mobility in the FST (Kokkinidis, Zacharko, & Anisma, 1986). Thus, the Forced Swim Test is one way of measuring depression-like behaviors in animal models.

Another behavioral test measuring depressive-like behaviors in animals is the Tail Suspension Test (Steru, Chermat, Thierry, & Simon, 1985). Similar to the Forced Swim Test, the tail suspension test measures immobility as an indicator of learned helplessness. In the Tail Suspension Test, animals are hung by their tails and suspended in the air. The amount of time they spend moving and immobile is measured (Cryan, Mombereau, & Vassout, 2005; Steru et al., 1985). The test is considered valid because when animals are treated with antidepressants, the amount of time spent immobile decreases, indicating a decrease in depressive-like behaviors (O'Leary & Cryan, 2009). Moreover, the Tail Suspension Test serves as another good measure of depressive-like behavior in animals.

Finally, the Sucrose Preference test is a tool that measures anhedonia (Papp, Willner, & Muscat, 1991), another common feature of major depressive disorder and PPD (APA, 2013). The test takes advantage of the fact that healthy rodents prefer 1–2% sucrose water over regular water when given the choice between the two. Thus, when given access to two bottles (i.e., one with pure water and one with 1–2% sucrose), animals that demonstrate a reduced consumption of sucrose-containing water in comparison to controls are said to exhibit a decreased preference for the sugar water which is indicative of anhedonia-like behavior (Navarre, Laggart, & Craft,
The validity of this measure comes from reversal of the behavioral change found in animals treated with antidepressants (Papp et al., 1991).

**Quantifying anxious behaviors.** Anxious-like behaviors can also be observed in animal models. One such measure is the Open Field Test. In the test, an animal is placed in the center of an open field, a walled enclosure that prevents escape (Gould & Kovacsics, 2009). Animals that spend more time around the perimeter are considered less exploratory and thereby more anxious than those that spend more time in the center of the field (Stanford, 2007). Another measure for anxiety-like behaviors is the Elevated Plus Maze. In this test, the animal is placed in the center of a plus-shaped apparatus suspended above the ground. Two of the arms of the shape are open and two are walled. Animals who spend more time in the closed arms are considered less exploratory and thereby more anxious than those who spend a greater amount of time in the open arms (Ming & Shinn-Yi, 2016). Both tests have been validated using pharmacology (Pellow, Chopin, File, & Briley, 1985; Seibenhener & Wooten, 2015; Walf & Frye, 2007) and can therefore be used to evaluate the anxious-like behavior of animal models in the postpartum.

**Ovarian-steroid-withdrawal model for pregnancy and the postpartum.** In addition to these behavioral tests, several models for postpartum depression have been developed in animal models. Some of the most popular include the chronic corticosterone treatment model which seeks to mimic the hypercortisolism found in women with PPD and the gestational stress model which takes advantage of the fact that stress during pregnancy is a risk factor for developing PPD and PPA (Ming & Shinn-Yi, 2016). For the purposes of the present study, the ovarian-steroid-withdrawal model, which replicates the rise and dramatic drop of estrogen and progesterone during human pregnancy, will be discussed in depth.
In 2001, researchers Galea, Wide, and Barr developed the ovarian-steroid-withdrawal model for postpartum depression in rats. In this procedure, female rats are ovariectomized and, once healed, are injected subcutaneously each day with either a vehicle or hormone combination for 27 days, the length of typical rat gestation. The control group receives injections of a vehicle, typically an oil, for the entire 27 days. Both the hormone-sustained (i.e., “pregnant” + estradiol benzoate) group and the hormone-withdrawn (i.e., “pregnant”) group receive a low dose of estradiol benzoate combined with a high dose of progesterone for 16 days. On days 17 through 24, both experimental groups receive a high dose of estradiol benzoate without progesterone. This regimen continues for the hormone-sustained groups, while the hormone-withdrawn group is administered the vehicle for days 24 through 27, which is considered the postpartum (Galea et al., 2001). This is supposed to mimic the estradiol and progesterone levels observed in pregnancy, as researchers believe that the dramatic drop in these hormones at birth is at least partially responsible for the development of postpartum mood disorders.

Evidence in support of this model for PPD was established as 3 days after hormone withdrawal, the withdrawn rats show more depressive-like behaviors as seen by their increased immobility and decreased struggling and swimming in the Forced Swim Test than the hormone-sustained or control rats (Galea et al., 2001). This suggests that estradiol may alleviate depressive-like symptoms in the postpartum model of PPD in rats. However, it is important to note that the researchers did not find a reduction in anxiety-like behavior in the hormone-withdrawn group. In fact, they found that this group spent more time crossing through the center of the open field than both the control and hormone-sustained groups in the Open Field Test (Galea et al., 2001). This suggests that the rats that underwent the hormone drop during the
postpartum period experienced fewer anxiety-like symptoms during this time in the model. Thus, there is evidence to support the ovarian-steroid-withdrawal model for PPD, but not for PPA.

Several studies have since utilized this model to further test its validity. Research has confirmed the finding by Galea et al. (2001) that the hormone-withdrawn females display depressive-like behavior as seen by their increased immobility in the Forced Swim Test than controls (Green, Barr, & Galea, 2009; Schiller, O'Hara, Rubinow, & Johnson, 2013; Stoffel & Craft, 2004). In addition, the hormone-withdrawn group also displays greater depressive-like symptoms as indicated by their decreased consumption of sucrose-containing water in the Sucrose Preference Test on days 2 and 3 postpartum (Green et al., 2009) and 3 weeks postpartum (Navarre et al., 2010). Research has also confirmed the finding by Galea et al. that the hormone-withdrawn rats do not display any difference in their anxiety-like behaviors than the control or hormone-sustained groups as behavior on the Elevated Plus Maze did not differ between groups (Stoffel & Craft, 2004).

Interestingly, the prolonged depressive-like behavior in the “postpartum” is only found in female rats who undergo the hormone simulated pregnancy and not in female rats who had previously been pregnant naturally (Navarre et al., 2010). This suggests that withdrawal from pregnancy levels of estradiol is responsible for the development of depressive-like symptoms in the “postpartum” period since the hormone simulated pregnancy regimen mainly simulates estradiol withdrawal. However, there are no differences in depressive-like behavior as measured by the Sucrose Preference Test between previously pregnant and non-pregnant rats. Together, this suggests that hormones other than estradiol that change during pregnancy and the postpartum may be responsible for buffering negative mood (Navarre et al., 2010). Oxytocin may be involved given that it is a hormone that rises and falls during this period.
Furthermore, research recently conducted in hamsters contradicts the findings of Galea et al. Mainly, the researchers found no difference between the hormone-withdrawn and hormone-sustained groups in depressive-like behaviors as measured by the sucrose preference test (Amaral, 2017; Bodie, 2017; D’Antonio, 2017; Lee, 2017). The researchers indicated that this may be due to the fact that their study used Syrian hamsters while the model by Galea et al. (2001) was developed in rats. This is significant because Syrian hamsters only have a 16–18 day gestation length as compared to the 26–27 day length for rats (Bond, 1945; Galea et al., 2001; Viswanathan, & Davis, 1992). Thus, the hamsters may have been insufficiently exposed to the ovarian hormones due to their shorter gestation, resulting in a lack of depression-like symptoms.

Interestingly, it is also possible that this model was merely simulating hormonal and behavioral changes associated with pregnancy in Syrian hamsters as opposed to postpartum depression. This is supported by the finding that hormone-withdrawn hamsters spend more time in center of open field test indicating that these hamsters are experiencing less anxiety-like symptoms than hormone-sustained hamsters (Amaral, 2017; Bodie, 2017; D’Antonio, 2017; Lee, 2017). As a matter of fact, reduced anxiety-like behavior during the postpartum is typical in both rats (Agrati, Zuluaga, Fernández-Guasti, Meikle, & Ferreira, 2008; Lonstein, 2005; Macbeth, Gautreaux, & Luine, 2008) and humans (Lonstein, 2007; Macbeth & Luine, 2010) in comparison to controls. This makes evolutionary sense in that decreased anxiety makes it more likely for the mother to feel comfortable leaving the nest to forage for food (Macbeth & Luine, 2010). Moreover, these findings suggest that the hormone simulated pregnancy developed by Galea et al. (2001) applied to a hamster model may actually simulate typical pregnancy as opposed to postpartum depressive- or anxiety-like behavior.
Oxytocin in animal models of pregnancy and the postpartum. Oxytocin also plays a role in animal models of postpartum mood disorders. In fact, the same researchers who used the hormone stimulated pregnancy in hamsters also found that the hormone-withdrawn animals had significantly more oxytocin producing neurons in the paraventricular nucleus than the hormone-sustained hamsters (Amaral, 2017; Bodie, 2017; D’Antonio, 2007; Lee, 2017). This is further support for the claim that hormone injections given in the model by Galea et al. (2001) are mimicking typical pregnancy in hamsters as increased oxytocin immunoreactive levels are elevated in the PVN during the normal postpartum period (Caldwell, Greer, Johnson, Prange, & Pedersen, 1987). It is also important to note that although some oxytocin is also produced by the supraoptic nucleus (SON) of the hypothalamus (Insel & Shapiro, 1992), there is no change in oxytocin-producing neurons in this region between hormone-sustained and hormone-withdrawn hamsters (Foster, Heaton, & Been, 2017). Furthermore, another recent study examining oxytocin in the PVN in relation to PPD in rats using a gestational stress model found that the rats with depression-like symptoms in the postpartum displayed a decrease of mRNA and peptide levels of oxytocin only in PVN and not the SON. These depressive-like behaviors were reversed when oxytocin was injected into the PVN (Wang et al., 2018). Together, these studies indicate that the oxytocin-producing neurons in the PVN play a role in both the reduction of anxious-like behaviors seen in healthy postpartum hamsters and the increase in depression-like behaviors seen in postpartum depressed rats.

