# $Comparison \ of \ pharma cological \ and \ non-pharma cological \ antidepressants \ in \ postpartum$

## depression: outcomes of mother and male and female offspring

by

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## Abstract

Postpartum depression (PPD) is a psychiatric illness that affects approximately 15% of mothers, negatively impacting mental health during the postpartum as well as increasing risk for future depression. For the developing child, untreated PPD is associated with several adverse outcomes including increased risk for depression, anxiety, and poor cognition particularly in boys. However, treating PPD is complicated because pharmacological antidepressants like fluoxetine (FLX) may function differently within the physiological conditions of the postpartum period. Additionally, these drugs are active in breast milk, directly reaching the infant and potentially influencing neurodevelopment. The long-term effects of neonatal antidepressant exposure are unclear. Alternatively, non-pharmacological antidepressants such as exercise are generally beneficial for maternal and fetal health; however, its potential as an antidepressant in the postpartum and its long-term effects on offspring are unclear. To investigate this, this thesis used a rat model of PPD in which dams are treated with high levels of corticosterone (CORT; primary rat glucocorticoid) and compared how different types of antidepressants affected dams and adult male and female offspring. In chapter 2, maternal postpartum FLX prevented CORT-induced disruptions in maternal care but was unable to prevent CORT-induced depressive-like behaviour or reductions in hippocampal neurogenesis. In chapter 3, maternal postpartum FLX increased anxiety-like behaviour, impaired hypothalamic-pituitary-adrenal (HPA) axis negative feedback, and increased hippocampal neurogenesis in adult male but not female offspring. In chapter 4, maternal exercise did not prevent CORT-induced disruptions in maternal care but it prevented CORT-induced depressive-like behaviour and increased hippocampal neurogenesis. While neither antidepressant alone increased maternal neurogenesis, the combination of both treatments

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increased neurogenesis. In chapter 5, maternal exercise increased hippocampal neurogenesis in dorsal hippocampus but maternal postpartum FLX reduced it. However, exposure to maternal postpartum FLX prevented the neurogenic effect of maternal exercise. Maternal exercise facilitated HPA axis negative feedback in males but impaired in females. Collectively, these data indicate that antidepressants can have dynamic effects on endophenotypes of PPD, emphasizing the need for further research in PPD. Furthermore, male and female offspring development is differentially sensitive to these maternal antidepressant interventions, highlighting the importance of studying sex differences in neurodevelopment.

# Lay Summary

Postpartum depression (PPD) affects 15% of mothers, and adversely affects emotional and cognitive development in children. The effectiveness of prescribed antidepressants may be compromised during the postpartum and these drugs remain active in breast milk, raising concerns about infant exposure to antidepressants. Alternatively, exercise is beneficial for maternal and fetal health, but its effectiveness in PPD is unclear. In this dissertation, a rat model of PPD was used and outcome measures such as behaviour and the production of new brain cells (neurogenesis) was examined. For mothers, fluoxetine (Prozac) treatment improved maternal care while exercise improved behaviour and neurogenesis. Adult male offspring were vulnerable to fluoxetine exposure, exhibiting changes in stress regulation and neurogenesis. Maternal exercise promoted neurogenesis in male and female offspring. Collectively, these data indicate that different interventions have dynamic effects on maternal outcome as well as adult offspring development, underscoring the importance of studying PPD treatment.

### Preface

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# List of Abbreviations

- ABC avidin-biotin complex
- ABN arched back nursing
- ACTH adrenocorticotropic releasing hormone
- ANOVA analysis of variance
- ANCOVA analysis of covariance
- BDNF brain derived neurotrophic factor
- CA1 cornu ammonis 1
- CA3 cornu ammonis 3
- CORT corticosterone
- CRH corticotropin-releasing hormone
- CYP-cytochrome P
- DAB-3,3'-diaminobenzidine
- DCX doublecortin
- DSM-5 Diagnostic and statistical manual of mental disorders, 5<sup>th</sup> edition
- EPM elevated plus maze
- FLX-fluoxetine
- FST forced swim test
- FST1 forced swim test, test session 1
- FST2 forced swim test, test session 2
- HPA hypothalamic-pituitary-adrenal
- I.P. intraperitoneal

- MDD major depressive disorder
- NSF novelty suppressed feeding
- OFT open field test
- $PBS-phosphate-buffered\ saline$
- PD postnatal day
- PPD postpartum depression
- S.C. subcutaneous
- SSRI selective serotonin reuptake inhibitors
- SEM standard error of the mean

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# Dedication

This thesis is dedicated to those advocating for women's rights. To all those actively fighting for women's rights in the political realm, the scientific realm, or in the community – your advocacy for equality is inspiring and vital for making the world a better place for all.

### **Chapter 1: Introduction**

### 1.1 Sex differences in major depression

Major depressive disorder (MDD) is serious neuropsychiatric disease that affects approximately 20% of the population and is the leading cause of disability and disease burden in the world (Greenberg et al., 2003; Ferrari et al., 2013). MDD is heterogenous, with the patient population displaying prolonged depressed mood and/or anhedonia as well as several possible permutations in weight gain or loss, altered sleep patterns, motor abnormalities such as lethargy or agitation, fatigue, feelings of guilt or worthlessness, inability to concentrate, and suicidality (American Psychiatric Association, 2013). MDD can be a highly recurrent disorder depending on genetic background, index episode, and severity of depression (Burcusa and Iacono, 2007). Further, MDD is highly co-morbid with other psychiatric illnesses such as anxiety disorders and substance abuse (American Psychiatric Association, 2013) and can present with medical illnesses such as cancer, stroke, and cardiovascular disease (Halaris, 2009; Pan et al., 2011; Kang et al., 2015).

Women are twice as likely to develop MDD in comparison to men (Gutiérrez-Lobos et al., 2002), and this phenomenon has been consistently documented across cultures (Seedat et al., 2009). Importantly, this sex difference emerges during adolescence (Nolen-Hoeksema and Girgus, 1994) and is most apparent during the reproductive years (i.e. 25-59 years; Gutierrez-Lobos et al., 2002), pointing to the possibility that changes in female-specific physiology may be influential in risk for MDD in women. In addition to prevalence, there are sex differences in presentation of MDD. Women with MDD are more likely to endorse symptoms such as depressed mood, sleep disturbances, somatic and gastrointestinal complaints, and changes in

weight than men (Angst et al., 2002; Schuch et al., 2014). Furthermore, women with depression are more likely to present with either co-morbid anxiety or have greater lifetime prevalence of anxiety than men (Young et al., 2009; Schuch et al., 2014). However, men with major depression are more likely to present with co-morbid alcohol or substance abuse and endorse symptoms related to anhedonia (Angst et al., 2002; Schuch et al., 2014). These sex difference in MDD are also present in the diagnosis of subtypes of MDD: MDD with melancholic features includes anhedonia, insomnia, weight loss, and motor agitation whereas MDD with atypical features includes increased appetite, hypersomnia, and lethargy (Gold and Chrousos, 2002). Women are more likely than men to present with either atypical and melancholic depression, although a greater proportion of men have melancholic depression whereas a greater proportion of women to have atypical depression (Hildebrandt et al., 2003). These subtypes of depression differ in their biological, inflammatory, and metabolic correlates (Lamers et al., 2013). Given these sex differences, it is imperative to study how MDD is different between males and females.

#### **1.1.1 Definition of postpartum depression**

According to the DSM-5, depression with a peripartum onset is a subtype of MDD that involves all the criteria of MDD with the distinguishing feature that it occurs during pregnancy or within the first four weeks of giving birth (American Psychiatric Association, 2013). However, it should be noted that this definition is controversial for several reasons. First, it still is debated whether depression arising during motherhood should be considered a distinct disorder from depression arising outside of motherhood. Support for distinguishing maternal depression from MDD stems from argument that motherhood is accompanied by numerous biological changes that trigger depression in a distinct population of women vulnerable to these particular changes. For example, exposure to a hormone-simulated pregnancy and withdrawal

from these hormones increased depressive symptoms in women who previously suffered from PPD (Bloch et al., 2005), suggesting that hormone fluctuations typical of pregnancy and postpartum alter mood particularly in vulnerable women. Thus, depression in mothers may be linked to specific changes in maternal physiology and different from the etiology of MDD. However, previous personal history of MDD are strong predictors of depression in pregnancy and/or in the postpartum period (O'Hara and McCabe, 2013; Kettunen et al., 2014). This lends support to the argument that maternal depression is an extension of MDD manifesting in mothers. Indeed, if the same women who experience depression during motherhood also have a history of depression outside of motherhood, this may point to a degree of convergence in the biological underpinnings of MDD regardless of onset timing at least for this population of women. Additional research disentangling the biological factors distinguish PPD as a recurrent MDD episode versus de novo depression arising in the postpartum is necessary to accurately diagnose and treat PPD.

Second, depression can arise before parturition ("antepartum depression") or after parturition ("postpartum depression"; PPD). One of the strongest predictors of PPD is antepartum depression, with approximately 80% of antepartum cases persisting through the postpartum (O'Hara and McCabe, 2013; Suzuki and Eto, 2017). Nonetheless, it is possible for antepartum depression to occur in isolation and not persist after parturition, and it is also possible for PPD to occur any time within the first year of childbirth in women without previous history of depression. For this reason, "with peripartum onset" is a contested portion of the definition because it does not distinguish antepartum onset from postpartum onset. This distinction is crucial because the endocrine changes that may underlie depression in mothers differ greatly between pregnancy and postpartum (further discussed below). Because pregnancy and

postpartum are characterized by different endocrine profiles which may be related to depression etiology, then this distinction may be crucial also in diagnosis to inform better understanding of disease mechanism and subsequently treatment.

Finally, the temporal restriction of the first four weeks of the postpartum period is controversial. Contrary to the fifth edition of the DSM, the World Health Organization and Centers for Disease Control and Prevention have extended this definition to be onset within the first year of giving birth (Centers for Disease Control and Prevention (CDC), 2008; Geneva: World Health Organization, 2010). This temporal discrepancy in addition to the other issues surrounding the definition of PPD likely contribute to highly variable prevalence rates for PPD. Clinical screening for PPD may only happen once during the postpartum period (Farr et al., 2014). Coupled with inadequate screening throughout the first postpartum year, especially in low-income communities, these issues likely contribute to a potential underestimation of PPD prevalence (Hansotte et al., 2017). Based on current evidence, it is estimated that approximately 10-15% of mothers suffer from PPD (Kornstein, 2002; Goodman, 2007; Wisner et al., 2013; Woody et al., 2017). As in the case with MDD, maternal depression can be recurrent and persist beyond postpartum period for months or even years (Goodman, 2007). Indeed, untreated PPD results in a four-fold increased risk for depression after the postpartum period, resulting in a lifelong burden of disease (Josefsson and Sydsjö, 2007). Women with a history of depression are more likely to experience peripartum depression and carry a lifelong risk for depression outside of motherhood; in women with their index episode of depression occurring in the peripartum period, there is an increased risk of future MDD with peripartum depression onset (Cooper and Murray, 1995). Collectively, these controversies highlight issues surrounding the definition and subsequent issues in prevalence for maternal depression.

#### 1.1.2 Heterogeneity in PPD

As in the case of MDD, PPD is complicated by its heterogeneity, particularly with regards to symptomology, timing, and risk factors. In this way, studying PPD is complicated by factors that distinguish it from depression outside of motherhood (discussed in the section above) as well as by factors which contribute to variability within cases of PPD. Screening for PPD commonly uses the Edinburgh Postnatal Depression Scale, a well-validated 10-question self-report that can be administered with relative ease (Gibson et al., 2009). Notably, this screening tool focuses on symptoms related to mood, feelings of guilt, anxiety, sleep disturbances, and self-harm ideation but does not explicitly ask about somatic symptoms, symptoms related to thoughts of harming the infant, or agitation (Norhayati et al., 2015).

The symptomology of PPD is classified as the same symptoms of MDD with a peripartum onset. However, some features of MDD may present differently in mothers. For example, mothers with PPD are more likely to experience anhedonia related to social interactions, exhibiting loss of interest in the infant as well as withdrawing from social relationships and increased relationship difficulties (Perani and Slattery, 2014). Indeed, anhedonia and anxiety are prominent symptoms in women with depression emerging in the postpartum (Putnam et al., 2017). Symptoms associated with sleep also differ in PPD. For example, in women with history of depression, insomnia during third trimester is a strong predictor of PPD (Suri et al., 2017). Alternatively, hypersomnia is associated with PPD as a recurring episode of depression (Fisher et al., 2016). Additionally, there are increases in comorbidity of anxiety and PPD in comparison to women with MDD outside of the postpartum period (Hendrick et al., 2000), and there is a growing body of evidence investigating postpartum anxiety as a distinct mood disorder from PPD (Pawluski et al., 2017). Furthermore, depression

onset in the postpartum is generally associated with an increased risk of obsessive compulsive symptoms in comparison to women with depression onset before or during pregnancy (Fisher et al., 2016).

As discussed above, the recent version of the DSM defines maternal depression onset to occur during the first four weeks of the postpartum period, which is problematic because severity of mood fluctuations can vary considerably over the course of the first year within the postpartum period. For example, the postpartum blues are a transient form of mood disturbances that commonly occurs within three to ten days after giving birth, resolves without treatment intervention, and is relatively mild in severity (O'Hara and McCabe, 2013; O'Hara and Wisner, 2014). However, PPD is more persistent and severe than the postpartum blues and can arise any time within the first year after child birth (O'Hara and McCabe, 2013). In fact, the greatest incidence for hospitalization for postpartum mental disorders in within the first three months of the postpartum period (Munk-Olsen et al., 2006). Interestingly, trajectories of depression scores can fluctuate over the course of the postpartum period depending on history of depression. In women with depression onset in the postpartum, depression scores on the Edinburgh Postnatal Depression Scale are highest six weeks after giving birth, suggesting that this form of PPD may manifest earlier. This observation is particularly noteworthy because approximately 40% of mothers with PPD are experiencing their first bout of depression (Wisner et al., 2013), and therefore screening extending to first six weeks may be beneficial. However, in women with a history of persistent MDD, depression scores are slightly elevated throughout pregnancy and early postpartum, and the highest scores are observed one year after giving birth, indicating that persistent MDD may trigger PPD later in the postpartum period (Fredriksen et al., 2017). Finally, other mood disorders such as anxiety and obsessive-compulsive disorder can emerge alongside

PPD, and these co-morbidities can differ over the course of the postpartum period. Women with PPD are more likely to score higher on scales of anxiety and obsessive-compulsive disorder two weeks after childbirth in comparison to postpartum women without depression, and the increased co-morbidity of PPD with obsessive-compulsive disorder persisted six months after childbirth whereas the co-morbidity of PPD and anxiety did not significantly persist (Miller et al., 2015). Collectively, timing of PPD and its presentation can vary greatly over the course of the postpartum period.

Certain risk factors for PPD are also informative on how trajectories differ for mood disruptions during the postpartum. As in the case of MDD, factors such as early life adversity and abuse, lack of social support, migration status, low socioeconomic status, and family history of psychiatric illness also increase risk for PPD (Gaillard et al., 2014; O'Hara and Wisner, 2014; Wosu et al., 2015; Kettunen and Hintikka, 2017). Intriguingly, these risk factors can dynamically influence timing of PPD. For example, while early life adversity can increase risk for PPD as a form of recurrent depression or as de novo depression, low socioeconomic status is specifically associated with PPD as an episode of recurrent depression (Fredriksen et al., 2017; Kettunen and Hintikka, 2017). A strong predictor of PPD is depression during pregnancy (O'Hara and McCabe, 2013) as well as history of depression and discontinuing medication during pregnancy (Cohen et al., 2006a). Furthermore, depression onset in pregnancy predicts more severe PPD (Postpartum Depression: Action Towards Causes and Treatment (PACT) Consortium, 2015). Medical concerns can also be a factor in risk and timing of PPD as early PPD (within 5 days of childbirth) is associated with pre-term birth and neonatal hospitalization whereas later PPD (after 6 weeks) is associated with obstetric complications (Warzecha et al., 2016).

### 1.2 Endocrine factors and PPD

Over the course of pregnancy and postpartum, there are numerous endocrine changes (Pawluski, Brummelte, Barha, Crozier, & Galea, 2009) which could contribute to the etiology of PPD (Hendrick et al., 1998; Bloch et al., 2003). During pregnancy, high concentrations of steroid and peptide hormones are secreted from the placenta, an endocrine gland generated during pregnancy (Hendrick et al., 1998). After parturition and into the postpartum period, ovarian hormone levels plummet with the expulsion of the placenta and remain low throughout most of the lactation period while glucocorticoid levels remain high with lactation (Brett and Baxendale, 2001). Some of these endocrine changes are illustrated in Figure 1-1. The following sections will discuss how these steroid hormones fluctuate during pregnancy and postpartum as well as how perturbations to these endocrine systems and lactation contribute to the etiology of PPD. However, there are other endocrine changes derived from the placenta that are not covered in this dissertation but are discussed elsewhere (e.g. Paskova et al., 2012).



**Figure 1-1 Diagram of steroid hormone changes over the course of pregnancy and postpartum.** Reprinted with permission from Pawluski et al., 2009

### 1.2.1 Stress hormones and MDD

Stress is defined as any real or perceived threat to homeostasis of either a physical or psychological nature (Cai-Lin and Zi-Zhen, 2002). Upon facing a stressor, the body rapidly releases epinephrine and norepinephrine peripherally from the adrenal medulla as well as in the central nervous system to promote the "fight-or-flight" response (see Figure 1-2). Norepinephrine has been implicated in MDD as serotonin-norepinephrine reuptake inhibitors can be used to treat MDD although its causative role in MDD is limited (Anand and Charney, 2000).

The hypothalamic-pituitary-adrenal (HPA) axis is also activated upon exposure to a stressor (Figure 1-2). When an organism is exposed to stress the parvocellular cells of the paraventricular nucleus of the hypothalamus release corticotropin-releasing hormone (CRH). CRH diffuses across the median eminence and binds to its receptors in the anterior pituitary gland, stimulating release of adrenocorticotropic hormone. Adrenocorticotropic hormone (ACTH) is secreted into the circulatory system and can bind to melanocortin receptor 2 on the

adrenal glands. Upon binding in the adrenal gland, adrenocorticotropic hormone stimulates synthesis and release of glucocorticoids from the zona fasciculata of the adrenal cortex. Glucocorticoids are steroid hormones that stimulate glucose production in the liver, mobilizing energy stores to cope with the present stressor. Glucocorticoids can transiently increase insulin resistance (to increase plasma glucose levels for energy use) and suppress inflammation to also promote stress coping. To terminate further release of glucocorticoids, these glucocorticoids can bind to glucocorticoid receptors in the hypothalamus and pituitary gland as well as in limbic regions such as the hippocampus, exerting negative feedback and suppressing terminating the HPA axis response. Glucocorticoids refer to a class of steroid hormones release from the adrenal cortex that stimulate liver glucose production; the primary glucocorticoid in humans is cortisol, and the primary glucocorticoid in rats is corticosterone (CORT) (Bourke et al., 2012; Handa and Weiser, 2014)



Figure 1-2 Diagram of HPA axis and epinephrine/norepinephrine release.

Stress is endorsed as a preceding factor in approximately 90% of cases of depression as reported by individuals suffering from MDD (Angst et al., 2002). Indeed, chronic exposure to stress and HPA axis stimulation are linked to development of depression (Tennant, 2002). Patients with MDD exhibit perturbations to the HPA axis, specifically elevated basal cortisol levels, disrupted diurnal cortisol secretion patterns, and HPA negative feedback dysregulation (Parker et al., 2003; Ising et al., 2007; Schüle, 2007; Stetler and Miller, 2011). The HPA negative feedback system can be tested via administration of dexamethasone, a potent synthetic glucocorticoid that suppresses cortisol secretion in healthy but not depressed individuals (Carroll et al., 1968; Heuser et al., 1994; Ising et al., 2007). Alleviation of depression as a result of chronic antidepressant treatment is coincident with or slightly precedes normalization of HPA negative feedback dysregulation via antidepressant treatment is more closely related to remission in women than men (Binder et al., 2009). Collectively, this points to aberrant HPA axis negative feedback as a potential biomarker of MDD.

Additional support linking HPA axis dysregulation with MDD stems from Cushing's disease and its association with increased depression. Cushing's disease is characterized by hypersecretion of cortisol and impaired HPA axis negative feedback in response to dexamethasone (Nieman, 2015). The etiology of a majority of Cushing's disease cases is tumors of the pituitary gland resulting in adrenocorticotropic hormone stimulation in excess or of the adrenal gland resulting in excess cortisol secretion (Morgan and Laufgraben, 2013; Starkman, 2013). This aberrant pituitary gland activity drives high levels of glucocorticoid production in the adrenal glands as well as reductions in corticotrophin-release hormone (Gold and Chrousos, 2002). Patients with Cushing's disease exhibit mood disturbances such as irritability and

depressed mood, with a majority also diagnosed with psychiatric illness including generalized anxiety (Starkman & Schteingart, 1981; Starkman, 2013). These patients also exhibit weight gain, sleep disturbances, fatigue, and impaired concentration, and these symptoms overlap with MDD (Starkman, 2013). Furthermore, Cushing's disease is more prevalent in women than in men, mirroring the sex-specific vulnerability of MDD in women (Starkman and Schteingart, 1981).

#### **1.2.2** Stress hormones, motherhood, and PPD

Basal levels of glucocorticoids gradually increase over the course of pregnancy, and HPA axis activity is attenuated (Brunton and Russell, 2008), largely due to the influence of the placenta. The placenta secretes corticotrophin releasing hormone, with the highest levels secreted towards the end of pregnancy and to stimulate parturition (Brunton and Russell, 2008). Placental secretion of corticotrophin releasing hormone suppresses the maternal HPA axis by reducing maternal corticotrophin releasing hormone levels (Perani and Slattery, 2014). After parturition and expulsion of the placenta, corticotrophin-releasing hormone plummet levels and remain low. During the postpartum and lactation, basal levels of glucocorticoids are sustained at high levels (Brunton & Russell, 2008; Neumann et al., 1998). The increased basal glucocorticoid levels are due to the increased metabolic demand of lactation as maternal glucocorticoid levels normalize with weaning (Voogt et al., 1969; Windle et al., 2013). Lactation decreases corticotrophinreleasing hormone levels in the paraventricular nucleus of the hypothalamus (Fischer et al., 1995) which contributes to reduced HPA axis activity during the postpartum period (Lonstein, 2007; Neumann et al., 1998; Neumann, 2003; Shanks et al., 1999). During the postpartum, the diurnal CORT rhythm is also disrupted as lactation maintains glucocorticoids at sustained, high levels (Lightman et al., 2001). These modifications to the endogenous glucocorticoid system are

crucial during the postpartum period for the induction and maintenance of maternal behaviour (Rees et al., 2004; Graham et al., 2006). This attenuation in postpartum HPA axis activity is also regulated by the neuropeptide oxytocin, which is released during parturition and contributes to mother-infant bonding (Numan and Young, 2016). Indeed, both the presence of pups and lactation are associated with increased brain levels of oxytocin (Figueira, Peabody, & Lonstein, 2008; Neumann, Koehler, Landgraf, & Summy-Long, 1994; Neumann & Landgraf, 1989; Neumann, Porter, Landgraf, & Pittman, 1994). Oxytocin is also important for milk production, and cessation of breastfeeding has been investigated as a potential mechanism contributing to PPD (Kim et al., 2014b; Moura et al., 2016). Indeed, breastfeeding is beneficial for maternal mood as it has been linked to reduced anxiety and reduced cortisol release in response to acute stressors in breastfeeding mothers (Heinrichs et al., 2001). However, it should be noted that it is unclear whether cessation from breastfeeding underlies development of PPD, or whether PPD results in reduced maternal care including cessation of breastfeeding (Dennis and McQueen, 2009; Stuebe et al., 2013; Kim et al., 2014b). Additional research is necessary to ascertain the relationship between PPD, breastfeeding, and oxytocin.

Pregnancy and postpartum are characterized by sustained high flattened levels of glucocorticoids in both humans and rodents (Magiakou et al., 1996; Lightman et al., 2001; Pawluski et al., 2009a), which is a similar hormone profile observed in depressed patients (Stetler and Miller, 2011). For this reason, there has been a considerable amount of research investigation regarding whether perturbations to the maternal glucocorticoid system underlie PPD. One proposed mechanism of PPD is that the abrupt loss of placental corticotrophinreleasing hormone signaling at the time of parturition precipitates depression in vulnerable women, with women suffering from PPD more likely to experience a greater magnitude of

corticotrophin-releasing hormone withdrawal (Meltzer-Brody et al., 2011). Indeed, corticotrophin-releasing hormone administration results in a blunted adrenocorticotropic hormone release in women with PPD in comparison to postpartum women without MDD (Magiakou et al., 1996). Dysregulation of the HPA axis and cortisol secretion have also been implicated in clinical studies of PPD (Jolley et al., 2007; Seth et al., 2016). Women who previously suffered from PPD reported more depressive symptoms and showed greater cortisol responses after exposure to a hormone-simulated pregnancy (Bloch et al., 2005), suggesting that in vulnerable women, the HPA axis and mood are altered in response to pregnancy hormones. In rodents, models of maternal depression commonly involve chronic stress exposure such as chronic restraint stress (Hillerer et al., 2011; Haim et al., 2014; Leuner et al., 2014), chronic intruder stress (Carini et al., 2013), or chronic CORT administration (Brummelte, Pawluski, & Galea, 2006; Brummelte & Galea, 2010; Workman, Brummelte, & Galea, 2013). These models not only induce depressive-like behaviour in tests such as the forced swim test, but they also reduce quality of maternal care and time spent with the pups (Brummelte et al., 2006; Brummelte & Galea, 2010; Carini et al., 2013; Workman et al., 2013), mirroring the phenotype of PPD in women.

#### **1.2.3** Fluctuations in sex hormones and MDD

In addition to stress hormone hypothesis, another prominent neuroendocrine hypothesis emphasizes the role of gonadal hormones in MDD (Hammarström et al., 2009). Within the female population, risk for MDD can fluctuate over the lifespan and is coincident with large fluctuations in hormones. As mentioned previously, the sex difference in depression incidence emerges during puberty (Nolen-Hoeksema and Girgus, 1994) and starts to decline around the average age of menopause (Gutiérrez-Lobos et al., 2002). Later in life, the perimenopausal

period can last 10 years prior to menopause and is associated with increased vulnerability to depression, regardless of women with or without a previous history of depression (Freeman et al., 2004; Cohen et al., 2006b). In female rats, loss of circulating ovarian hormones via ovariectomy or withdrawal from ovarian hormone-simulated pregnancy has been causally linked to increased depressive-like behaviour (Galea et al., 2001; Green and Galea, 2008; Green et al., 2009; Mahmoud et al., 2016). Interestingly, withdrawal from ovarian hormone-simulated pregnancy increased depressive symptoms in women with a history of PPD, indicating that fluctuations in ovarian hormones may precipitate mood disturbances in vulnerable women (Bloch et al., 2000). Furthermore, in women, pharmacologically reducing circulating levels of sex hormones via administration of gonadotropin-hormone agonist increased depressive symptoms in women as well as attenuated reward-processing activity in the amygdala and functional connectivity between the hippocampus and cingulate cortex (Fisher et al., 2017; Macoveanu et al., 2016). Interestingly, of women treated with the gonadotropin-hormone agonist, those with the highest induced depressive symptoms also exhibited hyperactivity in the anterior insula in response to emotional stimuli in comparison to the women with lower depressive scores (Henningsson et al., 2015). This suggests that in women, loss of sex hormone signaling can interact with emotional sensitivity to affect brain activity. Collectively, these data indicate that fluctuations in ovarian hormones may contribute causally to depression in females.

Although the glucocorticoid hypothesis and gonadal hormone hypothesis have been discussed thus far as separate hypotheses, it should be noted that the HPA axis interacts with the hypothalamic-pituitary-gonadal (HPG) axis, which regulates gonadal hormone secretion. The interaction between the HPA and HPG axis also is also relevant for MDD etiology. Generally, in adult subjects, testosterone inhibits HPA axis activity whereas estradiol potentiates HPA axis

activity (Goel et al., 2014a). The HPA axis can also exert inhibitory control over the HPG axis with chronic stress suppressing sexual behaviour in both males and females (Toufexis et al., 2014). Development of the HPA axis is tied to the maturation of the HPG axis (Romeo, 2010); the increased risk for MDD as well as the emerging sex difference in MDD during puberty may be related to the maturation of the HPA-HPG interactions (Angold and Costello, 2006).

### **1.2.4** Fluctuations in sex hormones and PPD

During pregnancy, estradiol and progesterone rise to high levels and then remain low after parturition (Shaikh, 1971; Rosenblatt et al., 1988; Pawluski et al., 2009a). During reproduction, these large fluctuations in steroid and peptide hormones may influence risk for depression (Bloch et al., 2003; Hendrick et al., 1998). In addition to changes in HPA axis hormones across pregnancy and the postpartum, estradiol levels are elevated throughout the third trimester but drop dramatically after parturition, leading to the hypothesis that an "estradiolwithdrawal state" during the first few weeks after parturition contributes to PPD ((Hendrick et al., 1998; Bloch et al., 2003; Macoveanu et al., 2016; Fisher et al., 2017). Indeed, this has been modelled in preclinical research as withdrawal from a hormone-simulated pregnancy induced depressive-like symptomology in female rats (increased immobility in the forced swim test and sucrose anhedonia; (Galea et al., 2001; Green et al., 2009) and reduced neurogenesis in the hippocampus (Green and Galea, 2008). Clinical studies in women have also confirmed that transient suppression of circulating gonadal hormones via pharmacological manipulations is also associated with neural phenotypes of depression such as attenuated reward processing (Macoveanu et al., 2016), altered serotonin transporter binding (Frokjaer et al., 2015), and altered functional connectivity between regions such as the amygdala, cingulate cortex, and hippocampus (Fisher et al., 2017).
#### **1.3 PPD and Antidepressant Efficacy**

One of the first theories regarding the etiology of MDD was the monoamine theory, positing that reduced monoamine transmission (i.e. serotonin, norepinephrine, and depression) contributes to the neuropathology of MDD. This theory is supported by a majority of antidepressant drugs targeting the monoamine system, including SSRIs. Although the mechanism of how SSRIs exert their therapeutic effect is still under investigation, the most rapid effect of SSRIs is increased extracellular serotonin in brain areas including the hippocampus (Popa et al., 2010). Interestingly, non-pharmacological antidepressants also increase serotonin levels as exercise can increase levels of serotonin in the hippocampus under basal and stress conditions (Wang et al., 2013; Wen et al., 2014), further supporting the role of serotonin in antidepressant effects. Finally, it should be noted that serotonin has several widespread effects including regulation of hippocampal neurogenesis (Alenina and Klempin, 2015) and interacting with low levels of ovarian hormones to influence depressive symptoms (Frokjaer et al., 2015).

The monoamine theory of depression may also be relevant for PPD as serotonin levels are low in postpartum dams in comparison to nulliparous female rats (Desan et al., 1988), which may contribute for a heightened risk for depression. However, the role of serotonin in the maternal brain is complex. While serotonin levels are lower in the hippocampus (Desan et al., 1988), serotonin turnover is higher in the medial preoptic area of the hypothalamus in postpartum rats in comparison to nulliparous rats (Lonstein, Dominguez, Putnam, De Vries, & Hull, 2003) and is implicated in maternal behaviour such as maternal aggression (da Veiga et al., 2011; Heiming et al., 2013). Furthermore, variants of genes associated with serotonin reuptake (*5-HTTLPR*) and serotonin synthesis (*TPH*) have been associated with risk for PPD although

other factors such as concurrent stressful life events may be an important moderating factor (Couto et al., 2015). Thus, serotonin may contribute to risk for PPD although further research is necessary to ascertain the extent of its mechanistic contribution to etiology of PPD.

## 1.3.1 Efficacy of SSRIs for treating PPD

Mothers suffering from depression can be prescribed the same antidepressants that are used to treat major depression in men and women. Treating maternal depression has been complicated by a poor understanding of depression at this time as well as the unique physiology of women during this time. Indeed, treating maternal depression is challenging because systematic reviews indicate that antidepressant efficacy is limited in pregnancy and postpartum (De Crescenzo et al., 2014). However, it should be noted that there are few clinical trials specifically investigating antidepressant efficacy specifically in the context of postpartum physiology. Of these few trials, there is some evidence that SSRIs may be beneficial early in the treatment regimen with SSRI treatment reducing depressive scores in comparison to counselling at 4 weeks into the intervention period but not 18 weeks into the intervention (Sharp et al., 2010). Other studies have found that SSRIs in comparison to placebo had no significant benefit on depressive scores in mothers also undergoing psychotherapy (Appleby et al., 1997; Bloch et al., 2012) and another trial found that SSRI offered no significant benefit over placebo on depressive scores although there was a high attrition rate (Yonkers et al., 2008). Studies demonstrating SSRI efficacy in the postpartum are difficult to interpret as there are no placebo control groups (Misri, Reebye, Corral, & Milis, 2004; Wisner et al., 2006) or differences in initial depression scores (Hantsoo et al., 2014). Overall, it is particularly problematic that there are few clinical trials investigating antidepressant efficacy in the postpartum, and this inconclusive set of clinical trials underscores the importance of studying how antidepressants function in mothers.

Given the alterations in the hormonal and neural profiles over the course of pregnancy and postpartum, it is plausible that the capacity for these antidepressants (either pharmacological or non-pharmacological) to function is altered. Altered antidepressant efficacy may be in part due to the endocrine alterations in maternal physiology affecting pharmacokinetics such as drug metabolism. For example, use of oral contraceptives and exogenously increasing ovarian hormones levels can reduce activity of CYP1A2, an important enzyme for clearing drugs from the system (Hilli et al., 2008). In addition to CYP1A2, there are numerous cytochrome enzymes such as CYP3A4, CYP2D6, and CYP3c19 that are also heavily impacted by oral contraceptive use and influence drug action (Kokras et al., 2011). For this reason, women prescribed antidepressants like FLX require a lower dose while taking oral contraceptives as higher doses result in drug accumulation in the body (Damoiseaux et al., 2014). In addition to changes in these enzymes, total blood volume, metabolic, and body weight change markedly over the course of pregnancy and postpartum which can influence pharmacokinetics of antidepressants. Presently, it is unclear how the physiological factors interact with antidepressant efficacy during pregnancy and postpartum and is actively being researched (Avram et al., 2016). From rodents, there is evidence that FLX is metabolized faster in female mice than in male mice (Hodes et al., 2010). Also, gonadal hormones can facilitate some of the effects of FLX to offset stress-induced depressive phenotypes in either male or female rats (Mahmoud et al., 2016; Wainwright et al., 2016b) which may contribute to the potentially altered efficacy in mothers at this time. Collectively, these differences highlight the importance of evaluating antidepressant efficacy specifically during motherhood as it may different that efficacy outside of motherhood.

