ΔFosB and the Nucleus Accumbens: Explanations for Peripartum Mood Disorders

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Abstract

Current research fails to determine the underlying neurological explanations of peripartum mood disorder (PMD) and its many behavioral components. In particular, peripartum anxiety behavior has been found to have increased rates compared to depression-like behavior. Yet, research efforts continue to concentrate on understanding postpartum depression. This has led to a significant gap in current literature, and its inability to explain peripartum anxiety and PMD as a whole. Therefore, the present study focused on peripartum anxiety-like behavior, and its relationship with changes in nucleus accumbens (NAc) neuroplasticity, specifically the transcription factor ΔFosB. Utilizing a hormone-simulated pregnancy model, we assessed anxiety behaviors in 21 female mice using the Elevated Plus Maze and Open Field Test. Prior to behavioral testing, mice were injected with one of two viral vectors, leading either to inhibited or undisturbed ΔFosB accumulation. This allowed us to investigate how ΔFosB impacts the exhibition of peripartum anxiety. Our trends suggest that ΔFosB accumulation during pregnancy is most impactful on peripartum anxiety-like behavior, but estrogen withdrawal after delivery is more influential on the postpartum anxiety behavior phenotype. These results are the first step towards gaining a comprehensive understanding of PMD’s manifestation in the brain, and contributes to a growing body of literature supporting increased educational and diagnostic tools to respond to peripartum anxiety-related disorders.

Keywords: ΔFosB, nucleus accumbens, peripartum, mood disorders, anxiety
ΔFosB and the Nucleus Accumbens: Explanations for Peripartum Mood Disorders

The most prevalent of all psychiatric conditions is anxiety and its related disorders (Fairbrother, Janssen, Antony, Tucker, & Young, 2016). Significant gender differences exist within the prevalence of anxiety such that women are one and a half times more likely to suffer from an anxiety disorder than men (Fairbrother et al., 2016). This gender difference can be further exacerbated during the challenging and exciting experience of pregnancy, which brings increased risks of mental health disorders for women. During and after pregnancy, women have an increased vulnerability to anxiety and its related disorders. More specifically, perinatal anxiety disorder disrupts between 9-22% of pregnant and postpartum women, and at least 10-15% of women report experiencing postpartum depression (PPD) (Ali, 2018; Fairbrother et al., 2016; Haim, Sherer, & Leuner, 2014; Pawluski, Lonstein, & Fleming, 2017; Robertson, Grace, Wallington, & Stewart, 2004). In addition, “baby blues,” a milder form of depression, are the most common mental health complication for postpartum women, which has an estimated prevalence of 30-75% (Robertson et al., 2004). Postpartum anxiety (PPA), PPD, and their related disorders are heavily comorbid, and are often referred to together as peripartum mood disorders (PMD) (Haim et al., 2014; Pawluski et al., 2017). These common complications of pregnancy require significant attention from the science community due to their high prevalence and potential hinderance on the health outcomes of peripartum women.

Peripartum mood disorders can have considerable impacts on the health of women and their children. Short term implications include reduced success in breastfeeding (Dennis, 2006). Furthermore, Rahman et al. (2004) reported higher rates of diarrheal episodes in a sample of infants whose mothers were diagnosed with PPD. Disrupted emotional attachment between a mother and her infant is a complication of PMD that comprises both short and long term
repercussions (Ali, 2018). Long term neurobiological consequences of PPD and anxiety can be seen in delayed cognitive and social development of infants, as well as increased growth retardation (Ali, 2018; Rahman, Iqbal, Bunn, Lovel, & Harrington, 2004). Children of mothers who suffered PPA also carry increased risk of developing anxiety themselves during adulthood (Ali, 2018). Lastly, concerns regarding infant abuse exist among women with PPA, a detrimental social impact of PMD that can cause negative neurobiological and mental health outcomes in children (Nayak & Milner, 1998). Overall, the consequences of PMD implicate both the mother and her child’s health in processes spanning past the postpartum period, further demonstrating the importance of addressing PMD within research.

**Current Definitions of Peripartum Mood Disorders**

The current clinical characterization and diagnoses of mood disorders that impact peripartum women do not address the significance of PPA within PMD. The existing diagnoses of PMD rely on the diagnostic criteria of major depressive disorder (MDD), obsessive compulsive disorder (OCD), and generalized anxiety disorder (GAD). Postpartum depression is characterized within MDD. However, no specific diagnosis exists for PPA or its other related disorders. Instead, PPA is quantified under PPD and MDD. In some instances, PPA is explained using the definition of an anxiety disorder that occurs outside of the peripartum period such as OCD or GAD (Ali, 2018; Drevets, Price, & Furey, 2008; Fairbrother et al., 2016; Pawluski et al., 2017). This is problematic because it leads to the inability to develop screening measures, treatment plans, and to assess the prevalence of PPA. Furthermore, recent meta-analyses have distinguished larger rates of PPA related disorders than PPD. Researchers attribute this discrepancy to the absence of a diagnostic criteria for PPA, demonstrating another concern regarding the limited availability of PMD diagnosis and the importance of addressing PPA
individually (Ali, 2018; Fairbrother et al., 2016; Matthey, Barnett, Howie, & Kavanagh, 2003; Pawluski et al., 2017). This observation is yet to be attended to in clinical settings; PPD continually gains priority over PPA throughout research and diagnosis. Due to the need for more accuracy when discussing PPD and PPA, research has also suggested to address both conditions at once by referring to the issue as the greater matter of PMD (Matthey et al., 2003). This proposal has many limitations, including its inability to encourage research on the symptoms of PPD and PPA independently. Additionally, it is integral that we understand the characteristics of both conditions prior to researching them in order to perpetuate accurate descriptions and understanding of PPD and PPA. Lastly, increased focus must be placed on PPA within the research context in order to distinguish the condition as significant enough to obtain its own, more specific, diagnostic criteria outside of PPD and MDD.

Postpartum depression’s diagnosis is more thorough than PPA but carries its own challenges. The DSM-V describes PPD’s symptoms using characteristics synonymous of MDD, with the exception of PPD being unique in its timing. The DSM-V specifies that symptoms of a major depressive episode are expected to arise during pregnancy or within four weeks of delivery in order to be diagnosed as MDD with postpartum implications (Association, 2013; Pawluski et al., 2017). Women experiencing PPD commonly report sad mood, restlessness, agitation, and impaired concentration. In contrast, the DSM-V requires symptoms such as lost interest or pleasure, lowered appetite, sleep changes, and other feelings that are not always reported by women with PPD. In addition, some women experience PPD symptoms starting months after the delivery of their child. These inconsistencies within the arrival and description of symptoms has generated disputes about the DSM’s accuracy of diagnosing PPD (Stuart, Couser, Schilder, O’hara, & Gorman, 1998). Ultimately, the current diagnostic criteria for PPD doesn’t account for...
the variability in women’s experiences. However, there has been a significant clinical response to PPD such that the Edinburgh Postnatal Depression Scale was developed, and is the most common tool to measure the symptoms of PPD (Matthey et al., 2003). Lastly, research on PPD has distinguished that the condition is often comorbid with PPA, and is strongly predicted by a prenatal history of depression or anxiety (Pawluski et al., 2017). These research findings demonstrate the complicated relationship between PPD and PPA symptoms and etiology. Therefore, more research is needed on the topic of PPA to explain its individual characteristics and determine the differences among PPD and PPA.

The description of PPA symptoms vary significantly from PPD and the other mental health disorders it’s commonly diagnosed under, developing an argument for why the condition demands more research and attention. Women experiencing PPA and its related disorders report excessive worry or concern that cannot be controlled, disturbing thoughts, impulses, and behaviors. Specific apprehensions tend to focus around a mother’s infant, with paranoia manifesting about the child’s safety and vulnerability. Although these concerns are normal to have, excessive levels contribute to unhealthy anxiety in the mother. Other symptoms of PPA and its related disorders are synonymous to GAD, panic disorder (PD), OCD, and even post-traumatic stress disorder (PTSD). Severe displays of PPA can lead to women feeling debilitated by panic attacks, obsessions, trauma regarding childbirth, sleep disturbance, and even fear of dying (Ali, 2018). As stated previously, PPA and its related disorders are typically diagnosed similarly to PPD: under the umbrella of MDD. However, in some instances the anxiety symptoms are categorized within GAD and OCD (Ali, 2018; Pawluski et al., 2017). Based on this research, there is a clear need to address PPA as its own condition with proper diagnostic criteria, and begin to discern the biological differences between PPA and PPD.
Current Explanations of Peripartum Mood Disorders

From an evolutionary perspective, one can justify the existence of peripartum anxiety by arguing that it heightens awareness and reactivity towards infants in new mothers. Increasing such sensitivity ideally enhances infant quality of life, and can even reinforce bond formation between mother and child (Kim, Strathearn, & Swain, 2016; Laurent, Stevens, & Ablow, 2011). However, as previously discussed, PPA and PPD symptoms can be immensely harmful to mothers, new infants, and their families. Therefore, current research utilizes neurological approaches to better understand the incidence of PMD, and pinpoint how the hormonal changes during the peripartum period contribute to anxiety and depressive-like behaviors.

Neurological Changes. The maternal caregiving brain network provides a neurobiological approach to understanding how abnormalities in the brain contribute to perinatal mental health disorders. The network is considered an important aspect in explaining PMD because symptoms of depressed or anxious mothers often include disruption of maternal caregiving behaviors. For example, increased irritability and decreased attentiveness towards infants can be found in women struggling with PMD, and result in mothers responding to their infant’s needs less consistently and intuitively (Ali, 2018). The maternal caregiving network exists between a variety of different brain structures, notably the medial preoptic area (mPOA), ventral tegmental area (VTA), and periaqueductal gray (Pawluski et al., 2017). The mPOA contains receptors for nearly all steroid and peptide hormones that activate maternal behaviors (Numan & Insel, 2006). The VTA is integral for dopamine projections throughout the brain’s limbic and cortical areas, thus an important part of the reward circuitry (Ranaldi, 2014). Lastly, the periaqueductal gray exists between the forebrain and brain stem to integrate behavioral responses to stressors or pain (Benarroch, 2012). However, the mPOA is the main player in the
maternal brain by using its neurons to project to other regions of the brain involved in the salience, executive or attention, and reward and attachment networks. The salience network requires projections from the ventral and ventromedial prefrontal cortex, as well as connectivity between the amygdala (AMG) and anterior insula. The network responsible for executive function and attention utilizes projections from the ventral striatum and medial prefrontal cortex (mPFC). The reward network also involves the dopamine reward system, including the ventral striatum and prefrontal cortex (Kinsley & Lambert, 2006; Lovic & Fleming, 2015; Moses-Kolko, Horner, Phillips, Hipwell, & Swain, 2014; Pawluski et al., 2017). The nucleus accumbens (NAc) is a crucial component of this dopamine reward system, involving dopamine neurons to signal motivation and positive reinforcement throughout many of the circuits that contribute to the maternal caregiving network (Haim et al., 2014). It’s evident that the regions involved in the maternal caregiving network are complexly integrated, indicating many opportunities for connectivity to transgress and possibly contribute towards the presentation of PMD.