Efferents of Interest from the Paraventricular Nucleus of the Hypothalamus

Given the established involvement of oxytocin-producing neurons in the paraventricular nucleus in modulating anxiety- and depressive-like behavior in the postpartum, it is important to understand where these oxytocin neurons may be firing. Although target projections of oxytocin-
producing neurons remain unmapped (McHenry, Rubinow, & Stuber, 2015), there are several efferents of the PVN that are likely to be involved given that they are traditionally hormone-sensitive regions and implicated in maternal behavior. These regions are the medial amygdala (MeA), nucleus accumbens (NAc), bed nucleus of the stria terminalis (BNST), and raphe nuclei.

**Medial amygdala.** The MeA is known to contain oxytocin receptors and also plays a central role in maternal behavior (Barrett & Fleming, 2011; Numan et al., 2010), making the region a likely target for oxytocin signaling following birth. In fact, birth induces an increase in oxytocin receptor mRNA expression in the MeA compared to pre-parturition. Interestingly, the receptor levels returned to the levels observed in virgin rats in the early postpartum (Meddle et al., 2007). This suggests that there is oxytocin receptor plasticity in this region in response to parturition in healthy, non-depressed animals. In addition, decreased oxytocin receptor mRNA expression in the MeA is correlated with poorer maternal care and lactation, as well as heightened maternal anxiety (Murgatroyd et al., 2015). This suggests that below average oxytocin receptor expression in the MeA may play a role in postpartum mood disorders.

**Nucleus accumbens.** The NAc is another hormone-sensitive region implicated in maternal and anxious behaviors, particularly in relation to postpartum mood disorders (Haim, Sherer, & Leuner, 2014). One study using a gestational stress model to induce PPD-like symptoms found that the gestationally-stressed rats experienced an increase in depressive-like behaviors throughout the postpartum. More importantly, this stress reduced the structural plasticity within the postpartum NAc in that stressed mothers had shorter dendrites and fewer branch points in comparison to controls (Haim et al., 2014). This plasticity in the postpartum NAc suggests that the region is altered in response to hormone fluctuations during this period, possibly including oxytocin. Additionally, lower levels of oxytocin receptors in the NAc are
correlated with poorer consolidation of maternal memory or a reduced onset of maternal responsiveness (D’Cunha, King, Fleming, & Lévy, 2011). Together, these findings suggest that the NAc may be implicated in postpartum mood disorders.

**Bed nucleus of the stria terminalis.** The BNST is another potential candidate for oxytocin receptor alteration in the postpartum due to its involvement in maternal behavior (Barrett & Fleming, 2011; McHenry et al., 2015) and anxiety regulation (Adhikari, 2014; Hammack, Richey, Watkins, & Maier, 2003). Specifically, inactivation of the BNST results in a reduction of maternal behavior as indicated by deficits in a separation/reunion test (Perrin & Lévy, 2007), suggesting that the BNST plays a role in the maintenance of maternal behavior. In addition, the BNST is also believed to mediate longer-lasting and slower-onset responses to sustained threats (Walker, Toufexis, & Davis, 2003). The BNST is also a hormone- and neurotransmitter-sensitive region (Barrett & Fleming, 2011) with several studies confirming an increase in oxytocin receptors in the region following birth and into the early postpartum period in both ewes (Broad et al., 1999) and rats (Insel, 1990). Given the role of the BNST in both maternal and anxious behaviors, as well as the presence of oxytocin receptors in response to birth, it is possible that the BNST may be involved in the etiology of postpartum mood disorders.

**Raphe nuclei.** Finally, oxytocin receptors are also abundantly present in the raphe nuclei, another region involved in maternal and anxious behaviors. First, lesions to the median raphe nucleus result in low incidence of maternal behavior in rats as compared to controls. Interestingly, rats with lesions to the dorsal raphe nucleus did not see this effect (Yurino, Tsukahara, Korányi, & Yamanouchi, 2001), suggesting that the median raphe nucleus and not the dorsal raphe nucleus may be partially responsible for maternal behavior. Second, oxytocin binding sites are located in the raphe nuclei. Specifically, about one half of the neurons involved
in modulating serotonin release in the raphe nucleus have oxytocin receptor sites (Yoshida et al., 2009), suggesting that oxytocin may play a role in serotonin release. Introduction of oxytocin within the raphe nucleus facilitates serotonin release and also reduces anxiety-like behavior. This anxiolytic effect is reversed when a serotonin receptor agonist is introduced (Onaka, Takayanagi, & Yoshida, 2012; Yoshida et al., 2009). Thus, the role of oxytocin in reducing anxious behavior in the raphe nuclei, as well as the region’s involvement in maternal behavior, make this region a possible regulator in the development of postpartum mood disorders.

The Present Study

Previous research has demonstrated that the dramatic rise and fall of ovarian hormones that occurs at parturition likely plays a role in the etiology of postpartum mood disorders. Additionally, there is evidence of an increase in oxytocin-producing neurons in the PVN following hormone withdrawal. We believe that the MeA, NAc, BNST, and raphe nuclei may be influenced by these alterations given that the regions are traditionally hormone-sensitive and implicated in maternal behavior. Put together, these findings lead to the hypothesis that estrogen withdrawal during the postpartum period could lead to changes in signaling between the oxytocin-producing neurons of the paraventricular nucleus and oxytocin-receiving neurons in the MeA, NAc, BNST, and/or raphe nuclei. If disrupted, these neuronal pathways could be implicated in the depressive- and/or anxiety-like behavior observed during the postpartum period. Experiment 1 will examine the oxytocin receptor density in these PVN efferents between hormone-withdrawn, hormone-sustained, and control groups to begin testing this hypothesis.

Finally, a pilot study (Experiment 2) will further examine the previous finding that hormone withdrawal increases oxytocin-producing neurons in the PVN. Specifically, the PVN of hormone-withdrawn and hormone-sustained animals will be stained with Fluoro-Jade C, a
marker of neurodegeneration (Schmued et al., 2005), to qualitatively assess whether neurons in the region are dying. Given that hormone-withdrawn animals have been shown to have more oxytocin-producing neurons in this region, we hypothesize that hormone-sustained animals will show greater cell death than hormone-withdrawn animals, which may account for the abundance of oxytocin neurons in the PVN of withdrawn animals.

**Method**

**Experiment 1**

**Subjects.** Twenty-four adult female Syrian hamsters (*Mesocricetus auratus*) weighing approximately 110 g were purchased from the Charles River Laboratories (Wilmington, MA, USA) at approximately 60 days of age and used as subjects in the present study. Eight hamsters were in the hormone-sustained group, eight in the hormone-withdrawn group, and eight in the control group. The animals were housed individually in a temperature-controlled room in standard 46 x 24.5 x 20 cm opaque polycarbonate solid bottom cages with aspen bedding for building nests. Temperature in the room was held at a consistent range (approximately 22°C), and the hamsters were maintained on a reversed light cycle of 14 hours light to 10 hours dark (lights off at 10:00 h). All behavioral testing occurred during the dark phase. Food and water were available to all animals *ad libitum*. All animal procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and relevant Haverford College policies. All efforts were made to minimize the number of animals used and their suffering.

**Materials.**

**Open Field Test.** The apparatus consisted of a 40.5 x 40.5 cm² arena. Fluorescent lights provided overhead illumination. The arena was closed in by 30 cm gray walls. Locomotor
activity over a ten minute period was recorded by a video camera positioned approximately 127.5 cm above the apparatus.

*Elevated Plus Maze.* The maze consisted of two open arms, 51 x 11.5 cm, and two enclosed arms, 51 x 11.5 x 39.5 cm, with an open roof, arranged such that the two open arms were opposite to each other. The maze was elevated to a height of 73 cm. Locomotor activity over a 5 minute period was recorded by a video camera suspended above the apparatus by a selfie stick.

**Procedure.**

*Ovariectomies.* All hamsters were bilaterally ovariectomized to remove their endogenous source of gonadal hormones. Specifically, the hamsters were anesthetized (2–5% isoflurane vaporized in 100% oxygen). Their flanks were shaved, and alternating gauze with ethanol and betadine was used to sterilize the surgical sites. Hamsters were then moved to a sterile surgical site and anesthesia was maintained via a nose cone. Bilateral flank incisions were made, and ovaries were removed via cauterization of the uterine horn. Their muscle was closed with sutures, and their skin closed with wound clips. To minimize post-operative pain and infection, butorphanol (5 mg/kg) and baytril (10 mg/kg), respectively, were administered for 3 days post-operatively.

*Hormone administration.* One week after ovariectomies, hormone regimens were started based on the procedure from Galea et al. (2001). All hamsters were administered daily hormone injections at approximately 09:00 h over a 17-day period. Hamsters in the control group received a cottonseed oil vehicle (0.1 ml) daily. Both the hormone-sustained and hormone-withdrawn hamsters were given a low dose (2.5 µg) of estradiol benzoate and a high dose (4 mg) of progesterone dissolved in a cottonseed oil vehicle daily on days 1-12. On days 13-17, both the
hormone-sustained and hormone-withdrawn hamsters received a daily high dose of estradiol benzoate (50 µg) dissolved in the vehicle. Days 18-21 were considered the postpartum period, and hamsters in the hormone-withdrawn condition were given the cottonseed oil vehicle with no estradiol in order to mimic the drop in estrogen that occurs with birth, while those in the hormone-sustained group continued to receive daily injections of a high dose (50 µg) of estradiol benzoate to prevent the effects of estrogen withdrawal.

**Behavioral measures.** During the postpartum (i.e., days 18-21), the hamsters were tested in a pseudo-randomized order in the Open Field Test and Elevated Plus Maze to measure their anxiety-like behavior. The behavioral testing order (open field vs. elevated plus) was counterbalanced between experimental groups. This order gave each hamster a day without testing in between each behavioral test. All behavioral testing took place during the first five hours of the dark phase, and behavioral equipment was cleaned with 70% ethanol after each trial.

