ΔFosB Induction in D1 Versus D2 Dopamine Neurons in the Nucleus Accumbens Following a Hormone-Simulated Pregnancy: An Exploratory Study

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Abstract

Postpartum mood disorders (PMD) are a worldwide health concern, yet the neurobiological etiology is still widely unknown. In the present study we replicated the hormone-simulated pregnancy method developed by Liisa Galea in a novel transgenic mouse model. This approach allowed us to measure long-term genomic changes in dopaminergic plasticity in medium spiny neurons (MSNs) within the nucleus accumbens (NAc) through the transcription factor ΔFosB. We used 16 ovariectomized female transgenic mice that had fluorescent reporter molecules coupled to either D1 or D2 receptor-containing neurons; this allowed us to differentially visualize the activity of ΔFosB in each of the neuronal subtypes following a hormone-simulated pregnancy. We found a significant increase in the expression of ΔFosB in D2-MSNs in the NAc core of hormone-withdrawn animals relative to hormone-sustained animals. These neurobiological changes did not correspond with measures of anxiety in either an Elevated Plus Maze or Open Field Test. Furthermore, we found no significant changes in ΔFosB expression in D2-MSNs in the NAc shell nor in D1-MSNs in the NAc core and shell. By further understanding the influence that hormonal changes throughout pregnancy have on neurological systems, we can identify the systems that may be involved in pathological cases to develop better and more direct treatment and diagnosis options for PMD.

Key words: postpartum mood disorder, nucleus accumbens, dopamine, medium spiny neuron, D1, D2, ΔFosB
Postpartum Depression and Anxiety Disorders

Postpartum depression (PPD) and postpartum anxiety disorders (PADs) are grouped together in the DSM-V as a subcategory of major depressive disorder (MDD) and can only be distinguished by the fact that the onset of symptoms occurs in pregnancy or within four weeks of delivery (American Psychiatric Association, 2013). This presentation of postpartum related mood disorders is widely disputed in the literature for being insufficient and negligent. First, women may experience the onset of depressive or anxious symptoms as late as 30 weeks postpartum at which point the DSM-V no longer considers it a postpartum related disorder (Andrews-Fike, 1999; Stuart, Couer, Schilder, O’Hara, & Gorman, 1998). Second, PPD is used as a specifier for all postpartum related mood disorders despite the fact that not all women experience depressive symptoms in their pathology and many experience the onset of anxious or obsessive-compulsive symptoms (Reck et al., 2008; Stuart et al., 1998). Finally, research has begun to uncover that the biological and behavioral characteristics of PPD and PADs do not map on exactly to those of MDD, suggesting that more research is needed in order to understand the potentially unique neurobiological underpinnings of PPD and PADs.

Prevalence. Many women suffer from PPD and PADs worldwide, and yet pathology in the peripartum period is severely understudied and the neurobiological foundations are still largely unknown. A comprehensive report that measured maternal attitudes and experiences before, during, and after pregnancy found that 11% of women across 27 states in the United States experience PPD, which is equivalent to one in every nine women (Ko, Rockhill, Tong, Morrow, & Farr, 2017). Despite the concentration of research in high-income countries, many
studies have found that the trends in maternal depression are universal, affecting approximately 10-20% of women in the world and up to almost 60% of women in some countries (Halbreich & Karkun, 2006; Shidhaye & Giri, 2014). Parsons, Young, Rochat, Kringelbach, and Stein (2012) determined that there is a higher prevalence of PPD in low- and middle-income countries, which is significant since these countries are home to 90% of the world’s children.

Although PPD is the popularized term and depressive symptoms frequently occur comorbidly with other postpartum related mood disorders, research has found that more women experience incidents of anxiety disorders than depressive disorders following pregnancy (Reck et al., 2008; Stuart et al., 1998). This observation in the literature is not consistent with the clinical diagnosis or treatment of disorders in the postpartum period, which continue to prioritize PPD (American Psychiatric Association, 2013; Andrews-Fike, 1999; Reck et al., 2008; Stuart et al., 1998). Matthey, Barnett, Howie, and Kavanagh (2003) suggest that we move to a standard use of the term postpartum mood disorder (PMD) instead of PPD in order to more accurately represent the difficulties that new parents face and to promote a more detailed direction for future research in understanding the underlying contributors to these disorders.

**Impact.** The impact of PMD extends beyond the psychological hardship that one can imagine a new mom experiences trying to care for a newborn while feeling depressed and/or anxious. There are also tangible physical consequences for the mother including the development of serious sleep disorders, drastic mood swings, changes in appetite, and even thoughts or attempts of suicide or infanticide, which can result in devastating impacts on the child and family (Ghaedrahmati, Kazemi, Kheirabadi, Ebrahimi, & Bahrami, 2017; Yonkers, Vigod, & Ross, 2012). Mothers with PMD tend to be less sensitive to their child’s needs, which corresponds to less responsivity in infants, potentially compromising caregiving activities such as feeding.
practices, sleep routines, and well-child visits, which in turn can have significant impacts on the child’s development (Deave, Heron, Evans, & Emond, 2008; Field, 2010; Society CP, 2004). Weakened mother-infant interactions correspond with delays in cognitive, behavioral, and academic development from infancy through adolescence and potentially into adulthood (Deave et al., 2008; Society CP, 2004).

Due to the lack of research in the field, it is difficult to separate out the specific biological and environmental factors that contribute to the disproportionate occurrence of developmental delays in children of mothers with PMD; there is, however, some preliminary evidence to suggest that both play a significant role. Deave et al. (2008) determined that at least some of effects of child developmental delays are attributable to depressive symptoms during pregnancy, which would indicate either a genetic or biological influence of depression on child development in utero. Other research, however, suggests that infants as young as 6 months old are significantly more likely to have right frontal EEG asymmetry, a signature associated with negative affect and depressed mood, only if they spend upwards of 50% of their day with a mother with PMD (Wen et al., 2017). The authors suggest that these changes are likely a result of decreased mother sensitivity in early infant development. Understanding how mood disorders in pregnancy may influence undesirable maternal behavior and possibly poor child development in the womb ought to be a priority for future research considering the prevalence of pathology surrounding the pregnancy period.

It is important to note that PMD also affects some men, but at a significantly lower rate than for women (Matthey et al., 2003). The high rates of PMD in both men and women suggest that the external stress of having a child is an important risk factor in predicting the onset of PMD; however, the substantially higher rates in women indicate that there is likely a significant
biological component that corresponds with carrying and birthing the child. Focusing research on this significant but understudied biological component to PMD may provide insight into the distinctiveness of PMD from other mood disorders.

Temporal, Behavioral, and Neurological Distinctiveness of PMDs

Much of what is known about PMD comes from the abundance of research on generalized mood disorders such as MDD. The similarities in the symptomology and in many of the neurological components of PMD and non-pregnancy related mood disorders suggest that they are closely related. Importantly, women with a history of MDD or an anxiety disorder are significantly more susceptible to developing PMD, but there is no significant correlation between the development of PMD and a history of a mood disorder in men (Bloch et al., 2000; Matthey et al., 2003; Robertson, Grace, Wallington, & Steward, 2004). This observation supports the theory that the distinct hormonal changes that women undergo throughout pregnancy and into the postpartum period contribute to long-term changes in the brain that may lead to the onset of PMD in susceptible women. Despite the close relationship between PMD and other mood disorders, preliminary studies have begun to uncover distinct temporal, behavioral, and neurological components of PMD, suggesting that pregnancy related mood disorders ought to be diagnosed and treated differently from other mood disorders.

Temporal differences. It is unclear if a woman is more vulnerable to being diagnosed with a mood disorder during the postpartum period than at any other period in her life, but the overwhelming prevalence of mood disorder diagnoses in this period suggests that it is at least one of the more vulnerable times. Research using a controlled design suggests that women are undoubtedly at a higher risk for experiencing depressive symptomatology and poor social adjustment in the peripartum period than non-childbearing women, even if they are not officially
diagnosed with a mood disorder (O’Hara, Zekoski, Philipps, & Wright, 1990; Stowe & Nemeroff, 1995). Furthermore, despite the clinical similarities between PMD and non-pregnancy related mood disorders, PMD is triggered by the experience of pregnancy in a subgroup of susceptible women (Bloch et al., 2000). This time-specific vulnerability to the onset of mood disorders implicates biological reproductive factors in the development of postpartum affective changes.

**Behavioral differences.** The developmental delays that are differentially seen in children of mothers with PMD are likely at least partially attributable to the specific infant-directed consequences realized by mothers diagnosed with a PMD. Mothers with mood disorders tend to be significantly more irritable in interactions with their child, less engaged, and less emotionally sensitive and warm in the first three months following parturition (Lovejoy, Graczyk, O’Hare, & Neuman, 2000; Society CP, 2004). Beyond influencing these more general maternal behaviors, researchers have also found specific infant-directed behaviors that are limited to depressed mothers. Kaplan, Bachorowski, Smoski, and Hudenko (2002) found that depressed mothers tend to use a less rhythmic style when speaking with their infant, which may be a potential basis for impaired child-development in children of mothers with PMD. Furthermore, while mothers suffering from MDD tend to report detached and withdrawn behaviors, mothers with PMD report irritation and intrusion with their child (Pawluski et al., 2017; Fleming et al., 1988). The influence that PMD has on maternal behaviors indicates that there may be specific neurological correlates to these behaviors, which ought to be considered separately from those of non-pregnancy related mood disorders.

**Neurological differences.** Given that there are temporal and behavioral distinctions between PMD and general mood disorders, it is presumed that there are also neurological
differences. This impression is consistent with research that suggests the behavioral similarities between the two disorders are reflected in neurological parallels. In fact, researchers have focused primarily on these similarities and have largely ignored the possibility of differences. Brain-imaging studies suggest that abnormal neural activities play a significant role in the psychopathology of PPD, consistent with MMD. For instance, using functional magnetic resonance imaging (fMRI), researchers have determined that depressed mothers in a resting state have significantly altered posterior cingulate, medial frontal, and temporal gyrus regional homogeneity which are all areas important in self-regulation, empathy, and emotion that have also been implicated in MDD (Wang, Wang, Liu, Ming, & Zhang, 2011). Researchers have begun to build off of these studies using similar brain imaging techniques to define the neurological differences between the disorders that are reflected in the unique temporality and behaviors associated with PMD, since they are largely unknown.

Primarily using fMRI, researchers have discovered that the infant-directed behavioral consequences in mothers with PMD correspond with specific neurological adaptations. Moses-Kolko et al. (2010) used a negative emotional face-matching paradigm to examine cortical neural activity and connectivity in response to negative emotional stimuli in depressed mothers relative to healthy mothers. As is suggested in the literature, they found that depressed mothers had significantly lower levels of attachment to their infants. Neurologically, these behaviors corresponded with lower activity in response to face blocks in depressed mothers relative to healthy mothers, such that subjects with greater severity of depressive and anxious symptoms had reduced left amygdala activity and diminished connectivity from the left dorsomedial prefrontal cortex (DLPFC) to the left amygdala reference region. DLPFC activity and connectivity to the amygdala may represent an important neural mechanism specific to PPD.
Other researchers used fMRI to determine the brain areas involved in a mother recognizing her infant’s cries. Typically, mothers demonstrate strong and specific brain responses to their own infant’s cries; however, less sensitive mothering is associated with less activation in some areas such as the right frontal pole and the inferior frontal gyrus, which may be other important regions in the etiology of PMD (Musser, Kaiser-Laurent, Ablow, 2012; Noriuchi, Kikuchi, & Senoo, 2008).