Understanding the relationships between the maternal caregiving network’s various brain regions provides insight to the biological underpinnings of PMD. Firstly, deficits to these networks unsurprisingly have consequences on mothers and their exhibited behaviors towards their children. Decreased AMG and insula connectivity is associated with increased depression in mothers (Wonch et al., 2016). In addition, damage to the executive function network decreases the quality of motherly behavior (Barrett & Fleming, 2011; Gonzalez, Jenkins, Steiner, & Fleming, 2012; Pawluski et al., 2017). The brain regions involved in the maternal caregiving network are also subjected to significant changes during and after pregnancy; studies have distinguished altered synaptic plasticity throughout the Nac, hippocampus, and AMG in the maternal brain (Haim et al., 2014; Pawluski et al., 2017). Additionally, structural changes in the
Nac occur throughout the postpartum period. Abnormalities in the Nac’s structural changes and neuroplasticity alterations during the peripartum period are associated with PPD behavior (Haim et al., 2014). More specifically, decreased activation and size of the Nac has been found in patients with mood disorder (Salgado & Kaplitt, 2015). Finally, several of the brain structures that are integral in the maternal caregiving networks contain hormone receptors, leading to their vulnerability to hormonal changes that are seen during pregnancy (Pawluski et al., 2017). These hormonal changes and their implications will be discussed later in this review.

Researchers also use methodologies such as fMRI to understand PMD’s presentation in the brain. Research in 2011 found evidence of less resting-state activity throughout the left frontal lobe, yet increased activity in the right frontal lobe of women with PPD. (Xiao-juan, Jian, Zhi-hong, Yan, & Shi-wei, 2011). Furthermore, women with PPD show weaker connectivity between the AMG, anterior cingulate cortex, dorsal lateral prefrontal cortex, and hippocampus compared to postpartum women not experiencing depression (Chase, Moses-Kolko, Zevallos, Wisner, & Phillips, 2014; Pawluski et al., 2017). Many of these brain regions are implicated within the maternal caregiving network, providing potential insight as to how alterations in these brain regions impact maternal behavior. Recent research utilizing fMRI and infant-related cues has led to an improved understanding of the connection between variations in brain activity and the behavioral changes in mothers with PPD. These studies require parents to be shown an image of their child’s face, and assess the neural responsiveness in the parents. Infant related cues have been found to have altered responses in depressed mothers, such that women experiencing depression or anxiety will have decreased activity in specific brain regions in response to an infant-related emotional cue but increased activity in response to non-infant emotional cues (Barrett & Fleming, 2011; Gingnell et al., 2015; Pawluski et al., 2017).
It is important to note that research distinguishing the interaction of PPA with the maternal caregiving network and other brain regions is limited. The majority of research focuses on depression and its effects on the brain. This perpetuates the need for more studies to acknowledge the importance of PPA within PMD and how its manifestation in the brain varies from PPD in order for us to gain a thorough understanding of the effects of PMD on one’s neurobiology.

**Hormonal Changes.** Pregnancy requires exposure to abnormally high levels of hormones. Each phase of pregnancy carries its own specific behavioral and biological changes. Moreover, before and after delivery there is a rapid change in estradiol and progesterone levels, the primary hormones involved in initiating and maintaining pregnancy. Specifically, estradiol and estriol, forms of estrogen, rise by 100-fold, and progesterone increases 10-fold by the end of pregnancy in comparison to levels seen during menstrual cycles outside of pregnancy. This vast increase in estrogen and progesterone is due to their increased production by the placenta. Therefore, once the placenta is removed after birth, hormone levels plunge back to prefollicular levels within five days (Hendrick, Altshuler, & Suri, 1998). Figure 1 demonstrates the dramatic variation in hormones that women undertake during pregnancy (Martin & Behbehani, 2006). Other hormones such as oxytocin and prolactin are integral in maternal care behavior like breast feeding and attachment (Grieb, Tierney, & Lonstein, 2017; Russell, Douglas, & Ingram, 2001).

The sudden drop in estrogen and progesterone levels after delivery is considered one explanation of the changes in mood and affect that are seen in PMD (Bekku & Yoshimura, 2005; Bloch, Daly, & Rubinow, 2003; Hendrick et al., 1998; Martin & Behbehani, 2006; Schiller, Meltzer-Brody, & Rubinow, 2015; Shimizu et al., 2003; Sohrabji, Miranda, & Toran-Allerand, 1994). The influence of hormones on the brain is due to the ability of ovarian hormones to pass
through the blood-brain barrier, which allows them to bind to receptors and alter gene transcription and expression (Martin & Behbehani, 2006). Because of the enormous increase and subsequent fall in hormone levels, there must be implications for equally vast changes in the brain which have behavioral implications and possibly impact PMD incidence. Thus, research on the relationship among the large hormone drop and changes in the maternal brain carry potential for understanding the presentation of PMD.

Researchers have studied the connection between hormones and PMD using animal models, with their findings suggesting a concrete association but further need to understand how and why hormones are so influential. Animal models are currently the most effective way to study the relationship between hormones and PMD due to great limitations when working with human subjects. More specifically, animal are easier to manipulate and provide more experimental possibilities. A common methodology involves ovariectomizing rodents and studying how injections of estrogen and progesterone impact their brain and behavior. Research utilizing these methods have found that reproductive hormones influence almost every biological system implicated in PPD (Schiller et al., 2015). Ovariectomy decreases and estradiol treatment increases brain-derived neurotrophic factor (BDNF) levels throughout the forebrain and hippocampus of female rats (Sohrabji et al., 1994). BDNF is a growth factor within the brain that promotes neuron growth and survival. Shimizu et al. demonstrated the important role BDNF plays within depression, and possibly PMD, among humans; they found decreased BDNF levels in those experiencing depression, and an increase in BDNF when the brain is introduced to antidepressants (Shimizu et al., 2003). Progesterone is similar to estradiol such that the hormone has also been found to be essential to perinatal brain changes; progesterone has been found to influence neurotransmission (Finocchi & Ferrari, 2011). Furthermore, research suggests that
progesterone up-regulates BDNF in the hippocampus and cerebral cortex (Pluchino et al., 2013). This research solidifies the impact perinatal hormones carry on the neuroplasticity of brain regions involved in the maternal caregiving network.

The relationship between perinatal hormones and brain alterations also has behavioral implications. The removal of estradiol in rats facilitates behavioral changes that are similar to depression; rats show increased despair and decreased appetite (Bekku & Yoshimura, 2005). If estradiol is reintroduced to the brain, it acts as an antidepressant when administered to rats experiencing despair during a forced swim test (Walf, Rhodes, & Frye, 2004). Walf, Rhodes, and Frye (2004) further determined that these changes in depressive behavior can be mapped to selective actions of estrogen receptors in the VTA, a key structure of the maternal caregiving network. Alterations in maternal rat behaviors such as lactation, pup-licking, and attention to pups has also been linked to changes of ovarian hormone levels. In particular, increases in postpartum progesterone and estradiol limit the display of maternal rat behaviors during the weaning period of pups (Grieb et al., 2017). Lastly, research continues to demonstrate that the reproductive hormones involved in pregnancy are fundamental throughout emotion processing, arousal, cognition, and motivation (Russell et al., 2001; Schiller et al., 2015). Thus, it’s logical that abnormalities in these hormone levels lead to irregular brain changes, possibly contributing to the risk of PMD.

**Hormone Withdrawal Model**

Hormone models in rodents support the theory that peripartum hormones play a crucial role in PMD, otherwise termed the “estradiol withdrawal” hypothesis (Galea et al., 2008; Galea, Wide, & Barr, 2001; Green, Barr, & Galea, 2009; Schiller et al., 2015). In 2001, Galea et al. demonstrated that a hormone-simulated pregnancy can mimic the hormonal fluctuations seen in
pregnancy. This model requires ovariectomizing rodents in order to remove their endogenous source of hormones, and then treating them with regimens of estradiol and progesterone injections over the same amount of days as a rat pregnancy. During the first 16 days, rats obtained a high daily dose of progesterone and low daily dose of estradiol. Days 17-22 provided rats with increased daily estradiol dosages, and progesterone levels were lowered. On the 23rd and last day of the injections, half of the sampled rats experienced an abrupt drop to similar levels as seen in postpartum due to their estradiol treatment concluding; this condition is termed “hormone-withdrawal.” The other half of the rats had sustained estradiol injections and acted as a control or “hormone-sustained” condition for later comparison.

The hormone-withdrawal model has been used to study the behavioral and neurological changes that occur during the peripartum period. With depression specifically, researchers have demonstrated that rodents who experience the hormone-withdrawal condition have increased depression-like behaviors (Galea et al., 2001; Stoffel & Craft, 2004). Most of these studies utilize the behavioral assessment called the Forced Swim Test to measure depressive symptoms such as learned helplessness in animal models. One study found an association between rats subjected to the hormone-withdrawal condition and a decrease in sucrose consumption, or appetite (Green et al., 2009). These studies also established that postpartum estradiol treatment decreases depressive behaviors; hormone-sustained rodents continually had less learned helplessness and performed better on the Forced Swim Test.