**Open Field Test.** On days 18 and 21 (i.e., days 1 and 4 postpartum, respectively), the hamsters were tested in the Open Field Test apparatus. Locomotor activity over a 10 min period was recorded by a video camera positioned above the apparatus. The videotapes were analyzed using the Noldus EthoVision-XT (Noldus Information Technology, the Netherlands) behavioral tracking software for both non-specific locomotor behavior (e.g., distance traveled, velocity) and anxiety-like behavior (i.e., amount of time spent in the center of the open field, amount of time spent in the periphery, difference between time spent in the periphery and time spent in the center).

**Elevated Plus Maze.** On days 19 and 20 (i.e., days 2 and 3 postpartum, respectively), the hamsters were tested in the Elevated Plus Maze apparatus. Locomotor activity over a 5 min period was recorded by a video camera positioned above the apparatus. The videotapes were
analyzed using the Noldus EthoVision-XT system (Noldus Information Technology, the Netherlands) for anxiety-like behavior (i.e., amount of time spent in the open arms, amount of time spent in the closed arms, difference between time spent in the closed and open arms).

**Tissue histology.** Twenty-four hours after the last day of behavioral testing (i.e., day 22), all animals were euthanized via anesthetized rapid decapitation. Their brains were then extracted, flash frozen on dry ice, and stored at −20°C.

**Oxytocin receptor autoradiography.** Flash frozen brains were sent to our collaborators in Dr. Elliott Albers’ lab at Georgia State University where they were sectioned for preparation and execution of autoradiography. An optical density analysis was performed by individuals blind to the experimental condition of the subjects to quantify oxytocin receptor density in our regions of interest.

Specifically, oxytocin receptor binding was determined with the $^{125}$I-labeled ornithine vasotocin analog Vasotocin, d(CH$_2$)$_3$[Tyr(Me)$_2$,Thr$^4$,Orn$^8$,$^{125}$I]Tyr$_9$-NH$_2$ (Perkin-Elmer). The tissue was thawed, dried, and then fixed in 0.1% paraformaldehyde for 2 min. The slides were then rinsed twice for 10 min each in buffer (50 mM Tris, pH 7.4) and were then incubated in tracer buffer (0.35 mM bacitracin, Sigma-Aldrich, St. Louis, MO, USA; 0.015 mM bovine serum albumin, Sigma-Aldrich, St. Louis, MO; 100 nM $^{125}$I vasotocin analog) for 1 hr. The slides were then rinsed twice for 5 min each and then for 35 min with agitation in buffer (50 mM Tris, 21 mM MgCl). All incubations and washes were performed at room temperature. Finally, the slides were dipped in 4°C deionized water and allowed to dry. The slides and a $^{14}$C standard calibration strip (American Radiolabeled Chemicals, St. Louis, MO, USA) were loaded into autoradiography cassettes and exposed to film (Kodak, Rochester, NY, USA) for 7 days at room temperature (A. Ross, personal communication, April 6, 2018).
Densitometry analysis was performed using Scion Image software (NIH, Bethesda, MD, USA) and a lightbox (Imaging Research, Inc., Ontario, Canada) attached to a camera (Panasonic, Newark, NJ, USA). 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 dorsal raphe nuclei sections, which were analyzed along the midline of the brain. With the exception of the MeA, a 0.35 mm\(^2\) 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 6, 2018).

**Statistical analyses.** All data was analyzed using SPSS 24.0 (SPSS Inc., Chicago, IL, USA) for Windows and significance was determined as \(p < 0.05\). Specifically, one-way between-subjects ANOVAS were performed to examine mean group differences in oxytocin receptor cell density within the four efferents. Additionally, one-way between-subjects ANOVAS were performed to examine mean group differences in anxiety-like and non-specific locomotor behavior as measured by the Open Field Test and Elevated Plus Maze between hormonal conditions.

**Experiment 2**

**Subjects.** Tissue previously collected from 16 adult female Syrian hamsters who underwent hormone-simulated pregnancy were used in the present experiment (see Amaral, 2017; Bodie, 2017; D’Antonio, 2017; Lee, 2017). Eight hamsters were in the hormone-sustained

**Procedure.**

**Ovariectomies, hormone administration, and tissue histology.** Ovariectomies, hormone-simulated pregnancy and tissue histology was previously performed using methods detailed in Amaral (2017), Bodie (2017), D’Antonio (2017), and Lee (2017). Briefly, all hamsters were bilaterally ovariectomized to remove their endogenous source of gonadal hormones and, one week after ovariectomies, hormone regimens were started. On days 1-12, both the hormone-sustained and hormone-withdrawn hamsters were given a low dose of estradiol benzoate and a high dose of progesterone dissolved in a cottonseed oil vehicle daily. On days 13-17, both the hormone-sustained and hormone-withdrawn hamsters received a daily high dose of estradiol benzoate dissolved in the vehicle. On days 18-21, hamsters in the hormone-withdrawn condition were given the cottonseed oil vehicle with no estradiol in order to mimic the drop in estrogen that occurs with birth, while those in the hormone-sustained group continued to receive daily injections of a high dose of estradiol benzoate to prevent the effects of estrogen withdrawal (Amaral, 2017; Bodie, 2017; D’Antonio, 2017; Lee, 2017).

On postpartum day 5 (i.e., day 22 of the hormone-simulated pregnancy), after a series of behavioral tests were completed (see Amaral, 2017; Bodie, 2017; D’Antonio, 2017; Lee, 2017), all animals were sacrificed via intracardial perfusion. The brains were removed from the skull and postfixed in 4% paraformaldehyde. Each brain was then cut into 40 µm coronal sections using a cryostat. All brains were then stored in cryoprotectant at −20°C (Amaral, 2017; Bodie, 2017; D’Antonio, 2017; Lee, 2017).
**Fluoro-Jade C staining.** Fluoro-Jade C (FJC), a dye that can sensitively and selectively bind to degenerative neurons (Schmued et al., 2005), was used to examine dying neurons in the brains of hormone-withdrawn and hormone-sustained hamsters. Brain sections containing the PVN were removed from cryoprotectant, washed six times in phosphate buffered saline (PBS) for 5 min each, and mounted on VWR Superfrost Plus Micro Slides using double distilled water. Slides were air-dried in the dark overnight, and subjected to FJC staining. Slides were immersed in a 100 mL solution containing 1% NaOH in 80% ethanol for 5 min. They were then rinsed for 2 min in 70% ethanol and for 2 min in double distilled water. Slides were incubated in 0.06% potassium permanganate solution for 10 min. Following a water rinse for 2 min, slides were transferred to the FJC staining solution and stained for 10 min. The FJC solution was obtained by first making a 0.01% stock solution of FJC dye (Histo-Chem, Inc., Jefferson, Arkansas, USA) in double distilled water and then adding 1 mL of that stock solution to 99 mL of 0.1% acetic acid. Slides were then washed in double distilled water three times each for 1 min and air-dried in the dark overnight. After clearing in xylene for at least 1 min and coverslipping with DPX Mountant for histology (Sigma-Aldrich, St. Louis, MO, USA), sections were examined under a Nikon Eclipse 80i confocal microscope. Nikon imaging software (EZ C1 Gold Version 3.90) was used for visualizing FJC staining and images were captured for demonstration.

**Qualitative analyses.** Fluoro-Jade positive cells were examined in 9 brain sections containing the PVN by researchers blind to the experimental (i.e., hormonal) conditions associated with each sample. Five images were from the hormone-withdrawn group and four from the hormone-sustained group. Images were qualitatively examined for FJC+ neurons. Specifically, tissue samples were categorized into low (i.e., 0-2), intermediate (i.e., 3-9), and high (i.e., 10+) numbers of FJC+ neurons.
Results

Experiment 1

Behavioral measures.

Open Field Test.

Anxiety-like behavior. No significant differences in the total time spent in the periphery of the open field \(F(2,23) = .35, p = .71\), total time spent in the center of the open field \(F(2,23) = .27, p = .76\), or difference in time spent in the periphery and center \(F(2,23) = .294, p = .748\) were found between hormonal conditions (see Table 1). This suggests that there are no differences in anxiety-like behavior as measured by the Open Field Test between the groups.

Non-specific locomotor behavior. A significant difference between hormone conditions was found in the average velocity of the animals during the Open Field Test \(F(2,23) = 3.639, p = .044\). Tukey’s HSD post hoc analysis revealed that the hormone-withdrawn hamsters \((M = 4.95, SD = 1.33)\) had significantly lower mean velocities than the hormone-sustained hamsters \((M = 7.66, SD = 2.58;\) Figure 1). The mean velocity of animals in the control condition \((M = 6.33, SD = 1.91)\) did not significantly differ from either the hormone-withdrawn or hormone-sustained groups (see Table 2). In addition, the total distance moved between the hormone-withdrawn and hormone-sustained animals was trending towards significance \(F(2,23) = 3.366, p = .054\). Tukey’s HSD post hoc analysis revealed that the hormone-withdrawn hamsters \((M = 2955.93, SD = 791.95)\) traveled significantly shorter distances than the hormone-sustained hamsters \((M = 4515.97, SD = 1545.37;\) Figure 2). Again, the distance traveled among the control animals \((M = 3753.01, SD = 1150.56)\) did not significantly differ from either the hormone-withdrawn or hormone-sustained groups (see Table 3). Together, this suggests that non-specific locomotor behavior is altered following hormone withdrawal.
**Elevated Plus Maze.**

Anxiety-like behavior. A significant difference between hormone treatment groups was found in the amount of time spent in the open arms of the Elevated Plus Maze \[F(2,23) = 6.08, p = .008; \text{Table 4}\]. Tukey’s HSD post hoc analysis revealed that the hormone-withdrawn hamsters spent significantly less time in the open arms \((M = 49.83, SD = 24.21)\) than the hormone-sustained hamsters \((M = 108.76, SD = 42.97)\) and control group \((M = 98.66, SD = 38.55; \text{see Table 5 and Figure 3})\). In addition, the difference between the time spent in the closed arms and open arms between the hormone treatment groups was significant \[F(2,23) = 3.71, p = .042; \text{Table 6}\]. However, Tukey’s HSD post hoc analysis revealed no significant differences between the groups, though some differences were trending towards significance such that hormone-withdrawn animals have a greater difference between time spent in the closed arms and time spent in the open arms than hormone-sustained animals (see Table 7 and Figure 4). No significant difference in the time spent in the closed arms was found between groups (see Table 8 and Figure 3). Thus, contrary to the hypotheses, this suggests that the hormone-withdrawn hamsters exhibit more anxiety-like behavior than the hormone-sustained or control groups.