Research suggests that beyond infant-directed consequences, there are also non-infant associated neurological changes that are specific to postpartum related mood disorders. Silverman et al. (2007) measured activity in the amygdala of subjects diagnosed with PPD following exposure to words that implied threat. The subjects with PPD demonstrated significantly lower levels of activation as opposed to the increased levels of activity observed in comparable studies using subjects with MDD (Fiorelli et al., 2015). Furthermore, studies have determined differences in neurological responses to non-infant emotional cues, such that individuals with PPD show decreased activation in the amygdala and striatum while individuals with MDD show increased activation in these areas (Drevets, 2000; Palusi, Lonstein, & Fleming, 2017). One group measured glutamate concentration in the frontal cortex of women with PPD and found a slight increase, which directly opposes the results in studies on MDD (McEwen et al., 2012). While the limited current research is not enough to generate a completely distinctive neurobiological profile for PMD, there is still evidence to suggest that the neural mechanisms involved in PMD may differ from those of non-postpartum related mood disorders. These differences are likely a product of the biological changes involved in pregnancy and parturition that result in novel cognitive, behavioral, and biological states for new mothers.
The Biology of Postpartum

The biological processes that women undergo throughout pregnancy are generally understood. Yet, there are still significant gaps in knowledge that prevent a clear understanding of why some but not all women develop PMD, and further why the times at which women develop PMD are so varied. Reproductive hormones are a good place to start for determining a potential trigger of PMD as they are in a constant state of flux throughout pregnancy and are known to play a major role in influencing basic emotional processing, arousal, cognition, and motivation, which are all factors implicated in PMD (Schiller, Brody-Meltzer, & Rubinow, 2015). Even slight changes in endogenous hormone cycles are associated with mood changes, so it is important to study the reproductive period where there are substantial biological and behavioral changes occurring in each phase: during pregnancy, at parturition, and postparturition where lactation occurs (Barth, Villringer, & Sacher, 2015; Russel, Douglas, & Ingram, 2001). Although all women undergo comparable hormonal changes throughout the reproductive cycle, not all women experience the same behavioral and affective changes. In each of these stages in the cycle, hormones are responsible for promoting and maintaining the health and survival of both the mother and child, but it is clear that these hormonal changes may also have disadvantageous influences in susceptible, hormone sensitive, women (Russel et al., 2001).

Hormones involved in pregnancy. The principle hormones involved in both initiating and maintaining pregnancy are progesterone and estrogen, the former of which helps create a healthy endometrial environment to promote implantation and the latter of which is essential in the development of sex characteristics. Both of these hormones undergo drastic changes throughout pregnancy and into the postpartum period (see Figure 1). By the end of the third trimester, plasma estrogen has risen approximately 50-fold higher than maximal menstrual cycle
levels and progesterone has risen 10-fold higher, at which point these hormones drop drastically to early follicular phase levels within the first few days postpartum (Bloch, Daly, and Rubinow, 2003; Hendrick, Altshuler, & Suri, 1998). These hormonal changes are directly responsible for the initiation and sustention of pregnancy and they also play a critical role in modulating the expression of other significant hormones throughout pregnancy such as prolactin and oxytocin, both of which play a direct role in breastfeeding, lactation, and maternal care behavior (Grieb, Tierney, & Lonstein, 2017; Kloet, Voorhuis, Boschma, & Elands, 1986; McCarthy, 1995; Neumann, 2003; Russell et al., 2001).

Research suggests that the prolonged exposure to estrogen and progesterone prior to parturition are critical for the effectiveness of oxytocin and lactogenic hormones in producing maternal behavior (Grieb et al., 2017; Kloet et al., 1986; McCarthy, 1995; Neumann, 2003; Russell et al., 2001). Rats that undergo a simulated pregnancy using estrogen and progesterone demonstrate parental behavior quickly upon exposure to pups without manipulating any other variables, while it takes rats that do not undergo the simulated pregnancy a longer period of time to demonstrate parental behavior and only with repeated exposure to pups (Russel et al., 2001). Grieb et al. (2017) isolated the influence of progesterone and found that specifically a prolonged exposure to progesterone into the postpartum period prevents the emergence of maternal care behaviors, while the drop off may instigate the emergence of these behaviors. Increasingly elevated levels of progesterone into mid-to-late lactation limits some maternal care behaviors, which may prep the young for weaning, suggesting that progesterone concentrations are especially significant in impacting temporary mothering instincts.

It is largely understood that progesterone and estrogen play a significant role in the onset of maternal care behaviors; however, there are other behaviors that are also related to the
survival of mother and child, such as anxiety and stress responses, which are significant for understanding the full range of influence that hormonal changes have on adaptive parental behavior (Neumann, 2003). For example, oxytocin is known to play significant psychological roles in preparing a mother to care for her infant by producing a stress reduced state and promoting attachment behaviors (Neumann, 2003; WHO, 2009). This reduction of anxiety in the postpartum period is a potential protective measure for new moms so that they don’t get overwhelmed when exposed to stressors (Perani & Slattery, 2014; Slattery & Neumann, 2008). Mothers also demonstrate a decreased aggressive response toward their newborns immediately after birth and an increased positive aggressive response toward intruders (Bosch, 2013). Both of these maternal responses have logical adaptive benefits, which support survival and health of both the newborn and the mother; however, as a result of hormonal changes, studies have found that animals tend to be more vulnerable to developing depressive symptoms as well, which doesn’t have an obvious adaptive advantage (Galea, Wide, & Barr, 2001; Green, Barr, & Galea, 2009).

While PPD does not affect the majority of women, postpartum blues develops in 50% to 70% of all women within the first 2 weeks following childbirth. Consistent with the symptoms of PPD women may experience crying spells, insomnia, depressed mood, fatigue, and poor concentration, but unlike with PPD the symptoms do not persist (Andrews-Fike, 1999). The prevalence of postpartum blues is consistent with research on animal models, which has found that undergoing a hormone-simulated pregnancy induces depressive-like symptoms (Galea et al., 2001; Green et al., 2009). While it is possible that these changes serve an adaptive function, the depressive component is likely the inadvertent result of hormonal changes throughout the postpartum period.
reproductive cycle, and more specifically the fluctuations of estrogen and progesterone, which have far-reaching impacts in all phases the reproductive cycle.

Based on differential findings between anxious and depressive behaviors across most women, it is clear that the peripartum period is an unpredictable and relatively unstable period of time for mothers that may correspond with decreased anxiety and increased depressive symptoms in a healthy sample. The advantageous trend toward reduced anxiety, however, doesn’t extend to all women as evidenced by the strong prevalence of anxiety disorders in the postpartum period. Some research suggests that this may be the result of differential effects of progesterone and estrogen concentrations on anxious and depressive phenotypes. Generally, abrupt decreases in progesterone are associated with higher levels of anxiety, while decreased concentrations of estrogen are associated with lower levels of anxiety (Gulinello, Gong, & Smith, 2002; Marcondes, Miguel, Melo, & Spadari-Bratfisch, 2001). Both estrogen and progesterone exist in reduced concentrations at parturition, which indicates that their low concentrations may balance out the experience of anxiety. Similarly, Schiller (2011) was unable to find an anhedonic response by withdrawing estrogen and progesterone together from high concentrations, but estrogen withdrawal alone did produce an anhedonic response, which suggests that the simultaneous withdrawal of estrogen and progesterone may serve the function of balancing out potential depressive symptoms as well as anxious symptoms. Taken together, these results indicate the necessity of a relatively ideal balance of progesterone and estrogen activity in order to produce a healthy non-depressed or anxious phenotype.

Overall, this research implicates estrogen and progesterone as significant factors involved in producing maternal behaviors and affective states throughout pregnancy and the postpartum period. Specifically the sudden and drastic changes in cycles of reproductive hormones are a
good place to start for understanding the potential biological factors that contribute to the development of PMD. Comparable hormonal fluctuations have been implicated in other disorders as well such as premenstrual dysphoric disorder and postmenopausal depression, both of which exclusively affect women and result in mood changes similar to those seen in PMD (Wharton, Gleason, Olson, Carlsson, & Asthana, 2012). For instance, women are more likely to suffer from mood disorders than men particularly during times in which estrogen levels are in a state of flux (Lorsche et al., 2018). Furthermore, researchers have been unable to find a significant consistent relationship between hormone concentrations and the development of PMD, but studies that focus on the withdrawal of reproductive hormones have found associations with either increased or decreased anxiety and depression (Gulinello et al., 2002; Hendrick et al., 1998; Schiller, 2011; Schiller et al., 2015).

**Hormone withdrawal model.** Galea et al. (2001) developed a method for modeling the impact of these particular hormonal changes during pregnancy on behavior and the brain. The researchers manipulated hormonal changes in rats across their standard 23-day gestation period by approximately mimicking the changes in progesterone and estrogen throughout pregnancy and into the postpartum period (see Figure 2). For the first term (days 1-16) of the simulated pregnancy all rats were given a high dose of progesterone daily and a low dose of estrogen. Moving into the second term (days 17-22), progesterone levels were dropped off and estrogen levels were elevated. Finally, on day 23, half of the rats were given cottonseed oil to simulate the hormonal drop off at parturition, while half continued to be treated with estrogen in order to serve as a point of comparison between hormonal withdrawal and sustention.

Researchers that use this particular model of hormone withdrawal and sustention find results that reflect the typical behavioral changes observed in humans and animals following an
actual pregnancy, such as decreased anxiety as measured by animal behavior in an Elevated Plus Maze and Open Field Test and increased depression as measured by learned helplessness behavior on a Forced Swim Test (Foster, Heaton, & Been, 2017; Galea et al., 2001).

Furthermore, Galea et al. (2001) found that the continued treatment of estradiol into the simulated postpartum period decreased depressive symptoms, which is consistent with studies on both animal and human models that find treatment with estradiol in estrogen deficient mothers can reduce depression following an actual pregnancy (Ahokas, Kaukoranta, Whalbeck, & Aito, 2001; Marcondes et al., 2001). Given that this hormone-simulated pregnancy model produces comparable behavioral results to those of healthy mothers following an actual pregnancy, the model is useful for beginning to explore the neurological changes that occur due to fluctuations in reproductive hormones.

It is accepted that the experience of pregnancy impacts the brain in order to mentally prepare a mother for caring for an infant, but we also know that pregnancy may have pathological influences on the brain as is suggested by the neurological distinctiveness of PMD from other mood disorders. Thus, it is assumed that hormonal changes throughout pregnancy likely influence both advantageous and pathological neurological changes, which researchers have begun to study more directly using animal models and controlled hormone-simulated pregnancy. In particular, researchers have found that reproductive hormones are implicated in neuroplasticity and can have both short-term and long-term effects on brain structure and functioning (Barth et al., 2015, Galea et al., 2008; McEwen, 1985). They have also been found to act differently in adult male and female brains likely due to unique environmental pressures imposed on men and women for reproduction and survival, which is a potential explanation for why men and women are differentially susceptible to developing mood disorders (Yoest,
Cummings, & Becker, 2014). More specifically, women’s increased risk for developing mood disorders is presumed to be associated with fluctuating estrogen levels during sensitive periods such as menopause, pre-menstruation, and pregnancy, which is supported by evidence that the regulation of mood is linked to neural structures that are rich in estrogen receptors and/or neurotransmitters that are highly sensitive to estrogen regulation (Wharton et al, 2012).

One system that is of particular interest with respect to understanding the influence of reproductive hormones on the brain during pregnancy is the mesolimbic dopamine pathway: it is affected by estrogen differentially in female animal models and it has been implicated in models of anxiety and depression to the extent that some researchers believe the influence of estrogen on cognition, mood, and motivated behaviors may be mediated by dopaminergic function (Barth et al., 2015; Bazzett & Becker, 1994; Yoest et al., 2014). While researchers have uncovered dopamine as a significant factor involved in understanding the etiology of mood disorders, few researchers have studied the direct acute and chronic effects that hormone activity in the reproductive cycle have on dopaminergic function, which could help uncover novel neurological underpinnings of PMD.