The relationship between hormone-withdrawal and anxiety-like behaviors is a little less clear. The studies previously mentioned, including Galea et al. (2001), found no significant associations between anxiety behavior and hormone-withdrawal. However, the procedural implementation of anxiety measures may have impacted their results; anxiety was measured via
the Open Field Test following two days of forced swim testing in the same population of rats by Galea et al. In comparison, previous research points to late pregnancy resulting in increased anxiety-like behaviors which were measured using the Elevated Plus Maze (Neumann, Johnstone, et al., 1998; Neumann, Torner, & Wigger, 1999; Neumann, Wigger, Liebsch, Holsboer, & Landgraf, 1998). The Elevated Plus Maze is a reliable assessment of anxiety when tested in a single trial for up to five minutes. However, habituation of the maze can occur and decrease the validity of the test if repeated within-subjects testing is utilized, or if trials last more than five minutes. (Schrader, Taylor, Lowery-Gionta, & Moore, 2018). More recent hormone-simulated pregnancy research has also utilized the Elevated Plus Maze, and found increased anxiety behaviors in mice subjected to estrogen-withdrawal (Zhang et al., 2016). This study concluded that postpartum estrogen withdrawal limits hippocampal neurogenesis in mice that present with anxious and depressive behaviors (Zhang et al., 2016).

Based on the findings from studies utilizing hormone-simulated pregnancy methodology, pregnancy has obvious implications on postpartum behavior and changes in the brain. However, more research is needed to uncover the neurological mechanisms of PMD and its various symptomology including anxiety. In addition, few studies have expanded our understanding on the neurobiological implications of peripartum hormone fluctuations. Most of our understanding on this topic is fueled by laboratory rodent models (Pawluski et al., 2017). Animal models such as what’s seen in Galea et al. (2001) provide the opportunity to investigate the role of hormonal changes on the neuroplasticity that occurs during and after pregnancy (Galea et al., 2008).

**The Nucleus Accumbens**

The NAc is an ideal brain location to study PMD due to its previously mentioned role within the reward circuit, maternal caregiving network, and implications in disorders such as
addiction, depression, and anxiety outside of the peripartum period (Holmes & Fam, 2013; Salgado & Kaplitt, 2015). The NAc is a key component of the ventral striatum (Grueter, Robison, Neve, Nestler, & Malenka, 2013). The NAc consists of a core and shell, the cells of which are 90% medium spiny neurons (MSNs) divided into Dopamine-1 (D1) and Dopamine-2 (D2) neuron types. The D1 receptors project to the midbrain, whereas the D2s mostly project to the ventral pallidum where it influences dopamine (DA) neurons. These DA neurons are what drive the natural reward system in the brain. For example, drugs of abuse increase DA in the NAc and even alter its plasticity (Salgado & Kaplitt, 2015). Additionally, DA neurons are sensitive to estrogen, suggesting associations between hormonal fluctuations, the reward circuit, and the NAc (Calipari et al., 2017). The bidirectional relationship between DA and estrogen is so significant that research suggests that DA can regulate many of the hormone-related behavioral changes seen in women. Menstrual disorders, for example, are coordinated with impaired dopaminergic systems (Barth, Villringer, & Sacher, 2015).

Several studies have demonstrated that the NAc plays a role in mood disorders (Barrot et al., 2002; Chen, Rada, Bützler, Leibowitz, & Hoebel, 2012; Huang, Wu, Chen, Manji, & Chen, 2003; Salgado & Kaplitt, 2015; Sequeira et al., 2012). Sequeria et al. (2012) observed abnormalities in the NAc of people who were diagnosed with an extreme mood disorder and had suicidal behaviors. In particular, a diminished neuroprotective response to stress was found in various areas of the brain, including the NAc. In mice, altered activity and morphology of NAc neurons is associated with increased depression (Huang et al., 2003). On a molecular level, enhances in cAMP response element binding protein (CREB) expression of the NAc shell increase anxiety-related behavior measured by the elevated plus maze (Barrot et al., 2002). CREB has been defined in past research as an important transcription factor that regulates
neuroplasticity and can impact neuropathological conditions (Yu & Rasenick, 2012, p. 2). From a structural perspective, decreased size of the left NAc and less NAc activation is linked to mood disorder (Baumann et al., 1999; Heller et al., 2009). Ultimately, research has demonstrated that a relationship exists among anxiety or depression, and abnormalities within the NAc. This provides a base framework for further investigation of the neurobiological explanations for PMD.

The NAc is also an imperative member of the mesolimbic pathway. The mesolimbic dopamine pathway is a focus of our research due to associations between abnormal pathway functioning and mood disorders; the pathway is involved in depression, anxiety, stress response, and stress (Grueter et al., 2013; Nestler & Carlezon, 2006). The pathway bridges the NAc to the VTA, which are brain regions of the maternal caregiving network. Disruptions in the mesolimbic dopaminergic projections from the VTA to the NAc are implicated in contributing to depressive symptoms and decreased DA release (Drevets et al., 2008; Haim et al., 2014). Furthermore, research demonstrates that stress and depression stimulates the mesolimbic dopamine pathway, thus regulating depressive-like behaviors (Nestler & Carlezon, 2006). In conclusion, there is an obvious relationship between the mesolimbic dopamine pathway, hormones, and maternal behavior that demands further attention. Hormone-simulated pregnancy provides an opportunity to investigate this connection and explain the behavioral alterations seen in women experiencing PMD.

ΔFosB

The NAc also comprises ΔFosB, a transcription factor that mediates gene expression changes throughout the brain (Grueter et al., 2013). Transcription factors bind to DNA, altering what genes are turned on and off. More specifically, environmental stimuli that trigger changes
in ΔFosB levels implement genetic changes in the induction of ΔFosB in the NAc’s D1 and D2 MSNs. This implies that epigenetic events have the capacity to alter chromatin structure through the use of ΔFosB, and can carry long term implications on behavior as seen in mood disorders (Sun, Kennedy, & Nestler, 2013). ΔFosB enhances neuron growth and connectivity within the NAc, acting as the main source of neuroplasticity in the NAc (Grueter et al., 2013; Nestler, Kelz, & Chen, 1999). A large body of research demonstrates that the effects of ΔFosB are long term, and relevant for understanding the manifestation of drug abuse, depression, and chronic stress (Hamilton et al., 2018; McClung et al., 2004; Nestler, 2008; Nestler, Barrot, & Self, 2001; Saurer, Ijames, & Lysle, 2009; Vialou et al., 2010). Regarding drug abuse, increasing evidence argues that ΔFosB is a “molecular switch” for drug addiction; it carries the ability to convert the initial responses seen in the brain after repetitive drug administration into the long term adaptations in brain plasticity that explain drug addiction (Nestler et al., 1999). However, research regarding ΔFosB’s function within depression and chronic stress provides more relevant material that might aid in understanding how ΔFosB relates to mood disorders.

Research suggests that ΔFosB expression and depressive behaviors are strongly related (Brown, Ye, Bronson, Dikkes, & Greenberg, 1996; Vialou et al., 2010). The influence of ΔFosB on depression can be seen among the decreased amounts of ΔFosB found in depressed individuals. ΔFosB also affects the success of antidepressant administration. Specifically, antidepressants are more productive when there is increased ΔFosB accumulation in the brain (Vialou et al., 2010). Repeated administration of antidepressants also proliferates ΔFosB. Research conducted by Brown et al. (1996) discovered a possible link between maternal behavior and ΔFosB as well; they discovered that limiting ΔFosB expression in the preoptic area causes less nurturing behavior in mice and presumably other mammals as well. Based on the
relationship among ΔFosB, depression, and maternal behavior one might suggest that ΔFosB also has implications and impact on PPD.

Chronic stress and its related experiences also have a connection to ΔFosB accumulation, as previously noted. Following exposure to chronic stress in the form of foot shocks, an increase in ΔFosB can be found in the NAc and dorsal raphe of rodents (McClung et al., 2004). Furthermore, resilient mice have been found to have the greatest induction of ΔFosB within the NAc core and shell. More specifically, mice that demonstrate less learned helplessness behaviors in a chronic foot shock paradigm have higher levels of ΔFosB induction. Additional research points to reduced ΔFosB activity in the NAc causing a reduction in positive and adaptive responses to stress (Nestler, 2008; Vialou et al., 2010). In conclusion, several forms of stress including restraint stress, unpredictable stress, and foot shock stress induce ΔFosB in the NAc when studied within animal models (McClung et al., 2004). Therefore, it is clear then that ΔFosB represents an adaptive, active coping mechanism within the brain that buffers the development of learned helplessness, or depression.

No research was found that pertained to anxiety-like symptoms and its connection to ΔFosB levels. However, research shows a correlation between chronic stress and anxiety-like behavior such that exposure to stressors can generate anxiety in both rodents and humans (Matuszewich et al., 2007; Vyas & Chattarji, 2004). Therefore, we can conclude that a link may also exist between ΔFosB and anxiety behavior, a topic that our research aims to address and build upon.

The Present Study

Summary of Rationale. PMD is a common experience in a woman’s life. However, severe manifestations of anxiety and depression-like behaviors can lead to long term alterations
in child, maternal, and familial health outcomes (Rahman et al., 2004). Although PMD isn’t rare, it’s poorly understood, as represented in the diagnostic criteria that fails to address PPA specifically (Ali, 2018; Pawluski et al., 2017). Furthermore, PPA carries higher prevalence than PPD, yet there has been a limited focus on postpartum anxiety-related behaviors, with most research attending to understand PPD (Ali, 2018; Fairbrother et al., 2016). Therefore, our research aims to concentrate on anxiety-like symptoms during the postpartum phase to build on anxiety-related literature and add to the discourse on neurological underpinnings of PPA.

Current literature attributes PMD to hormonal fluctuations seen during and after pregnancy (Martin & Behbehani, 2006; Schiller et al., 2015). More specifically, estrogen increases at levels generously above menstruation right before parturition. These levels drop post-birth, creating a risk factor for PMD. The hormone-simulated pregnancy animal model created by Galea et al. (2001) is a popular methodology that we intend to use in order to study this drop in hormones and its effects on the brain and behavior. In addition, previous work in our lab has implemented the hormone-simulated pregnancy methodology in mice and hamsters, finding results that support the theory that the significant drop in hormones may contribute to PMD (Carson, 2018). However, further explanation is needed of the neurobiological alterations that take place during this postpartum drop, specifically within neuroplasticity. Consequently, we hope to expand this research and investigate the effects of the postpartum drop in hormones on the neuroplasticity that leads to transformations in maternal behavior.

The maternal caregiving network provides us with a framework for understanding the brain functioning required for healthy maternal behavior (Haim et al., 2014; Pawluski et al., 2017). Within the maternal caregiving network is the NAc, which is a crucial aspect of the mesolimbic dopaminergic pathway and reward circuitry of the brain as well (Grueter et al.,
Since all of these brain routes diverge at the NAc, it delivers us with an ideal brain region to study the effects of hormone-simulated pregnancy on neuroplasticity. The NAc has been overlooked in MPD research, yet provides a novel explanation to unhealthy postpartum behaviors (Salgado & Kaplitt, 2015). Additionally, the NAc holds DA neurons that are sensitive to estrogen, as well as ΔFosB.