**Oxytocin receptor density.**

**Medial amygdala.** No significant effect of hormone condition on oxytocin receptor density in the MeA was found \[F(2, 21) = 1.87, p = .170; \text{Table 9}\]. Thus, the oxytocin receptor density in the MeA does not appear to be influenced by estrogen withdrawal during the postpartum period (see Table 10 and Figure 5).

**Nucleus accumbens.** Two areas in the NAc were examined: the shell and the core. No significant effect of hormone condition on oxytocin receptor density in either the shell of the NAc \[F(2, 21) = 1.74, p = .201; \text{Table 11}\] or the core of the NAc was found \[F(2, 21) = 1.13, p = \]
Thus, the oxytocin receptor density in the NAc shell (see Table 13) or core (see Table 14) does not appear to be influenced by estrogen withdrawal during the postpartum period (see Figure 6).

**Bed nucleus of the stria terminalis.** No significant effect of hormone condition on oxytocin receptor density in the BNST was found \( F(2, 21) = 1.75, p = .199; \text{Table 15}\). Thus, the oxytocin receptor density in the BNST does not appear to be influenced by estrogen withdrawal during the postpartum period (see Table 16 and Figure 7).

**Raphe nucleus.** There was a significant effect of hormone condition on oxytocin receptor density in the dorsal raphe nuclei \( F(2, 21) = 4.351, p = 0.026; \text{Table 17}\). Tukey’s HSD post hoc analysis revealed that the mean oxytocin receptor density score for the hormone-withdrawn condition \( (M = 275.8, SD = 105.6) \) was significantly higher than the control condition \( (M = 156.3, SD = 87.3; P = 0.033) \). Oxytocin receptor density in the hormone-sustained condition \( (M = 173.0, SD = 65.7) \) did not significantly differ from the hormone-withdrawn and oil conditions (see Tables 18-19 and Figures 8-9). These results suggest that the sudden drop in estrogen following hormone-simulated pregnancy influences oxytocin receptor density such that there are more oxytocin receptors in the dorsal raphe nucleus following hormone withdrawal in comparison to the control condition.

**Experiment 2**

**Neurodegeneration in the PVN.** Qualitative analyses revealed that the hormone-withdrawn animals had more images categorized as having high numbers of FJC+ neurons than the hormone-sustained group, contrary to the hypothesis (see Figure 10). This preliminarily suggests that more neurons are degenerating following hormone withdrawal than in hormone-sustained animals.
**Discussion**

**Experiment 1**

The goal of the first experiment was to replicate and add to previous behavioral and neuroendocrinological findings using the hormone-simulated pregnancy in a hamster model. In further support of previous research using the Open Field Test to measure anxiety-like behavior following hormone-simulated pregnancy (Amaral, 2017; Bodie, 2017; D’Antonio, 2017; Lee, 2017), we expected to show that levels of anxiety-related behavior would decrease in the withdrawn group as compared to the sustained and control groups following hormone-simulated pregnancy. Specifically, we wanted to replicate the finding that hormone-withdrawn animals spend more time in the center of the Open Field Test than hormone-sustained or control hamsters. In addition, we predicted that hormone-withdrawn animals would spend more time on the open arms of the Elevated Plus Maze than the hormone-sustained or control groups. Contrary to our hypotheses, the results showed no significant differences between the hormonal conditions in anxiety-like behavior as measured by the Open Field Test. However, there were significant differences in non-specific locomotor behavior on the Open Field Test such that hormone-withdrawn hamsters moved shorter distances and at slower velocities than the hormone-sustained hamsters. Results from the Elevated Plus Maze revealed that the hormone-withdrawn animals spent less time in the open arms of the maze than the hormone-sustained and control groups, suggesting that, contrary to our hypothesis, hormone withdrawal results in greater anxiety-like behavior.

In regards to the neuroendocrinological testing, we wanted to build on previous findings that hormone-withdrawn animals show greater oxytocin-producing neurons in the PVN by examining possible regions where these neurons may be projecting. Given evidence supporting
that several PVN efferents (i.e., the MeA, NAc, BNST, and raphe nuclei) are traditionally hormone-sensitive and implicated in maternal behavior, we hypothesized that these regions may also be influenced by estrogen withdrawal during the postpartum period. We identified three possible outcomes based on maintaining homeostatic balance that could have resulted from our experimental manipulation given the exploratory nature of our study. Mainly, we expected oxytocin receptor density expression in one or more of the PVN efferents of interest to decrease, increase, or remain unchanged in the hormone-withdrawn group in comparison to the hormone-sustained and control groups. Additionally, we expected oxytocin receptor levels to be lowest in the control condition since these animals have no endogenous production of estrogen and it is known that estrogen facilitates oxytocin receptor expression (Broad et al., 1999, Haim et al., 2014, Hiller, 2004; Insel, 1990, Meddle et al., 2007).

In support of our hypotheses, we found a significant increase in oxytocin receptor density in the dorsal raphe nuclei of hormone-withdrawn animals in comparison to control animals. This finding is in support of one the possible outcomes, suggesting that estrogen withdrawal may have altered the brain such that there is an increase in oxytocin receptors in the raphe nuclei. No differences in oxytocin receptor density in the dorsal raphe nuclei were found between the hormone-withdrawn and hormone-sustained animals or the hormone-sustained and the control animals. However, as was expected, the control animals did have the lowest density of oxytocin receptors among the three groups. Put together, these findings suggest that the rise in estrogen and progesterone during pregnancy is itself not enough to significantly alter oxytocin receptor density in the dorsal raphe; rather, the rise followed by the sudden drop in estrogen following hormone-simulated pregnancy is needed for the increase in oxytocin receptor density in this region. Additionally, hormone condition did not have a significant effect on oxytocin receptor density in the
density in the medial amygdala, nucleus accumbens core, nucleus accumbens shell, or bed nucleus of the stria terminalis.

**Altered non-specific locomotor behavior following hormone withdrawal.** Contrary to our hypotheses, the Open Field Test revealed no differences in anxiety-like behavior between the hormone treatment groups; however, the hormone-withdrawn animals did have reduced non-specific locomotor behavior such that they had slower velocities and traveled shorter distances than the hormone-sustained animals. This suggests that hormone withdrawal results in altered non-specific locomotion. This altered locomotive behavior could explain why no differences between hormone treatment groups in anxiety-like behavior as measured by the Open Field Test were noted. Because there were significant differences in the locomotor ability of the animals, further analyses of time spent in the periphery and center of the arena could be skewed due to inactivity instead of treatment effects (Seibenhener & Wooten, 2015). In other words, it is possible that no differences in time spent in the center or periphery of the open field were noted between the hormone treatment groups because the hormone-withdrawn hamsters were highly inactive and thus moved between the areas less than the hormone-sustained animals.

Moreover, it is also possible that the Open Field Test does not capture postpartum anxiety-like behavior in hamsters. When rodents are initially placed into the open field, they are expected to stay along the periphery of the arena. This tendency to stay close to the walls, known as thigmotaxis, typically decreases as the rodent remains in the field. When an animal does not demonstrate this propensity to explore, it is interpreted as a measure of anxiety-like behavior (Simon, Dupuis & Costentin, 1994). While this measure has been validated using pharmacology (Seibenhener & Wooten, 2015), previous research has found that thigmotaxis does not differ between naturally pregnant and postpartum mice (Leppänen, Ravaja, & Ewalds-Kvist, 2008),
despite the fact that most laboratory rodents do experience decreases in anxiety-like behavior following birth as humans do (Agrati & Lonstein, 2016). Thus, it is possible that the Open Field Test does not capture postpartum changes in anxiety-like behavior in hamsters.

**Anxiogenic behavior following hormone withdrawal.** Contrary to our hypotheses, the Elevated Plus Maze demonstrated that the hormone-withdrawn hamsters display more anxiety-like behavior than both the hormone-sustained and control groups. This increase in anxious-like behavior following estrogen withdrawal contradicts evidence showing that females typically display less anxious behavior immediately after birth (Agrati et al., 2008; Agrati & Lonstein, 2016; Lonstein, 2005; Lonstein, 2007; Macbeth et al., 2008; Macbeth & Luine, 2010). However, when Galea, Wide, and Barr (2001) developed the ovarian-steroid-withdrawal regimen, which the present protocol was based on, they found increases in depressive-like behavior in hormone-withdrawn rats, suggesting that the regimen is a model for postpartum depression. While the group did not find increased anxiety-like behavior in the hormone-withdrawn rats (Galea et al., 2001) as was found in the present study, the hormone regimen may affect behavior differently in a hamster model. Thus, if our anxiogenic finding in hormone-withdrawn hamsters is replicated, it is possible that the hormone-simulated pregnancy used in the present study may be modeling postpartum anxiety in hamsters.

Another possible explanation of this unexpected finding is that the reduced activity of the hormone-withdrawn animals influenced the results. While poor video quality of the Elevated Plus Maze trials led to the inability to measure non-specific locomotor behavior of the different hormone treatment groups, altered activity levels were found between the hormone treatment groups as measured by the Open Field Test. Thus, it is possible that the diminished locomotor ability of the hormone-withdrawn hamsters skewed the amount of time spent in the arms of the
Elevated Plus Maze. In other words, the hormone-withdrawn hamsters may have spent less time in the open arms of the maze because they were not moving as much as the hormone-sustained and control hamsters. This would mean that the hormone-withdrawn animals are not necessarily displaying more anxious-like behavior, but rather exploring less overall than the hormone-sustained and control hamsters.

