Postpartum oxytocin receptor plasticity in Syrian hamsters:

Implications for the treatment of peripartum mood disorders

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Abstract

Peripartum mood disorders, if left untreated, result in negative outcomes for both the mother and child. Despite these severe consequences, the neurobiology of peripartum mood disorders is not well understood. The present study aims to build upon previous research investigating the role of oxytocin in neuroplastic and behavioral changes during the peripartum period. Past work found a significant increase in oxytocin-immunoreactive neurons in the PVN as a result of hormone withdrawal in a hormone-simulated pregnancy model conducted in Syrian hamsters. Using the same model, we studied post-synaptic plasticity: specifically, we assessed which PVN efferents, if any, experienced changes in oxytocin receptor expression using receptor autoradiography. The regions of interest in this study were medial amygdala (MA), nucleus accumbens (NA), bed nucleus of the stria terminalis (BNST), and the raphe nuclei as each of these four areas are heavily involved in the production of maternal behavior. There was a significant increase in OTR density in the dorsal raphe in the hormone withdrawn group as compared to oil control. Changes in OTR density in the dorsal RN, which regulates serotonergic activity and anxiety- and depression-related behavior in humans and rodents, may play a role in the development or sustainment of peripartum mood disorders.

*Keywords:* peripartum, mood, oxytocin, autoradiography, postpartum depression
Having a baby is commonly regarded as one of the most joyous and meaningful events in an individual’s life. Images of mothers in various states of euphoria are widely publicized to the point that the “glow” of motherhood has become a cultural expectation. It may come as a surprise to hear that upwards of 20 percent of women experience mild to severe mood disruptions during the peripartum period (Pawluski, Lonstein, & Fleming, 2017). In fact, of the four million women who are or will become pregnant in 2018, around six hundred thousand will be diagnosed with postpartum depression (PPD), a disorder characterized by depressive symptoms within four to six weeks of birth (World Health Organization, 1992). These numbers are probably underestimated as most mothers are reluctant to admit their depressive or anxious state during a time of expected happiness (Marcus, 2009). This is a serious problem since postpartum mood disorders often result in negative outcomes for both the mother and child. For the child, this can include emotional, cognitive, and behavioral consequences (Beck, 1998). In mothers, mood disorders can lead to increased suicidality and even, in extreme cases, infanticide (Chaudron, 2003; Spinelli, 2004). Despite these severe consequences, the neurobiology of peripartum mood disorders is not well understood. Further research into the neurobiological changes associated with typical human pregnancy and postpartum mood is vital as it will increase knowledge, diagnosis, and successful treatment of peripartum mood disorders in the general population.

The DSM-V defines postpartum depression as an episode of major depressive disorder (MDD) with a peripartum-onset specifier. A woman must meet criteria for MDD during pregnancy or within four weeks of delivery to be diagnosed with PPD (American Psychiatric Association, 2013). Importantly, the DSM-V definition of PPD includes any heightened levels of
anxiety. Similarly, the ICD-10 defines PPD as depression in association with the peripartum period with a strict cut-off at 6 weeks postpartum (World Health Organization, 1992). Symptoms of PPD include sleep disorders, mood swings, changes in appetite, fear of injury, sadness and crying, a sense of doubt, difficulty concentrating, lack of interest in daily activities, and thoughts of death and suicide (Chaudron, 2003; Ghaedrahmati, Kazemi, Kheirabadi, Ebrahimi, & Bahrami, 2017; Pawluski et al., 2017). This is in addition to infant-related symptoms such as a weak attachment to the baby and a fear of harming the baby (Ghaedrahmati et al., 2017). Many common depressive symptoms (sleep, energy, and appetite change) can be misinterpreted as normative experiences of pregnancy and postpartum, which is a large contributor to the number of undiagnosed cases of PPD. Notably, neither the DSM-5 nor the ICD-10 contains a specific definition of postpartum anxiety (PPA), a lesser known but still prevalent peripartum mood disorder. PPA is characterized by heightened levels of anxiety with cognitive and behavioral patterns similar to generalized anxiety disorder and obsessive-compulsive disorder (Pawluski et al., 2017). Unfortunately, all postpartum mood disorders are generally diagnosed as postpartum depression regardless of whether they are rooted in anxiety- or depression-related behavior. Therefore, further discussion of PPD in this paper and throughout the literature broadly encapsulates peripartum mood disorders, which includes any or all anxiety- and depression-related behaviors that arise during the peripartum period.

This terminology is important as we turn to recent studies on underlying risk factors of peripartum mood disorders. Risk factors of PPD are expressed at the social, psychological, biological, and obstetric levels. Social factors include relationship with spouse and general social support. Mothers who reported a poor marital relationship on average displayed more depressive symptoms than those mothers who felt their spouse was adequately supporting them (Norhayati,
Hazlina, Asrenee, & Emilin, 2015; Serhan, Ege, Ayranví, & Kosgeroglu, 2013). Similar results were reported for mothers who did not feel they were receiving suitable social support from their surrounding family and friends (Serhan et al., 2013). At the psychological level, one of the greatest risk factors for peripartum mood disorders is a previous history of depression and/or anxiety. Any expression of anxious or depressive behavior, ranging from third-trimester sadness to a diagnosable disorder, significantly increases the likelihood of a PPD diagnosis (Ghaedrahmati et al., 2017; Hartmann, Mendoza-Sassi, & Cesar, 2017; Norhayati et al., 2015). This notably includes any previous instances of PPD during a past pregnancy (M. Bloch et al., 2000). Additional psychological factors that increase the risk of peripartum mood disorders include a negative attitude towards pregnancy (Ghaedrahmati et al., 2017; Norhayati et al., 2015), anxiety about motherhood (Serhan et al., 2013), history of sexual abuse, and low self-esteem (Ghaedrahmati et al., 2017).

There are several biological risk factors that may contribute to these psychological influences. Though these factors are not well understood, many researchers agree that a younger maternal age increases the likelihood of developing PPD (Ghaedrahmati et al., 2017; Hartmann et al., 2017). PPD may also be linked to heritable genetic factors (Landsman, Aidelman, Smith, Boyko, & Greenberger, 2017). Finally, obstetric factors play a large role in the mother’s mood during the peripartum period. In general, unexpected events during birth that cause the mother to become stressed may increase the risk of peripartum mood disorder. Risky pregnancy procedures such as cesarean sections, prenatal deliveries, and other complications during delivery heighten the chance of depressive symptoms in the mother in the days following birth. Negative affect may also occur if the birthing process differs from the mother’s expectations, such as the mother needing to give birth at the hospital when she wanted a home birth (Ghaedrahmati et al., 2017).
Together, these social, psychological, biological, and obstetric risk factors in a future mother’s life may affect the chance of developing serious peripartum mood disruption. It is by studying these risk factors that we may more clearly understand the neurobiological underpinnings of peripartum mood disorders.

Limited research into peripartum mood disorders has resulted in several different pharmacological treatments for PPD. The first class of treatments is antidepressants. Selective serotonin reuptake inhibitors (SSRIs) are traditionally prescribed to treat PPD due to their effectiveness in alleviating the symptoms of MDD. Kim et al. (2014) report that the administration of SSRIs improves mood and increases remission of depressive symptoms in mothers with PPD. However, there are serious concerns about the use of SSRIs and other antidepressants in women who are pregnant or breastfeeding. SSRIs are able to cross the placenta during gestation, which may influence fetal development. These drugs are also found in significant quantities in the breastmilk of women who were prescribed antidepressants. On average, around 30 percent of SSRI-exposed infants are impacted by levels of monoamine inhibitors. Short term effects include respiratory distress, temperature instability, feeding difficulties, jitteriness, irritability, and sleep problems, but these symptoms tend to disappear up to two weeks after birth (Olivier et al., 2013). Long term effects are much more serious: Wisner et al. (2009) found low Apgar scores in SSRI-exposed infants. Nielsen, Ljungdalh, Nielsen, Nørgård, and Qvist (2017) reported a correlation between SSRI exposure and congenital malformations, specifically Hirschsprung’s disease, a deformity of the gastrointestinal tract. SSRIs are therefore insufficient in treating postpartum depression as the risks of harming the infant often outweigh the benefits from the point of view of the mother.
Apprehensions about the impact of antidepressants on the development of the infant has led to an increase in alternative pharmacological treatments for peripartum mood disorders, including hormone therapy. The goal of hormone therapy is to target the flux of gonadal hormones inherent in pregnancy as this flux may be related to increased peripartum mood disruption. Estradiol therapy, a type of hormone therapy, is shown to be effective in treating symptoms of depression: women with PPD who were treated with estrogen skin patches showed greater and more rapid improvements in their depressive symptoms when compared to a placebo group (Gregoire, Kumar, Everitt, Henderson, & Studd, 1996). In a separate study that focused on major depression, researchers found that 19 out of 23 severely depressed women reached remission within two weeks of treatment with sublingual estradiol (Ahokas, Kaukoranta, Wahlbeck, & Aito, 2001). Though hormone therapy does seem to be effective in alleviating depressive symptoms, there is still concern about treating women in peripartum, who need to maintain healthy levels of gonadal hormones to promote gestation and subsequent lactation, with external doses of estradiol. Although hormone therapy seems to be an option for those mothers who do not wish to take antidepressants during the peripartum period, it is not able to target peripartum mood disorder symptoms without risking the health of the infant.

With these concerns in mind, many individuals with peripartum mood disorders have turned to nonpharmacological alternatives to alleviate their symptoms. Peripartum activities such as childbirth classes and epidural labor analgesia are correlated with lower risks of PPD (Ding, Wang, Qu, Chen, & Zhu, 2014). This is most likely due to the anxiolytic effects of reducing uncertainty and pain at the moment of delivery. Other therapies aimed at general wellbeing – psychotherapy, parenting classes, dietary supplements, exercise – are also effective in treating
PPD (Pawluski et al., 2017). Again, these activities have anxiolytic properties that may counteract the risk factors of heightened stress during peripartum. An additional factor that may contribute to alleviating PPD symptoms is physical contact with one’s infant. Tactile input from one’s child can affect almost all maternal behaviors, which may include anxiety- and depression-related behaviors (Pawluski et al., 2017). Related to this fact, mothers suffering from peripartum mood disorders touch their infants less and do less breastfeeding. Breastfeeding is associated with a reduction in PPD symptoms (Figueiredo, Canário, & Field, 2014), perhaps due to the increased level of contact between mother and child. From this, one could infer a potential nonpharmacological treatment for PPD may be to encourage breastfeeding. However, breastfeeding is not a viable option for all mothers. In fact, difficulties with breastfeeding in early postpartum can lead to increased risk for peripartum mood disorders (Tait, 2000; Ystrom, 2012). Therefore, it may be more beneficial to encourage other general tactile interactions in new mothers.