**A Unique Neurobiological Approach for Studying PMD**

The present study aims to fill the gap in PMD research by experimentally determining if hormone withdrawal following a hormone-simulated pregnancy is associated with long-term and short-term impacts on dopamine activity in the nucleus accumbens (NAc). The NAc is a brain region of particular interest with respect to depression and addiction that has been largely overlooked in research on PMD (Salgado & Kaplitt, 2015). Explorations on impacts that pregnancy has on this region in a healthy model could set the groundwork for identifying a novel
region implicated in pathological cases, which could then be targeted for more direct diagnosis and treatment of PMD.

**The NAc.** The NAc is a specialized component of the striatal complex located in the ventral striatum, a region essential to mediating motivational and emotional processes to the extent that it is considered the neural center of reward and learning (Russo & Nestler, 2013; Shirayama & Chaki, 2006). It also has many distinct characteristics that make it the principal region of interest for the present study: it has significant control over the biological drives necessary for survival and reproduction, it is implicated in many neurological psychiatric disorders including depression and anxiety, and it has been found to be both sensitive to hormone-mediated changes and prone to plasticity (Russo, Dietz, Mumitriu, Malenka & Nestler, 2010; Saigusa, Takada, Baker, Kumar, & Stephenson, 1997; Salgado & Kaplitt, 2015; Shirayama & Chaki, 2006; Thompson & Moss, 1994). Taken together, these factors make it a promising target in the etiology of PMD since prolonged exposure to estrogen and progesterone with subsequent withdrawal could have significant acute and chronic effects on the region.

With respect to pathology, the NAc appears to be highly sensitive to neurochemical alterations due to environmental factors such as chronic stress, drug exposure, and drug withdrawal, all of which can produce a depressive or anxious phenotype (Levita, Hoskin, & Champi, 2012; Shirayama & Chaki, 2006; Sturm et al., 2003). The depressive phenotype corresponds with a reduced volume of the left NAc, and furthermore, depressed individuals fail to successfully sustain NAc activity over time on tasks, which corresponds with an anhedonic behavioral response (Baumann et al., 1999; Heller et al., 2009). With respect to addiction, reduced activity within the VTA-to-NAc dopamine projection corresponds with negative emotional signs of withdrawal such as irritation, anxiety, anhedonia, and dysphoria (Radke &
Gewirtz, 2012). The sensitivity of the NAc to prolonged stress and drug exposure with subsequent withdrawal suggests that it may also be sensitive to exposure of other neurochemical alterations such as prolonged elevated hormone exposure throughout pregnancy with sudden withdrawal at parturition.

Of particular significance in the context of this study, the NAc is a region with a high concentration of both dopamine neurons and estrogen receptors, which makes it a significant region to target for understanding potential estrogen-mediated dopamine changes (Heinsbroek et al., 2017). Researchers have noted that estrogen receptor overexpression in the NAc promotes a pro-resilient phenotype to depression and stress responses in mice (Lorsche et al., 2018). Given that estrogen receptors are activated by estrogen, this would indicate an adaptive advantage to pro-resilient transcriptional changes that upregulate estrogen receptors in the NAc in the presence of stress. What is more, this may indicate that estrogen positively influences resilient behavior via dopaminergic transmission to the NAc in a subset of women with estrogen receptor overexpression (Lorsche et al., 2018). In the limited research that has been conducted directly in mothers with PMD in this region, researchers have observed diminished striatum activity, which is complemented by findings of decreased dopamine in the striatum in depressive mice postpartum (Avraham et al., 2017; Silverman et al., 2007). These findings together strongly suggest that the pathology of PMD is related to dysfunction of the reward/motivational pathway; however, few researchers have specifically examined the influence of estrogen-mediated changes that may inform pro- or anti-resilient phenotypes in the NAc in response to hormonal changes throughout pregnancy. Thus, it is very important that we develop an understanding of both the advantageous and pathological effects that hormone fluctuations may have on this region in
order to gain insight into neurological mechanisms implicated in pregnancy related mood disorders.

**The mesolimbic dopamine pathway.** The NAc is also a critical target in the present study because it plays an important role in the mesolimbic dopamine pathway, which is implicated in addiction, natural reward, stress response, anhedonia, and anxiety (Chien, Rada, Bützler, Leibowitz, & Hoebel, 2012; Grueter, Robison, Neve, Nestler, & Malenka, 2013; Zarrindast & Khakpai, 2015). The mesolimbic dopamine pathway extends from the ventral tegmental area (VTA) to the NAc. This pathway is the primary focus of the present study because alterations to the system are directly implicated in animal models of depression and maternal caregiving behavior, both of which are directly and indirectly mediated by reproductive hormones (Bazzett & Becker, 1994; Bridges, 2015; Küppers, Ivanova, Karolczak, & Beyer, 2000; Saigusa et al., 1997; Stern and Keer, 1999; Thompson & Moss, 1994; Yoest et al., 2014).

Altogether, understanding the influence of a hormone-simulated pregnancy on changes in the relationship between the NAc and the mesolimbic dopamine pathway may be especially important for understanding the development of infant-directed behavioral consequences observed in women with PMD.

Due to the lack of research on the specific influence of pregnancy on the mesolimbic dopamine pathway, it is valuable to pull from more heavily researched areas that could help inform our understanding of how drastic hormonal fluctuations may also have a neurological and behavioral effect on this region. For instance, Yoest et al. (2014) studied the modulatory impact of estradiol on dopamine systems as it relates to compulsive drug use and found that estradiol significantly modulates dopamine systems in females, but not males, likely through both acute and chronic neurological changes that vary directly with gonadal hormone fluctuations. In
general, disruption of normal estrogen transmission has an inhibitory influence on dopaminergic signaling in the mesolimbic pathway, while enhancement of estrogen transmission corresponds with more active dopaminergic signaling (Barth et al., 2015; Leranth et al., 2000; Yoest et al., 2014). Yoest et al. (2014) also determined that these estrogen modulated dopaminergic changes are directly associated with subjective effects of stimulant drugs in women, such that women more rapidly develop compulsive drug use than men. These subjective effects are even further regulated by specific estrogen fluctuations: women tend to report that when estradiol is lower during the menstrual cycle, the subjective experience of stimulant drugs are more similar to those reported by men, while at times when estradiol is higher the subjective experience is reported as being enhanced, likely as a result of corresponding dopamine signal enhancement.

These estrogen-modulated changes in motivated behaviors correspond directly with alterations within mesolimbic dopamine systems in female rats. While the research conducted by Yoest et al. (2014) specifies motivated behaviors related to compulsive drug use and subjective stimulant experience, these changes are also associated with naturally motivated behaviors. Fluctuating estrogen levels mediate adaptive sexual behaviors that promote increased sexual motivation and decreased motivation for food when a woman is more likely to conceive (Yoest et al., 2014). Based on this heavy dependence of motivated behaviors on estrogen-modulated dopamine activity, it is important to acknowledge the potential changes that occur due to the more drastic hormone fluctuations around the peripartum period. For instance, it is significant to understand more deeply the interaction between estrogen withdrawal following sustained estrogen elevation on motivated behaviors as regulated via dopaminergic function.

**Dopamine neurons.** The ability of the mesolimbic dopamine pathway to regulate motivated behaviors is dependent on its high concentration of neurons with dopamine receptors.
The activation of dopamine neurons in the NAc via the mesolimbic dopamine pathway directly predicts reward delivery, which is likely related to the neurotransmitter’s functional involvement in motor control, learning, motivation, reward and decision-making (Barth et al., 2015; Yoest et al., 2014). Furthermore, research indicates that hormone-related behavioral changes are directly mediated by dopamine activity, which has been largely determined through studies on women during periods outside of pregnancy when hormones are also fluctuating. For instance, the dopaminergic system is impaired in women with premenstrual dysphoric disorder, which is a condition that a subpopulation of women experiences in response to premenstrual hormone changes (Barth et al., 2015). In fact, several neurological disorders that have gender specific consequences are linked to abnormal dopaminergic function. Barth et al. (2015) propose that, “a better understanding of the interaction between sex hormones and dopaminergic neurotransmission could help to improve pharmacological treatment regimens for these diseases and significantly impact women’s mental health” (p. 6).

The presence of estrogen both acutely and chronically influences dopaminergic neuron stimulation and inhibition, either by directly targeting dopamine neurons or by indirectly stimulating neural branching and expression of enzymes involved in dopamine synthesis (Küppers et al., 2000). Estrogen and progesterone may also indirectly impact the mesolimbic dopamine pathway through its positive influence on oxytocin. Oxytocin interacts closely with the neural pathways responsible for processing motivationally relevant stimuli, such that oxytocin may exert some of its social-behavioral effects through its impact on motivational networks (Love, 2015). Another indirect influence of estrogen on this pathway is the regulatory influence of estradiol on dopamine responsivity in the medial preoptic area (mPOA). The mPOA is a region that contains one of the highest concentrations of cells expressing sex-steroid hormone
receptors including progesterone and estrogen receptors, and it has been found to moderate cocaine responsiveness in the NAc via dopaminergic neurons in the VTA (Tobiansky et al., 2016). Taken together, the varied direct and indirect influences of ovarian hormones on dopaminergic function makes it a likely target for understanding neurological changes influenced by the reproductive cycle.

Due to the countless number of interactions and processes occurring simultaneously in the brain, the relationship between dopaminergic function and behaviors is very complicated. Some researchers have determined that dopamine depletion induces anxious and depressive symptoms; while others have found that both acute and long-term stressors produce an increased anxious state that corresponds with increased dopaminergic sensitivity and activity in the NAc (Leranth et al., 2000; Radke & Gewirtz, 2012; Yorgason et al., 2013; Zarrindast & Khakpai, 2015). These conflicted findings reflect the intricacies of the relationship between neural states and behavioral outcomes. In the context of the present study, it is prudent to prioritize the research that is associated directly with dopaminergic function in the environment of reproductive and hormonal states.

High levels of estrogen with subsequent withdrawal are associated with dopaminergic sensitization, which may provide insight into the emergence of maternal care-giving behaviors peripartum (Afonso, Shams, and Fleming, 2013; Barth et al. 2015; Yoest et al., 2014). Afonso et al. (2013) found a succession of results that suggest hormone withdrawal following pregnancy is essential to normal maternal rearing patterns through dopaminergic mediation. Primarily, they determined that mothers who underwent either an actual pregnancy or a hormone-simulated pregnancy had lower basal dopamine activity relative to control virgin females. Additionally, they found that presenting a pup to a mother results in a surge of dopamine activity, with lower
baseline concentrations corresponding directly to more extreme dopamine activation in response to pup presentation. Thus, the research suggests that hormone withdrawal produces a relatively depleted dopamine environment, which is exceedingly reactive and sensitive to external maternal cues. This interaction is highly adaptive in an evolutionary context due to the enhanced survival advantage for the offspring with improved mothering instincts and skills. In order to understand more clearly the complex interaction between hormones, dopaminergic function, and behavioral outcomes it is important to acknowledge and identify the deeper complexities of this system, some aspects of which may even work antagonistically.

