Blocking Oxytocin Receptors in the Dorsal Raphe Nucleus of Syrian Hamsters

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Abstract

Almost 20% of women suffer from peripartum depression and/or anxiety which has negative ramifications for the health of both mother and baby. Despite the gravity of this public health problem, effective treatments are lacking due to an incomplete understanding of the complex neurobiological mechanisms of these conditions. Previous research suggests that the intense fluctuations of ovarian hormones that occur in pregnancy and postpartum may render women particularly vulnerable to mood disturbances in this period. In particular, changes in oxytocin signaling in the brain, mediated by estrogen and progesterone, may contribute to the etiology of peripartum depression and anxiety. Previous work from our lab found increases in oxytocin-producing neurons in the paraventricular nucleus (PVN) of the hypothalamus and oxytocin receptors in the dorsal raphe nucleus (DRN), as well as increased anxiety-like behavior following a hormone simulated pregnancy. Given these results, this experiment sought to examine the effects of blocking oxytocin receptors in the DRN on anxiety behavior following a hormone-simulated pregnancy. Results from this pilot study suggest that blocking oxytocin signaling with an oxytocin receptor antagonist in the DRN during the postpartum period may impact anxiety-like behavior in hamsters. Further research is necessary to confirm the effects of OTA in the DRN during the peripartum period, but these results suggest that oxytocin signaling could be an important mechanism in peripartum anxiety and a potential route for the development of treatments.
Blocking Oxytocin Receptors in the Dorsal Raphe Nucleus of Syrian Hamsters

Postpartum Depression and Anxiety

Pregnancy and the postpartum period are often viewed with expectations of maternal bliss. However, these are times of intense changes at the behavioral, physical, and social levels for new mothers, which can render women particularly vulnerable to psychological struggles (Brummelte & Galea, 2010; Brunton & Russell, 2008; Hendrick, Altshuler, & Suri, 1998). Anywhere between 50-85% of women experience “baby blues” which spans approximately the first ten postpartum days. Symptoms include crying, anxiety, sadness, and dysregulated sleep, but this typically resolves with time without treatment (Cohen et al., 2010). A smaller but significant subset of women experience more persistent postpartum affective disorders including postpartum anxiety (PPA) and postpartum depression (PPD) (Fairbrother, Janssen, Antony, Tucker, & Young, 2016; Le Strat, Dubertret, & Le Foll, 2011).

Though these peripartum mood disorders certainly occur at significant rates, issues of reporting and diagnosis specifications make the exact prevalence uncertain. Estimates of postpartum depression incidence range anywhere from 7-19% of parturient women, while estimates of postpartum anxiety range from 8-12%, and approximately 2-5% of women suffer from both PPA and PPD (Fairbrother, Janssen, Antony, Tucker, & Young, 2016; Goodman, Watson, & Stubbs, 2016; Le Strat, Dubertret, & Le Foll, 2011; O’Hara, 2009; Vesga-López et al., 2008). Measurements of the incidence of these disorders vary greatly due to methodological heterogeneity with differences in research populations, measurement scales, and diagnostic criteria (Brummelte & Galea, 2016; Goodman, Watson, & Stubbs, 2016; O’Hara & McCabe, 2013). Furthermore, rates may be underestimated due to underreporting of PPD and PPA symptoms due to stigma or lack of effective screening (Hendrick, 2003).
Postpartum depression is not identified as a separate condition in the Diagnostic and Statistical Manual of Mental Disorders, but instead is classified by the standards of Major Depressive Disorder with an onset in the peripartum period which is classified as anytime from pregnancy through the fourth week postpartum (American Psychiatric Association, 2013). Though there is evidence to suggest differences in etiology and neurobiology of MDD and PDD, research has found that they are largely symptomologically compatible. Symptoms across the two conditions include lassissitude, changes in appetite, feelings of worthlessness, and sleep disturbance with additional incidences of mood lability and fluctuations and over-concern about the infant specific to PPD (O’Hara & McCabe, 2013). Additionally, research suggests that women with postpartum, as opposed to non-postpartum, depression are more likely to present with anxious features and may take longer to respond to pharmacological treatment (Hendrick, Altshuler, Strouse, & Grosser, 2000). Postpartum anxiety is not formally defined by the DSM-5. It is characterized by the general symptoms of general anxiety disorder such as intense concern and worry, sleep disturbances, irritability, and fatigue (American Psychiatric Association, 2013). These symptoms pose significant challenges in daily activity for women who suffer from PPA and PPD and their children and families, making this an important public health concern.

Given the prevalence of PPD and PPA, some research has sought to identify social and demographic categories of women that face an increased likelihood of developing these conditions. Women with a history of psychiatric disorders, particularly episodes of MDD or GAD show increased rates of postpartum mood disorders such as PPD and PPA (Goodman, Watson, & Stubbs, 2016; Le Strat, Dubertret, & Le Foll, 2011; Martini et al., 2015; O’Hara & McCabe, 2013; Vesga-López et al., 2008). Darcy et al. found that in a sample of working mothers with infants, mothers who reported a younger age of motherhood, being a minority race, living in poverty, being unmarried, or having less than a college education were more likely to
experience major depressive symptoms in the postpartum period. Potentially stressful
demographic factors such as unstable relationships, insecure socioeconomic status, and
unplanned pregnancy are consistently correlated with increased incidence of postpartum
depression and anxiety (Le Strat, Dubertret, & Le Foll, 2011; Martini et al., 2015; Norhayati, Nik
found that the temperament traits of harm avoidance and self-directedness were significantly
higher in women with PPD than healthy postpartum controls, suggesting that some personality
factors may also render women more vulnerable to postpartum mood disorders. These pre-
existing stressors and predispositions may only augment the detrimental symptoms that affect
women with postpartum mood disorders.

The impacts of postpartum anxiety and depression reach beyond the mother’s affective
symptoms. Research has found that mothers with PPD demonstrate insensitivity to infant
distress, hostile parenting styles, internalizing, externalizing, and lack of engagement with the
infant (Goodman et al., 2011; Murray, Cooper, & Fearon, 2014). These disturbances in mother-
infant bonding can have serious implications for the child’s development and mental health. PPD
symptoms can also impact several physical health and safety practices. Maternal depressive
symptoms are correlated with early cessation of breastfeeding despite evidence that continuation
of breastfeeding is associated with lower depression scores (Figueiredo, Canário, & Field, 2014).
Mothers with PPD are less likely to keep their children up to date with wellness visits and
vaccinations and may have decreased attention to safety practices in the home (Field, 2010).

Effects of postpartum mood disorders can also extend past mother-child relations to
impact the child’s physical and cognitive health and development. Research has found that
maternal postpartum depression leads to an increased likelihood of the child being underweight
and having stunted first year growth and impaired growth into early childhood (Fárias-Antúnez,
Xavier, & Santos, 2018). Some evidence suggests that maternal depression is associated with increased gastrointestinal distress and greater levels of pain and discomfort in offspring (Darcy et al., 2011). Children of mothers with postpartum mood disorders demonstrate atypical emotional processing and attentional behaviors in early development (A. Porto, L. Nunes, & Nelson, 2016). Further into childhood, children of mothers with PPD showed lower ego-resiliency, social competence, and decreased verbal competency in early school-age development (Kersten-Alvarez et al., 2012). The diversity of these implicated outcomes highlights how postpartum depression and anxiety can harm mother and child in both the immediate and long-term future.

Despite these profound effects, consistently effective treatments for postpartum depression and anxiety are lacking. Many women are reluctant to utilize common drug treatments for major depressive symptoms. Selective serotonin reuptake inhibitors (SSRIs) have been found to increase risk of delayed motor and cognitive development, ADHD, and heart defects in infants when taken during pregnancy (Pariante, 2015). Similarly, some evidence suggests children of women taking SSRIs while breastfeeding face developmental disturbances (Wisner, Perel, & Findling, 1996). Furthermore, research into the efficacy of antidepressant use for postpartum depression has generated inconclusive results (Sharma & Sommerdyk, 2013). Some studies suggest that women with postpartum depression respond more slowly and less effectively to pharmacological treatments (Hendrick, Altshuler, Strouse, & Grosser, 2000). Evidence is mixed for the efficacy of psychotherapy, a common non-drug treatment of depressive symptoms. In a study by O’Hara et al. (2000) women receiving interpersonal psychotherapy demonstrated significant improvement in depressive symptoms. However, other studies have found that psychotherapy had no significant impacts on maternal behavior, infant development, and later childhood outcomes (Murray, Cooper, Wilson, & Romaniuk, 2003). Further research is necessary to elucidate both the efficacy of treatment and potential risk factors.
for mother and child in the psychopharmacological treatments of affective disorders in the peripartum period. In the interim, many women forgo treatment altogether, creating a public health problem (Cohen et al., 2010).

**Potential Mechanisms of PPD and PPA: Hormonal Fluctuations in Pregnancy and Postpartum**

There are several possible explanations in the peripartum mood disorder literature ranging from the genetic to systemic levels. Given the unique patterns of hormonal fluctuations in the peripartum period, many suggested etiologies start from an understanding of these changes and how they may implicate certain aspects of brain and behavior (Bloch et al., 2000; Hendrick, Altshuler, & Suri, 1998). Research has pointed to several possible mechanisms based on the hormonal changes that occur in pregnancy and the postpartum period. Evidence suggests that women face an increased susceptibility to affective disorders surrounding periods of hormonal fluctuation including puberty, reproduction, and menopause (Schiller, Meltzer-Brody, & Rubinow, 2015; Soares & Zitek, 2008). This has been suggested as a potential explanation for why women experience depressive episodes at significantly higher rates than men starting in the adolescent period (Albert, 2015; Altemus, Sarvaiya, & Epperson, 2014). Pregnancy and the postpartum period represent some of the most dramatic hormonal changes that a woman can experience in her lifetime (Brummelte & Galea, 2010). Unsurprisingly, this coincides with increased prevalence of depressive and anxiety symptoms (Fairbrother, Janssen, Antony, Tucker, & Young, 2016; Wisner et al., 2013). Therefore, examinations of the trajectory of hormonal changes across pregnancy and postpartum has informed several hypotheses of potential mechanisms of postpartum depression and anxiety. The following sections identify some key hormones that may be involved in PPD and PPA, how they fluctuate across pregnancy and the
postpartum period, and how these changes may play a role in the development of postpartum affective disorders.