## **1.3.2** Efficacy of exercise for treating PPD

Exercise, particularly aerobic exercise, is considered an antidepressant for major depression in humans (Schuch et al., 2016). However, while the antidepressant effect has been robustly observed in humans, the underlying mechanism of this antidepressant effect has been difficult to ascertain in the MDD population. This is due in part to heterogeneity of MDD pathology itself as well as to the highly variable amounts of exercise with low attendance rates unable to provide antidepressant relief and subsequently changes in MDD biomarkers (Schuch et al., 2016). This issue of low compliance represents an issue in studying exercise in treating MDD: because the disease itself is characterized by loss of motivation, lethargy, and fatigue, the disease itself can be a barrier in patients complying with an energy-demanding intervention, especially in those suffering from severe depression (Salmon, 2001; Flegal et al., 2007; Roberts and Bailey, 2011). Thus, there has been interest in using exercise as an adjunct antidepressant intervention to augment pharmacotherapy (Trivedi et al., 2011; Legrand and Neff, 2016).

Given the substantial changes in endocrine and neural profiles that naturally occur over the course of pregnancy and postpartum regardless of depression status, it is plausible that the capacity for exercise to benefit maternal mood and the brain is compromised. Few clinical studies have examined the efficacy of exercise specifically in the treatment of maternal depression or PPD. A meta-analysis by Daley, Jolly, & MacArthur, 2009 analyzed 5 small trials of women at risk with PPD (but not clinically diagnosed PPD) and found that exercise reduced depression scores specifically when a study using low intensity exercise and social support was used in the analysis (Armstrong and Edwards, 2003). Exclusion of that study from the metaanalysis resulted in exercise no longer significantly positively impacting depression scores in these women (Daley et al., 2009). Recently, Daley et al., 2015 found that in women diagnosed

with PPD, a six-month exercise intervention of moderate-intensity thrice per week (including consultation phone calls) significantly reduced depression scores at six months postpartum by not at twelve months postpartum. Others have also investigated the preventative effects of exercise on peripartum mood disruptions and found that there is curvilinear relationship such that the greatest proportion of mothers recovering from depression exercised 1-3 per week and fewer women recovered from depression when exercising either more or less often than that (Sexton et al., 2012). Thus, the efficacy of exercise in PPD may be altered during the peripartum period but perhaps use of a multimodal antidepressant intervention may be particularly useful in mothers.

In rodents, the behavioural antidepressant effects of exercise have been observed in models of MDD such as the injections of CORT (Yau et al., 2014), social isolation (Hong et al., 2015), genetic manipulations such as the Flinders Sensitive Line (Bjørnebekk et al., 2010), and chronic unpredictable stress (Lapmanee et al., 2013). Exercise may be exerting its antidepressant effect by enhancing hippocampal neurogenesis (Yau et al., 2011; Yau et al., 2014), increasing trophic factors (Kiuchi et al., 2012), and increasing serotonin transmission (Klempin et al., 2013). Interestingly, there is evidence that voluntary running wheel activity can fluctuate over the course of the estrous cycle with the highest levels occurring during proestrus when ovarian hormones are high (Anantharaman-Barr and Decombaz, 1989). In contrast, when ovarian hormones are low such as after ovariectomy or after parturition, voluntary running wheel activity is low in rodents (Bick-Sander et al., 2006; Park et al., 2016). For this reason, it is importantly to investigate the antidepressant effects of voluntary exercise within the context of ovarian hormone levels and fluctuations and is studied in Chapter 4.

## 1.4 Effects of PPD and antidepressants on child outcome

Several forms of postnatal early life stress, such as sexual, physical, or emotional abuse as well as parental loss, neglect, or mental illness can have detrimental effects on child emotional outcome such as increased risk for adult mood and anxiety disorders (Famularo, Kinscherff, & Fenton, 1992; Heim & Nemeroff, 2001; Pelcovitz et al., 1994). In fact, research suggests that depression is harder to remit if the individual has a history of early childhood adversity such as physical abuse (Fuller-Thomson et al., 2014). Interestingly, the connection between persistent hyperreactivity of the HPA axis and MDD has been more reliably found in individuals with history of early life adversity regardless of current psychopathology (Heim, Mletzko, Purselle, Musselman, & Nemeroff, 2008). This suggests that early life adversity may shape HPA axis activity and underlie a predisposition for depression (Heim et al., 2008; Pariante and Lightman, 2008).

#### **1.4.1** Effects of untreated PPD on child outcome

In rodents, disruptions to the early postnatal environment have also been linked to depressive-like behavior in animal models of depression. One of the most common postnatal paradigms used is maternal separation in which pups are removed from the dam for three hours per day during the postnatal period (Schmidt et al., 2011). Maternal separation during the first two postnatal weeks or the entire postnatal period (from birth to weaning) increased immobility in the forced swim test in adult male and female rats (Lee et al., 2007; Aisa et al., 2008; Lajud et al., 2012). Maternal separation lasting the entire postnatal period elevated basal CORT in adult females (Aisa et al., 2008). Interestingly, maternal separation during the first two postnatal weeks resulted in exaggerated ACTH secretion after restraint (Liu, Caldji, Sharma, Plotsky, &

Meaney, 2000) but not after exogenous CRH in male rats (Wigger and Neumann, 1999), suggesting that maternal separation may have a more potent effect on sensitivity of the pituitary gland to different stressors in male rats. Another model of early life adversity, such as the CORT-induced model of PPD, also induces long-term effects on offspring outcome and will be discussed below (section 5.2). Together, these data indicate that reduced maternal care represents a form of early life adversity that impacts the long-term risk for depressive-like behaviour and disturbances to HPA axis.

It should be noted that both antepartum and maternal depression, referring to depression occurring during pregnancy or within the first year of childbirth, can have dynamic effects on child outcome depending on depression timing. Depression during pregnancy in the mother increased emotional responses in infant boys, but not girls (Gerardin et al., 2011) and increased risk for depression in adolescence (Pawlby et al., 2009; Pearson et al., 2013). Maternal depression, referring to depression spanning pregnancy and postpartum, can critically affect cognitive and emotional development of children (Goodman & Gotlib, 1999). Thus, maternal depression at various times can profoundly influence child development and represents a risk for behavioral and cognitive disturbances. Notably, this is another reason highlighting the importance of distinguishing depression with antepartum or postpartum onset as this may be influential in how child outcome is disrupted. With regards to potential mechanisms, antepartum depression can influence child outcome via fetal programming effects whereas depression arises after parturition can indirectly program child outcome via disruptions in maternal care; depression spanning both pregnancy and postpartum may be influential via a mix of both programming and early life environment effects. Furthermore, whether antidepressant intervention began during pregnancy or postpartum could also influence child outcome.

#### **1.4.2** Effects of treating PPD with SSRIs on child outcome

Treating mothers with SSRIs is complicated because antidepressant efficacy may be compromised in the postpartum (discussed in section 2.6). If antidepressants are not providing relief for the mother, this can be problematic for infant outcome as the PPD remains unresolved and can adversely affect child development (discussed in section 3.1). Furthermore, antidepressants, such as FLX, and its metabolites, such as norFLX, can remain active in breast milk and directly reach the infant (Wisner, Perel, & Findling, 1996). Neonatal exposure to SSRIs is associated with adverse outcomes in the infant such as reduced weight gain (Chambers et al., 1999), reelin levels (Brummelte et al., 2013), psychomotor scores during the first year (Santucci et al., 2014a) as well as increased colic (Lester, Cucca, Andreozzi, Flanagan, & Oh, 1993), hypertension (Chambers et al., 2006), and risk for autism (Boukhris et al., 2016). However, maternal SSRI use is also associated with enhanced infant readiness to interact with their mother (3 mo infants - Weikum, Mayes, Grunau, Brain, & Oberlander, 2013), accelerated perceptual development (6 mo and 10 mo infants - (Weikum, Oberlander, Hensch, & Werker, 2012a), and improved executive function (6 yo children Weikum, Brain, et al., 2013). Although the later studies (Weikum et al., 2012a; Weikum, Brain, et al., 2013; Weikum, Mayes, et al., 2013) suggest a seemingly beneficial result of maternal SSRI use, it is unclear whether these effects of maternal SSRI use are advantageous in the long term or precede negative behavioral outcomes that emerge later in life, i.e. beyond the time the infant is dependent on the mother (and consequently exposed to SSRI). To date, it remains unclear from the clinical literature whether the effects of neonatal antidepressant exposure outweighs the adverse effects of PPD on child outcome, and this uncertainty is one of the primary reasons why mothers are reluctant to seek and adhere to pharmacological antidepressants. Unfortunately, clinical evidence is limited to

infancy and childhood, and this impedes both the mother and clinician in making the best decision.

To this end, rodent studies can assess how certain windows of SSRI exposure affect adult male and female offspring outcome. For example, in examining anxiety-like behaviour, perinatal FLX exposure has highly variable effects depending on timing of exposure, dose of FLX, and sex of the offspring. FLX treatment (25 mg/kg) from prenatal day 15 through postpartum day 12 had no effect on anxiety-like behaviour in comparison to control treatment in adult female mice ( Kiryanova, Meunier, & Dyck, 2017) but reduced anxiety-like behaviour in adult male mice (Kiryanova et al., 2016). However, prenatal stress followed by maternal postpartum FLX treatment (5 mg/kg) had no significant effect on anxiety-like behaviour relative to controls in either adult male offspring (Boulle et al., 2016b) or female offspring (Boulle et al., 2016a). Thus, depending on the model of maternal adversity, timing of FLX exposure, and sex of the adult offspring, different anxiety phenotypes can be observed. In addition to altered anxiety phenotypes, maternal FLX exposure has been associated with altered circadian rhythm (Kiryanova et al., 2017b), increased aggression in male mice (Kiryanova et al., 2016; Svirsky, Levy, & Avitsur, 2016), and increased depressive-like behaviour in adult female rats (Boulle et al., 2016a). Thus, different forms of affective behaviour can be sensitive to the effects of maternal FLX exposure depending on sex.

Neonatal exposure to FLX is particularly important to investigate because serotonin is an important trophic factor during development (Mazer et al., 1997; Yan et al., 1997). During the postpartum period, refinement of serotonin-related circuitry as well as peak synaptogenesis in the brain occurs in the offspring, and these developmental processes are sensitive to exogenous serotonin and experiential factors such as maternal care (Kepser and Homberg, 2015). As

previously mentioned, maternal SSRI use can increase milk concentrations of FLX and norFLX (Wisner et al., 1996). However, given the ability of all of these antidepressants to alter mood as well as endogenous serotonin levels in the mother, it is plausible that maternal exposure to either of these agents can potentially affect either indirectly (via maternal care) or potentially directly (via milk content) and subsequently offspring outcome.

## **1.4.3** Effects of treating PPD with exercise on child outcome

Exercise is encouraged for mothers because metabolic diseases in mothers are associated with adverse effects in offspring. Obesity in mothers is associated with increased risk of the child developing obesity themselves (Lau et al., 2014; Mourtakos et al., 2015) and risk for cardiovascular disease in adulthood (Reynolds et al., 2013). Gestational diabetes in mothers is also associated with metabolic dysfunction in children, increasing risk for daughters to also develop gestational diabetes and perpetuating the risk for metabolic disease across generations (Fraser and Lawlor, 2014). While there is caution warranted against strenuous exercise, exercise is considered to be beneficial for fetal health (Hinman et al., 2015).

It is unclear how maternal exercise factors into the conflict between untreated PPD and treated PPD. The effects of exercise itself do not appear to be harmful on fetal growth (Tomić et al., 2013) and may be beneficial for the aforementioned risks of metabolic disease. However, it is not clear whether this intervention is sufficient in alleviating the altered maternal caregiving behaviour typical of PPD nor is it clear whether maternal exercise offsets the effects of PPD on child outcome. To some extent, maternal exercise has been associated with reduced anxiety-like behaviour in rat pups (Aksu et al., 2012; Haydari et al., 2014) although these findings did not persist when a model of prenatal stress was also present (Lee et al., 2016). For this reason, it is

imperative to evaluate how exercise functions in a model of PPD and examine offspring outcome and this is empirically studied in Chapter 4.

## 1.5 Neurobiology of MDD and PPD: focus on the hippocampus

The hippocampus is a subcortical, bilateral structure located in the medial temporal lobe at the inferior horn of the lateral ventricles. The hippocampus is composed of the following subfields which interlock in a C-shape with laminar organization: the cornu ammonis (CA) 1, CA2, CA3, and CA4, and the dentate gyrus. The entorhinal cortex and subiculum can be considered to be part of the hippocampal formation. Intra-hippocampal activity is connected via the tri-synaptic circuit (Figure 1-3). Afferent inputs to the hippocampus enter via the entorhinal cortex (layer 2) synapse with the granule cells in the dentate gyrus, forming the perforant path. Axons from granule cells in the dentate gyrus synapse with the CA3 pyramidal neurons, forming mossy fibers. Axons from CA3 pyramidal neurons synapse with CA1 pyramidal neurons, forming the Schaffer collaterals. Axonal fibers from the CA1 subfield form the efferent path of



Figure 1-3 Diagram of tri-synaptic hippocampal circuit. MF: Mossy fibers; PP: Perforant path; SC: Schaffer collaterals

the hippocampus via the subiculum, the primary outflow of the hippocampal circuitry (Amaral et al., 2007; Neves et al., 2008).

The dentate gyrus is composed of three layers: the molecular layer, the granule cell layer, and the polymorphic layer (or hilus). The molecular layer is relatively free of cell bodies and contains the dendrites of granule cells. During development, stem cells located in the hilus give rise to granule cells, which are densely packed in the granule cell layer (Amaral et al., 2007). The granule cell layer forms approximately at gestation day 20. Production of granule cells begins approximately at gestation day 20 and reaches peak levels at postpartum day 6 (Altman and Bayer, 1990). By the end of the postpartum period (between postpartum day 20 and 30), neurogenesis is mostly confined to the subgranular zone (Altman and Bayer, 1990). A substantial amount of neurogenesis continues after the postnatal period and continues in the adult dentate gyrus. In adulthood, neural stem cells or progenitor cells divide in the subgranular zone and differentiate into either neurons or glia. The newly differentiated neurons migrate from the subgranular zone into the granule cell layer. As the neuron matures, dendritic processes extend into the molecular layer and dendritic branching becomes more complex and make synaptic contact to the CA3 subfield of the hippocampus (Amaral et al., 2007).

Doublecortin (DCX) is an endogenous, microtubule-associated protein important for neuronal migration. Disrupted DCX expression results in disorganized neuronal migration and cortical laminar heterotopia (des Portes et al., 1998; Gleeson et al., 1998). DCX is also a marker of neuronal differentiation as it is only expressed in neurons (Brown et al., 2003). DCX is expressed for up to twenty-one days in immature neurons of rats, coinciding with proliferation, differentiation, and migration of neurons in the dentate gyrus in adult rats. Maturity of DCXexpressing neurons can be distinguished by characterizing the morphology of these cells (Figure 1-4). Proliferative DCX-expressing (type 1) neurons have short, plump processes. Intermediate DCX-expressing (type 2) neurons have few, thin dendritic branching in the molecular layer.

Post-mitotic DCX-expressing (type 3) neurons have complex dendritic branching (Plümpe et al., 2006). DCX is considered a marker of immature neurons as its expression wanes as granule cells mature and begin to express NeuN; furthermore, DCX is not co-expressed with markers of mature neurons such as calbindin (Brown et al., 2003; von Bohlen und Halbach, 2007). Because of its long-lasting expression, quantifying DCX expression is a useful measure of how chronic treatments (such as chronic CORT, FLX, or exercise) influence neurogenesis. DCX-expressing neurons also express glucocorticoid receptors (Fitzsimons et al., 2008), so these cells will be vulnerable to the effects of high CORT levels. Another advantage in examining DCX-expression is that it is an endogenously expressed protein and quantifying its expression does not require additional injections or questions on timing of injections for exogenous methods such as the use of bromodeoxyuridine or other thymidine analogs (Brown et al., 2003; Plümpe et al., 2006; Taupin, 2007).



Figure 1-4 Diagram of DCX morphology. Reprinted with permission from Workman et al., 2015

Interestingly, the hippocampus is a heterogenous structure with the dorsal and ventral subdivisions expressing differences in function and neurogenic properties. The topographic gradient of dorsal-ventral axis is present at the time of birth. With regards to function, dorsal hippocampus has been implicated in cognition and spatial memory. Dorsal hippocampus projects to cingulate cortices related to spatial navigation, and dorsal hippocampus has the greatest density and sensitivity of place cells (Jung et al., 1994; Cenquizca and Swanson, 2007; Fanselow and Dong, 2010a). Ventral hippocampus projects to olfactory bulb, amygdala, and prefrontal cortex (Cenquizca and Swanson, 2007; Fanselow and Dong, 2010a). Ventral hippocampus also has place cells which encode a larger place field (Jung et al., 1994). Density of glucocorticoid receptors is higher in the dorsal hippocampus than in ventral hippocampus (Robertson et al., 2005). Ventral hippocampus is functionally related to regulation of affective behaviour and stress. Although dorsal hippocampus is predominantly associated with spatial navigation and cognition and ventral hippocampus is predominantly associated with affective behaviour, it should be noted that these functions are not exclusive. Dorsal hippocampal activity can affect anxiety-like behaviour (Bannerman et al., 2002; Rezayat et al., 2005), and ventral hippocampal activity can be involved in spatial memory (Loureiro et al., 2012; Workman et al., 2015a).

It should be noted that many brain areas connected to the dorsal and ventral hippocampus are also implicated in the neurobiology of MDD, including the amygdala, prefrontal cortex, and cingulate cortex (Nestler et al., 2002). Moreover, neural processes besides hippocampal neurogenesis, such as production of trophic factors such as brain derived neurotrophic factor (BDNF) and neuroinflammation, are also implicated in MDD (Roy et al., 2014). This thesis will primarily focus on the contributing role of hippocampal neurogenesis with further justification

provided in the sections below; the potential contributing role of other brain regions and processes are discussed in section 6.6.

The hippocampus is implicated in the neurobiology of depression (Vakili et al., 2000; Nestler et al., 2002; McKinnon et al., 2009). A meta-analysis determined that depressed patients have a smaller hippocampal volume after 2 years with depression (McKinnon et al., 2009). Antidepressant use protects against hippocampal volume loss primarily in women (Vakili et al., 2000). Furthermore, prolonged high cortisol levels reduce hippocampal volume as evident in Cushing's patients: they exhibit hypercortisolemia and smaller hippocampal volumes, which is reversed (10% hippocampal volume increase) when cortisol levels are normalized (Starkman et al., 1999). Thus, the reduced hippocampal volume seen in depressed patients may be the result of prolonged hypersecretion of CORT.

#### 1.5.1 Adult hippocampal neurogenesis and MDD

Animal models of depression reliably reduce hippocampal neurogenesis including chronic unpredictable stress (Hawley et al., 2012; Wainwright et al., 2016a), chronic CORT administration (Brummelte and Galea, 2010a), ovarian hormone withdrawal (Green and Galea, 2008; Zhang et al., 2016), social defeat stress (Lehmann et al., 2013), prenatal stress (Morley-Fletcher et al., 2011), and early maternal separation (Mirescu et al., 2004). Reducing adult hippocampal neurogenesis via transgenic manipulation in male mice resulted in HPA axis negative feedback dysregulation, increased immobility in the forced swim test under no stress conditions, and increased sucrose anhedonia (Snyder et al., 2011a). Conversely, increasing adult hippocampal neurogenesis via transgenic manipulation in male mice protected against CORTinduced anxiety-like behaviour (elevated plus maze), depressive-like behaviour (tail suspension test), but did not affect HPA axis activity (Hill et al., 2015). Reduction of hippocampal neurogenesis via irradiation (Surget et al., 2011) or pharmacological administration of MAM itself did not induce depressive-like behaviour but rather exacerbated the effects of chronic unpredictable stress in male rats (Bessa et al., 2009). In humans, depression reduced progenitor cells (Boldrini et al., 2012). Thus, reduced hippocampal neurogenesis may precipitate depressive-like behaviour or exacerbate depressive-like behaviour under certain conditions.

Many studies show that antidepressant administration can increase hippocampal neurogenesis in adult male rodents. This includes SSRIs such as FLX as well as nonpharmacological antidepressants such as exercise and electroconvulsive treatment (van Praag et al., 1999; Tanti and Belzung, 2013). This neurogenic effect of pharmacological antidepressants has also been observed in human post-mortem tissue (Boldrini et al., 2012), and was found to a greater extent in women (Epp et al., 2013). Other studies suggest that the antidepressant effect of SSRIs such as FLX are dependent on the presence on adult hippocampal neurogenesis. For example, abolishment of hippocampal neurogenesis via irradiation rendered FLX ineffectual in rescuing the sucrose anhedonia and HPA axis negative feedback dysregulation induced by chronic unpredictable stress (Surget et al., 2011). Reducing hippocampal neurogenesis prevented FLX effect on sucrose anhedonia and latency to feed (Santarelli et al., 2003). Importantly, Bessa et al., 2009 also revealed that FLX reduced latency to feed in NSF only in animals with intact hippocampal neurogenesis, suggesting that this is a neurogenic-dependent test of antidepressant efficacy. NSF is also a test of anxiety-like behaviour (Bodnoff et al., 1989), and others have indicated an important role of neurogenesis in anxiety (Petrik et al., 2012; Hill et al., 2015). Collectively, these data highlight the importance of hippocampal neurogenesis as a marker of depression as well as a potential mechanism underlying antidepressant efficacy.

## 1.5.2 Motherhood affects hippocampal structure

Given that prolonged periods of stress hormone exposure and fluctuations in sex hormones impact the brain, it may not be surprising that there are numerous changes in the brain that occur during pregnancy and postpartum. In fact, during pregnancy, total brain volume shrinks by 8% and reverts to its original volume by 6 months postpartum (Oatridge et al., 2002). Hippocampal volume is reduced during pregnancy and postpartum, and with a partial recovery to pre-pregnancy volume 2 years after giving birth (Hoekzema et al., 2017). Furthermore, these pregnancy-induced alterations are present at the cellular level of the hippocampus as pregnancy results in suppressed apoptosis without affecting cell proliferation (Pawluski, Barakauskas, & Galea, 2010). However, after parturition, hippocampal cell proliferation and survival of new neurons are suppressed (Leuner, Mirescu, Noiman, & Gould, 2007; Pawluski & Galea, 2007). This postpartum reduction in cell proliferation is due to elevated circulating CORT because surgical removal of adrenal glands prevented this reduction in cell proliferation (Leuner et al., 2007). Survival of new neurons is also reduced during lactation in primiparous but not biparous dams, although this effect appears to be driven by pregnancy alone as primiparous rats who had their pups removed 24 h after giving birth still showed the reduction in new neuron survival at the 22 days postpartum (Pawluski & Galea, 2007). In addition to altered neurogenesis, there are transient increases in dendritic spine density in the CA1 region of the hippocampus during the early postpartum period (Kinsley et al., 2006). However, by the end of the postpartum period, primiparous rats (first-time mothers) exhibit reductions in CA1 and CA3 dendritic spine density. This reduction is not observed in multiparous (multiple mothering experiences), suggesting that number of reproductive experiences can also impact structural plasticity of the hippocampus (Pawluski and Galea, 2006). Together, these alterations in hippocampal neurogenesis and

plasticity may be influential in risk for depression as well as impact how efficacious antidepressants are in mothers.

There are relatively few studies examining the neurobiology of depression in mothers. From clinical studies, increased monoamine oxidase-A binding in the prefrontal cortex and anterior cingulate cortex was observed in mothers with PPD in comparison to non-depressed mothers within 18 months of childbirth (Sacher et al., 2010). Additionally, mothers with PPD exhibit increased glutamate levels in the medial prefrontal cortex (McEwen et al., 2012), in line with findings that glutamate is dysregulated in MDD (Gerhard et al., 2016). Treating dams with high levels of CORT diminished hippocampal cell proliferation and reduced dendritic complexity in CA3 subfield of the hippocampus (Brummelte & Galea, 2010b; Workman et al., 2013). Furthermore, inducing withdrawal from hormone-simulated pregnancy reduced adult hippocampal neurogenesis in female rats (Green and Galea, 2008). These preclinical studies indicate that the hippocampus is involved in models of PPD.

#### **1.5.3** Developmental stress affects hippocampal neurogenesis

The hippocampus is highly sensitive to perturbations in the early postnatal environment. Indeed, alterations in maternal care and early life adversity are influential on offspring hippocampal development (Korosi et al., 2012; Lajud and Torner, 2015). For this reason, maternal mood disorders such as PPD may be considered a form of early life adversity for the developing child and hippocampal development. In humans, children exposed to maternal depression since birth exhibit no significant change in hippocampal volume (Lupien et al., 2011) whereas adult hippocampal volume is vulnerable to the effects of childhood maltreatment (Chaney et al., 2014). Although PPD itself was not associated with altered hippocampal volume in children, others have reported that there are sex differences in how self-reported low maternal

bonding was associated with small hippocampal volume in women but not in men (Buss et al., 2007).

Postnatal hippocampal neurogenesis (i.e. neurogenesis occurring from birth through weaning) is crucial for hippocampal development, and a majority of granule cells forming the dentate gyrus are born during this time (Altman and Bayer, 1990). Maternal separation lasting the first two postnatal weeks reduced cell proliferation, but not the survival of new neurons in the dentate gyrus, in adult male rats, which was reversed by adrenalectomy (Mirescu et al., 2004). Maternal deprivation for 24 h on postnatal day 3 enhanced number of immature neurons in male rats but diminished number of immature neurons in female rats at weaning (Oomen et al., 2009). However, by adulthood, the number of immature neurons and survival of new neurons was diminished in the ventral hippocampus of adult male rats but not in female rats (Oomen et al., 2011). This suggests that males experience more dynamic and long-lasting changes to neurogenesis than females in response to maternal separation. This observation is particularly interesting because it is possible that males are more vulnerable the females in response to early stress exposure whereas females are more vulnerable later in life (Gobinath et al., 2015).

#### **1.6** Animal models of MDD and PPD

Animal models of depression can provide insight into the neurobiological underpinnings of depression. The strength of these animal models can be evaluated based on the face validity (referring to how close the induced phenotype approximates symptoms of MDD in humans), predictive validity (referring to antidepressant efficacy), and construct validity (referring to whether the theoretical or etiological basis for MDD is viable) (Willner, 1984; Willner and Mitchell, 2002). Although it is not possible to model all the symptoms associated with MDD, it

is possible to quantify depressive-like endophenotypes in rodents including changes in body mass, changes in activity, anhedonia, passive coping, and changes in sleep. Moreover, animal models of MDD can test causative roles physiological features of MDD, such as aberrant HPA axis activity or reduced hippocampal neurogenesis, in relation to these symptoms.

#### 1.6.1 CORT-injection model of MDD

Numerous rodent models of MDD exist with the majority capitalizing on the wellestablished relationship between stress and MDD (Yan, Cao, Das, Zhu, & Gao, 2010). Some of these stress-based models of MDD include chronic exposure to a singular form of stressor (such as restraint stress) (Liu et al., 2016; O'Mahony, Clarke, Gibney, Dinan, & Cryan, 2011; Sun, Sha, & Xu, 2015), chronic exposure to multiple stressors in an unpredictable series (Tanti et al., 2013; Wainwright et al., 2016a), early life stress via separating pups repeatedly from the mother (van Zyl et al., 2016), social defeat (Hollis and Kabbaj, 2014), or chronic CORT administration to induce hypercortisolemia (Kalynchuk et al., 2004; Gregus et al., 2005; Johnson et al., 2006). Each of these models have their advantages and weaknesses. Arguably, the CORT-induced model of MDD is most useful when mechanistically testing how elevated levels of CORT (the end-product of the HPA axis) are related to the symptomology of MDD. Other models of MDD may be problematic as repeated episodes of stress exposure such as restraint stress exposure can result in habituation of the HPA axis (Gregus et al., 2005; Herman, 2013). However, chronic CORT administration is advantageous such that the exogenous delivery of CORT levels ensures that the rodents maintain chronically elevated CORT levels throughout the experiment. Chronic CORT administration at a dose of 40 mg/kg has been shown to induce the following depressivelike endophenotypes in both sexes: increased depressive-like behaviour in the forced swim test (Kalynchuk et al., 2004; Gregus et al., 2005; Johnson et al., 2006), reduced body mass (Johnson et al., 2006), dysregulated HPA axis activity (Johnson et al., 2006), reduced hippocampal cell proliferation (Brummelte and Galea, 2010a), and reduced density of immature neurons in the hippocampus (Brummelte and Galea, 2010a).

It should be noted that chronic CORT administration can be applied via subcutaneous implantation of CORT pellets, dissolving CORT into the drinking water, or injections of CORT itself; each of these methods has advantages and weaknesses. For example, while subcutaneous implantation of CORT pellets results in continual release of CORT, the dose cannot be adjusted for any CORT-induced changes in body mass after surgical implantation nor is it possible to control or ensure continual drug release throughout the experiment after implantation. Furthermore, there is evidence the ability of chronic antidepressant exposure to increase neurogenesis in the hippocampus is observed only when lability of CORT secretion is intact but not when CORT levels are persistently flattened which is resultant from CORT pellet implantation (Huang and Herbert, 2006). Alternatively, dissolving CORT in the drinking water of rodents is non-invasive and does not require additional handling. However, as in the case of CORT pellet implantation, it is not possible to control the dose of CORT as the amount of water consumed can be variable or even reduced. Daily CORT injections can control for body mass changes and adjust dose accordingly but is minimally invasive by involving repeated injection stress. Each of these routes of CORT administration has been implicated in endophenotypes of MDD in male rodents (Kalynchuk et al., 2004; Murray et al., 2008; Donner et al., 2012). However, in female rats, daily injections of CORT reduced body mass, increased serum CORT levels after twenty-five days of exposure, increased passive coping in the forced swim test, and reduced number of DCX-expressing cells in the ventral dentate gyrus whereas CORT pellets were not able to induce these MDD-like endophenotypes (Kott et al., 2016). While CORT in

drinking water reduced number of DCX-expressing cells in the ventral dentate gyrus, it did not affect the other MDD-like endophenotypes in females (Kott et al., 2016). Thus, to effectively study CORT-induced MDD in females, the optimal route is via daily injection.

## 1.6.2 CORT-injection model of PPD

Our laboratory adapted the CORT-induced model of MDD to dams (i.e. rat mothers) as a model of PPD. Treating dams with high levels of CORT throughout the postpartum period reliably disrupts quality of maternal care by increasing time spent away from the nest and reducing time spent nursing in the early postpartum (Brummelte & Galea, 2010b; Brummelte, Lieblich, & Galea, 2012; Brummelte et al., 2006; Workman et al., 2013). Importantly, dams voluntarily disengage from maternal care without completely depriving pups of maternal care. During the postpartum period, dams are normally highly motivated to interact with pups and pup interaction can be rewarding (Lee, Clancy, & Fleming, 2000; Mattson, Williams, Rosenblatt, & Morrell, 2003). Because postpartum CORT treatment reduces time spent with the pups, high levels of CORT may be inducing a form of anhedonia relevant to the postpartum period. Furthermore, unlike other models of postnatal adversity which forcibly separate pup from the dam, CORT administration in the dam results in voluntary disengagement from pup interactions which more closely approximates disrupted maternal care in PPD (Lovejoy et al., 2000). Treating dams with high levels of CORT increased passive coping or depressive-like behavior at the end of the postpartum period (time spent immobile in the forced swim test, another MDDlike endophenotype further discussed below). Postpartum CORT treatment to the dams also interrupts hippocampal integrity with decreased dendritic complexity and hippocampal neurogenesis at the end of the postpartum period relative to control dams (Brummelte & Galea, 2010b; Brummelte et al., 2006; Workman et al., 2013). Finally, postpartum CORT treatment

predominantly affects male but not female offspring outcome, mirroring the sex-specific effects of PPD on child outcome. In male offspring, postpartum CORT treatment reduced hippocampal cell proliferation at weaning (Brummelte et al., 2006) and increased anxiety-like behavior in adolescence without disrupting female outcomes (Brummelte et al., 2012). Thus, adapting the CORT-induced model of MDD to the postpartum period has good face validity (reduced maternal care, increased maternal passing coping behaviour, disrupted offspring behaviour) as well as construct validity (elevated levels of glucocorticoids, reduced hippocampal plasticity) for modelling PPD. This thesis aims to determine predictive validity within this model with regards to SSRIs and exercise as well as evaluate how these treatments could influence outcome in the dam (Chapters 2 and 4) and offspring (Chapter 3 and 5). The following sections with discuss the behavioural and physiological tests that were used to address these studies.