Moreover, it is important to use some caution when interpreting the present behavioral results. Given concerns regarding the reliability of findings from both the Open Field Test and the Elevated Plus Maze due to technical difficulties during video collection in the present study, we analyzed existing data from another experiment that we had collected using the same hormonal manipulation for hormone-withdrawn and hormone-sustained groups under better circumstances. These results showed no differences in anxiety-like behavior as measured by the Elevated Plus Maze between hormone-withdrawn and hormone-sustained hamsters. This suggests that the reliability of the behavioral results of the present study may be limited and should be reproduced in future studies.

Nonetheless, the behavioral findings in the present study, while not ideal, are still valuable. In considering the findings that hormone-withdrawn animals demonstrate an increase anxiety-like behavior in the present study but, in a subsequent cohort, there is no significant effect of hormone condition on behavior in the elevated plus, it is also possible that the behavioral effect following a hormone-simulated pregnancy is simply not a robust one. In other words, the anxiety-like behavior may appear following the hormone treatment in some cases and not others, as is evidenced by the increase in anxiety-like behavior in the hormone-withdrawn hamsters in the present study and not for the hormone-withdrawn hamsters in the unpublished data. In this case, another more robust measure of anxiety-like behavior for hamsters following a
hormone-simulated pregnancy should be found for future studies that hope to test the effect of neuroendocrine manipulations on postpartum anxiety-like behavior.

**Importance of temporality for the MeA, NAc, and BNST.** It is possible that no alterations in oxytocin receptor density based on the different hormonal conditions were found in the MeA, NAc, and BNST due to the day of the postpartum period that these regions were examined in the present study. In other words, it is possible that any changes in oxytocin receptor density occur closer to birth or later in the postpartum period than was measured in the present study, which could account for the lack of visible alterations between hormonal groups during day 5 of the postpartum. For instance, Meddle et al. (2007) investigated oxytocin receptor expression and activation perinatally in proestrus virgin, 21- and 22-day pregnant, parturient (90 minutes from the first pup birth), and postpartum rats (only 4 to 12 hours after birth). The researchers found an increase in oxytocin receptor mRNA expression in the MeA only during parturition as compared to pre-parturition (Meddle et al., 2007). Thus, while there is evidence that oxytocin receptor density is altered in the MeA immediately following birth, it appears that this effect may only be present in the minutes following parturition and not in the days that follow. Further, Terenzi and Ingram (2005) found that while lactating (i.e., days 5-8 postpartum) rats had a larger proportion of oxytocin-responsive neurons in the MeA compared with virgin or pregnant animals, the postpartum rats did not show alterations in the density of oxytocin binding sites. Thus, even while changes in oxytocin neurons are noted in the later postpartum period, oxytocin receptor density in particular may not be altered during that time.

In addition, while we hypothesized that the NAc may express an alteration in oxytocin receptor density following hormone withdrawal due to the region’s established involvement in maternal behavior (D’Cunha et al., 2011; Haim et al., 2014), it may be that this region also
experiences changes in oxytocin receptor density at times that were not measured in this study. For instance, D’Cunha et al. (2011) demonstrated how oxytocin receptors in the NAc shell play an important role in the consolidation of maternal memory. However, half of the rats were administered oxytocin antagonists either 15 minutes or 1 hour following a period of pup exposure on the first day postpartum, while the maternal behavioral tests were obtained between days 11 and 22 postpartum (D’Cunha et al., 2011). Thus, it appears that, again, the action of oxytocin may be most critical in the early days of the postpartum given that blocking oxytocin receptors during this time altered behavior. Nonetheless, it cannot be gathered from the study whether similar behavioral outcomes would be found following antagonistic action in later days of the postpartum (e.g., day 5). As such, the temporality of oxytocin receptor changes, if any, following hormone withdrawal is unknown.

Finally, we also believed that oxytocin receptor density in the BNST may be influenced by hormone withdrawal following a hormone-simulated pregnancy, though this hypothesis was also based on findings showing changes in the early postpartum period. For instance, Meddle et al. (2007) looked at the BNST and found its peak oxytocin receptor mRNA expression to be at parturition, but that this level returned to that of virgin rats within the first 12 hours following birth. Further, Insel (1900) showed that oxytocin receptor binding in the BNST was only increased on day 1 of the postpartum period for rats and not on day 6. Finally, while Broad et al. (1999) found an increase in oxytocin receptor density in the region following birth and into the early postpartum period in ewes, the latest postpartum measurement was only 30 minutes after birth. Thus, it is likely that any changes in oxytocin receptor density in the BNST return to normal by the fifth day postpartum, as was captured in the present study.
Overall, given the dramatic change in hormones during the perinatal period, it is likely that each day of the postpartum period experiences grand shifts in hormone-sensitive regions. As previous research has shown, alterations in some regions such as the MeA, NAc, and BNST may only occur in the early hours or days of the postpartum period. This was particularly true when examining changes in oxytocin within these regions. However, alterations in other regions may still be present in later days of the postpartum, such as the raphe nucleus as was found in the present study.

**Increase in oxytocin receptor density in the raphe nuclei following hormone withdrawal.** Autoradiography results demonstrated a significant increase in oxytocin receptor density expression in only one of the examined PVN efferents, the raphe nuclei. Specifically, oxytocin receptor density was significantly increased in the hormone-withdrawn group compared to the control group, but not to the hormone-sustained group. This increase in oxytocin receptor density suggests that the dorsal raphe nucleus becomes more sensitive to oxytocin following a hormone-simulated pregnancy and subsequent hormone withdrawal (i.e., a simulated postpartum period). Possible explanations for this finding will be subsequently discussed.

**Oxytocin-serotonin pathway.** One possible explanation of this finding is that oxytocin modulates the release of serotonin in the dorsal raphe nuclei following hormone withdrawal in order to modulate peripartum anxiety. The dorsal raphe nucleus contains the largest group of serotonin-producing neurons in the brain (Jacobs & Azmitia, 1992) and thus provides the majority of serotonergic input to structures in the limbic system involved in regulating emotional responses to stress and anxiety (Paul & Chen, 2017). For instance, it is well-established that increasing serotonin, otherwise known as 5-hydroxytryptamine (5-HT), levels via selective 5-HT reuptake inhibitors results in a potent decrease in levels of anxiety, an effect underlying many
anxiolytic drugs (Carr, Schechter, & Lucki, 2011). Importantly for the present study, approximately half of the serotonin neurons in the raphe nucleus have oxytocin receptor sites in rats (Yoshida et al., 2009), suggesting that oxytocin plays a role in modulating serotonin release.

In fact, research has demonstrated that oxytocin infusion within the raphe nucleus not only modulates serotonin release but also results in behavioral alterations. Specifically, oxytocin infusion within the raphe nucleus enables serotonin release and also reduces anxiety-like behavior among male rats (Yoshida et al., 2009). Importantly, the anxiolytic actions of oxytocin are reduced when a serotonin receptor antagonist is introduced, supporting the idea that oxytocin reduces anxiety by facilitating serotonin release (Onaka et al., 2012; Yoshida et al., 2009).

However, one key caveat of this study is that this relationship was found within the median raphe nucleus, whereas the present study examined the dorsal raphe nucleus.

Another group of researchers looked in both the dorsal and median raphe nuclei to test whether oxytocin acts on neurons in these regions to influence serotonergically-mediated anxiety-like, aggressive and parental care behaviors in mice (Pagani et al., 2015). When oxytocin receptor expression was eliminated in the serotonergic raphe neurons, the male mice showed deficits in aggression in comparison to controls, but, unexpectedly, no alterations in anxiety-like behavior were found between the groups for either males or females. The researchers suggested that this may be due to methodological limitations in that the elimination of oxytocin receptors may have initiated compensatory mechanisms to adjust for the deletion. Because the mice were bred for generations without these oxytocin receptors, they may rely on other non-serotonergic neurons in the raphe nuclei that contain oxytocin receptors to exert their effect (Pagani et al., 2015). In addition, it is important to note that females were tested for anxiety-like behavior in the postpartum more than one week following birth (Pagani et al., 2015) when differences in
anxiety-like behavior may no longer be measurable. Thus, it is possible that methodological limitations or late postpartum testing prevented any anxiety-like behaviors from being witnessed during the postpartum.

Moreover, neuroplastic changes in the dorsal raphe of rats in the early postpartum period of rats further support this theory that increased oxytocin receptor density in the region modulates serotonin release in the postpartum. Holshbach and Lonstein (2016) found that raphe cells born in the early postpartum period are less likely to survive to the late postpartum period than cells born in late pregnancy that survive to reach the early postpartum period (Holshbach & Lonstein, 2016). This suggests that young neurons in the dorsal raphe nuclei are subject to greater change during the early postpartum period. Importantly, the changes observed in newborn cell survival across the different peripartum time points are also associated with simultaneous changes in the pathway for serotonin synthesis and metabolism. Specifically, early-postpartum rats have higher dorsal raphe nuclei levels of the serotonin precursor, 5-HTP, and serotonin metabolite, 5-HIAA, compared to the late-postpartum rats or virgin rats (Holshbach & Lonstein, 2016). This increase in serotonin starting product during the early postpartum could mean that there is a reserve for serotonin, which may be released in the future to sustain the necessary reduction in anxiety following estrogen withdrawal from pregnancy.

While studies in humans have not been nearly as thorough as in animal models, there is some evidence to support that oxytocin modulates serotonin release in human subjects, particularly during the peripartum period. First, researchers found that average 5-HT concentrations were higher and correlated with lower average anxiety scores in late (i.e., the third-fourth and sixth-seventh weeks postpartum), but not early (e.g., first week), postpartum than in the third trimester or non-pregnant controls (Sekiyama et al., 2013). This indicates that
the serotonin system plays an important role in the regulation of anxiety in healthy postpartum women. Further, based on the finding that oxytocin modulates the release of serotonin by activating receptors in the raphe of rats (Yoshida et al., 2009), another group researchers wanted to determine whether there is a link between oxytocin and serotonin receptors in human subjects (Marazziti et al., 2011). They found that the administration of fenfluramine, a serotonergic agonist, to healthy human subjects increases levels of plasma oxytocin and increases the binding potentials of two peripheral markers for serotonin, suggesting that oxytocin does indeed influence the serotonin system in humans. While these studies have not linked this finding to particular brain regions, it is likely that the raphe is involved based on the high number of serotonergic neurons in the region in humans (Hornung, 2003).