**Ovarian hormones.** Two primary hormones involved in pregnancy, delivery, and the postpartum period are estrogen and progesterone. Estrogens and progesterone are produced by the ovaries and the placenta and rise sharply over the course of pregnancy. Estrogen is involved in fetal development and the growth of the mother’s uterus (Hendrick, Altshuler, & Suri, 1998). Estradiol and estriol, biologically active forms of estrogen, increase by 100 and 1000-fold respectively during pregnancy due to production from fetal metabolic activity (Hendrick, Altshuler, & Suri, 1998). Progesterone is involved in the smoothing of the uterine muscle wall and softens cartilage and joints in preparation for delivery (Bridges, 2015). Progesterone levels increase 10-fold by the third trimester (Hendrick, Altshuler, & Suri, 1998). With the removal of the placenta at birth, both estrogen and progesterone levels decrease drastically in the postpartum period, reaching pregravid levels by the fifth postpartum day (Bloch, Daly, & Rubinow, 2003).

Given their functional significance in gestation, delivery, and maternal care behavior and their dramatic fluctuations in the peripartum period, estrogen and progesterone have been implicated as potential mediators of PPD and PPA. The ‘ovarian-steroid-withdrawal hypothesis,’ suggests that postpartum depression is in some part caused by the steep decrease in ovarian hormones following delivery. Some of the first evidence for this potential mechanism came from a study by Bloch et al. (2000) that simulated pregnancy levels of hormones in euthymic women, some with a history of postpartum depression and some without, and then withdrew these steroids. In this study, women with a history of postpartum depression showed significantly more depressive symptoms after hormone withdrawal compared to controls. Similarly, Frokjaer et al. (2015) manipulated estradiol levels in healthy females and found that withdrawal of estradiol was associated with depressive symptoms. These findings together suggest that estrogen and
progesterone play a role in postpartum depression, and that women with a history of postpartum depression might be particular sensitive to these fluctuations.

From a clinical perspective, postpartum depression patients with low levels of serum estradiol have been successfully treated with 17beta-estradiol (Ahokas, Kaukoranta, Wahlbeck, & Aito, 2001). Though human studies are still limited, animal models using hormone-simulated pregnancy in rats have also found that estradiol treatments reduce depressive-like symptoms (Galea, Wide, & Barr, 2001; Green, Barr, & Galea, 2009). Evidence from rodent models will be explored in subsequent sections. The evidence of depressive symptoms following hormone withdrawal and the examples of successful amelioration of depressive symptoms using estradiol suggest that the fluctuations of ovarian hormones is an important factor in the development of postpartum depression.

**Oxytocin.** Oxytocin is a neuropeptide primarily synthesized in the paraventricular nucleus (PVN) of the hypothalamus which is implicated in the delivery, lactation, and the regulation of maternal social behaviors (Bridges, 2015; Insel, 2010; Leng, Meddle, & Douglas, 2008; Lonstein, 2007). Peripheral oxytocin is secreted from the posterior pituitary into the bloodstream and is responsible for the uterine contractions of parturition and the milk-let down reflex (Leng, Meddle, & Douglas, 2008). Oxytocin is also projected centrally to several neural targets including the caudal brainstem and the spinal cord, acting as a neurotransmitter (Lee, Macbeth, Pagani, & Young, 2009). In most women, plasma oxytocin levels increase over the course of pregnancy, peaking with delivery, and decreasing in the postpartum period but this pattern is highly variable (Jobst et al., 2016; Prevost et al., 2014). One explanation for oxytocin fluctuations, is that oxytocin signaling is mediated by ovarian hormones. Evidence suggests that oxytocin receptor expression is modulated by estrogen which fluctuates dramatically in the peripartum period (Acevedo-Rodriguez, Mani, & Handa, 2015; Kimmel, Clive, & Gispen, 2016).
Based on its relationship with estrogen and critical role in facilitating childbirth, lactation, and an array of maternal behaviors, oxytocin has been studied as a potential factor in the modulation of postpartum depressive and anxiety behaviors (Kim et al., 2013). Some evidence suggests that central oxytocin moderates the stress response, thereby exerting an anxiolytic effect (Leng, Meddle, & Douglas, 2008; Neumann, Torner, & Wigger, 1999). Additionally, increased oxytocin during childbirth targets several areas of the brain implicated in maternal care including the supraoptic nucleus of the hypothalamus, medial preoptic area of the hypothalamus, bed nucleus of the stria terminalis, olfactory bulbs, the raphe nuclei, and the medial amygdala (Lee, Macbeth, Pagani, & Young, 2009; Leng, Meddle, & Douglas, 2008). Though the mechanisms of central oxytocin in the peripartum are not particularly well understood, some research has examined plasma oxytocin levels as a potential predictor of depression and anxiety symptoms in the postpartum period. Skrundz et al. (2011) found that lower plasma oxytocin levels in mid-pregnancy predicted depressive symptoms at 2 weeks postpartum while controlling for various sociodemographic and birth-outcome variables. Jobst et al. (2016) also tracked plasma oxytocin levels across pregnancy and the postpartum, finding that while oxytocin levels increased in all women from late pregnancy to 6 months postpartum, these levels decreased for women with postpartum depressive symptoms from the 38th week of pregnancy to 2 days postpartum. Though plasma oxytocin levels do not provide much information about the exact neurobiological mechanisms, these findings suggest that differences in oxytocin levels are likely a factor in postpartum affective disorders.

**Cortisol and HPA axis dysregulation.** It has been suggested that changes in estrogen may alter HPA function which renders this system vulnerable to dysregulation in the peripartum (Schiller, Meltzer-Brody, & Rubinow, 2015). During pregnancy, cortisol levels rise to almost three times nonpregnant levels due to both the rise in maternal estrogen levels and placental
secretion of CRH (Jung et al., 2011; Mastorakos & Ilias, 2003). In late pregnancy, the increased plasma cortisol levels feed back to the hypothalamus to downregulate the maternal secretion of CRH. This leads to hyporesponsivity of the HPA axis to stress stimuli in late pregnancy (Kammerer, Adams, Castelberg, & Glover, 2002). In the postpartum period, the HPA axis maintains this hyporesponsivity for up to three weeks while plasma cortisol levels return to pre-pregnancy levels (Duthie & Reynolds, 2013; Hendrick, Altshuler, & Suri, 1998; Mastorakos & Ilias, 2003).

In addition to its changes in the peripartum period, HPA axis dysregulation is one of the most prominent biological changes in cases of non-postpartum depression and anxiety (Brummelte & Galea, 2010; Nestler et al., 2002). External sources of stress affect hormone levels in the HPA axis and impact the feedback mechanisms of this system. In both major depression and postpartum depression, high life stress or previous trauma are factors that increase risk (Le Strat, Dubertret, & Le Foll, 2011; Martini et al., 2015; Yim et al., 2009). These factors together have led some researchers to suggest that HPA dysregulation plays a role in the etiology of postpartum depression.

Several studies have found dysregulation of the HPA axis in postpartum depression. Jolley et al. (2007) compared HPA axis regulation of adrenocorticotropic hormone (ACTH) and cortisol in depressed and non-depressed postpartum women. In a healthy HPA axis, rising ACTH levels trigger an increase in cortisol levels; in depressed postpartum women, however, there was no relationship in these levels, with higher ACTH and lower cortisol levels than controls at six and twelve weeks postpartum (Jolley, Elmore, Barnard, & Carr, 2007). Some research suggests that higher levels of placental CRH during pregnancy is a significant predictor of PPD symptoms 2-3 months postpartum, though others have failed to replicate these results (Yim et al., 2009). During a hormone-simulated pregnancy, women with a history of PPD had higher depression
symptoms and greater cortisol responses than healthy controls (Bloch et al., 2005). Though there are still many gaps in the understanding of HPA axis function in pregnancy and postpartum, research suggests that differences HPA axis hormones and how they interact with other systems may impact postpartum depressive and anxiety symptoms.

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

Studies of the neurobiological mechanisms of postpartum depression and anxiety in humans are limited due to methodological and sample issues. Studying the underlying endocrine and neurotransmitter differences in the postpartum brain requires more invasive techniques and manipulations. However, some studies have utilized the less invasive technique of functional magnetic resonance imaging (fMRI) to study the neural correlates of postpartum depression and anxiety. Some fMRI studies have examined resting state functional connectivity or the default mode network which is involved in emotional regulation and cognition without stimuli. This system, including the posterior and anterior cingulate cortices (ACC), precuneus, and parietal cortex, has been connected with some of the behavioral symptoms of depression (Chase, Moses-Kolko, Zevallos, Wisner, & Phillips, 2014; Pawluski, Lonstein, & Fleming, 2017). These studies have found diminished resting-state connectivity between the anterior cingulate cortex, dorsolateral prefrontal cortex, amygdala, and certain areas of the hippocampus for subjects with postpartum depression compared to healthy controls (Chase, Moses-Kolko, Zevallos, Wisner, & Phillips, 2014; Deligiannidis et al., 2013).