#### **1.6.3** Behavioural test – Forced swim test

FST is a putative measure of behavioural despair or depressive-like behaviour. This test is typically a two-day procedure in rats (Porsolt et al., 1977a, 1977b). First, rats are placed into a cylindrical tank of water for fifteen minutes followed by a test session twenty-four hours later in which rats are returned to the cylindrical tank of water for five minutes. During this test session, time spent immobile, swimming, and climbing are quantified (Slattery and Cryan, 2012). Increased time spent immobile is considered to be indicative of passive coping. Furthermore, antidepressant treatment reduces time spent immobile including FLX treatment (Detke et al., 1995; Surget et al., 2011; Wainwright et al., 2016a) and exercise (Han, Lee, & Leem, 2015; Lapmanee et al., 2013; Yau et al., 2014). Moreover, alterations in swimming behaviour can be indicative of altered serotonergic tone whereas alterations in climbing behaviour can be indicative of altered noradrenergic tone (Detke et al., 1995). Reductions in immobility are

subject to a variety of interpretations, including the possibility that reduced immobility represents deliberate disengagement from active behaviour like swimming and climbing to conserve energy by maintaining an immobile posture or reflects learning acquisition (Slattery and Cryan, 2012). However, time spent immobile is sensitive to pharmacological antidepressant exposure, with SSRIs, tricyclic antidepressants, and selective-norepinephrine reuptake inhibitors reducing time spent immobile, indicating that this is a useful test of predictive validity (Petit-Demouliere et al., 2005). Females show more immobility in males, and this sex difference is apparent only after puberty (Goel and Bale, 2009), an effect that may be related to sex differences in basal CORT levels (Viau, 2002). This time course of sex-specific vulnerability to depression is in line with clinical evidence that females are more likely to suffer from depression as men (Gutierrez-Lobos et al., 2002), strengthening the use of forced swim test as an assay of depressive-like behaviour. Furthermore, time spent swimming is associated with serotonergic tone, and time spent climbing is associated with noradrenergic tone, providing insight into altered neurotransmission (Detke et al., 1995). Many well-validated models of MDD use increased immobility as an index of depressive-like behaviour including chronic unpredictable stress, chronic CORT administration, and social defeat stress (Galea et al., 2001; Kalynchuk et al., 2004; Gregus et al., 2005; Johnson et al., 2006; Hollis and Kabbaj, 2014; van Zyl et al., 2016; Wainwright et al., 2016a)

## **1.6.4** Behavioural test – Novelty suppressed feeding

The NSF test can be used as a measure of anxiety-like behaviour as well as a measure of antidepressant efficacy. In a novel environment, rats exhibit neohypophagia, or an aversion to entering open or unfamiliar environments to feed. In this test, rats are food deprived for approximately sixteen hours in order to motivate animals to feed. Rats are then placed in a novel

arena with a piece of rat chow or a Froot Loop in the center. Rats are then forced to resolve the anxiogenic conflict between entering the novel environment to consume food. Latency to feed is used as an index of anxiety-like behaviour. Indeed, this measure is sensitive to acute anxiolytic drug treatment (Bodnoff et al., 1989). Interestingly, reductions in latency to feed as a result of chronic antidepressant treatment is dependent of the presence of intact hippocampal neurogenesis (Santarelli et al., 2003; Bessa et al., 2009; David et al., 2009). Ablation of adult hippocampal neurogenesis prevents antidepressant-induced reductions in latency to feed.

#### **1.6.5** Behavioural test – Dexamethasone suppression test

Dexamethasone is a potent synthetic glucocorticoid. Endogenous glucocorticoids can bind to either mineralocorticoid or glucocorticoid receptors, and both receptors are present throughout the central nervous system. Glucocorticoid receptors are predominantly occupied during stress responses when endogenous glucocorticoid levels are high (Reul et al., 1990). Dexamethasone selectively binds to glucocorticoid receptors, stimulating negative feedback system of the HPA axis and suppressing glucocorticoid release. However, negative feedback is perturbed in depressed individuals as dexamethasone administration does not effectively suppress cortisol secretion (Carroll et al., 1968; Heuser et al., 1994; Ising et al., 2007).

In humans, the dexamethasone suppression test is typically conducted by administering a single oral dose of dexamethasone followed by a challenge of corticotropin-releasing hormone; serum cortisol levels are measured prior and after the administration of CRH to measure the effectiveness of dexamethasone to suppress CRH-induced cortisol levels as a measure of HPA negative feedback (Ising et al., 2007). In rats, the DEX suppression test is typically conducted by administering dexamethasone and measuring suppression of stress-induced (via restraint stress or corticotrophin releasing hormone challenge) CORT levels. In this regard, poor DEX suppression

of HPA axis activity would indicate impairments in HPA negative feedback and can be informative as an endophenotype of depression. Furthermore, chronic exposure to antidepressants and remission from MDD coincides with normalization of HPA axis negative feedback in the dexamethasone suppression test (Ising et al., 2007), adding support the use of this test as a biomarker of depression. It should be noted that there are sex differences in basal and stress-induced levels of CORT (Goel et al., 2014a). This thesis will examine how exposure to CORT-induced PPD as well as different types of antidepressants (FLX and/or exercise) influence adult male and female HPA axis negative feedback in the dexamethasone suppression test.

## 1.7 Thesis overview and objectives

The following experiments described in this thesis investigate the effects of a pharmacological antidepressant (FLX) in comparison to a non-pharmacological antidepressant (exercise) on endophenotypes of CORT-induced depression in the dam: maternal behaviour, depressive-like behaviour in the forced swim test, HPA axis activity, and hippocampal neurogenesis. Further, we assessed how these maternal antidepressant interventions affect adult male and female offspring outcome in terms of anxiety-like behaviour, HPA axis negative feedback, and hippocampal neurogenesis in adulthood. The overarching hypothesis of this thesis was that antidepressant efficacy is altered in the postpartum period and that a combination of both treatments (FLX and exercise) would be optimal for the mother and be linked to the fewest adverse effects for adult offspring.

Chapter 2: To determine the efficacy of a pharmacological antidepressant (FLX) on endophenotypes of CORT-induced PPD in dams, including maternal behaviour, depressive-like behaviour, and hippocampal neurogenesis.

We hypothesized that postpartum CORT treatment would reduce quality of maternal care, increase depressive-like behaviour in the forced swim test, and reduce hippocampal neurogenesis. Furthermore, we hypothesized that postpartum FLX treatment would alleviate these CORT-induced endophenotypes of PPD.

Chapter 3: To determine the developmental effects of maternal postpartum treatment with a pharmacological antidepressant (FLX) on adult male and female offspring outcome, including anxiety-like behaviour, HPA axis negative feedback, and hippocampal neurogenesis.

We hypothesized that maternal postpartum FLX treatment would adversely affect anxiety-like behaviour, HPA axis negative feedback, and hippocampal neurogenesis in the adult offspring. Furthermore, we hypothesized that maternal postpartum FLX treatment would differentially affect the sexes with males more vulnerable to the effects of maternal postpartum CORT and/or FLX than females. Chapter 4: To compare the efficacy of a non-pharmacological antidepressant (exercise) alone or in conjunction with a pharmacological antidepressant (FLX) on endophenotypes of CORT-induced PPD in dams, including maternal behaviour, depressive-like behaviour, and hippocampal neurogenesis.

We hypothesized that maternal exercise would prevent CORT-induced disruptions in maternal care, depressive-like behaviour in the FST, and hippocampal neurogenesis. Furthermore, we hypothesized that the combination of maternal exercise and postpartum FLX treatment would be more efficacious than FLX alone in alleviating these CORT-induced endophenotypes of PPD.

Chapter 5: To compare the efficacy of a non-pharmacological antidepressant (exercise) alone or in conjunction with a pharmacological antidepressant (FLX) on adult male and female offspring outcome, including anxiety-like behaviour, HPA axis negative feedback, and hippocampal neurogenesis.

We hypothesized that maternal exercise would have fewer adverse effects than maternal postpartum FLX exposure on adult offspring outcome in terms of anxiety-like behaviour, HPA axis negative feedback, and hippocampal neurogenesis in the adult offspring. Furthermore, we hypothesized that either antidepressant alone or in combination would affect both sexes differently with adult male offspring more vulnerable to these maternal interventions than female offspring.

# Chapter 2: Parity modifies the effects of FLX and corticosterone on behavior, stress reactivity, and hippocampal neurogenesis<sup>1</sup>

# 2.1 Introduction

Women are more likely than men to be diagnosed with depression during the reproductive years (Kessler et al., 1994; Gutiérrez-Lobos et al., 2002). Depression is associated with higher resting cortisol concentrations (Parker et al., 2003) and impaired HPA axis negative feedback (Ising et al., 2007). Although impaired HPA function may not be evident in all depressed patients, it is associated with melancholic depression (Lamers et al., 2013) and a meta-analysis indicates that HPA axis hyperactivity is a common feature of major depression (Stetler and Miller, 2011). PPD is a subtype of major depression and affects approximately 15% of women (Goodman, 2007). PPD associated with perturbations in the HPA axis beyond what are considered typical maternal adaptations. For instance, women with PPD or postpartum blues have higher cortisol concentrations compared with postpartum women who were not depressed (Lommatzsch et al., 2006; Okano & Nomura, 1992; Taylor, Littlewood, Adams, Doré, & Glover, 1994). Additionally, women with a history of PPD had higher cortisol responses to corticotropin releasing hormone during a hormone-simulated pregnancy (Bloch et al., 2005). Collectively, these data suggest that low mood during the postpartum is related to HPA dysregulation.

HPA axis tone and responses change throughout the postpartum as the diurnal rhythm of cortisol becomes flattened (Lightman et al., 2001), CORT concentrations are high, and HPA axis

<sup>&</sup>lt;sup>1</sup> Workman, J.L., Gobinath, A.R., Kitay, N.F., Chow, C., Brummelte, S., Galea., L.A. (2016) Parity modifies the effects of fluoxetine and corticosterone on behavior, stress reactivity, and hippocampal neurogenesis. *Neuropharmacology*, 105:443-53. Doi: 10.1016/j.neuropharm.2015.11.27.

responses to stressors are suppressed (Lightman, 1992; Torner et al., 2002). Similarly, in postpartum women, basal cortisol levels are elevated (Abou-Saleh et al., 1999) but nursing suppresses HPA axis responses to stressors (Altemus et al., 1995; Heinrichs et al., 2001). Thus, reproductive state may alter the susceptibility to stress- or CORT-related affective changes. Despite being associated with a greater risk for mood disorders (O'Hara and McCabe, 2013; Russell et al., 2013), the postpartum may also confer resilience to stress-related responses. For instance, the postpartum transiently reduces anxiety-like behavior (Lonstein, 2005, 2007), but does not affect immobility in the forced swim test (Molina-Hernández and Téllez-Alcántara, 2001; Craft et al., 2010). Nevertheless, dams may be differentially susceptible to stress- or glucocorticoid-induced changes in behavior as, in addition to hyporesponsiveness of the HPA axis, dams have reduced corticosteroid binding globulin (Pawluski et al., 2009b) and glucocorticoid receptor binding in the hippocampus (Meaney et al., 1989). Thus, we sought to determine whether nulliparous and postpartum rats would differ in their responses to chronic high CORT exposure.

The hippocampus contains a high density of glucocorticoid receptors (Aronsson et al., 1988; McEwen, Weiss, & Schwartz, 1968; Morimoto, Morita, Ozawa, Yokoyama, & Kawata, 1996) and undergoes structural changes in response to prolonged high glucocorticoid levels (McEwen & Magarinos, 2001). In both males and females, high CORT administration compromises hippocampal plasticity and increases immobility in the forced swim test (Kalynchuk et al., 2004; Brummelte et al., 2006; David et al., 2009; Seedat et al., 2009; Brummelte and Galea, 2010b). Chronic stress or CORT reduce hippocampal neurogenesis in males, dams, and nulliparae (Brummelte and Galea, 2010a, 2010b; Hillerer et al., 2013), but see also (Hillerer et al., 2014) and reduce CA3 pyramidal cell complexity in males, dams, and

nulliparae (Galea et al., 1997; Workman et al., 2013a). Further, patients with depression have smaller hippocampi (McKinnon et al., 2009). Thus, the hippocampus is affected in depression and this may manifest from compromised structural plasticity due to chronically elevated glucocorticoids in depression. Interestingly, reductions in hippocampal cell complexity (Pawluski and Galea, 2006) and neurogenesis (Darnaudéry, Dutriez, Viltart, Morley-Fletcher, & Maccari, 2004; Leuner, Mirescu, Noiman, & Gould, 2007; Pawluski & Galea, 2007; Workman, Raineki, Weinberg, & Galea, 2015) in postpartum rats are also present without any additional manipulations and may alter vulnerability to stress or treatment responses during the postpartum. Further, high CORT (40 mg/kg/day) during the postpartum increases immobility in the forced swim test, reduces maternal care, and reduces cell proliferation and dendritic branching in the hippocampus of postpartum rats (Brummelte & Galea, 2010b; Brummelte et al., 2006; Workman et al., 2013), but the extent to which parity alters responses to glucocorticoids has not been investigated.

FLX is a SSRI prescribed for depression, including PPD (Epperson et al., 2003). Antidepressants restore HPA axis negative feedback in some individuals with depression (Ising et al., 2007; Schüle et al., 2009) and hippocampal structural plasticity in both stressed and unstressed males and nulliparae (Duman, 2004; Wainwright and Galea, 2013), but few studies have examined parous females for antidepressant effects on the brain. Antidepressants can alleviate symptoms of PPD within the first 4 weeks after giving birth (Hantsoo et al., 2014), but systematic reviews suggest that antidepressants may not confer adequate efficacy later in the postpartum (Sharma and Sommerdyk, 2013; De Crescenzo et al., 2014; Molyneaux et al., 2014). Antidepressants also reinstate HPA axis negative feedback in some individuals with depression (Ising et al., 2007), which may occur through increases in hippocampal neurogenesis (Dranovsky

and Hen, 2006; Snyder et al., 2011b). Thus, we sought to determine whether FLX would differentially reverse glucocorticoid-induced immobility in the forced swim test and protect hippocampal neurogenesis in nulliparous and postpartum rats. We hypothesized that CORT would reduce maternal care, increase immobility, and reduce neurogenesis in the dentate gyrus. Additionally, we hypothesized that FLX would reverse deficits in maternal care but that FLX would exert greater effects on neurogenesis and CORT concentrations in nulliparous compared with postpartum rats.

# 2.2 Methods

Sixty-four female 2-month old Sprague-Dawley rats were purchased from Charles River. All rats were initially housed in same-sex pairs in transparent polycarbonate cages ( $24 \times 16 \times 46$  cm) with aspen chip bedding and *ad libitum* access to standard rat chow (Jamieson's Pet Food Distributors Ltd, Delta, BC, Canada) and water. All rats were exposed to a 12:12 light/dark cycle (lights on at 7:00 am) in temperature- and humidity-controlled rooms and were allowed 7 days to acclimate to the facility. Rats were randomly assigned to groups (n = 7 - 8 per group; see Figure 2-1 for timeline). Thirty-two rats were mated and the other 32 were assigned to be nulliparous controls that were not exposed to males or pups. All procedures for nulliparae occurred concurrently with those of dams (with the exception of breeding and maternal observations) and nulliparae were single housed throughout the experiment. One dam was euthanized, yielding n = 7 in the oil-saline group. All protocols were in accordance with ethical guidelines set by the Canada Council for Animal Care and were approved by the University of British Columbia Animal Care Committee.

# **Breeding Procedures**

Breeding was conducted as previously described (Workman et al., 2013b). All dams were single housed in a separate room and remained undisturbed until parturition with the exception of cage changes and weighing. Day of birth was considered postpartum day (PD) 0. On PD 1, litters were culled to 5 males and 5 females, and weighed.

## Drug Preparation

Rats received CORT (40 mg/kg, s.c.) or 1 ml/kg vehicle (sesame oil and 10% EtOH, s.c.) and either FLX (FLX, 10 mg/kg, i.p.) or 1 ml/kg vehicle (physiological saline and 10% DMSO, i.p). FLX (Sequoia Research Products, Pangbourne, UK) and an emulsion of CORT (Sigma-Aldrich, St. Louis, MO, USA) were prepared every 3 days. This dose of CORT was chosen because it induces a depressive-like phenotype (Kalynchuk et al., 2004; Gregus et al., 2005), reduces hippocampal neurogenesis (Brummelte and Galea, 2010a, 2010b) and results in similar CORT concentrations in response to a physiological stressor in lactating rats (Yeh, 1984). Injections began on PD 2 and lasted until PD 23 and were administered between 11 a.m. and 2 p.m.

#### Maternal Observations

Maternal behaviors were scored 3 times per day from PD 2 - 8 at least 1 h after the previous observation or after injections. Within a 75-min period, each dam was observed once every 3 min for the following behaviors: licking pups, licking pups while nursing, arched-back nursing (ABN), blanket nursing, passive nursing (nursing while supine or on side), retrieving, nest building (i.e., moving bedding and nesting material), eating, drinking, self grooming, sleeping, and off nest (Myers et al., 1989; Liu et al., 1997; Champagne et al., 2001).

Behavioral responses to swim stress were assessed using the FST (Cryan et al., 2005; Galea et al., 2001; Porsolt et al., 1978; Porsolt et al., 1977). FST was conducted as previously described (Workman et al., 2013). FST 1 lasted 15 min and FST 2 (24 h later) lasted 5 min. Fecal boli were counted and water was changed between rats. All sessions were recorded and scored for time spent swimming, climbing, and immobile (making only movements necessary to keep the head above water) using BEST Collection Software (Educational Consulting, Inc., Hobe Sound, FL, USA) by an observer unaware of treatment conditions.

#### Blood and Tissue Collection

Vaginal lavages were conducted daily for 8 days prior to perfusion as previously described (Workman, Crozier, Lieblich, & Galea, 2013) to determine whether cycling varied between treatments and to determine whether estrous phase would alter the proportion of proliferative cells (Tanapat et al., 1999; Rummel et al., 2010) or FST performance (Kokras et al., 2015). After FST 2, rats were placed back into colony rooms and 90 min later, 100 µl of blood (to determine CORT concentrations following swim stress) was collected via tail vein puncture within 3 min of moving the cage. Blood sampling took place approximately 24 h following the final injection. Offspring were weaned and used in another study (Gobinath et al., 2016). After blood sampling, rats were given an overdose of Euthanyl. Blood was collected via cardiac puncture to determine serum levels of estradiol and rats were perfused with 60 ml cold physiological saline followed by 120 ml cold 4% paraformaldehyde. Adrenal glands were collected and weighed. Brains were extracted and placed in 4% paraformaldehyde at 4 °C overnight and then transferred to 30% sucrose in phosphate buffer at 4 °C. Brains were rapidly

FST

frozen, sectioned coronally using a freezing microtome (Leica, Richmond Hill, ON, Canada) at 40 µm, and stored in antifreeze (ethylene glycol/glycerol; Sigma) at -20 °C until processing. *Radioimmunoassays* 

Blood samples were kept at 4 °C for 24 h and centrifuged for 15 min. Serum was stored at -20 °C until radioimmunoassay. Samples were run in duplicate according to the manufacturer's instructions. CORT and estradiol concentrations were detected using a double antibody <sup>125</sup>I radioimmunoassay kit (MP Biomedicals, Orangeburg, NY, USA) and Ultrasensitive <sup>125</sup>I radioimmunoassay kit (Beckman Coulter, Mississauga, Ontario, Canada; respectively). The intra-assay coefficients of variation were less than 5% for CORT and less than 15% for estradiol. *Immunohistochemistry* 

Briefly, between PBS washes, sections were treated with 0.3% hydrogen peroxide (30 min) and transferred into primary antibody solution: 1:1000, goat anti-doublecortin (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Twenty-four h later, sections were washed and transferred to secondary antibody solution: 1:500, rabbit anti-goat (Vector Laboratories, Burlington, ON, Canada) for 24 h. Then washed sections were incubated in ABC complex (ABC Elite Kit; 1:1000; Vector) for 4 h and washed in 0.175 M sodium acetate buffer. Finally, sections were developed using diaminobenzidine in the presence of nickel (DAB Peroxidase Substrate Kit, Vector), mounted, dehydrated, and coverslipped (Workman et al., 2015a; Workman et al., 2015b).

#### Microscopy

All analyses were conducted by an experimenter who was blind to treatment conditions. To quantify number of immature neurons, we selected every 20<sup>th</sup> section in dorsal and ventral hippocampal sections within limits previously described (Brummelte and Galea, 2010a). DCX- expressing cells were exhaustively counted in each of these sections at 1000x magnification. Cells were averaged per section for analysis. DCX-expressing cells were also categorized in developmental stages. A total of 50 cells per rat were randomly selected from the dorsal (n = 25) and the ventral (n = 25) hippocampus. Cells were selected if they were relatively isolated (i.e., not clumped or overlapping) and if they were within the granule cell layer. Cells were classified in one of three developmental stages based on morphological attributes: (1) proliferative if they had no processes or short, plump processes; (2) intermediate if they had one process; or (3) postmitotic if they had branching dendrites reaching the molecular layer (Plümpe et al., 2006; Workman, Chan, & Galea, 2015; Workman, Raineki, et al., 2015)

## Statistical Analyses

All data (unless specified below) were analyzed using factorial ANOVA with parity (nulliparous, postpartum), hormone (oil, CORT), and drug (saline, FLX) as between-subjects factors. Maternal behaviors were averaged and analyzed using ANOVA with hormone and drug as between-subjects factors. Adrenal masses were analyzed (as above) both as absolute mass and relative mass (mg/g body mass). Estradiol concentrations were analyzed using ANCOVA with proestrus (yes, no) as the covariate. DCX-expressing cells and cell maturity were analyzed using repeated measures ANOVA with hippocampal region (dorsal, ventral) as the within-subjects factor and an additional within-subjects factor for cell maturity (proliferative, intermediate, post-mitotic). Fisher's protected LSD were conducted following a significant *F*. Limited pairwise comparisons were conducted based on specific *a priori* hypotheses. Effects are considered significant when  $P \le 0.05$ . Partial  $\eta^2$  and Cohen's *d* are included for all significant effects. All data were analyzed using Statistica 12 (StatSoft, Inc., Tulsa, OK, USA).
# 2.3 Results

CORT and FLX reduced body mass in offspring; FLX reduced body mass in nulliparae only

CORT reduced body mass in offspring at weaning (main effect of hormone:  $F_{1,25} = 8.93$ , P = 0.006,  $\eta_P^2 = 0.26$ ). FLX also reduced body mass at weaning (main effect of drug:  $F_{1,25} = 5.73$ , P = 0.024,  $\eta_P^2 = 0.19$ ). In adults, CORT reduced body mass taken at the end of the study, just prior to perfusions (main effect of hormone:  $F_{1,55} = 15.31$ , P < 0.001,  $\eta_P^2 = 0.22$ ). Additionally, FLX reduced body mass in nulliparae (P = 0.012, d = 0.77), but not in dams (P = 0.51; drug by parity interaction:  $F_{1,55} = 4.97$ , P < 0.03,  $\eta_P^2 = 0.08$ ). Saline-treated dams also weighed less than saline-treated nulliparae at the end of the study (P = 0.025, d = 0.82).

# FLX reversed CORT-induced disruptions in maternal care

CORT reduced arched back nursing (ABN; main effect of hormone:  $F_{1,27} = 5.79$ , P = 0.02,  $\eta_P^2 = 0.18$ ; Figure 2-1B) but among CORT-treated rats, FLX increased ABN (planned comparison: P = 0.005, d = 1.9, main effect of drug:  $F_{1,27} = 11.16$ , P = 0.002,  $\eta_P^2 = 0.29$ ). FLX reduced passive nursing (main effect of drug:  $F_{1,27} = 4.14$ , P = 0.05,  $\eta_P^2 = 0.13$ ; Figure 2-1C) and nest building (main effect of drug:  $F_{1,27} = 5.24$ , P = 0.004,  $\eta_P^2 = 0.27$ ; Figure 2-1D). CORT increased self-grooming (main effect of hormone:  $F_{1,27} = 4.23$ , P = 0.05,  $\eta_P^2 = 0.13$ ; Figure 2-1E), whereas FLX reduced self-grooming within CORT-treated rats (planned comparison: P = 0.011, d = 1.63, main effect of drug:  $F_{1,27} = 7.5$ ,  $\eta_P^2 = 0.22$ , P = 0.01). CORT increased off nest frequency in saline-treated rats (planned comparison: P = 0.036, d = 1.04), whereas in CORT-treated rats, FLX reduced off nest frequency (planned comparison: P = 0.018, d = 1.11; Figure 2-1F).



Figure 2-1 Experimental timeline and effects of maternal postpartum CORT and FLX treatments on maternal behaviour. A) Timeline of experimental events. One week after arrival, rats were randomly assigned to either parous or nulliparous groups. Half of the rats were bred and the other half remained isolated from males and pups. Injections (either oil or CORT and either saline or FLX) for all rats began on the same day (which coincided with postpartum day [PD] 2 for parous rats) and occurred daily until PD 23. Maternal observations occurred PD 2 - 8. Vaginal lavages occurred the last 8 days of the experiment, which coincided with PD 17 - 24. The final two days of the experiment, FST was conducted and 90 min following the second FST, blood was collected via tail vein puncture and rats were perfused. B) Mean percent + SEM behaviors during maternal observations. CORT reduced arched-back nursing (ABN) in saline-treated rats (main effect of CORT: P = 0.02) and among CORT-treated rats, FLX increased ABN ( $\dagger$  effect of FLX within CORT condition, Fisher's LSD: P =(0.005). C) FLX reduced passive nursing (§ main effect of drug: P = 0.05) and D) nest building (§ main effect of drug: P < 0.004). E) CORT increased self-grooming in saline-treated rats (main effect of CORT: P = 0.05), and in CORT-treated rats FLX reduced self-grooming ( $\dagger$  effect of drug within CORT condition, Fisher's LSD: P =0.011). F) In saline-treated rats, CORT increased off nest (@ effect of hormone within saline condition, Fisher's LSD: P = 0.036), and among CORT-treated rats, FLX reduced off nest († effect of drug within CORT condition, Fisher's LSD: P = 0.018). n=7-8/group. Reprinted with permission from Workman et al., 2016.

CORT increased immobility whereas parity reduced immobility in saline-treated rats

In the second FST, CORT increased time immobile regardless of FLX or parity (P < 0.001; main effect of hormone:  $F_{1,55} = 36.5$ , P < 0.001,  $\eta_p^2 = 0.4$ ; Figure 2-2A). Regardless of hormone treatment, parity reduced time immobile in saline-treated rats, (P = 0.018, d = 0.74), but not in FLX-treated rats (P = 0.55; drug by parity interaction:  $F_{1,55} = 4.91$ , P = 0.031,  $\eta_p^2 = 0.08$ ). Compared with saline, FLX did not alter time immobile in either nulliparae or dams (Ps > 0.12). CORT decreased swimming regardless of FLX or parity (main effect of hormone:  $F_{1,55} = 38.29$ , P < 0.001,  $\eta_p^2 = 0.41$ ; Figure 2-2B). Among saline-treated rats, parity increased swimming regardless of CORT (P = 0.005, d = 0.88) but not in FLX-treated rats (P = 0.88; drug by parity interaction:  $F_{1,55} = 4.19$ , P = 0.046,  $\eta_p^2 = 0.07$ ). CORT decreased the latency to immobility (main effect of hormone:  $F_{1,55} = 20.96$ , P < 0.001,  $\eta_p^2 = 0.28$ , Figure 2-2C), but there were no effects of drug or parity on latency to immobility (Ps > 0.13). Hormone, drug, and parity did not alter percentage of climbing behavior in FST 2 (Ps > 0.26, Table 2-1).



Figure 2-2 Effects of postpartum CORT and FLX treatments on FST2 behavior. Mean percent + SEM behaviors in the second FST. A) CORT increased time immobile regardless of FLX or parity (\* main effect of CORT: P < 0.001). Additionally, in saline-treated rats parity reduced time immobile (§ effect of parity within saline condition, Fisher's LSD: P = 0.05). B) CORT reduced time swimming regardless of FLX or parity (\* main effect of CORT: P < 0.001). Additionally, in saline-treated rats, parity increased swimming (§ effect of parity within saline condition, Fisher's LSD: P = 0.01). C) CORT reduced the latency to immobility (\* main effect of hormone: P < 0.001). n=7-8/group. Reprinted with permission from Workman et al., 2016.

In the first FST, CORT significantly increased immobility (main effect of hormone:  $F_{1,55}$  = 14.7, P < 0.001,  $\eta_p^2 = 0.21$ ) and decreased swimming (main effect of hormone:  $F_{1,55} = 11.88$ , P = 0.001,  $\eta_p^2 = 0.18$ ). FLX and parity significantly interacted to alter latency to immobility in the first FST ( $F_{1,55} = 7.91$ , P = 0.007,  $\eta_p^2 = 0.13$ ). Specifically, among saline treated rats, dams took longer to reach first immobility compared with nulliparae (P < 0.001, d = 1.41). In dams, FLX reduced the latency to immobility compared with saline (P = 0.005, d = 0.62). CORT also significantly reduced the latency to first immobility in FST 1 (main effect of hormone:  $F_{1,55} = 12.44$ , P < 0.001,  $\eta_p^2 = 0.18$ ). Hormone, drug, and parity did not alter percentage of climbing behavior in FST 1 (Ps > 0.1, Table 2-1).

			FST1			FST 2	
			Latency	Immobilit y	Swimming	Climbing	Climbing
OIL	SAL	Nulli	177.6 ± 22.88	62.74 ± 3.73	$29.13\pm2.3$	$8.1\pm2.27$	$9.83 \pm 2.57$
		Dam	323.64 ± 35.7†	54.54 ± 5.49	38.21 ± 6.38	7.23 ± 1.15	$5.7 \pm 2.8$
	FLX	Nulli	232.42 ± 31.28	$55.23 \pm \\ 6.66$	$36.64 \pm 6.22$	8.1 ± 1.43	$\begin{array}{c} 10.57 \pm \\ 3.84 \end{array}$
		Dam	$256.92 \pm 28.9$	54.56 ± 5.19	$38.02 \pm 5.13$	7.39 ± 1.44	$7.77\pm3.46$
CORT	SAL	Nulli	106.45 ± 15.9*	73.14 ± 2.74*	20.16 ± 3.55*	6.61 ± 1.61	$7.65\pm2.85$
		Dam	247.15 ± 50.89*†	63.83 ± 5.64*	30.52 ± 4.79*	5.61 ± 1.25	8.26 ± 3.9
	FLX	Nulli	156.72 ± 23.07*	69.06 ± 3.66*	$23.48 \pm 3.55*$	$7.4\pm0.72$	$8.93 \pm 2.23$
		Dam	178.1 ± 20.27*§	71.46 ± 2.54*	$\begin{array}{c} 24.42 \pm \\ 2.03 \ast \end{array}$	$4.1 \pm 1.48$	$1.31\pm0.92$

 Table 2-1 Effects of CORT and FLX treatments on FST1 behavior and FST2 climbing. Mean ± SEM behaviors

 in FST 1 and climbing in FST 2. \* significant main effect of CORT; † significantly greater than saline-treated

 nulliparae; § significantly lower than saline-treated dams. Reprinted with permission from Workman et al., 2016.

CORT and parity reduced neurogenesis, whereas FLX increased ventral hippocampal neurogenesis

Compared with oil, CORT reduced DCX-expressing cells in both the dorsal and the ventral hippocampus of nulliparae (P < 0.001, d = 2.13; P < 0.001, d = 4.97, respectively) and dams (P = 0.014, d = 1.39; P < 0.001, d = 2.31, respectively; hormone by parity by region interaction:  $F_{1,54} = 10.03$ , P < 0.003,  $\eta_P^2 = 0.16$ ; Figure 2-3B&C). Additionally, parity reduced DCX-expressing cells in the dorsal (P = 0.003, d = 1.3) and ventral hippocampus (P < 0.001, d = 1.9) of oil-, but not CORT-treated rats (Ps > 0.34).

FLX increased DCX-expressing cells in the ventral hippocampus of both oil- and CORT treated rats (oil: P = 0.03, d = 0.36; CORT: P = 0.04, d = 0.9), but not in the dorsal hippocampus

(oil: P = 0.16; CORT: P = 0.1; hormone by drug by region interaction:  $F_{1,54} = 3.94$ , P = 0.05,  $\eta_p^2 = 0.07$ ). Finally, FLX increased DCX-expressing cells in the ventral hippocampus in nulliparae (planned comparison: P < 0.003, d = 0.34), but not dams (planned comparison: P = 0.29).

*CORT* interacted with parity to alter immature neuron maturation; *FLX* accelerated immature neuron maturation

In nulliparae, CORT increased the percent of proliferative (P < 0.001, d = 1.72) and intermediate cells (P < 0.001, d = 1.54) but reduced the percent of post-mitotic cells (P < 0.001, d = 2.33; hormone by parity by cell stage interaction:  $F_{2, 102} = 5.55$ , P = 0.005,  $\eta_P^2 = 0.1$ ; Figure 2-3D-F). In dams, CORT reduced the percent of post-mitotic cells only (P = 0.024, d = 1.17). Among oil-treated rats, parity increased percent of proliferative cells (P = 0.025, d = 0.87) and decreased percent of post-mitotic cells (P = 0.004, d = 0.96). FLX reduced the percent of cells in the intermediate stage (P = 0.027, d = 0.56) and increased the percent of cells in the post-mitotic stage (P = 0.027, d = 0.38; drug by cell stage interaction:  $F_{2, 102} = 3.42$ ; P = 0.036,  $\eta_P^2 = 0.06$ ).



**Figure 2-3 Effects of postpartum CORT and FLX treatments on neurogenesis and maturation of DCXexpressing cells.** A) Representative photomicrograph of DCX-expressing cells in the suprapyramidal blade of the dorsal dentate gyrus. Mean + SEM DCX-expressing cells per section of B) the dorsal dentate gyrus and C) the ventral dentate gyrus. In the dorsal and ventral dentate gyrus, CORT reduced DCX-expressing cells in both nulliparae (*@* effect of CORT within nulliparous condition, Fisher's LSD: *Ps* < 0.001) and dams (^ effect of CORT within postpartum condition, Fisher's LSD: *Ps* < 0.001). Parity also reduced the number of DCX-expressing cells in the dorsal and ventral dentate gyrus (# effect of parity within region comparisons, Fisher's LSD: *Ps* ≤ 0.003). Compared with saline, FLX increased DCX-expressing cells in the ventral hippocampus in oil- (\*\**P* = 0.03) and CORT-treated rats (†*P* = 0.04). Compared with saline, FLX increased DCX-expressing cells in the ventral hippocampus of nulliparae only (§ effect of FLX within nulliparous condition and hippocampal region, Fisher's LSD: *P* < 0.001). D – F) Mean + SEM percent of DCX-expressing cells in different stages of development. CORT increased the percent of cells in the D) proliferative and E) intermediate stages in nulliparae only (\* effect of CORT within nulliparous condition, Fisher's LSD: Ps < 0.001). CORT reduced the percent of F) postmitotic cells in both nulliparae and dams (\* effect of CORT within reproductive condition, Fisher's LSD:  $Ps \le 0.024$ ). Finally, in oil-treated rats, parity increased the proportion of proliferative cells (# effect of parity within oil condition, Fisher's LSD: P = 0.025) and reduced the proportion of postmitotic cells (# effect of parity within oil condition, Fisher's LSD: P = 0.004). Finally, regardless of drug or hormone exposure or parity, FLX reduced the percent of cells in the intermediate stage and increased the percent of cells in the postmitotic stage (§ effect of FLX within cell stage comparison, Fisher's LSD: Ps = 0.027). n=7-8/group. Reprinted with permission from Workman et al., 2016.