In sum, peripartum mood disorders, including PPD and PPA, are a potentially life-threatening class of disorders that are underdiagnosed in the US population. Their anxiety- and depression-related symptoms are detrimental not only to the health of the mother, but can result in developmental setbacks in the child. Current treatments are informed by the fairly limited information on risk factors for postpartum depression and are somewhat effective, though their mechanisms of action place the child’s development at risk. Research on novel pharmacological targets is needed to more effectively treat peripartum mood disorders. With this in mind, we now discuss what is known about the neurobiology of peripartum mood disorders with the intent to identify potential therapeutic targets.
The Neurobiology of Peripartum Mood Disorders

For an occurrence as common as pregnancy, there is shockingly little information on normal peripartum neurobiology. The research that exists has been conducted in both human and animal models with an emphasis on maternal behavior and mother-child interactions. In the case of peripartum mood disorders, studies have focused on using previous work regarding MDD to inform their investigation of PPD as the symptoms of MDD and PPD are quite similar. We now compare and contrast peripartum mood disorder research in human and animal models and highlight several potential causal theories of PPD that have resulted from this work.

Theories of peripartum mood disorders in humans. Pregnancy is characterized by dramatic changes throughout the human body, including the brain. Over the course of the peripartum period, levels of gonadal steroid hormones, serotonin, and oxytocin fluctuate wildly in response to the needs of the mother and fetus (Stamatakis et al., 2014). As a result, a number of behavioral changes are observed in the mother, including a reduced level of anxiety (Macbeth, Gautreaux, & Luine, 2008). However, dysregulation of these symptoms may result in abnormal behavior. As MDD, a disorder that shares many physical characteristics with PPD, is known to be the result of some combination of changes in neurotransmitter, hormonal, genetic, and psychosocial factors, it therefore makes sense to investigate these factors in PPD as well (Kalia, 2005). As a result, several hypotheses were developed in the attempt to draw causal lines between these numerous physiological factors and peripartum mood disorders in humans.

Monoamine deficiency hypothesis. Monoamines are arguably the most well-known neurochemical factor in depressive behavior in humans. However, very little research has been done on typical levels of monoamines during human pregnancy. Serotonin is thought to regulate neurodevelopmental processes in the fetus via the placenta during gestations (Kliman et al.,
2018). Furthermore, norepinephrine and serotonin decrease during late pregnancy and rise steadily in early postpartum. This is coupled with significant changes in dopamine throughout pregnancy: dopamine sharply increases before birth, then decreases in postpartum (Jonathan & Munsick, 1980). These changes in monoamines have been connected to several cognitive and behavioral changes, including variations in the mother’s social cognition and attachment style (Hoekzema et al., 2017). Taking into account the role of monoamines in both depressive symptoms and typical pregnancy, it would be reasonable to conclude that monoamine neurotransmitter changes could be involved in the development of peripartum mood disorders.

One of the most important targets for mood disorder research during any period is serotonin. Serotonin levels are regulated in the brain at the synaptic level using serotonin transporter 5-HTT. The short polymorphism in this transporter is associated with lower levels of synaptic serotonin and higher risk for developing depressive disorders later in life (Yim, Tanner Stapleton, Guardino, Hahn-Holbrook, & Dunkel Schetter, 2015). In one study of 274 women who were at risk for PPD, carriers of the short 5-HTT polymorphism were more likely to develop PPD (Binder et al., 2010). This is supported by research that found serotonin levels are significantly lower in women with postpartum depression (Yildiz et al., 2017). In contrast, a similar study by Pinheiro et al. (2013) found that it was the long 5-HTT allele that was associated with heightened PPD risk in conjunction with stress during pregnancy. It is clear that serotonin and its related transporters play at least a correlational role in the development of postpartum mood disorders.

Similarly, dopamine is implicated in the development of depressive symptoms in the peripartum period in humans. Dopamine levels increase in the nucleus accumbens as a result of maternal behavior in typical mothers (Numan, 2007). Levels of dopamine are found to be even
higher in mothers with PPD, potentially leading to lower levels of D2/3 receptors in the nucleus accumbens in an attempt to maintain homeostasis (Luo, Zheng, Zhou, & Pi, 2007; Moses-Kolko et al., 2012). Though the relationship between dopamine and peripartum mood disorders is not completely clear, dopamine seems to be related to peripartum mood disorders via its regulation in the nucleus accumbens.

**Ovarian-steroid-withdrawal hypothesis.** A second theory of the development of peripartum mood disorders in humans focuses on ovarian hormones. During a typical pregnancy, ovarian hormones such as estrogen and progesterone rise before birth, then drop dramatically with the expulsion of the placenta. This subsequent drop in ovarian hormones is implicated in postpartum mood disruption (Bloch, Daly, & Rubinow, 2003; Yim et al., 2015). Ovarian hormones, which are regulated via the hypothalamic-pituitary-gonadal (HPG) axis, are tied generally to mood in humans. For example, there is an increased likelihood of depressive symptoms during the transition to menopause, when estrogen is low, and a decreased likelihood of depressive symptoms after menopause, when estrogen has increased (Freeman et al., 2004). Estrogen and progesterone may be tangentially involved in increased anxiety- and depression-related behavior at the onset of puberty due to changes in ovarian hormones (Holder & Blaustein, 2014). As ovarian hormones are related to both pregnancy and mood, it would make sense for there to be some relationship between hormones and postpartum mood disorders. A landmark study by Bloch et al. (2000) simulated two hormonal conditions related to the peripartum period in women with and without a history of PPD. Five of the eight women with a history of PPD developed significant mood symptoms during the simulated hormone withdrawal. Women who did not have a history of PPD were not affected. This study strongly supports the hypothesis that
ovarian hormone withdrawal after birth plays a role in mood disruption during the postpartum period in humans.

Ovarian hormones may also modulate mood via their interaction with the hypothalamic-pituitary-adrenal (HPA) axis. While the HPG axis is involved in reproductive behaviors, the HPA axis regulates stress responses in the body using glucocorticoids such as cortisol. The HPA axis is tightly linked to depressive behavior. Dysregulation of the HPA axis is the most prominent endocrine change seen in major depression (Ising et al., 2007). This dysregulation can be treated using antidepressants, resulting in improved mood and alleviation of depressive symptoms in humans (Ising et al., 2007). Glucocorticoids and the HPA axis also play a role in the peripartum period: during pregnancy and postpartum, humans mothers experience sustained high flattened levels of glucocorticoids (Glynn, Davis, & Sandman, 2013). Brummelte and Galea (2016) observe that this hormone profile is similar to that seen in depressed patients. It seems strange that glucocorticoid levels in typical pregnancy would lead directly to depression. Instead, these results may suggest a heightened vulnerability to depressive behavior during the peripartum period due to normal changes in the HPA axis. However, if these typical fluctuations in glucocorticoids were to become abnormal during peripartum, mothers may start to show the depressive symptoms associated with HPA axis dysregulation. Importantly, the HPA axis and HPG axis regulate one another (Dismukes, Johnson, Vitacco, & Shirtcliff, 2015): if either axis is out of sync we can expect ovarian hormone imbalance, glucocorticoid disparity, or both, resulting in mood disruption. For these reasons, ovarian hormones and their interaction with the HPA axis is an important factor in researching and treating peripartum mood disorders.

**Animal models of peripartum mood disorders.** An individual’s experience of pregnancy includes a host of cognitive stressors and biological factors that render each birth
unique. However, it is impossible to control for these factors in a human participant study for both ethical and practical reasons – one cannot empirically manipulate the environment of a human over their lifespan, for example. The solution to this problem lies in animal research.

Animal models provide the opportunity to ask questions about more simple versions of complex human problems. Many key elements of human pregnancy are preserved across our mammalian cousins. Researchers can use animal models to isolate elements of pregnancy such as hormonal fluctuations and gestational features while still maintaining an empirical experiment. The answers to these simpler questions form the basis of our understanding of complex problems in humans such as peripartum mood disorders. We now turn to two such animal models to determine how they may inform future research on peripartum mood disorders.

**Gestational stress model.** The gestational stress model of peripartum mood disorder strives to emulate depression-related behavior in pregnant animals by exposing them to stress. The resulting animal shows strong symptoms of depression while experiencing gestational changes due to pregnancy. Methods of stressing animals vary among studies: some researchers restrain the animal for one hour/day for days 10 to 20 of gestation, while others have used one week of chronic restraint stress three times a day (Brummelte & Galea, 2010). Regardless of the method, stressing an animal during the peripartum period affects neurobiological change consistent with what is known about postpartum mood disorders. For instance, gestational stress alone increases serotonin metabolism in the cingulate cortex in rats; treatment with fluoxetine, a SSRI, normalizes these effects (Gemmel et al., 2016). Additionally, gestational stress is found to induce structural modifications in the postpartum nucleus accumbens, an important facet of the monoamine deficiency hypothesis in humans (Haim, Sherer, & Leuner, 2014). Though stress is not the only contributor to the development of PPD, the gestational stress model successfully
measures one significant risk factor and provides valuable information on the underlying
neurobiological changes in peripartum mood disorders.

**Hormone-withdrawal model.** The hormone-withdrawal model in animals is based on the
ovarian-steroid-withdrawal hypothesis in humans. The goal of the model is to use animal models
to experimentally isolate the drop in ovarian hormones that occurs during the peripartum period,
as these changes in hormone levels are implicated in the development of peripartum mood
disorders in humans. Pregnancy is simulated in ovariectomized female animals via daily
hormone injections. These injections follow the course of hormones over a typical pregnancy for
the animal model. At “birth,” hormones are completely withdrawn, resulting in the steep drop in
estrogen and progesterone that is seen in humans (Galea, Wide, & Barr, 2001).

In the first experiment using the hormone-withdrawal model, female rats were placed in
one of two conditions: hormone-withdrawn (experienced the drop of hormones at “birth”) vs.
hormone-sustained (no drop in hormones; maintained high estrogen levels after “birth”). Females
in the hormone-withdrawn group displayed more depressed behavior overall during the
“postpartum” period: they had decreased mobility in the forced swim test and decreased sucrose
preference. The researchers took this to mean that the hormone withdrawal in mammals had
directly caused PPD (Galea et al., 2001). Further investigation in this lab found that hormone
withdrawal led to reduced neurogenesis in the hippocampus, a phenomenon also found in human
MDD (Brummelte & Galea, 2010; Green & Galea, 2008). These results confirmed that
fluctuations in hormones in the peripartum period could cause neurobiological changes similar to
that in other depressive disorders.

Several questions about this model arise upon closer examination of their experimental
groups. It is concerning that the majority of females in the hormone-withdrawal condition
demonstrated depressive symptoms. The withdrawal condition actually models typical pregnancy, which is not characterized by anxiety- or depression-related behavior. Future studies using this model would benefit from first studying typical pregnancy in the hormone withdrawal condition. After understanding how the hormone withdrawal affects normal neurobiology in the brain during the peripartum period, researchers may be able to disrupt these circuits and measure the resulting behavior. It is possible that an underlying factor of peripartum mood disorders is a disruption in typical hormone regulation in females during a time that is already known for its tumultuous hormone levels.