**D1 versus D2 dopamine receptors.** The ability of the NAc to regulate behavior via dopamine concentration is largely due to the isolation of D1 and D2 dopamine receptors in either direct or indirect pathway medium spiny neurons (MSNs) (Gerfen & Surmeier, 2011; Muir et al., 2017). Research suggests that there are molecular, anatomical, and physiological differences between these two neural subtypes, which would indicate that they likely motivate differential—and sometimes even contrasting—behavioral outcomes (Gerfen & Surmeier, 2011; Heinsbroek et al., 2017). In particular, research suggests that D1 receptors stimulate while D2 receptors inhibit adenylyl cyclase activity, which in-turn influences intracellular signaling in opposite directions (Yawata, Yamagushi, Danjo, Hikida, & Nakanishi, 2012). Indeed, the behavioral outcomes that we are interested in for the present study are differentially motivated by activity of D1 and D2 dopamine MSNs. Since D1 and D2 receptors are intermingled in the NAc such that they are indistinguishable geographically, it is necessary to actively differentiate between these neuronal subtypes based on function and structure in order to understand the full impact of dopamine activity on behavioral outcomes.
The activation of these neuronal subtypes differentially regulates the expression of both maternal behaviors and stress responses. When D2 activity is inhibited using a D2 receptor antagonist, maternal behaviors such as pup retrieval and grooming are severely disturbed, which would suggest that D2 receptor activity is activated postpartum to encourage the onset of maternal behaviors (Keer & Stern, 1999). Given that reproductive hormone activity is also known to directly promote or demote maternal behavior, it is likely that the activity of D2-MSN receptors is moderated by reproductive hormone activity (Grieb et al., 2017; Kloet et al., 1986; McCarthy, 1995; Neumann, 2003; Russell et al., 2001). With respect to mood, Muir et al. (2017) revealed that individual differences in depressive response to chronic stress are also mediated differentially by D1- and D2-MSN activity in the NAc. Differentiating between the activities of these neuronal subtypes could provide insight into why chronic stress may cause only a subset of individuals to become depressed and by extension why only a subset of women may develop PMD. Mice that develop depressive-like symptoms after chronic social-defeat stress show distinct changes in D1- and D2-MSN activity, such that increased baseline D1-MSN activity, but not D2-MSN activity, predicts resilience to chronic stress (Muir et al., 2017). This finding is consistent with research that suggests directly stimulating D1-MSN activity, but not D2-MSN activity produces resilient behavioral outcomes (Francis et al., 2015). Perhaps distinguishing between these two neuronal subtypes in studies related to hormone withdrawal will provide insight into the consistency of the pro-resilient influence of D1-MSN activity. Research that fails to distinguish between these two neuronal subtypes may overlook valuable insight into neurological correspondences with behavior.

In terms of the research that has already been conducted to understand the direct connection between hormone activity and dopamine neuron subtype, researchers have
determined that estrogen down-regulates D2 neuron binding and only in females (Yoest et al., 2014). This finding would suggest that low levels of estrogen promote D2 neuron binding, which complements research that indicates D2-MSN sensitivity is associated with the emergence of maternal behaviors (Keer & Stern, 1999). Unfortunately, research also suggests that a hypersensitivity of D2-MSNs is associated with anxious and depressive symptomatology, which stresses the complexity of the neurological interactions occurring throughout pregnancy and may provide a rationale for the abundance of mood disorders diagnosed during such a delicate time (Wieck et al., 1991).

**Delta FosB (ΔFosB).** In order to gain further insight into the specific changes that occur in the brain following a hormone-simulated pregnancy, it is important to identify a relevant molecular target that is present in dopamine neurons in order to measure particular neurological changes. ΔFosB is a promising molecular target for measuring neuroplasticity in the NAc and more specifically within dopamine MSNs. As a transcription factor, changes to the induction of ΔFosB would suggest potential long-term genetic changes in the induction of ΔFosB in D1- and D2-MSNs within the NAc. Epigenetics suggests that environmental cues, such as hormonal fluctuations, can influence stable changes in chromatin structure, which results in the sustained altered expression of a gene. Furthermore, these transcriptional changes influence cellular physiology and circuit level signaling, which have profound effects on long-term behavioral outcomes including those of psychiatric disorders such as depression and anxiety (Lorsche et al., 2018; Sun, Kennedy, & Nestler, 2013).

Neuroplasticity in the NAc is largely dependent on ΔFosB, which promotes synaptic plasticity through its promotion of neuronal connectivity and growth (Grueter et al., 2012; McClung et al., 2004). In fact, ΔFosB has been used as a direct biomarker for measuring
activation states of the reward circuitry system (Nestler, 2008). Relevant to the present study, these long-term effects on plasticity are implicated in the expression of maternal behavior and in models of depression (Brown, Ye, Bronson, Dikkes, & Greenberg, 1996; Hamilton et al., 2017; Vialou et al., 2010). Specifically, the genetic modification of mice such that they have an inactive fosB gene results in severe deficits in postpartum maternal care behavior in mice, which suggests that the activity of ΔFosB is significant in the emergence of mothering instincts (Brown et al., 1996). Furthermore, the induction of ΔFosB in the nucleus accumbens mediates chronic social defeat stress in both animal and human models. In a postmortem study, ΔFosB concentrations in the nucleus accumbens were 50% lower in depressed individuals (Vialou et al., 2010). The researchers also determined that ΔFosB induction in nucleus accumbens of mice is associated with resilience to chronic stress and its presence is necessary for successful antidepressant treatment, such that when ΔFosB induction is blocked antidepressants are no longer effective (Vialou et al., 2010).

It is possible that ΔFosB induction is a significant marker of acute and chronic neurological changes following a hormone-simulated pregnancy due to the fact that dopaminergic activation is directly related to ΔFosB induction, such that chronic dopaminergic activation in the NAc stimulates ΔFosB induction (Nestler, Barrot, & Self, 2001). Prior to parturition the body is flooded with high concentrations of estrogen, which is associated with increased dopaminergic accumulation, so it is possible that this period stimulates ΔFosB induction in the NAc producing long-term epigenetic changes in the region leading up to estrogen withdrawal (Hendrick et al., 1998; Yoest et al., 2014). Given that dopaminergic function is also acutely impacted by hormonal changes such that estrogen deprivation results in decreased dopamine-MSN maintenance, it is possible that hormone withdrawal results in distinct
patterns of ΔFosB induction as compared to a hormone sustained condition (Barth et al., 2015; Leranth et al., 2000). De Pauli et al. (2014) observed unique patterns of ΔFosB induction in sensitized mice associated with the withdrawal period from ethanol, which might suggest that dopamine sensitization from estrogen withdrawal produces distinct patterns of markers of plasticity as well.

In an unpublished pilot study, Foster et al. (2017) used immunohistochemistry on tissue from Syrian hamsters that had been treated using the hormone withdrawal model developed by Galea et al. (2001) to look at the expression of ΔFosB in MSN-dopamine receptors in the NAc. They did not find any significant differences between animals in the hormone-withdrawn and hormone-sustained conditions. As outlined above, it is possible that the lack of results was due to the examination of induction of ΔFosB induction in dopamine neurons in general without distinguishing between D1 and D2 receptors. Research related to stress response suggests that stress susceptibility is oppositely regulated by ΔFosB induction in D1- and D2-MSNs, such that an increased presence of ΔFosB due to histone acetylation may be a potential mediator of non-depressive symptoms in D1-MSNs and depressive symptoms in D2-MSNs, while decreased presence of ΔFosB due to histone methylation may be a potential mediator of depressive symptoms in D1-MSNs and non-depressive symptoms in D2-MSNs (Hamilton et al., 2017).

A healthy postpartum phenotype following hormone withdrawal is characterized by a decreased stress-response, so it is possible that ΔFosB induction in postpartum women corresponds with the stress-resilient phenotype: an increased concentration of ΔFosB in D1-MSNs and a decreased concentration of ΔFosB in D2-MSNs relative to a hormone-sustained condition (Hamilton et al., 2017; Neumann, 2003; WHO, 2009). This pattern would be consistent with the emergence of maternal care patterns based on D2-MSN sensitivity. For instance,
researchers have observed that amplified D2 receptor sensitivity is associated with maternal caregiving behaviors (Keer & Stern, 1999). The accumulation of ΔFosB has been found to suppress the D2 receptor activity in some brain regions, which is potentially consistent with the influence of high estrogen levels on increased ΔFosB induction and decreased D2-MSN activity (Ohnishi et al., 2011). Estrogen withdrawal on the other hand upregulates D2 neuron binding in females, which is associated with the emergence of maternal care in response to the presence of the offspring (Keer & Stern, 1999; Ohnishi et al., 2011; Yoest et al., 2014). Thus, the estrogen withdrawal period may result in decreased ΔFosB induction in D2-MSNs corresponding with an increased D2 receptor sensitivity.

Altogether, ΔFosB is differentially expressed in D1- and D2-MSNs in the NAc in response to many different stimuli including drugs, antidepressant medication, and social defeat stress, which makes it a promising molecular target for the present study in measuring NAc plasticity in response to hormone withdrawal following prolonged hormonal treatment (Lobo et al., 2013). Furthermore, the fact that it influences both maternal and depressive behavioral outcomes indicate that it could be an important target for uncovering neurological correlates in the development of PMD (Lobo et al., 2013). In particular, clinically, if we can reproduce the resilient transcription phenotype in patients, it has the potential to provide relief from the symptoms of PMD as a novel therapeutic approach (Lorsche et al., 2018). Given the complexities of the system there is a wide range of possible ΔFosB induction patterns in a hormone-sustained versus hormone-withdrawn condition, which is why it is important to conduct an experimental trial to uncover the actual outcomes that occur in practice.
Summary of the Rationale for the Present Study

PMD influences women, children, and families worldwide and yet research has largely overlooked its distinct temporal, behavioral, and neurological components that indicate pregnancy related mood disorders are categorically distinct from non-pregnancy related mood disorders. Due to this lack of attention, diagnosis and treatment options for PMD are limited to those for MDD and generalized anxiety disorders. Focusing on the typical biological transformations that women undergo throughout pregnancy and into the postpartum period could provide important insight into the neurological sites that are implicated specifically in PMD, which could promote more direct diagnosis and treatment options.

In particular, research suggests that estrogen and progesterone are crucial for promoting maternal caregiving behaviors and are related to both depressive and anxious phenotypes. Given that they also undergo drastic changes throughout the reproductive cycle, they may be an essential trigger of PMD (Bloch et al., 2003; Hendrick et al., 1998). Galea et al. (2001) developed a method for modeling the impact of these hormonal changes during pregnancy on behavior and the brain, replicating the maternal behaviors and affective responses that are typically seen in both human and animal models following pregnancy. Using a hormone-simulated pregnancy model researchers can effectively study the direct isolated impacts that reproductive hormones have on neurological and behavioral outcomes.

Prior research on the etiology of depressive and anxiety disorders implicates dopaminergic activity from the VTA to the NAc via the mesolimbic dopamine pathway as a significant neurological correlate. Nonetheless, dopaminergic function in this region has been largely understudied in research specific to PMD, despite the fact that dopamine activity throughout the mesolimbic dopamine pathway is highly sensitive to gonadal hormone-mediated
changes in women (Bazzett & Becker, 1994; Bridges, 2015; Küppers et al., 2000; Saigusa et al., 1997; Stern & Keer, 1999; Thompson & Moss, 1994; Yoest et al., 2014). It is likely that the region undergoes modifications throughout pregnancy and into the postpartum period in both typical and pathological cases. Furthermore, it is possible that the transcription factor ∆FosB serves as a molecular target for measuring plasticity in dopamine neurons differentially in D1 and D2 dopamine neuron subtypes in the NAc. Both maternal behaviors and depression are differentially motivated by induction of ∆FosB in D1 and D2 dopamine MSNs, which makes it necessary to distinguish between these neuronal subtypes in order to understand the full impact of dopamine activity on behaviors postpartum (Brown et al., 1996; Hamilton et al., 2017). Taken together, the research suggests that this unique neurobiological approach to studying PMD may provide more nuanced detail into the neurological effects of pregnancy on women so that we can begin studying the more specific etiology of PMD.

The Present Study

The present study is a preliminary model for gaining insight into how hormone fluctuations throughout pregnancy may induce neurological changes that correspond with distinct behavioral outcomes. The study expands on the unpublished pilot study conducted by Foster et al. (2017), which did not find any significant differences between induction of ∆FosB in dopamine MSNs within the NAc of hamsters in hormone-withdrawn and hormone-sustained conditions following a hormone-simulated pregnancy. We used transgenic mice that were genetically engineered to be able to differentially view the presence of ∆FosB in D1 receptor and D2 receptor MSNs, since they have been found to differentially respond to the increased or decreased presence of ∆FosB (Hamilton et al., 2017; Lobo et al., 2013). In order to conduct the
study, we also adapted the estrogen withdrawal model to simulate pregnancy over the course of a mouse’s typical 21-day gestation period.