# FLX reduced CORT concentrations despite increasing relative adrenal mass in nulliparae only; CORT increased CORT concentrations and reduced adrenal mass

FLX reduced CORT concentrations in nulliparae (P < 0.001, d = 1.1) but not dams, (P = 0.87, drug by parity interaction:  $F_{1,52} = 6.09$ , P = 0.017,  $\eta_P^2 = 0.1$ ; Figure 2-4A). Parity reduced CORT concentrations in saline- (P = 0.003, d = 0.69), but not FLX-treated rats (P = 0.54). Finally, CORT increased CORT concentrations (main effect of hormone:  $F_{1,52} = 73.18$ , P < 0.001,  $\eta_P^2 = 0.58$ ).

CORT decreased relative adrenal mass in FLX- (P < 0.001, d = 3.34) and saline-treated rats (P < 0.001, d = 3.85, hormone by drug interaction:  $F_{1,55} = 4.81$ , P = 0.033,  $\eta_p^2 = 0.08$ ; Figure 2-4B). FLX increased relative adrenal mass in oil- (P < 0.001, d = 1.54) but not CORT-treated rats (P = 0.83). Additionally, FLX increased relative adrenal mass in nulliparae (P < 0.001, d =0.69) but not dams (P = 0.24; drug by parity interaction:  $F_{1,55} = 5.7$ , P = 0.02,  $\eta_p^2 = 0.09$ ). CORT also reduced absolute adrenal mass in both FLX- (P < 0.001, d = 4.52) and saline-treated rats (P < 0.001, d = 4.47; hormone by drug interaction:  $F_{1,55} = 7.19$ , P = 0.009,  $\eta_p^2 = 0.12$ ). Additionally, FLX increased absolute adrenal mass in oil- (P < 0.001, d = 1.44), but not CORT-treated rats (P = 0.74).



Figure 2-4 Effects of postpartum CORT and FLX treatments on serum CORT and adrenal mass. A) Mean + SEM CORT concentrations 90 min following the FST. CORT increased CORT concentrations regardless of FLX or parity (\* main effect of CORT: P < 0.001). Among nulliparae, FLX reduced CORT concentrations compared with saline (‡ effect of FLX within nulliparous condition, Fisher's LSD: P < 0.001). Additionally, in saline-treated rats, dams had lower CORT concentrations compared with nulliparae (§ effect of parity within saline condition, Fisher's LSD: P = 0.003). B) Mean + SEM relative adrenal mass. CORT reduced adrenal mass regardless of FLX or parity (\* main effect of CORT: P < 0.001). Compared with saline, FLX increased relative adrenal mass in oil-treated rats (@ effect of FLX within oil condition, Fisher's LSD: P < 0.001). Finally, in nulliparae, FLX increased relative adrenal mass compared with saline (‡ effect of FLX within nulliparous condition, Fisher's LSD: P < 0.001). Finally, in nulliparae, FLX increased relative adrenal mass compared with saline (‡ effect of FLX within nulliparous condition, Fisher's LSD: P < 0.001). Finally, in nulliparae, FLX increased relative adrenal mass compared with saline (‡ effect of FLX within nulliparous condition, Fisher's LSD: P < 0.001). n=7-8/group. Reprinted with permission from Workman et al., 2016.

# CORT suppressed estrous cyclicity in nulliparae; CORT and FLX altered estradiol concentrations in dams but not nulliparae

In nulliparae, CORT reduced the proportion of rats displaying normal estrous cycles in saline- (P < 0.001) and FLX-treated rats (P = 0.003). In saline-treated rats, CORT increased estradiol concentrations in dams (P = 0.011, d = 1.25), but not nulliparae (P = 0.41; hormone by drug by parity interaction:  $F_{1,53} = 4.5$ , P = 0.039,  $\eta_P^2 = 0.08$ ; Table 2-2). FLX reduced estradiol concentrations in CORT- (P < 0.001, d = 1.84) but not oil-treated dams (P = 0.58). Proestrus was not a significant covariate (P = 0.7).

	Nulliparous		Postpartum		
	Oil	CORT	Oil	CORT	
SAL	$24.05 \pm 3.52$	33.34 ± 3.71	26.87 ± 4.71*	58.68 ± 12.81	
FLX	$16.29 \pm 2.06$	$17.6 \pm 1.93$	$40.43 \pm 19.52$	13.09 ± 3.19*	

 Table 2-2 Effects of postpartum CORT and FLX treatments on serum estradiol. Mean ± SEM serum estradiol

 concentrations (ng/ml) at perfusion. \*significantly lower than CORT-, saline-treated dams. Reprinted with

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# 2.4 Discussion

Here we show that FLX restored maternal behaviors of CORT-treated dams to control levels but was not sufficient to reduce immobility in the FST. However, FLX increased DCXexpressing cells in the ventral hippocampus of nulliparae, but not dams and reduced CORT concentrations in nulliparae, but not dams. Thus, parity is a significant factor in the effects of FLX on depressive-like behavior, neuroplasticity, and HPA axis responses following acute stress. Although dams were less sensitive to FLX, parity reduced immobility and CORT concentrations following the FST and limited the effects of CORT on immature neuron maturation, suggesting that the postpartum period may shield females from the detrimental effects of chronic CORT or acute stress (such as the FST) on behavior, HPA axis responses, and neuroplasticity. These data enhance our understanding of how parity modifies responses to antidepressants and glucocorticoids and are relevant to postpartum mood disorders.

# FLX reversed CORT-induced decrements in maternal care.

CORT impaired maternal care by reducing arched-back nursing and increasing off-nest and self-grooming behaviors, consistent with prior research using CORT or stress (Brummelte & Galea, 2010b; Brummelte et al., 2006; Nephew & Bridges, 2011; Workman et al., 2013). Our findings also corroborate other studies showing exogenous glucocorticoids also impair maternal care in marmosets (Saltzman and Abbott, 2009) and reduce nest attendance in birds (Angelier et al., 2009). These conserved responses to high glucocorticoids in across maternal animals indicate that high glucocorticoids may represent a physiological signal to disengage from offspring investment and engage in survival (Wingfield, 2003; Love et al., 2013). However, the effects of CORT on maternal care are dose dependent as lower doses of CORT maintain maternal care (Casolini et al., 2007).

Although prior studies demonstrate that dexamethasone or chronic stress during the postpartum period, impair lactational competency (Vilela and Giusti-Paiva, 2011; Carini and Nephew, 2013), the contributions of dams' ability to lactate to nursing behaviors and pup development in this study are unknown. However, both CORT and FLX treatment to dams significantly attenuated pup body mass gain (although CORT and FLX did not significantly interact). Thus, CORT and FLX might interfere with milk letdown, although it is difficult to

separate the effects of CORT and FLX on lactation versus the effects on pup growth in this study.

In our study, FLX returned ABN, off-nest behavior, and self-grooming in CORT-treated dams to levels similar to control dams. FLX reduced passive nursing, suggesting that dams receiving FLX opted for more active as opposed to passive nursing postures. Maternal behaviors are, in part, dependent on serotonin neurotransmission (Lerch-Haner et al., 2008). In a prior study, FLX (5 mg/kg/day) increased arched-back nursing and reduced passive nursing in postpartum unstressed rats, but not dams that received gestational stress (Pawluski et al., 2012), suggesting pregnancy stress may inhibit the effect of lower doses of FLX on maternal care. Our data indicate that a higher dose of FLX (10 mg/kg/day) is sufficient to restore decrements in maternal care associated with concurrent, high CORT, suggesting that higher doses of FLX may be needed to overcome detrimental effects of either stress or high glucocorticoids during reproductive time points in females.

# Parity reduced immobility in and CORT concentrations following the FST.

Parity increased swimming and reduced time immobile in the second FST in salinetreated rats. Similarly, parity also extended the latency to immobility in the first FST in salinetreated rats. Parity-related reductions in immobility were not present in FLX-treated rats. Although prior research suggests parity does not alter immobility in the FST (Walker et al., 1995; Molina-Hernández and Téllez-Alcántara, 2001; Lavi-Avnon et al., 2005; Craft et al., 2010) the present data suggest that aspects of the late postpartum period may protect against a depressive-like behavioral phenotype. Prior work indicates that the effects of parity on locomotor behavior are equivocal (Craft et al., 2010; Pawluski, Lieblich, & Galea, 2009). Although we did

not assess locomotor activity in this study and cannot rule out an effect of locomotor behavior on parity-related changes in immobility, past studies using this same model demonstrate no difference in locomotor activity following CORT administration. Thus, locomotor behavior does not likely account for CORT-induced immobility in the FST.

CORT increased depressive-like behavior, consistent with prior studies (Kalynchuk et al., 2004; Brummelte et al., 2006). In males, glucocorticoid signaling mediates immobility in the FST (Báez and Volosin, 1994; Korte et al., 1996). Thus, postpartum reduction in immobility in the FST may be attributable to hyporesponsiveness of the HPA axis demonstrated in prior studies (Toufexis et al., 1999; Tu et al., 2005), but could also be related to reduced circulating corticosteroid binding globulin concentrations (Pawluski et al., 2009) or glucocorticoid receptor binding in the hippocampus in dams (Meaney et al., 1989).

Further, parity reduced CORT concentrations following acute swim stress (FST), suggesting that parous rats in our study either mounted lower HPA axis responses to the stressor or potentially recovered more quickly, as sampling was done 90 minutes after the second exposure to the FST. The suppressive effect of parity on the HPA axis, however, was not evident in FLX-treated dams. Despite having hyporesponsive HPA axes, primiparous rats typically have higher baseline CORT concentrations, which might depend on time of day and postpartum day (Pawluski et al., 2009). Thus, the precise time course of and relative change in CORT concentrations following swim stress in the present study is not known.

FLX reduced CORT concentrations in nulliparae only and inhibited the beneficial effects of parity in the FST.

FLX reduced CORT concentrations in nulliparae, but not dams following the FST, suggesting that FLX could be reducing CORT responses to acute swim stress or facilitating HPA axis recovery following stress. Prior work indicates that antidepressants facilitate negative feedback of the HPA axis (Keck, 2006; Ising et al., 2007). However, future studies are necessary to determine whether FLX alters HPA axis responses and negative feedback differently in dams and nulliparae. In healthy women, the early postpartum is associated with impaired HPA negative feedback as dexamethasone failed to suppress cortisol levels in the majority of postpartum women (Maes et al., 1992). Surprisingly, FLX inhibited the beneficial effects of parity in the FST as dams only had lower immobility and CORT concentrations if they were treated with saline. These data suggest that the later postpartum period may render antidepressants less effective at least on HPA axis responses to acute swim stress, potentially due to chronically low estradiol characteristic of the postpartum period. This stands in contrast to the early effects of FLX on maternal behavior, and may indicate that differences in the hormonal milieu at specific postpartum time points may alter the efficacy of SSRIs. FLX also did not significantly alter immobility in the FST in either nulliparous or postpartum females, which is not entirely surprising given that the effects of antidepressants on immobility in females can depend on estrous cycle phase and dose of SSRI (Kokras et al., 2015). Treatment regimen (acute, chronic) may also play a role as well as relative estradiol levels as effectiveness of some SSRIs appears to wane after 14 d of treatment or with concentrations of estradiol (Estrada-Camarena et al., 2008). Furthermore, in postpartum women, an SSRI alleviated depression if depression occurred within 4 weeks after parturition (Hantsoo et al., 2014). This latter point is intriguing,

because in the present study postpartum females responded behaviorally to FLX early in the postpartum (i.e., maternal care) but did not respond in the FST that was conducted later postpartum. This might mirror the findings in a prior study in mice indicating that SSRI treatment for 5 d reduced immobility in the FST on day 10 postpartum (Jury et al., 2015). Thus it may be the case that FLX alleviates depressive-like behaviors early in injection protocol, but not after 21 days of exposure perhaps due to hormonal milieu during the postpartum or loss of efficacy in females (Kokras et al., 2015).

As expected, CORT reduced both absolute and relative adrenal mass. The suppressive effect of CORT on adrenal mass likely represents that high exogenous CORT suppresses endogenous HPA axis activity via negative feedback mechanisms leading to adrenal atrophy. Additionally, FLX increased both relative and absolute adrenal mass in oil-treated rats, suggesting changes in adrenal mass following CORT or FLX administration are not due to changes in body mass. Parity increased relative adrenal mass, but not absolute adrenal mass, suggesting that changes in relative adrenal mass following reproductive experience may reflect changes in body mass. Indeed, dams lost weight throughout the postpartum period compared with nulliparae (but only if they were treated with saline) and CORT reduced body mass regardless of drug or parity. These data also suggest that changes with adrenal mass do not always reflect stress-related glucocorticoid output, as FLX increased relative adrenal mass but reduced CORT concentrations in nulliparae. CORT and parity reduced neurogenesis and FLX increased neurogenesis in the ventral dentate gyrus only in nulliparae.

In the present study, we found that FLX increased DCX-expressing cells in the ventral, but not dorsal, dentate gyrus of both oil- and CORT-treated rats. The ventral hippocampus is involved in stress and anxiety (Fanselow and Dong, 2010b; O'Leary and Cryan, 2014). Thus, regionally specific effects of FLX have greater implications for mood and stress regulation and indeed, some studies show that antidepressants preferentially increase neurogenesis in the ventral hippocampus (Tanti and Belzung, 2013; O'Leary and Cryan, 2014). Further, we also found that FLX increased ventral hippocampal neurogenesis in nulliparae only. These data may explain why FLX reduced CORT concentrations in nulliparae only, as new neurons buffer HPA axis responses to stressors (Snyder et al., 2011b). In the present study, CORT reduced neurogenesis regardless of parity, consistent with prior work (Brummelte and Galea, 2010a, 2010b). However, we also demonstrate (via Cohen's d) that CORT exerted a larger effect in the ventral hippocampus than the dorsal, suggesting that neurogenesis in the ventral hippocampus is more sensitive to glucocorticoids. Prior work demonstrates that glucocorticoids regulate cell proliferation, survival, and differentiation in males (Cameron & Gould, 1994; Gould, Cameron, Daniels, Woolley, & McEwen, 1992; Wong & Herbert, 2006; Wong & Herbert, 2004). Further, our prior work indicates that chronic high CORT suppresses cell proliferation in dams (Brummelte and Galea, 2010b). Thus, changes in DCX expression could reflect differences in cell proliferation, survival, and differentiation, although some sex differences (Hillerer et al., 2013) or differences attributable to reproductive phase might be factors as well. Finally, parity independently suppressed both dorsal and ventral hippocampal neurogenesis, consistent with previous work in primiparous rats (Pawluski & Galea, 2007; Workman, Raineki, et al., 2015).

CORT interacts with parity to delay immature neuron development, whereas FLX accelerates immature neuron development.

In nulliparae, CORT shifted the percent of DCX-expressing cells toward more immature, proliferative and intermediate cells and away from postmitotic cells. Although dams had a lower percent of more mature postmitotic cells if they were treated with CORT, there was no significant effect of CORT on proliferative or intermediate cells. In male rats, CORT also delays maturation of DCX-expressing cells (Lussier et al., 2013; Workman et al., 2015a). However, the present data further suggest that parity may protect against some the deleterious effects of high CORT on new neuron maturation, as there was no effect of CORT on proliferative cells and CORT exerted a greater effect on postmitotic cells in nulliparae.

Additionally, FLX shifted DCX-expressing cells toward a more mature, postmitotic stage of development. These data suggest that in females, FLX accelerates the development of immature neurons, as previously demonstrated in males (Wang et al., 2008). Finally, parity increased the proportion of proliferative and reduced the proportion of postmitotic cells, as we have previously shown (Workman, Raineki, et al., 2015)

CORT suppressed estrous cyclicity in nulliparae and CORT increased estradiol in dams but not nulliparae

CORT reduced the proportion of nulliparae displaying normal estrous cycles, consistent with prior work using high CORT (Brummelte and Galea, 2010a) and exposure to chronic stressors (Konkle et al., 2003; Baker et al., 2006; Herzog et al., 2009). Also, CORT increased, but FLX normalized estradiol concentrations in dams, but not nulliparae. Because we collected blood samples following the FST, it is possible that drug and hormone interacted with stress of

testing or anesthetic administration leading to an increase or different rate of recovery in estradiol concentrations in some groups. Indeed, acute stressors increase (Shors et al., 1999; Martínez-Mota et al., 2011), whereas chronic stressors decrease estradiol concentrations in nulliparae (Galea et al., 1997). In pregnant rats and mice, however, chronic stress increased estradiol concentrations (MacNiven et al., 1992; Misdrahi et al., 2005). Thus, parity may also modify how the hypothalamic-pituitary-gonadal axis responds to stressors or high CORT.

Notably, FLX blocked CORT-induced increase in estradiol concentrations in dams but not in nulliparae. Numerous studies point to a bidirectional relationship between serotonin and the HPG axis, (Matuszczyk et al., 1998; Rehavi et al., 2000; Mennigen et al., 2008; Barth et al., 2015). Additionally, FLX reduced serum estradiol concentrations in ovariectomized rats treated with estradiol benzoate (Taylor, Farr, Klinga, & Weiss, 2004) suggesting that FLX may also modify metabolic pathways of estrogens. Thus, in our study, FLX may have suppressed pathways that mediate estradiol synthesis and release or increased estradiol metabolism.

# Conclusions

Maternal postpartum CORT reduced maternal care, neurogenesis, and increased depressive like behavior. However, FLX reversed deficits in maternal care in CORT-treated dams, but was not sufficient to reduce depressive-like behavior in either nulliparae or dams. FLX increased neurogenesis in the ventral hippocampus and promoted recovery of stress-induced CORT concentrations in nulliparae only, suggesting that dams may be less sensitive to antidepressants. Notably, parity reduced depressive-like behavior and promoted recovery of CORT following stress, despite reducing neurogenesis. CORT altered immature neuron development to a greater extent in nulliparae. These data indicate that whereas the postpartum

confers resilience to chronic glucocorticoids and acute stressors, dams during the postpartum were also more resistant to the effects of FLX on neurogenesis and the HPA axis. Taken together, these data indicate that parity modifies the effects of CORT and FLX on behavior, HPA function, and the hippocampus. This study suggests it is imperative to consider reproductive experience and is essential for understanding susceptibility to and treatment of postpartum mental illness. Chapter 3: Maternal postpartum corticosterone and FLX differentially affect adult male and female offspring on anxiety-like behavior, stress reactivity, and hippocampal neurogenesis<sup>2</sup>

# 3.1 Introduction

According to the DSM-5, perinatal depression is defined as depression during pregnancy and the early postpartum (American Psychiatric Association, 2013). As with MDD, one of the most common treatments for perinatal depression is pharmacological antidepressants, such as SSRIs (Oberlander et al., 2006; Kim et al., 2014a). As more women receive antidepressants to treat perinatal depression, the population of children who have been exposed to antidepressants during the perinatal period also increases (Oberlander et al., 2006). However, maternal SSRI use may be problematic as SSRIs such as fluoxetine (Prozac) can cross the placental barrier (Hendrick et al., 2003) and pass into breast milk (Wisner et al., 1996; Weissman et al., 2004a), potentially affecting the developing offspring. Indeed, perinatal SSRI exposure is associated with adverse outcomes in the infant such as reduced weight gain (Chambers et al., 1999), levels of reelin required for normal brain development (Brummelte et al., 2013), psychomotor scores during the first year (Santucci et al., 2014b), increased hypertension (Chambers et al., 2006), cardiac defects (Malm et al., 2011), and risk for autism (Croen et al., 2011). However, the negative effects of perinatal fluoxetine may outweigh the detrimental effects of untreated maternal depression on child development. Specifically, children of mothers with postpartum

<sup>&</sup>lt;sup>2</sup> Gobinath, A.R., Workman, J.L., Chow, C., Lieblich, S.E., Galea, L.A. (2016) Maternal postpartum corticosterone and fluoxetine differentially affect adult male and female offspring on anxiety-like behaviour, stress reactivity, and hippocampal neurogenesis. *Neuropharmacology*, 101: 165-78. Doi: 10.1016/j.neuropharm.2015.09.001.

depression (PPD) are more likely to develop depression, anxiety, and attention deficits even long after the mother's depression has remitted (Pilowsky et al., 2006; Murray et al., 2011). Thus, the potential therapeutic effect of maternal SSRIs may mitigate these negative effects on child development. In fact, maternal SSRI use is associated with enhanced infant readiness to interact with their mother (3 mo infants; Weikum, Mayes, Grunau, Brain, & Oberlander, 2013), accelerated perceptual development (6 mo and 10 mo infants; (Weikum, Oberlander, Hensch, & Werker, 2012)), and improved executive function (6 yo; (Weikum et al., 2013a)). However, it is unclear whether the effects of maternal fluoxetine are advantageous in the long term or precede negative behavioral outcomes that emerge later in life. This study aims to fill this gap.

Preclinical research investigating the long term effects of perinatal fluoxetine on emotional behavior has yielded mixed results, likely due to methodological differences including timing and method of administration. For example, direct administration of fluoxetine to pups during the postnatal period increased anxiety-like behavior (Yu et al., 2014), while maternal exposure to fluoxetine (gestation and lactation) resulted in no significant effect on anxiety-like behavior in adult offspring (Lisboa et al., 2007; Francis-Oliveira et al., 2013). Additionally, direct administration of fluoxetine to pups during the postnatal period decreased depressive-like behavior in adult rats (Mendes-da-Silva et al., 2002) whereas maternal fluoxetine (gestation and postpartum) increased depressive-like behavior in adult female but not male mice offspring (Lisboa et al., 2007). In addition, the current state of research examining neonatal fluoxetine exposure is hindered by a general lack of preclinical research investigating maternal fluoxetine exposure within a model of depression or PPD. Because mothers typically use SSRIs to treat depression, there is a need for preclinical research to address how maternal fluoxetine influences offspring within a concurrent model of depression or stress in order to contribute valid

conclusions regarding the use of SSRIs to treat PPD. To this end, there are a few studies examining how gestational stress followed by maternal postpartum fluoxetine normalizes immobility in the forced swim test in adolescent male and female offspring (Rayen et al., 2011) as well as blunts serum corticosterone (CORT; primary glucocorticoid in rats) levels in adolescent male, but not female, offspring (Pawluski et al., 2012). However, gestational stress did not result in a depressive phenotype in the dam in this study (Pawluski et al., 2012), so it is unclear whether these results can be interpreted as modeling maternal depression. Moreover, it is unknown how modeling depression and antidepressant treatment occurring exclusively in the postpartum affect offspring development. This is an important problem to investigate because approximately 40% of perinatal depression arises solely in the postpartum period (Wisner et al., 2013) and treatment and outcome for mother and child differ depending on the timing of depression onset (Cooper and Murray, 1995). Thus, there is a need to study postpartum antidepressant treatment in animal models of depression based on postpartum and antenatal depression, respectively.

The hippocampus exhibits morphological alterations long after exposure to developmental stress (Korosi et al., 2012; Loi et al., 2014). Although maternal depression does not predict significant changes in hippocampal volume in children (Lupien et al., 2011), childhood maltreatment (Chaney et al., 2014) and low maternal bonding (Buss et al., 2007) are associated with reduced hippocampal volume in adulthood, which both may be present in PPD. Reduction in hippocampal volume can be attributed to a number of factors such as lower levels of hippocampal neurogenesis. Broadly speaking, stress reduces adult hippocampal neurogenesis depending on age at the time of stress exposure and sex of the subject (Gobinath et al., 2015). For example, maternal deprivation diminished expression of doublecortin (an endogenous

protein expressed in immature neurons) in adult male but not female rat offspring (Oomen et al., 2010; Oomen et al., 2011). Furthermore, adult hippocampal neurogenesis may play an important role in the etiology of mood-related disorders such as depression (DeCarolis and Eisch, 2010; Eisch and Petrik, 2012), as well as regulation of the HPA axis (Snyder et al., 2011b). Despite evidence that antidepressants can normalize HPA axis activity (Ising et al., 2007) and increase hippocampal neurogenesis (Malberg et al., 2000; Santarelli et al., 2003; Boldrini et al., 2009; Epp et al., 2013), little is known about how maternal fluoxetine affects HPA axis and adult neurogenesis in the hippocampus of offspring beyond the time they are exposed to the drug. Maternal postpartum fluoxetine reversed the detrimental effects of prenatal stress on hippocampal doublecortin expression in both male and female adolescent rat offspring (Rayen et al., 2011). However, by adulthood, maternal postpartum fluoxetine only diminished doublecortin expression after prenatal stress exposure, particularly in adult male offspring (Rayen et al., 2015). Thus, hippocampal neurogenesis represents a neurobiological intersection of developmental exposure to stress, antidepressants, and adult behavioral outcomes and will be investigated in the present study.

We have previously shown that chronic CORT administered to the dam postpartum increases maternal depressive-like behavior and diminishes maternal care (Chapter 2; Brummelte & Galea, 2010b; Brummelte et al., 2006; J. L. Workman et al., 2013; J L Workman et al., 2016). Interestingly, maternal postpartum CORT decreases hippocampal cell proliferation in male offspring at weaning (Brummelte et al., 2006) and increases anxiety-like behavior in adolescent male, but not female, offspring (Brummelte et al., 2012). However, it is unclear whether these sex differences or effects on offspring brain and behavior persist when the dam is exposed to concurrent maternal antidepressant exposure. The present study investigates whether high levels

of maternal postpartum CORT and concurrent fluoxetine administered to dams differentially affect adult male and female offspring outcome at the behavioral (anxiety- and depression-like behavior, locomotion), endocrine (HPA axis dysregulation), and neural (doublecortin expression) levels. We hypothesized that maternal postpartum fluoxetine would negatively affect behavior, HPA axis regulation, and hippocampal neurogenesis in the affected adult offspring. Further, we expect that both sexes will be differentially affected by maternal postpartum fluoxetine and CORT.

#### 3.2 Methods

#### Animals

Thirty-two adult female Sprague-Dawley rats (2 - 3 months old) and 16 adult male Sprague-Dawley rats (2 - 3 months old, Charles River) were initially housed in same-sex pairs in opaque polyurethane bines  $(24 \times 16 \times 46 \text{ cm})$  with aspen chip bedding. Rats were maintained in a 12 h: 12 h light/dark cycle (lights on at 7:00 a.m) and given rat chow (Jamieson's Pet Food Distributors Ltd, Delta, BC, Canada) and tap water ad libitum. All protocols were in accordance with ethical guidelines set by Canada Council for Animal Care and were approved by the University of British Columbia Animal Care Committee.

# **Breeding Procedures**

For breeding, males were single housed and two females and one male were paired daily between 5:00 and 7:00 pm. Females were vaginally lavaged each morning between 7:30 and 9:30 am and samples were assessed for the presence of sperm. Upon identification of sperm, females were considered pregnant, weighed, and single housed into clean cages with autoclaved paper towels and an enrichment tube.

One day after birth (birth day = postnatal day 0), all litters were culled to 5 males and 5 females. If there were not enough males or females in one litter, pups were cross-fostered from a dam that gave birth the same day. If there were not enough pups available to support a 5 male and 5 female litter, then dams maintained a sex-skewed or smaller litters (this happened twice with both being in the CORT/saline group). Dams were randomly assigned to one of four treatment groups: 1) CORT/FLX; 2) CORT/saline; 3) Oil/FLX; 4) Oil/saline. Beginning on postpartum day 2, dams received two daily injections of either subcutaneous CORT (40 mg/kg) or sesame oil (1 ml/kg) and intraperitoneal FLX (10 mg/kg) or saline (1 ml/kg) for 22 consecutive days. The effects of maternal postpartum CORT/saline on depressive-like behavior were verified in the dam (Chapter 2; Workman et al., 2016), and data investigating maternal outcome will be published separately (Workman et al., 2016). Dams received both injections in succession between 11 A.M. and 2 P.M. Pups were weaned on postpartum day 24 and pairhoused with an unrelated, same-sex cage mate whose mother received the same treatment. No more than 2 males and 2 females were taken from each litter for the behavioral tests. Besides weekly cage changing, offspring remained undisturbed until behavioral testing.

# Drug preparation

An emulsion of CORT (Sigma-Aldrich, St. Louis, MO, USA) was prepared every 2-3 days by mixing CORT with ethanol and then adjusting with sesame oil to yield a final concentration of 40 mg/ml of CORT in oil with 10% ethanol. The dose was chosen because it reliably induces a depressive-like phenotype in dams, impairs maternal care, and affects offspring development (Brummelte et al., 2006, 2011, 2012; Brummelte and Galea, 2010b; Workman et al., 2013a). Fluoxetine (Sequoia Research Products, Pangbourne, UK) was prepared every 2-3 days by dissolving in dimethyl sulfoxide (DMSO; Sigma Aldrich) and adjusting with

0.9% saline to yield a final concentration of 10 mg/ml fluoxetine in saline with 10% DMSO. This dose of fluoxetine was chosen based on work illustrating that this dose increased brain derived neurotrophic factor and cell proliferation in the hippocampus and amygdala after 21 days of injections in both male and female rodents (Hodes et al., 2010). Control dams were given two vehicle injections: "oil" consisted of 10% ethanol in sesame oil to control for the CORT injections, and "saline" consisted of 10% DMSO in 0.9% saline to control for the fluoxetine injections.

For the dexamethasone suppression test, a solution of dexamethasone (Sigma Aldrich) was prepared 1-2 days prior to the test by dissolving dexamethasone in propylene glycol and adjusted to yield a final dose of 50 ug/kg dexamethasone in propylene glycol. This dose and timing of dexamethasone injection were chosen based on previous studies (Cole et al., 2000). *Behavioral Testing* 

Beginning at postnatal day  $65 \pm 2$ , 6-10 male and female rats per group underwent behavioral testing (EPM, OFT, FST, and NSF). Based on the four maternal treatments described above, rats from each of the following groups (60 rats total) were utilized: Adult male Oil/Saline offspring, n=8; Adult male Oil/FLX offspring, n=6; Adult male CORT/Saline offspring, n=6; Adult male CORT/FLX offspring, n=10; Adult female Oil/Saline offspring, n=9; Adult female Oil/FLX offspring, n=6; Adult female CORT/Saline offspring, n=6; Adult female CORT/FLX offspring, n=9. Behavioral tests were conducted in the same order for all the animals with 48 h between each test. Behavioral testing occurred at 9:00 A.M. each day under dim light conditions (approximately 12 lux). Twenty-four h after the final behavioral test, rats underwent a dexamethasone-suppression test under standard bright light conditions (approximately 180 lux). Seventy-two h after dexamethasone suppression test, all rats were perfused and brain tissue was collected. For an overview of experimental procedures, please refer to Figure 3-1.



Figure 3-1 Experimental Timeline. Reprinted with permission from Gobinath et al., 2016.

#### EPM

The EPM was used to evaluate anxiety-like behavior in male and female offspring. Briefly, the apparatus consists of two open arms bisected by two closed arms (arm length: 50 cm; arm width: 10 cm; arm wall height: 40 cm). Rats were placed into the center of the apparatus, facing the open arm. Each test session lasted 5 min and was video recorded. The apparatus was cleaned using a 15% vinegar solution between each testing session to remove any odors or waste. The numbers of entries (all four paws entering an arm) into the open arm and closed arm as well as time (in seconds) spent in the open arm and closed arm were analyzed. Ratio of time spent in the closed arm versus the open arms and center was used as an index of anxiety as previously described (Brummelte et al., 2012).

# OFT

The OFT was used to assess general locomotor activity as previously described (Brummelte et al., 2006). The apparatus, a 90 x 90 x 40 cm square arena divided into 16 squares of equal dimension, was placed in a dimly lit room. Rats were placed in the apparatus facing the

same corner and video recorded for 10 min. The apparatus was cleaned using a 15% vinegar solution between each testing session to remove any odors or waste. A line crossing was defined as all four paws crossing a gridline (Brummelte et al., 2006). Total number of line crossings was used as an index of general locomotion.

# FST

Approximately 48 h after OFT, rats were tested in the forced swim test to assess depressive-like behavior. A glass cylindrical tank (45 x 28 cm) filled to a depth of approximately 30 cm of tap water  $25 \pm 1$ °C. For the first session, rats were placed into the water for 15 min. The second session took place 24 h later and rats were placed into the water for 5 min. Water was replaced between each rat. An observer blind to treatment conditions scored the sessions for percent time spent swimming, climbing, or immobile using BEST Collection Software (Educational Consulting, Inc., Hobe Sound, FL, USA).

### NSF

Approximately 48 h after forced swim test, rats were tested for anxiety-like behavior in the novelty suppressed feeding paradigm. In this test, rats must resolve an anxiogenic conflict of entering the center of arena to access a morsel of chow after being food deprived (Bodnoff et al., 1989; Santarelli et al., 2003; Bessa et al., 2009; Leuner et al., 2010). Food was removed from rats' cages 16 h prior to testing to incite motivation to consume food during the test. Each rat was placed in a square arena (60 x 60 cm) facing the right corner. Latency to feed was recorded in seconds as an index of anxiety-like behavior. The trial was terminated either after the rat began to eat or after 10 min if the rat did not eat. Lab chow was added to the cages after testing, and food consumption was measured in each cage 1 h after test to assess whether feeding behavior was altered by maternal postpartum CORT or FLX.