**Link between PVN and raphe nuclei.** Interestingly, serotonergic fibers originating from both the dorsal and medial raphe nuclei have been found to project toward neurons in the paraventricular and supraoptic nuclei of the hypothalamus (Sawchenko, Swanson, Steinbusch, & Verhofstad, 1983). Further, the distribution of serotonin-labeled fibers follows that of oxytocin-labeled cells in the paraventricular and supraoptic nuclei of the hypothalamus (Emiliano, Cruz, Pannoni, & Fudge, 2007). Serotonin is also able to modulate the release of oxytocin via either exogenous 5-HT or by 5-HT agonists infused centrally in the hypothalamus (Jørgensen, Riis, Knigge, Kjaer, & Warberg, 2003). Thus, it is possible that the oxytocin-serotonin pathway is cyclical in that increased oxytocin from the PVN modulates serotonin release in the dorsal raphe which then acts on oxytocin receptors in the PVN.

**Strengths and limitations.** The present study has several strengths that are important to consider. To the best of our knowledge, this is the first study to examine oxytocin receptor density in various PVN efferents following hormone-simulated pregnancy. Given previous
research finding an increase of oxytocin-producing neurons in the PVN, it is important to know where these neurons may be releasing oxytocin, as the linkage may be implicated in postpartum mood disorders. To be clear, the present study did not aim to establish a causal link between the increased oxytocin-producing neurons in the PVN and receptor changes in PVN efferents. Rather, we sought to further explore changes in oxytocin-receptor density in specific regions in the brain following hormone-simulated pregnancy to begin examining that hypothesis.

Another strength of the present study is the addition of a non-hormonal control group and multiple behavioral measures over varying days of the postpartum. Previous research using a hormone-simulated pregnancy model in hamsters had compared hormone-withdrawn animals to hormone-sustained ones (Amaral, 2017; Bodie, 2017; D’Antonio, 2017; Lee, 2017). While this is helpful when teasing apart the effect of estrogen withdrawal, it does not allow us to measure the effect of the rise in pregnancy hormones. Thus, the addition of an oil control group allows for a more complete picture of changes occurring during the peripartum period. Further, the use of two different measures of anxiety-like behavior allows for more robust behavioral assay. Given the breadth of symptoms associated with PPA in humans (APA, 2013; Pawluski et al., 2017), we would expect animal models of the disorder to experience a range of anxiety-like behaviors as well. Put another way, animal models may not express anxiety-like behavior on every established measure of such behavior since humans also vary in the symptoms of the disorder that they experience. The use of multiple behavioral assays allows researchers to examine a greater range of these anxiety-like symptoms. Additionally, conducting these behavioral tests over four consecutive days during the postpartum allows for the potential for time course analysis which can more precisely shed light on variations in behavior for each day of this hormonally variable time.
The use of a hormone-simulated pregnancy model in the present study has both strengths and limitations. First, by simulating pregnancy we were able to have strict control over the hormone levels of each of our subjects. Such control would not be achievable if the animals had been pregnant naturally, since individual variations exist in the hormone levels of estrogen and progesterone during this time. In addition, simulating pregnancy does not result in pups, so fewer animals are needed to be sacrificed in order to address the research question at hand. However, this strength is also a potential weakness or rather an alteration to the model. Because the females did not give birth to pups, the mothers were not able to interact with their offspring, which has been shown to influence oxytocin in the brain (Galbally, Lewis, IJzendoorn, & Permezel, 2011). What’s more, while altered oxytocin was found in the brain of hormone-withdrawn animals in the present study, given the inconsistencies in behavioral findings following the hormone-simulated pregnancy regimen, it is possible that maternal context matters more to the model than was initially thought. Specifically, it is possible that the hormone treatment alters oxytocin in the brain, which then primes the animal for specific behaviors only when certain conditions are met. In the case of a simulated pregnancy, one of these conditions may be when the animal is in the presence of maternally salient stimuli such as pups. In the absence of such a stimulus, the animal would not display the expected behavior even though the hormones have altered the brain in preparation for the animal to respond. Thus, it is possible that, without maternally salient stimuli, the hormone-simulated pregnancy as it was used in the present study is modeling a late pregnancy disruption as opposed to a typical healthy pregnancy and postpartum. In further support, the hormone regimen used in the study also only altered estrogen and progesterone throughout the simulated pregnancy, despite the fact that many hormones including cortisol, prolactin, and vasopressin are known to change throughout this
period (Hendrick et al., 1998). Therefore, the absence of maternally salient stimuli and of other fluctuating pregnancy hormones are potential limitations of the present study.

Finally, another limitation of the present study had to do with technical difficulties regarding the behavioral tracking software used to measure anxiety-like behavior. Unfortunately, poor video quality due to off-center camera positioning and dark shadows led to difficulties in consistently detecting the animal in both the Elevated Plus Maze and Open Field Test. In many cases, hamsters were only partially tracked throughout the video, deeming our data less reliable than we had hoped.

**Future directions.** Based on the results and limitations of this experiment, there are several possible routes for future research. In regards to behavioral measures, efforts should be made to reproduce the hormonal conditions and behavioral tests across the first several days of the postpartum to validate the present findings. Hopefully, the use of higher quality videos will result in greater confidence in the results of those measures. Further, additional measures examining maternal behavior may also be included to test whether the hormone-simulated pregnancy, as it was used in the present study, results in typical maternal behavior expression. These measures of maternal behavior could include tests for nest building behavior, pup retrieval, or kyphosis (a stereotyped spinal posture that rodents enact while lactating; Nichita, Şereş & Coman, 2010). This would help to clarify whether the hormone-simulated pregnancy is modeling a typical or abnormal pregnancy and postpartum in hamsters.

In regards to the neuroendocrinological finding of an increase in oxytocin receptor density in the dorsal raphe nuclei, future research should use pharmacology to target these receptors and measure resulting changes in behavior. If an oxytocin receptor agonist is used, the amount of oxytocin to the region would increase. Based on findings that increased oxytocin to
the region can facilitate anxiolytic behavior, we would expect an oxytocin receptor agonist to decrease anxiety-like behavior. What’s more, if an oxytocin receptor antagonist is used, the amount of oxytocin to the dorsal raphe nuclei would decrease. If involved in postpartum mood disorders, we would expect this to produce more depressive- and/or anxious-like behavioral changes.

Finally, future research could examine whether oxytocin is modulating serotonin release in the dorsal raphe nuclei during the postpartum. One way to explore this relationship would be to disrupt oxytocin-producing neurons in the PVN through an oxytocin receptor antagonist and measure serotonergic output in the dorsal raphe nucleus. If disruption of oxytocin in the PVN results in decreased serotonin in the dorsal raphe nuclei, it would suggest that oxytocin in the PVN does modulate the release of serotonin in the dorsal raphe nuclei. Measures of anxiety-like behavior could also be obtained to see whether disrupting this pathway results in changes in behavior.

**Experiment 2**

The second experiment preliminarily examined neurodegeneration in the PVN using Fluoro-Jade C to begin to uncover the underlying processes behind previous findings of more oxytocin-producing neurons in the PVN of hormone-withdrawn animals in comparison to hormone-sustained animals. It was hypothesized that the hormone-sustained animals would show higher numbers of FJC+ neurons than hormone-withdrawn animals. This would indicate that neurons were in the process dying in the PVN of hormone-sustained animals which could account for the lower number of oxytocin-producing neurons found in this group. Importantly, this hypothesis was based on the notion that it is more energetically favorable for neurons to die than to generate, and thus we would expect cell death among the sustained group as opposed to
cell generation among the withdrawn group. Contrary to our hypothesis, higher numbers of FJC+ neurons were visualized in the PVN of the hormone-withdrawn animals, suggesting that neurons in that region are in the process of dying following estrogen withdrawal. However, it should be noted that these results are preliminary and future analyses are needed to more precisely determine neurodegenerative occurrences during this period. Nonetheless, the present findings suggest that hormone withdrawal is followed by some neurodegeneration, and if future analyses were to confirm this, it would have several interesting implications.

**Temporality of oxytocin production.** One possible explanation for the unexpected finding is that the increase in oxytocin-producing neurons observed in the PVN of hormone-withdrawn animals in previous research is only temporary. In this case, the higher numbers of FJC+ neurons in the PVN of hormone-withdrawn animals could be the beginning of oxytocin cell death. In support of this, researchers have shown that while there are great elevations of oxytocin following childbirth in humans, plasma oxytocin levels return to their pre-partum levels about an hour following delivery (Nissen, Lilja, Widström, & Uvnás-Moberg, 1995). While plasma oxytocin levels are not necessarily indicative of central levels, it is possible that fluctuations in plasma levels of oxytocin interact with those in the brain (Bell, Erickson, & Carter, 2014). Thus, the increased neurodegeneration found in the PVN of hormone-withdrawn animals in the present study could result from declining oxytocin neurons in the region by day 5 of the postpartum.

**Other neuronal cell death to make room for oxytocin.** Another possible explanation for the finding is that neuronal cell death occurs in the PVN of hormone-withdrawn animals in order to make room for increased oxytocin-producing ones. This would be a type of neuronal pruning such that neurons in the PVN would degenerate in order to become an area more specialized for
oxytocin production. While this is merely conjecture, there is evidence of both neurogenesis (Gregg et al., 2007) and neurodegeneration (Hoekzema et al., 2017) during and following pregnancy, suggesting that some neurons may be dying in order to make room for new neurons. This would explain the findings of both increased cell death and increased oxytocin-producing neurons in the PVN following hormone withdrawal, and could make the brain region more specialized for maternal functions.