ΔFosB acts as a transcription factor that holds the potential to alter long-term gene expression (Nestler et al., 1999). It also has an established relationship with depression and chronic stress; ΔFosB is highly induced after exposure to chronic stressors. Depression also increases ΔFosB accumulation (Hamilton et al., 2018; Sequeira et al., 2012). Due to its affiliations with depression and chronic stress, ΔFosB appears to play an integral role in buffering toxic stress and prepping maternal coping. Therefore, ΔFosB is a fitting molecular target for measuring changes in neuroplasticity of the NAc core and its DA neurons as seen in our previous lab work.

Prior research in our lab has explored ΔFosB expression in MSNs in the NAc while utilizing the hormone-withdrawal model of Galea et al. (2001) with mice and hamsters. Our findings from past research with mice and hamster models supports the theory that the extreme peripartum drop in hormones during delivery is related to PMD, and specifically anxiety-related postpartum behavior as seen in the Elevated Plus Maze (Carson, 2018). Our past research also demonstrated that ΔFosB accumulation can mediate this experience such that an increase in ΔFosB decreases anxiety behaviors or the duration an animal spends in the open arms of the Elevated Plus Maze. It was also discovered that estrogen-withdrawn mice had significantly more ΔFosB in the NAc core than shell, aiding in our future assessment of ΔFosB accumulation. Moving forward, our research suggests that an increase in ΔFosB expression is necessary for
postpartum mental health, and abnormal activation may be related to mood disorders seen in the postpartum stage. Therefore, this study aimed to inhibit ΔFosB in the NAc core, in hopes of discerning whether ΔFosB is related to anxiety-related behavior subsequent to hormone-simulated pregnancy and to extend the previous research in our lab.

**Research Questions and Hypothesis.** The current study explores the relationships between estrogen withdrawal postpartum, changes in the neuroplasticity of the NAc, and anxiety-like behaviors. These dynamics were assessed through utilizing Galea et al.’s (2001) hormone-simulated pregnancy animal model adapted for a mouse’s 21-day gestation period. Comparisons between estradiol-sustained and withdrawn anxiety-like behaviors provided us with insight on how significant the drop in estrogen after pregnancy affects PMD. We know from previous work that ΔFosB has increased induction in animals experiencing the hormone-withdrawn condition. Thus, we inhibited ΔFosB induction with a viral mediated gene transfer to confirm ΔFosB’s influence on the neuroplasticity of the NAc in order to build on past research. We utilized the Elevated Plus Maze to assess anxiety behavior in regards to the duration that a mouse spends time in the open or closed arms of the maze. In addition, the Open Field Test served as an ideal counter-measure in regards to its ability to provide information about the animals’ locomotor activity, and reaffirm that findings from the Elevated Plus Maze were not due to low locomotor ability.

If ΔFosB is truly an adaptive coping mechanism that is required for normal behavioral and neurological changes postpartum, then we anticipated seeing the largest increase in anxiety-like behaviors during the Elevated Plus Maze in hormone withdrawn mice with inhibited ΔFosB accumulation. We expected to see no differences in open field behavior between the mice in each study condition. Comparing the anxiety behavior between hormone sustained mice with ΔFosB
inhibition and mice without ΔFosB inhibition that experience hormone withdrawal provided crucial insight in regards to how ΔFosB accumulation and the postpartum hormone drop interact and contribute to PMD. More specifically, if the mice with ΔFosB inhibition that undergo hormone sustainment exhibit increased anxiety-like behaviors compared to estrogen withdrawn mice with normal ΔFosB accumulation, then it suggests that the role of ΔFosB accumulation is more influential than the postpartum drop in estrogen in promoting PMD. Therefore, this study offers necessary evidence to decipher the role of ΔFosB in the postpartum brain, and gauge the importance of the postpartum hormone drop on PMDs, particularly anxiety-related behaviors.

Methods

Subjects

This study utilized 32 adult, female, C57-Black6 mice. Mice were purchased at 8 weeks old from Charles River Laboratories (Wilmington, MA, USA). The mice were housed in pairs in 28 cm x 17 cm x 12 cm polycarbonate cages with aspen bedding and nesting material. Each mouse cage was supplied with one, three ounce Diet HydroGel Recovery pack (Clear H2O, Portland, ME, USA), and ad libitum access to food and water. A reversed light cycle of 12:12 light to dark was implemented. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC).

Procedure

Ovariectomy. All 32 mice underwent ovariectomy (OVX) before hormone-simulated pregnancy. This procedure eliminated the source of endogenous hormones, allowing us to manipulate hormone levels of the mice as an independent variable. Mice were anesthetized prior to surgery using 4-5% isoflurane aerosolized in oxygen. Next, the flanks of the mice were shaved and sterilized with three alternating scrubs of 70% ethanol and betadine. After moving the mouse
to a sterile surgical field, anesthesia was maintained throughout surgery using a nose cone with 2-3% isoflurane aerosolized in oxygen. A toe-pinch test confirmed the animal’s sedation before beginning surgery. Mice were also given an injection of 0.1 mL of Meloxicam (Boehringer Ingelheim, St. Joseph, MO, USA) before surgery. Once sedated, we made bilateral flank incisions and removed the ovaries using cauterization of the uterine horn. The muscle wall was closed using absorbable suture (Ethicon, Inc. Somerville, NJ, USA) and the skin was closed with wound clips. Mice were injected with 0.1 mL of Meloxicam for three days post-surgery.

**ΔFosB Inhibition.** Approximately one week after ovariectomy, mice were given bilateral intracranial injections of adeno-associated virus (AAV) targeting the NAc. Animals were randomly assigned to one of two vector conditions: a control or experimental vector, both gifted to us by the Nestler laboratory at Mt. Sinai School of Medicine, NYC, USA. While the control vector did not contain an active gene for transfer, the experimental vector was engineered to contain JunD. Overexpression of JunD forms competitive heterodimers with FosB in the brain. This inhibits the production of ΔFosB by preventing transcription of AP-1, the gene that FosB transcribes to produce ΔFosB (Nestler, 2008). This process of inhibition is called a viral mediated gene transfer. Both the experimental and control vector carried Green Fluorescent Protein Maker (GFP) in order to facilitate injection localization later.

On injection day, mice were anesthetized prior to injection using 4-5% isoflurane aerosolized in oxygen. Once anesthetized, top of each mouse’s scalp was shaved and sterilized with three alternating scrubs of ethanol and betadine. Afterwards, mice were placed in the stereotaxic frame, where anesthesia was maintained throughout surgery using a nose cone attached to the stereotaxic frame with 2-3% isoflurane aerosolized in oxygen. Mice were then injected with 0.1 mL of Meloxicam before an incision along the sagittal plane of the mouse skull.
was made stretching from the eyes to the ears of the mouse. The eyes of the mice were then covered with Puralube Vet Ointment (Dechra Veterinary Products, Overland Park, KS, USA) to decrease post-surgery eye discomfort. The bregma and lambda coordinates were then measured to ensure the skull was level. After any necessary leveling, the coordinates for the NAc (bregma 1.4 mm; lateral 1.0 mm; ventral 4.4 mm) were used to mark the skull to aid in drilling.

Approximately 0.5 μl of virus was bilaterally injected into the brain using a 10 μl Hamilton syringe under stereotaxic control. The injection needle was withdrawn five minutes after its insertion. Following injection, the skin was closed with wound clips and the animal was returned to its cage. Mice were injected with additional painkiller (0.1 mL of Meloxicam) for three days post-surgery.

Half of the mice (n = 16) were randomly placed in the experimental vector group, and received the experimental vector injections that limit the accumulation of ΔFosB. The other half (n = 16) received the control vector that only includes GFP. Of these two groups of 16 mice, they were again split into groups of eight for the hormone-simulated pregnancy. The subject conditions were such that eight mice were in the hormone-withdrawn experimental vector condition, eight in the hormone-withdrawn control vector, eight in the hormone-sustained experimental vector, and eight placed in the hormone-sustained control vector. Table 1 demonstrates the distribution of mice within the study conditions.

**Hormone Simulated Pregnancy.** Mice began hormone-simulated pregnancy one week following the AAV injections. Our model of hormone-simulated pregnancy is based on the methodology seen in Galea et al. (2011) but adapted for the mouse animal model in which gestation period lasts 21 days. All 32 mice received daily subcutaneous hormone injections at 9am. The early pregnancy period was simulated in the first 14 days. All mice received 0.5-μg of
estradiol and 0.8-mg of progesterone dissolved in 0.1 mL of cottonseed oil to mimic the low estradiol and high progesterone hormone levels of early pregnancy. Days 15-22 acted as late pregnancy, when estradiol levels are high and progesterone levels are low. Again, all mice received the same dosage of 10-μg estradiol dissolved in 0.1 mL of cottonseed oil. The first day of the postpartum period is day 23. This day initiates the hormone sustained and withdrawn conditions as previously mentioned as a part of our independent variables. Therefore, mice either received additional estradiol injections in cottonseed oil until day 26 in the hormone-sustained condition, or they received hormone-free cottonseed oil injections after day 23 and were withdrawn from estradiol if assigned to the hormone-withdrawal group.

**Behavioral Testing.** On the third day of the simulated postpartum period, we began conducting the behavioral tests. Mice underwent behavioral testing for anxiety behavior while one condition of mice were still experiencing sustained estrogen levels. The order of testing the animals in each condition was randomized and counterbalanced throughout the day to ensure that mice from each of the conditions did not display behavior based on the time of testing. The tests used to measure anxiety levels include the Elevated Plus Maze and Open Field Test.

**The Elevated Plus Maze.** The Elevated Plus Maze is a widely used and reliable measure of anxious behavior in animal models. The structure used in this test is two intersecting walkways in the shape of an “X” raised 73 cm above the ground. Within the “X,” there are two open and two closed arms. Each arm is 51 cm long and 11.5 cm wide. The closed arms have solid black walls that are 39.5 cm tall along the walkway, whereas the open arms have transparent walls with a small 1.0 cm lip along them. There is a 10 cm x 11 cm square opening where the two walkways diverge that allows a mouse to stand. Lower levels of anxiety are associated with the animal spending more time in the open arms of the maze. Higher levels of
anxiety limit the animal from exploring the open arms, leading to more time spent inside the closed arms (Carola, D’Olimpio, Brunamonti, Mangia, & Renzi, 2002; Walf & Frye, 2007).