Additional fMRI research has examined neural correlates of postpartum depression in the presence of emotionally-valanced and infant-specific cues (Silverman et al., 2011, 2007; Wonch et al., 2016). Evidence suggests that subcortical motivation-related regions of the brain including the striatum, amygdala, hypothalamus and hippocampus, and cortical regions involved in social cognition, including the ACC, insula, and frontal cortex, interact with endocrine systems to
moderate stress and maternal behaviors in response to infant stimuli (Swain, Kim, & Ho, 2011). Several studies have found decreased striatal response to rewarding stimuli in mothers with postpartum depression (Moses-Kolko et al., 2011; Silverman et al., 2007). Additionally, higher levels of postpartum depression and anxiety symptoms are correlated with decreased amygdala activation to infant cries and other negatively valanced stimuli (Laurent & Ablow, 2012; Moses-Kolko et al., 2010; Silverman et al., 2011). These findings, though limited, indicated that there are some unique neural correlates of postpartum depression compared to healthy postpartum women and cases of non-postpartum depression. However, in order to gain a better understanding of the neurobiological mechanisms of postpartum affective disorders, researchers have turned to more invasive methods that are only feasible in animal models.

**Quantifying Depressive and Anxiety Behavior in Animal Models**

**Measures of depressive behavior.** As animals cannot self-report depressive symptoms or exhibit psychosocial symptomology in the same ways as humans, researchers have developed behavioral tests to capture various aspects of depressive like behavior. One common measure is the Forced Swim Test, which was developed as a measure of depressive-like despair that was responsive to antidepressant treatments (Porsolt, Le Pichon, & Jalfre, 1977). In this model, rats are placed in an inescapable cylinder of water and forced to swim for a predetermined test time. Active behavior (swimming and climbing) and passive behavior (immobility) are scored as measures of depressive-like despair and learned-helplessness which are both common symptoms of major depression and postpartum depression (APA, 2013.; Hendrick, Altshuler, Strouse, & Grosser, 2000; Slattery & Cryan, 2012). The Forced Swim Test has been validated within other animal models (Can et al., 2012) as well as by the effects of antidepressant drugs on performance (Lucki, 1997). Though some critiques (eg: Molendijk & de Kloet, 2015) suggest that this test is
more of a measure of an adaptive switch from active to passive behavior to conserve energy, consistent results suggest that it is one of the most reliable measures of depressive behavior.

The Sucrose Preference Test is another common measure used in animal models of depression which measures anhedonia, or lack of pleasure, another common symptom of PPD and major depression (APA, 2013). Animals are given the choice between 1-2% sucrose water and regular water, and their consumption is recorded (Navarre, Laggart, & Craft, 2010). This model was developed based on the observation that healthy rats typically exhibit a preference for sucrose water over regular water, while rats exposed to chronic stress demonstrate decreased consumption of the sucrose water (Papp, Willner, & Muscat, 1991). The application of this model to depression has been validated by studies that show that antidepressant treatments reverse the anhedonic decrease in sucrose preference (Muscat, Papp, & Willner, 1992).

**Measures of anxiety behavior.** Like depressive behavior, anxiety behavior is measured in animal models using behavior testing techniques. One of the most common models is the Open Field Test in which an animal is placed in the center of a walled open field and locomotor activity is measured (Stanford, 2007). Originally developed by Hall (1932) as a measure of motivation, this test is based on the understanding that rodents are averse to brightly lit open spaces, therefore increased time spent in the center of the maze has been associated with decreased anxiety (Stanford, 2007). Measurements of distance moved, time spent moving, and locations of movements are commonly used to assess not only anxiety but also general locomotion. This can be useful in ascertaining that previous treatments that the animal underwent, such as surgery or drug injections, are not simply reducing general locomotive abilities (Gould, Dao, & Kovacsics, 2009; Stanford, 2007). A similar, but slightly more complex model of anxiety behavior is the Elevated Plus Maze. Originally developed by Pellow et al. (1985), in this test, animals are placed in a plus-shaped maze with two open and two enclosed
arms and their time spent in each of the arms is recorded. Anxious rats spend enter the open arms fewer times and significantly less time in the open arms of the maze (Pellow, Chopin, File, & Briley, 1985; Walf & Frye, 2007). Both of these measures of anxiety have been validated by tests of anxiolytic drugs that reduce the demonstrated anxiety behaviors (Pellow & File, 1986; Stanford, 2007; Walf & Frye, 2007).

**Animal models of postpartum depression and anxiety**

Due to the sampling and methodological limitations of human studies of postpartum anxiety and depression, researchers have turned to animal models to attempt to uncover the etiology and deeper neural mechanisms of these disorders. Researchers have developed several different animal models of postpartum depression and anxiety based on potential etiologies. The chronic corticosterone treatment model, developed by Brummelte et al. (2010), mimics the increased cortisol levels observed in major depression by treating pregnant and postpartum rats with either low or high doses of corticosterone. This model demonstrates that high levels of corticosterone in the postpartum period increased depressive behaviors as measured by the forced swim test and measures of maternal care (Brummelte & Galea, 2010). Other models focus on the stress-induced components of depressive and anxiety-like behavior. Researchers have developed a chronic social stress model (Nephew & Bridges, 2011), gestational stress model (Hillerer, Reber, Neumann, & Slattery, 2011), and learned helplessness model (Kurata, Morinobu, Fuchikami, Yamamoto, & Yamawaki, 2009) that attempt to capture the psychological symptoms of postpartum depression (Ming & Shinn-Yi, 2016). One of the most commonly studied models is the hormone withdrawal model utilizing a hormone-simulated pregnancy which will be utilized in this study and therefore discussed more fully.

**Ovarian Steroid Withdrawal Model.** Of particular relevance to this study is the hormone withdraw model, which is based on the hypothesis that the dramatic changes in ovarian
hormones in pregnancy and the postpartum period contribute to postpartum depression (Bloch, Daly, & Rubinow, 2003; Bloch et al., 2000). Galea et al. (2001) were the first to develop this hormone-simulated pregnancy model in Wistar rats. Female rats are ovariectomized to remove the endogenous source of ovarian hormones and injected daily with doses of estradiol benzoate and progesterone to mimic the hormonal levels of a typical pregnancy. In the first 16 days, the rats receive lower doses of estradiol benzoate and higher doses of progesterone; days 17 to 23 the estradiol increases as occurs in late pregnancy. Animals are then divided into two groups: The hormone-withdrawn group stops receiving these injections for days 24-27, simulating the drastic postpartum drop, while the hormone-sustained group continues to receive hormone injections (Galea, Wide, & Barr, 2001). In this way, one can differentiate between whether the dramatic rise and subsequent drop in ovarian hormones is necessary for changes in brain and behavior, or if the rise alone is sufficient.

During the simulated postpartum period, the rats underwent behavior testing for depressive symptoms using the forced swim test and open field test. Hormone-withdrawn rats show increased immobility and decreased struggling and swimming behaviors in the forced swim test compared to hormone-sustained and control rats (Galea, Wide, & Barr, 2001). These findings suggest that withdrawal from these ovarian hormones may contribute to depressive symptoms in the postpartum period. However, the hormone-withdrawn rats were more active in the open field test than hormone-sustained and control rats, suggesting that rats in this simulated postpartum may have fewer anxiety-like behaviors.

Several studies have utilized this hormone-simulated pregnancy model to study the mechanisms of postpartum depression. Research has replicated the findings of the forced swim test with hormone-withdrawn rats showing increases in immobility relative to controls at varying time intervals in the simulated postpartum period (Fernandez, Grizzell, Philpot, & Wecker, 2014;
Schiller, O’Hara, Rubinow, & Johnson, 2013; Stoffel & Craft, 2004). Further extensions have utilized the sucrose-preference model of anhedonic behavior to test for depressive-symptoms in the simulated postpartum. Green et. al (2009) found that rats in the postpartum period following a hormone-simulated pregnancy display decreased sucrose consumption compared to ovariectomized controls. Similar to Galea et. al (2009), research has found that rats in the simulated postpartum do not show increased anxiety behavior as measured by the Elevated Plus Maze (Stoffel & Craft, 2004).

In a study comparing hormone-simulated postpartum rats and rats who had previously been pregnant, Navarre et al. (2010) found that while rats in the hormone-simulated postpartum displayed decreased sucrose preference, previously pregnant rats showed only a slight and short-lasting decrease in sucrose preference. As previously mentioned, there are many other hormonal changes that occur in conjunction with the dramatic drop in estradiol during pregnancy and the postpartum period, such as changes in oxytocin and cortisol. Navarre et. al (2010) suggest that these changes may moderate the negative affective changes of this postpartum period.

Recent research in our lab has also adapted the hormone simulated pregnancy model to Syrian hamsters (Amaral, 2017; Bodie, 2017; D’Antonio, 2017; Lee, 2017). Unlike Galea et al., this study found no significant difference between hormone-sustained and hormone-withdrawn hamsters in the sucrose-preference test. Additionally, further research with the hormone-simulated pregnancy model in Syrian hamsters found no change in the open field test, but increased anxiety in the elevated plus maze (Benedetto, 2018; Heaton 2018; Levine 2018; Taveras, 2018). These differences in these results may be in part due to the differences in pregnancy length between rats, which have a 26-27 day gestation, and hamsters, which have a 16-18 day gestation (Galea, Wide, & Barr, 2001; Viswanathan & Davis, 1992).

**Neurobiological Mechanisms of Oxytocin**
Oxytocin production is affected by the dramatically fluctuating estrogen levels during pregnancy and the postpartum period, making it a subject of interest in the study of postpartum mood disorders (Hendrick, Altshuler, & Suri, 1998; Kim, 2013; Moura, Canavarro, & Figueiredo-Braga, 2016). Various studies have found that oxytocin-deficient mice exhibit anxiety-like behavior and reduced maternal behaviors, suggesting that oxytocin activation plays a role in behaviors implicated in PPD and PPA (Mantella, Vollmer, Li, & Amico, 2003; Rich, deCárdenas, Lee, & Caldwell, 2014). While human studies are limited to studies of plasma levels of oxytocin and intranasal administration, animal models can reach specific neural targets. Using an intracerebroventricular infusion of an oxytocin antagonist, Neumann et al. (1999) found that endogenous oxytocin moderated the responses of the HPA axis to stressors and affected anxiety behavior differently for virgin and peripartum-state rats. This study measured corticosterone and corticotropin secreted into the blood following this antagonist infusion, allowing for both a central and peripheral perspective in the role of the HPA axis.