**Heightened state of plasticity.** Independent of oxytocin, it is also possible that neuronal cell death occurs following hormone withdrawal because the peripartum period is a time of great neuroplastic change. In fact, there appear to be both short- and long-term changes in the brain throughout this time. For example, brain size has been found to decrease in women during the peripartum period with pre-pregnancy brain size recovering in about six months following birth (Oatridge et al., 2002). More long-term changes include reductions in gray matter volume in specific regions of the brain, mainly in the cerebral cortex, that are still present during a two-year follow up (Hoekzema et al., 2017). As the researchers suggest, this reduction in gray matter could have resulted from changes in the number of neurons in those areas (Hoekzema et al., 2017), which could explain the increased in neurodegenerative cells among the hormone-withdrawn animals in the present study.

Importantly, this reduction, whether through increased neuronal death or otherwise, is not necessarily detrimental. In fact, researchers have suggested that the female brain may undergo further maturation or specialization of the neuronal networks during the peripartum period (Hoekzema et al., 2017). This would be adaptive, since motherhood brings many new demands such as recognizing the needs of her dependent offspring or establishing a mother-infant bond.
Therefore, instances of neuronal cell death, such as our finding in the PVN, following pregnancy could be a result of adaptive pruning.

**Strengths, limitations, and future directions.** To the best of our knowledge, this is the first study to preliminarily examine neurodegeneration in the PVN following hormone-simulated pregnancy. Other studies have noted selective neuron loss in the PVN (Manaye et al., 2005), but not in the postpartum period. While our findings do suggest that neurons are dying in the PVN following hormone withdrawal, our study was preliminary and no statistical analyses were conducted. Thus, further quantitative analysis is needed to reproduce and extend these results. If this finding is statistically confirmed, future research could work to double-label tissue from both hormone-withdrawn and hormone-sustained animals with oxytocin and FJC. This would shed light on whether oxytocin neurons in the PVN are dying or whether other neurons are responsible for the increased neurodegeneration.

**Concluding Remarks**

The purpose of the present study was to gain a better understanding of the peripartum period in order to identify mechanisms that may be involved in postpartum mood disorders. Specifically, we wanted to replicate and expand upon previous research showing that the dramatic rise and fall of ovarian hormones results in an increase in oxytocin-producing neurons in the PVN and decreased anxiety-like behavior. The first experiment sought to examine regions in the brain where the increased oxytocin may be firing. We found an increase in oxytocin receptor density in the dorsal raphe nucleus, a PVN efferent and region known to release serotonin and thus facilitate anxiolytic effects. Contrary to our hypothesis, behavioral results demonstrated that hormone-withdrawn animals exhibit decreased non-specific locomotor behavior as measured by the Open Field Test and increased anxiety-like behavior as measured by
the Elevated Plus Maze. However, unpublished data addressing technical difficulties experienced while analyzing the behavioral data in the present study suggest that the reliability of the present results are limited and should be interpreted with caution. The second experiment sought to explore whether neurodegeneration was responsible for the decreased oxytocin-producing neurons found in hormone-sustained animals. Unexpectedly, we found evidence of cell death in hormone-withdrawn animals, suggesting that some neuroplasticity may be taking place in the region. Overall, these two experiments add to our understanding of the brain and behavior following hormone-simulated pregnancy in hamsters, which may inform our understanding the postpartum period in humans.

Nonetheless, it is important to emphasize that knowledge of the neurobiological changes during the postpartum period and, consequently, postpartum mood disorders is by no means extensive. Much research is still needed on the postpartum period before we can begin to understand how this system is disrupted so that new therapies to treat peripartum mood disorders can be proposed. Only then will the adverse consequences of postpartum mood disorders for both mothers and their offspring be prevented. Given the observed changes in oxytocin-producing neurons in the PVN, oxytocin receptor density in the raphe nuclei, and anxiety-like behavior following hormone-simulated pregnancy, many routes for further research are available. We believe that disrupting oxytocin signaling pathways, possibly between the PVN and dorsal raphe nuclei, that occur in healthy, non-depressed hamsters may alter anxiety-like symptoms in hormone-withdrawn animals, thus demonstrating a possible mechanism of PPA. If this is found to be the case, oxytocin may meet the demand for a new therapeutic target to treat the prevalent and detrimental postpartum mood disorder.
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Table 1

One-Way Between-Subjects Analysis of Variance of Time Spent in Periphery, Time Spent in Center, and the Difference Score in the Open Field Test

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
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<td>217.90</td>
<td>.35</td>
<td>.7121</td>
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<td>13130.95</td>
<td>625.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>13566.76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Time in Center</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between</td>
<td>2</td>
<td>359.02</td>
<td>179.51</td>
<td>.27</td>
<td>.76</td>
</tr>
<tr>
<td>Within</td>
<td>21</td>
<td>13846.39</td>
<td>659.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>14205.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Difference Score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between</td>
<td>2</td>
<td>1478.69</td>
<td>739.35</td>
<td>.29</td>
<td>.75</td>
</tr>
<tr>
<td>Within</td>
<td>21</td>
<td>52855.70</td>
<td>2516.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>54334.39</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* Time in periphery, time in center, and difference score did not differ between hormone conditions. Difference score was time spent in center of the arena subtracted from time spent in the periphery. Time measured in seconds (s).
Table 2

*Tukey’s HSD Post-Hoc Multiple Comparison Analyses of Average Velocity Between Groups 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>Hormone-Sustained</td>
<td>-1.33</td>
</tr>
<tr>
<td></td>
<td>Hormone-Withdrawn</td>
<td>1.38</td>
</tr>
<tr>
<td>Hormone-Sustained</td>
<td>Control</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>Hormone-Withdrawn</td>
<td>2.71*</td>
</tr>
<tr>
<td>Hormone-Withdrawn</td>
<td>Control</td>
<td>-1.37</td>
</tr>
<tr>
<td></td>
<td>Hormone-Sustained</td>
<td>-2.71*</td>
</tr>
</tbody>
</table>

*Note.* Hormone-withdrawn hamsters have significantly slower mean velocities than hormone-sustained hamsters in the Open Field Test. Velocity measured in centimeters per second (cm/s).  

*p < .05*
Table 3

*Tukey’s HSD Post-Hoc Multiple Comparison Analyses of Total Distance Moved Between Groups 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>Hormone-Sustained</td>
<td>-762.96</td>
</tr>
<tr>
<td></td>
<td>Hormone-Withdrawn</td>
<td>797.08</td>
</tr>
<tr>
<td>Hormone-Sustained</td>
<td>Control</td>
<td>762.96</td>
</tr>
<tr>
<td></td>
<td>Hormone-Withdrawn</td>
<td>1560.05*</td>
</tr>
<tr>
<td>Hormone-Withdrawn</td>
<td>Control</td>
<td>-797.08</td>
</tr>
<tr>
<td></td>
<td>Hormone-Sustained</td>
<td>-1560.05*</td>
</tr>
</tbody>
</table>

*Note.* Hormone-withdrawn hamsters travel significantly less than hormone-sustained hamsters in the Open Field Test. Distanced moved measured in centimeters (cm). *p < .05
Table 4

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. Total time spent in the open arms of the Elevated Plus Maze significantly differs between hormone conditions. Time measured in seconds (s). *p < .05
Table 5

*Tukey’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></td>
<td></td>
</tr>
<tr>
<td>Hormone-Sustained</td>
<td>-10.10</td>
<td>.84</td>
</tr>
<tr>
<td>Hormone-Withdrawn</td>
<td>48.82*</td>
<td>.03*</td>
</tr>
<tr>
<td>Hormone-Sustained</td>
<td>10.10</td>
<td>.84</td>
</tr>
<tr>
<td>Hormone-Withdrawn</td>
<td>58.93*</td>
<td>.01*</td>
</tr>
<tr>
<td>Hormone-Withdrawn</td>
<td>-48.82*</td>
<td>.03*</td>
</tr>
<tr>
<td>Hormone-Sustained</td>
<td>-58.93*</td>
<td>.01*</td>
</tr>
</tbody>
</table>

*Note.* Hormone-withdrawn hamsters spend significantly less time in the open arms of the Elevated Plus Maze than hormone-sustained and control hamsters. Time measured in seconds (s). *p < .05
Table 6

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. The difference in total time spent in the open arms subtracted from the total time spent in the closed arms of the Elevated Plus Maze significantly differs between hormone conditions. Time measured in seconds (s). *p < .05
Table 7

*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>Hormone-Sustained</td>
<td>8.79</td>
</tr>
<tr>
<td>Control</td>
<td>Hormone-Withdrawn</td>
<td>-77.31</td>
</tr>
<tr>
<td>Hormone-Sustained</td>
<td>Control</td>
<td>-8.79</td>
</tr>
<tr>
<td>Hormone-Sustained</td>
<td>Hormone-Withdrawn</td>
<td>-86.11</td>
</tr>
<tr>
<td>Hormone-Withdrawn</td>
<td>Control</td>
<td>77.32</td>
</tr>
<tr>
<td>Hormone-Withdrawn</td>
<td>Hormone-Sustained</td>
<td>86.11</td>
</tr>
</tbody>
</table>

*Note.* No significant differences between specific hormone treatment groups were found for the difference in total time spent in the open arms subtracted from the total time spent in the closed arms of the Elevated Plus Maze. Hormone-withdrawn hamsters trending towards having a significantly greater difference between time spent in the open arms and closed arms than hormone-sustained and control hamsters. Time measured in seconds (s). †p < .1
Table 8

*One-Way Between-Subjects Analysis of Variance of Total Time Spent in the Closed 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>4140.66</td>
<td>2070.33</td>
<td>1.46</td>
<td>.26</td>
</tr>
<tr>
<td>Within Groups</td>
<td>21</td>
<td>29852.18</td>
<td>1421.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>33992.84</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* Total time spent in the closed arms of the Elevated Plus Maze did not differ between hormone conditions. Time measured in seconds (s).
Table 9

One-Way Between-Subjects Analysis of Variance of Oxytocin Receptor Density within the Medial Amygdala

<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>105410.01</td>
<td>52705</td>
<td>1.87</td>
<td>.179</td>
</tr>
<tr>
<td>Within Groups</td>
<td>21</td>
<td>28147.04</td>
<td>28147.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>696497.78</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* Oxytocin receptor density in the MeA did not differ between hormone conditions. Mean oxytocin receptor densities measured in disintegrating units per min per mg tissue (dpm/mg).
### Table 10

*Mean Oxytocin Receptor Density in the Medial Amygdala Between Hormone Treatment Groups*

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>M</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>589.25</td>
<td>261.52</td>
</tr>
<tr>
<td>Hormone-Sustained</td>
<td>8</td>
<td>521.14</td>
<td>56.03</td>
</tr>
<tr>
<td>Hormone-Withdrawn</td>
<td>8</td>
<td>427.58</td>
<td>113.62</td>
</tr>
</tbody>
</table>

*Note.* Mean oxytocin receptor densities measured in disintegrating units per min per mg tissue (dpm/mg).
Table 11

*One-Way Between-Subjects Analysis of Variance of Oxytocin Receptor Density within the Nucleus Accumbens Shell*

<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>109209.90</td>
<td>54604.95</td>
<td>1.74</td>
<td>.20</td>
</tr>
<tr>
<td>Within Groups</td>
<td>21</td>
<td>660641.79</td>
<td>31459.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>769851.69</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* Oxytocin receptor density in the NAc shell did not differ between hormone conditions.