Mice were placed in the maze and given five minutes to explore. A camera was placed above the center of the plus maze to record the behavior. The Ethovision XT animal tracking software designed by Noldus Industries was used to analyze each video of animal behavior. The software quantitatively measures mouse movements, and provides values for velocity, total distance traveled, time spent in open arms, time spent in closed arms, and time spent in the open box in the center of the maze.

**The Open Field Test.** The Open Field Test acts as another reliable measure to assess animal anxiety and motor activity. The test requires a square box with the dimensions 40.5 cm x 40.5 cm x 30 cm. There is no top to the box for the purpose of being able to look into the box and observe the animals. Each mouse received five minutes of uninterrupted time in the box. Lower levels of animal anxiety are associated with more time spent in the center of the box. Higher anxiety levels are associated with more time spent in the periphery (Carola et al., 2002; Choleris, Thomas, Kavaliers, & Prato, 2001). Furthermore, this test measures general locomotor activity to balance the measurement of the Elevated Plus Maze. More specifically, it is important to discern whether mice are limited in movement due to exhaustion and inability to move, or anxiety. A video camera, placed on a tripod above the box, recorded each trial. The Ethovision XT software by Noldus Industries was again used to measure animal movements and generate values of velocity, total distance traveled, and time spent in the center versus box periphery.

**Animal Sacrifice.** All mice were sacrificed via intracardial perfusion on the fifth day of the simulated postpartum period. First, the mice were anesthetized with 0.1 mL beuthanasia-D which was administered via intraperitoneal injection. Once it was guaranteed that the animal
reached a surgical plane of anesthesia, we performed intracardial perfusion under a fume hood; a small lateral incision was made through the abdominal wall under the rib cage. This incision was enlarged by cutting away the ribs and lifting up the flap, exposing the heart for perfusion. Next, a needle was passed through the posterior left ventricle of the heart and clamped in place using a hemostat. The needle during this phase was connected to a peristaltic pump. Another incision was made in the right atrium with scissors such that once the perfusion buffer was pumped through the needle, the solution was sent throughout the animal’s body by the bloodstream before exiting through the right atrium. The pump line was moved into a 4% paraformaldehyde fixative once the liquid ran clear and the animal’s liver was void of blood. The brains were then removed manually using scissors and rangeurs after fixation. The brains were stored in a paraformaldehyde solution in the refrigerator until tissue sectioning.

**Neural Measures.**

*Tissue Sectioning and Histology.* Each brain was adhered to a brain block using Optimal Cutting Temperature (OCT) Compound (Sakura Finetek, Torrance, CA, USA) to remove the olfactory and cerebellum with a razor blade. The remaining brain was then coronally sectioned into 40-μm sections using a manual cryostat set at -20°C. The sections were stored in cryoprotectant at -20°C until staining.

The tissue histology used immunofluorescence to label ΔFosB expression in dopamine neurons of the NAc through GFP labeled MSNs. Sections consisting of a significant part of the NAc were selected for tissue histology, determined using a mouse stereotaxic atlas (Paxinos & Franklin, 2004). First, brain sections were washed in 25 mM phosphate buffered saline (PBS) five times, with each wash lasting five minutes. Then they were incubated for 48 hours at room temperature in a solution of antibodies in PBS with 0.1% Triton-X comprised of: a rabbit-anti
FosB (1:000, Cell Signaling Technology, Danvers, MA, USA), and a goat-anti GFP antibody (1:5000, Abcam, Cambridge, UK). Two days later, the brain sections were given five additional five minute washes in PBS before their last incubation in secondary antibodies (anti-rabbit-Alexa 594, anti-goat-Alexa 488, Jackson Immunoresearch Lab, West Grove, PA, USA). This final incubation period lasted one hour in a dark room, after which we washed the tissue three times for five minutes in PBS. Once the washing was complete, tissue sections were mounted on a slide. ProLong Gold Antifade Mountant (Life Technologies Corporation, Eugene, OR, USA) was used during cover slipping.

**Confocal Microscopy.** Confocal microscopy was used to assess the success of the site of the AAV injections in the NAc. Photomicrographs were taken at 10X, and focused on the anterior commissure of both the left and right hemisphere. Photomicrographs were sorted based on strong versus weak staining for future exclusion criteria.

**Statistical Analysis.** Two by two factorial ANOVAs were conducted to compare the influence of hormone and vector condition on the anxiety behavior of the mice in the Elevated Plus Maze in terms of time spent in the open and closed arms, and in the Open Field Test in regards of time spent in the inside or outside zone of the open field. We performed additional 2x2 factorial ANOVAs to assess the influence of hormone and vector condition on the locomotor activity as measured in the Open Field Test, including the values of velocity, total distance traveled, and time spent in the outside and inside zones of the open field. We also completed paired samples t-tests within groups to look for patterns in the amount of time spent in the closed versus open arms of the Elevated Plus Maze, and the outside versus inside zones of the Open Field Test.
Results

Eight mice were lost during this study due to complications during surgery. Therefore, two mice were housed individually for part of the study. The results of the injection sites further eliminated three animals: two due to weak staining, and one because of a missed injection site. Our final sample size consisted of 21 animals: hormone sustained, control GFP vector \((n = 4)\); hormone withdrawn, control GFP vector \((n = 5)\); hormone sustained, experimental JunD vector \((n = 6)\); hormone withdrawn, experimental JunD vector \((n = 6)\).

Elevated Plus Maze

We generated two values for the elevated plus maze: duration in the closed arms, and duration in the open arms. The 2x2 factorial ANOVA performed on the closed arms duration found no significant results (Table 2), however the interaction of hormone and vector condition approached significance, \(F(1,17) = 3.41, p = 0.082\) (Figure 2). The main effect of vector condition, \(F(1,17) = 1.46, p = 0.243\), did not vary based on experimental JunD vector \((M = 223.85, SD = 62.79)\) or control GFP vector \((M = 188.46, SD = 99.95)\). There was also no significant effect of hormone condition on the duration of mice in the closed arms of the maze measured in seconds, \(F(1,17) = 1.12, p = 0.289\), such that there was no significant difference between the hormone withdrawal condition \((M = 220.93, SD = 43.41)\) and the hormone sustained condition \((M = 195.21, SD = 109.25)\).

Similar statistics were gathered regarding the open arms duration (Table 3). The interaction effect between hormone and vector condition neared significance as it yielded an F ratio of \(F(1,17) = 4.12, p = 0.059\) (Figure 3). No significant effect of vector condition was found, \(F(1,17) = 2.25, p = 0.152\), such that experimental JunD vector group \((M = 25.45, SD = 28.52)\) and control GFP vector group \((M = 55.22, SD = 77.25)\) did not significantly differ from each
other either. No significant effect of hormone condition on duration in the open arms was found as well, $F(1,17) = 1.37, p = 0.257$, within hormone withdrawal ($M = 29.67, SD = 28.22$) or hormone sustained conditions ($M = 47.59, SD = 75.72$).

Lastly, we conducted paired samples t-tests to compare the duration spent in the open and closed arms within each condition. A significant difference in seconds spent in the closed ($M = 211.14, SD = 55.89$) versus the open arms ($M = 35.07, SD = 37.51$) was found in the JunD hormone-withdrawn mice; $t(5) = 5.18, p = 0.004$. We also found significance among the duration spend in the closed ($M = 236.57, SD = 71.84$) and open arms ($M = 15.82, SD = 12.68$) of the JunD hormone-sustained animals; $t(5) = 6.54, p = 0.001$. There was a significant difference in time spent in the open ($M = 27.46, SD = 14.77$) and closed arms ($M = 230.18, SD = 20.73$) for the GFP hormone-withdrawn group, too; $t(5) = 15.02, p < 0.000$. Finally, the GFP hormone-sustained group had no significant difference between their duration in the open ($M = 79.39, SD = 100.85$) or closed arms ($M = 144.57, SD = 120.99$); $t(4) = 0.69, p = 0.528$.

**Open Field Test**

We also performed a 2x2 factorial analysis of variance on the influence of the hormone versus the vector group on the duration of mice spending their time on the center or peripheral spaces of the open field (Table 4). The interaction effect of the vector and hormone condition was insignificant, $F(1,17) = 0.28, p = 0.605$. All effects were also statistically insignificant; vector condition carried no main effect on the duration of mice in the center of the open field as measured in seconds, $F(1,17) = 0.004, p = 0.949$, meaning there were no significant differences between the control ($M = 97.34, SD = 70.42$) and experimental vector conditions ($M = 98.33, SD = 80.78$). Additionally, there was no main effect of hormone condition on duration in the
center of the field, $F(1,17) = 0.02, p = 0.887$, and no significant difference among sustained ($M = 96.71, SD = 86.51$) and withdrawn ($M = 98.99, SD = 66.38$) mice.

Statistical insignificance was also found when looking at the duration (sec) spent in the peripheral or outside zone of the open field (Table 5); again, the interaction between vector and hormone groups was insignificant, $F(1,17) = 0.45, p = 0.513$. Vector condition yielded an F ratio of $F(1,17) = 0.02, p = 0.893$, indicating an insignificant difference between control ($M = 187.91, SD = 71.45$) and experimental vector conditions ($M = 184.31, SD = 83.71$). No main effect of hormone condition was discovered either, $F(1,17) = 0.00, p = 0.992$ such that sustained ($M = 184.06, SD = 89.80$) and withdrawn ($M = 187.48, SD = 67.34$) hormone conditions did not significantly contrast each other.

The 2x2 factorial ANOVA assessing the variance within the velocity of the mice during the open field test also found nothing significant (Table 6); the interaction effect was insignificant, $F(1,17) = 0.02, p = 0.898$. Vector condition had no main effect on velocity, $F(1,17) = 0.08, p = 0.778$, such that both control GFP vector ($M = 5.30, SD = 1.32$) and experimental JunD vector ($M = 5.04, SD = 1.78$) were insignificantly different. Hormone condition also had no significant effect, $F(1,17) = 3.40, p = 0.083$ including no difference between withdrawn ($M = 5.77, SD = 1.46$) and sustained ($M = 4.49, SD = 1.46$) conditions.