**Research questions.** The primary goal of the present study was to determine whether or not hormone withdrawal at parturition corresponds with a significant change in ΔFosB expression in dopamine MSNs in the NAc relative to hormone sustention and whether these changes concur with particular behavioral outcomes associated with anxiety and depression. We aimed to determine whether the inability of previous researchers to find a direct association with hormone withdrawal and ΔFosB expression in dopamine neurons can be explained by the differential expression of ΔFosB in D1- and D2-MSNs in the NAc.

**Hypothesis #1.** Given that postpartum adaptations tend to result in increased calmness and reduced stress responses in mothers in order to ensure the survival of the offspring we anticipated that mice in the hormone-withdrawn condition would exhibit decreased anxiety in the Open Field Test and Elevated Plus Maze relative to mice in the hormone-sustained condition (Perani & Slattery, 2014; Slattery & Neumann, 2008). When this particular hormone-stimulated pregnancy model developed by Galea et al. (2001) was applied to rats and hamsters, researchers observed decreased anxiety in the hormone-withdrawal condition as measured by Elevated Plus and Open Field tests (Foster et al., 2017; Galea et al., 2001). Thus, we anticipated that we would replicate these findings in a novel mouse model.

**Hypothesis #2.** We anticipated that we would see neurobiological correlates that correspond with a phenotype that is more resilient to stress in the hormone-withdrawn condition relative to the hormone-sustained condition due to the consistent finding in the literature that estrogen withdrawal is associated with reduced anxiety (Neumann, 2003; Perani & Slattery, 2014; Slattery & Neumann, 2008; WHO, 2009). Literature on social defeat stress suggests that
stress resilience may be mediated by an increased presence of ΔFosB in D1-MSNs and a 
decreased presence of ΔFosB in D2-MSNs (Hamilton et al., 2017). As such, we anticipated that 
in a typical hormone-withdrawal model, we would see results consistent with pro-resilience to 
social defeat stress such that there would be an increased presence of ΔFosB in D1-MSNs and 
decreased presence of ΔFosB in D2-MSNs relative to the hormone-sustained condition. 
Decreased ΔFosB induction in D2-MSNs following hormone withdrawal would also be 
consistent with findings that suggest increased D2-MSN activity marks the emergence of 
maternal caregiving behaviors (Keer & Stern, 1999). ΔFosB concentration is inversely associated 
with D2-MSN activity, such that estrogen withdrawal may decrease ΔFosB concentration, thus 
enhancing D2-MSN sensitivity in preparation for rearing (Keer & Stern, 1999; Ohnishi et al., 
2011; Yoest et al., 2014).

It is important to note that since the typical behavioral state following hormone 
withdrawal at parturition is characterized by a complex combination of decreased anxiety and 
increased depressive episodes, we anticipated that the pattern of ΔFosB induction in dopamine 
neuron subtypes could resemble a combination of the stress resilient and stress susceptible 
phenotypes, however, there was not enough research to support any particular pattern.

Methods

Transgenic Mice

We were gifted 16 fluorescently labeled transgenic mice from the Nestler laboratory at 
the Mt. Sinai School of Medicine. Half of the mice were genetically engineered to have 
fluorescently labeled red D1 neurons with tdTomato marker and the other half had fluorescently 
labeled green D2 neurons with green fluorescent protein marker (GFP). This labeling paired with 
staining techniques allowed us to differentially visualize the presence of ΔFosB in D1- versus
D2-MSNs. The mice were pair-housed with one mouse from each experimental group in 28 cm x 17 cm x 12 cm cages. Eight of the mice were randomly assigned to the hormone-sustained condition and eight to the hormone-withdrawn condition, such that each of the experimental conditions had four mice with the fluorescently labeled red D1 neurons and four mice with the fluorescently labeled green D2 neurons.

Procedure

Ovariectomy. Prior to initiating the hormone-simulated pregnancy, all 16 mice underwent surgery to remove their ovaries in order to isolate and control hormonal secretion by eliminating the natural endogenous fluctuations of gonadal hormones produced by ovaries. The ovariectomies took place with the mice at a surgical plane of anesthesia—they were initially anesthetized using 4-5% isoflurane aerosolized in oxygen until sedated after which point the anesthesia was maintained throughout the surgery using a nose cone with 2-3% isoflurane aerosolized in oxygen. Prior to moving the animal to a sterile surgical field, we shaved their flanks and sterilized the groomed area with ethanol and betadine. After successfully administering a toe-pinching test to ensure the animal was sedated, we then made bilateral flank incisions and removed the ovaries using cauterization of the uterine horn from the flank fat pad. We then closed the muscle wall with suture and the skin with wound clips. We injected the mice with an antibiotic to prevent infection (0.1 mL of 10-mg/kg baytril) and a painkiller to moderate any discomfort (0.1 mL of meloxicam). The antibiotic and painkiller were administered daily up until the third day postoperative.

Hormone-simulated pregnancy. We adapted the model of hormonal manipulation designed by Galea et al. (2001) for a mouse’s 21-day gestation period. Following four days of recovery from the ovariectomies, all 16 mice received 9 am daily hormone injections. Early
pregnancy, which is characterized by low estradiol and high progesterone, was simulated in the first 13 days. During this period, both conditions received 0.1 mL of 2.5-µg estradiol and 0.1 mL of 4-mg progesterone in cottonseed oil. The second term, days 14-20, is characterized by high estradiol and low progesterone, and both conditions received 0.1 mL of 50-µg estradiol in cottonseed oil. On the first day of the postpartum period (day 21) the eight mice assigned to the hormone-sustained condition continued receiving injections of elevated estradiol in cottonseed oil, while the eight mice assigned to the hormone-withdrawn condition received injections of cottonseed oil through day 24 when the animals were sacrificed.

**Behavioral testing.** All behavioral tests were conducted on the third day of the simulated postpartum period (day 23). During testing, half of the animals were in a state of hormone withdrawal and the other half had sustained elevated estrogen concentrations. Two different tests were conducted over the course of the day to measure anxiety levels in each of the animals. In order to control for behavioral results that could be specific to the time of testing or to the order in which the animals completed each test, we counterbalanced the order of testing throughout the day and assigned the mice to pseudo-randomized testing groups, ensuring that mice from each of the conditions were evenly mixed into each of the testing groups.

**Open Field Test.** The Open Field Test is a commonly used measure of anxious behaviors in animal research. Generally, lower levels of anxiety have been associated with more time spent in the center of the enclosure, while higher anxiety levels have been associated with more time spent in the periphery; however, it is also important to consider other behaviors such as grooming, speed of movement, and total distance traveled while the animal is the enclosure as it may provide additional insight into the animal’s internal state (Seibenhener & Wooten, 2015; Kalueff & Tuohimaa, 2004). The Open Field Test apparatus resembled a square box with the
dimensions 40.5 cm x 40.5 cm x 30 cm. The top of the box was removed such that we could easily see into the enclosure to observe the animals. The test was administered over the course of 10 minutes per animal. We placed a video camera secured on a tripod above the box and filmed each of the trials so that we could keep track of the mice’s movement and behavior on film. We then applied the Ethovision XT animal tracking software designed by Noldus Industries to each of the videos, which quantitatively measured the mice’s movements to provide a value for velocity, total distance traveled, and time spent in the center versus the periphery of the enclosure.

**Elevated Plus Maze.** The Elevated Plus Maze is another widely used measure for anxious behaviors in animal models. The mice were placed in the center of a cross raised 73 cm above the ground for 5 minutes. The plus structure consisted of two open and two closed arms, each 51 cm long and 11.5 cm wide. The walls of the closed arms extended 39.5 cm and the open arms had a 1.0 cm lip along them. In the center of the apparatus, where the arms meet, there was a 10 cm x 11 cm square space large enough for the animal to stand. We placed a camera above the arms using a selfie-stick to stabilize it so that the movement and behavior of the mice could be documented and tracked. Lower levels of anxiety are associated with more time spent in the open arms of the maze, while higher levels of anxiety are associated with more time spent in the closed arms (Kalueff & Tuohimaa, 2004; Walf & Frye, 2007). We applied the Ethovision XT animal tracking software designed by Noldus Industries to each of the videos, which quantitatively measured the mice’s movements to provide a value for velocity, total distance traveled, and time spent in the open arms, closed arms, and neutral zone of the enclosure.

**Animal sacrifice.** On the fourth day of the postpartum period (day 24), all 16 mice were sacrificed using intracardial perfusion under a fume hood. Prior to initiating the procedure, we
anesthetized the animal with 0.1 mL beuthanasia-D administered via intraperitoneal injection. After ensuring that the animal had reached a surgical plane of anesthesia, we made a small lateral incision through the abdominal wall beneath the rib cage, which was then expanded to expose the heart by cutting away the ribs and lifting up the flap. We then passed a needle connected to a source of perfusion buffer through the posterior left ventricle of the exposed heart into the ascending aorta and clamped it in place using a hemostat. We made an incision in each animal’s right atrium with scissors. The perfusion buffer was pumped through the needle, effectively pumping the solution throughout the animal’s body via the bloodstream and out through the cut right atrium. Once the liquid ran clear and the animal’s liver appeared clear of blood we moved the pump line into a 4% paraformaldehyde fixative, which was then pumped through the body until the animal was stiff and fixed. The brains were removed manually with scissors and rongeurs immediately following fixation and were stored in a paraformaldehyde solution for 24 hours post-fixing before being transferred to a 30% sucrose cryoprotectant solution. We stored the brains in this solution at -20°C until sectioning.

**Tissue sectioning.** We placed each brain in a brain block in order to remove the olfactory bulbs and cerebellum cleanly with a razor blade. The rest of the brain was coronally sectioned into 40-µm sections using a manual cryostat set at -20°C. The sections were stored at -20°C in cryoprotectant until staining.

**Tissue histology.** The tissue histology was performed using immunofluorescence to double-label ΔFosB expression in both D1 and D2 receptor-containing neurons: D1 was visualized through RFP labeled MSNs and D2 was visualized through GFP labeled MSNs. We used a representative coronal section from each animal containing a consistent section of the NAc (see Figure 3). After selecting the representative sections from each animal using a mouse
brain atlas designed by Paxinos and Franklin (2004), we washed the brain sections five times for five minutes per wash in 25 millimolar PBS. The sections were then incubated for 24 hours at room temperature in a solution of primary antibodies in 0.1% triton-X with the following concentrations: in D1 animal tissue we used a 1:750 concentration of goat anti-RFP antibody (Rockland, 200-101-397); in D2 animal tissue we used a 1:5000 concentration of goat anti-GFP antibody (abcam, 5450); and in both D1 and D2 animal tissue we used a 1:1000 concentration of rabbit anti-FosB antibody (abcam, 184938). We performed five additional five-minute washes in PBS before incubating the tissue at room temperature in the dark for one hour in a solution of secondary antibodies in 0.1% triton-X with the following concentrations: in D1 animal tissue we used a 1:500 concentration of Alexa Fluor 488 goat to anti-rabbit antibody (Jackson ImmunoResearch, 111-545-144) and a 1:500 concentration of Alexa Fluor 594 donkey to anti-goat antibody (Jackson ImmunoResearch, 705-585-147); and in D2 animal tissue we used a 1:500 concentration of Alexa Fluor 594 donkey to anti-rabbit antibody (Jackson ImmunoResearch, 711-585-152) and 1:500 concentration of Alexa Fluor 488 donkey anti-goat antibody (Jackson ImmunoResearch, 705-546-147). We washed the tissue five final times before mounting the tissue wet on slides in a solution of 50% Mili-Q water and 50% PBS. The tissue was coverslipped on the slide with ProLong Gold Antifade Mountant.