Altogether, the neurobiology of postpartum mood disorders is incredibly complex and potentially involves several key monoamine, endocrine, and neuropeptide systems. Research in humans shows that though major depression and postpartum depression are separate diagnoses, there may be some overlap in underlying neurobiological processes. This is confirmed by the effective use of SSRIs, hormone therapy, and oxytocin administration in alleviating depressive symptoms for both MDD and PPD. However, these treatments still fall short of alleviating distress in a majority of cases. This is especially salient when the harmful effects of SSRIs on infant development are taken into account. In animals, research has focused on experimentally isolating potential causal targets for peripartum mood disorders. Though much progress has been made, little is known about what the brain looks like during a typical pregnancy, let alone how it differs in individuals with peripartum mood disorders. We now turn towards the literature to investigate a specific neurobiological target that may both meet the demand for risk-free treatment for peripartum mood disorders and reveal more about the neural underpinnings of pregnancy.
The Role of Oxytocin in Postpartum Mood Disorders

Oxytocin was first discovered in 1954. Since then, it has been implicated in many social behaviors, including mating, pair bonding, social affiliations, maternal behaviors, and olfactory memory (Gimpl & Fahrenholz, 2001; Pedersen, 2004). Oxytocin is produced in two regions of the brain: the supraoptic nucleus (SON) and the paraventricular nucleus (PVN) of the hypothalamus. The SON sends oxytocin to the periphery while the PVN projects oxytocin to central brain regions. On the postsynaptic side of oxytocin communication sits the oxytocin receptor (OTR), a typical class 1 G-protein-coupled receptor. Genetic variation in OTR expression in both humans and rodents is linked to individual variation in social cognition, including problems with empathy, trust, emotional regulation, and stress reactivity (Russell & Brunton, 2017). It is clear that oxytocin is involved in the same social processes that are disrupted in mothers suffering from postpartum mood disorders. Therefore, this characteristic anxiety and inability to connect with the infant may be regulated by levels of oxytocin in the body. However, central oxytocin and peripheral oxytocin play vastly different roles in the homeostatic environment of the body: central OT is a neurotransmitter, while peripheral OT serves as a hormone. With this in mind, we move to investigate peripheral and central oxytocin to determine their respective impacts on peripartum mood.

Peripheral oxytocin. Research in humans shows that peripheral oxytocin plays a large role in pregnancy, delivery, lactation, and maternal caregiving (Grammatopoulos & Hillhouse, 1999; Phaneuf et al., 1997). As a hormone, oxytocin increases uterine sensitivity before labor, stimulates uterine contractions, and pushes the baby towards the cervix during delivery (Kimmel et al., 2016; Russell & Brunton, 2017). Animal research shows peripheral oxytocin is also a strong inhibitor of the HPA axis in nulliparous animals. There is no such inhibition in pregnant
or postpartum rats, suggesting pregnancy changes the way oxytocin interacts with the HPA axis (Neumann et al., 2000). In this way, peripheral oxytocin could have an impact on maternal mood in both human and animal models.

If peripheral oxytocin is so involved in the peripartum period, it would make sense to target oxytocin in initial peripartum mood disorder research. In fact, Skrundz, Bolten, Nast, Hellhammer, and Meinlschmidt (2011) found that mothers with lower serum levels of oxytocin were more likely to be diagnosed with PPD after delivery. However, these results may be due to the natural anxiolytic effects of oxytocin, as seen in rodent research (Neumann & Landgraf, 2012). Therefore, human individuals with naturally lower levels of oxytocin may be predisposed to depressive behavior at any time of their lives. With this in mind, lower serum oxytocin levels before birth may not be an inherent characteristic of peripartum mood disorders in humans. In fact, recent research using the hormone-withdrawal model in hamsters found that central-projecting oxytocin, and not peripheral-projecting oxytocin, was impacted in the peripartum period. Specifically, there were greater numbers of oxytocin immunoreactive cells in the PVN in the hormone-withdrawal group as compared to a hormone-sustained condition. No change was found in the SON, suggesting peripheral-projecting oxytocin neurons are not regulated by the drop in ovarian hormones at birth (Amaral, 2017; Bodie, 2017; D’Antonio, 2017; Lee, 2017). As a result, we turn our focus towards the potential role of central oxytocin in peripartum mood disorders.

Central oxytocin. Although neuropeptides such as central oxytocin have long been recognized for their involvement in cardiovascular disease (Chen, Lu, Tang, Li, & Gao, 2015), food intake (Nelson, Gilbert, & Cline, 2015), and diabetes (Qureshi, Galiveeti, Bichet, & Roth, 2014), they have only recently been considered in research on maternal behavior. Central
oxytocin is highly involved in pair bonding, maternal behavior, conspecific trust, and enhanced salience of socially-relevant stimuli in both human and animal models (Marlin & Froemke, 2017; Pedersen, 2004). Blocking central OTR in the medial preoptic area (mPOA) impairs maternal care in lactating rats (Bosch, Pförsch, Beiderbeck, Landgraf, & Neumann, 2010). Additionally, chronic infusion of oxytocin at the beginning of lactation increases the frequency of arched-back nursing (Hillerer, Reber, Neumann, & Slattery, 2011). Similar results are seen in humans, such that oxytocin is vital for the milk-letdown reflex and potentiates positive maternal behavior (Yoshihara, Numan, & Kuroda, 2017). It is therefore reasonable to conclude that some suite of maternal behaviors is dependent on oxytocin in the brain.

Central oxytocin is also implicated in the study of depression- and anxiety-related behaviors. Oxytocin agonists have consistently shown an anxiolytic effect in male and female rodents during periods of “robust activation,” including lactation and sexual activity (Bale, Davis, Auger, Dorsa, & McCarthy, 2001; Neumann & Slattery, 2016). No such effects are seen in stress-free animals, suggesting that oxytocin is only involved in decreasing stress past a certain threshold of anxiety (Neumann & Slattery, 2016). Similar results are seen in rodent literature on depression, including PPD. Exogenous oxytocin administration decreases depressive behaviors in rats such that rats treated with oxytocin spend less time floating in a forced swim test (Loyens et al., 2013). However, using oxytocin to decrease depression-related behaviors in humans has mixed results. A pilot study investigating the effects of chronic, rather than single-dose, intranasal oxytocin in depressed human males found that depressed individuals in the oxytocin group actually showed anxiogenesis (MacDonald et al., 2013). This is in contrast to research that suggests depletion of oxytocin via DNA methylation results in increased risk of depressive behaviors in humans (Bell et al., 2015). In PPD literature, Lonstein, Maguire,
Meinlschmidt, and Neumann (2014) found that mothers display an increase in oxytocin release in response to reproductive stimuli, subsequently reducing anxiety- and depression-like behaviors. This is in contrast with Mah, Van Ijzendoorn, Smith, and Bakermans-Kranenburg (2013), who conducted a randomized, double-blind, placebo-controlled, within-subject clinical study in 25 postnatally depressed mothers. Mothers who received oxytocin were sadder and more likely to describe their babies as difficult but reported that the quality of their relationship with their baby was more positive. Though there is no general conclusion on the effects of oxytocin in depression treatment, it is clear oxytocin plays a role in anxiety- and depression-related behavior, specifically during the peripartum period. This research suggests that a deficiency in central oxytocin could be linked to the development of peripartum mood disorders in humans and animal models.

**Ovarian hormone regulation of oxytocin.** The function and physiological regulation of the central oxytocin system is largely steroid dependent: estrogen up-regulates OTR expression, while progesterone is inhibitory (Gimpl & Fahrenholz, 2001; Kimmel et al., 2016; Russell & Brunton, 2017). Peripheral oxytocin and estrogen interact in a variety of environments, including bone marrow to facilitate skeletal recovery (Colaianni et al., 2012). A majority of research in animal models is focused on the interaction between central oxytocin and estrogen receptors. Estrogen receptor beta (ERβ) and oxytocin are both present in the PVN where they seem to modulate HPA reactivity and the display of anxiety-related behavior in rodents (Kudwa, McGivern, & Handa, 2014). Furthermore, oxytocin regulates estrogen receptor alpha (ERα) expression within the mPOA and ventromedial hypothalamus (VMH) during development (Perry, Paramadilok, & Cushing, 2009). In support of these results, Cao et al. (2014) found that Mandarin vole pups who experienced paternal deprivation had reduced OTR and ERα mRNA
expression in the medial amygdala and nucleus accumbens, two brain regions heavily implicated in social behavior. Less research has been conducted studying the relationship between progesterone and oxytocin. General consensus states levels of oxytocin has little effect on progesterone levels in both rodents and humans but doses of progesterone decrease levels of OTR (Brozos et al., 2012; Vallet, Lamming, & Batten, 1990; Wirth et al., 2015). Taken together, ovarian hormones and oxytocin interact in both the periphery and the brain to have a profound effect on mood and behavior.

In conclusion, oxytocin is an important mediator of mood and behavior in human and animal models. Central oxytocin in particular seems to play an important role in the development and continuation of depression- and anxiety-related behaviors. This is perhaps caused by the regulation of oxytocin by ovarian hormones, which are dysregulated during pregnancy and postpartum. Further investigation into how ovarian hormones regulate oxytocin in specific brain regions may reveal important information about the development of peripartum mood disorders.

**Brain Regions Implicated in Postpartum Mood Disorders**

Oxytocin is projected centrally to many brain areas involved in the production of social behavior including regions in the midbrain and brainstem (Althammer & Grinevich, 2017). In particular relevance to the study of the peripartum period are PVN efferents involved in maternal behavior such as the medial amygdala (MA), nucleus accumbens (NAc), bed nucleus of the stria terminalis (BNST), and raphe nuclei (Althammer & Grinevich, 2017; Marlin & Froemke, 2017). In fact, levels of OTR expression in these regions are correlated with the quality of maternal behavior in rodents (Russell & Brunton, 2017). We believe further study of the impact of peripartum ovarian hormone withdrawal on OTR levels in these brain regions will provide both
information on typical neural plasticity during pregnancy and illuminate potential targets for future peripartum mood disorder research.