We also performed a 2x2 factorial analysis of variance for the distance traveled during the open field test (Table 7). The interaction effect was insignificant, $F(1,17) = 0.12, p = 0.748$. In addition, the vector condition had an insignificant main effect on the distance mice traveled in the open field, $F(1,17) = 0.24, p = 0.632$, such that the distance traveled in the control vector group ($M = 1591.23, SD = 395.38$) did not significantly differ from the experimental vector group ($M = 1474.85, SD = 535.36$). Hormone condition had no significant main effect, yet
almost reached significance, $F(1,17) = 4.36, p = 0.052$. Thus, distance traveled did not significantly vary based on hormone withdrawal ($M = 1729.63, SD = 439.20$) or sustainment ($M = 1299.33, SD = 418.22$).

Paired samples t-tests were also conducted for each animal condition to compare the amount of time each animal group spent in the center versus the peripheral of the open field. There was no significant difference in the duration spent in the outside ($M = 196.28, SD = 64.83$) or inside ($M = 91.53, SD = 63.15$) of the open field for animals in the JunD-withdrawn group; $t(5) = -2.01, p = 0.101$. The JunD animals that experienced hormone sustainment also had an insignificant difference in time spent in the peripheral ($M = 172.34, SD = 104.26$) or center ($M = 105.14, SD = 101.27$) of the open field; $t(5) = 0.81, p = 0.454$. No significant difference was found between seconds spent in the outside ($M = 168.20, SD = 71.55$) or center ($M = 116.37, SD = 71.44$) of the open field for GFP hormone-withdrawn animals; $t(5) = 0.89, p = 0.415$. Lastly, an insignificant difference in duration in the inside ($M = 77.94, SD = 62.78$) compared to the outside ($M = 207.32, SD = 64.84$) was found in the GFP-sustained mice; $t(4) = -2.27, p = 0.085$.

**Discussion**

This is the first study that we are aware of to assess ΔFosB accumulation in the NAc via a hormone-simulated pregnancy in mice. Thus, our novel methods contribute to a huge lack of research on PMD and its neurological underpinnings within the topics of neuroplasticity and peripartum hormone fluctuations. The results we obtained from this study help to determine the importance of ΔFosB expression within the manifestation of PMD. More specifically, our research suggests several trends in regards to the influence of ΔFosB expression and hormone-withdrawal in the brain during pre and postnatal periods. Before discussing these findings in detail, it’s important to recognize the low statistical power in our results; due to our small sample
sizes, we were underpowered to detect statistical differences when assessing the relationship between hormone or vector conditions and anxiety behavior. Nevertheless, our findings carry many implications in understanding how ΔFosB impacts peripartum anxiety behaviors.

**Interpreting Behavioral Data**

Initially, our results were not congruent with previous findings suggesting that rodents exhibit increased anxious behavior after exposure to an elevated-estrogen drop (Galea et al., 2008, 2001; Zhang et al., 2016). Nevertheless, although insignificant via the ANOVA, a noticeable trend exists between the anxiety-like behavior of the GFP hormone sustained group and the three other conditions (Figure 4). The GFP-sustained mice clearly spent less time in the closed arms and more in the open arms compared to the GFP-withdrawn JunD-sustained, and JunD-withdrawn mice. Additionally, every animal condition except the GFP-sustained mice spent significantly more time in the open arms of the plus maze than the closed arms. This big picture perspective of our results suggests that both the dramatic drop in estrogen seen in postpartum and ΔFosB inhibition in the NAc increase anxiety-like behavior. However, we need to compare each condition more closely in order to understand when ΔFosB inhibition makes the largest impact in behavior and how it interacts with hormone-withdrawal.

When focusing on the results of the hormone conditions, our findings seem to imply that the high estrogen levels during pregnancy are related to low anxiety behaviors during pregnancy as measured in the Elevated Plus Maze. Furthermore, estrogen withdrawal was continuously found to lead to an anxious behavioral phenotype in the Elevated Plus Maze no matter what vector condition the mice were in. In particular, mice in the hormone withdrawal condition, regardless of their vector category, spent more time in the closed arms of the plus maze than animals in the hormone sustained condition. Overall, the sustained and withdrawn hormone
conditions had noticeably different amounts of time spent in the open arms too, even when ignoring their vector condition; hormone withdrawn mice spent less time in the open areas than the sustained group. These results suggest that the postpartum estrogen drop has significant implications on the postpartum anxiety behavior phenotype.

This trend regarding the influence of the estrogen drop continues when comparing the results of the GFP-withdrawn and GFP-sustained mice. These two conditions provided us with a baseline example of how hormone withdrawal impacts behavior. Since both GFP groups had uninterrupted ΔFosB accumulation, the GFP-withdrawn mice represented a typical postpartum estrogen drop experience during their behavioral testing, whereas the GFP-sustained mice experienced the equivalent of a late stage pregnancy while tested for anxiety behavior. The GFP-withdrawn mice showed more anxiety-like behavior than the GFP-sustained mice such that they spent more time in the closed arms than the open arms of the Elevated Plus Maze. These findings are supported by previous research conducted in the Been lab and other research groups (Carson, 2018; Neumann et al., 1999; Zhang et al., 2016). Overall, the differences in duration between hormone groups may be statistically insignificant, but they point out the underlying impact that estrogen has on anxiety behaviors during peripartum period and possibly PMD. Lastly, since we used methodology from Galea et al. (2001), our study supplements current literature that supports the hormone-withdrawal model as an accurate way to study the effects of the postpartum estrogen drop on the brain and behavior (Green et al., 2009; Stoffel & Craft, 2004; Zhang et al., 2016).

Next, we compared the results of the GFP-withdrawn animals to the JunD-withdrawn animals in order to investigate how the JunD vector impacted anxiety-like behavior after the postpartum estrogen drop. Since one can assume that the GFP-withdrawn mice experienced a
baseline postpartum experience compared to other mice, they acted as an ideal comparison against the other conditions. Yet, the GFP-withdrawn mice spent slightly more time in the closed arms and less time in the open compared to the JunD-withdrawn animals. This was unexpected because the JunD-withdrawn animals were not only subjected to the estrogen drop, but they also had decreased ΔFosB accumulation. Therefore, we predicted that the JunD-withdrawn mice would have the highest demonstration of anxiety. The comparison between these two groups leads us to consider whether ΔFosB accumulation is less influential on postpartum anxiety behavior than the postpartum estrogen drop.

In order to better understand the role of ΔFosB, we looked for differences in anxiety behavior between both groups of mice that obtained the JunD vector. This provided us with a rudimentary perspective towards the effects of ΔFosB inhibition during late pregnancy and postpartum anxiety-like behavior. Both JunD hormone sustained and withdrawn mice demonstrated significantly more activity in the closed versus the open arms of the plus maze. Nevertheless, when comparing the time each group spent in the open arms, it appears as though the JunD hormone sustained mice showed a less anxious phenotype than the JunD-withdrawn animals. Although statistically insignificant, this trend suggests that the JunD-sustained mice had more anxiety, and that the postpartum estrogen drop carries more influence on the postpartum anxiety phenotype than ΔFosB inhibition. As mentioned previously, this is an unpredicted finding – we hypothesized that the JunD-withdrawn mice would exhibit the most anxiety behavior, supporting the idea that ΔFosB accumulation is a crucial component in limiting postpartum anxiety behaviors. This insinuates that ΔFosB accumulation is more influential before the postpartum period, and proposes that the anxiety phenotype is prevented by the ΔFosB expression during pregnancy.
Our final conclusions in regards to the role of ΔFosB on the anxiety phenotype can be drawn while interpreting the results of the JunD-sustained and GFP-sustained conditions. In specific, the GFP-sustained mice gave us insight to the anxiety levels during the end of pregnancy, without any ΔFosB inhibition. In contrast, the JunD-sustained mice experienced high estrogen levels seen in the end of pregnancy while having limited ΔFosB accumulation. It’s interesting to note that the JunD-sustained mice spent the most time in the closed arms and the smallest amount of time in the open arms. In comparison, the GFP-sustained animals spent somewhat similar amounts of time in the closed versus open arms. This could be interpreted as the JunD-sustained group exhibiting more anxiety behavior than the GFP-sustained mice. If so, it suggests that ΔFosB accumulation must take place during pregnancy in order for it to act as a buffer against anxiety-like behavior after pregnancy. In the case of the JunD-sustained group, it’s possible that ΔFosB was unable to accumulate during the late stage of pregnancy while estrogen levels increased, therefore, the JunD-sustained mice were unable to prevent the high anxiety phenotypes associated with PMD behavior. If true, then this carries implications for the role of ΔFosB during pregnancy and points to ΔFosB’s ability to promote anti-anxiety behavior if it properly accumulates prior to delivery.

In contrast to the Elevated Plus Maze, the results from the Open Field Test supported our hypotheses; we expected no significant differences between the hormone and vector conditions and their behavior in the Open Field Test. With the exception of a significant variance in velocity among the hormone conditions, we found no significant differences between the hormone or vector conditions in regards to their locomotor activity. It is extremely unlikely that such a finding within the vector variable has any implications on the interpretations of our results. Thus, our prediction that all animals would demonstrate similar locomotor functioning was confirmed.
by our data. Our findings also support the use of the Open Field Test as a more valuable assessment of locomotor activity than anxiety-like behavior. More specifically, there were no trends found in terms of which conditions spent more time in the inside or outside zone of the open field. This demonstrated that the Elevated Plus Maze was more successful at measuring anxiety-like behavior than the Open Field Test, suggesting that the Open Field Test is best utilized as a baseline evaluation of locomotor functioning.

**Neurological Implications for PMD**

Firstly, our findings add to a long list of studies which have determined that estrogen exposure carries many neurological implications, especially in regards to the postpartum estrogen drop associated with PMD (Bekku & Yoshimura, 2005; Bloch et al., 2003; Galea et al., 2001; Green et al., 2009; Hendrick et al., 1998; Martin & Behbehani, 2006; Schiller et al., 2015; Sohrabji et al., 1994; Stoffel & Craft, 2004). In specific, estrogen can have long lasting impacts on the brain, such that estradiol treatment has been found to enhance BDNF levels (Sohrabji et al., 1994). Additionally, current research has found evidence that estrogen exposure increases hippocampus plasticity (Barha & Galea, 2010; Galea et al., 2008; Workman, Barha, & Galea, 2011). Our research builds upon these findings by demonstrating that increases in estrogen during pregnancy, or exposure to the postpartum estrogen drop, leads to changes in NAc neuroplasticity, or ΔFosB accumulation. Such fluctuations in estrogen levels can be considered contributing factors towards the neurological alterations seen in PMD, meanwhile displaying the complicated relationship between estrogen and neuroplasticity.