**Confocal microscopy.** We used confocal microscopy to visualize and count the number of cells in the NAc that were double-labeled in yellow such that they simultaneously expressed either green ΔFosB and red RFP in D1 neurons or red ΔFosB and green GFP in D2 neurons. The tissue was imaged using a confocal microscope that displayed images on a computer through Nikon’s EZ-C1 Freeviewer software (version 3.90), which could simultaneously display both the red and green labels. Pictures were taken at 20X from the medial side of the anterior commissure.
on both the left and right hemisphere. We counted the double-labeled yellow cells within a consistently sized sample box just adjacent to the anterior commissure for the NAc core and just adjacent to that for the NAc shell while blind to the experimental condition of the mice (see Figures 3, 6, and 7).

Results

Behavioral Results

The behavioral data for both Open Field and Elevated Plus Maze testing were acquired through EthoVision XT behavioral tracking software, which provided information about the average velocity and average time spent in specific locations of each of the arenas. An unpaired t-test was used to statistically measure mean level differences between hormone-withdrawn and hormone-sustained animals based on different behavioral measures.

Open Field Test. We calculated a difference score for each animal based on total time in seconds spent in the periphery versus total time spent in the center of the open field. Over the course of the ten minute trial, we found no significant difference in the amount of time spent in the periphery versus the center of the enclosure between withdrawn ($M=82.9$, $SD=203$) and sustained animals ($M=64.7$, $SD=203$); $t(14)=-.582$, $p=.570$. We also conducted separate independent samples t-test measures to compare withdrawn and sustained animals in the first five minutes and second five minutes of the behavioral test separately in order to account for the fact that animals may get accustomed to the arena, which could correspond with reduced anxiety over time. We found no significant difference in the amount of time spent in the periphery versus the center in the first five between withdrawn ($M=11.7$, $SD=97.7$) and sustained animals ($M=46.4$, $SD=109$); $t(14)=-.669$, $p=.514$. We also found no significant difference in the second
five minutes between withdrawn \((M=-5.49, \ SD=136)\) and sustained animals \((M=18.3, \ SD=109)\); \(t(14)=-.385, \ p=.706\).

In addition to the measures of total time spent in the periphery versus in the center, we also performed an independent samples t-test to compare average velocity in centimeters per second and total distance traveled in centimeters during the open field test between hormone-withdrawn and hormone-sustained animals in order to gain insight into emergence of other unique behavioral outcomes. Over the course of the ten minute trial, we found no significant difference in the mean velocity between hormone-withdrawn \((M=3.34, \ SD=.550)\) and hormone-sustained animals \((M=3.16, \ SD=.904)\); \(t(14)=.493, \ p=.630\). Over a ten minute trial, there was also no significant difference in the total distance traveled between hormone-withdrawn \((M=2001, \ SD=333.5)\) and hormone-sustained animals \((M=1879, \ SD=537.7)\); \(t(14)=.547, \ p=.593\).

Based on these results, our hypothesis that animals in the hormone-withdrawn condition would exhibit significantly decreased anxiety symptoms in Open Field Test measures was not supported for any of our testing variables (see Table 1).

**Elevated Plus Maze.** We also used an independent samples t-test to compare behavioral trends in the Elevated Plus Maze between hormone-withdrawn and hormone-sustained animals. We calculated a difference score for each animal based on total time in seconds spent in the closed arms versus total time spent in the open arms of the maze. Over a five minute trial, we found no significant difference in the time spent in the periphery versus the center between hormone-withdrawn animals \((M=132, \ SD=41.2)\) and hormone-sustained animals \((M=157, \ SD=63.2)\); \(t(14)=-.943, \ p=.361\). In order to gain further insight into emergence of other unique behavioral differences, we also calculated measures for average velocity in centimeters per second and total distance traveled in centimeters. We found no significant difference in velocity
between hormone-withdrawn ($M=7.62$, $SD=3.70$) and hormone-sustained animals ($M=3.70$, $SD=2.61$), $t(14)=0.90$, $p=.930$. Additionally, we found no significant difference in the total distance traveled between withdrawn ($M=2232$, $SD=1075$) and sustained animals ($M=2188$, $SD=757.5$), $t(14)=.95$, $p=.392$. Thus, the data did not support our hypothesis that animals in the hormone-withdrawn condition would exhibit decreased anxiety symptoms in Elevated Plus Maze measures (see Table 2).

**Neurological Results**

We calculated the neurological data by averaging the number of double-labeled ΔFosB in either D1 neurons expressing RFP or D2 neurons expressing GFP from the left and right medial anterior commissure at 20X magnification. We also did a separate calculation for the core and the shell of the NAc for each animal using a brain atlas to approximate the distinction (see Figure 3). We applied an independent samples t-test to the data to compare mean level differences in ΔFosB expression in either D1 or D2 neurons between the hormone-sustained and hormone-withdrawn conditions. The results are represented graphically (see Figures 4 and 5) as well as visually in confocal photomicrographs (see Figures 6 and 7).

**D1 transgenic mice.** We were only able to collect data from five of the eight D1 mice because two animals from the sustained condition and one animal from the withdrawn condition did not express RFP. We found no significant difference (see Figures 4 and 6) in ΔFosB expression in D1 neurons within the NAc core between the hormone-sustained ($M=8.000$, $SD=7.071$), and hormone-withdrawn condition ($M=13.33$, $SD=5.485$), $t(3)=0.9641$, $p=0.4061$. We also found no significant difference (see Figures 5 and 6) in ΔFosB expression in D1 neurons within the NAc shell between hormone-sustained ($M=15.00$, $SD=2.828$) and hormone-withdrawn animals ($M=10.67$, $SD=5.346$), $t(3)=1.019$, $p=0.3834$. This data did not support our hypothesis.
that ΔFosB induction would be higher in D1 neurons of hormone-withdrawn animals relative to hormone-sustained animals.

**D2 transgenic mice.** We were only able to collect data from six of the eight D2 mice, because one mouse from each of the experimental conditions did not express GFP. We found a significant difference (see Figures 4 and 7) in ΔFosB expression in D2 neurons within the NAc core between hormone-sustained ($M=12.33$, $SD=1.756$) and hormone-withdrawn animals ($M=19.00$, $SD=3.606$) $t(4)=2.879$, $p<0.05$, such that mice in the hormone-withdrawn condition had significantly higher ΔFosB expression in D2 neurons as compared to hormone-sustained mice. We found no significant difference (see Figures 5 and 7) in ΔFosB expression in D2 neurons within the NAc shell between hormone-sustained ($M=13.17$, $SD=5.107$) and hormone withdrawn mice ($M=19.67$, $SD=7.67$), $t(4)=1.211$, $p=0.2925$. The significantly higher ΔFosB expression in D2-MSNs of the NAc core in hormone-withdrawn mice is contradictory to our hypothesis.

**Discussion**

The present study presents a novel model for simulating pregnancy hormonally in mice that has primarily been conducted in hamsters and rats. Using this model, we were able to emphasize hormone withdrawal as the primary experimental variable to determine whether or not neurological and behavioral changes that occur around the peripartum period are attributable to hormonal fluctuations—and in particular estrogen withdrawal—at parturition. While this model is not ideal for measuring the true hormonal experience of pregnancy in terms of an accurate co-fluctuation of estrogen and progesterone, the model does isolate estrogen withdrawal as a primary candidate for behavioral changes related to anxiety and depression in the postpartum period. There is evidence that the regulation of mood is linked to neural structures
that are rich in estrogen receptors and/or neurotransmitters that are highly sensitive to estrogen regulation (Wharton et al, 2012). Thus, despite the fact that the model lacks some of the hormonal and physical characteristics of an actual pregnancy, it is an important first step in outlining the relationship between estrogen withdrawal and neurological/behavioral changes.

Sample Limitations

Before discussing the findings, it is important to acknowledge that due to sample size limitations in the present study we cannot draw conclusions about the precise influence of estrogen withdrawal following a hormone-simulated pregnancy on ΔFosB induction in D1-versus D2-MSNs. However, based on some significant results and other potential trends in the data, we can posit a likely relationship between ΔFosB and dopamine neurons in the NAc that is mediated by estrogen withdrawal following prolonged estrogen exposure. Moving forward in studies with transgenic animals in general it is important to have a large sample size and to be mindful of running tests for fluorescence on multiple subjects before determining whether or not a stain is viable. Working with transgenic animals can be inconsistent as there are individual differences in the occurrences of animals silencing foreign genes (Liu, 2013). In the present study three mice silenced RFP expression in D1-MSNs and two mice silenced GFP expression in D2-MSNs, which significantly reduced our sample and prolonged the process of determining a viable double-label staining technique that would express the transgenic mutation. A larger sample could account for these limitations and ought to be run in order to more credibly expand on the findings in the present study.

No Change in Anxiety Behaviors Between Hormone Conditions

Inconsistent with previous findings conducted in hamsters and rats, we found no statistically significant differences in behavioral outcomes between hormone-sustained and
hormone-withdrawn animals based on measures of velocity, total movement, and time spent in peripheral versus exposed areas of the Open Field Test and the Elevated Plus Maze arenas (Foster et al., 2017; Galea et al., 2001). These results may suggest that this particular hormone-simulated pregnancy model does not translate well to a mouse model, especially in terms of producing anxiety-like behaviors that are consistent with an actual pregnancy. Given that it is a novel test it would be prudent to run a trial with a larger cohort of animals in order to determine more conclusively whether or not anxiety behaviors differ in mice following this particular hormone-simulated pregnancy. We had access to only a small cohort of animals (N=16), so we were unable to perform an additional control condition in which some of the animals were not subject to a hormone-simulated pregnancy as another point of comparison between-subjects. Furthermore, we did not take a baseline measurement of anxiety for each animal to conduct within-subjects analyses. Due to the potential of individual differences between mice, future studies should conduct a baseline anxiety measure for each animal prior to initiating the hormone simulated pregnancy or at different points throughout the trial to get a better sense of how the behavior is changing within-subjects upon hormone withdrawal.

**ΔFosB Expression in Dopamine MSNs within the NAc Core and Shell**

**Increased ΔFosB expression in D2-MSNs within the NAc core.** We found a statistically significant difference in ΔFosB induction in D2 neurons between hormone-sustained and hormone-withdrawn mice such that following four days of either estrogen withdrawal or continued estrogen sustention, mice in the hormone-withdrawn condition had significantly higher ΔFosB induction in D2 neurons within the core of the NAc (M=19.00, SD=3.606) than mice in the hormone-sustained condition (M=12.33, SD=1.756), t(4)= 2.879, p<0.05. The emergence of a statistical significance within such a small sample suggests that there is a strong
relationship between estrogen withdrawal and ΔFosB mediated D2-MSN activity in the NAc core. These data do not support our initial hypothesis and suggest that we should revise our view to include a more nuanced perspective of D2 neurons in the NAc that would explain a correspondence between ΔFosB expression and increased D2-MSN activity.