Further studies have targeted specific brain areas with oxytocin receptor antagonists. This model, which is utilized in the present study, allows for researchers to examine changes in behavior when the increased oxytocin signaling of the postpartum period is disrupted. Figueira et al., (2008) studied the anxiety behavior of postpartum and virgin rats when injected with an oxytocin receptor antagonist into the ventrocaudal periaqueductal gray (cPAGv). Blocking these oxytocin receptors significantly increased anxiety behavior in the elevated plus maze for postpartum rats only, and subsequent oxytocin infusions reversed this effect (Figueira, Peabody, & Lonstein, 2008). Given the proximity of the cPAGv to the DRN, these findings could be related to the same population of cells containing oxytocin receptors that are of particular interest in the present study. A similar study using a gestational stress model focused on the paraventricular nucleus (PVN). This study found that rats exhibiting depressive symptoms in the
postpartum period had decreased levels of mRNA and peptide levels of oxytocin in the PVN. Injecting oxytocin in the PVN reversed these depressive behaviors, suggesting a potential antidepressant role for oxytocin particularly in the PVN (Wang, 2018). The role of the PVN is particularly relevant to the previous work of the Been lab and the extensions explored in this study.

Recent research adapting the hormone-simulated pregnancy model to Syrian hamsters found significantly more oxytocin immunoreactive neurons in the paraventricular nucleus of the hypothalamus in hormone-withdrawn hamsters compared to hormone-sustained hamsters (Amaral, 2017; Bodie, 2017; D’Antonio, 2007; Lee, 2017). Though central oxytocin is also produced in the supraoptic nucleus of the hypothalamus (SON), research found no difference in oxytocin immunoreactive neurons in the SON between hormone-withdrawn and hormone-sustained hamsters (Foster, Heaton, & Been, 2017). These findings further validate the importance of oxytocin producing neurons specifically in the PVN in the moderation of anxiety and depressive behaviors in the postpartum period.

Further research extended these findings to examine the oxytocin-receptor (OXTR) density in estrogen-sensitive oxytocin efferents of the PVN (Benedetto, Heaton, Levine, Travers, Ross, Albers, & Been, 2018). Using the same Syrian hamster model, this research found that hormone-withdrawn hamsters had significantly greater OXTR density in the dorsal raphe nucleus that hormone-sustained or controls. Furthermore, the hormone-withdrawn females demonstrated increased anxiety behavior in the elevated plus maze (Benedetto, Heaton, Levine, Travers, Ross, Albers, & Been, 2018). Importantly, more than 50% of serotonin (5HT) neurons in the DRN contain oxytocin receptors, leading the researchers to hypothesize a possible oxytocin-serotonin pathway in the modulation of postpartum anxiety. In another study of oxytocin receptor mechanisms in anxiety behavior, Yoshida et al. (2009) used a mouse model
where the oxytocin receptor gene was replaced with a variant of yellow fluorescent protein, Venus cDNA. Examining the Venus expression, researchers found that about 50% of tryptophan hydroxylase-immunoreactive neurons in the raphe-nucleus were positive for Venus expression. This suggests that oxytocin may modulate the release of serotonin in this brain region. Additionally, this study found that infusions of oxytocin stimulated serotonin release in the median raphe nucleus and reduced anxiety behaviors; a 5-HT2A/2C receptor antagonist reversed the anxiolytic effects, suggesting that oxytocin receptor activation in serotonergic neurons is necessary for the anxiety-reducing effects of oxytocin (Yoshida et al., 2009). Taken together, these findings suggest that postpartum estrogen withdrawal may increase oxytocin signaling between the PVN and the raphe nuclei, thereby affecting serotonin release and affecting anxiety behaviors.

The Present Study

Research in both human and animal models suggests that the significant increase and subsequent drastic decrease in ovarian hormones in pregnancy and the postpartum period contributes to the development of postpartum affective disorders. Oxytocin has been specifically implicated as an important moderator of anxiety-like and depressive-like behaviors both within and outside of the postpartum period. Previous research in our lab found an increase in oxytocin immunoreactive neurons in the PVN in the hormone-withdrawn condition following a hormone-simulated pregnancy in Syrian hamsters (Amaral, 2017; Bodie, 2017; D’Antonio, 2007; Lee, 2017). Further extensions also found an increase in OXTR in the DRN, an estrogen sensitive oxytocin efferent of the PVN. These findings were coupled with an increase in anxiety behavior in the hormone-withdrawn condition (Benedetto, Heaton, Levine, Travers, Ross, Albers, & Been, 2018). One potential explanation is that the sudden estrogen withdrawal in the postpartum...
period increases oxytocin signaling between the PVN and the DRN, thereby moderating 5HT release and leading to changes in anxiety behavior.

Given the increased signaling in this PVN-DRN pathway and previous research suggesting that oxytocin receptor activation is implicated in moderation of anxiety behavior (Figueira, Peabody, & Lonstein, 2008; Wang, 2018; Yoshida et al., 2009), this study will explore the effects of blocking oxytocin receptors in the DRN in the peripartum period on anxiety-like behavior. Using a hormone-simulated pregnancy model in Syrian hamsters, this study will measure changes in anxiety behavior in hormone-withdrawn and hormone-sustained animals treated with an oxytocin receptor antagonist in the DRN. We hypothesize that increased oxytocin receptor activation in the DRN in the peripartum period leads to increased anxiety-like behavior. Therefore, we predict that blocking oxytocin receptors in the DRN will lead to decreased anxiety behavior in the simulated postpartum.

**Method**

**Subjects**

32 adult female Syrian hamsters were purchased from the Charles River Laboratories (Wilmington, MA, USA) to be used as subjects in this study. Hamsters were divided into four groups based on hormone condition and oxytocin receptor antagonist condition. The four groups included: hormone-sustained with OTA, hormone sustained without OTA, hormone-withdrawn with OTA, and hormone-withdrawn without OTA. All hamsters were housed individually in the Haverford College vivarium in accordance with protocols for laboratory animal use. Temperature in the housing room was held at approximately 22 degrees Celsius and lights are on a reverse light-dark cycle (14 dark:10 light). Food and water were available *ad libitum*. Testing occurred in the dark phase for all animals. Procedures were carried out according to the Guide
for the Care and Use of Laboratory Animals and approved IACUC protocols, and all efforts were made to minimize discomfort and suffering of the animals.

**Procedure**

**Ovariectomy.** All hamsters were bilaterally ovariectomized to remove the endogenous source of ovarian hormones. Hamsters were administered butorphanol (5 mg/kg) and anesthetized with 2-5% isoflurane in 100% oxygen. Surgical sites on the flank were shaved and sterilized using alternating scrubs of 70% ethanol and betadine. A small incision was made with scissors and forceps above the flank gland to reach the muscle wall. Then another small incision moved through the muscle wall to reach the underlying fat pad attached to the ovary. The ovary was cauterized using a heated hemostat and removed with a scalpel. The fat pad was replaced underneath the muscle wall. The muscle wall was sutured using two double surgical knots, and the skin incision closed using one staple. This surgical procedure was repeated for the other ovary. Hamsters were monitored for 60 minutes following surgery and received post-operative analgesic (torbugesic 10 mg/ml) injections for three days.

**Cannulation.** After a recovery period of at least 1 week, each hamster underwent stereotaxic surgery to insert a cannula targeting the dorsal raphe nucleus (DRN) in order to administer either the OTA or vehicle control. Hamsters were anesthetized with 5% isoflurane in 100% oxygen, the surgical site at the skull was shaved and sterilized using alternating scrubs of 70% ethanol and betadine. The hamster was placed into a stereotaxic frame and secured using ear bars while anesthesia was maintained with 2.5-5% isoflurane in 100% oxygen through a nose cone. A pre-operative analgesic (torbugesic 10 mg/ml) was administered and a toe pinch confirmed sedation every 10 minutes. An incision was made in the scalp, and the lateral muscles were retracted to visualize bregma. After confirming skull leveling, the stereotaxic arm was moved to the DRN coordinates from bregma (-4.5 mm AP, -1.8 mm ML). One hole was drilled
at the DRN coordinates and two additional holes were drilled slightly anterior to the DRN coordinates on either side for bone screws to provide additional support for the skull cap. The cannula was lowered under stereotaxic control to the dorsal-ventral coordinates (-2.3 mm DV). The cannula and skull screws were covered with dental cement to form the skull cap. Subjects were fitted with dummy cannulas that do not extend beyond the guide cannulas. The scalp was closed around the cannulas using surgical staples. Hamsters were monitored for one hour following surgery and post-operative analgesic injections were administered for 3 days.

**Hormone-simulated pregnancy.** At least 1 week following cannulation surgeries, hamsters began a subcutaneous hormone injection schedule based on the hormone-simulated pregnancy model of Galea et al. (2001). On days 1-12 all hamsters received a daily low dose (2.5 μg) of estradiol benzoate and a daily high dose (4 mg) of progesterone dissolved in a cottonseed oil vehicle. On days 13-17, hamsters received an increased dose of estradiol benzoate (50 μg) in cottonseed oil. For the simulated postpartum on days 18-21, hamsters in the hormone-withdrawn condition received the cottonseed oil vehicle with no estradiol and hamsters in the hormone sustained condition continued with the higher estradiol dose.

**Drug treatment.** On days 20-21 of simulated postpartum period, hamsters received an intracranial injection of either 250 nanoliters of oxytocin receptor antagonist (90 micromolar, a gift from Elliott Albers) or 250 nanoliters of sterile saline through their cannula. Hamsters were briefly anesthetized with 5% isoflurane in 100% oxygen and OXTR antagonist or saline was administered via an injection needle that extended 3mm below the guide cannula attached to an automated syringe infusion pump. Half of the hamsters in the hormone-withdrawn and hormone-sustained groups were randomized to the OXTR antagonist and half to the vehicle control condition.
Behavior testing. Ten minutes after the drug or vehicle injections, hamsters underwent behavior testing to measure anxiety-like behavior. Behavior testing order was pseudo-randomized and balanced between experimental groups. Each hamster performed one test on a given day on postpartum days 20-21.