**Medial amygdala.** The MA is typically involved in sexually dimorphic functions such as aggression, sexual behavior, and integration of olfactory information (Hines, Allen, & Gorski, 1992). In general, oxytocin in the amygdala is anxiolytic: it reduces activation of fear circuitry and works to disconnect the amygdala from the brainstem. This regulation of the MA by oxytocin seems to play a role in maternal behavior by reducing aggression and aversion to offspring (Russell & Brunton, 2017). Further study on the role of the MA in maternal behavior has revealed a connection between the MA and dam-pup interaction. Lee, Clancy, and Fleming (2000) found that lesioning the amygdala in postpartum rats decreased their bar-pressing for the delivery of rat pups, a measure of motivation for pup contact. Furthermore, rats in an amygdala lesion condition showed deficits in maternal response in the home cage and retrieval of pups in the testing chamber. There are several potential reasons for decreased dam-pup interaction in an amygdala lesion study. First, if the amygdala is lesioned and therefore unable to receive anxiolytic input from its afferents, the dams may be experiencing increased aversion to and aggression towards their pups, thereby decreasing their motivation to retrieve them via bar press and interact with them in the home cage. A second possibility is that the amygdala lesion disrupted the olfactory system of the mother such that they are unable to properly recognize their pups as the MA plays a role in integrating olfactory information (Hines et al., 1992). Regardless, the MA clearly plays a role in the interaction between mother and child (Akbari et al., 2013; Oxley & Fleming, 2000). Further investigation of how this brain region, specifically its interaction with oxytocin, changes over the course of pregnancy may reveal how oxytocin impacts peripartum mood disorders.
**Nucleus accumbens.** The NAc is part of a larger circuit of brain regions that regulate appetitive components of maternal behavior, such as licking and grooming and retrieval. Numan and Stolzenberg (2009) postulate that the mPOA and BNST, when hormonally primed, activate the ventral tegmental area (VTA). This leads to increased mesolimbic dopamine input to the shell of the NAc, resulting in a change in maternal behavior. VTA dopamine input to the NAc seems to be essential for active maternal behaviors (Hansen, Harthon, Wallin, Löfberg, & Svensson, 1991; Numan et al., 2005; Numan & Stolzenberg, 2009). This is in comparison to behaviors such as nursing which are characterized as passive. Oxytocin modulates the activity of this system by acting at different nodes in the circuit, including the mPOA, NAc, and VTA. We can measure the impact of oxytocin in the NAc by recording levels of dopamine in relation to several maternal behaviors in rodents. Dopamine levels in the NAc change when a postpartum mother interacts with her young. For example, licking and grooming behaviors increase the level of extracellular dopamine. In fact, licking/grooming can be predicted by an increase in dopamine before the onset of the behavior (Champagne et al., 2004). This is supported by pharmacological manipulation: administering a dopamine uptake blocker to postpartum females increases both the amount of dopamine in the NAc and the frequency of licking/grooming behavior (Champagne et al., 2004). Completely lesioning the shell of the NAc disrupts pup retrieval and licking/grooming, further confirming the role of the NAc in active maternal behaviors (Keer & Stern, 1999). With these results in mind, the present study aims to investigate how OTR expression in this area is impacted by estrogen withdrawal.

**Bed nucleus of the stria terminalis.** While the NAc regulates active maternal behavior, the BNST is involved in more passive actions. The BNST is a node in the limbic system that is associated with aggression regulation, similarly to the MA. Broadly, we know that lesions to the
BNST disrupt maternal behavior (Numan & Stolzenberg, 2009). The BNST seems to be essential for both the onset and maintenance of these behaviors. A minimal level of corticotropin-releasing factor receptor (CRF-R) activation in the medial-posterior BNST is necessary for maternal behavior in rats (Klampfl, Brunton, Bayerl, & Bosch, 2016; Numan & Stolzenberg, 2009). In addition, stress during postpartum increases cFos expression in the BNST. From this, the authors interpreted a potential role for the BNST in the integration of threat cues and their meaning into long-term effects on expression of maternal care (Kenny, Wright, Green, Mashoodh, & Perrot, 2014). Of all of these roles, the central function of the BNST seems to be lactation and nursing in postpartum mothers. The anterior-dorsal BNST modulates arched-back nursing, but not maternal motivation, aggression, or anxiety in rats (Klampfl et al., 2016). We also see an increase in OT and vasopressin binding in the BNST during lactation (Bosch et al., 2010). In comparison to the NAc, the BNST seems to be involved in activities that are triggered by the postpartum period but are not necessarily active behavioral choices – mothers do not get to choose if they lactate, but theoretically can choose to lick their young. With this in mind, it would not be surprising to see no change in oxytocin in the BNST during postpartum mood disorder. Mothers would still be physically modified to take care of their child but suffer cognitive blocks regarding motivation and aversion to infant stimuli. In other words, peripartum mood disorders affect more active aspects of maternal behavior rather than passive behaviors such as nursing. Regardless, the BNST clearly plays an important role in the peripartum period. Further research into the neuroplastic changes in this region may highlight targets for future peripartum mood disorder study.

Raphe nuclei. The raphe nuclei are the main source of serotonin in the central nervous system. Serotonin is implicated in a wide variety of physiological elements including mood,
appetite, and sleep. Interestingly, serotonin and the raphe nuclei are also implicated in maternal behavior regulation, potentially through their relationship with the PVN. In 2009, Yoshida et al. found that of all serotonergic neurons in the raphe nuclei, about half were positive for OTRs. Pharmacological manipulations revealed that OT infusion increased serotonin release in the medial raphe nucleus, resulting in reduced anxiety-related behavior in mice. Furthermore, infusion of a serotonin antagonist blocked all anxiolytic effects of the oxytocin influx. From these results we can interpret that OTR activation in serotonergic neurons in the raphe nuclei mediates anxiolytic effects of 5-HT. This has important implications for the study of postpartum mood disorders. As stated previously, there are increased levels of oxytocin in the PVN after birth during a typical pregnancy in mammals (Amaral, 2017; Bodie, 2017; D’Antonio, 2017; R. H. Lee, 2017). Increased levels of oxytocin would lead to heightened OTR activation, potentially in the raphe nuclei, which would then upregulate serotonin release. The impact on mood would be drastic, decreasing anxiety and increasing resilience in the face of stressors during birth. Additionally, it is well documented that SSRIs are an effective treatment of postpartum depression symptoms (Kim et al., 2014). Therefore, a decrease in the level of central oxytocin at birth (compared to a typical pregnancy) followed by lower levels of serotonin could be a potential model for peripartum mood disorders. SSRIs, then, are an effective treatment as they correct serotonin deficits that resulted from changes in oxytocin.

This theory is further supported by research into the role of serotonin in maternal behaviors. Serotonin and the raphe nuclei seem to play a similar role to the NAc as serotonin is critical to regulating nurturing behavior such as grooming or pup retrieval. Mothers with a Pet-1 knockout, a gene responsible for serotonin synthesis, have severely reduced pup survival. This is attributed to a reduction in maternal nurturing behavior rather than problems with nursing as the
pups had milk in their stomachs (Lerch-Haner, Frierson, Crawford, Beck, & Deneris, 2008). The authors further speculate that as serotonin is also involved in aggression, regulation of the serotonin system via oxytocin in the raphe nuclei may impact maternal responsiveness and aggressive behavior. Support for this theory comes from Angoa-Pérez et al. (2014), who found that rodent mothers with genetic depletion of brain serotonin displayed sustained aggression that could not be affected by other behaviors. This was in addition to a reduction in pup survival, increased cannibalization, and poor pup retrieval, huddling, and nest construction. The raphe nuclei and serotonin are therefore an important area for the research of peripartum mood and maternal behavior regulation.

Each of these four brain regions – the MA, NAc, BNST, and RN – plays an important role in regulating maternal behaviors that are often impacted in mothers with peripartum mood disorders. However, most of the research on these areas involves lesioning the region in order to measure impacted behaviors. In order to better understand the potential role for PVN efferents in peripartum mood, we must first elucidate their neurobiology during typical pregnancy in mammals. With this information we can we begin to highlight targets that may be dysregulated in PPD.

The Present Study

The present study aims to build upon previous research investigating the role of oxytocin in neuroplastic and behavioral changes during the peripartum period. These are exploratory studies as little research has been conducted examining the relationship between estrogen withdrawal and central oxytocin at birth in mammals. For this reason, we chose to conduct these studies using a hormone-simulated pregnancy in a Syrian hamster model as seen in previous
work. This allows us a greater amount of control over hormone levels, environmental confounds, and other factors inherent in human studies.

We propose two experiments that further elucidate the role of oxytocin and estrogen in the peripartum period. Experiment 1 is developed from past work studying oxytocin producing-neurons during the peripartum period. There is an elevated number of oxytocin-immunoreactive (OT-ir) neurons in the PVN following hormone withdrawal in a hormone-simulated pregnancy model conducted in Syrian hamsters (Amaral, 2017; Bodie, 2017; D’Antonio, 2017; R. H. Lee, 2017). We are now looking at the post-synaptic side: specifically, we are interested in which PVN efferents, if any, are experiencing change in OTR expression. Though there are many PVN efferents in the central nervous system, we have chosen to investigate the MA, NAc, BNST, and the RN as each of these four areas are heavily involved in maternal behavior. As there is an increase in oxytocin in the PVN at day five postpartum, and increased oxytocin is known to decrease OTR expression to maintain homeostasis, we generally expect to see a decrease in OTR density and a decrease in anxiety-related behaviors in the estrogen-withdrawn group as compared to estrogen-sustained and oil control.

The second experiment is a direct continuation of work by Amaral, Bodie, D’Antonio, and Lee (2017). These researchers used a hormone-simulated pregnancy in female hamsters to determine that there is a higher number of OT-ir neurons in the PVN in the hormone withdrawn condition as compared to the hormone sustained condition five days postpartum. The goal of Experiment 2 is to determine the immediate cause of this difference in OT-ir neurons. Using the tissue from this previous study, we work to elucidate if this effect is due to cell degeneration in experimental animals that did not experience hormone withdrawal.
Method

Experiment 1

Subjects. Twenty-four adult female Syrian hamsters (*Mesocricetus auratus*) were purchased from Charles River Laboratories (Wilmington, MA, USA) at approximately 60 days of age. Subjects were housed in polycarbonate cages (46 x 24.5 x 20 cm). All animals were maintained on a reversed 14-hour light/10-hour dark photoperiod (lights off at 10 a.m.). All behavioral testing occurred during the dark phase. The animal room was maintained at a controlled temperature and humidity. Food and water were available *ad libitum*. All animal procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Haverford College Institutional Animal Care and Use Committee. Maximal efforts were made to minimize the number and relative suffering of animals in this study.

Procedure.

Ovariectomy. To ensure endogenous fluctuations in ovarian hormones were eliminated, all 24 hamsters underwent ovariectomy. Anesthesia was induced using 2.5 to 5% isoflurane aerosolized in oxygen. Animals were weighed, then had their flanks shaved and sterilized with ethanol and betadine. After transfer to the sterile surgical field, anesthesia was maintained via nosecone. Bilateral flank incisions were made and ovaries were removed via cauterization of the uterine horn. The muscle wall was closed with suture and the skin closed with wound clips. Analgesic (butorphanol, 5 mg/kg) and antibiotic (baytril, 10 mg/kg) were administered before surgery and for three days postoperative.
**Hormone-simulated pregnancy.** Hormone injections began one week after ovariectomies were completed. Every morning at approximately 0900 h for 21 days vehicle or hormone was injected subcutaneously into each hamster. The control group \((n = 8)\) was administered 0.1 mL of cottonseed oil daily for 21 days. Both the hormone-sustained and hormone-withdrawn groups \((n = 8 \text{ per group})\) received a low dose of estradiol (2.5 µg) and a high dose of progesterone (4 mg) dissolved in cottonseed oil for days 1 through 11, thus simulating early pregnancy. On days 12 through 16, hormone-sustained and hormone-withdrawn conditions received only a high dose of estradiol (50 µg) with no progesterone, simulating late pregnancy. On day 17, we simulated “birth” in the hormone-withdrawn group by rescinding all hormone treatments: they received only oil through day 24 (day 5 “postpartum”). In contrast, the hormone-sustained group continued to receive a high dose of estradiol through day 24. Hormone levels for each stage of the peripartum period are based on previous research on the hormone-simulated pregnancy in rats (Galea et al., 2001).

**Tissue histology.** All hamsters were euthanized on day 5 “postpartum” via anesthetized rapid decapitation followed by brain extraction. Brains were immediately flash frozen in dry ice and sent to Dr. Amy Ross at Georgia State University for processing. Four sets of 20 µm coronal sections were collected via cryostat. One set of tissue was used for autoradiographic localization of OTRs.