Based on our results, it’s plausible that neuroplasticity in the NAc, in the form of ΔFosB, plays an influential role during pregnancy in moderating peripartum anxiety levels. As a central aspect of the mesolimbic dopamine pathway, abnormal NAc functioning carries many
associations with depression, anxiety, and stress behaviors (Grueter et al., 2013; Nestler & Carlezon, 2006). All of these conditions are common characteristic of PMD; as discussed in the introduction, PMD involves a wide variety of symptoms including paranoia, irritability, sadness, and other behaviors seen in mood disorders outside of the peripartum period. Furthermore, the mesolimbic dopamine pathway acts as a connection between the NAc and the VTA, which is a connection bridging two members of the maternal caregiving network, as well (Haim et al., 2014). These pathways between the NAc, VTA, and other brain regions involved in the mesolimbic dopamine network have been found to activate during stress in order to limit depressive behaviors (Drevets et al., 2008; Haim et al., 2014; Nestler & Carlezon, 2006). Deficits to these networks have consequences on the behaviors of new mothers such that research in the past as found an increase in PPD if there are alterations in NAc neuroplasticity (Haim et al., 2014). Based on our results, deprivation of ΔFosB in the NAc is a specific neural deficit that can induce anxiety behaviors, and adds to the current literature focusing on how abnormal NAc function contributes to PMD.

Our findings suggest that if unable to accumulate during pregnancy, ΔFosB isn’t capable of providing a protective buffer against the anxiety-like and other PMD-related behaviors seen after the postnatal estrogen drop. ΔFosB has previously been determined as an important source of neuroplasticity in the NAc, with implications in the manifestation of depression and chronic stress (Hamilton et al., 2018; McClung et al., 2004; Nestler, 2008). This study adds anxiety to the list of behaviors associated with ΔFosB. Furthermore, current literature suggests that ΔFosB may moderate maternal behaviors and adaptive responses to stress (Brown et al., 1996; Vialou et al., 2010). Due to the conclusions from this study and many others, it’s clear that ΔFosB plays an instrumental role in active, coping, neurological mechanisms in the brain that buffer the
development of depression and anxiety-like behaviors. Pregnancy and motherhood provides a valuable time period to test this association between ΔFosB and mood disorders, leaving us with the outlook that ΔFosB plays a crucial role during the peripartum period as well. Overall, this study demonstrates an existing link between ΔFosB accumulation and PMD, opening the door for future studies to further explore when during the peripartum time period FosB is most significant.

**Clinical Implications for PMD**

In the present study, we aimed to distinguish how hormone-withdrawal impacts anxiety-related peripartum behaviors. In contrast, previous research has mainly focused on the depressive-like behaviors after hormone-withdrawal and within PMD. Our investigation into anxiety and its manifestation during the peripartum period found engaging evidence that anxiety is a very significant experience pre and postpartum. This carries many implications in regards to the clinical perspectives towards PPA and PMD. More specifically, recent metanalyses distinguished larger rates of peripartum anxiety than depression-related disorders (Ali, 2018; Fairbrother et al., 2016; Matthey et al., 2003; Pawluski et al., 2017). Yet, PPD has traditionally gained more priority than PPA in clinical and research settings, leading to inaccurate understandings and diagnoses of PMD, and the inability to clinically treat anxiety disorders during peripartum period. Therefore, our investigation into anxiety-like behavior demonstrates its prevalence during the peripartum period, and relevance to the greater topic of PMD, thus explicitly pointing out a need for a clinical response to the prevalence of PPA.

The only diagnostic material currently implemented to assess and treat PMD-related behavior merely addresses postpartum depression. In particular, the Edinburgh Postnatal Depression Scale is routinely utilized in clinical settings and research studies looking at human
postpartum depression (Matthey et al., 2003). However, the Edinburgh scale doesn’t include measures of anxiety, thus failing to account for the wide range of symptoms and experiences involved in PMD. Moving forward, it seems obvious that anxiety is an important factor of PMD based on previous research and the findings from the present study. Thus, our study is calling for increased awareness and response from the medical community in recognizing PPA in the form of developing more comprehensive diagnostic tools for PMD that include anxiety-related behavior.

Not only is it important to involve PPA symptoms within clinical assessment tools, but it is also imperative that clinical reactions to peripartum anxiety incorporate accurate educational material to benefit new mothers. Stigma towards new mothers unable to experience a typical peripartum experience is common, and is detrimental to the way in which women look for help to address their stress and mental health (Logsdon, Wisner, & Pinto-Foltz, 2006; Pinto-Foltz & Logsdon, 2008). In order to fully respond to the rates of PMD, medical care professionals need to be informed about the diversity in PMD’s manifestation, with the hopes that it will encourage decreases in stigma and promote positive, healthy maternal experiences (Logsdon, Foltz, Scheetz, & Myers, 2010). Likewise, providing new mothers with educational material has been found to benefit their psychological well-being months after delivery (Ho et al., 2009). A recent study by Garg, Morton, & Heneghan (2005) found that most hospitals educate newly delivered women on the topic of PPD, but that mothers thought it would have been more helpful to gain information many times throughout pregnancy and after delivery. Again, this study points out the clinical focus on postpartum depressive-like behaviors, and not anxiety or peripartum symptoms. Therefore, in the future, this educational material should include an overview of the symptoms
associated with PMD, including anxiety, as well as encourage PMD education throughout the peripartum period.

Limitations

One of the central limitations in the present study involves the sample size, as previously mentioned. Due to surgery complications, a significant amount of the mice for this study were lost and led to low statistical power in terms of numbers in each study condition. With many of our statistical tests, we found trends in the anxiety behavior. However, we are unable to draw concrete causation between ΔFosB inhibition, hormone manipulation, and anxiety behavior.

The injection sites in the NAc may have contributed to an inconsistent effect of the JunD virus due to varying sensitivity in the NAc shell versus core. Previous research in the Been lab found that significantly more FosB is present in the NAc core in animals that experience estrogen withdrawal compared to mice that undergo estrogen sustainment (Carson, 2018). No difference was found in the NAc shell. These findings might point to variance in JunD sensitivity between the core and shell of the NAc depending on which subsection of the NAc our injections hit. This could impact the strength of the ΔFosB inhibition or expression. Additionally, we were unable to verify that the JunD vector completely knocked down ΔFosB expression due to complications with the red immunofluorescence staining. This carries significant implications on our results – although we were able to verify our injection sites utilizing the staining for GFP, it was impossible to confirm the difference in ΔFosB expression between the subject conditions. Nevertheless, previous work in the Nestler lab have confirmed the effectiveness of the JunD vector, therefore we had no concerns regarding the success of the viral mediated gene transfer (Berton et al., 2009; Ribeiro et al., 2018).
The behavioral data left us with concerns regarding a “ceiling effect” existing in the levels of anxiety-like behavior. In particular, three out of the four groups had similar means for duration spent in closed arms of the Elevated Plus Maze. The similarity of their results suggest that some mice might have been showing the maximal duration in the closed arms before completely adapting to the maze. If true, the varied levels of anxiety between the different experimental conditions were impossible to discern, leading to limitations in understanding the full range of effects from the JunD injections and hormone fluctuations. Another complication in the behavioral data occurs in our inability to assess a baseline level of anxiety in mice before the hormone-simulated pregnancy; due to limitations of the behavioral measures, we had to limit exposure time of the Open Field Test and Elevated Plus Maze to one trial per animal.

Lastly, we wanted to recognize the general limitation that hormone simulated pregnancy is not fully equivalent to the experience of pregnancy. Namely, many peripartum changes cannot be replicated through hormone simulated pregnancy as well as the hormone withdrawn animals experienced a postpartum period without pups, which is an immensely unrealistic postpartum experience. In addition, many other hormones outside of estrogen have significant fluctuations during pregnancy, including corticotropin-releasing hormone (CRH) and progesterone (Cizza, Gold, & Chrousos, 1997; Lawrie, Herxheimer, & Dalton, 2000). Nevertheless, current research is conflicting in regards to the influence of progesterone on PMD, and limited research has investigated the relationship between PMD and CRH levels. Therefore, although our model of hormone simulated pregnancy is not altogether an accurate representation of pregnancy, it provides a reliable methodology to isolate estrogen and probe its role in PMD.
**Future Directions**

The present study contributed to establishing the roles of the NAc, ΔFosB, and the postpartum estrogen drop in the presentation of anxiety-like behaviors, and thus added to a growing body of literature on PMD and its neuro-correlates. Future studies can refine the methodologies and limitations of our research to better evaluate ΔFosB, expand on the roles of other brain regions, and include additional measures to aid in assessing PMD-related behaviors. In regards to ΔFosB accumulation, it was impossible in this study to confirm complete FosB inhibition in the NAc due to staining issues. In the future, confirmation and quantification of ΔFosB levels in the NAc of each animal would be beneficial when visualizing the full effects of the JunD vector. In addition, next steps might involve quantifying the different injection sites in terms of targeting the NAc core or shell; depending on the injection target, the NAc may have different sensitivity to the JunD virus. This validation would aid in the exclusion and inclusion of animals in each study condition and enhance the statistical significance of our results.

Many other brain regions besides the NAc engage in the mesolimbic dopamine network and the maternal caregiving network, thus providing possibilities for future studies to uncover additional puzzle pieces in understanding the neural framework of PMD. At the center of all hormones activating maternal behaviors is the mPOA and bed nucleus of the stria terminalis (BNST). Both structures are sexually dimorphic, sensitive to estrogen, as well as are decidedly integral in maternal and motivational behavior (Adhikari, 2014; Numan & Stolzenberg, 2009; Stamatakis et al., 2014; Xu et al., 2012). The BNST, in particular, has been found to play a role in anxiety circuitry (Adhikari, 2014; Davis, Walker, Miles, & Grillon, 2010). Together, both brain regions receive a wide array of input cues from throughout the brain, and respond using bidirectional networks to communicate back to various brain structures including the VTA,
which projects to the NAc (McHenry, Rubinow, & Stuber, 2015). In regards to FosB, the BNST has been found to have increased FosB-containing neurons six hours after new mother rats are exposed to their young pups (Numan & Numan, 1995, 1997; Numan, Numan, Marzella, & Palumbo, 1998). Outside of the peripartum phase, the BNST is associated with the autonomic and behavioral responses to stress that results in anxiety behavior (Walker, Toufexis, & Davis, 2003). Furthermore, Walker, Toufexis, & Davis (2003) suggest that the BNST is involved in long-lasting responses to sustained stress. This may be relevant to the postpartum period, when animals are exposed to new and sustained levels of stress due to the pressures of taking care and adapting to new pups. In conclusion, these characteristics of the BNST and its functionality with the mesolimbic dopamine pathway of the VTA and NAc make it a favorable brain region to investigate when understanding the neural manifestation of PMD. Future directions might focus on postpartum FosB expression in the BNST, such that FosB might have more influence during pregnancy in the NAc, but more influence after pregnancy in the BNST.