Open Field Test. On days 20 and 21, hamsters were tested in the Open Field Test apparatus to assess anxiety behavior and general locomotive function. Hamsters were placed in a 40.5 x 40.5 cm² arena enclosed by 30cm gray walls for 5 minutes. Tests were recorded from above using a video camera and analyzed using Noldus Ethovision behavior tracking software. Anxiety measures included the time spent in the center vs. time spent in the periphery. General locomotive measures examined total distance traveled and velocity. Measures of general locomotive ability from the open field test help to validate that activity, or lack thereof, in the elevated plus maze is due to changes in anxiety level and not simply due to deficits in motor control following surgery. A representative heat map from the Open Field Test is presented in Figure 1.

Elevated Plus Maze. On days 20 and 21, hamsters were tested in the Elevated Plus Maze consisting of two open arms, 51 x 11.5 cm, and two enclosed arms, 51 x 11.5 x 39.5 cm, with an open roof, and open arms opposite each other. The animal was placed in the center of the arena and activity was recorded for 5 minutes using Noldus Ethovision behavior tracking software. The test was terminated if the animal jumped from the maze. Anxiety behaviors were analyzed using Noldus Ethovision behavior tracking software. Behaviors for analysis included time spent in the open arms, which is indicative of lower anxiety and time spent in the closed arms, which is indicative of higher anxiety. As in the open field test, general locomotive measures will examine distance traveled and velocity. A representative heat map from the Elevated Plus Maze is presented in Figure 2.
**Brain Sectioning and Histology.** 24 hours after behavior testing on day 21, hamsters were euthanized via an overdose of sodium pentobarbital (Sleepaway, 0.3 mL), and brains were extracted for histological verification of cannula placement. Prior to brain extraction, 250 nanoliters of black India Ink was infused through the cannula to mark the location of the cannula and injection site. Following extraction, brains were post-fixed in a 4% paraformaldehyde prior to sectioning. The brain was blocked using a brain matrix, removing the cerebellum and olfactory bulbs to create a flat surface. The brain was then mounted on a cryostat chuck using optimal cutting temperature (OCT) compound and left to freeze in the cryostat for ten minutes. 40 µm coronal sections were taken throughout the entire brain. Tissue samples from around the dorsal raphe nucleus were transferred to a culture dish with PBS using a paint brush and mounted onto slides. Slides were examined under a light microscope to verify the location of the cannula in the DRN. A representative image of successful cannula placement in the DRN shown via ink infusion staining performed post-mortem is presented in Figure 1. Representative images of successful cannula placement in the DRN using images from a stereotaxic atlas of the hamster brain are presented in Figures 6-8.

**Statistical Analyses.** Data were analyzed using SPSS with significance determined at $p < 0.05$. 2 x 2 factorial ANOVAS (hormone condition x drug condition) were used to examine mean group differences in anxiety-like behavior and locomotive behaviors in the Open Field Test and Elevated Plus Maze between the hormonal and drug conditions. Given the limited statistical power of the 2x2 ANOVA due to small sample size, within-group t-tests were conducted for further analyses of potential trends in this pilot data. T-tests examined differences in the time spent in the periphery versus the center in the Open Field Test and differences in time spent in the open and closed arms for the Elevated Plus Maze.

Results
From the initial sample of 32 subjects, 1 hamster did not survive the stereotaxic cannulation surgery. 8 hamsters lost their skull caps during the recovery and hormone simulated pregnancy periods and therefore could not receive infusions of OTA or vehicle prior to behavior testing. These animals were included in the vehicle groups based on their hormone condition for analysis. Histological lesion verification revealed that the DRN was successfully targeted in 20 of the 31 hamsters. The 11 hamsters with missed cannulations were excluded from final analyses. The final analysis included \( n = 20 \) hamsters with 4 experimental groups based on hormone and drug condition: Sustained/Antagonist (S/A) \( (n = 3) \), Sustained/Vehicle (S/V) \( (n = 5) \), Withdrawn/Antagonist (W/A) \( (n = 3) \), Withdrawn/Vehicle \( (n = 9) \). ANOVA tables

**Behavior Measures**

**Open field test.**

**Non-specific locomotor behavior.** There was no significant interaction effect, \( F(1,19) = .108, p = .747 \) for velocity. There was no significant main effect of hormone group (Withdrawn: \( M = 2868.33, SD = 1087.01 \); Sustained: \( M = 2353.27, SD = 1362.87 \), \( F(1,19) = .1.30, p = .271 \) or drug treatment (OTA: \( M = 3321.84, SD = 1046.59 \); Vehicle: \( M = 2379.64, SD = 1178.91 \), \( F(1,19) = 3.23, p = .091 \) for velocity (See Table 1). There was no significant interaction effect, \( F(1,19) = .003, p = .960 \) for distance moved. There was no significant main effect of hormone group (Withdrawn: \( M = 9.76, SD = 3.78 \); Sustained: \( M = 7.74, SD = 4.23 \), \( F(1,19) = 1.96, p = .181 \) or drug treatment (OTA: \( M = 11.02, SD = 3.45 \); Vehicle: \( M = 8.06, SD = 3.98 \), \( F(1,19) = 2.93, p = .106 \) for distance moved (See Table 2).

**Anxiety-like behavior.** There was no significant interaction effect, \( F(1,19) = 1.28, p = .275 \) for the duration of time spent in the periphery. There was no significant main effect of hormone group (Withdrawn: \( M = 181.69, SD = 31.48 \); Sustained: \( M = 180.92, SD = 25.14 \), \( F(1,19) = .101, p = .755 \) or drug treatment (OTA: \( M = 184.62, SD = 34.89 \); Vehicle: \( M = 179.99 \),
SD=26.92), $F(1,19)=.186, p = .672$ for the duration of time spent in the periphery (See Table 3). There was no significant interaction effect, $F(1,19)=.489, p = .490$ for the duration of time spent in the center square of the open field. There was no significant main effect of hormone group (Withdrawn: $M=105.31$, $SD=31.41$; Sustained: $M=100.13$, $SD=19.91$), $F(1,19)=.317, p = .581$ or drug treatment (OTA: $M=97.99$, $SD=33.66$; Vehicle: $M=105.49$, $SD=24.57$), $F(1,19)=.305, p = .589$ for the duration of time spent in the center square of the open field (See Table 4). There was no significant interaction effect, $F(1,19)=.866, p = .366$ for the difference score for time spent in the center and periphery. There was no significant main effect of hormone group (Withdrawn: $M=76.38$, $SD=62.38$; Sustained: $M=80.79$, $SD=44.56$), $F(1,19)=.195, p = .628$ or drug treatment (OTA: $M=86.63$, $SD=67.30$; Vehicle: $M=74.51$, $SD=50.78$), $F(1,19)=.245, p = .628$ for the difference score for time spent in the center or periphery (See Table 5).

Exploratory paired samples t tests showed that for the W/A group, the mean time spent in the periphery ($M=173.99$ s) was greater than time spent in the center of the field ($M=106.94$ s) but this difference was not significantly different, $t(2)=1.44, p = .286$. For the W/V group the mean time spent in the periphery ($M=184.26$ s) was significantly greater than time spent in the center of the field ($M=104.77$ s), $t(2)=3.93, p = .004$. For the S/A group the mean time spent in the periphery ($M=195.24$ s) was greater than time spent in the center of the field ($M=89.045$) but this difference was not significantly different, $t (2)=3.04, p = .094$. For the S/V group the mean time spent in the periphery ($M=172.33$ s) was significantly greater than time spent in the center of the field ($M=106.79$ s), $t(4)=4.99, p = .008$ (See Figure 3).

**Elevated Plus Maze.**

**Non-specific locomotor behavior.** There was no significant interaction effect, $F(1,19)=.784, p = .389$ for distance moved. There was no significant main effect of hormone group (Withdrawn: $M=1538.24$, $SD=782.37$; Sustained: $M=1578.44$, $SD=768.47$), $F(1,19)=.056, p =
.816 or drug treatment (OTA: M=1953.11, SD=390.80; Vehicle: M=1383.31, SD=820.32),
\[ F(1,19) = 2.64, p = .124 \] for distance moved (See Table 6). There was no significant interaction
effect, \[ F(1,19) = .872, p = .364 \] for velocity. There was no significant main effect of hormone
group (Withdrawn: M=5.22, SD=2.58; Sustained: M=5.28, SD=2.59), \[ F(1,19) = .040, p = .845 \] or
drug treatment (OTA: M=6.54, SD=1.33; Vehicle: M=4.69, SD=2.73), \[ F(1,19) = .2.56, p = .129 \]
for velocity (See Table 7).

**Anxiety-like behavior.** There was no significant interaction effect, \[ F(1,19) = .493, p = .492 \] for the duration of time spent in the open arms. There was no significant main effect of
hormone group (Withdrawn: M=107.79, SD=66.82; Sustained: M=106.41, SD=92.40), \[ F(1,19) = .143, p = .711 \]
or drug treatment (OTA: M=126.05, SD=59.75); Vehicle: M=99.18, SD=82.28),
\[ F(1,19) = .400, p = .536 \] for the duration of time spent in the open arms (See Table 8). There were
no significant interaction effect, \[ F(1,19) = .301, p = .591 \] for the duration of time spent in the
closed arms. There was no significant main effect of hormone group (Withdrawn: M=98.68,
SD=38.11; Sustained: M=109.12, SD=63.82), \[ F(1,19) = .402, p = .535 \] or drug treatment (OTA: 
M=99.72, SD=46.99; Vehicle: M=104.19, SD=51.05), \[ F(1,19) = .035, p = .855 \] for the duration of
time spent in the closed arms (See Table 9). There was no significant interaction effect, \[ F(1,19) = .456, p = .509 \]
for the difference score for time spent in the open arms versus the closed arms.
There was no significant main effect of hormone group (Withdrawn: M=-9.12, SD=94.49;
Sustained: M=2.72, SD=154.09), \[ F(1,19) = .254, p = .621 \] or drug treatment (OTA: M=-26.33,
SD=106.04; Vehicle: M=5.02, SD=125.50), \[ F(1,19) = .231, p = .638 \] for the difference score for
time spent in the open arms versus the closed arms (See Table 10).