# Dexamethasone Suppression Test

Approximately 48 h after novelty suppressed feeding, rats were tested for HPA axis negative feedback using the dexamethasone suppression test. Dexamethasone was administered to all rats subcutaneously 90 min prior to a 30 min restraint stressor. Tail blood samples were collected at the beginning of restraint (t=0), the end of restraint (t=30), and 1 h after cessation of restraint (t=90).

## Tissue Collection

Approximately 72 h after dexamethasone suppression test, rats were weighed and then given an overdose of Euthanyl. Rats were perfused with 60 ml cold 0.9% saline followed by 120 ml cold 4% paraformaldehyde. Brains were extracted and postfixed using 4% paraformaldehyde overnight at 4°C. Brains were then transferred to 30% sucrose in phosphate buffer at 4°C until they sank to the bottom. Brains were rapidly frozen with dry ice and sectioned using a freezing microtome (Leica, Richmond Hill, ON, Canada) at 40 µm and collected in series of 10. Sections were stored in antifreeze (ethylene glycol/glycerol; Sigma) and stored at -20°C until processing. *CORT Assay* 

Blood samples were stored overnight at 4°C to allow blood to clot completely. Blood was then centrifuged at 10,000 g for 15 min. The serum was collected and stored at -20 °C until radioimmunoassay. Total CORT (bound and free) was measured using the ImmuChem Double Antibody 125I radioimmunoassay Kit (MP Biomedicals, Solon, OH, USA). The antiserum crossreacts 100% with CORT, 0.34% with deoxycorticosterone, 0.05% with cortisol, and does not cross-react with dexamethasone (<0.01%). All reagents were halved and samples run in duplicate.

#### DCX Immunohistochemistry

Sections were rinsed 5 x 10 min in 0.1 M PBS, treated with 0.3% hydrogen peroxide in dH<sub>2</sub>O for 30 min, and incubated at 4 °C in primary antibody solution: 1:1000, goat anti-DCX (Santa Cruz Biotechnology, Santa Cruz, CA, USA) with 0.04% Triton-X in PBS and 3% normal rabbit serum for 24 h. Sections were then rinsed 5 x 10 min in 0.1 M PBS and transferred to a secondary antibody solution with 1:500, rabbit anti-goat (Vector Laboratories, Burlington, ON, Canada) in 0.1 M PBS for 24 h at 4°C. Then, sections were washed 5 x 10 min in 0.1 M PBS and incubated in ABC complex (ABC Elite Kit; 1:1000; Vector) for 4 h. Sections were then washed in 0.175 M sodium acetate buffer 2 x 2 min. Finally, sections were developed using diaminobenzidine in the presence of nickel (DAB Peroxidase Substrate Kit, Vector), mounted on slides, and dried. Sections were then counterstained with cresyl violet, dehydrated, and coverslipped with Permount (Fisher).

DCX-expressing cells were quantified in 3 dorsal sections (-2.76 mm to -4.68mm below bregma) and 3 ventral sections (-5.52 mm to -6.60 mm below bregma) using the 40x objective using an Olympus CX22LED brightfield microscope. Areas of these sections were quantified using ImageJ (NIH, Bethesda, MD, USA) and used for density calculations (number of cells per mm<sup>2</sup>). To determine the maturity of doublecortin-expressing cells, 100 cells positively labeled for doublecortin were randomly selected in the ventral hippocampus because ventral hippocampus is associated with stress regulation and affective behaviors (Fanselow & Dong, 2010). Two hundred cells positively labeled for doublecortin (100 dorsal and 100 ventral) were randomly selected and categorized as either proliferative (no process or short process), intermediate (medium process with no branching), or post-mitotic (strong dendrite branching in

the molecular layer or delicate dendritic tree branching present in the granule cell layer) based on previously published criteria (Plümpe et al., 2006; Workman, Chan, & Galea, 2015; see 3-7A-C).

#### Data Analyses

Data collected from the elevated plus maze test, open field test, and novelty suppressed feeding task were analyzed using ANOVA with sex, maternal postpartum CORT, and maternal postpartum FLX as between-subjects factors. Behavior in the elevated plus maze was analyzed using repeated measures ANOVA with arm of maze (closed and open arm) as the within-subjects factor. Behavior in open field test was analyzed using repeated measures ANOVA with area of maze (center, periphery) as within-subjects factor. Behavior in the forced swim test was analyzed using repeated measures ANOVA with behavior (percent time climbing, swimming, and immobile) as the within-subjects factor. CORT concentrations from the dexamethasone suppression test were analyzed using repeated measures ANOVA with time (t=0, beginning of restraint; t=30, end of restraint; t=90, 1 h after restraint ended) as the within-subjects factor. The density of doublecortin-expressing cells was analyzed using repeated measures ANOVA with region (dorsal, ventral) as the within-subjects factor. Morphology of doublecortin-expressing cells was analyzed using repeated measures ANOVA with region (dorsal, ventral) and type of cell (proliferative, intermediate, post-mitotic) as the within-subjects factor. Post hoc comparisons used Newman-Keuls. Because we had hypotheses that there would be interactions between sex, CORT, and FLX, a priori comparisons were subjected to Bonferroni corrections. All data were analyzed using Statistica software (v. 9, StatSoft, Inc., Tulsa, OK, USA). All effects were considered statistically significant if  $p \le 0.05$ , trends are discussed if  $p \le 0.10$ .

# 3.3 Results

Maternal postpartum FLX increased anxiety-like behavior in the elevated plus and novelty suppressed feeding task in adult male, but not female, offspring

In the elevated plus maze, maternal postpartum FLX increased the ratio of time spent in the closed arms versus open arms + center in comparison to maternal postpartum saline in adult male (*a priori*; p=0.023), but not female offspring (p=0.946; figure 3-2A). Overall males had a higher ratio of time spent in the closed arms versus open arms + center compared to females (main effect of sex; p=0.027). There was a trend for maternal postpartum FLX to increase ratio of time spent in closed arms versus open arms + center compared to maternal saline in adult male, but not female, offspring (interaction between sex and FLX; F(1, 52)=2.78; p=0.099) but no other significant main or interaction effects (all p's > 0.10). Males spent more time in the closed arms in comparison to females (interaction between arm of maze and sex; F(1, 52)=867.7; p=0.02; Table 3-1). There were no other significant main or interaction effects for time in open and closed arms (all p's>0.14). Females had more arm entries into closed arms in comparison to males (interaction between arm of maze and sex; F(1, 52)=13.21; p<0.001) regardless of maternal postpartum CORT or FLX. Maternal postpartum CORT increased closed arm entries compared to maternal postpartum oil (interaction between arm, maternal postpartum CORT, maternal postpartum FLX; F(1, 52)=4.76; p=0.034; Table 3-1) within saline exposed offspring (p=0.05) but not FLX exposed offspring (p=0.83). There were no other significant main or interaction effects for arm entries (all p's>0.11).

		Mean percent time in the open arms ± SEM	Mean percent time in the closed arms ± SEM	Mean open arm entries ± SEM	Mean closed arm entries ± SEM
Male	Maternal OIL/SAL	$3.04\pm0.95$	86.92 ± 2.73*	$2.50\pm0.73$	$8.63 \pm 0.68$
	Maternal CORT/SAL	$3.94\pm2.31$	$83.44 \pm 4.22*$	$1.83\pm0.40$	10.67 ± 1.38*
	Maternal OIL/FLX	$3.22 \pm 1.42$	$92.44 \pm 2.37*$	$1.17\pm0.48$	$8.33\pm0.56$
	Maternal CORT/FLX	$6.47\pm3.11$	$86.80 \pm 3.95*$	$1.60 \pm 0.40$	$9.30\pm0.79$
Female	Maternal OIL/SAL	$9.33 \pm 3.23$	$72.26 \pm 4.50$	$2.00\pm0.55$	$10.44 \pm 0.85$
	Maternal CORT/SAL	$4.50 \pm 1.61$	82.83 ± 3.13	$1.17\pm0.40$	$12.17 \pm 0.95*$
	Maternal OIL/FLX	$4.06 \pm 1.33$	$78.94 \pm 2.51$	$1.33\pm0.49$	$12.17 \pm 1.35$
	Maternal CORT/FLX	$6.89 \pm 3.11$	$79.70 \pm 4.56$	$1.44 \pm 0.34$	$11.00 \pm 0.41$

Table 3-1 Effects of maternal postpartum CORT and FLX exposure on EPM behaviours in adult male and female offspring. Mean  $\pm$  SEM of additional variables in the elevated plus maze. Males overall spent more time in the closed arms and made fewer closed arm entries (p<0.05). Maternal CORT/saline increased closed arm entries in comparison to maternal oil/saline (p<0.05). Reprinted with permission from Gobinath et al., 2016.

In the novelty suppressed feeding task, maternal postpartum FLX increased latency to feed compared with maternal postpartum saline in adult male (*a priori*; p=0.023), but not female offspring (p=0.801; Figure 3-2B). Females had longer latencies to feed than males (main effect of sex; p<0.001) and there was a trend for maternal postpartum FLX to increase latency to feed in comparison to maternal postpartum saline in adult males only (interaction between maternal FLX and sex; F(1,52)=3.08; p=0.085). There were no other significant main or interaction effects (p's > 0.086). Lastly, males ate more than females within an hour of returning to their home cage (main effect of sex; p<0.001; Table 3-2).



**Figure 3-2 Effects of maternal postpartum CORT and FLX exposure on anxiety-like behaviour in adult male and female offspring.** Anxiety-like behavior as measured by (A) ratio of time spent in closed arms compared to center and open arms (mean + SEM) in elevated plus maze and (B) latency to feed (mean + SEM) in novelty suppressed feeding task. Maternal postpartum FLX increased ratio of time spent in the closed arms versus open arms and center of elevated plus maze and increased latency to feed in novelty suppressed feeding task in comparison to maternal postpartum saline in adult male offspring only. Dashed line in (B) represents end of test session (600 seconds). \* denotes p<0.05. n=6-10/group/sex. Reprinted with permission from Gobinath et al., 2016.

	Males*	Females
Maternal OIL/SAL	$23.00 \pm 3.65$	8.40 ± 1.97
Maternal CORT/SAL	$22.00 \pm 5.69$	$7.67 \pm 0.67$
Maternal OIL/FLX	$17.33 \pm 1.45$	$4.33 \pm 2.33$
Maternal CORT/FLX	$18.50 \pm 0.65$	8.40 ± 0.93

Table 3-2 Mean ( $\pm$  SEM) food consumption 1 h after novelty suppressed feeding task. Males overall ate morethan females within an hour of being returned to their home cage (\*p<0.001). Reprinted with permission from</td>Gobinath et al., 2016.

Maternal postpartum CORT increased total locomotor activity and peripheral crossings in adult male, but not female, offspring in the open field test. Maternal CORT/FLX decreased peripheral crossings.

Maternal postpartum CORT increased total crossings in the open field test compared to maternal postpartum oil in adult male (*a priori*; p=0.002), but not female offspring (p=0.383; Figure 3-3A). Females made more total crossings than males (main effect of sex; p<0.001) and maternal postpartum CORT increased total crossings in comparison to maternal postpartum oil controls (main effect of maternal postpartum CORT; p=0.003). There were no other significant main or interaction effects were present for total crossings (all p's > 0.077).

Maternal postpartum CORT increased peripheral crossings in comparison to maternal postpartum oil in adult males (p<0.0001) but not adult females (p=0.40; interaction between area, sex, and maternal CORT; F(1, 52)=4.28; p=0.04; figure 3-3B). Furthermore maternal postpartum FLX decreased peripheral crossings in comparison to maternal postpartum saline (interaction between area, maternal CORT and maternal FLX; F(1, 52)=4.73; p=0.034; figure 3-3B) only within the CORT-exposed offspring (p=0.032) but not oil-exposed offspring (p=0.24). There were no significant differences in center crossings (p's>0.22). Animals spent a higher percent time in the periphery of the open field than in the center (main effect of area; p<0.0001). There were no other statistically significant main or interaction effects for percent time in periphery or center (all p's>0.09; Table 3-3).

		Mean percent time in the periphery ± SEM (*)	Mean percent time in the center $\pm$ SEM
Male	Maternal OIL/SAL	96.17 ± 1.34	$3.83 \pm 0.47$
	Maternal CORT/SAL	$95.29\pm3.29$	$4.71 \pm 1.34$
	Maternal OIL/FLX	$97.45\pm0.66$	$2.55\pm0.66$
	Maternal CORT/FLX	$94.30\pm0.86$	$5.69\pm0.86$
Female	Maternal OIL/SAL	$94.79\pm0.91$	$5.21 \pm 0.91$
	Maternal CORT/SAL	$94.86\pm0.64$	$5.14\pm0.64$
	Maternal OIL/FLX	$96.01 \pm 1.09$	$3.99 \pm 1.09$
	Maternal CORT/FLX	$96.20\pm0.62$	$3.80 \pm 0.62$

Table 3-3 Effects of maternal postpartum CORT and FLX exposure on OFT behaviour in adult male and

female offspring. All animals spent more time in the periphery than in the center of the open field (\*p<0.001).

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**Figure 3-3 Effects of maternal postpartum CORT and FLX exposure on OFT behaviours in adult male and female offspring.** Locomotor behavior as measured by total crossings (mean + SEM) in open field test (n=6-10/group/sex). Maternal postpartum CORT increased ambulation in adult male offspring only (A). Maternal postpartum oil-exposed males had fewer peripheral crossings in comparison to maternal postpartum CORT-exposed males and oil-exposed females. Maternal postpartum CORT/fluoxetine diminished peripheral crossings in comparison maternal postpartum CORT/saline (B). There were no significant effects on center crossings (see inset in B). Reprinted with permission from Gobinath et al., 2016.

Maternal postpartum FLX increased time spent swimming in the forced swim test in both adult male and female offspring

Maternal postpartum FLX increased time spent swimming compared with maternal postpartum saline, regardless of maternal postpartum CORT during day 2 of the forced swim test (interaction between maternal FLX and behavior type; F(2, 104)=4.497; p=0.013; Figure 3-4). There were no other significant main or interaction effects on any other forced swim test behaviors (all p's > 0.146). To determine if this effect on swimming behavior was affected by day, we further analyzed percent time swimming with a repeated measures ANOVA using day

(day 1, day 2) as a within factor. Maternal postpartum FLX increased percent time spent swimming regardless of day, sex or maternal postpartum CORT (main effect of FLX; F(1,52)=5.721, p=0.02; Figure 4B). Additionally, animals had a higher percent swimming on day 2 than day 1(main effect of day; F(1, 52)=176.75, p<0.0001; Figure 3-4B).

		Mean percent time climbing ± SEM	Mean percent time immobility ± SEM	Mean percent time swimming ± SEM
Male -	Maternal OIL/SAL	29.22±2.09	34.26±4.00	36.52±3.82
	Maternal CORT/SAL	30.63±4.26	36.54±3.98	32.84±5.02
	Maternal OIL/FLX	22.99±3.21	43.63±4.01	33.38±5.08*
	Maternal CORT/FLX	19.33±1.90	33.70±4.90	46.97±5.28*
- Female -	Maternal OIL/SAL	25.16±3.46	33.72±5.31	44.12±5.88
	Maternal CORT/SAL	34.66±7.55	27.65±3.88	37.69±7.18
	Maternal OIL/FLX	31.10±2.00	18.14±4.98	50.75±4.31*
	Maternal CORT/FLX	28.51±1.82	28.85±5.37	42.65±5.53*

Table 3-4 Effects of maternal postpartum CORT and FLX exposure on FST1 behaviours in adult male and

female offspring. Maternal postpartum FLX increased percent time swimming in comparison to maternal

postpartum saline during day 1 of the forced swim test (\*p<0.001). Reprinted with permission from Gobinath et al.,

2016.



**Figure 3-4 Effects of maternal postpartum CORT and FLX exposure on FST2 behaviours in adult male and female offspring.** Percent time spent swimming, climbing, and immobile (mean + SEM) in forced swim test in both males and females (n=6-10/group/sex). \* denotes p<0.05. Reprinted with permission from Gobinath et al., 2016.

Maternal postpartum FLX impaired HPA axis negative feedback only in adult male offspring in the dexamethasone suppression test. Maternal postpartum CORT enhanced HPA axis negative feedback in both adult male and female offspring.

Male and female offspring were analyzed separately, due to the well-established sex differences in HPA axis regulation (Viau, 2002). In adult male offspring, maternal postpartum FLX exaggerated male offspring CORT release at t=30 in comparison to maternal saline male controls (interaction between time and maternal FLX; F(2,22)=8.05; p=0.002; Figure 3-5A). Furthermore, in adult male offspring, maternal postpartum CORT blunted serum CORT release at t=30 in comparison to maternal postpartum oil male controls (interaction between time and maternal CORT; F(2,22)=4.74; p=0.019; Figure 3-5B). Similarly, in adult female offspring, *a priori* comparisons revealed that maternal postpartum CORT blunted serum CORT release at t=30 in comparison to maternal postpartum oil in the adult female offspring (p=0.014; Figure 3-50).

5D). No other significant main or interaction effects were present in the female offspring (all p's





**Figure 3-5 Effects of maternal postpartum CORT and FLX exposure on HPA axis negative feedback in adult male and female offspring.** Serum CORT (mean ± SEM) during dexamethasone suppression tests in males (A-C) and females (D). Maternal postpartum FLX exaggerated CORT release after restraint stress in comparison to maternal postpartum SAL in adult male offspring (A). Maternal postpartum CORT blunted serum CORT release after restraint stress in comparison to maternal postpartum oil in adult male offspring (B). All four maternal experimental groups are displayed for the male offspring (C) and female offspring (D). Only t=30 was greater than all other time points. Solid black line represents 30 min of restraint stress. \* denotes p<0.05. n=6-10/group/sex. Reprinted with permission from Gobinath et al., 2016.

Maternal postpartum FLX and maternal postpartum CORT increased the density of DCXexpressing cells in dorsal hippocampus but not ventral hippocampus in adult male offspring. Males had a higher proportion of proliferative doublecortin-expressing cells in comparison to females.

Maternal postpartum FLX increased the density of dorsal, but not ventral, DCXexpressing neurons compared to maternal saline in adult males (interaction between region, sex, and maternal postpartum FLX; F(1, 51)=3.97; p=0.05; Figure 3-6A). However this effect was driven by the male offspring also exposed to maternal postpartum CORT/FLX (a priori; p=0.01) but not in maternal oil/FLX group (p=0.79). Intriguingly, the opposite effect was seen in females such that maternal postpartum FLX tended to decrease the density of dorsal DCX-expressing immature neurons in adult females compared to maternal postpartum saline controls (p=0.07; Figure 3-6A). Maternal postpartum CORT increased density of dorsal DCX-expressing cells in male offspring in comparison to maternal CORT-exposed female offspring (p<0.001) and to maternal postpartum oil control males (a priori: p=0.023, interaction between region, sex, and maternal CORT; F(1,51)=4.367; p=0.042; Figure 3-6B). Maternal postpartum CORT diminished the density of DCX-expressing cells in the dorsal hippocampus in the adult females in comparison to maternal postpartum oil (p=0.017). There was also significant interaction between region and maternal CORT (p=0.033), main effects of region (p <0.001) and sex (p=0.033) but no other significant main or interaction effects (all p's>0.21).



Figure 3-6 Effects of maternal postpartum CORT and FLX exposure on offspring hippocampal neurogenesis. Maternal postpartum FLX increased density of dorsal DCX-expressing cells (mean + SEM) in the adult male offspring compared to maternal saline in the maternal CORT group only (p<0.01). However maternal postpartum FLX tended to decrease the density of DCX-expressing cells in the adult female offspring in the dorsal hippocampus compared to controls (p<0.07) (A). Maternal postpartum CORT increased the density of DCX expression in males (p<0.02) but decrease it in the females (p<0.017) in comparison to maternal postpartum oil. There was no significant effect of either sex or maternal postpartum FLX in the ventral hippocampus (see inset). \* denotes p<0.05, # denotes p<0.10. B, representative photomicrograph of adult male offspring exposed to maternal postpartum saline; C, representative photomicrograph of adult male offspring exposed to maternal fluoxetine, scale bar = 100  $\mu$ m; D, representative photomicrographs of dorsal hippocampus, scale bar = 100  $\mu$ m; E, representative photomicrographs of ventral hippocampus. n=6-10/sex/group. Reprinted with permission from Gobinath et al., 2016.

We also examined the phenotype of the doublecortin-expressing cells in both the dorsal and ventral dentate gyrus. Males and females had significantly more proliferative doublecortinexpressing cells in comparison to females, regardless of region (p=0.01; interaction between sex and type of cell; F(2, 100)=274.3; p=0.05; figure 3-7D). There was a trend for doublecortin morphology to differ based on region (F(2, 100)=2.69; p=0.07; figure 3-7D) and a main effect of doublecortin morphology with more proliferative cells compared to the other two types of cells and more intermediate cells than post-mitotic cells (all p<0.0002) but no other significant effects (p>0.4).



**Figure 3-7 Morphology of DCX-expressing cells.** Examples of doublecortin-expressing cells at the proliferative (A), intermediate (B), and post-mitotic stage (C). All offspring expressed a greater proportion of proliferative doublecortin-expressing cells in comparison to intermediate or post-mitotic cells. There was a trend for males to express more proliferative doublecortin-expressing cells than females in the dorsal hippocampus. \* denotes p<0.05; # denotes p<0.10. n=6-10/sex/group. Reprinted with permission from Gobinath et al., 2016.

#### Maternal postpartum CORT/FLX diminished body mass of adult male offspring

In adult male offspring only, maternal postpartum Oil/FLX increased body mass in comparison to maternal postpartum Oil/Saline whereas maternal postpartum CORT/FLX diminished mass in comparison to maternal postpartum CORT alone or FLX alone (interaction between sex, CORT, and FLX; F(1, 52)=5.120; p=0.028; Table 5). As expected, adult males weighed more than the adult females (main effect of sex; p<0.001). No other significant main or interaction effects were present (all p's > 0.081).

	Males	Females
Maternal OIL/SAL	$503.75 \pm 10.95$	$309.89\pm9.38$
Maternal CORT/SAL	533.50 ± 11.05	283.50 ± 17.23
Maternal OIL/FLX	$554.17 \pm 18.30^*$	298.33 ± 11.20
Maternal CORT/FLX	$486.20 \pm 15.63^*$	$283.56 \pm 3.88$

 Table 3-5 Effects of maternal postpartum CORT and FLX exposure on body mass in adult male and female

 offspring. Maternal postpartum FLX alone increased body mass in comparison to maternal postpartum saline in the

 adult male offspring. Additionally, maternal postpartum CORT/FLX significantly diminished body mass in adult

 male offspring in comparison to maternal postpartum CORT or FLX alone. \* denotes p<0.05. n=6-10/group/sex.</td>

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Density of doublecortin expression in ventral hippocampus was positively correlated with percent time spent in closed arms of the elevated plus maze in maternal postpartum CORT/FLX male offspring.

Among the maternal postpartum CORT/FLX-exposed male offspring, time spent in the closed arms of the elevated plus maze was positively associated with density of ventral hippocampus doublecortin expression (r=0.832; p=0.01; Figure 8). All other variables were either not significant after correcting for multiple correlations or when outliers in correlations were removed.



**Figure 3-8 Ventral hippocampal neurogenesis correlated with anxiety-like behaviour.** Density of doublecortinexpressing cells in ventral hippocampus was positively associated with percent time in the closed arm of the elevated plus maze in male offspring of CORT/fluoxetine-treated dams. n=6-10/sex/group. Reprinted with permission from Gobinath et al., 2016.

#### 3.4 Discussion

Here we show that maternal exposure to fluoxetine during the postpartum period can have long-lasting effects on anxiety-like behavior, HPA axis negative feedback, and hippocampal neurogenesis in adult offspring. In adult male offspring, maternal postpartum fluoxetine increased anxiety-like behavior in the elevated plus maze and novelty suppressed feeding test and density of doublecortin-expressing cells in the dorsal hippocampus. Maternal postpartum fluoxetine also impaired HPA axis negative feedback in males. Perhaps not surprising, both adult male and female offspring from maternal postpartum fluoxetine-treated dams exhibited increased swimming behavior in the forced swim test, indicative of enhanced serotoninergic tone (Detke et al., 1995). Maternal postpartum CORT enhanced HPA axis negative feedback, increased locomotor behavior and increased hippocampal doublecortinexpressing cells in adult male offspring. Perhaps the most striking finding in our study is that the majority of effects of maternal postpartum CORT and fluoxetine were seen in adult male offspring. This is consistent with many studies that indicate that males may be more susceptible to perturbations during early development (Stevenson et al., 2000; Kent et al., 2012). Collectively, these data reveal that maternal postpartum fluoxetine has long-lasting effects on anxiety-like behaviors, the HPA axis, and neuroplasticity in male offspring. *Maternal postpartum fluoxetine increased anxiety-like behavior in adult male offspring, but not female offspring, regardless of maternal postpartum CORT exposure* 

Maternal postpartum fluoxetine increased anxiety-like behavior in adult male but not female offspring in the elevated plus maze and the novelty suppressed feeding tests. This increase in anxiety-like behavior was not due to differences in locomotor activity as maternal postpartum fluoxetine did not affect total crossings in the open field test in either adult male or female offspring. This is in line with similar studies showing that either prenatal fluoxetine exposure (Olivier et al., 2011) or direct administration of fluoxetine to mice pups (postnatal days 2-21; (Yu et al., 2014) increases latency to feed in the novelty suppressed feeding test. However, our results are the first to show that fluoxetine increases anxiety-like behavior in adult male offspring when administered to nursing dams, even with concurrent CORT exposure (a model of postpartum stress/depression). In women, maternal postpartum fluoxetine increases breast milk concentration of both fluoxetine and its active metabolite norfluoxetine (Wisner et al., 1996). Therefore, it is possible that in nursing offspring, maternal fluoxetine exposes the developing brain to high levels of serotonin and subsequently disturbs development of the serotonin system. Indeed, developmental disturbances to the serotonin system, such as genetically knocking out the serotonin transporter or the 5HT-1a receptor, are associated with increased anxiety-like behavior (Lira et al., 2003; Lo Iacono & Gross, 2008; respectively), which is consistent with our findings. The relationship between perinatal exposure to fluoxetine and anxiety-like behavior may be

related to abnormal activity of the serotonin reuptake transporter and 5-HT1a receptor, both of which are implicated in the etiology of anxiety (SERT: Sen, Burmeister, & Ghosh, 2004; 5-HT1a: Heisler et al., 1998; Ramboz et al., 1998). Although we did not find an effect of maternal postpartum fluoxetine in adult female offspring in elevated plus maze, possible effects of maternal postpartum fluoxetine on anxiety-like behavior in the novelty suppressed feeding test could have been obscured by a ceiling effect, as most females did not feed in the 10 minute trial. Further studies need to optimize this test for female rats by food depriving for longer, extending the length of the trial, or offering more palatable food (Machado et al., 2013). Moreover, in adult mice, females metabolize fluoxetine faster than males (Hodes et al., 2010; McNamara et al., 2010). Given this sex difference in fluoxetine exposure due to metabolism, it is likely that developmental fluoxetine exposure had a more potent effect on the males than in the females, resulting in larger effects of developmental fluoxetine in males than females. Finally, our results are also consistent with previous work with this model of PPD that have found that maternal postpartum CORT does not increase anxiety-like behavior in adult male or female offspring (Brummelte et al., 2006).

It should be noted that lower doses of maternal fluoxetine have been shown to not significantly affect anxiety-like behavior in either male or female offspring (7.5 mg/kg/day: Lisboa et al., 2007; 5 mg/kg/day: (Francis-Oliveira et al., 2013). Additionally, differences in timing of fluoxetine administration may contribute to differences in anxiety-related outcomes as both Lisboa et al., 2007 and Francis-Oliveira et al., 2013 exposed dams during gestation and postpartum whereas the current study exposed dams only in the postpartum. These lower doses of fluoxetine may not be sufficient to alter offspring development, or there may be differences in offspring outcome if dams are treated with fluoxetine throughout gestation as well as postpartum.

Furthermore, higher doses of maternal fluoxetine (25 mg/kg/day) during mid-gestation (prenatal day 15) through postpartum (postnatal day 12) decreased anxiety-like behavior in adult male (Kiryanova and Dyck, 2014) and female mice (McAllister et al., 2012). Together, this highlights the importance of dose and timing of fluoxetine as crucial methodological factors when evaluating effects of maternal fluoxetine on offspring outcome (Kiryanova et al., 2013). *Maternal postpartum fluoxetine increased serotonin-mediated behavior (swimming) in the forced swim test in both adult male and female offspring* 

In the present study, maternal postpartum fluoxetine increased percent time spent swimming, but not percent time spent immobile or climbing, in the forced swim test in both adult male and female offspring. Increased swimming behavior is indicative of increased serotonin activity (Detke et al., 1995). Thus, our findings suggest that maternal postpartum fluoxetine increased serotonin-mediated behavior in both adult male and female offspring. This may not be surprising given the aforementioned evidence that maternal postpartum fluoxetine increases milk concentration of fluoxetine (Wisner et al., 1996). It should be noted that another study did not find a significant effect on swimming behavior after maternal postpartum fluoxetine (Rayen et al., 2011). However, there were differences between studies in terms of dose and administration (Rayen et al., 2011: 5 mg/kg via osmotic mini-pump) as well as age at testing (Rayen et al., 2011: adolescence). Maternal postpartum fluoxetine did not significantly alter immobility, which is inconsistent with studies showing that maternal fluoxetine (7.5 mg/kg/day) increased immobility in adult female but not male mice offspring (Lisboa et al., 2007). However, dose and species differences could account for this discrepancy, as forced swim test outcomes differ between mice and rats (Slattery and Cryan, 2012). Our results confirm previous work with this model of PPD in which maternal postpartum CORT did not significantly affect depressive-like

behavior of adult offspring in the forced swim test (Brummelte et al., 2006, 2012). Our findings show that maternal postpartum fluoxetine exerts enduring changes in serotonin-related behavior, which may manifest from disturbances to the developing serotonin system following developmental exposure to fluoxetine.

### Maternal postpartum fluoxetine impaired HPA negative feedback whereas maternal postpartum CORT enhanced HPA negative feedback in adult male offspring

In adult male offspring, maternal postpartum fluoxetine exaggerated stress-induced increase in serum CORT concentrations whereas maternal postpartum CORT blunted stressinduced increase in serum CORT concentrations in the dexamethasone suppression test. To our knowledge, no studies have examined the effects of maternal fluoxetine on HPA axis negative feedback in offspring. One study found maternal postpartum fluoxetine blunted serum CORT in adolescent male but not female rat offspring although samples were collected at the time of perfusion (Pawluski, Rayen, et al., 2012), complicating whether this reflects a basal or stressinduced measure as anesthetics can rapidly increase CORT levels (Wu et al., 2015). Clinical findings indicate that prenatal fluoxetine increased corticosteroid-binding globulin levels in neonates (Pawluski, Brain, Underhill, Hammond, & Oberlander, 2012) and blunted evening levels of serum cortisol in 3 month old infants (Oberlander et al., 2008). Developmental fluoxetine also may alter HPA axis negative feedback by affecting limbic structures that regulate HPA axis activity. For instance, maternal postpartum fluoxetine diminished hippocampal glucocorticoid receptor density in adolescent male but not female rat offspring (Pawluski, Rayen, et al., 2012). Additionally, maternal fluoxetine during gestation and lactation enhanced activation (Fos expression) in the basolateral amygdala and medial amygdala after restraint stress in adult female but not male rat offspring (Francis-Oliveira et al., 2013). Both the hippocampus and

amygdala are sources of limbic control over the HPA axis (Herman and Cullinan, 1997) and could therefore contribute to differences in HPA axis negative feedback. Sex differences in stress circuits may underlie these effects of maternal fluoxetine on HPA axis in males. Although we did not find an effect of maternal postpartum fluoxetine on adult female HPA axis activity, it is possible that our dose of dexamethasone was not sufficient to elicit group differences in CORT concentrations. Basal and stress-induced activity of the HPA axis are generally higher in females compared with males and as seen in our data (compare Figure 5C with 5D; (Goel et al., 2014b). Thus, a higher dose of dexamethasone for females may be necessary to optimally assess HPA axis negative feedback (Osborn et al., 1996).

Interestingly, maternal postpartum CORT blunted serum CORT concentrations in adult male and female offspring. Previous work with using this model showed that after 1 h of restraint, maternal postpartum CORT did not significantly alter serum CORT concentrations in either adult male or female offspring (Brummelte et al., 2006, 2012). This suggests that maternal postpartum CORT results in developmental disturbance specific to negative feedback of the HPA axis. This may be related to the fact the maternal postpartum CORT results in increased brain and serum CORT content in the offspring (Brummelte et al., 2011). Alternatively, maternal postpartum CORT could indirectly affect the developing HPA axis via diminished quality of maternal care (Brummelte et al., 2006, 2012). Indeed, maternal separation (a similar model of maternal stress/neglect) blunted HPA axis activity in juvenile (Litvin et al., 2010) and adolescent male rats (Ogawa et al., 1994). Additionally, clinical evidence suggests that children under conditions of extreme parental neglect in Romanian orphanages exhibit blunted diurnal cortisol release (Carlson and Earls, 1997). Thus, early life adversity, such as maternal postpartum CORT, can induce permanent disruptions to the HPA axis of both male and female offspring.

## Maternal postpartum fluoxetine increased density of doublecortin-expressing cells in the dorsal hippocampus of adult male offspring

Maternal postpartum fluoxetine increased density of doublecortin-expressing cells in the dorsal dentate gyrus in adult male offspring. A prior study also showed that maternal postpartum fluoxetine slightly increased density of doublecortin-expressing cells in adult male offspring and decreased it in the adult female offspring (Rayen et al., 2015). However, our results suggest that after behavioral testing, maternal postpartum fluoxetine stimulates doublecortin expression in the dorsal (but not ventral) dentate gyrus, and only in the adult male offspring. This difference might be attributed to a higher dose of fluoxetine (10 mg/kg) than Rayen et al., 2015 (5 mg/kg). Regardless, maternal fluoxetine appears to increase doublecortin expression in adult males although this may be mitigated by prenatal stress (Rayen et al., 2015), but not by maternal postpartum CORT. Indeed, the increase immature neurons due to maternal fluoxetine was only evident in the male offspring of CORT-treated dams. Although the exact mechanism of how maternal fluoxetine influences adult hippocampal neurogenesis in the offspring is not well understood, there are many possible explanations: serotonergic influences, changes in maternal care, or increased environmental enrichment via behavioral testing.