Previous research consistently suggests that increased D2 activity in the NAc is essential in the promotion of maternal behaviors (Keer & Stern, 1999). Yoest et al. (2014) found that acute estrogen treatment generally down-regulates D2 receptor binding, however, over time estrogen treatment increases the number of D2 neuron binding sites in the NAc core. This seemingly incongruent combination of estrogen-mediated effects on D2 receptors suggests that while estrogen is circulating, D2 receptors accumulate in the NAc core without actively binding such that once estrogen is withdrawn they are highly sensitive due to the increased receptor sites that have now been stimulated to bind. This is consistent with findings from Bazzett and Becker (1994) who found even more specifically that in states of reduced circulating estrogen during a rat’s normal endogenous cycle (such as during diestrus) there is a greater ratio of high/low affinity states of striatal D2-MSNs than for elevated estrogenic states (such as during proestrus). They were even able to replicate the increased ratio of high/low affinity states experimentally with a condition in which animals were ovariectomized, significantly decreasing circulating estrogen (Bazzett & Becker, 1994). In D2 receptors a high-affinity state corresponds with a high affinity for agonists, which is associated with increased binding and activity (Graf-Guerrero et al., 2009). This research taken together suggests that estrogen-withdrawal at parturition may enhance maternal caregiving behavior through a significantly increased ratio of high/low affinity D2-MSNs, which effectively corresponds with increased sensitivity and efficiency of D2-MSN agonist binding.
It is important to note that the changes in D2 affinity outlined above occur with acute estrogen exposure. Furthermore, all of the evaluations of dopaminergic activity outlined were also relative to a receptor-specific view at a given time. Given that in the present study we were interested in evaluating stable markers of plasticity with chronic exposure and withdrawal, it is important that we distinguish between the different functions of ΔFosB (our particular target marker of plasticity) over time in order to gain insight into the underlying molecular mechanisms that influence receptor-level changes. McClung and Nestler (2008) found that ΔFosB acts as a transcriptional repressor at some sites with acute short-term treatments and a transcriptional activator with more chronic treatments. Specifically ΔFosB is a stable variant of the FosB gene, which accumulates under chronic conditions following the accumulation of other Fos family proteins in acute stimulation (McClung & Nestler, 2008). Song et al. (2014) determined that the withdrawal period following prolonged exposure to drugs is necessary for producing D2-MSN plasticity, which is associated with addictive behavioral outcomes corresponding with impaired reward and motivational functions. Hormonal exposure may act similarly in that the withdrawal period following prolonged exposure could increase plasticity and sensitization of D2-MSNs in the NAc, as is potentially mediated by differential ΔFosB induction in neuronal subtypes within sub-NAc regions (Grueter et al., 2013). This increased sensitivity in D2 neurons may also be important for predicting the high incidence of depressive symptoms in the early stages of the postpartum period as ΔFosB expression in D2 neurons is generally associated with a stress susceptible phenotype in social defeat models (Hamilton et al., 2017).

**No change in ΔFosB expression in D2-MSNs within the NAc shell.** We found no significant difference in ΔFosB expression in D2 neurons within the NAc shell between hormone-withdrawn ($M=19.67$, $SD=7.67$) and hormone-sustained mice ($M=13.17$, $SD=5.107$).
Due to our very small sample size with only three animals in each condition, these results are inconclusive and ought to be examined more closely in a larger sample in order to more accurately hone in on the non-statistically significant trend that emerged between the means. While the lack of significance due to the high level of variation within groups suggests that these mean differences could be due to chance, the mean trend is consistent with findings from the NAc core such that there was a higher mean level of ΔFosB in the hormone-withdrawn condition relative to the hormone-sustained condition (see Figure 4). In such a small sample the lack of significant results could indicate that there is no relationship between hormone withdrawal and ΔFosB expression in D2-MSNs within the NAc shell, that the relationship between hormone withdrawal and ΔFosB expression in D2-MSNs within the NAc shell is weaker than within the NAc core, or that there were more individual difference variations within the measurements from the NAc shell that were magnified by the small sample.

The fact that we found no significant change in the expression of ΔFosB in D2-MSNs within the NAc shell, but we did find significant changes within the NAc core, generally suggests that there is a more complicated relationship between D2 neuron activity and ΔFosB induction patterns within different sub-regions of the NAc than was proposed in the hypotheses, such that they ought to be examined separately (Keer & Stern, 1999; Ohnishi et al., 2011; Yoest et al., 2014). Researchers have determined that estrogen withdrawal up-regulates D2 neuron binding in the NAc in females, which is associated with the emergence of maternal care in response to the presence of the offspring; however these studies did not differentiate between the core and the shell of the NAc (Keer & Stern, 1999; Ohnishi et al., 2011; Yoest et al., 2014). Additional research indicates that lesions of the NAc shell and not the core are associated with disruptions of maternal behaviors including maternal memory and pup-retrieval, which suggests
that it is important to distinguish between neuronal changes in the core and the shell of the NAc, particularly in interpreting behavioral outcomes (Li & Fleming, 2003a; Li & Fleming, 2003b). Additionally, the NAc core and NAc shell project to distinct regions of the brain, which suggests that they may have differential modes of action (Francis & Lobo, 2017).

Beyond the fact that the shell and the core motivate unique behavioral outcomes, there are also distinctions in the electrophysiological and synaptic properties of D2 neurons within different neural sub-regions (Grueter et al., 2013). While Ohnishi et al. (2011) found that ΔFosB overexpression is associated with decreased D2-MSN activity within some brain regions such as the subthalamic nucleus, Grueter et al. (2013) found that specifically within the NAc shell ΔFosB overexpression is associated with the un-silencing of synapses on D2-MSNs. If the mean trend from the present study were to hold up in a replication with a larger animal cohort such that ΔFosB expression significantly increased in D2-MSNs within the NAc shell following hormone withdrawal, then this may indicate that estrogen withdrawal un-silences synapses in D2-MSNs on the shell via ΔFosB mediated pathways, which would correspond with the emergence of maternal behavior as D2-MSN synapses become activated (Keer & Stern, 1999; Ohnishi et al., 2011; Yoest et al., 2014).

**No change in ΔFosB expression in D1-MSNs within the NAc core or shell.** We found no significant differences in ΔFosB expression in D1 neurons within the NAc core or shell following hormone withdrawal. The sample from D1 mice was more limited than D2 mice such that the results were based on neurological data from only two animals in the sustained group and three animals in the withdrawn group. As such, these results are inconclusive and further research would need to be conducted in order to uncover reliable trends.
Based on our experimental model, and if we generalize our analysis to include the NAc as whole, there are three possible final outcomes to the replicated present study in a greater sample size: we see only a change of ∆FosB induction in D2-MSNs, which would be consistent with the results of the present study; we see a congruent change of ∆FosB induction in D1- and D2-MSNs, which would be inconsistent with the lack of significant results but consistent with the trend in mean differences from the NAc shell; or we see an incongruent change of ∆FosB induction in both D1- and D2-MSNs, which would be inconsistent with the lack of significant findings and but consistent with the trend in mean differences from the NAc core.

**Implications of no change in ∆FosB expression in D1-MSNs.** If we found that consistent with the present study there are no significant changes in ∆FosB expression in D1-MSNs following hormone withdrawal, then the primary conclusion may be that ∆FosB is selectively overexpressed in D2 neuronal subtypes in the NAc following estrogen withdrawal. This result may indicate that we need to look at other transcriptional markers to understand long-term regulation of D1-MSNs in the NAc. Two transcription factors in particular—cAMP-responsive element binding protein (CREB) and brain-derived neurotrophic factor (BDNF)—are associated with neuronal substrate activity and plasticity in the mesolimbic dopamine pathway (Lu, Nagappan, & Lu, 2014; Sakamoto, Karelina, & Obrietan, 2011). CREB is a necessary component of synaptic plasticity and neuronal protection in the NAc, such that the dysregulation of CREB is associated with neuropathological conditions (Dong et al., 2006; Sakamoto, Karelina, & Obrietan, 2011). Likewise, BDNF is especially important in synapse regulation and has been found to have differential expression in the NAc core and shell, which motivate unique behavioral outcomes (Li et al., 2013). Dysregulation of BDNF signaling is also associated with neuropathological conditions including depression and memory degenerative disorders.
(Sakamoto et al., 2011). Either factor would be a promising target for understanding long-term genomic changes that may occur in D1-MSNs in the nucleus accumbens beyond ΔFosB.

Another possible explanation for a lack of significant changes in ΔFosB expression in D1-MSNs could be that the highly stable structure of ΔFosB is differentially expressed in D1-MSNs versus D2-MSNs following chronic and acute estrogen treatments in a time-specific manner (Nestler et al., 2001). The methodology of the present study was limited in that it only examined behavioral and neurological outcomes at one point following hormone withdrawal, which eliminates the ability to study time-specific changes in ΔFosB. As such, we could only conclude that after four days following hormone withdrawal, ΔFosB induction in D1 neurons did not differ between hormonal conditions. It is possible that there are unique time-dependent induction patterns that occur following hormone withdrawal in dopamine neuron subtypes that were not captured in the present study.

A final explanation involves potentially magnified individual differences in a small sample. Abrahao, Quadros, and Souza-Formigoni (2011) used ethanol treatments and found that there are naturally occurring individual differences in development of sensitization to ethanol between testing mice, which corresponded with individual variability in D1-MSN expression in the NAc. Only mice that were prone to ethanol-sensitization demonstrated hypersensitive D1-MSN activity in the NAc, and this sensitization was in-turn dependent on the activation of NAc D1-MSNs. It is possible that there were individual differences in the susceptibility to estrogen sensitization between mice in our sample. If these individual differences exist, then it could provide an important avenue through which to study stress-resilience and susceptibility in postpartum females based on D1-MSN susceptibility to estrogen sensitization, which may be associated with PMD resilience (Peña, Neugut, Calarco, & Champagne, 2014). It may also be
indicative of the high rates of comorbidity between PMD and non-pregnancy related mood disorders, such that individuals may be predisposed to stress-susceptibility mediated by D1-MSN sensitivity in the NAc (Bloch et al., 2000; Matthey et al., 2003; Robertson et al., 2004).

**Implications of increased ΔFosB expression in D1-MSNs.** If we find that ΔFosB expression in D1-MSNs is congruently increased along with D2-MSN activity following hormone withdrawal, then the results would be consistent with research that suggests the increased reward sensitization to maternal cues is directly associated with ΔFosB induction in general, regardless of its expression in particular neuronal subtypes (Afonso et al., 2013; Barth et al. 2015; Yoest et al., 2014). Furthermore, it would support Nestler’s (2008) hypothesis that ΔFosB could be used as a biomarker for assessing reward circuitry activation.

In terms of reward motivated behaviors, this result would be consistent with research that suggests increased D1 activity is associated with maternal behaviors and stress-resilience, since ΔFosB induction in D1-MSNs generally up-regulates D1-MSN activity (Hamilton et al., 2017; Keer & Stern, 1999; Numan et al., 2005; Peña et al., 2014). Researchers have found that the infusion of D1 receptor antagonists in the NAc to block D1 receptor activity, concurrently blocks pup retrieval behavior (Keer & Stern, 1999; Numan et al., 2005). Additionally lactating females who are naturally more engaged in grooming behaviors demonstrate increased density of D1 receptors in the NAc (Peña et al., 2014). As it relates to emotional states in the postpartum period, this outcome may be indicative of the complicated and variable degree of stress-resilience in mothers in the postpartum period. Hamilton et al. (2017) determined that stress resilience corresponds with an increased presence of ΔFosB in D1-MSNs and a decreased presence in D2-MSNs. These findings together would be consistent with research that suggests an elevated dopamine tone in the NAc is associated with a heightened motivational drive, which
could act to both promote maternal behaviors as well as protect against chronic-stress through action and reward motivation in healthy mothers (Peña et al., 2014). Ultimately, this finding would suggest a combination of a stress-resilient phenotype with increased ΔFosB in D1-MSNs and a stress-susceptible phenotype with increased ΔFosB in D2-MSNs, which reflects the vulnerability to mood disorders following parturition in order to stimulate the emergence of maternal-specific caregiving behaviors.

**Implications of reduced ΔFosB expression in D1-MSNs.** A reduced expression of ΔFosB in D1-MSNs would be consistent with a stress-susceptible phenotype in terms of both D1 and D2 dopaminergic function in the NAc, however it would be inconsistent with research that suggests maternal behaviors are dependent on D1-MSN expression (Hamilton et al., 2017). Thus, it is important to evaluate the result through perspective of both models in order to gain a more in depth understanding of the potential interactions that may occur to produce this type of incongruent expression of ΔFosB in D1-MSNs versus D2-MSNs.