Exploratory paired samples \( t \) tests showed that for the W/A group the mean time spent in
the open arms (M=147.43 s) was greater than time spent in the closed arms (M=84.47 s) but this
difference was not significantly different, \( t(2) = .972, p = .433 \). For the W/V group the mean time
spent in the open arms ($M=94.584$ s) was less than time spent in the closed arms ($M=103.40$ s), but this difference was not significant, $t(8)=-.302$, $p=.770$. For the S/A group the mean time spent in the open arms ($M=104.67$ s) was less than time spent in the closed arms ($M=114.96$ s) but this difference was not significantly different, $t(2)=-.166$, $p=.883$. For the S/V group the mean time spent in the open arms ($M=107.45$ s) was greater than time spent in the closed arms ($M=105.62$ s), $t(4)=.022$, $p=.984$, but this difference was not significant. (See Figure 4).

**Discussion**

The goal of the present study was to examine the effect of blocking oxytocin receptors in the dorsal raphe nucleus during the peripartum period in Syrian hamsters. Previous research in our lab found an increase in oxytocin receptors in the dorsal raphe nucleus, an estrogen sensitive oxytocin efferent of the paraventricular nucleus following a hormone-simulated pregnancy and hormone withdrawal simulating the peripartum period. These findings were coupled with an increase in anxiety behavior in the hormone-withdrawn condition (Benedetto, Heaton, Levine, Travers, Ross, Albers, & Been, 2018). Based on these findings, we hypothesized that increased oxytocin receptor in the DRN in the peripartum period may lead to increased anxiety-like behavior in the peripartum period. We therefore predicted that blocking oxytocin receptors in the dorsal raphe nucleus during the simulated postpartum period would lead to decreased anxiety behavior in the hormone withdrawn animals.

Due to a small sample ($n=20$), this study was not statistically powered to detect significant between-group differences in measures of anxiety-like behavior with a 2x2 ANOVA. Therefore, we decided to approach this as pilot data and conducted further analyses using within-group t-tests for measures of anxiety in the Open Field and Elevated Plus Maze. We hoped to use these tests to detect potential patterns of the effects of the OTA.
In the Open Field Test, we compared time spent in the periphery of the field (near the wall) and time spent in the center of the open field. Within-group t-tests revealed that all groups spent more time in the periphery than in the center of the field which is indicative of an overall anxious phenotype. However, this difference was only significant in the groups that received the vehicle infusion, regardless of hormone condition. While hamsters in the two OTA groups still demonstrated an overall pattern of more time spent in the periphery than the center of the open field, these differences were smaller and not statistically significant (See Figure 1). This distinction between OTA and vehicle groups may suggest that administration of OTA reduces anxiety regardless of hormone condition.

Within-group t-tests for the Elevated Plus maze examined differences between time spent on the open arms and time spent on the closed arms of the maze. While none of the comparisons reached statistical significance at the p<0.05 level, there was a marginal trend in the Withdrawn/Antagonist group towards spending more time in the open arms of the maze (See Figure 2). This is suggestive of lower anxiety levels in this group of hamsters in the simulated peripartum period after receiving the OTA. In previous studies from our lab, animals in the simulated peripartum period exhibited increased anxiety-like behaviors as measured by a greater mean time spent in the closed arms of the elevated plus maze (Benedetto, Heaton, Levine, Traveras, Ross, Albers, & Been, 2018). The trend in the anxiety behavior of the Withdrawn/Antagonist group found in the present study would suggest that blocking oxytocin receptors in the DRN in the peripartum period may be able to reverse this increase in anxiety in the peripartum period.

The overall patterns observed in within-group t-tests for both the Open Field Test and the Elevated Plus Maze may suggest that blocking oxytocin receptors in the dorsal raphe nucleus may decrease anxiety-like behaviors in the peripartum period. The lower anxiety patterns are not
specific to hormone condition in anxiety-like measures of the Open Field. However, this trend seems specific to the hormone withdrawn hamsters in measures of anxiety in the Elevated Plus Maze, which is a more robust measure of anxiety behavior. Taking the findings of this experiment as pilot data, expanding this sample in future cohorts could provide some interesting insights into the role of oxytocin signaling in the DRN in the modulation of peripartum anxiety.

We see two potential paths that could emerge from increasing the sample in a future cohort and reaching a higher level of statistical power. In one scenario, the pattern of reduced anxiety-like behavior in OTA groups could become more pronounced and reach statistical significance. Alternatively, increasing statistical power could reveal that there are in fact no significant differences in anxiety behavior following administration of an OTA. In this scenario the trends demonstrating lower overall anxiety-like behaviors in the OTA groups would disappear, suggesting that there is no effect of blocking oxytocin receptors in the DRN in the peripartum period. While the trends found in this pilot data are suggestive of some effect of the OTA treatment, both of these potential outcomes are worth consideration.

**Blocking Oxytocin Receptors in the DRN May Reduce Anxiety-Like Behavior in the Peripartum Period**

In support of our hypothesis, within-group t-tests indicated a pattern of reduced anxiety-like behavior in hamsters treated with an oxytocin receptor antagonist (OTA). This pattern was significant independent of hormone condition in anxiety measures of the Open Field Test but appears to be specific to the hormone withdrawn condition in the measures from the Elevated Plus Maze. Previous studies from our lab found an increases of oxytocin immunoreactive neurons in the PVN and increases in oxytocin receptor density in the DRN in the peripartum period following a hormone simulated pregnancy as well as increased anxiety behavior in the Elevated Plus Maze in hormone-withdrawn animals (Amaral, 2017; Bodie, 2017; D’Antonio,
2007; Lee, 2017; Benedetto, Heaton, Levine, Travers, Ross, Albers, & Been, 2018). This increased oxytocin signaling from the PVN to the DRN paired with the increase in anxiety behavior suggests that blocking oxytocin receptors in the DRN could lead to decreased anxiety behavior as was suggested by the trends in our data.

This increase in anxiety-like behavior in the peripartum period is counter to some previous evidence that reduced anxiety is typical immediately following parturition in both rats and human females (Lee, Macbeth, Pagani, & Young, 2009; Lonstein, Maguire, Meinlschmidt, & Neumann, 2014). Longitudinal self-report studies have found decreases in anxiety in the postpartum period in humans, but these patterns are highly variable and dependent upon pre-existing mood conditions and stress exposure (Dipietro, Costigan, & Sipsma, 2008; Heron et al., 2004). There is evidence of reduced anxiety in the postpartum period in rats, but these findings are generally dependent upon both time and exposure to maternal stimuli. Agrati et al. (2008) found that both lactating postpartum dams and virgin female rats conditioned to maternal behavior demonstrate reduced anxiety and fear responses. Similarly, Lonstein et al. (2005) found that lactating rats demonstrated reduced anxiety behavior in the first postpartum week, but this reduction was mediated by exposure to pups prior to testing. Therefore, the reduced anxiety responses found in postpartum rats seems highly dependent upon maternal stimuli which is not present in a hormone-simulated postpartum. Therefore, the role of hormonal fluctuation in the mediation of postpartum anxiety behavior cannot be clearly determined from this evidence.

When Galea, Wilde, and Barr (2001) first developed the hormone-simulated pregnancy model, they observed increases in depressive-like but not anxiety-like behavior in hormone-withdrawn rats. The application of the hormone-simulated pregnancy model to study peripartum anxiety in hamsters is still fairly novel and the findings of increased anxiety in the peripartum period as in Benedetto et al. (2018) are highly variable across different models.
Though much of the previous literature suggests an anxiolytic effect of increases in oxytocin signaling, there is some evidence that oxytocin receptor signaling may be differentially involved in the modulation of anxiety during pregnancy and the peripartum period. In a study comparing the effects of an oxytocin receptor antagonist on anxiety in virgin, pregnant, and lactating rats, intracerebral infusion of OTA prior to behavior testing resulted in decreased anxiety behavior in the Elevated Plus Maze in pregnant and lactating rats, but not virgin females (Neumann, Torner, & Wigger, 1999). These findings offer some support for our hypothesis and the potential observed trends. However, the relationship must be interpreted with caution as this study used a real pregnancy model and OTA was not targeted specifically to the DRN. If the trends found in our data were confirmed with results from future cohorts, it would provide support for the fairly novel hypothesis that increased oxytocin signaling from the PVN to the DRN is related to increases in anxiety behavior.

**Oxytocin-serotonin pathway.** One proposed mechanism for this effect of oxytocin signaling on peripartum anxiety is the oxytocin-serotonin pathway. A majority of the serotonin (5HT) neurons in the DRN contain oxytocin receptors, and serotonin has been shown to have anxiolytic effects both within and outside of the peripartum period (Binder, Newport, & Zach, 2009; Frokjaer et al., 2015). Furthermore, there is significant evidence that oxytocin signaling modulates the release of serotonin and its anxiolytic effects, making this pathway a prime target for potential intervention (Yoshida et al., 2009). However, this study found that increasing oxytocin through infusions to the raphe nucleus lead to serotonin release and reduced anxiety behavior which is not completely concordant with the proposed mechanisms of the trends found in our lab (Yoshida et al., 2009). If further studies confirm the finding that blocking oxytocin receptors in the DRN does in reduce anxiety behavior this could indicate that the increased oxytocin signaling specifically in the DRN is somehow differently involved in serotonin release
such that such markedly increased levels of signaling actually reduce serotonin release thereby increasing anxiety behavior.