**Measures.**

**Behavioral measures.** Anxiety-related behavior was measured using an elevated plus maze and the open field test. Behavioral testing was conducted on days 1 through 4 postpartum. The order of tests was counterbalanced across experimental groups. Within each test group, the order of subjects was pseudorandomized to account for differences in experimenter behavior.
over time. All recordings were analyzed using EthoVision XT behavior tracking software (Noldus Information Technology, Wageningen, The Netherlands).

*Elevated plus maze.* Elevated plus maze behavioral tests were conducted on days 2 and 3 postpartum. The elevated plus maze is a valid and reliable measure of anxiety-related behavior in rodents. Rodents who spend more time on the open arms of the EPM are presumed to be less anxious (Kalueff & Tuohimaa, 2004). We focused specifically on time spent on open arms vs. time spent on closed arms. We also monitored nonspecific locomotor activity such as velocity (cm/s), distance traveled (cm), and any extraneous behaviors such as attempting to climb the walls.

Hamsters were brought into the experimental room individually and placed in the center of the plus maze. The plus maze used in these experiments is made of plastic and consists of two open arms (51 cm long x 11.5 cm wide) and two closed arms (51 cm long x 11.5 cm wide, with 40 cm high walls) that extend from a central platform (10 x 11 cm) elevated 73 cm above the floor. The experimenter was in the room with the animal. They made observations and recorded behavior throughout the test. Animals spent a total of 10 minutes in the EPM. The maze was thoroughly cleaned with ethanol after each test.

*Open field test.* Open field tests were conducted on days 1 and 4 postpartum. The open field test is also a valid and reliable measure of anxiety-related behavior in rodents. Rodents who spend more time in the middle of the field are presumed to be less anxious than animals who stay on the edges of the field because rodents tend to be afraid of open spaces (Kalueff & Tuohimaa, 2004). The focus of this behavioral measure is time spent the center vs. periphery of the open field. Just as in the EPM, we monitored nonspecific locomotor activity and any extraneous behaviors.
Hamsters were again brought in individually and placed in the center of the open field. The field used in these experiments consists of an opaque plastic box (40.5 x 40.5 cm, with 30 cm high walls) with an open top. The experimenter was in the room with the animal. They made observations and recorded the same nonspecific locomotor behavior throughout the test. Animals spent a total of 5 minutes in the open field. The field was thoroughly cleaned with ethanol after each test.

**Autoradiography.** Dr. Amy Ross of the Albers lab at Georgia State University conducted autoradiography on our behalf to measure density of OTRs in the MA, NAc, BNST, and RN. OT receptor binding was determined with the $^{125}$I-labeled ornithine vasotocin analog Vasotocin, $d(CH_2)_5[Tyr(Me)^2, Thr^4, Orn^8][^{125}I]Tyr^9-NH_2$ (Perkin-Elmer). The tissue was allowed to thaw and dry. It was fixed in 0.1% paraformaldehyde for 2 minutes. Slides were then rinsed twice for 10 minutes each in buffer (50 mM Tris, pH 7.4) and incubated in tracer buffer (0.35 mM bacitracin, Sigma-Aldrich, St. Louis, MO; 0.015 mM bovine serum albumin, Sigma-Aldrich, St. Louis, MO; 100 nM $^{125}$I vasotocin analog) for 1 hour. Slides were rinsed twice for 5 minutes each and then for 35 minutes 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 C$^{14}$ standard calibration strip (American Radiolabeled Chemicals, St. Louis, MO) were loaded into autoradiography cassettes and exposed to film (Kodak, Rochester, NY) for 7 days at room temperature.

An experimenter blind to the conditions of the subjects conducted an optical density analysis to quantify OTR density in our regions of interest. Densitometry analysis was performed using Scion Image software (NIH, Bethesda, MD) and a lightbox (Imaging Research, Inc., Ontario, Canada) attached to a camera (Panasonic, Newark, NJ). Standard curves were created
using the C\textsuperscript{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 sections, which were analyzed along the midline of the brain (Figures 1, 2, 3, and 4). With the exception of the MA, a 0.35 mm\textsuperscript{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 MA in order to measure dorsal and ventral MA simultaneously. Background binding was subtracted from this measurement. Optical densities were calculated as disintegrating units per minute per mg tissue (dpm/mg; Ross, 2018).

**Statistical analysis.** All statistical analyses were conducted using SPSS (IBM Predictive Software). To measure between-group hormone condition differences for each behavioral task, one-way ANOVAs with hormone condition as the independent variable were conducted for each behavioral measure, followed by Tukey’s post hoc tests. Autoradiography data were analyzed using one-way ANOVAs with hormone condition as the independent variable, followed by Tukey’s post hoc tests when appropriate, to determine the effects of hormone condition on OTR density in the MA, NAc, BNST, and RN. Statistical significance for all analyses was determined as \( p \leq 0.05 \).

**Experiment 2**

For a complete rendition of the subjects and procedure that produced the tissue in Experiment 2, please reference Amaral (2017), Bodie (2017), D’Antonio (2017), and Lee (2017).

**Subjects.** This study included 16 adult female Syrian hamsters, identical in age, species, and origin to all hamsters in this paper. All animal procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Haverford
College Institutional Animal Care and Use Committee. As before, maximal efforts were made to minimize the number and relative suffering of animals in this study.

**Procedure.** Animal care, ovariectomy, and hormone-simulated pregnancy procedures were identical to those described in Experiment 1. Hamsters were euthanized on day 5 postpartum via i.p injection of [0.2 mL of Sleepaway (Fort Dodge Animal Health)] and transcardially perfused with 25 mM phosphate buffered saline (PBS, pH = 7.2) for 3 minutes at a flow rate of 25 mL/minute, followed by 4% paraformaldehyde in 25 mM PBS for 20 minutes. After perfusion, brains were removed, post-fixed for 1 hour in 4% paraformaldehyde in PBS and placed in a 10% sucrose solution in PBS overnight at 4 degrees Celsius. All tissue was stored in cryoprotectant in December 2016 (Been, Staffend, Tucker, & Meisel, 2013).

**Fluoro-Jade C staining for cell degeneration.** To visualize degenerative neurons, brain sections containing the PVN were mounted on VWR Superfrost Plus Micro Slides, air-dried in the dark overnight, and subjected to Fluoro-Jade C (FJC) staining (Histo-Chem, Inc.). The slides were first immersed in a 100 mL solution containing 1% NaOH in 80% ethanol for 5 minutes. They were then rinsed for 2 minutes in 70% ethanol and for 2 minutes in double distilled water, then incubated in 0.06% potassium permanganate solution for 10 minutes. Following another water rinse for 2 minutes, slides were transferred to the FJC staining solution and stained for 10 minutes. FJC staining solution was composed of 1 mL stock solution (0.01% FJC dye in ddH2O) in 99 mL 0.1% acetic acid. Slides were then washed in double distilled water three times each for 1 minute and then air-dried in the dark overnight. After clearing in xylene and coverslipping with DPX mountant for histology, sections were examined under a Nikon Eclipse 80i fluorescence microscope.
Qualitative analysis. A qualitative analysis of the stained tissue was conducted to determine trends of FJC positive neurons in the PVN. Tissue sections were categorized into one of three groups of FJC positive neurons: low (0-2 neurons), intermediate (3-9 neurons), and high (10+ neurons). An example section from each group is found in Figure 1. All analyses were conducted by experimenters blind to the conditions of the study.

Results

Experiment 1

Elevated plus maze. One-way between-subjects ANOVAs were conducted to determine the impact of hormone condition on time spent on open and closed arms of the EPM. There was a significant effect of hormone condition on time spent on the open arms of the EPM \( F(2, 21) = 6.082, p = 0.008 \); Table 1). Tukey’s HSD post hoc revealed that animals in the hormone withdrawn condition spent significantly less time on the open arms of the EPM than both the hormone sustained condition and oil control (Table 2). In contrast, there was no significant effect of hormone condition on time spent in the closed arms of the EPM \( F(2, 21) = 1.456, p = 0.256 \); Table 1). These results suggest heightened levels of anxiety in the hormone withdrawn condition as compared to the hormone sustained and oil control conditions (Figure 2).

A difference score was calculated by subtracting time spent on the open arms from time spent on the closed arms. This difference score mitigates individual differences in behavioral data and is therefore a more accurate representation of our EPM results. There was a significant effect of hormone condition on difference score \( F(2, 21) = 3.71, p = 0.04 \); Table 1). However, Tukey’s HSD post hoc reveal that difference scores were only marginally higher in the withdrawn condition as compared to the control and sustained conditions (Table 3). This
suggests there is a strong trend towards increased time spent in the closed arms of the EPM in the hormone withdrawn condition (Figure 3).

**Open field test.** We again conducted one-way ANOVAs to determine the impact of hormone condition on behavioral measures in the OFT. Interestingly, we found a significant difference in velocity between hormone conditions \( F(2, 21) = 3.639, p = 0.044 \). Tukey’s HSD post hoc shows that velocity is higher in animals in the hormone sustained condition as compared to hormone withdrawn (Table 4, Figure 4). Fittingly, hormone condition also had a significant effect on total distance moved during the OFT \( F(2, 21) = 3.366, p = 0.054 \) such that animals in the hormone sustained condition moved significantly farther than animals in the hormone withdrawn condition (Tukey’s HSD, Table 5, Figure 5). There was no significant effect of hormone condition on total time spent on the edge or middle of the OFT, nor was there an effect on the difference score (edge minus middle; Table 6).

**Autoradiography.** A one-way between-subjects ANOVA was conducted for each brain region to compare the effect of hormone condition on OTR density for hormone withdrawn, hormone sustained, and oil control groups. For a full description of mean densities per brain region, please see Table 7. There was a significant effect of hormone condition on OTR density in the dorsal RN for the oil and hormone withdrawn conditions \( F(2, 21) = 4.351, p = 0.026; \) Table 8]. Post hoc comparisons using the Tukey HSD test indicated that the mean score for the hormone withdrawn condition \((M = 275.8, SD = 105.6)\) was significantly higher than the oil condition \((M = 156.3, SD = 87.3; p = 0.033)\). The hormone sustained condition \((M = 173.0, SD = 65.7)\) did not significantly differ from the hormone withdrawn and oil conditions. There were no significant effects of hormone condition on OTR density in the MA, NAc, or BNST (Tables 9, 10, and 11). Exemplary autoradiographs for the four brain regions from each condition can be
found in Figure 6, 7, 8, and 9. Taken together, these results suggest the sudden drop in estrogen at “birth” in female hamsters has an impact on OTR density such that OTR density is higher in the dorsal RN after hormone withdrawal in comparison to the oil condition.

**Experiment 2**

A qualitative analysis of the stained tissue was conducted to determine trends of FJC positive neurons in the PVN. A majority of tissue in the low range of numbers of FJC positive cells was from animals in the sustained condition, while a majority of tissue in the intermediate to high ranges was in the withdrawn condition. If future analyses were to confirm these results, they would suggest that estrogen withdrawal has an impact on cell degeneration in the PVN such that there is increased cell degeneration in the PVN after a rise and fall in estrogen over the peripartum period.