One explanation for how maternal postpartum fluoxetine could have disrupted adult offspring hippocampal neurogenesis is that fluoxetine present in the milk directly affected serotoninergic regulation of hippocampal neurogenesis. Indeed, direct administration of fluoxetine to pups enhanced CA1 hippocampal dendritic spine density (Zheng et al., 2011) and hippocampal brain derived neurotrophic factor content in adult male mice (Karpova et al., 2009). This enhanced hippocampal plasticity with fluoxetine exposure is in line with findings that adult exposure to chronic fluoxetine administration stimulates hippocampal neurogenesis in adult male

rats (Malberg et al., 2000; Huang and Herbert, 2006; David et al., 2009). Thus, our findings that maternal fluoxetine enhances adult hippocampal neurogenesis parallel findings from studies directly exposing pups to fluoxetine. Direct administration of fluoxetine to pups also diminished serotonin terminals in the dentate gyrus in adult male rats (Silva et al., 2010). This supports the possibility that early exposure to fluoxetine itself from the dam may disrupts serotonergic regulation of hippocampal neurogenesis in the offspring.

Another alternative explanation for how maternal postpartum fluoxetine could have disrupted adult offspring hippocampal neurogenesis is that fluoxetine indirectly affected offspring hippocampal development via alterations in maternal care. In a complimentary study maternal postpartum fluoxetine reversed CORT-induced reductions in maternal care (Workman et al., 2016). Therefore, it is possible that the positive effect of maternal postpartum fluoxetine on maternal care resulted in enhanced neurogenesis. This is in line with findings that higher maternal care (licking and grooming) increases hippocampal plasticity in adult offspring whereas lower maternal care reduces it (Moore and Morelli, 1979; Bredy et al., 2003; Liu et al., 2016). Interestingly, males and females were differentially affected by maternal fluoxetine exposure. Given that there are sex differences in the amount of maternal care pups receive (Moore and Morelli, 1979), it is possible that maternal fluoxetine further skewed the amount of attention male and female pups receive and may explain the opposing effects of maternal fluoxetine on adult offspring hippocampal neurogenesis.

Another reason for maternal fluoxetine to selectively increase the density of immature neurons in males is that behavioral testing constituted exploration of a variety of different apparatuses over 10 days and may have an enriching component. Doublecortin is expressed for up to 21 days after the cell divides in rats (Brown et al., 2003), and it is possible that behavioral

testing altered doublecortin expression. Environmental enrichment increases doublecortin expression in adult male and female rodents (Leal-Galicia et al., 2007; Ramirez-Rodriguez et al., 2014). Interestingly, environmental enrichment increased number of early immature neurons expressing doublecortin in adult male mice selectively in the septal (dorsal) region of the hippocampus (Tanti et al., 2013). This is consistent with our results that maternal postpartum fluoxetine increased doublecortin expression exclusively in the dorsal hippocampus. Therefore, it is possible that maternal postpartum fluoxetine in combination with the enrichment present in the battery of behavioral tests increased neurogenesis specifically in the male offspring. Alternatively, it is possible that the stress of multiple behavioral and neuroendocrine tests diminished doublecortin expression, except in the male subjects exposed to maternal postpartum fluoxetine. Generally, stress reduces hippocampal neurogenesis but is sex- and stressordependent (Gobinath et al., 2015). In adult male rodents, fluoxetine can reverse the stressinduced reduction in hippocampal neurogenesis (Malberg and Duman, 2003; Warner-Schmidt and Duman, 2006). Therefore, it is possible that maternal postpartum fluoxetine buffered against the stress of multiple behavioral tests in the adult male offspring but not in the other experimental conditions.

We also found that maternal postpartum CORT increased density of doublecortinexpressing cells in adult male offspring and diminished the density of doublecortin-expressing cells in adult female offspring. As in the case of maternal postpartum fluoxetine, it is possible that this effect of maternal postpartum CORT can be explained by increased environmental enrichment or stress resulting from behavioral testing. Developmental stress is typically associated with detrimental effects on hippocampal plasticity (Gobinath et al., 2015). However, early life stress in the form of maternal deprivation (Oomen et al., 2010) or low maternal care

(Champagne et al., 2008; Bagot et al., 2009) enhanced long term potentiation of adult-born granule cells in the dentate gyrus only in the presence of CORT. This suggests that early life adversity could promote hippocampal plasticity under mildly stressful conditions, such as multiple behavioral tests. Moreover, there are sex differences in how early life adversity affects the adult hippocampus (Gobinath et al., 2015), which may explain the opposing effects of maternal postpartum CORT on offspring doublecortin expression in this study.

We did not find a significant effect of maternal postpartum CORT or fluoxetine on doublecortin-expression in either males or females in the ventral hippocampus. This was surprising because we observed significant effects of these maternal treatments on anxiety-like behavior and HPA axis negative feedback regulation, and both affective behavior and HPA axis function are associated with ventral hippocampus activity (Fanselow and Dong, 2010b). However, we did observe that among the males exposed to both maternal CORT and fluoxetine, doublecortin expression selectively in the ventral hippocampus was correlated with increased anxiety-like behavior in the elevated plus maze. This suggests that developmental exposure to CORT and fluoxetine may have long-term effects on the association between immature neurons of the ventral hippocampus and neural circuits underlying anxiety-like behavior. *Maternal CORT and concurrent fluoxetine during the postpartum period attenuated body mass* 

#### in adult male offspring

Our results indicate that maternal postpartum CORT and concurrent fluoxetine diminished body mass only in adult male offspring, consistent with a study using prenatal dexamethasone followed by maternal postpartum fluoxetine in adult males (Nagano et al., 2012). This is consistent with findings that maternal fluoxetine diminished body mass in neonatal rats (da-Silva et al., 1999) and infants (Chambers et al., 1999). However, maternal postpartum

fluoxetine *alone* increased body mass in adult male offspring. Perinatal exposure to SSRIs (including fluoxetine) is also associated with being overweight in boys but not girls (7 y; Grzeskowiak et al., 2013). Collectively, this suggests that maternal SSRI use can impact body mass of offspring differently depending on developmental time point and whether exposure occurred exclusively during the prenatal, postnatal, or both periods of development.

#### Conclusions

Our data indicate that maternal postpartum fluoxetine can have long-lasting effects on behavioral, endocrine, and neural outcomes of adult offspring in a sex-specific manner. Specifically, adult male offspring were more vulnerable to the effect of maternal postpartum fluoxetine than the female offspring with regards to anxiety-like behavior, HPA axis negative feedback regulation, and hippocampal neurogenesis. However, both adult male and female offspring exhibited more serotonin-dependent behavior in the forced swim test. Finally, maternal postpartum CORT was associated with blunted HPA activity in adult male and female offspring. Collectively, these findings bear implications for treating mothers with pharmacological antidepressants and highlight the importance of studying the consequences of maternal pharmacology on both male and female offspring.

#### Conflicts of interest

The authors have nothing to declare.

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# Chapter 4: Voluntary running influences the efficacy of FLX in a model of postpartum depression<sup>3</sup>

#### 4.1 Introduction

Physical exercise is beneficial for mood (Rethorst and Trivedi, 2013), but its ability to benefit mood during motherhood is unclear. Indeed, exercise increases neurotrophic factors, neuroplasticity, and hippocampal neurogenesis, which may underlie the numerous benefits of exercise on mental well-being (Voss et al., 2013). However, hippocampal volume, trophic factors, and neurogenesis are reduced during pregnancy and lactation (Hoekzema et al., 2017; Leuner et al., 2007; Pawluski & Galea, 2007). Therefore, it is possible that the capacity for exercise to benefit mood and neuroplasticity is compromised during motherhood. It is imperative to evaluate the efficacy of antidepressant interventions, including exercise, in treating depression specifically in mothers. Approximately 15% of mothers suffer from PPD (Wisner et al., 2013) with 75% of women with maternal depression experiencing their first ever episode of depression (Fisher et al., 2016). Treatment options for PPD are currently limited, and addressing this limitation is necessary for the health of the mother and child. To this end, the following study compares the antidepressant effects of exercise alone or in conjunction with pharmacological antidepressant FLX in a rat model of PPD.

<sup>&</sup>lt;sup>3</sup> Gobinath A.R., Richardson, R. J., Chow, C., Workman, J.L., Lieblich, S.E., Barr, A.M., Galea, L.A.M. (2017). Voluntary running influences the efficacy of FLX in a model of postpartum depression. *Neuropharmacology*, 128: 106-118. Doi 10.1016/j.neuropharm.2017.09.017.

Systematic reviews evaluating efficacy of postpartum use of SSRIs suggest that these drugs are no more effective than placebo in mothers by 3-6 months postpartum (Yonkers et al., 2008; De Crescenzo et al., 2014). Other antidepressants like ketamine (Xu et al., 2017) may have limited preventative benefits in the postpartum. Nonetheless, approximately 6% of mothers use antidepressants after childbirth (Smolina et al., 2015). SSRIs are problematic during pregnancy and the postpartum because they can cross the placenta and remain active in breast milk, potentially affecting infant development (Weissman et al., 2004b). Whether developmental SSRI exposure outweighs the adverse effects of maternal depression on child development remains unclear. For this reason, some mothers hesitate to seek antidepressant treatment for PPD, and some women preemptively disengage from prescription antidepressant use during pregnancy (Prady et al., 2016), risking potential relapse in depression. Alternatively, women may be more compliant in using non-pharmacological antidepressants such as exercise due to fewer side effects and concerns for infant outcome. However, systematic reviews of clinical studies investigating the effects of exercise during pregnancy have been inconclusive regarding whether prenatal exercise or postnatal exercise prevents PPD (Dennis, 2004; Ersek and Brunner Huber, 2009; Daley et al., 2015; Hinman et al., 2015). Both exercise and FLX are well-validated antidepressants outside of pregnancy and postpartum periods but may have altered efficacy during this time. Thus, this study aims to comprehensively evaluate how the combination of both treatments in comparison to either exercise or FLX alone affects maternal outcome in terms of maternal care, HPA axis activity, affective behavior, and hippocampal neurogenesis in a rat model of PPD.

Our laboratory has developed a model of PPD in which a high dose of CORT (primary glucocorticoid in rats) is administered daily during the postpartum period, modelling index depression episode in the postpartum, which accounts for 40% of cases of maternal depression (Fisher et al., 2016). This CORT-induced model of depression capitalizes on the well-established relationship between stress and depression (Angst et al., 2002) including PPD (Seth et al., 2016). Postpartum CORT treatment induces a PPD-like phenotype by reducing time spent with the offspring, increasing depressive-like behaviour, and reducing hippocampal neurogenesis and plasticity (Brummelte et al., 2006; Brummelte and Galea, 2010b; Workman et al., 2013a). We recently reported that maternal postpartum FLX treatment prevented CORT-induced disruptions in maternal care early in the postpartum but was unable to rescue CORT-induced depressive-like behaviour, flattening of HPA axis activity, or reductions in hippocampal neurogenesis in dams (Workman et al., 2016). To expand upon these findings, we sought to determine whether exercise (voluntary running) would prevent CORT-induced depressive-like endophenotypes in dams or potentially boost the efficacy of FLX in the postpartum. We hypothesized that exercise alone, or in conjunction with FLX, would alleviate CORT-induced disruptions in maternal care, depressive-like behavior, HPA axis activity, and hippocampal neurogenesis in dams.

#### 4.2 Methods

#### Animals

Sixty adult female Sprague-Dawley rats (~2 months old) and fifteen adult male Sprague-Dawley rats (2 months old, Charles River, QC) were given rat chow (Jamieson's Pet Food Distributors Ltd, Delta, BC, Canada) and tap water *ad libitum*. Rats were individually housed in transparent polyurethane bins (27 x 25 x 20 cm) with aspen chip bedding and maintained in a 12 h: 12 h light/dark cycle (lights on at 7:00 a.m.). All protocols were in accordance with ethical



**Figure 4-1 Experimental timeline** 

guidelines set by Canadian Council on Animal Care and were approved by the University of British Columbia Animal Care Committee. The timeline of the experiment is depicted in Figure 4-1.

#### Exercise Treatment

Upon arrival in the facility, female rats were randomly assigned to either standard housing with no running wheel ("no exercise;" n=30) or with access to running wheels ("exercise;" n=30; Med Associates Inc., VT, USA) and groups were housed in separate colony rooms. Running wheel activity was recorded daily and in total dams were given access to the running wheel for 8 weeks (± 4 days, due to the variability in length of time to conceive). Dams had voluntary access to running wheel during pre-conception, pregnancy, and postpartum periods in order to: 1) prevent additional postpartum stress (besides CORT treatment) of introducing running wheel during the postpartum, 2) prevent any stress associated with removal

of the wheel at any point, and 3) allow sufficient acclimation to engage in running wheel activity, which can be sensitive to ovarian hormone changes (Park et al., 2016).

#### **Breeding Procedures**

Breeding was conducted as previously described (Workman et al., 2016). During pregnancy, dams were left undisturbed besides weekly weighing and cage-changing. One day after birth (birth day = postpartum day 0), all litters were culled to 4 males and 4 females.

#### Drug Preparation and Treatment

Dams were randomly assigned to one of four treatment groups within both exercise and non-exercise conditions: 1) CORT/FLX (exercise, n=8; non-exercise, n=9); 2) CORT/saline (exercise, n=8; non-exercise, n=8); 3) oil/FLX (exercise, n=7; non-exercise, n=7); 4) oil/saline (exercise, n=7; non-exercise, n=6). Beginning on postpartum day 2, dams received two daily injections of either CORT (40 mg/kg, s.c.) or sesame oil (1 ml/kg and 10% EtOH, s.c.) and FLX (10 mg/kg, i.p.) or saline (1 ml/kg and 10% DMSO) for 24 consecutive days. Dams received both injections in succession between 8:30 A.M. and 10:30 A.M. CORT (Sigma-Aldrich, St. Louis, MO, USA) and FLX (Sequoia Research Products, Pangbourne, UK) were prepared as previously described (Workman et al., 2016). The CORT dose reliably induces a depressive-like phenotype in dams and impairs maternal care (Brummelte et al., 2006; Brummelte and Galea, 2010b; Workman et al., 2013a). The FLX dose increased brain derived neurotrophic factor, and hippocampal cell proliferation after 21 days of injections in both sexes (Hodes et al., 2010).

#### Maternal Behavior

Maternal observations were conducted twice per day from PD 2 – 8. Observations occurred at least 2 h after injections or after previous observation. Each observation lasted for 10 min and duration of the following behaviors were scored: nursing (including arched back nursing, blanket nursing, and passive nursing), licking, nursing and licking, self-grooming, and off nest. Time spent running during maternal observation was also quantified in the exercise groups. Data was summated across all observations.

#### FST

Dams were tested in the FST on postpartum day 22 (FST1) and 23 (FST2) to assess antidepressant efficacy as previously described (Workman et al., 2016). Behavioral testing began approximately at 12:30 p.m. Briefly, dams were placed into clean water for 15 min on postpartum day 22 and for 5 min on postpartum day 23. Sessions were scored blinded for percent time spent immobile during the first and across five minutes using BEST Collection Software (Educational Consulting, Inc., Hobe Sound, FL, USA). We scored the first minute as well as the total of five minutes due to the fact that minute by minute changes are seen in the FST with greater differences in immobility observed early in the test session (des Portes et al., 1998; Mezadri et al., 2011); additionally, because blood was collected after FST1, we reasoned that this may affect immobility in all animals (see below).

#### Blood Collection and Serum CORT Assay

Immediately after FST1, a blood sample was collected via the tail vein to measure stressinduced levels of CORT. During recovery, rats were returned to home cages, and another blood sample was collected 2 h later. Rats in the exercise condition were not allowed access to running wheel during these two hours to prevent exercise-induced elevations in CORT,

Blood samples were stored overnight at 4°C, centrifuged at 10,000 g for 15 min and serum collected and stored at -20 °C until radioimmunoassay. Total CORT (bound and free) was measured in duplicate using the ImmuChem Double Antibody 125I radioimmunoassay Kit (MP Biomedicals, Solon, OH, USA). The antiserum cross-reacts 0.05% with cortisol, and does not cross-react with dexamethasone (<0.01%). Intra-assay coefficient of variation was 5%.

#### NSF

Rats were tested for anxiety-like behavior in the novelty suppressed feeding paradigm as previously described (Mahmoud et al., 2016) 24 h after FST2. In this test, rats must resolve an anxiogenic conflict of entering the center of arena to access a Froot Loop after 16 h of food deprivation (Bessa et al., 2009). Behavioral testing began approximately at 11:00 a.m. Latency to feed was recorded as an index of anxiety-like behavior. The trial was terminated either after the rat began to eat or after 10 min which ever came first. Home cage food consumption was measured for one h after the test.

#### Tissue Collection and Immunohistochemistry

Twenty-four h after novelty suppressed feeding test, rats were given an overdose of sodium pentobarbital and perfused with 60 ml cold 0.9% saline followed by 120 ml cold 4% paraformaldehyde. Adrenal glands were collected and weighed. Brains were extracted, postfixed overnight at 4°C, and transferred to 30% sucrose in phosphate buffer at 4°C. Brains were rapidly frozen with dry ice and sectioned coronally at 40 µm using a freezing microtome (Leica, Richmond Hill, ON, Canada). Sections were stored in series of 10 in antifreeze (ethylene glycol/glycerol; Sigma) at -20°C until processing.

Immunohistochemistry for doublecortin (DCX), an endogenous marker of immature neurons (Brown et al., 2003), was conducted as described (Gobinath et al., 2016). Briefly, between PBS rinses, sections were treated with 0.3% hydrogen peroxide (30 min) and incubated at 4 °C for 24 h in primary antibody solution (1:1000, goat anti-DCX; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Sections were rinsed and incubated in a secondary antibody solution (1:500, rabbit anti-goat; Vector Laboratories, Burlington, ON, Canada) at 4°C for 24 h. Then, rinsed sections were incubated in ABC complex (ABC Elite Kit; 1:1000; Vector) for 4 h. Sections were developed using diaminobenzidine in the presence of nickel (DAB Peroxidase Substrate Kit, Vector), and counterstained with cresyl violet.

#### Microscopy

DCX-expressing cells were quantified in 3 dorsal sections (-2.76 mm to -4.68mm below bregma) and 3 ventral sections (-5.52 mm to -6.60 mm below bregma) using the 40x objective using an Olympus CX22LED brightfield microscope. Areas of these sections were quantified using ImageJ (NIH, Bethesda, MD, USA) and used for density calculations (number of cells per mm<sup>2</sup>). Additionally, 50 DCX-positive cells in dorsal (n=25) and ventral (n=25) dentate gyrus were randomly selected and classified as either proliferative, intermediate, or post-mitotic as previously described (Gobinath et al., 2016).

#### Data Analyses

Litter characteristics were analyzed using independent t-tests. Data from maternal observations, FST, and novelty suppressed feeding test were analyzed using ANOVA with exercise, CORT, and FLX as between-subjects factors. CORT concentrations after the FST were analyzed using repeated measures ANOVA with time as the within-subjects factor. The density of DCX-expressing cells was analyzed using repeated measures ANOVA with region (dorsal, ventral) as the within-subjects factor. Post hoc comparisons used Newman-Keuls. *A priori* comparisons were subjected to Bonferroni corrections. Post-hoc analyses of running data categorized dams as either high or low runners using a median split. All data were analyzed using Statistica software (v. 9, StatSoft, Inc., Tulsa, OK, USA). All effects were considered statistically significant if  $p \le 0.05$ .

#### 4.3 Results

#### Running was reduced during the final week of gestation and with postpartum FLX

Before drug treatment, dams maintained consistent levels of running from pre-conception through the second week of gestation (p's > 0.45). Dams ran less during the third week of gestation in comparison to previous time points (p's < 0.001; main effect of week). Due to the high variability in running we categorized dams as either high or low running using a median split on amount of running before drug treatment began (Figure 4-1A), and subsequent post-hoc analyses were conducted with high and low running as a variable. However, because this resulted in an unbalanced design due to skewed sample sizes within these groups data are presented as exploratory analyses.

Running wheel activity increased from the second to the third week of the postpartum period in saline-treated dams (p < 0.001). However, this increase was not observed in FLX-treated dams [p = 0.47; interaction between postpartum week and FLX; F (2, 52) = 8.24; p < 0.001; Figure 4-2B; Table 4-1].



**Figure 4-2 Individual differences in running wheel activity** A) Individual running data based on average revolutions per day before drug treatment began. Individual dams were categorized as "high running" or "low running" based on a median split (n=15/group). B) During pre-conception and gestation periods, high running dams consistently ran significantly more than low running dams. Regardless of high or low running, dams voluntarily ran at similar levels during pre-conception and first two weeks of gestation. Dams ran less during the third and final week of gestation in comparison to earlier periods. After parturition and regardless of drug treatment, all dams ran at comparable levels during the first two postpartum weeks. During the third week of the postpartum, FLX reduced running in comparison to saline in high runners only.n=6-9/group. \* p < 0.05; FLX: fluoxetine; HR: high running; LR: low running.

		Pre- Conception	<u>Gestation</u> – Week 1	Gestation Week 2	<u>Gestation</u> – Week 3	<u>Postpartum</u> – Week 1	Postpartum – Week 2	Postpartum – Week 3
High Running	Oil/ Saline	21297 ± 2281	22702 ± 1872	21051 ± 2037	9063 ± 1046	4571 ± 464	3214 ± 359	7135 ± 1083*
	Oil/ FLX					3010 ± 619	2361 ± 1039	$2405 \pm 109$
	CORT/Saline					4849 ± 1946	3091 ± 1102	7896 ± 2163*
	CORT/FLX					2429 ± 653	$1892\pm403$	2203 ± 372
Low Running	Oil/ Saline	10912 ± 1561	11879 ± 1150	11069 ± 1150	3948 ± 572	2364	2126	2365
	Oil/ FLX					2452 ± 414	1165 ± 312	$2728\pm506$
	CORT/Saline					3010 ± 1117	3166 ± 673	$4095\pm529$
	CORT/FLX					3198 ± 895	2595 ± 732	2314 ± 425

Table 4-1 Mean running wheel revolutions  $\pm$  SEM. Voluntary running wheel activity increased from the postpartumweek 2 to postpartum week 3 in saline-treated dams but not FLX-treated dams. n=6-9/group. \* p < 0.05 CORT:</td>Corticosterone; FLX: Fluoxetine.

Postpartum FLX under sedentary or high running conditions prevented CORT-induced reductions in time spent nursing and increases in time spent off nest

Postpartum CORT decreased time spent nursing in comparison to controls (p = 0.04), and concurrent FLX prevented this reduction in CORT-treated dams [p < 0.01; interaction between CORT and FLX; F (1, 52) = 4.132, p = 0.05; Figure 4-3A]. No other significant or main effects were present (all p's > 0.2). Follow-up analyses indicate that postpartum FLX significantly increased time spent nursing in high running dams only, indicating greater efficacy with FLX in high runners [p < 0.001; interaction between running amount/FLX; F (2, 48) = 5.42; p = 0.008; Figure 4-3C].

*A priori* comparisons reveal that postpartum CORT/FLX decreased time off nest in comparison to postpartum CORT alone [p < 0.01; interaction between CORT/FLX; F (1, 52) = 2.83, p = 0.098; Figure 4-3B.] Follow-up analyses indicate that postpartum FLX significantly decreased time spent off nest in comparison to saline in high running dams only [p = 0.003; interaction between running amount/FLX; F (2, 48) = 3.43; p = 0.04, Figure 4-3D].

Neither postpartum CORT nor FLX treatment interacted with time spent in the running during maternal observations (all p's > 0.08). There were no significant main or interacting effects of maternal exercise, CORT, and FLX treatment on time spent licking (all p's > 0.10). No exercise/CORT-treated dams spent significantly more time nursing + licking in comparison to no exercise/oil-treated dams [p=0.05; interaction between exercise and CORT; F (1, 52) = 4.67; p = 0.04]. There were no other significant main or interacting effects of maternal exercise, CORT, or FLX treatment on time spent nursing + licking. Postpartum FLX treatment reduced time spent self-grooming in comparison to saline (main effect of FLX; p = 0.009). There were no other significant main or interacting effects, CORT, and FLX treatment on time spent self-grooming (all p's > 0.06). These data are summarized in Table 4-2.

		Mean % time licking ± SEM	Mean % time nursing + licking ± SEM	Mean % time self- grooming ± SEM
	<b>Oil/Saline</b>	$0.4 \pm 0.1$	$10.2\pm2.1$	$9.9 \pm 1.1$
<b>T</b>	Oil/FLX	$0.3 \pm 0.2$	$10.5\pm1.9$	7.9 ± 1.3 *
Exercise	CORT/Saline	$0.6 \pm 0.2$	$8.7 \pm 2.1$	$10.7\pm1.5$
	CORT/FLX	$0.2\pm0.1$	$7.7 \pm 1.4$	$6.8 \pm 1.2*$
	Oil/Saline	$0.3 \pm 0.1$	$7.1 \pm 1.0$	$12.8\pm1.8$
No	Oil/FLX	$0.2\pm0.1$	$8.8\pm1.3$	$7.5 \pm 1.8*$
Exercise	CORT/Saline	$0.8 \pm 0.3$	$13.4 \pm 3.0*$	$12.8\pm2.0$
	CORT/FLX	$0.5\pm0.2$	$11.2 \pm 2.9*$	$11.2 \pm 2.0*$

Table 4-2 Mean percent time spent licking, nursing + licking, and self-grooming ± SEM. Maternal exercise,

CORT, and FLX treatment did not significantly affect time spent licking. Non-exercising/CORT-treated dams spent more time nursing + licking in comparison to non-exercising/oil-treated dams. Postpartum FLX treatment reduced time spent self-grooming in comparison to saline. n=6-9/group. \* p < 0.05; CORT: Corticosterone; FLX: Fluoxetine.



**Figure 4-3 Effects of maternal exercise, CORT, and FLX treatment on maternal behaviour.** A) Regardless of exercise, CORT treatment reduced time spent nursing in comparison to vehicle. FLX increased time spent nursing in comparison to saline in CORT-treated dams. B) Regardless of exercise, CORT/FLX-treated dams spent less time off nest in comparison to CORT/saline-treated dams. C) FLX increased time spent nursing in comparison to saline in high running dams but not low running dams. D) FLX reduced time spent off nest in comparison to saline in high running dams but not low running dams. In figures 4-3C and 4-3D, data from the non-exercising dams are presented again for reference. n=6-9/group. \* p < 0.05; CORT: corticosterone; FLX: fluoxetine; HR: high running; LR: low running.

Exercise reduced immobility while postpartum CORT or FLX increased immobility in FST2

Exercise decreased time spent immobile in comparison to no exercise [main effect of exercise, F(1, 49) = 19.97; p < 0.001; Figure 4-4A]. Postpartum CORT and postpartum FLX increased time spent immobile in comparison to controls [main effect of CORT, F(1, 49) = 10.06; p = 0.003; main effect of FLX; F(1, 49) = 11.53; p = 0.001; Figure 4-4A]. There were no significant effects of running amount (p's > 0.27). We used body mass as a covariate to account for its influence on immobility, and it did not significantly alter our results with main effects of exercise, CORT and FLX still evident.

Across the 5 min session, the main effect of exercise to reduce time spent immobile persisted (p = 0.03; Table 4-3). However, there were no longer any significant effects of either CORT or FLX or any significant interactions (all p's > 0.16).

	No Exercise	Exercise*
Oil/Saline	24.4%	13.0%
Oil/FLX	20.5%	20.0%
CORT/Saline	22.4%	16.7%
CORT/FLX	31.0%	15.6%

Table 4-3 Mean percent time immobile over the entire 5 min session of FST2  $\pm$  SEM. Maternal exercise maintained its significant effect to reduce time spent immobile over the course of FST2. However, the effects of postpartum CORT and FLX were not apparent in analyzing the entire 5 min session. \* p < 0.05; CORT: Corticosterone; FLX: Fluoxetine.

Maternal postpartum CORT impaired, while FLX treatment facilitated, CORT recovery after stress in the CORT-treated dams

Immediately after FST1, both oil-treated and CORT-treated dams showed similar levels of serum CORT (p = 0.10). However, 2 h after FST1, CORT-treated dams maintained elevated serum CORT levels in comparison to oil-treated dams [p < 0.001; interaction between time and CORT; F (1, 43) = 6.831, p = 0.02; Figure 4-4B]. *A priori* comparisons revealed that 2 h after forced swim test, serum CORT levels were significantly lower in oil/saline, oil/FLX, and CORT/FLX dams (p's < 0.001) but not CORT/saline dams [p = 0.13; interaction between time, CORT, and FLX; F(1, 42) = 1.08; p = 0.07; Figure 4-4B]. No other significant main or interacting effects were present (all p's > 0.11). There were no significant effects of running amount (p's > 0.12).

#### Postpartum FLX treatment increased anxiety-like behaviour in NSF

FLX treatment increased latency to feed in NSF in comparison to saline (main effect of FLX; p = 0.047; Figure 4-4C). No other main or interaction effects were present (p's > 0.29). FLX increased latency to feed only among high running dams (p < 0.001) but not low (p = 0.9) or no running dams [p = 0.14; interaction between running amount and FLX; F (2, 48) = 4.4; p = 0.018; Figure 4-4D].

FLX-treated dams and CORT-treated dams consumed significantly less food 1 h after testing than saline-treated dams (main effect of FLX; p = 0.01; main effect of CORT; p = 0.004; Table 4-4). No other main or interaction effects were present (p's > 0.521). We used food consumption as a covariate for the NSF data: there was no significant effect of the covariate (p = 0.13) although it did render the effect of postpartum FLX treatment to be a trend (p = 0.07).


Figure 4-4 Effects of maternal exercise, CORT, and FLX treatment on FST behaviour, HPA axis, and NSF behaviour. A) Postpartum CORT and postpartum FLX independently increased time spent immobile in FST2 in comparison to oil and saline, respectively. Exercise reduced time spent immobile in comparison to no exercise. B) Immediately after FST1, all dams reached similar levels of stress-induced serum CORT regardless of CORT, FLX, or exercise. After 2 h, CORT-treated dams maintained elevated serum CORT levels in comparison to oil-treated dams. There was a slight but significant effect of FLX to reduce serum CORT levels in comparison to saline among CORT-treated dams. C) FLX increased latency to feed in comparison to saline in novelty suppressed test. D) FLX increased latency to feed in comparison to saline in high running dams but not low or no running dams. n=6-9/group. \* p < 0.05; CORT: corticosterone; FLX: fluoxetine; HR: high running; LR: low running. It should be noted that the main effects of FLX are denoted with \* by FLX in the legend.

	SAL	FLX*
No Exercise – OIL	32.7 ± 2.2	29.6 ± 2.9
Exercise – OIL	32.0 ± 2.9	28.3 ± 1.9
No Exercise – CORT	28.9 ± 1.3	23.9 ± 1.4
Exercise - CORT	27.6 ± 2.8	23.5 ± 2.3

 Table 4-4 Mean (± SEM) food consumption 1 h after novelty suppressed feeding test

Postpartum CORT reduced density of DCX-expressing cells whereas exercise and FLX together increased DCX in exercising dams; high running increased density of DCX-expressing cells in ventral hippocampus

FLX increased density of DCX-expressing cells in exercising dams (compared to all other groups p's < 0.03) but decreased the density of DCX-expressing cells in non-exercising dams [p = 0.007; exercise/FLX interaction; F (1, 54) = 3.93; p = 0.05; Figure 4-5B].

Exercise increased density of DCX-expressing cells in ventral (p<0.001) but not dorsal dentate gyrus [p = 0.14; region/exercise interaction; F (1, 54) = 12.41; p < 0.001; Figure 4-5C]. In ventral hippocampus, high running increased density of DCX-expressing cells compared to low (p < 0.001) or no running dams [p < 0.001; region/running amount interaction; F (2, 48) = 6.7; p = 0.003; Figure 4-5D]. There were no significant differences in dorsal hippocampus (p's > 0.42).

Postpartum CORT decreased density of DCX-expressing cells in both dorsal (p < 0.001; Figure 5E) and ventral regions (p < 0.001; Figure 4-5F). The density of DCX-expressing cells was greater in dorsal compared to the ventral dentate gyrus in CORT-treated [p < 0.001] but not oil-treated dams, [p = 0.27; interaction between region/CORT; F (1, 54) = 4.37, p = 0.04].

#### Maternal Postpartum CORT decreased the proportion of post-mitotic DCX-expressing cells

Of saline-treated dams, maternal postpartum CORT tended to decrease proportion of post-mitotic DCX-expressing cells in comparison to oil regardless of hippocampal region [p = 0.06; type, CORT, and FLX interaction; F (2, 108) = 3.80, p = 0.03; Figure 4-5G] with no other significant main or interaction effects (p's > 0.05).

Amount of running during the postpartum positively correlated with density of DCX-expressing cells in the ventral hippocampus only in control dams

Average running wheel activity positively correlated with density of DCX-expressing cells in the ventral hippocampus among oil/saline-treated dams (r = 0.81; p = 0.02; Figure 4-5H) but not among dams of the other drug treatments (p's > 0.40). No other dependent variables significantly correlated with time spent running.