**Implications of these findings.** If the trends in our data reached significance with an expansion of the cohort, it would imply that blocking oxytocin receptors in the dorsal raphe nucleus can have an anxiolytic effect in the peripartum period. This would implicate the increased oxytocin signaling between the PVN and the DRN in the simulated peri-partum period as a mechanism of peripartum anxiety. Given that much of the previous research in peripartum mood disorders has found that increasing oxytocin signaling has anxiolytic effects in both rodents and humans, this finding would be relatively novel (Figueira, Peabody, & Lonstein, 2008; Mantella, Vollmer, Li, & Amico, 2003; Neumann, Torner, & Wigger, 1999; Wang, 2018; Yoshida et al., 2009). However, the exact mechanisms of oxytocin signaling as it relates to serotonin release and anxiety are not well understood. Therefore, this result could indicate a novel potential route for treatment of peripartum anxiety through the influence of oxytocin signaling in the DRN.

**Blocking Oxytocin Receptors in the DRN May Not Reduce Peripartum Anxiety-like Behavior**

Alternatively, increasing the statistical power may reveal that there is in fact no significant effect on anxiety-like behavior of blocking oxytocin receptors in the dorsal raphe nucleus during the simulated peri-partum period. While behavior data from the Open Field test suggests that OTA may reduce anxiety behavior, it is entirely possible that with an increase in statistical power, this difference would disappear, instead confirming the overall trend of an anxious phenotype regardless of hormone or drug condition (Figure 2). The patterns found in the Elevated Plus Maze are even more tenuous as none of these trends reach statistical significance. While within-group trends suggest that the Withdrawn/Antagonist group has lower levels of
anxiety, this is purely speculative and not statistically significant. Expansion of the sample with further cohorts may reduce this difference further and eliminate the trend, suggesting no difference in anxiety across the four conditions.

In recent work from our lab, hamsters in the hormone-withdrawn simulated peripartum period demonstrated increased anxiety as measured by the Elevated Plus Maze (Benedetto, Heaton, Levine, Traveras, Ross, Albers, & Been, 2018). It is possible that the results from Benedetto et al. (2018) were not robust or were potentially implicated by reduced overall locomotor function that was also observed in the hormone-withdrawn hamsters in this study. Other studies using hormone-simulated pregnancy models in rats have also found no significant differences in anxiety-like behavior in the elevated plus maze based on hormone condition (Figueira, Peabody, & Lonstein, 2008; Galea, Wide, & Barr, 2001). Further research is necessary to validate consistent patterns of peripartum anxiety behavior during hormone withdraw in the hormone-simulated pregnancy model in hamsters.

Assuming the findings of increased anxiety in hormone-withdrawn hamsters are in fact a robust measure of peripartum anxiety, finding no significant difference in anxiety behavior following infusion of an OTA in the DRN would suggest that the increased oxytocin signaling between the PVN and the DRN is not the primary neurobiological mechanism of peripartum anxiety. Though we hypothesized that the increased oxytocin signaling between the PVN and the DRN in hormone-withdrawn hamsters was related to increased anxiety-like behaviors, there is significant evidence suggesting that higher levels of central oxytocin signaling may have anxiolytic effects.

Rodent models have been able to further examine the role of central oxytocin in specific brain regions implicated in its production and signaling. Figueira et al. (2010) found that infusion of an oxytocin receptor antagonist into the ventrocaudal periaqueductal gray lead to increased
anxiety behavior in peripartum rats but not diestrous virgins. Furthermore, after inducing anxiety behavior via separation from their litters, dams treated with an infusion of oxytocin into the cPAGv demonstrated decreases in anxiety behavior. These findings suggest that disrupting increased oxytocin signaling present in the peripartum period may in fact increase anxiety. In support of the potential role of oxytocin in the moderation of peripartum mood, Wang et al (2018) found that an injection of oxytocin into the PVN of peripartum rats reversed previously observed increases in depressive symptoms. If further extensions of our cohort found no decreases in anxiety behavior, this would indicate that blocking the increased oxytocin signaling does not have anxiolytic effects as we hypothesized. This would fit more closely with the previous literature suggesting that increased oxytocin may have anxiolytic or anti-depressant effects.

**Oxytocin-serotonin pathway.** We hypothesized that the increase in oxytocin signaling between the PVN and the DRN observed in the simulated peripartum period may have implicated serotonin 5-HT signaling, leading to the observed increases in anxiety-like behavior in the hormone-withdrawn hamsters (Benedetto, 2018; Heaton 2018; Levine 2018; Taveras, 2018). If a further expansion of our sample revealed no significant effect of the OTA in the DRN, this would suggest that increased oxytocin signaling between the PVN and the DRN is not disrupting the release of serotonin 5-HT in a way that causes increased anxiety-like behavior.

As discussed previously, Yoshida et al. (2009) provide some evidence to suggest increased oxytocin signaling may have anxiolytic effects through its effects on serotonin. This study found that over 50% of serotonin neurons in the raphe nucleus were modulated by oxytocin and infusion of oxytocin within the median raphe nucleus increased serotonin release and lead to decreased anxiety behavior in rats. Furthermore, infusion of a 5-HT2A/2C receptor antagonist blocked these anxiolytic effects. However, this study specifically examined infusions
of oxytocin in the median raphe nucleus, whereas we observed increased signaling in the dorsal raphe nucleus. Furthermore, this study did not address postpartum neuroplasticity of oxytocin receptor density in these areas. These changes in neural signaling could be important in the modulation of anxiety behavior and explain some of the variability in behavior found across studies.

While research in humans has not been able to study this signaling relationship in specific regions of the brain, the prevalence of serotonin neurons with oxytocin receptors in the dorsal raphe nucleus suggest that this region is likely a key contributor. Additionally, there is evidence of neuroplasticity in the dorsal raphe during the peripartum period that may have important implications for this pathway. Holschbach and Lonstein (2017) found that neurons in the dorsal raphe undergo significant changes in the early postpartum period that are accompanied by alterations in the mechanisms of serotonergic signaling. Though the exact mechanisms are undetermined, it seems likely that these neuroplastic changes throughout the peripartum period could play a role in oxytocin-mediated serotonergic signaling that could affect anxiety. Importantly, these changes were also influenced by maternal care experience, which could explain differences in our study given that the peripartum period was modeled after a hormone-simulated pregnancy.

**An alternative explanation: HPA axis dysregulation.** In addition to the lack of natural maternal stimuli, the hormone-simulated pregnancy model also does not account for neuroendocrine changes that occur in pregnancy and the postpartum period other than the fluctuation in ovarian hormones. As discussed previously, HPA axis functioning undergoes significant changes during pregnancy and the postpartum period. Cortisol levels increase up to three times nonpregnant levels in response to both fluctuations in estrogen levels and placental secretion of CRH (Jung et al., 2011; Mastorakos & Ilias, 2003). This feedback leads to a
hyporesponsivity of the HPA axis in late pregnancy which persists through several weeks postpartum as cortisol levels return to normal (Kammerer, Adams, Castelberg, & Glover, 2002). This HPA hyporesponsivity is hypothesized to be adaptive in the modulation of anxiety as a mother deals with the stress of labor, delivery, and early childcare. However, there is some evidence of HPA axis dysregulation in postpartum depression and anxiety where PPD and PPA are associated with higher cortisol responses in the postpartum period (Jolley, Elmore, Barnard, & Carr, 2007).

In particular, there is increasing evidence that oxytocin may play a role in the modulation of HPA axis responsivity and anxiety behavior in pregnancy and the postpartum period. In rat models, anxiety and stress responses show decreases in late pregnancy and the early postpartum period which is accompanied by increases in oxytocin mRNA and oxytocin receptor binding in some brain regions (Windle et al., 2006). Results from our lab also found increased oxytocin immunoreactive neurons in the PVN and oxytocin receptor increases in the DRN in Syrian hamsters, suggesting that changes in oxytocin signaling are consistently present in models of pregnancy and the postpartum period. Given these concurrent alterations, there is reason to hypothesize that changes in oxytocin signaling in pregnancy and the postpartum period may influence anxiety through interactions with HPA axis responses.

Neumann et al. (1999) found that endogenous oxytocin exerted differential effects on corticotropin and corticosterone secretion in virgin female rats compared to those in the postpartum period. While treatment with an oxytocin antagonist significantly reduced anxiety behavior in the Elevated Plus Maze in pregnant and lactating rats, there were no effects on virgin females. These findings suggest that intracerebral oxytocin has independent effects on the HPA axis and oxytocin-serotonin pathways modulated by ovarian hormone levels. Windle et al. (2006) used a progesterone withdrawal model of late pregnancy in rats, finding that this led to
decreases in anxiety-like behavior and stress reactivity which are implicated in adaptive maternal behaviors. Furthermore, the anxiolytic effects of this hormonal fluctuation were reversed with the infusion of an oxytocin antagonist suggesting a mediating role of central oxytocin (Windle et al., 2006). While the exact mechanisms of central oxytocin signaling and the HPA axis in the postpartum period are not fully understood, it is possible that this relationship may be related to peripartum mood disturbances in response to ovarian hormone fluctuations.

**Strengths and Limitations**

There are several important limitations to our study that should be considered in the interpretation of our findings. ANOVAs were not sufficiently statistically powered to detect significant between-group effects of hormone and drug condition due to our reduced cohort of \( n=20 \) hamsters and only 3 hamsters in each OTA treatment group. Treating this as pilot data, within-group t-tests allowed for observational analysis of potential patterns of within-group behavior, but only two of these tests reached significance. Despite these limitations, our findings provide a useful indication of trends in anxiety behavior to serve a basis for further expansions of the study.