In terms of a stress-susceptibility model, this result would potentially provide insight into new mothers’ increased likelihood of developing mood disorders; however, given that in general mothers tend to experience reduced anxiety symptoms following parturition, researchers would need to examine parallel processes that may work to counterbalance this increased stress-susceptibility in a healthy sample (O’Hara et al., 1990; Perani & Slattery, 2014; Slattery & Neumann, 2008; Stowe & Nemeroff, 1995). The present study is focused in on a very specific neurological region and within that region the expression of a single transcriptional factor expressed directly in D1 and D2 neurons, so it may be important to take a more holistic perspective on the interactions that are occurring within this neural system in order to better understand the emergence of particular behavioral outcomes following pregnancy. It should be
noted that the vast majority (up to 95%) of the NAc—established as the reward center of the brain—is composed of MSNs, which are distinguished by their expression of either D1 or D2 dopamine receptors (Soares-Cunha et al., 2016). This finding suggests that even when evaluating the etiology of reward-motivated behaviors on a holistic level, it is still important to focus on the unique behaviors associated with dopaminergic activity in the NAc (Russo & Nestler, 2013; Shirayama & Chaki, 2006).

The present study focused specifically on estrogen-mediated ΔFosB expression in these two neuronal subtypes, however, it is certainly not giving us the whole story. In particular, research suggests that oxytocin is a vital hormone in the production of both the calm and stress-reduced state in mothers following birth as well as in the initiation of infant attachment behaviors (Kendrick, 2000). Further research on neurological mechanisms of oxytocin suggest that it has a high interface with both estrogenic and dopaminergic activity (Shahrokh, Zhang, Diorio, Gratton, & Meaney, 2010). In general for a healthy model, oxytocin release is associated with decreased anxiety and susceptibility to fear (Cochran, Fallon, Hill, M., & Frazier, 2013). Guzmán et al. (2013), however, found that oxytocin can also have fear and anxiety enhancing effects in susceptible individuals. The implications of reduced ΔFosB expression in D1-MSNs and D2-MSNs following hormone withdrawal in conjunction with research on the interaction between oxytocin and dopaminergic function, suggest that this outcome could be consistent with individual susceptibility models for depression as mediation by oxytocin. Specifically, in a healthy model oxytocin may interact with dopamine to incur stress-resilience even within a typically stress-susceptible dopaminergic state induced by ΔFosB. This relationship between oxytocin and dopaminergic function would also be consistent with research that suggests estrogen and progesterone are critical components in the modulation of oxytocin, which is
directly associated with breastfeeding, lactation, and maternal caregiving (Grieb, et al., 2017; Kloet, et al., 1986; McCarthy, 1995; Neumann, 2003; Russell et al., 2001).

Unfortunately, despite the fact that oxytocin could potentially satisfy the stress-susceptibility model, it is limited in its ability to explain how ΔFosB expression decrease in D1 neurons (which is generally associated with reduced D1 activity) is related to the propagation of maternal instincts (which is generally associated with increased D1 activity) (Keer & Stern, 1999; Numan et al. 2005; Ohnishi et al., 2011). Maternal behavior is dependent on the mutual interaction between oxytocin and dopamine activity in the NAc, such that if either one is inhibited there are severe deficits in the emergence of maternal caregiving behaviors (Shahrokh et al., 2010). Thus, if the model of the present study were to produce this particular result, it would be important to return to a more region-specific analysis within the core and shell of the NAc in order to gain a more nuanced understanding of the big picture. Additionally, it would likely still be informative to examine other transcriptional makers such as CREB and BDNF in order to establish a more comprehensive model of the long-term regulation of D1-MSNs in the NAc from chronic estrogen states.

Interpreting the Behavioral and Neurological Results Together

We found no statistically significant differences in anxiety-like behaviors between conditions as measured by two common anxiety measures, which would suggest that the behaviors tested did not correspond with the neurological changes found between hormone conditions in the NAc core of D2 mice. While future research should replicate the present study in a larger cohort of animals in order to determine whether the lack of statistically significant results could be accounted for by the small sample size, it is also possible that increased ΔFosB expression in D2 neurons of the NAc core in hormone-withdrawn mice corresponded with other
behaviors that were not measured in this particular study such as depression, stress susceptibility, and/or emergence of maternal behaviors.

Previous research has found increased depressive symptoms in women following parturition, which makes it an important measure for determining the viability of the pregnancy simulation (Galea et al., 2001). Unfortunately, we were unable to perform depressive or stress-susceptible measures at the time the study was conducted. Future studies should incorporate a Tail Suspension Test to measure the state of learned helplessness, which is central to depression and stress susceptibility. In this test, the time that the mouse continues to struggle is associated with depression, such that the mice that give up sooner are considered more helpless and therefore more depressed (Landgraf, Long, Der-Avakian, Streets, & Welsh, 2015). The Sucrose Preference Test is an additional measure for depression in animal models that uncovers incidents of anhedonia, which is often associated with depression (Rygula et al., 2005). In addition to emotional states, previous researchers have used hormonal models to provoke maternal care behaviors, which is an important measure of validity for a hormone-simulated pregnancy model (Grieb et al., 2017; Russell et al., 2001). These particular measures may be more valid indicators of behavioral changes that correspond with unique dopaminergic states. Researchers frequently focus on the relationship between \( \Delta FosB \) induction in D1 versus D2 neurons as it relates to stress resilience and susceptibility, which may be more directly measured by depressive behavioral tests such as the Tail Suspension Test (Hamilton et al., 2017; Landgraf et al., 2015). Furthermore, given that the study involves the simulation of pregnancy, it would be insightful to measure the influence of hormone-mediated neurological changes on preparing a mother to provide for her offspring.
Future Directions

Accounting for Limitations in the Present Study

The present study was an important pilot for establishing the existence of a relationship between estrogen withdrawal and dopaminergic activity in reward pathways as mediated by a stable transcriptional marker of plasticity; however, there are still many ways in which the findings from the present study could be refined in order to gain a clearer understanding of the underlying significance of the changes in ΔFosB induction following hormone withdrawal. Research from addiction models indicate that ΔFosB is a highly stable molecular switch that can persist for weeks following activation, which conveys long-term neural and behavioral plasticity (Nestler et al., 2001). In the present study, all of the brains were fixed in the fourth day following estrogen withdrawal. Our results would suggest that four days of estrogen withdrawal following sustained estrogen exposure was enough to produce stable ΔFosB changes in D2-MSNs, however, future research should incorporate additional experimental conditions that provide a more detailed representation of ΔFosB activity throughout the pregnancy. For instance, it would be interesting to compare between-subjects data from animals perfused at different times throughout the hormonal process (e.g. prior to hormone treatment, following estrogen rise, and on different days following hormone withdrawal). This research would provide insight into the time-specific changes in ΔFosB induction, which would be important for predicting behavioral outcomes.

The present study did not establish any correlations between neurological states and behavioral outcomes. Future studies ought to replicate the present study with a larger cohort of animals in order to incorporate more experimental conditions and to account for the variability in individual differences. Additionally, researchers should consider incorporating additional
behavioral measures in order to determine whether or not the neurological changes confer with depressive symptoms, stress-susceptibility, or maternal behaviors.

**Additional Future Research Considerations**

The results from a more conclusive replication of the present study would determine the directions that future researchers could take in order predict behavioral outcomes from direct neurological manipulations. The approach to manipulating neurological states is dependent on whether there is a congruent change in both D1- and D2-MSNs following hormone withdrawal, there is an incongruent change in D1- and D2-MSNs, or there is only a change in D2-MSNs and no change in ΔFosB induction in D1-MSNs. If ΔFosB increases in both D1- and D2-MSNs, then researchers could use viral mediated gene transcription adino-associated viruses (AAvs) to artificially up-regulate or down-regulate the concentration of ΔFosB to determine if it results in corresponding behavioral changes. For example, if researchers determine that increased ΔFosB expression in both neuronal subtypes following hormone withdrawal is associated with the emergence of maternal-care behaviors, then it would be predicted that directly up-regulating ΔFosB in the NAc would produce corresponding maternal outcomes. If there is an incongruent change in ΔFosB expression in D1- and D2-MSNs, such that there is a decrease of ΔFosB in D1-MSNs and an increase of ΔFosB in D2-MSNs, then researchers could engineer a strain of transgenic mice for the use of Cre dependent expression to selectively target either D1 or D2 neurons in the NAc to determine whether or not it results in a corresponding behavioral changes. This same method could be used if the only significant change is in D2-MSNs and there is no change in ΔFosB expression in D1-MSNs. Ultimately, these additional studies would provide a more clear and direct understanding of the influence of ΔFosB expression in dopamine neurons.
on behavioral outcomes, such as to establish particular causal relationships that could then be used to develop PMD specific drug treatments.

**Concluding Remarks**

There is a notable stigmatization of mental illness in our society, especially for mothers who are expected to be happy and consumed by their newborn, but instead feel depressed and/or anxious. While there is still a long way to go, we hope that this exploratory research will be a step in the right direction for more directly defining the influence of pregnancy on neurological and behavioral outcomes. By further understanding the normative influence that hormonal changes throughout pregnancy have on neurological systems, we can identify the systems that may be involved in pathological cases to develop novel diagnostic and treatment options for PMD.
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**Table 1**

*Independent samples t-test for equality of means in Open Field Test measures between hormone-withdrawn and hormone-sustained conditions*

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<td>.570</td>
<td>-59.1</td>
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<td>Score in Second-Half</td>
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<td>.630</td>
<td>.184</td>
<td>.374</td>
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<td>Total Distance Traveled</td>
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<td>.593</td>
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</table>
Table 2

Independent samples t-test for equality of means in Elevated Plus Maze measures between hormone-withdrawn and hormone-sustained conditions

<table>
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<th>Sig. (2-tailed)</th>
<th>Mean Difference</th>
<th>Std. Error Difference</th>
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<td>.926</td>
<td>44.3</td>
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</table>
Figure 1. Graph representing the trend of estrogen and progesterone levels after the first 6 weeks of pregnancy into postpartum at approximately 40 weeks (adapted from Martin and Mehbehani, 2006).
Figure 2. Graph that visually represents the trend of hormone treatments used in the present study to simulate a pregnancy in mice (adapted from Galea et al., 2001).
Figure 3. Representative section of the brain from which neurological results were calculated. Double-labeled ΔFosB and RFP in D1 transgenic mice and GFP in D2 transgenic mice were counted in the left and right hemisphere of the NAc core (red boxes) and the NAc shell (blue boxes). The counting boxes are drawn on Figure 21 from the mouse brain atlas developed by Paxinos and Franklin (2004).
Figure 4. Bar graph depicting mean-level differences in the NAc core of ΔFosB expression in both D1 and D2 transgenic mice between hormone-withdrawn and hormone-sustained conditions.
Figure 5. Bar graph depicting mean-level differences in the NAc shell of ΔFosB expression in both D1 and D2 transgenic mice between hormone-withdrawn and hormone-sustained conditions.
Figure 5. Confocal photomicrographs of 40-µm brain sections from the NAc core and shell in D1 receptor transgenic mice at 20X magnification. In the images displayed ΔFosB is labeled in green, D1 receptor neurons are labeled in red, and the D1 receptor neurons expressing ΔFosB are yellow. Double-labeled neurons (yellow) within the sampling boxes were counted and averaged between the right and left hemisphere of the NAc core and shell respectively and the results were compared between hormonal conditions.
Figure 6. Confocal photomicrographs of 40-µm brain sections from the NAc core and shell in D2 receptor transgenic mice at 20X magnification. In the images displayed ΔFosB is labeled in red, D2 receptor neurons are labeled in green, and the D2 receptor neurons expressing ΔFosB are yellow. Double-labeled neurons (yellow) within the sampling boxes were counted and averaged between the right and left hemisphere of the NAc core and shell respectively and the results were compared between hormonal conditions.