Mean oxytocin receptor densities measured in disintegrating units per min per mg tissue (dpm/mg).
Table 12

One-Way Between-Subjects Analysis of Variance of Oxytocin Receptor Density within the Nucleus Accumbens Core

<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>71054.85</td>
<td>35527.42</td>
<td>1.13</td>
<td>.34</td>
</tr>
<tr>
<td>Within Groups</td>
<td>21</td>
<td>660641.79</td>
<td>31353.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>769851.69</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. Oxytocin receptor density in the NAc core did not differ between hormone conditions.

Mean oxytocin receptor densities measured in disintegrating units per min per mg tissue (dpm/mg).
Table 13

Mean Oxytocin Receptor Density within the Nucleus Accumbens Shell Between Hormone Treatment Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>M</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>329.20</td>
<td>272.75</td>
</tr>
<tr>
<td>Hormone-Sustained</td>
<td>8</td>
<td>173.73</td>
<td>71.53</td>
</tr>
<tr>
<td>Hormone-Withdrawn</td>
<td>8</td>
<td>202.98</td>
<td>121.94</td>
</tr>
</tbody>
</table>

*Note.* Mean oxytocin receptor densities measured in disintegrating units per min per mg tissue (dpm/mg).
Table 14

*Mean Oxytocin Receptor Density within the Nucleus Accumbens Core Between Hormone Treatment Groups*

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>M</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>343.64</td>
<td>265.31</td>
</tr>
<tr>
<td>Hormone-Sustained</td>
<td>8</td>
<td>218.77</td>
<td>44.61</td>
</tr>
<tr>
<td>Hormone-Withdrawn</td>
<td>8</td>
<td>240.86</td>
<td>147.24</td>
</tr>
</tbody>
</table>

*Note.* Mean oxytocin receptor densities measured in disintegrating units per min per mg tissue (dpm/mg).
Table 15

One-Way Between-Subjects Analysis of Variance of Oxytocin Receptor Density within the Bed Nucleus of the Stria Terminalis

<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>50191.44</td>
<td>2549.72</td>
<td>1.75</td>
<td>.20</td>
</tr>
<tr>
<td>Within Groups</td>
<td>21</td>
<td>306249.71</td>
<td>14583.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>357169.16</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. Oxytocin receptor density in the BNST did not differ between hormone conditions. Mean oxytocin receptor densities measured in disintegrating units per min per mg tissue (dpm/mg).
Table 16

*Mean Oxytocin Receptor Density within the Bed Nucleus of the Stria Terminalis Between Hormone Treatment Groups*

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>M</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>279.73</td>
<td>98.50</td>
</tr>
<tr>
<td>Hormone-Sustained</td>
<td>8</td>
<td>166.96</td>
<td>82.28</td>
</tr>
<tr>
<td>Hormone-Withdrawn</td>
<td>8</td>
<td>220.20</td>
<td>165.16</td>
</tr>
</tbody>
</table>

*Note.* Mean oxytocin receptor densities measured in disintegrating units per min per mg tissue (dpm/mg).
Table 17

One-Way Between-Subjects Analysis of Variance of Oxytocin Receptor Density within the Dorsal Raphe Nucleus

<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>67005.49</td>
<td>33501.74</td>
<td>4.35</td>
<td>.03*</td>
</tr>
<tr>
<td>Within Groups</td>
<td>21</td>
<td>161686.80</td>
<td>7699.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>228692.29</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note. Oxytocin receptor density in the raphe nuclei significantly differs between hormone conditions. Mean oxytocin receptor densities measured in disintegrating units per min per mg tissue (dpm/mg). *p < .05*
Table 18

*Tukey’s HSD Post-Hoc Multiple Comparison Analyses of Oxytocin Receptor Density within the Dorsal Raphe Nucleus 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>Hormone-Sustained</td>
<td>-16.76</td>
</tr>
<tr>
<td></td>
<td>Hormone-Withdrawn</td>
<td>-119.52*</td>
</tr>
<tr>
<td>Hormone-Sustained</td>
<td>Control</td>
<td>16.76</td>
</tr>
<tr>
<td></td>
<td>Hormone-Withdrawn</td>
<td>-102.76</td>
</tr>
<tr>
<td>Hormone-Withdrawn</td>
<td>Control</td>
<td>119.52*</td>
</tr>
<tr>
<td></td>
<td>Hormone-Sustained</td>
<td>102.76</td>
</tr>
</tbody>
</table>

*Note.* Hormone-withdrawn hamsters have significantly greater oxytocin receptor density than control hamsters. Oxytocin receptor densities measured in disintegrating units per min per mg tissue (dpm/mg). *p < .05
### Mean Oxytocin Receptor Density within the Dorsal Raphe Nucleus Between Hormone Treatment Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>M</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>156.25</td>
<td>87.35</td>
</tr>
<tr>
<td>Hormone-Sustained</td>
<td>8</td>
<td>173.01</td>
<td>65.73</td>
</tr>
<tr>
<td>Hormone-Withdrawn</td>
<td>8</td>
<td>275.77</td>
<td>105.59</td>
</tr>
</tbody>
</table>

*Note.* Mean oxytocin receptor densities measured in disintegrating units per min per mg tissue (dpm/mg).
Figure 1. Graph depicting mean differences in velocity of animals during the Open Field Test between hormonal conditions. Hormone-withdrawn hamsters have significantly slower velocities than hormone-sustained hamsters. Velocity measured in centimeters per second (cm/s). Error bars represent standard deviation. *p < .05
Figure 2. Graph depicting mean differences in total distance moved during the Open Field Test between hormonal conditions. Hormone-withdrawn hamsters move significantly less than hormone-sustained hamsters. Distance moved measured in centimeters (cm). Error bars represent standard deviation. *p < .05
Figure 3. Graph depicting mean differences in time spent in the open arms (solid) and time spent in the closed arms (dotted) on the Elevated Plus Maze between hormonal conditions. Hormone-withdrawn hamsters spend significantly less time on the open arms than hormone-sustained and control hamsters. Time measured in seconds (s). Error bars represent standard deviation. *p < 0.05
Figure 4. Graph depicting the difference in time spent in the open arms subtracted from time spent in the closed arms of the Elevated Plus Maze between hormonal conditions. Hormone-withdrawn hamsters trending towards having a significantly greater difference between time spent in the open arms and closed arms than hormone-sustained and control hamsters. Time measured in seconds (s). Error bars represent standard deviation. †p < .1
Figure 5. (A) Representative image of oxytocin receptor density within the medial amygdala (depicted by the box) in hormone-withdrawn animals. Oxytocin receptor density in the MeA did not differ between hormone conditions. (B) Analysis domains for optical density of the MeA (depicted by the box) were taken from a stereotaxic atlas of the hamster brain (Morin & Wood, 2001).
Figure 6. (A) Representative image of oxytocin receptor density within the nucleus accumbens core (left box) and shell (right box) in hormone-withdrawn animals. Oxytocin receptor density in the NAc did not differ between hormone conditions. (B) Analysis domains for optical density of the NAc core (depicted by the left box) and the NAc shell (depicted by the right box) were taken from a stereotaxic atlas of the hamster brain (Morin & Wood, 2001).
Figure 7. (A) Representative image of oxytocin receptor density within the bed nucleus of the stria terminalis (depicted by the box) in hormone-withdrawn animals. Oxytocin receptor density in the BNST did not differ between hormone conditions. (B) Analysis domains for optical density of the BNST (depicted by the box) were taken from a stereotaxic atlas of the hamster brain (Morin & Wood, 2001).
Figure 8. Graph depicting mean differences in oxytocin receptor density in the dorsal raphe nuclei between hormonal conditions. Hormone-withdrawn hamsters have significantly greater oxytocin receptor density than control hamsters. Oxytocin receptor densities measured in disintegrating units per min per mg tissue (dpm/mg). Error bars represent standard deviation. *p < .05
Figure 9. Representative images of oxytocin receptor density within the dorsal raphe nucleus (depicted by the box) in control (A), hormone-sustained (B), and hormone-withdrawn (C) animals. Significantly greater oxytocin receptor density was found within the raphe of hormone-withdrawn animals as compared to control animals. (D) Analysis domains for optical density of the raphe nucleus (depicted by the box) were taken from a stereotaxic atlas of the hamster brain (Morin & Wood, 2001).
Figure 10. Representative images of FJC+ staining (FJC+ neurons indicated by red arrow) in the PVN of hormone-withdrawn (A) and hormone-sustained (B) animals. Hormone-withdrawn animals show more images categorized as having high numbers of FJC+ neurons than the hormone-sustained group. (C) Analysis domains for FJC+ imaging of the PVN (depicted by the box) were taken from a stereotaxic atlas of the hamster brain (Morin & Wood, 2001).