Outside of these limitations to our analyses, the design of this experiment had some notable strengths and weaknesses. By using a hormone-simulated pregnancy model, we were able to have complete control over estrogen and progesterone levels whereas a natural pregnancy model introduces some endogeneity with other hormonal factors. Additionally, the females did not give birth to pups, which effectively limits the number of animals sacrificed. However, the hormone-simulated pregnancy model also has some important limitations. While we were effectively able to simulate the rise and fall of estrogen and progesterone observed in pregnancy and the peripartum period, this does not account for other hormonal fluctuations that occur in this period. Cortisol, ACTH, prolactin, and vasopressin levels have all been shown to change in this
period which could have interactive effects with levels of ovarian hormones, oxytocin, or serotonin that could impact anxiety-like behaviors (Hendrick, Altshuler, & Suri, 1998). Additionally, this model removes the factor of maternal behaviors in the peripartum period. In a natural pregnancy model, dams typically interact with their pups and engage in hormonally mediated behaviors including lactation and maternal care. These behaviors are not only mediated by oxytocin, but also have feedback effects that influence further neuroendocrine processes (Bridges, 2015). Therefore, the changes in oxytocin signaling observed in previous studies may be more affected by the conditional presence of maternal stimuli than can be accounted for in the hormone-simulated pregnancy model. The absence of maternal stimuli and the role of other hormones could implicate the validity of this model in assessing peripartum behavior.

Implanting a cannula directly into the DRN allowed us to specifically target the large population of oxytocin-receptors in this area of the brain which has been implicated in oxytocin-dependent serotonergic signaling that affects anxiety (Mottolese, Redouté, Costes, Bars, & Sirigu, 2014; Yoshida et al., 2009). Therefore, any results can be attributed to this specific mechanism of blocking the increased oxytocin signaling from the PVN to the DRN previously observed in the simulated peripartum period. However, this does not clearly address the potential mechanism of the oxytocin-serotonin pathway as we have no measure of 5-HTT serotonin levels. If the behavioral data trends were to reach significance with expansion of future cohorts, the findings could effectively demonstrate that blocking this specific population of oxytocin receptors in the DRN can lead to reduced anxiety following a hormone-simulated pregnancy which provides a very specific mechanism to target for potential treatments.

**Conclusions and Future Directions**

The goal of this study was to examine the role of increased oxytocin signaling between the PVN and the DRN found in previous studies in peripartum anxiety. Specifically, we tested
the effect of blocking oxytocin receptors in the dorsal raphe nucleus following a hormone-simulated pregnancy with the hypothesis that this would lead to decreased anxiety-like behavior as measured by the Open Field Test and Elevated Plus Maze. While the behavioral results of this experiment were not sufficiently statistically powered for between-group analysis, within-group t-tests show some within-group patterns in anxiety-like behavior that could provide a basis for further exploration. Behavior results from the Open Field Test suggest that the OTA has a potentially anxiolytic effect regardless of hormonal condition, which may emerge as a significant difference with increased statistical power. In the Elevated Plus Maze, the hormone-withdrawn animals treated with the OTA demonstrated a non-significant trend of lower anxiety levels as measured by time spent in the open arms of the maze. Previous studies have found that hormone-withdrawn hamsters demonstrated higher levels of anxiety-like behavior (Benedetto, 2018; Heaton 2018; Levine 2018; Taveras, 2018). Therefore, this finding, if replicated with significance could indicate that blocking the increased oxytocin signaling between the PVN and DRN in the peripartum period may be able to effectively reduce anxiety.

However, this pilot data only allows for the observation of patterns within the data. This data should be expanded with future cohorts to explore this relationship further. As previously mentioned in the discussion of this paper, we see two potential results of expanding the cohort of this experiment. The trends found in this data may be confirmed, indicating that blocking oxytocin receptors in the DRN leads to decreased anxiety behavior following a hormone simulated pregnancy; alternatively, these differences could disappear, indicating that blocking this oxytocin signaling pathway has no effect on peripartum anxiety. Either result would provide valuable information for continued research into the neural mechanisms of peripartum mood disorders and potential treatments. If this mechanism is significant, further studies should examine the role of the oxytocin-serotonin pathway to understand how blocking these receptors
influences serotonin levels. Alternatively, if the OTA treatment has no significant behavioral effects, further research could examine other targets of oxytocin signaling within the brain. Furthermore, future research should aim to address more fully the roles of other hormonal fluctuations and the influence of maternal stimuli feedback as these may implicate the behavioral results of hormone-simulated pregnancy models. In particular, the changes in oxytocin signaling found in our lab may in fact influence peripartum mood through its relationship with HPA axis function. The relationship between ovarian hormone withdrawal, oxytocin signaling, and HPA axis responsivity in pregnancy and the postpartum period warrants further exploration. Particularly, hormone-simulated pregnancy models may be expanded to account for and examine these concurrent fluctuations as much of the present literature studies one specific mechanism. Research should continue to refine these methods to provide the best possible model to enhance our understanding of the neurobiological mechanisms of the peripartum period in humans.

Though the present study only presents pilot data, it provides some context for further exploration of the potential role of oxytocin receptor activation in the DRN as a neural mechanism of peripartum anxiety. Continued exploration of these mechanisms could provide a more comprehensive understand of the neurobiological mechanism of peripartum mood regulation and provide a basis for therapeutic interventions involving this specific mechanism. Our current understanding of the peripartum period and the immense changes in hormones, brain structures, and behavior that are involved in this intense period of a woman’s life are far from comprehensive. Postpartum depression and anxiety have estimated prevalence rates of up to 20%, yet treatment options are still insufficient for the management of these complex disorders that can have profoundly detrimental effects for both mother and offspring (Le Strat, Dubertret, & Le Foll, 2011; O’Hara & McCabe, 2013). Our study provides a basis for further exploration of oxytocin’s role in peripartum anxiety. With continued expansion and increased understanding of
these mechanisms, this could lead to novel therapeutic strategies involving oxytocin signaling for
the treatment of postpartum mood disorders.
References


blocking oxytocin receptors in the drn

https://doi.org/10.1210/jc.2004-1388


Lonstein, J. S. (2005). Reduced anxiety in postpartum rats requires recent physical interactions with pups, but is independent of suckling and peripheral sources of hormones. *Hormones and Behavior, 47*(3), 241–255.


Tables and Figures

Table 1. (Analysis of Variance)

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Note.—MS = Mean squares, effect size = η2 or partial η2. *p < .05. †p < .01. ‡p < .001.

Table 2. (Analysis of Variance)

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Note.—MS = Mean squares, effect size = η2 or partial η2. *p < .05. †p < .01. ‡p < .001.

Table 3. (Analysis of Variance)

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Note.—MS = Mean squares, effect size = η2 or partial η2. *p < .05. †p < .01. ‡p < .001.
Table 4. (Analysis of Variance)

*Two-Way Analysis of Variance of Duration in Open Area by Hormone-Group and Injection Type For OFT*

<table>
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Note.—MS = Mean squares, effect size = η² or partial η². *p < .05. †p < .01. ‡p < .001.

Table 5. (Analysis of Variance)

*Two-Way Analysis of Variance of Difference Between Duration in Closed-Arms and Open-Arms by Hormone-Group and Injection Type for OFT*

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Note.—MS = Mean squares, effect size = η² or partial η². *p < .05. †p < .01. ‡p < .001.
Table 6. (Analysis of Variance)

Two-Way Analysis of Variance of Total Distance Moved by Hormone-Group and Injection Type For EPM

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<td>Error</td>
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<td>566417.621</td>
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Note.—MS = Mean squares, effect size = $\eta^2$ or partial $\eta^2$. *p < .05. †p < .01. ‡p < .001.

Table 7. (Analysis of Variance)

Two-Way Analysis of Variance of Velocity by Hormone-Group and Injection Type For EPM

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<td>Hormone Group</td>
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Note.—MS = Mean squares, effect size = $\eta^2$ or partial $\eta^2$. *p < .05. †p < .01. ‡p < .001.

Table 8. (Analysis of Variance)

Two-Way Analysis of Variance of Open-Arms Duration by Hormone-Group and Injection Type for EPM

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Note.—MS = Mean squares, effect size = $\eta^2$ or partial $\eta^2$. *p < .05. †p < .01. ‡p < .001.
Table 9. (Analysis of Variance)

Two-Way Analysis of Variance of Closed-Arms Duration by Hormone-Group and Injection Type for EPM

<table>
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<td>Error</td>
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Note.—MS = Mean squares, effect size = $\eta^2$ or partial $\eta^2$. *p < .05. †p < .01. ‡p < .001.

Table 10. (Analysis of Variance)

Two-Way Analysis of Variance of Difference Between Duration in Closed-Arms and Open-Arms by Hormone-Group and Injection Type for EPM

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Note.—MS = Mean squares, effect size = $\eta^2$ or partial $\eta^2$. *p < .05. †p < .01. ‡p < .001.
Figure 1. Representative heat map of the Open Field Test. Blue color represents areas with less hamster activity during the 5 minute test. Red color represents areas of higher activity. This heatmap shows a higher concentration of time spent towards the periphery and away from the open center which is typical of an anxious phenotype.
Figure 2. Representative heat map of the Elevated Plus Maze. Blue color represents areas with less hamster activity during the 5 minute test. Red color represents areas of higher activity. This heatmap shows a higher concentration of time spent in the open arms which is indicative of lower anxiety.
Figure 3. Graph depicting within-group differences in mean time spent near the wall and time spent in the open center of the Open Field Test. There is an overall pattern of an anxious phenotype, but these differences are only significant for W/V and S/V groups. Mean time is measured in seconds and the error bars represent standard deviation.
Figure 4. Graph depicting within-group differences in mean time spent in the open and closed arms of the Elevated Plus Maze. The W/A group demonstrates a non-significant pattern of decreased anxiety. Mean time is measured in seconds and the error bars represent standard deviation.
Figure 5. Representative image of successful cannula placement in the DRN shown via ink infusion staining performed post-mortem.
Figure 6. Representative image of successful cannula placement in the DRN (depicted by green circles) using an image from a stereotaxic atlas of the hamster brain (Morin and Wood, 2001).
Figure 7. Representative image of successful cannula placement in the DRN (depicted by green circles) using an image from a stereotaxic atlas of the hamster brain (Morin an Wood, 2001).
Figure 8. Representative image of successful cannula placement in the DRN (depicted by green circles) using an image from a stereotaxic atlas of the hamster brain (Morin and Wood, 2001).