#### Figure 4-5 Effects of maternal exercise, CORT, and FLX treatment on neurogenesis in dams. A)

Photomicrograph of a post-mitotic DCX-expressing cell. B) FLX reduced density of DCX-expressing cells in comparison to saline vehicle in non-exercising dams. However, the combination of exercise and FLX increased density of DCX-expressing cells in comparison to either exercise or FLX alone. C) Exercise increased density of DCX-expressing cells in comparison to no exercise in ventral hippocampus only. D) The neurogenic effect of exercise was significant only in high runners; high running increased density of DCX-expressing cells in comparison to no exercise in ventral hippocampus only. D) The neurogenic effect of exercise was significant only in high runners; high running increased density of DCX-expressing cells in comparison to no or low running in ventral hippocampus only. E) CORT reduced density of DCX-expressing cells in comparison to no exercise in ventral hippocampus. Exercise increased density of DCX-expressing cells in comparison to no exercise in ventral hippocampus. G) CORT reduced proportion of type 3 (post-mitotic) DCX-expressing cells in comparison to vehicle-treated dams, there was a positive correlation in amount of average postpartum running wheel activity and density of DCX-expressing cells in ventral hippocampus. n=6-9/group. \* p < 0.05; CORT: corticosterone; DCX+: DCX-expressing; FLX: fluoxetine; HR: high running; LR: low running. For post-mortem analyses: 1) CORT/FLX (exercise, n=8; non-exercise, n=9); 2) CORT/saline (exercise, n=6).

*Exercise reduced body mass during pregnancy; postpartum CORT reduced body mass by the end of the postpartum* 

During pregnancy, all dams gained body mass (main effect of week; p = 0.001) but exercise dams maintained a lower body mass than non-exercising dams (main effect of exercise; p = 0.001; Table 4-3). As expected, maternal postpartum CORT reduced body mass of the dams in comparison to oil treatment (main effect of CORT; p < 0.001) but no other significant main or interaction effects were present (p's > 0.08).

#### Exercise did not affect litter parameters

Exercise had no significant effects on litter outcome [number of pups: p = 0.36, litter body mass: p = 0.16; litter sex ratio: p=0.45; Table 4-5]. There were no significant effects of running amount (p's > 0.35).

Exercise increased relative adrenal mass among oil/saline dams whereas FLX increased relative adrenal mass among non-exercising/oil dams

Exercise increased relative adrenal mass in comparison to no exercise among oil/saline treated dams (p < 0.001) but not under any drug/hormone condition (p's > 0.68). Postpartum CORT reduced relative adrenal mass in comparison to oil regardless of exercise or FLX exposure (p's < 0.001). Under no exercise/oil conditions, FLX treatment increased relative adrenal mass in comparison to saline [p<0.001; exercise, CORT, and FLX interaction; F (1, 50) = 5.33; p = 0.03; Table 4-5].

		Dams			Offspring				
		Body Mass G0 (g)	Body Mass G21 (g)	Body Mass P25 (g)	Relative Adrenal Mass (g/100 g bw)	# of pups	Ratio of male: female pups in birth litter	Litter mass at birth (g)	Litter mass on P22 (g)
Exercise	Oil/Saline	239 ± 3*	406 ± 7*	323 ± 16	0.31 ± 0.01*	14.6 ±0.5	0.9 ± 0.1	101.9 ± 3.2	559 ± 17
	Oil/FLX			336 ± 19	0.29 ± 0.02				523 ± 28
	CORT/Saline			276 ± 14*	0.11 ± 0.01				525 ± 32
	CORT/FLX			273 ± 7*	0.11 ± 0.01				423 ± 21
No Exercise	Oil/Saline	267 ± 7	431 ± 7	344 ± 15	0.22 ± 0.02	15.3 ± 0.5	$1.1 \pm 0.1$	107.9 ± 2.9	568 ± 13
	Oil/FLX			329 ± 8	$\begin{array}{c} 0.28 \pm \\ 0.02 \end{array}$				501 ± 21
	CORT/Saline			293 ± 10*	0.10 ± 0.01				504 ± 12
	CORT/FLX			274 ± 8*	0.12 ± 0.01				423 ± 12

Table 4-5 **Body mass during pregnancy and postpartum**. Exercising dams maintained lower body mass from the beginning to end of pregnancy in comparison to no exercise. At the time of perfusion, CORT treatment reduced body mass in comparison to oil and reduced relative adrenal mass in dams. Under vehicle conditions, exercise increased adrenal mass in comparison to no exercise. Under no exercise/oil conditions, FLX increased adrenal mass in comparison to saline. Exercise had no significant effect on number of pups, ratio of male:female pups, or mass of the

litter at the time of parturition. At the final week of the postpartum period, both maternal postpartum CORT as well as FLX independently reduced litter mass in comparison to oil and saline, respectively. n=6-9/group. CORT: Corticosterone; FLX: Fluoxetine; G0: day 1 of pregnancy (conception); G21: day 21 of pregnancy. \* denotes p < 0.05.

#### 4.4 Discussion

Few studies have investigated antidepressant efficacy in models of maternal depression, raising concerns regarding how to best treat PPD. Postpartum CORT reduced maternal care, increased depressive-like behavior, and reduced hippocampal neurogenesis, which is consistent with our prior results. Intriguingly, voluntary exercise, but not FLX treatment, prevented CORT-induced depressive-like behavior and increased hippocampal neurogenesis. Additionally, FLX alone or the combination of high maternal exercise and postpartum FLX prevented CORT-induced reductions in maternal care. These results suggest that voluntary exercise, particularly with greater running amount, could be used as an effective treatment or adjunct treatment with FLX. One caveat is that FLX and high running also increased anxiety-like behavior which is often an initial side effect of FLX in patients (Messiha, 1993). Collectively, these data suggest that exercise and FLX differentially impact CORT-induced endophenotypes of PPD (summarized in Table 4-6).

Endophenotype of PPD	CORT	FLX only	Exercise only	FLX + Exercise
Body mass	Ļ	Ļ	$\leftrightarrow$	$\leftrightarrow$
Loss of interest in litter (anhedonia)	1	Ļ	$\leftrightarrow$	↓ (high running)
HPA axis activity	1	↓ (recovery)	$\leftrightarrow$	$\leftrightarrow$
Depressive-like behaviour (forced swim test)	1	1	Ļ	Ļ
Hippocampal neurogenesis (density of DCX-expressing cells)	Ļ	Ļ	↑ (high running)	↑

Table 4-6 Summary of the effects of maternal postpartum CORT on endophenotypes of postpartum depression and how FLX, exercise, and FLX + exercise affected these measures under CORT-conditions. Maternal

postpartum FLX prevented disruptions in maternal care and serum CORT recovery after FST1. Maternal exercise also prevented disruptions in maternal care and depression-like behaviour in FST2. Further, FLX + exercise or high running alone increased hippocampal neurogenesis in comparison to controls. CORT: Corticosterone; DCX: doublecortin; FLX: Fluoxetine; FST1: forced swim test, day 1; FST2: forced swim test, day 2; HPA: hypothalamicpituitary-adrenal

 $\uparrow$ : increased; ↓: decreased;  $\leftrightarrow$ : no significant change

Postpartum FLX prevented CORT-induced disruptions in maternal care under sedentary and high running conditions; exercise did not impact litter parameters.

We replicated previous findings that FLX treatment prevented CORT-induced reductions in maternal care in sedentary conditions (Workman et al., 2016). Interestingly, voluntary exercise itself was not sufficient to prevent CORT-induced reductions in maternal care, but rather the combination of high levels of voluntary running and postpartum FLX treatment were sufficient. Possible mechanisms underlying this observation are changes in hippocampal plasticity (discussed below) or alterations in serotonin transmission. High intensity aerobic exercise but not low or moderate levels increased serum serotonin levels in humans (both men and women; Zimmer et al., 2016), and running is associated with increased brain serotonin in rodents (only males studied; Liu, Wu, Liu, Li, & Xie, 2017; Wang, Chen, Zhang, & Ma, 2013). Serotonin transmission can in part regulate maternal behavior. Therefore, modification of maternal behavior by FLX under sedentary and high running conditions may be indicative of altered serotonergic tone. Cortical and hippocampal serotonin levels are naturally lower during pregnancy and postpartum (Desan et al., 1988) and exposure to glucocorticoids may exacerbate this reduction (Bambico et al., 2009). Although the exact role of serotonin in maternal behaviour

is still under investigation, serotonin turnover is higher in the preoptic area of the hypothalamus, a region crucial for expression of maternal behaviour (Lonstein et al., 2003). Therefore, under high CORT conditions, it is possible that FLX preserves maternal behavior by increasing synaptic serotonin levels and that high running further bolsters serotonin transmission and subsequently the efficacy of FLX. Further research could explore the interaction between stress and serotonin in the postpartum brain, particularly the hippocampus and hypothalamus, to clarify the neural mechanism underlying CORT-induced perturbations to maternal behaviour. Although the experiment did not originally intend to distinguish high and low-running dams, an interesting observation is that neither CORT nor FLX significantly affected maternal care in low-running dams; future studies can attempt to characterize whether low intensity exercise is beneficial for maternal care or is not sufficient as an adjunct therapy to FLX.

Strenuous exercise could impair milk production (Treadway and Lederman, 1986) or alter milk nutrition content (Matsuno et al., 1999) in women, thereby impacting infant development. Although our study did not directly measure this, our findings indicate that exercise (regardless of high or low running) during gestation did not significantly affect size or sex ratio of birth litter. Furthermore, continuing to exercise after parturition did not affect pup growth by the time of weaning, suggesting that any effects of exercise on milk content were minimal. These observations are in line with similar findings that maternal exercise has little impact on infant growth in humans (Dewey et al., 1994; Su et al., 2007; Daley et al., 2012), lactational competency (Prentice, 1994) or pup growth in rodents (Rosa et al., 2012). Importantly, while exercise itself did not affect postnatal growth, it was not sufficient to prevent the negative effects of maternal postpartum CORT and FLX exposure on pup growth or maternal

body mass. Therefore, although exercise itself may not necessitate concern during breastfeeding, its limited efficacy in metabolic measures of dams and offspring may be important to note in cases of maternal depression or maternal use of FLX.

### Postpartum CORT treatment impaired serum CORT recovery after stress and FLX treatment improved recovery in the CORT-exposed dams

Maternal postpartum FLX facilitated serum CORT recovery after acute stress (FST1) in CORT-treated rats. Prior research suggests that antidepressant efficacy may be partly attributed to the ability of antidepressants to normalize HPA axis activity (Ising et al., 2007). The postpartum is characterized by higher basal levels of CORT, attenuated HPA axis activation, and lower CORT-binding-globulin levels (i.e. increased free CORT), and these alterations may contribute to mood disturbances in mothers (Seth et al., 2016). Although we previously found that FLX had no effect on CORT recovery 90 minutes after stress in CORT-treated dams, the means were in the predicted direction (Workman et al., 2016). In the present study maternal FLX improved CORT recovery 2 h after swim stress, which may be reflective of timing- or conditionspecific effects. The present study measured serum CORT recovery after FST1 whereas the previous study (Workman et al., 2016) measured CORT recovery after FST2 (the "test" session). Thus, it is possible that FLX facilitated CORT recovery in response to the initial acute stress exposure but not later after the second and shorter stress exposure. Importantly all dams had comparable stress-induced levels of serum CORT regardless of CORT treatment. Together with the stress recovery measure, this indicates that our dose of CORT increased and flattened HPA axis tone to a level relevant to postpartum female rat physiology, thus modelling hypercortsolemia and depression (Stetler and Miller, 2011) including PPD (Seth et al., 2016).

Previous research demonstrated that exercise increases basal levels of glucocorticoids and adrenal gland mass, particularly the adrenal cortex (Droste et al., 2007). Although the present study observed that exercise increased adrenal mass, exercise did not affect stress-induced or recovery serum CORT levels. However, it is possible that postpartum-induced glucocorticoid changes masked any exercise-induced changes in CORT and that exercise may have not been able to further stimulate HPA axis activity under the conditions of postpartum physiology. *Maternal exercise reduced depressive-like behaviour whereas maternal postpartum FLX increased anxiety-like behaviour and depressive-like behaviour* 

Exercise, regardless of CORT or FLX, reduced depressive-like behavior in FST2, consistent with previous findings in male and non-parturient female rodents (Lapmanee et al., 2013; Yau et al., 2014; Han et al., 2015a). Our findings are also consistent with clinical findings that exercise is efficacious for PPD (Armstrong and Edwards, 2003; Daley et al., 2015). However, an element of social support also accompanied those clinical exercise interventions, obscuring the degree to which depression relief was attributable to exercise. Findings from the present study indeed point to the role of exercise itself in reducing maternal depressive-like behavior. Notably, another possible explanation is that exercise increased overall cardiovascular and muscular fitness or reduced adipose tissue, rendering exercise dams more adept than sedentary dams in active coping behavior in FST2. Although these factors have not been explicitly studied in forced swim test, voluntary running in rats is beneficial for cardiovascular and skeletal muscle health (Pósa et al., 2015; Mobley et al., 2017) which may ultimately contribute to forced swim test performance. However, using body mass as a covariate still resulted in the main effect of exercise to reduce immobility in the FLX. Indeed, voluntary

running was substantially reduced during the postpartum, limiting the likelihood that robust cardiovascular fitness was involved. Moreover, if more running directly increased fitness, then it would then be reasonable to predict that high running dams would be the most fit. However, there were no significant effects of high or low running in the FST. These data collectively suggest that maternal exercise can reduce depressive-like behavior in this test.

Maternal FLX treatment increased both depressive-like behaviour and anxiety-like behavior (which was partially explained by reduced food consumption), partially consistent with other studies (Mahmoud et al., 2016; Pawluski et al., 2012). A lower dose of FLX also increased both anxiety-like and depressive-like behaviors in non-parous female rats (Mahmoud et al., 2016). Similarly, prenatal stress followed by postpartum FLX treatment of rat dams increased anxiety-like behavior in the elevated zero maze (Pawluski et al., 2012). Although SSRIs are prescribed to improve mood, few studies have tested whether antidepressants are efficacious in the postpartum, and of those conducted, the findings are inconclusive. Studies of antidepressant efficacy are limited by high attrition rates, differences in the baseline depression score, low sample size, and equal efficacy between SSRI and placebo treatment (Yonkers et al., 2008; Sharma and Sommerdyk, 2013; De Crescenzo et al., 2014; Hantsoo et al., 2014). Research from preclinical models, including the present study, supports the limited efficacy of long-term SSRI treatment in dams (Workman et al., 2016), unless dams are continuously exposed to the SSRI via osmotic mini-pump (Haim et al., 2016). Further, preclinical research has observed efficacy of FLX on maternal depressive-like behavior early in the postpartum on postpartum day 7 (Salari et al., 2016a). This is an interesting observation as the present study and previous work with this model (Workman et al., 2016) found early beneficial effects of FLX on CORT-induced

disruptions in maternal care (postpartum days 2-8). Thus, it is possible that SSRIs have a positive effect early in the postpartum but are ineffective with prolonged use, i.e. by the end of the postpartum period. Indeed, 57% of depressed patients who find antidepressant relief with SSRIs also relapse despite continual use of medication (Byrne and Rothschild, 1998), and the trajectory of this cyclical relief and relapse with SSRI treatment may be different in mothers. It should also be noted that the effect of postpartum FLX treatment was apparent early in FST2 but not over the entire duration of FST2; however, this partially contradicts findings from Workman et al., 2016, perhaps due to the additional stress (tail bleed) after FST1. PPD is a heterogenous disease with distinct symptom profiles depending on history of depression, timing of index episode, and presence of concurrent mood disorders (Postpartum Depression: Action Towards Causes and Treatment Consortium, 2015). An interesting avenue of future research would be to utilize rodent models to discern whether there are precise time windows and duration of exposure when SSRI intervention in a maternal depression model can yield positive effects on mood.

## The combination of exercise and FLX treatment increased neurogenesis, and high running alone increased neurogenesis in ventral hippocampus

Maternal exercise, specifically high running, increased neurogenesis in the ventral dentate gyrus of dams, consistent with studies in male and non-parturient female rodents (van Praag et al., 1999; Yau et al., 2014) and confirming this exercise-induced neurogenic effect in the postpartum. Interestingly, this increase occurred specifically in the ventral dentate gyrus, which is functionally relevant for stress regulation (Fanselow and Dong, 2010a). Interestingly, others have observed that exercise predominantly increases neurogenesis in dorsal but not ventral hippocampus in male rodents (Tanti & Belzung, 2013; Vivar et al., 2016), highlighting that

exercise affects different sub-regions of the hippocampus differently during motherhood. This combinatory effect of exercise and FLX was also observed in maternal behaviour, particularly in high runners, and perhaps contributed to preventing CORT-induced disruptions in maternal care. Because neurogenesis levels are low during the postpartum due to increased circulating glucocorticoids (Leuner et al., 2007; Chapter 2; Workman et al., 2016), it is possible that a more potent manipulation such as high running was necessary to bolster neurogenesis. Furthermore, the difference in voluntary running activity between high runners and low runners in the present study was greatest during pre-conception and gestation. After parturition, all the dams engaged in similar levels of running wheel activity. This suggests that for high runners, activity earlier in the experiment was more influential on hippocampal plasticity, resulting in increased density of DCX-expressing cells by the end of the postpartum. It is noteworthy that DCX expression can last for up to three weeks (Brown et al., 2003). Thus, while high running dams engaged in the most amount of running prior to parturition, high running likely had limited direct effects on DCX expression by the end of the postpartum period (approximately 3 weeks after parturition). However, running prior to parturition could have influenced DCX expression *indirectly* by the end of the postpartum period perhaps by increasing trophic factors that promoted generation of DCX-expressing cells. Further research could determine how running influenced the survival of cells generated prior to parturition in the postpartum hippocampus by using BrdU labelling; however, it should be noted that BrdU given during gestation could have cytotoxic effects for the developing offspring which may influence maternal outcomes. Further research could determine how running influenced the survival of cells generated prior to parturition in the postpartum hippocampus by using BrdU labelling; however, it should be noted that BrdU in the doses given

to adult rats (200mg/kg) can have cytotoxic effects for the developing offspring which may influence developmental and maternal outcomes and thus was not used in the present study.

Interestingly, FLX increased neurogenesis only under exercise conditions while FLX reduced neurogenesis under sedentary conditions. This interaction is important for two reasons. First, under sedentary conditions, FLX decreased neurogenesis in addition to increasing depressive-like and anxiety-like behavior in dams. Collectively, these observations appear to be contrary to the expected antidepressant effects of FLX, but they underscore the gaps in our understanding how antidepressant interventions specifically in the postpartum affect the maternal brain and behavior. Indeed, studies examining effects of SSRIs on the maternal hippocampus observe either no significant effect on neurogenesis (Chapter 2; Workman et al., 2016) or a neurogenic effect when the SSRI is administered continuously via osmotic mini-pump (Pawluski et al., 2012). As previously mentioned, there is limited evidence for postpartum antidepressant efficacy, and it would be reasonable to expect limited effects of SSRIs in the postpartum hippocampus. Secondly, exposure to both exercise and FLX increased neurogenesis in the postpartum hippocampus despite FLX reducing amount of voluntary running in the postpartum. Thus, it is possible that the high running earlier in the experiment with its commensurate elevated neurogenesis is required for the neurogenic attributes of postpartum FLX. This may be due to increased serotonergic tone because exercise during pregnancy increased density of serotonin-positive cells in the dorsal raphe nucleus by the end of the postpartum (Seo et al., 2013). Additional research is necessary to understand how interventions in pregnancy alter the postpartum brain. It is also plausible that within the normal conditions of postpartum physiology, including reduced neurogenesis and increased basal levels of glucocorticoids, a more robust

regimen of antidepressants may be necessary to elicit changes in the postpartum hippocampus. Given the concerns regarding maternal SSRI exposure on child development, it may be of interest for further research in PPD treatments to consider a multimodal approach (i.e. a combination of pharmacological and non-pharmacological) as opposed to higher doses of SSRIs or other pharmacological antidepressants. Indeed, there is evidence that cell survival in the dorsal raphe nucleus (a major source of serotonergic innervation) is reduced in postpartum rat dams, an effect mediated by presence of pups (Holschbach and Lonstein, 2017). Furthermore, (Banasr et al., 2001) showed that serotonin and estradiol interact to increase in cell proliferation in the dentate gyrus of adult female rats but more research should discern the dynamics between serotonin and hippocampal neurogenesis in the postpartum brain.

In conclusion, we show that exercise, but not FLX, can reduce maternal depressive-like behavior and that exercise coupled with FLX increased hippocampal neurogenesis. Further, FLX alone or in combination with high running prevented CORT-induced disruptions in maternal behavior. These independent and coordinated effects of an SSRI and exercise as antidepressants on CORT-induced endophenotypes of PPD highlight that maternal physiology is unique and underscore the importance of reproductive experience as a factor in translation of animal models of mood disorders. This study indicates that the postpartum affects efficacy of different types of antidepressant interventions and contributes a better understanding of maternal mood disorders such as PPD.

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#### Conflicts of interest

The authors have nothing to declare.

# Chapter 5: Maternal exercise increases while maternal FLX decreases adult neurogenesis of offspring<sup>4</sup>

#### 5.1 Introduction

Quality of maternal care is a crucial factor in the neurodevelopmental outcome of children (Kaffman and Meaney, 2007). Maternal mood disorders, such as postpartum depression (PPD), are not only devastating for maternal mental health, but can also perturb maternal caregiving (Lovejoy et al., 2000). PPD is associated with disengaged and withdrawal of maternal care and even hostility towards the infant (Lovejoy et al., 2000). Altered patterns of maternal behavior can influence child development and increase risk for adverse behavioral outcomes (Brummelte and Galea, 2016). Prescription antidepressants such as selective serotonin reuptake inhibitors (SSRIs) like fluoxetine (FLX; Prozac) can be prescribed for PPD. However, FLX remains active in breast milk and can directly reach the developing infant (Weissman et al., 2004a). Moreover, it is unclear if FLX is efficacious in alleviating mood disturbances and restoring maternal caregiving behaviors in women (Sharma and Sommerdyk, 2013). For these reasons, it is unclear whether risking neonatal FLX exposure outweighs potential therapeutic effects of FLX. Regardless, it is estimated that 6% of mothers utilize prescription antidepressants after birth (Smolina et al., 2015). Given the potential risks of pharmacological antidepressants on infant health, mothers may more inclined to use non-pharmacological antidepressant interventions such as exercise. However, whether maternal exercise interacts or prevents the

<sup>&</sup>lt;sup>4</sup> Gobinath A.R., Wong, S., Chow, C., Lieblich, S.E., Barr, A.M., Galea., L.A.M. (2017, under review). Maternal exercise increases while maternal fluoxetine decreases adult neurogenesis of offspring, *Psychoneuroendocrinology*.

long-term effects of PPD on offspring development remains unknown. The goal of the present study is to use a rodent model of PPD and compare how different types of maternal antidepressant exposure (FLX vs. exercise) affect long-term outcomes of adult male and female offspring.

Research attempting to ascertain whether risks of neonatal exposure to SSRIs outweigh the adverse effects of untreated PPD on child outcome is inconclusive. This ambiguity may be explained by methodological considerations because the effects of neonatal SSRI exposure depend on whether sex differences, timing of exposure, and concurrent maternal depression were analyzed. PPD is associated with increased risk for depression particularly in girls (LeMoult et al., 2015) and lower IQ particularly in boys (Azak, 2012). Given the sex differences in sensitivity to untreated PPD, it is reasonable to predict that these sex differences persist when examining how treating PPD affects offspring outcome. For example, maternal FLX exposure can impair the negative feedback system of the hypothalamic-pituitary-adrenal (HPA) axis in response to dexamethasone administration in adult offspring depending on these factors. Without a model of maternal depression, maternal FLX treatment throughout gestation and postpartum impaired HPA axis negative feedback in both adult male and female mice offspring (Avitsur et al., 2016; Avitsur, 2017). However, when using a rat model of PPD, maternal FLX treatment selectively in the postpartum impaired HPA axis negatively feedback only in adult male but not female offspring (Chapter 3; Gobinath et al., 2016). Impaired HPA axis negative feedback is biomarker of depression (Ising et al., 2007). Therefore, the translational implications of maternal SSRI use on risk for depression in offspring based on these preclinical findings may be sex-specific and more related to postpartum exposure. In clinical studies, maternal SSRI use has been associated

with increased risk for autism (Brown et al., 2017). However, this effect is also moderated by concurrent maternal depression (Viktorin et al., 2017) and is particularly linked to exposure during the first trimester (Brown et al., 2017; Sujan et al., 2017) although the role of sex is not yet clear. Collectively, these studies underscore the importance of sex, timing, and concurrent maternal adversity as factors in evaluating the conflict between risk of neonatal SSRI exposure versus untreated PPD on offspring behavioural outcome. The following study will compare how maternal postpartum FLX exposure treating a model of PPD selectively in the postpartum differentially impact adult male and female outcomes.

Unlike maternal FLX exposure, maternal exercise is relatively less controversial with generally beneficial effects reported on offspring outcome. For example, maternal exercise mitigates the risk for obesity and metabolic measures on offspring outcome (Blaize et al., 2015; Vega et al., 2015; Wasinski et al., 2015). Further, as in the case of maternal SSRI exposure, maternal exercise can affect offspring in a sex-specific manner. For example, maternal exercise prevented insulin resistance in male, but not female, offspring in response to maternal high-fat diet (Fernandez-Twinn et al., 2017). These beneficial effects of maternal exercise also include reduced anxiety-like behaviour in rat pups (both sexes; Aksu et al., 2012), increased hippocampal cell proliferation in adolescent rats (only females studied; postnatal day 36; Bick-Sander, Steiner, Wolf, Babu, & Kempermann, 2006), and number of neurons in the CA1 and CA3 subfields of the hippocampus in adult rats (both sexes; postnatal day 120; Dayi et al., 2012). However, these studies did not challenge the dams with a concurrent model of maternal depression. This point is particularly important because it remains unclear whether the beneficial effects of maternal exercise presented in the context of maternal depression. In dams exposed to

prenatal stress, maternal forced treadmill exercise did not prevent prenatal stress-induced anxiety-like behaviour in adult male offspring (Lee et al., 2016). This could indicate limitations of exercise to offset the effects of prenatal stress although the use of forced exercise may have also been stressful (Griesbach et al., 2012). Recently, we used a voluntary exercise intervention over the course of pre-conception, gestation, and postpartum in a CORT-induced rat model of PPD. We found that voluntary running did not prevent reductions in quality of maternal care, but it reduced maternal depressive-like behaviour in the forced swim test and increased neurogenesis in the dam with concurrent FLX treatment (Chapter 4; Gobinath et al., under revision). To further expand on the developmental effects of exercise we compared the effects of maternal postpartum FLX and/or maternal voluntary exercise to impact adult male and female outcomes.

To address whether the effects of maternal postpartum FLX and/or maternal exercise exposure would outweigh the effects of PPD, we used a rat model of CORT-induced PPD and observed adult male and female offspring for anxiety-like behaviour in the novelty suppressed feeding test, HPA axis negative feedback in the dexamethasone suppression test, and hippocampal neurogenesis (density DCX-expressing immature neurons). In this model of PPD, dams are treated daily with high levels of CORT from postpartum days 2 – 25 to induce a depressive-like phenotype. This methodology models index episode of depression occurring after parturition, which is characteristic of 40% of women with PPD (Fisher et al., 2016). Our laboratory has shown that maternal postpartum CORT treatment reduces voluntary engagement with the litter, increases maternal depressive-like behavior in the forced swim test (Chapter 2; Brummelte & Galea, 2010; Brummelte, Pawluski, & Galea, 2006; Workman et al., 2016; Workman, Brummelte, & Galea, 2013; Gobinath et al., under review). We have also shown that concurrent FLX treatment prevented CORT-induced disruptions in maternal care but also

increased anxiety-like behavior, HPA axis activity, and hippocampal neurogenesis after a battery of behavioral tests in adult male but not female offspring. The present study will expand on these findings by comparing how maternal exercise (i.e. access to voluntary running wheel from preconception through weaning) affected adult male and female offspring neurodevelopmental outcome. Because exercise is generally associated with beneficial effects for maternal mental and physical health, we hypothesized that maternal exercise alone or in conjunction with FLX would offset the developmental effects of maternal postpartum CORT exposure on offspring outcome. Further, we hypothesized that overall males would be more sensitive to these maternal treatments than females.

#### 5.2 Methods

#### Animals

Thirty-two adult female Sprague-Dawley rats (2 - 3 months old) and 16 adult male Sprague-Dawley rats (2 - 3 months old, Charles River) were initially housed in same-sex pairs in opaque polyurethane bines  $(24 \times 16 \times 46 \text{ cm})$  with aspen chip bedding. Rats were maintained in a 12 h: 12 h light/dark cycle (lights on at 7:00 a.m) and given rat chow (Jamieson's Pet Food Distributors Ltd, Delta, BC, Canada) and tap water ad libitum. For an overview of experimental procedures, refer to Figure 1. All protocols were in accordance with ethical guidelines set by Canada Council for Animal Care and were approved by the University of British Columbia Animal Care Committee. Please refer to Figure 5-1 for an experimental timeline.



**Figure 5-1 Experimental timeline** 

#### Exercise Treatment

Upon arrival in the conventional facility, female rats were randomly assigned to either standard housing conditions with no running wheel ("non-exercise;" n=32) or housing with voluntary access to running wheels ("exercise;" n=30; Med Associates Inc., VT, USA). Female rats in the exercise and non-exercise conditions were housed in separate colony rooms. Running wheel activity was recorded daily via an external electronic LCD counter. Dams could voluntarily run from arrival in the facility (pre-conception) throughout pregnancy and postpartum periods.

#### **Breeding Procedures**

One week after habituation to the facility (and exercise intervention), breeding procedures began as described in (Workman et al., 2016). One day after birth (birth day = postnatal day 0), all litters were culled to 4 males and 4 females (postnatal day 1). Beginning on postpartum day 2, all dams received two daily injections of either CORT (40 mg/kg) or sesame oil (1 ml/kg) and intraperitoneal FLX (10 mg/kg) or saline (1 ml/kg) for 24 consecutive days. The effects of maternal postpartum CORT/saline on maternal behavior and depressive-like behavior were verified in the dam and outcome of the dams will be published separately (Gobinath et al., under review). Pups were weaned on postpartum day 25 and pair-housed with an unrelated, same-sex cage mate whose mother received the same treatment. No more than 2 males and 2 females were taken from each litter for the behavioral tests. Besides weekly cage changing, offspring remained undisturbed until behavioral testing (age: 125-135 days old). Thus, a total of 158 offspring were used in this study with 16 experimental groups. Adult male and female of the following maternal treatments were used: 1) CORT/exercise/FLX (n=10/sex); 2) CORT/exercise/saline (n=10/sex); 3) CORT/no exercise/FLX (n=10/sex); 4) CORT/no exercise/FLX (n=10/sex); 7) oil/no exercise/FLX (n=10/sex); 8) CORT/no exercise/saline (n=10/sex); 7) oil/no

#### Maternal treatments

Within exercise and non-exercise conditions, dams were randomly assigned to one of four antidepressant treatment groups: 1) CORT/FLX; 2) CORT/saline; 3) Oil/FLX; 4) Oil/saline. Beginning on postpartum day 2, dams received two daily injections of either subcutaneous CORT (40 mg/kg) or sesame oil (1 ml/kg) and intraperitoneal FLX (10 mg/kg) or saline (1 ml/kg) for 22 consecutive days. The effects of maternal postpartum CORT/saline on depressive-like behavior were verified in the dam and data investigating maternal outcome are published separately (Gobinath et al., under review), Dams received both injections in succession between 8 A.M. and 10 A.M. Pups were weaned on postpartum day 25 and pair-housed with an unrelated, same-sex cage mate whose mother received the same treatment. Besides weekly cage changing, offspring remained undisturbed until behavioral testing in adulthood.

#### Drug preparation

An emulsion of CORT (Sigma-Aldrich, St. Louis, MO, USA) was prepared and delivered subcutaneously. The dose of 40 mg/kg was chosen because it reliably induces a depressive-like phenotype in dams, impairs maternal care, and affects offspring development (Brummelte et al., 2006, 2011; Brummelte and Galea, 2010b; Workman et al., 2013a, 2016). The control injection for CORT was "oil" and consisted of 10% ethanol in sesame oil. FLX (Sequoia Research Products, Pangbourne, UK) was also prepared and delivered intraperitoneally. The dose of 10 mg/kg was because it increased brain derived neurotrophic factor and cell proliferation in the hippocampus and amygdala after 21 days of injections in both male and female rodents (Hodes et al., 2010). The control injection for FLX was "saline" and consisted of 10% DMSO in 0.9% saline.

For the dexamethasone suppression test, a solution of dexamethasone (Sigma Aldrich) was prepared 1-2 days prior to the test by dissolving dexamethasone in propylene glycol and adjusted to yield a final dose of 50 ug/kg dexamethasone in propylene glycol in males and 100 ug/kg in females. This dose and timing of dexamethasone injection were chosen based on previous studies (Cole et al., 2000).

#### NSF test

Beginning at postnatal day  $125 \pm 5$ , rats (n=10/sex/group) underwent the novelty suppressed feeding test. Behavioral testing occurred at 9:00 A.M. each day under dim light conditions. In this test, rats must resolve an anxiogenic conflict of entering the center of arena to access a Froot Loop after being food deprived (Bodnoff et al., 1989; Santarelli et al., 2003; Bessa et al., 2009; Mahmoud et al., 2016). Food was removed from rats' cages 16 h prior to testing to 151 motivate food consumption during the test session. Each rat was placed in a square arena (60 x 60 cm) facing the right corner. Latency to feed was recorded in seconds as an index of anxietylike behavior. The trial was terminated either after the rat began to eat or after 10 min if the rat did not eat. Lab chow was added to the cages after testing, and food consumption was measured in each cage 1 h after test to assess whether feeding behavior was altered by maternal exercise, postpartum CORT, or postpartum fluoxetine exposure.

#### Dexamethasone Suppression Test

Approximately 48 h after novelty suppressed feeding, rats were tested for HPA axis negative feedback using the dexamethasone suppression test. Dexamethasone was administered to all rats subcutaneously 90 min prior to a 30 min restraint stressor. Males received a dose of 50 ug/kg and because of the well-documented sex differences in HPA axis activity (Goel et al., 2014b), females were given a higher dose that has been previously used in female rats (100 ug/kg; Mahmoud et al., 2016). Tail blood samples were collected at the beginning of restraint (t=0), the end of restraint (t=30), and 1.5 h after cessation of restraint (t=120).