**Discussion**

Despite evidence that peripartum mood disorders impact up to 20 percent of mothers every year, very little is known about their underlying neurobiological factors. Research in both humans and animal models has shown that the dramatic drop in estrogen at birth in mammals is related to depression- and anxiety-like behavior (M. Bloch et al., 2000; Galea et al., 2001). This hormone withdrawal is correlated with changes in OT-ir neurons in the PVN during postpartum, a natural connection as oxytocin is highly relevant to maternal behavior and the estrogen and oxytocin systems regulate one another (Amaral, 2017; Bodie, 2017; D’Antonio, 2017; Gimpl & Fahrenholz, 2001; R. H. Lee, 2017; Marlin & Froemke, 2017). The goal of the present study was to build on this research by investigating the role of oxytocin in changes in neuroplasticity and behavior during peripartum. This was achieved in two experiments focused on the postpartum
period: one examining change in OTR density in four efferents of the PVN; and the other exploring possibilities of cell degeneration in the PVN.

**Change in OTR Density in the Dorsal Raphe Nucleus**

OTR autoradiography was used to determine if estrogen withdrawal impacts OTR density in four regions of the brain related to maternal behavior. Of these four brain regions, only the dorsal RN showed any change in OTR density as a result of hormone withdrawal. Specifically, there was a significantly higher density of OTR in the hormone withdrawn condition as compared to the oil condition in the dorsal RN. These results suggest that neuroplasticity in the brain during the postpartum is related directly to both the rise and drop of estrogen over the course of pregnancy. The rise in estrogen and progesterone alone was not sufficient to induce changes in OTR density – otherwise we would expect see similar results in both the hormone withdrawn and sustained conditions.

A change in OTR density during normal pregnancy is consistent with literature as many factors over the peripartum period may influence receptors (Blanks, Shmygol, & Thornton, 2007; Pawlusi et al., 2017; Russell & Brunton, 2017). In this study, though our results show a change in OTRs in the RN, it is not clear in this study what is regulating their expression in the hormone withdrawn condition. One of the most obvious possibilities is estrogen. Estrogen withdrawal was the main independent variable in this study; therefore, our results are likely related to fluctuations in this system. OTR expression is not dependent on the estrogen system such that OVX rodents still have OTRs (Gimpl & Fahrenholz, 2001). This is confirmed by the present study: OVX hamsters in the oil condition had OTRs after 22 days without estrogen. However, a great amount of research has shown that estrogen upregulates OTR in the brain (Blanks et al., 2007; Gimpl & Fahrenholz, 2001; Kimmel et al., 2016). For example,
administering estrogen to OVX female rats increases central OTR binding sites and OTR mRNA (Blanks et al., 2007; Gimpl & Fahrenholz, 2001). This information helps interpret the results of our study. In both hormone conditions, estrogen dosing increased over the course of the hormone-simulated pregnancy, followed by a complete withdrawal at birth. We speculate that OTRs could have been increasing over the course of pregnancy in response to rising estrogen levels in the hormone conditions. Our results support this theory as OTR levels were higher in both hormone conditions than in the oil condition (which did not receive any estrogen and therefore would not be expected to show an increase in OTRs). However, if high levels of estrogen were all that was required for high levels of OTRs, we would expect the sustained group to have higher OTR density as compared to the withdrawn group at day 5 postpartum. This is because the sustained group is kept on high E and the withdrawn group has no E. However, the present study found that OTR density was lower in the sustained group compared to withdrawn in the dorsal RN at day 5 postpartum. Therefore, we speculate there is something specific about the drop in estrogen at birth that causes OTRs to, if not increase, maintain high density during postpartum in the withdrawn condition. Further research is needed to determine how estrogen may be regulating OTRs in the RN during the peripartum period.

Though the underlying mechanisms may be unclear, changes in OTR density in the dorsal RN in our typical pregnancy condition have large implications for our understanding of the neurobiology of peripartum mood disorders. Importantly, these results form a connection between the oxytocin system and the serotonin system during peripartum. The RN is home to about 80 percent of all central serotonin-producing neurons in the brain. Of these serotonergic neurons, about half have OTRs (Yoshida et al., 2009). One lab found that oxytocin infusion into the medial RN facilitated serotonin release in rats (Yoshida et al., 2009), suggesting that OTRs
upregulate the serotonergic system. Though the present study investigated the dorsal rather than the medial RN, both dorsal and medial RN neurons are serotonergic and include OTRs. Therefore, it is possible that oxytocin infusion into the dorsal RN would facilitate serotonin release in a similar fashion (Beck, Pan, Akanwa, & Kirby, 2004). It is reasonable to assume that typical pregnancy includes serotonin regulation during early postpartum as serotonin is a critical factor in maternal nurturing behavior such as grooming and pup retrieval, as well as downregulating aggression towards offspring (Angoa-Pérez et al., 2014; Lerch-Haner et al., 2008). In fact, research from Holschbach and Lonstein (2017) found that female rats in early postpartum had higher levels of serotonin precursor 5-HP and serotonin metabolite H-HIAA compared to late postpartum or diestrus virgins. It is therefore possible that serotonin release during early postpartum could be regulated by increased density of OTRs in the RN, resulting in typical maternal behavior during the postpartum period in female hamsters.

Serotonin also impacts anxiety- and depression-related behaviors, two distinct phenotypes of peripartum mood disorders. Depression is commonly associated with decreased serotonin metabolism and neurotransmission (Mahar, Bambico, Mechawar, & Nobrega, 2014; Yuan et al., 2015). Similarly, anxious behavior in humans, rodents, and crayfish is at least partially regulated by the serotonin system (Fossat, Bacqué-Cazenave, Deurwaerdère, Delbecque, & Cattaert, 2014; Marcinkiewcz et al., 2016; Zangrossi & Graeff, 2014) and it is well established that SSRIs are effective in improving mood and decreasing depressive symptoms in mothers with PPD (Kim et al., 2014). Just as maternal behavior could be a result of regulation of serotonin via OTRs in the dorsal RN, so too could OTRs be regulating anxiety- and depression-related behavior. There are several potential mechanisms for this regulation. As previously stated, Yoshida et al. (2009) found that infusion of oxytocin into the RN increased serotonin
release. This resulted in a significant anxiolytic effect in mice. Infusion of serotonin receptor antagonist blocked all anxiolytic effects, thereby showing that it was the oxytocin-induced serotonin release that was regulating anxious behavior. Regardless, it is very reasonable to speculate that changes in OTR density in the dorsal RN, which may modulate serotonergic activity and anxiety- and depression-related behavior, play a role in the development or sustainment of peripartum mood disorders.

Many questions arise from this conclusion that can be addressed by further research. One next step could be to manipulate or block the change in OTR density in the dorsal RN in the withdrawn condition. If this manipulation results in an anxiety- or depression-related phenotype one could further connect changes in OTR to the development of peripartum mood disorders. The timing of changes in OTR density is also unknown. It is possible OTRs fluctuate happens sometime during pregnancy, though if this were the case we would have expected to see similar changes in OTR density in the hormone sustained and withdrawn groups. We do not have a sense of when OTR density could change during postpartum, though it must at least begin to happen at day 5. Previous research has also shown an increase in oxytocin in the PVN as a result of hormone withdrawal (e.g. Amaral, 2017). One could potentially interfere with this increase in the PVN and see if this results in a change in OTR density in the dorsal RN, therefore at least partially confirming that these two phenomena interact with each other. These experiments, when coupled with the present findings, represent a step forward in understanding neuroplasticity during the peripartum period and may eventually lead to better treatment options for those who suffer with peripartum mood disorders.
Measures of Anxiety-Related Behavior

Experiment 1 had varying results as to the nature of anxiety-related behaviors in the three hormone conditions. In the EPM, animals in the withdrawn condition spent less time in the open arms of the maze than both sustained and control conditions. The withdrawn condition also had marginally larger difference scores, showing they spent less time on the open arms compared to time in the closed arms of the maze. These results suggest that animals in the withdrawn condition are more anxious than the rest of their cohort – therefore, estrogen withdrawal at birth in this animal model impacts anxiogenesis. The OFT did not confer with the results of the EPM. There were no significant measures of anxiety-related behavior across all three hormone conditions. Instead, we found animals in the sustained condition showed higher velocity and greater distance traveled than either withdrawn or control. Importantly, sustained showed the highest amount of locomotion (velocity and distance traveled) while withdrawn showed the lowest. There are two possible explanations for this phenomenon: either sustained estrogen led to a general increase in locomotion or estrogen withdrawal leads to a general decrease in locomotion. Further study is required to determine the directionality of this relationship.

Results from the EPM and OFT do not support our hypothesis that estrogen withdrawal leads to decreased anxiety during the postpartum. In fact, our data on the EPM aligns more closely with the model of postpartum depression suggested by Galea et al. (2001). In this study, the hormone-sustained condition of a hormone-simulated pregnancy was modeled after PPD and therefore expected to show heightened levels of depression or anxiety during postpartum. Rats in the hormone withdrawn condition showed a robust depressive phenotype during a forced swim test (FST): increased immobility and defecation and decreased struggling (Galea et al., 2001). Perhaps the same behavioral effect of estrogen withdrawal that Galea et al. observed during the
FST is reflected in our results of heightened anxiogenesis in the EPM. Several other studies conducted in a similar manner to both Galea et al. and the present study support the notion of either increased or constant anxiety throughout the peripartum (Neumann et al., 1998; Stoffel & Craft, 2004). Importantly, Galea et al. also found no significant anxious behavior in the OFT for any of the three hormone conditions. Therefore, though they do not agree, our OFT data do not necessarily discount the results of the EPM. It is possible that the EPM and OFT are not good measures of anxious behavior for this estrogen-based model, or that further understanding of how estrogen regulates anxious behavior is needed before interpreting behavioral data.

As for differences in velocity and distance traveled in the OFT, estrogen has been shown to impact locomotor activity. This is especially true in high stress environments such as an OFT or EPM. da Silva et al. (2014) showed estradiol replacement treatments increased locomotion in OVX rats in both the OFT and EPM. Galea et al. (2001) also reported a greater number of area crossings in the OFT for the withdrawn condition. Though these studies show opposite results from the current data, they do suggest that estrogen likely regulates anxiety-related locomotor responses via several neurotransmitters such as serotonin, dopamine, or GABA. Perhaps if estrogen is completely removed during the withdrawal, this regulation is completely desynchronized, resulting in unusual locomotor activity. Again, further research is needed to properly elucidate the relationship between estrogen withdrawal and locomotor activity in a hormone-simulated pregnancy.