Another potential model that may aid in understanding the expression of PMD-related behaviors involves CRH. It’s important to note that CRH receptors carry estrogen receptor binding elements as well, demonstrating the complicated relationship between these two hormones and pregnancy (Galea et al., 2001). Similarly to estrogen, CRH levels increase during the third trimester of pregnancy due to the hypothalamic-pituitary-adrenal (HPA) axis, which is hyperactive during late pregnancy (Magiakou et al., 1996). Once the placenta is removed after delivery, the body’s larger source of CRH is also eliminated. This forces the body to depend on hypothalamic CRH secretion in order to maintain steady amounts of CRH. However, estrogen deficiency or elevated glucocorticoid exposure threatens the HPA’s ability to produce CRH, which can cause a significant drop in the body’s levels of CRH. This sudden decline in CRH has
the potential to be another risk factor for brain changes that are related to PMD symptoms (Cizza et al., 1997). In particular, Cizza et al. (1997) found that women who developed postpartum emotional disorders had significant HPA axis suppression. Since the HPA axis is primarily associated with the body’s chronic stress response, dramatic changes in CRH excretion may be related to ΔFosB as well. More specifically, ΔFosB has previously been found to have increased expression in reaction to chronic stressors (Hamilton et al., 2018; Vialou et al., 2010). Therefore, it’s possible that peripartum ΔFosB accumulation is impacted by fluctuating levels of CRH and estrogen, wherein such instabilities may be forms of metabolic stressors on the body. If true, future models of pregnancy in rodents should include the alternating rates of CRH as an added variable in order to investigate if its significant postpartum drop contributes to the anxiety and depressive-like behaviors seen in PMD. This would provide us with another future direction to pursue in gaining a full understanding of how peripartum hormone changes lead to alterations in the brain, and PMD-related behaviors.

Research on PMD fully acknowledges the multidimensional nature of PMD symptoms including depressive and anxiety-like behaviors. In order to fully represent the range of behaviors seen in affective disorders, next models of the present study should include additional measures of depression and anxiety behaviors. Two supplementary tests that could be performed include the sucrose preference test, and the forced swimming test (Porsolt, Pichon, & Jalfre, 1977; Willner, Towell, Sampson, Sophokleous, & Muscat, 1987). Previous research has found overlaps in anxiety, chronic stress behaviors, and sucrose preference in rats and mice (Brenes Sáenz, Villagra, & Fornaguera Trías, 2006; Diaz Weinstein, Villafane, Juliano, & Bowman, 2013; Pothion, Bizot, Trovero, & Belzung, 2004). Additionally, the forced swim test is a continually verified measure of depressive behavior in rodents (Can et al., 2012; Petit-Demouliere, Chenu, &
In the future, research using both of these paradigms might be able to discern the differences in anxiety and depressive rodent behavior, and contribute to a more realistic and thorough assessment of PMD related behaviors.

Lastly, future studies should aim to understand the interaction between timing of ΔFosB accumulation and peripartum anxiety behaviors and build upon the findings in this study. As mentioned earlier, our results suggest that ΔFosB is most influential during pregnancy in order to impact peripartum anxiety-like behavior. The next step to evaluate this hypothesis would require assessing ΔFosB levels and anxiety behavior at various time points throughout pregnancy and the postpartum period. Although this would necessitate a significantly generous sample size, it would confirm when FosB accumulation takes place, and when animals are most vulnerable to the behavioral effects of neuroplasticity abnormalities.

**Conclusion**

This study is the first to assess ΔFosB accumulation in the NAc via a hormone-simulated pregnancy in mice. Thus, our novel methods contribute to a huge lack of research on PMD and its neurological underpinnings within the topics of neuroplasticity and postpartum hormone fluctuations. Through the manipulation of hormone withdrawal and sustenance, we were able to distinguish that the dramatic drop in estrogen during the postpartum period leads to an increase in anxiety behavior. Our results also point to the role of ΔFosB during pregnancy in decreasing peripartum anxiety behaviors. Overall, it appears that the estrogen drop after delivery is more influential on anxiety behavior phenotype than ΔFosB expression. However, ΔFosB accumulation seems to have a more significant effect on behavior during pregnancy. Future research should further examine the variable of timing in regards to ΔFosB accumulation and how it impacts anxiety and PMD symptomology. Finally, this study directs us towards being able
to paint a full neurological and behavioral picture of PMD and develop more successful diagnostic tools and educational material for new mothers.
References


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ΔFOSB, NAC, & PMD


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https://doi.org/10.1167/iovs.02-0198


ΔFOSB, NAC, & PMD

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https://doi.org/10.1016/j.cell.2011.12.018


https://doi.org/10.1016/j.psyneuen.2016.01.013
Tables and Figures

Table 1

Subject Conditions

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<th>Condition</th>
<th>Description</th>
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<tr>
<td>Condition 2</td>
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</tr>
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<td>Condition 3</td>
<td>Estrogen Withdrawal Experimental Vector</td>
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<td>Condition 4</td>
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Table 2

2x2 Factorial ANOVA for Elevated Plus Maze Closed Arms Duration

<table>
<thead>
<tr>
<th>Source</th>
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<th>MS</th>
<th>F</th>
<th>Sig.</th>
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<tbody>
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<td>.243</td>
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<tr>
<td>Hormone Condition</td>
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<td>7006.29</td>
<td>1.20</td>
<td>.289</td>
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<tr>
<td>Interaction of Vector x Hormone</td>
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<td>19933.50</td>
<td>3.41</td>
<td>.082</td>
</tr>
<tr>
<td>Error</td>
<td>99338.94</td>
<td>17</td>
<td>5843.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1044261.31</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: $R^2 = 0.234$, Adj. $R^2 = 0.099$, Sig. is two-tailed with $p < 0.05$

Table 3

2x2 Factorial ANOVA for Elevated Plus Maze Open Arms Duration

<table>
<thead>
<tr>
<th>Source</th>
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<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig.</th>
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<td>Hormone Condition</td>
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<td>Interaction of Vector x Hormone</td>
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<td>.059</td>
</tr>
<tr>
<td>Error</td>
<td>44046.78</td>
<td>17</td>
<td>2590.99</td>
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<td>Total</td>
<td>91898.23</td>
<td>21</td>
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Note: $R^2 = 0.281$, Adj. $R^2 = 0.154$, Sig. is two-tailed with $p < 0.05$
Table 4

2x2 Factorial ANOVA for Open Field Test Duration Spent in Inside Zone

<table>
<thead>
<tr>
<th>Source</th>
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<th>df</th>
<th>MS</th>
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<th>Sig.</th>
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<td>27.42</td>
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<td>.949</td>
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<td>Hormone Condition</td>
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<td>134.52</td>
<td>.02</td>
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<td>Interaction of Vector x Hormone</td>
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<td>.605</td>
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<tr>
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<td>109622.29</td>
<td>17</td>
<td>6448.37</td>
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<td></td>
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<tr>
<td>Total</td>
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<td></td>
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</tbody>
</table>

Note: $R^2 = 0.016$, Adj. $R^2 = -0.157$, Sig. is two-tailed with $p < 0.05$

Table 5

2x2 Factorial ANOVA for Open Field Test Duration Spent in Outside Zone

<table>
<thead>
<tr>
<th>Source</th>
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<td>.75</td>
<td>.00</td>
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<td>2030.95</td>
<td>.45</td>
<td>.513</td>
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<td>Error</td>
<td>114847.54</td>
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<tr>
<td>Total</td>
<td>843351.31</td>
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Note: $R^2 = 0.027$, Adj. $R^2 = -0.145$, Sig. is two-tailed with $p < 0.05$

Table 6

2x2 Factorial ANOVA for Open Field Test Velocity

<table>
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<tr>
<th>Source</th>
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<td>8.06</td>
<td>3.40</td>
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<tr>
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<td>.898</td>
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<td>Error</td>
<td>40.31</td>
<td>17</td>
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<td>Total</td>
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Note: $R^2 = 0.179$, Adj. $R^2 = 0.034$, Sig. is two-tailed with $p < 0.05$
Table 7

2x2 Factorial ANOVA for Open Field Test Total Distance Traveled

<table>
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<th>Source</th>
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<td>Interaction of Vector x Hormone</td>
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<tr>
<td>Error</td>
<td>3437425.35</td>
<td>17</td>
<td>202201.49</td>
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<td>Total</td>
<td>53293545.07</td>
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Note: $R^2 = 0.232$, Adj. $R^2 = 0.096$, Sig. is two-tailed with $p < 0.05$
Figure 1. Trajectories of estrogen and progesterone during pregnancy and postpartum (Martin & Behbehani, 2006).
Figure 2. A graph depicting the interaction between the vector and hormone condition. They had an insignificant interaction for duration (measured in seconds) in the closed arms of the Elevated Plus Maze.
Figure 3. A graph depicting the interaction between the vector and hormone condition. They had an insignificant interaction for duration (measured in seconds) in the open arms of the Elevated Plus Maze, as well.
Figure 4. A bar graph showing the mean duration (measured in seconds) spent in the open versus closed arms of the Elevated Plus Maze for each animal condition.
Figure 5. Confocal photomicrographs of 40-μm brain sections from the NAc of Animal #44, tissue #1. Photos taken at 10X magnification. Both images depict neurons with GFP-labeling, the left image is of the anterior commissure and NAc in the left hemisphere, the right image shows the anterior commissure and NAc in the right hemisphere.
Figure 6. Representative heat map of a mouse demonstrating increased anxiety-like behavior, or decreased activity in the open arms of the Elevated Plus Maze on the left. Similarly, the heat map on the right shows a mouse with decreased anxiety-like behavior, or increased activity in the open arms of the Elevated Plus Maze.