It is important to question why we are seeing an increase in anxious behavior in this animal model while studies in human mothers have reported such robust levels of anxiolysis during postpartum. Firstly, the present model of hormone-simulated pregnancy in rodents was not created to perfectly mimic a clinical human model of pregnancy, typical or otherwise.
Neither in the original model (Galea et al., 2001) nor in the current study were animals genuinely pregnant. There was no pup interaction, which has been shown to greatly impact serotonin, oxytocin, and estrogen regulation during postpartum (Akbari et al., 2013; Angoa-Pérez et al., 2014; Holschbach & Lonstein, 2017). And the gestational length, hormone profile, and environment are different. It is reasonable to find conflicting behavioral results in such different models of pregnancy. Secondly, the quality of data collection for both the EPM and OFT for Experiment 1 was quite poor. In response we ran an almost identical pilot study of the hormone-simulated pregnancy with only the withdrawn and sustained conditions and put the animals through the EPM. In contrast to the present study, the pilot showed no significant effects of hormone condition on any of the measurements reported in this paper. Therefore, we must state that due to problems with data collection we are not confident in the behavioral results of this study. In conclusion, there is evidence that behavior is impacted by changes in estrogen during the peripartum period. However, future studies and more consistent methods are needed in order to draw any concrete conclusions.

**Cell Degeneration in the PVN**

Fluoro-Jade C staining was used to determine if estrogen withdrawal impacts cell degeneration in the PVN during postpartum. Qualitative analysis reported an increased number of FJC positive neurons in the PVN of animals in the hormone withdrawn condition as compared to hormone sustained. If further research were to prove these results are statistically significant, a higher number of FJC positive neurons in the withdrawn condition would suggest that the rise followed by the drop in estrogen at birth increases cell degeneration in the PVN. Importantly, the rise in estrogen and progesterone over the course of pregnancy was not enough to induce degeneration – if this was the case, we would expect to see similar results in both the withdrawn
and sustained conditions. Instead, there is some aspect of neuroplasticity specific to the rise
followed by the drop in hormones that increase cell degeneration in the PVN.

Neuroplasticity is a well-studied phenomenon in peripartum literature. Motherhood has
been known to alter forebrain cytogenesis as well as cell death (Holschbach & Lonstein, 2017).
For instance, prolactin release stimulated the production of neuronal progenitors in the forebrain
subventricular zone (SVZ) of female mice during pregnancy. These progenitors become new
olfactory neurons in the olfactory bulb, a brain region critical for recognizing offspring in
rodents (Shingo et al., 2003). In a similar vein, serotonin has been shown to mediate estrogen
stimulation of cell proliferation in the adult dentate gyrus of the hippocampus during a hormone-
simulated pregnancy (Banasr, Hery, Brezun, & Daszuta, 2001). Serotonin and estrogen both
have receptors in the PVN – perhaps a similar relationship between estrogen and neuroplasticity
is occurring in the PVN during postpartum. This depends on the identity of the FJC positive
neurons. It is possible, though not confirmed, that these are oxytocin-producing neurons. Levels
of OT-ir neurons are elevated in the PVN on day 5 postpartum following hormone withdrawal
(e.g. Amaral, 2017). It has been suggested that estrogen receptors in the hypothalamus are
increased several days before birth in mammals (Meurisse et al., 2005). With this in mind,
estrogen could be upregulating oxytocin neurogenesis via elevated estrogen receptors sometime
directly before or after birth. In the present study, after five days postpartum, these heightened
levels of OT-ir neurons could be starting to degenerate. Perhaps after another week we would
expect the return to some baseline level of oxytocin neurons in the PVN.

Of course, the PVN is made up of more than just oxytocin neurons. It is just as likely that
the FJC positive cells are not the OT-ir neurons studied in Amaral, 2017. An alternate
explanation for the presence of FJC staining in the PVN is that cells are dying to make room,
physically or energetically, for the increase in OT-ir neurons by day 5 postpartum. Astrocytes are responsible for neuroenergetic regulation of cells as populations of neurons are constantly in competition for energy delivery, production, utilization, and storage (Bélanger, Allaman, & Magistretti, 2011; Pellerin et al., 2007). If oxytocinergic neurons need more energy for neurogenesis surrounding cells may enter a state of degradation via astrocyte regulation.

Though these two possible explanations for increased FJC staining in the withdrawn condition are plausible, it is just as likely that cell degeneration in the PVN is part of the wider picture of heightened neuroplasticity during the peripartum period. On a global scale, brains post-pregnancy show increased white matter plasticity via prolactin release to the point that pregnancy effectively reduces symptoms of demyelination in individuals with multiple sclerosis (Gregg et al., 2007). This is coupled with decreased gray matter specifically in regions related to maternal responses (Hoekzema et al., 2017). Changes in gray matter and white matter contribute to an overall reduction in brain size during and after pregnancy in first time mothers (Oatridge et al., 2002). Pregnancy induces changes in more than just neurons: changes in glia abound. For instance, astrocyte coverage of postsynaptic neurons is reduced in order to increase the number of new synaptic connections (Hillerer, Jacobs, Fischer, & Aigner, 2014). Perhaps the observed cell degeneration in the PVN is a result of glial cell death instead of neurons. Taken together, this literature suggests that there is a wide variety of possible explanations for cell degeneration in the PVN.

Regardless of the cause, the maternal brain is uniquely primed for maternal behavior and is able to conduct such behaviors in an efficient manner as a result of heightened neuroplasticity. It is reasonable to assume that dysregulation of this neuroplasticity would result in dysregulation of maternal behavior, which is a symptom of peripartum mood disorders. Related research has
been done on neuroplasticity in depression. Banasr et al. (2001) speculate that the regulation of neuroplasticity in the hippocampus is related to depressive symptoms. Current literature supports this theory. Specifically, individuals who experienced MDD or bipolar show posthumous selective neuronal loss in the PVN (Manaye et al., 2005). In the context of the present study, perhaps an animal model of postpartum depression may show similar increased levels of neurodegeneration in the PVN and therefore increased levels of FJC positive staining compared to typical pregnancy.

Many similar questions have arisen as a result of this experiment. One of the most pressing the confirmation of the current qualitative analysis with a full scale quantitative experiment. Once this is complete we can begin to identify these FJC positive neurons. Our current hypothesis is that cell degeneration in the PVN is related to the increased number of OT-ir neurons in the hormone withdrawn condition. We plan to double label oxytocin and FJC to determine if the neurons undergoing degeneration are in fact oxytocinergic. A second future experiment involves instead staining for neurogenesis: there may be an increased number of OT-ir neurons due to neuronal proliferation. We will perform a fluorescent IHC staining for oxytocin and doublecortin, a commonly-used marker of neurogenesis, to elucidate both the presence of neurogenesis in the PVN during postpartum as well as the identity of such cells. Furthermore, we intend to conduct PCR in tissue from the PVN for hormone sustained and hormone withdrawn conditions of a hormone-simulated pregnancy to confirm the increase of OT-ir neurons at the molecular level.

**Strengths and Limitations**

As with every research project, our study had its strengths and limitations. Firstly, the hamsters in this study were not actually pregnant. There are many aspects of pregnancy that,
though related to hormone levels, do not occur in a hormone-simulated pregnancy: copulation, gestation, birth, and mother-pup interactions. A genuine pregnancy model would allow for more ecological validity as well as the opportunity to study how maternal behavior after birth impacts the brain. However, a genuine pregnancy is harder to experimentally control and results in the sacrifice of numerous pups, something we are hesitant to do for a primarily exploratory study. In contrast, a hormone-simulated pregnancy allows for tight experimental control while preventing undue animal sacrifice. In fact, one of our greatest strengths was our experimental control of hormone levels across the hormone-simulated pregnancy. This permitted us to isolate and manipulate estrogen withdrawal at birth. Therefore, we know our data are a direct result of this independent variable. Without a hormone-simulated pregnancy, we could not be sure that our data was not the result of some other endogenous factor.

A second limitation is that the hormone doses used in the hormone-simulated pregnancy were not endogenous to Syrian hamsters. Doses of estradiol and progesterone in the present study were shown to simulate the endogenous hormone cycles seen in pregnancy in rats rather than in hamsters (Galea et al., 2001). As the average body weight of rats (approx. 250 g) is much greater than the average body weight of hamsters (approx. 150 g), and hamsters have higher naturally cycling levels of estradiol in comparison to rats, the hormone doses in the present study are most likely higher than what one would expect in a typical pregnant hamster. This being said, as this experiment is exploratory in nature, it is a reasonable strategy to use hormone levels that exceed the threshold needed to express maternal behavior in hamsters. Certainly, future research using more physiologically relevant levels or genuinely pregnant hamsters is needed to verify these results.
Our hormone-simulated pregnancy was based on a similar model developed by Galea et al. (2001). We endeavored to improve upon these methods in order to achieve the best results possible. Specifically, in Experiment 1 we chose to view the hormone withdrawn group as a model of typical pregnancy rather than as a model of postpartum depression. Every mother experiences a drop in estrogen but only 20 percent of women develop PPD. Therefore, the drop in estrogen is not inherently detrimental. We also added an oil control condition to better control for hormone levels during pregnancy that are shared by the hormone sustained and withdrawn conditions. Overall, we hope these modifications to an otherwise excellent model of hormone-simulated pregnancy in rodents will continue to serve the peripartum mood disorder literature.

**Conclusion**

The goal of the present study was to investigate the role of oxytocin in changes in neuroplasticity and behavior during the peripartum period. Using a hormone-simulated pregnancy, we were able to conclude that estrogen withdrawal leads to an increase in the density of OTRs in the dorsal RN of Syrian hamsters. Changes in OTR density in the dorsal RN, which regulates serotonergic activity and anxiety- and depression-related behavior in humans and rodents, may play a role in the development or sustainment of peripartum mood disorders. Related behavioral results, while inconclusive, support the theory that estrogen regulates locomotor function during peripartum. Furthermore, qualitative analysis revealed increased cell degeneration in the PVN of animals in the hormone withdrawn condition, suggesting that estrogen withdrawal also impacts neuroplasticity in typical pregnancy. Looking forward, we hope these findings contribute to a greater understanding of the role of hormones in neuroplastic regulation. And, above all, that this work will lead to better treatment options for those who suffer with peripartum mood disorders.


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Stamatakis, A., Kalpachidou, T., Raftogianni, A., Zografou, E., Tzanou, A., Pondiki, S., & Stylianoopoulou, F. (2014). Rat dams exposed repeatedly to a daily brief separation from the pups exhibit increased maternal behavior, decreased anxiety and altered levels of receptors for estrogens (ERα, ERβ), oxytocin and serotonin (5-HT1A) in their brain. *Psychoneuroendocrinology, 52C*, 212–228. https://doi.org/10.1016/j.psyneuen.2014.11.016
https://doi.org/10.1016/j.physbeh.2004.08.033


https://doi.org/10.1159/000381023


https://doi.org/10.1007/s00404-017-4313-0


Tables and Figures

Table 1

*One-Way ANOVAs Between Behavioral Measures and Hormone Condition*

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<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time in Closed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between</td>
<td>4140.66</td>
<td>2</td>
<td>2070.33</td>
<td>1.456</td>
<td>0.256</td>
</tr>
<tr>
<td>Within</td>
<td>29852.18</td>
<td>21</td>
<td>1421.53</td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td>33992.84</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Time in Open</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between</td>
<td>15888.05</td>
<td>2</td>
<td>7944.03</td>
<td>6.08</td>
<td>0.008*</td>
</tr>
<tr>
<td>Within</td>
<td>27429.29</td>
<td>21</td>
<td>1306.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43317.34</td>
<td>23</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>35921.60</td>
<td>2</td>
<td>17960.80</td>
<td>3.71</td>
<td>0.04*</td>
</tr>
<tr>
<td>Within</td>
<td>101780.61</td>
<td>21</td>
<td>4846.70</td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td>137702.21</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* Difference score was made up of time in open arms subtracted from time in closed arms of the EPM. Time in open arms of the EPM and difference score both significantly varied by hormone condition. There was no significant difference in time spent in closed arms of the EPM between hormone conditions. *p ≤ 0.05
Table 2

*Tukey’s HSD Post-Hoc for Total Time Spent in the Open Arms of the EPM Between Hormone Conditions*

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Difference</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Sustained</td>
<td>-10.10</td>
</tr>
<tr>
<td></td>
<td>Withdrawn</td>
<td>48.48</td>
</tr>
<tr>
<td>Sustained</td>
<td>Control</td>
<td>10.10</td>
</tr>
<tr>
<td></td>
<td>Withdrawn</td>
<td>58.93</td>
</tr>
<tr>
<td>Withdrawn</td>
<td>Control</td>
<td>-48.82</td>
</tr>
<tr>
<td></td>
<td>Sustained</td>
<td>-58.93</td>
</tr>
</tbody>
</table>

*Note.* Animals in the withdrawn condition spent significantly more time on the open arms of the EPM than animals in the control or sustained conditions. *p ≤ 0.05*
Table 3

*Tukey’s HSD Post-Hoc for the Difference Score of the EPM Between Hormone Conditions*

<table>
<thead>
<tr>
<th>Group</th>
<th>Group</th>
<th>Mean Difference</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Sustained</td>
<td>8.79</td>
<td>.97</td>
</tr>
<tr>
<td></td>
<td>Withdrawn</td>
<td>-77.31</td>
<td>.09†</td>
</tr>
<tr>
<td>Sustained</td>
<td>Control</td>
<td>-8.79</td>
<td>.97</td>
</tr>
<tr>
<td></td>
<td>Withdrawn</td>
<td>-86.11</td>
<td>.06†</td>
</tr>
<tr>
<td>Withdrawn</td>
<td>Control</td>
<td>77.32</td>
<td>.09†</td>
</tr>
<tr>
<td></td>
<td>Sustained</td>
<td>86.11</td>
<td>.06†</td>
</tr>
</tbody>
</table>

*Note.* Animals in the withdrawn condition had marginally larger differences between in time spent in open and closed arms of the EPM. † *p* ≤ 0.1; *p* ≤ 0.05
Table 4

Tukey’s HSD Post-Hoc for Velocity in the OFT

<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>Sustained</td>
<td>-1.33</td>
<td>.40</td>
</tr>
<tr>
<td>Withdrawn</td>
<td>1.38</td>
<td>.37</td>
</tr>
<tr>
<td>Sustained</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.33</td>
<td>.40</td>
</tr>
<tr>
<td>Withdrawn</td>
<td>2.71*</td>
<td>.04*</td>
</tr>
<tr>
<td>Withdrawn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-1.37</td>
<td>.37</td>
</tr>
<tr>
<td>Sustained</td>
<td>-2.71</td>
<td>.04*</td>
</tr>
</tbody>
</table>

*Note. *p ≤ 0.05
Table 5

*Tukey’s HSD Post-Hoc for Distance Traveled in the OFT*

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Difference (cm)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Sustained</td>
<td>-762.96</td>
</tr>
<tr>
<td></td>
<td>Withdrawn</td>
<td>797.08</td>
</tr>
<tr>
<td>Sustained</td>
<td>Control</td>
<td>762.96</td>
</tr>
<tr>
<td></td>
<td>Withdrawn</td>
<td>1560.05</td>
</tr>
<tr>
<td>Withdrawn</td>
<td>Control</td>
<td>-797.08</td>
</tr>
<tr>
<td></td>
<td>Sustained</td>
<td>-1560.05</td>
</tr>
</tbody>
</table>

*Note.* *p* ≤ 0.05
Table 6

*One-Way ANOVA Between Conditions for Time on Edge, Time in Middle, and Difference Score in OFT*

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>Mean Sq</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
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<td><strong>Time on Edge</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between</td>
<td>435.81</td>
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<td>217.90</td>
<td>0.348</td>
<td>0.710</td>
</tr>
<tr>
<td>Within</td>
<td>13130.95</td>
<td>21</td>
<td>625.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>13566.76</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Time in Middle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between</td>
<td>359.02</td>
<td>2</td>
<td>179.51</td>
<td>0.272</td>
<td>0.764</td>
</tr>
<tr>
<td>Within</td>
<td>13846.39</td>
<td>21</td>
<td>659.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14205.41</td>
<td>23</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>1478.69</td>
<td>2</td>
<td>739.35</td>
<td>0.294</td>
<td>0.748</td>
</tr>
<tr>
<td>Within</td>
<td>52855.70</td>
<td>21</td>
<td>2516.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* Difference score was time on edge subtracted from time in middle. Time on edge, time in middle, and difference score did not differ between hormone conditions. *p ≤ 0.05
### Table 7

*Mean OTR Density by Brain Region*

<table>
<thead>
<tr>
<th>Region</th>
<th>Group</th>
<th>n</th>
<th>M</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA</td>
<td>Oil</td>
<td>8</td>
<td>589.25</td>
<td>261.52</td>
</tr>
<tr>
<td></td>
<td>Sustained</td>
<td>8</td>
<td>521.14</td>
<td>56.03</td>
</tr>
<tr>
<td></td>
<td>Withdrawn</td>
<td>8</td>
<td>427.58</td>
<td>113.62</td>
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<tr>
<td>BNST</td>
<td>Oil</td>
<td>8</td>
<td>279.72</td>
<td>98.5</td>
</tr>
<tr>
<td></td>
<td>Sustained</td>
<td>8</td>
<td>166.96</td>
<td>82.28</td>
</tr>
<tr>
<td></td>
<td>Withdrawn</td>
<td>8</td>
<td>220.21</td>
<td>165.16</td>
</tr>
<tr>
<td>NAc Core</td>
<td>Oil</td>
<td>8</td>
<td>343.64</td>
<td>265.3</td>
</tr>
<tr>
<td></td>
<td>Sustained</td>
<td>8</td>
<td>218.77</td>
<td>44.61</td>
</tr>
<tr>
<td></td>
<td>Withdrawn</td>
<td>8</td>
<td>240.86</td>
<td>147.24</td>
</tr>
<tr>
<td>NAc Shell</td>
<td>Oil</td>
<td>8</td>
<td>329.20</td>
<td>272.75</td>
</tr>
<tr>
<td></td>
<td>Sustained</td>
<td>8</td>
<td>173.73</td>
<td>71.53</td>
</tr>
<tr>
<td></td>
<td>Withdrawn</td>
<td>8</td>
<td>202.98</td>
<td>121.94</td>
</tr>
<tr>
<td>Dorsal RN</td>
<td>Oil</td>
<td>8</td>
<td>156.25</td>
<td>87.35</td>
</tr>
<tr>
<td></td>
<td>Sustained</td>
<td>8</td>
<td>173.01</td>
<td>65.73</td>
</tr>
<tr>
<td></td>
<td>Withdrawn</td>
<td>8</td>
<td>105.59</td>
<td>37.33</td>
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</table>
Table 8

*One-Way ANOVA Between Oxytocin Receptor Density and Hormone Condition in the Dorsal RN*

<table>
<thead>
<tr>
<th>Group</th>
<th>SS</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>67005.49</td>
<td>2</td>
<td>33502.74</td>
<td>4.351</td>
<td>.026*</td>
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<tr>
<td>Within</td>
<td>61686.8</td>
<td>21</td>
<td>7699.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>228692.3</td>
<td>23</td>
<td>7699.37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* OTR density differed significantly based on hormone condition. *p ≤ 0.05*
Table 9

*One-Way ANOVA Between Oxytocin Receptor Density and Hormone Condition in the MA*

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>105410.01</td>
<td>2</td>
<td>52705</td>
<td>1.872</td>
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<tr>
<td>Within</td>
<td>591087.77</td>
<td>21</td>
<td>28147.03</td>
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</tr>
<tr>
<td>Total</td>
<td>696497.78</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* There was no significant difference in OTR density between hormone conditions. *p ≤ 0.05*
Table 10

One-Way ANOVA Between Oxytocin Receptor Density and Hormone Condition in the BNST

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>50919.44</td>
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<td>25459.72</td>
<td>1.746</td>
<td>.199</td>
</tr>
<tr>
<td>Within</td>
<td>306249.71</td>
<td>21</td>
<td>14583.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>357169.16</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. There was no significant difference in OTR density between hormone conditions. *p ≤ 0.05
### Table 11

*One-Way ANOVA Between Oxytocin Receptor Density and Hormone Condition in the NAc Core and Shell*

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NAc Core</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between</td>
<td>71054.85</td>
<td>2</td>
<td>35527.42</td>
<td>1.133</td>
<td>.341</td>
</tr>
<tr>
<td>Within</td>
<td>658417.03</td>
<td>21</td>
<td>3353.19</td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td>729471.88</td>
<td>23</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>NAc Shell</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between</td>
<td>109209.90</td>
<td>2</td>
<td>54604.95</td>
<td>1.736</td>
<td>.201</td>
</tr>
<tr>
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<td>660641.79</td>
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<td>31459.13</td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td>769851.69</td>
<td>23</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* There was no significant difference in OTR density between hormone conditions. *p ≤ 0.05*
Figure 1

*Exemplary Photos for FJC Qualitative Categories*

*Note.* A = Female 2, high (10+ FJC positive neurons); B = Female 6, intermediate (3-9 neurons); C = Female 9, low (0-2 neurons).
Figure 2

*Time Spent in the Open and Closed Arms of the EPM*

*Note. * $p \leq 0.05$
Figure 3

*Difference Between Time Spent in Open and Closed Arms For Each Hormone Condition*

Note. Animals in the withdrawn condition had marginally larger differences between in time spent in open and closed arms of the EPM. † \( p \leq 0.1; \) * \( p \leq 0.05 \)
Figure 4

*Mean Differences for Velocity in the OFT*

Note. *p ≤ 0.05*
Figure 5

*Mean Differences in Total Distance Traveled in OFT*

Note. *p ≤ 0.05*
Figure 6

*Autoradiographs of Oxytocin Receptor Density in the Dorsal Raphe Nucleus*

Note. (A) Oil control. (B) Hormone-sustained. (C) Hormone-withdrawn. (D) Atlas reference used to localize the dorsal raphe.
Figure 7

*Autoradiograph of Oxytocin Receptor Density in the Medial Amygdala*

*Note.* (A) Hormone-withdrawn. (B) Atlas reference used to localize the medial amygdala. The MA was scored bilaterally.
Figure 8

Autoradiograph of Oxytocin Receptor Density in the Nucleus Accumbens Shell and Core

Note. (A) Hormone-withdrawn condition. Left box is core, right box is shell. (B) Atlas reference used to localize the core and shell of the nucleus accumbens. The shell and core were scored bilaterally.
Figure 9

Autoradiograph of Oxytocin Receptor Density in the Bed Nucleus of the Stria Terminalis

Note. (A) Hormone-withdrawn condition. (B) Atlas reference used to localize the BNST. The BNST was scored bilaterally.