#### CORT Assay

Blood samples were stored overnight at 4°C to allow blood to clot completely. Blood was then centrifuged at 10,000 g for 15 min. The serum was collected and stored at -20 °C until radioimmunoassay. Total CORT (bound and free) was measured using the ImmuChem Double Antibody 125I radioimmunoassay Kit (MP Biomedicals, Solon, OH, USA). The antiserum crossreacts 100% with CORT, 0.34% with deoxycorticosterone, 0.05% with cortisol, and does not cross-react with dexamethasone (<0.01%). All reagents were halved and samples run in duplicate. Intraassay coefficient of variation was 6.4%.

#### Tissue Collection

Approximately 7 d after dexamethasone suppression test, rats were weighed and then given an overdose of Euthanyl. Rats were perfused with 60 ml cold 0.9% saline followed by 120 ml cold 4% paraformaldehyde. Brains were extracted and postfixed using 4% paraformaldehyde overnight at 4°C. Brains were then transferred to 30% sucrose in phosphate buffer at 4°C. Brains were rapidly frozen with dry ice and sectioned using a freezing microtome (Leica, Richmond Hill, ON, Canada) at 40  $\mu$ m and collected in series of 10. Sections were stored in antifreeze (ethylene glycol/glycerol; Sigma) and stored at -20°C until processing.

#### DCX Immunohistochemistry

DCX immunohistochemistry was conducted as previously described in Gobinath et al., 2016. Briefly, sections were rinsed in 0.1 M phosphate buffered saline (PBS) and incubated at 4 °C in primary antibody solution: 1:1000, goat anti-doublecortin (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Approximately 24 h later, sections were rinsed and transferred to a secondary antibody solution with 1:500, rabbit anti-goat (Vector Laboratories, Burlington, ON, Canada). Another 24 h later, sections were rinsed and incubated in ABC complex (ABC Elite Kit; 1:1000; Vector) for 4 h. Sections were then washed in 0.175 M sodium acetate buffer, developed using diaminobenzidine in the presence of nickel (DAB Peroxidase Substrate Kit, Vector), mounted on slides, and dried. Sections were then counterstained with cresyl violet, dehydrated, and coverslipped with Permount (Fisher).

#### Microscopy

DCX-expressing cells were quantified in 3 dorsal sections (-2.76 mm to -4.68mm below bregma) and 3 ventral sections (-5.52 mm to -6.60 mm below bregma) using the 40x objective using an Olympus CX22LED brightfield microscope. Areas of these sections were quantified using ImageJ (NIH, Bethesda, MD, USA) and used for density calculations (number of cells per mm<sup>2</sup>). To determine the maturity of doublecortin-expressing cells, 50 cells positively labeled for doublecortin were randomly selected in the ventral hippocampus because ventral hippocampus is associated with stress regulation and affective behaviors (Fanselow & Dong, 2010). Fifty cells positively labeled for doublecortin (25 dorsal and 25 ventral) were randomly selected and categorized as either proliferative (no process or short process), intermediate (medium process with no branching), or post-mitotic (strong dendrite branching in the molecular layer or delicate dendritic tree branching present in the granule cell layer) based on previously published criteria (Plümpe et al., 2006; Gobinath et al., 2016).

#### Data Analyses

Data collected from the novelty suppressed feeding task were analyzed using ANOVA with sex, maternal exercise, maternal postpartum CORT, and maternal postpartum FLX as between-subjects factors. CORT concentrations from the dexamethasone suppression test were analyzed using repeated measures ANOVA with time (t=0, beginning of restraint; t=30, end of restraint; t=120, 1.5 h after restraint ended) as the within-subjects factor. Results from males and females were analyzed separately in the dexamethasone suppression test because males and females were treated with different doses of dexamethasone. The density of DCX-expressing cells was analyzed using repeated measures ANOVA with sub-region (dorsal, ventral) as the

within-subjects factor. Morphology of doublecortin-expressing cells was analyzed using repeated measures ANOVA with sub region (dorsal, ventral) and type of cell (proliferative, intermediate, post-mitotic) as the within-subjects factor. Post hoc comparisons used Newman-Keuls. Because we had hypotheses that there would be interactions between sex, CORT, and FLX, a priori comparisons were subjected to Bonferroni corrections. All data were analyzed using Statistica software (v. 9, StatSoft, Inc., Tulsa, OK, USA). All effects were considered statistically significant if  $p \le 0.05$ .

#### 5.3 Results

Maternal exercise increased latency to feed under maternal postpartum oil- but not maternal CORT-exposed treatments

Exposure to maternal exercise increased latency to feed in the novelty suppressed feeding test in comparison to no maternal exercise (p = 0.007) or the combination of maternal exercise and exercise/postpartum CORT exposure [p = 0.005; interaction between maternal exercise and maternal postpartum CORT; F(1, 142) = 3.69; p = 0.05; Figure 5-2A]. No other significant of main interacting effects were present (all p's > 0.09). We used food consumption as a covariate for the novelty suppressed feeding data but while there was no significant effect of the covariate (p = 0.09) it did render the interaction between maternal exercise and maternal postpartum CORT to a trend (p = 0.08). Food consumption data is presented in table 5-1.

		No Maternal	Maternal
		Exercise	Exercise
	Oil/SAL	8.0 ± 0.2	$7.0\pm0.6$
Males	Oil/FLX	$6.5 \pm 0.5$	8.3 ± 0.4
	CORT/SAL	$8.5 \pm 0.8$	$6.8\pm0.7$
	CORT/FLX	$10.0 \pm 1.0$	$8.1\pm0.4$
	Oil/SAL	$3.2 \pm 0.5$	$2.3 \pm 0.4$
Females	Oil/FLX	$5.0\pm0.5$	$2.3\pm0.7$
	CORT/SAL	$3.4 \pm 0.3$	$3.5 \pm 0.5$
	CORT/FLX	$3.9 \pm 0.5$	$5.0 \pm 1.0$

Table 5-1 Mean ( $\pm$  SEM) food consumption per cage 1 h after novelty suppressed feeding task.



Figure 5-2 Maternal exercise increased latency to feed in the novelty suppressed feeding test. A)

Maternal exercise increased latency to feed in the novelty suppressed feeding test in comparison to controls or offspring exposed to both maternal exercise and maternal postpartum CORT (although this effect was rendered a trend after using food consumption as a covariate.) B) latency to feed from all 16 experimental groups are provided for reference. n=8-10/sex/group. \* p < 0.05.

In males, exposure to maternal exercise, postpartum CORT, or maternal postpartum FLX facilitated HPA axis negative feedback activity in the dexamethasone suppression test

In adult male offspring, exposure to either maternal exercise alone or maternal postpartum CORT alone reduced serum CORT release at the end of restraint stress in comparison to controls (p = 0.002, p = 0.003, respectively) in adult male offspring [interaction between time, maternal exercise, and maternal postpartum CORT; F (2, 136) = 3.15; p = 0.05; Figure 5-3A]. There were no other significant effects prior to restraint stress or during stress recovery.

*A priori* comparisons also revealed that exposure to either maternal exercise or maternal FLX alone blunted serum CORT release at the end of restraint stress in comparison to controls (p < 0.001, p = 0.012, respectively) in adult male offspring [interaction between time, maternal exercise, and maternal postpartum FLX; F(2, 136) = 2.83; p = 0.06; Figure 5-3B]. There were no other significant effects prior to restraint stress or during stress recovery.

In females, exposure to either maternal postpartum CORT facilitated HPA negative feedback in the dexamethasone suppression test but maternal exercise impaired HPA negative feedback.

In adult female offspring, maternal postpartum CORT exposure blunted serum CORT release at the end of restraint stress in comparison to maternal postpartum oil exposure [p = 0.03; interaction between time and maternal postpartum CORT; F (2, 14) = 3.68; p = 0.03; Figure 5-3C]. Additionally, maternal exercise increased serum CORT release in comparison to no maternal exercise (main effect of maternal exercise; F (1, 70) = 4.06; p = 0.05; Figure 5-3D].

There were no other significant or main effects prior to restraint stress or during stress recovery or any effects of maternal postpartum FLX (all p's > 0.2).



Figure 5-3 Effects of maternal exercise, CORT, and FLX on offspring HPA axis negative feedback. A)

Both maternal exercise and maternal postpartum CORT independently reduced serum CORT levels in comparison to control adult male offspring after 30 min restraint stress challenge. B) Both maternal exercise and maternal postpartum FLX reduced serum CORT levels in comparison to control adult male offspring after 30 min restraint stress challenge. C) Maternal postpartum CORT reduced serum CORT levels in comparison to control female offspring after 30 min restraint stress challenge. D) Maternal exercise increased serum CORT levels in comparison to control female offspring after 30 min restraint stress challenge. To maternal exercise increased serum CORT levels in comparison to control female offspring after 30 min restraint stress challenge. D) Maternal exercise increased serum CORT levels in comparison to control female offspring after 30 min restraint stress challenge. To maternal exercise increased serum CORT levels in comparison to control female offspring after 30 min restraint stress challenge. D) Maternal exercise increased serum CORT levels in comparison to control female offspring after 30 min restraint stress challenge. To maternal exercise increased serum CORT levels in comparison to control female offspring after 30 min restraint stress challenge. To maternal exercise increased serum CORT levels in comparison to control female offspring after 30 min restraint stress challenge. To maternal exercise increased serum CORT levels in comparison to control female offspring after 30 min restraint stress challenge. To maternal exercise increased serum CORT levels in comparison to control female offspring after 30 min restraint stress challenge. To maternal exercise increased serum CORT levels in comparison to control female offspring after 30 min restraint stress challenge. To maternal exercise increased serum CORT levels in comparison to control female offspring after 30 min restraint stress challenge.

Maternal exercise increased density of dorsal DCX-expressing cells whereas maternal postpartum FLX decreased density of dorsal DCX-expressing cells, regardless of sex

Maternal exercise increased density of DCX-expressing cells in comparison to no maternal exercise in dorsal hippocampus (p < 0.001) but not ventral hippocampus in adult offspring regardless of sex [p = 0.18; interaction between postpartum maternal exercise and region; F (1, 124) = 6.64; p = 0.01; Figure 5-4A].

Maternal FLX decreased density of DCX-expressing cells in comparison to maternal postpartum saline in dorsal (p < 0.001) but not ventral hippocampus regardless of sex [p = 0.81; interaction between maternal postpartum FLX and region; F(1, 124) = 7.27; p = 0.008; Figure 5-4B].

We had *a priori* hypotheses that sex would significantly interact with maternal CORT, FLX, and exercise. *A priori* analyses revealed that among saline-exposed male offspring, maternal exercise significantly increased density of DCX-expressing cells in comparison to no maternal exercise (of oil-exposed offspring: p = 0.048; of CORT-exposed offspring: p = 0.01). Among saline-exposed female offspring, maternal exercise increased density of DCX-expressing cells in comparison to no maternal exercise (p = 0.02) but concurrent exposure to maternal postpartum CORT prevented this increase (p = 0.92). Among FLX-exposed offspring, this neurogenic effect of exercise was not present in either sex [all p's > 0.25; interaction between sex, maternal exercise, maternal postpartum CORT, maternal postpartum FLX; F (1, 122) = 2.68; p = 0.10; Figure 5-4C].
Maternal exercise increased proportion of post-mitotic DCX-expressing neurons specifically in dorsal hippocampus

Maternal exercise increased proportion of post-mitotic DCX-expressing neurons in comparison to no maternal exercise in dorsal hippocampus (p = 0.007) but not ventral hippocampus [p = 0.86; interaction between region, type, and maternal exercise; F (2, 262) = 3.21; p = 0.04; Figure 5-4D].



**Figure 5-4 Effects of maternal exercise, CORT, and FLX on offspring hippocampal neurogenesis.** A) Maternal exercise increased density of DCX-expressing cells in comparison to no maternal exercise in dorsal but not ventral dentate gyrus. B) Maternal postpartum FLX decreased density of DCX-expressing cells in comparison to saline in dorsal but not ventral dentate gyrus. C) In male offspring, maternal exercise increased density of DCXexpressing cells in comparison to no maternal exercise unless offspring also were exposed to maternal postpartum FLX. In female offspring, maternal exercise increased density of DCX-expressing cells in comparison to no maternal exercise unless offspring also were exposed to maternal postpartum CORT or FLX. D) Maternal exercise increased proportion of post-mitotic or type 3 DCX-expressing cells in comparison to no maternal exercise. This effect was selectively in dorsal dentate gyrus not ventral dentate gyrus. \* p < 0.05. n=8-10/sex/group.

### 5.4 Discussion

Here we show that regardless of sex, maternal exercise increased density of DCXexpressing cells in the dorsal dentate gyrus but this neurogenic effect was prevented by concurrent maternal postpartum FLX exposure. While maternal exercise and FLX treatment had opposing effects on neurogenesis, exposure to either treatment had similar, facilitative effects on HPA axis negative feedback in adult male offspring. However, in adult female offspring, maternal exercise impaired HPA axis negative feedback while maternal postpartum FLX had no significant effect. Maternal postpartum CORT significantly attenuated HPA axis negative feedback in both adult male and female offspring, and it prevented the neurogenic effect of maternal exercise selectively in females. Perhaps paradoxically, maternal exercise increased anxiety-like behavior in adult male and female offspring but exposure to maternal postpartum CORT prevented expression of this anxiety-like behaviour. Collectively, these data highlight that pharmacological and non-pharmacological maternal interventions can independently or interact to yield diverse outcomes for adult male and female offspring development. These data are summarized in Table 5-2.

	CORT only		SSRI only		Exercise only		SSRI + Exercise	
	Males	Females	Males	Females	Males	Females	Males	Females
Anxiety-Like Behaviour	-	-	-	-	↑	<b>↑</b>	1	<b>↑</b>
HPA Axis Negative Feedback	1	↑	↑	-	↑	$\downarrow$	-	-
Dorsal hippocampal neurogenesis	-	-	Ļ	Ļ	¢	Ť	-	-

**Table 5-2 Summary of findings** 

# Maternal exercise increased neurogenesis in the dorsal hippocampus in adult offspring regardless of sex

In adult offspring, maternal exercise increased density of DCX-expressing cells in dorsal dentate gyrus, particularly increasing proportion of post-mitotic DCX-expressing cells. This is in line with the evidence that voluntary running in adult rats increases hippocampal neurogenesis in both male and female rodents (van Praag et al., 1999; Clark et al., 2011; Marlatt et al., 2012; Grégoire et al., 2014). Our findings expand upon these studies by demonstrating that offspring born to dams running prior to gestation, during gestation, and throughout lactation also exhibit increased hippocampal neurogenesis even though the offspring themselves did not run. Interestingly, another study found that mice dams running throughout pregnancy and postpartum transiently increased in hippocampal neurogenesis in juvenile pups (36 days old) but that maternal exercise had no significant effect on neuronal survival of the cells born at weaning (Bick-Sander et al., 2006). Others have shown that exercise during pregnancy (running or swimming) increased hippocampal neurogenesis in rat pups at the time of weaning (Lee et al., 2006; sex not examined) and in adult male and female rats (forced treadmill: Dayi et al., 2012). Our findings confirm that this neurogenic effect of maternal exercise persists in adult rats with voluntary running, with an emphasis on the enhanced survival of more mature DCX-expressing cells. As in the case of adult exercise-induced hippocampal plasticity, the mechanism underlying this neurogenic effect of developmental exposure to exercise may be related to increased trophic factors (Vivar et al., 2012). Different types of exercise during pregnancy increase hippocampal brain derived neurotrophic factor (BDNF) expression in rat pups at the time of birth (sex not examined; forced running: Parnpiansil, Jutapakdeegul, Chentanez, & Kotchabhakdi, 2003) and at weaning (swimming: Cowdry, 1992; forced running: Kim, Lee, Kim, Yoo, & Kim, 2007. However, it should be noted that this increase was transient as gestational exercise did not significant affect hippocampal BDNF mRNA levels in adult male offspring (Venezia et al., 2015). It is of interest in the present study that the neurogenic effect of maternal exercise was specific to the dorsal hippocampus, which may in turn affect cognition (Fanselow and Dong, 2010b). This may underlie findings that maternal running during pregnancy increased object recognition memory (Robinson & Bucci, 2014; females not studied) and acquisition of spatial memory in adult male offspring (Gomes da Silva et al., 2016; females not studied).

It is noteworthy that this neurogenic effect of maternal exercise on offspring was not present in the maternal postpartum FLX-exposed offspring of either sex. Indeed, exposure to maternal postpartum FLX alone decreased neurogenesis in the dorsal dentate gyrus regardless of sex. This is consistent with other studies indicating that developmental FLX reduced hippocampal BDNF mRNA levels in adult male and female offspring (Boulle et al., 2016; Boulle et al., 2016) as well as other measures of plasticity such as reelin (humans; Brummelte, Galea, Devlin, & Oberlander, 2013) and synaptophysin in females (CA3 hippocampal sub-field; (Rayen et al., 2015). Thus, in our study, it is possible that exposure to maternal postpartum FLX prevented the neurogenic effect of maternal exercise by diminishing expression of plasticityrelated proteins. Additionally, exposure to maternal postpartum CORT prevented the neurogenic effect of maternal exercise specifically in adult female offspring. Similarly, a different form of early life adversity (limited nesting/bedding) prevented the neurogenic effect of adult voluntary running specifically in dorsal hippocampus of adult female mice (male mice not studied; Abbink, Naninck, Lucassen, & Korosi, 2017). Collectively, these data indicate that exposure to multiple

maternal treatments can counteract each other and underlies the importance of testing treatments in models of disease. In our study, the positive effects of maternal exercise on offspring neurogenesis were eliminated when given in conjunction with maternal FLX regardless of sex. However, females appear to be more sensitive than males in that exercise (either developmental or adult exposure) does not stimulate neurogenesis after early life adversity exposure in a variety of paradigms.

# Maternal exercise improved HPA negative feedback in adult male offspring but impaired it in adult female offspring

In males, maternal exercise improved HPA axis negative feedback as measured by the dexamethasone suppression test, which is in line with similar studies using adult exposure to voluntary exercise (Campeau et al., 2010). Interestingly, a comparable reduction was mirrored in the adult male offspring of maternal postpartum CORT-treated dams, which is also a replication of Gobinath et al., 2016 (Chapter 3). It is interesting that maternal exercise and maternal postpartum CORT exposure resulted in similar reductions in HPA axis activity or improvements in HPA negative feedback as either maternal treatment can increase maternal glucocorticoid levels (Carlberg et al., 1996; Brummelte et al., 2012; Workman et al., 2016). During pregnancy, the effects of maternal exercise may be related to activity of the placental enzyme 11- $\beta$ -dehydrogenase. When this enzyme is blocked or saturated, it can reduce HPA axis activity (Welberg et al., 2000; Burlet et al., 2005) and increase glucocorticoid receptor expression in areas regulating the HPA axis such as amygdala (Welberg et al., 2000). During the postpartum, maternal glucocorticoids could have indirectly (via maternal care) or directly (via glucocorticoids in the milk) influenced HPA axis in both exercise- and CORT-exposed male

offspring. In our previous study, exercise did not significantly affect maternal care (Gobinath et al., under review), limiting its influence on offspring development in the present study. Although it is not clear whether the mechanism underlying maternal exercise and maternal postpartum CORT exposure overlap or are distinct, it is interesting that maternal exercise significantly increased hippocampal neurogenesis whereas maternal postpartum CORT had no significant effect on hippocampal neurogenesis. Adult hippocampal neurogenesis may function to regulate HPA axis negative feedback in adult male mice (Snyder et al., 2011b). Thus, it is possible that maternal exercise increased neurogenesis, which then contributed mechanistically to increased HPA axis negative feedback in males, whereas maternal postpartum CORT could have impacted other sites of HPA axis regulation, such as the hypothalamus, prefrontal cortex, or amygdala, to yield a similar outcome in males.

Interestingly, in females, maternal exercise impaired HPA negative feedback, which contrasts the findings in males. However, maternal postpartum CORT facilitated HPA axis activity, which was also observed in males and is consistent with Gobinath et al., 2016 (Chapter 3). Although the mechanism or functional relevance of maternal exercise impairing HPA axis negative feedback in females are unclear, it is noteworthy that males and females exhibited opposing responses in the dexamethasone suppression test in response to maternal exercise exposure. This may be related to sex differences  $in11-\beta$ -dehydrogenase, rendering males and females differentially sensitive to the effects of maternal exercise. This prenatal female-specific vulnerability is also observed in humans as prenatal synthetic glucocorticoid exposure increased peak cortisol levels in infant girls not boys (Alexander et al., 2012). However, because the dams in the present study exercised before pregnancy and after parturition, maternal exercise could

have also influenced female HPA axis development during at these times as well. Nonetheless, these findings highlight the importance of studying both sexes as males and females as both were differentially sensitive to the effects of maternal exercise.

#### Maternal exercise increased anxiety-like behavior regardless of sex

Under oil-exposed conditions, maternal exercise increased latency to feed, a measure of putative anxiety in rats, in the novelty suppressed feeding test in adult offspring. Based on this interpretation, our results suggest that maternal exercise surprisingly increased anxiety-like behaviour in adult offspring. This is partially contradictory to evidence that direct adult exercise reduces anxiety in adult rodents (Sciolino et al., 2012) and humans (Broman-Fulks et al., 2015; Fetzner and Asmundson, 2015). Nonetheless, there is evidence indicating that exercise can in fact increase anxiety-like behavior under altered developmental conditions such as in mice bred for high voluntary running wheel activity (Jónás et al., 2010). There are multiple reasons why an anxiogenic effect of maternal exercise was observed in adult offspring. First, it is possible that maternal developmental exercise exposure altered food-related motivation. Indeed, maternal exercise decreased food consumption after testing, and maternal exercise lead to decreased weight and upon using food consumption as a co-variate, the interaction between maternal exercise and maternal postpartum CORT was no longer statistically significant (statistical trend: p=0.08). Thus, perhaps a longer food deprivation could have been more motivating for the maternal exercise rats. Second, in comparison to the well-validated effects of how adult exposure to exercise affects adult expression of anxious phenotypes, there are relatively fewer studies examining the effects of exercise early in life (either maternal or developmental) on anxious phenotypes later in life. One study found a decrease in anxiety-like behavior in juvenile male

offspring as a result of gestational exercise exposure (elevated plus maze; (Haydari et al., 2014). Another study found an increase in anxiety-like behavior as a result of exercise during early adolescence in adolescent male rat offspring (elevated plus maze and open field tests; (Grace et al., 2009). Thus, it is possible that developmental exposure to exercise has age-specific effects to either increase or decrease anxious phenotypes in rodents. Finally, it is possible that lighting conditions are particularly important for assessing the effects of exercise on novelty suppressed feeding. While dim or bright light conditions evoke similar latencies to feed for exercising male rats, these conditions can yield different results in control male rats. In fact, in novelty suppressed feeding, an anxiolytic effect of exercise was observed in bright light conditions when it also provoked anxiety in controls; no significant effect of exercise was observed in dim light conditions (Schoenfeld et al., 2016). Future studies examining the effect of developmental exercise on anxiety-like behavior may want to consider different testing parameters, i.e. standard food for incentive, longer food deprivation, and/or bright light conditions.

### Maternal postpartum FLX reduced neurogenesis in the dorsal hippocampus of offspring

Maternal postpartum FLX reduced density of DCX-expressing cells in dorsal hippocampus regardless of sex in adult offspring. This reductive effect of maternal postpartum FLX is in line with similar findings in which a lower dose of maternal postpartum FLX (5 mg/kg) reduced brain derived neurotrophic factor IV and TrkB mRNA expression in the adult offspring hippocampus regardless of sex (Boulle et al., 2016; Boulle et al., 2016). Interestingly, this reductive effect of maternal postpartum FLX on offspring neurogenesis is consistent between the present study and Gobinath et al., 2016 (Chapter 3) in adult female offspring but opposes findings from Gobinath et al., 2016 (Chapter 3) in adult male offspring. Additionally,

we have shown that these effects are not present under cage control conditions as maternal postpartum FLX had no significant effect on DCX expression at this age in either sex. Collectively, this may point to the possibility that the effects of maternal postpartum FLX are context-specific (i.e. amount of behavioural testing versus cage control), particularly in males. To further support this point, in the previous study with the more intense battery of behavioural testing, all the offspring displayed increased DCX-expressing cells in the proliferative stage, potentially because the novel aspects of multiple environments stimulated neuronal proliferation. In the present study, however, maternal postpartum FLX had no significant effect on maturation of DCX-expressing cells, potentially because fewer behavioral tests were used. In this series of studies, pup exposure to FLX during the postnatal period, the time of greatest overall brain growth and synaptogenesis (Kepser and Homberg, 2015), may have plausibly altered the longterm plasticity potential in these offspring. Further research is necessary to support this idea, but this possibility may be important to future studies evaluating the effects of developmental FLX exposure to consider the moderating effects of how challenging the context is on the measured outcome. Nonetheless, these findings point to an important sex difference with regards to the relationship between maternal postpartum FLX and adult hippocampal neurogenesis: females consistently exhibit reduced neurogenesis after behavioural testing but males exhibit altered neurogenesis depending on amount of behavioural challenges prior to evaluation. This interaction between sex and concurrent exposure to challenging environments may be translationally relevant when evaluating the effect of maternal SSRI use on the outcomes of boys and girls.

#### Maternal postpartum FLX reduced HPA axis activity in adult male but not female offspring

Maternal postpartum FLX facilitated negative feedback in the dexamethasone suppression test in adult males. Although the direction of the effect opposes findings from Gobinath et al., 2016, it is also noteworthy that the sex-specific effects of maternal postpartum FLX on male offspring HPA axis are consistent across both studies. Interestingly, others have shown that a lower dose of maternal postpartum FLX reduced in glucocorticoid receptor density in the CA3 region of the hippocampus in adolescent male but not female rats (Pawluski et al., 2012c). Thus, it is possible that maternal postpartum FLX perturbed intrahippocampal activity and subsequently hippocampal regulation of HPA axis activity. Interestingly, adult treatment with the same dose of FLX reduced HPA axis activity in male mice bred for low reactivity to novelty but exaggerated HPA axis activity in mice bred for high reactivity to novelty (Surget et al., 2016). This may be relevant for comparing our present findings (relatively low amounts of novel exposure) and previous findings (Gobinath et al., 2016: relatively higher amounts of novel exposure). Thus, developmental background may interact with FLX exposure to affect HPA axis activity.

# Maternal postpartum FLX did not affect anxiety-like behaviour in either sex in the novelty suppressed feeding test

Maternal postpartum FLX exposure had no significant effects on anxiety-like behavior in the present version of the novelty suppressed feeding test although previous work in this model and dose of maternal postpartum FLX observed that maternal postpartum FLX increased latency to feed in adult male but not female offspring (Gobinath et al., 2016; Chapter 3). This discrepancy may also provide a nuanced insight into the developmental effects of FLX on anxiety phenotypes. First, in Gobinath et al., 2016 (Chapter 3), the incentive used in novelty suppressed feeding test was standard rat chow, and the present study used Froot Loops. Earlier exposure to the same dose of maternal FLX (gestation day 14 through postpartum day 7) reduced number of cells in the nucleus accumbens (involved in motivation) and density of serotonin transporters in the dorsal raphe nucleus, a source of serotonergic tone in the brain in rats (sex differences not analyzed; (Forcelli and Heinrichs, 2008). Thus, it is possible that developmental FLX exposure impacts the neural circuitry underlying motivation in a nuanced way – adult male offspring may be less motivated to venture into the open space with the benefit is relatively low (standard rat chow) but are more motivated for more palatable rewards (Froot Loops). Second, rat offspring in Gobinath et al., 2016 (Chapter 3) had already undergone elevated plus maze, open field test, and forced swim test prior to novelty suppressed feeding. In the present study, novelty suppressed feeding was the first and only behavioural test. Thus, another possibility is that conditions of higher stress and/or novelty exposure may elicit anxiety in FLX-exposed offspring that is not apparent under low stress/novelty exposure conditions. Third, rat offspring were approximately 125 d old in the present study and were approximately 75 d old in Gobinath et al., 2016 (Chapter 3). Although both ages qualify as adulthood in rats, it is possible that an anxious phenotype was observed earlier in the adulthood and with age, this effect was no longer apparent. It is noteworthy there are several subtypes of serotonin receptors which are involved in anxiety such as the 5HT-1A receptor (Albert et al., 2014) and 5HT-7 receptor (Hedlund, 2009). Developmental FLX exposure can impact this highly heterogenous system as well as interact with age and sex of offspring. Finally, it should be noted that experiments in Gobinath et al., 2016 (Chapter 3) were conducted in a barrier facility whereas the present study was conducted in a conventional facility. Although the exact mechanism underlying these facility differences is

unclear, expression of anxiety-like behaviour and HPA axis can be influenced by developmental exposure to sterile environments (Sudo et al., 2004) or environmental microbes in mice (Reber et al., 2016). For this reason, facility and environmental regulators of the bacterial flora are critical factors to include in future preclinical research and may partly explain some of the observed discrepancies here. These numerous factors may explain why certain windows of developmental FLX exposure result in a variety of anxiety phenotypes for males (Kiryanova et al., 2013).

#### **Conclusions**

These data collectively indicate that maternal exercise and maternal FLX exposure can differentially affect hippocampal neurogenesis, HPA axis negative feedback, and anxiety-like behaviour in adult offspring. Regardless of sex, maternal exercise increased hippocampal neurogenesis whereas maternal postpartum FLX reduced neurogenesis in the dorsal hippocampus, and maternal postpartum FLX exposure prevented the neurogenic effect of maternal exercise. Maternal exercise increased anxiety-like behaviour regardless of sex. However, maternal exercise facilitated HPA axis negative feedback in males but impaired it in females, while, maternal postpartum FLX selectively facilitated HPA axis negative feedback in males but had no significant effect in females. Notably, there were some discrepancies between previous findings with this model of PPD and maternal postpartum FLX treatment on male offspring outcome, potentially because of experimental conditions (i.e. testing conditions, facility, amount of testing, and age evaluated). This may have important implications for evaluating maternal SSRI exposure on male and female offspring outcome in the clinical setting. Collectively, these data suggest that both non-pharmacological and pharmacological antidepressants result in long-lasting effects on offspring outcomes and contribute to a better understanding of how treating PPD affects offspring development.

### Conflicts of Interest

The authors have nothing to declare.

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### **Chapter 6: General conclusions**

#### 6.1 Summary of experimental findings

The research in this dissertation compared a pharmacological antidepressant (FLX), a non-pharmacological antidepressant (exercise), or how a combination of both treatments would alter maternal and offspring outcomes in a CORT-induced rat model of PPD. Specifically, I evaluated PPD endophenotypes in the dam and the neurodevelopmental effects on her adult male and female offspring in terms of affective behaviour, HPA axis activity, and hippocampal neurogenesis. The major findings of this thesis are: 1) maternal postpartum FLX prevented CORT-induced disruptions in maternal care but did not prevent CORT-induced depressive-like behaviour (immobility in forced swim test) or reductions in neurogenesis (Chapter 1: Workman et al., 2016); 2) maternal postpartum FLX influenced anxiety-like behaviour, HPA axis negative feedback, and neurogenesis, predominantly in adult male offspring (Chapter 2: Gobinath, Workman, Chow, Lieblich, & Galea, 2016); 3) maternal exercise reduced depressive-like behaviour (immobility in forced swim test) and increased neurogenesis in conjunction with FLX but did not prevent CORT-induced reductions in maternal care (Chapter 3; Gobinath et al, under revision); 4) maternal exercise increased neurogenesis in adult offspring unless they were also exposed to maternal postpartum FLX (Chapter 4; Gobinath et al, under review). Collectively, these data indicate that FLX and exercise can differentially impact endophenotypes of PPD, suggesting that antidepressants function differently during the postpartum period. Additionally, these data indicate exposure to either pharmacological or non-pharmacological antidepressants bear long term effects on offspring anxiety-like behaviour, HPA axis negative feedback, and hippocampal neurogenesis, predominantly in males.

## 6.2 In a CORT-induced model of PPD, maternal postpartum FLX prevented CORTinduced reductions in maternal behaviour but did not rescue maternal depressive-like behaviour or hippocampal neurogenesis

As in the case of MDD, the first line of treatment for PPD includes SSRIs such as FLX. However, a growing body of literature suggests that SSRIs may have lower efficacy based on factors such as age and genotype (Jamerson et al., 2013; Shiroma et al., 2014; Mitchell et al., 2015). Further evidence from clinical research indicates that antidepressants may work differently during the postpartum (Sharma and Sommerdyk, 2013; De Crescenzo et al., 2014). The data from Chapter 2 indicated that postpartum FLX treatment prevented CORT-induced disruptions in maternal care but was unable to prevent CORT-induced depressive-like behaviour in the forced swim test as well as reductions in hippocampal neurogenesis. These data suggest that FLX efficacy is altered during the postpartum period and demonstrates that maternal postpartum FLX is efficacious in alleviated PPD-endophenotypes related to maternal care but is not efficacious in other metrics of PPD-endophenotypes, i.e. increased immobility in the forced swim test and reduced neurogenesis.

#### 6.2.1 Moderating factors of SSRI efficacy – endocrine factors

It is important to note that FLX had limited efficacy within this model of PPD which mechanistically tests the relationship between PPD and hypercortisolemia arising after parturition. While there is robust evidence linking MDD and hypercortisolemia (Stetler and Miller, 2011), there is less conclusive evidence connecting hypercortisolemia to PPD. Indeed, there have been observations that PPD is associated with high levels of circulating cortisol as well as with even lower levels of cortisol and no relationship between circulating cortisol and PPD (Bloch et al., 2005; Jolley et al., 2007; Seth et al., 2016). This inconsistency may be due to a variety of methodological reasons. For example, symptom severity of PPD can vary between hypocortisolemia and hypercortisolemia with hypercortisolemia being related to transient mood disturbances but hypocortisolemia being related to chronic depressive states (Seth et al., 2016). Thus, symptom severity may be an important moderator in the relationship between glucocorticoids and PPD that future studies may considering including in the analyses. Furthermore, breastfeeding increases circulating levels of glucocorticoids (Neumann et al., 1998; Brunton and Russell, 2008), and discontinuing breastfeeding is associated with PPD (Kim et al., 2014b; Moura et al., 2016). Moreover, glucocorticoid levels normalize with weaning (Voogt et al., 1969; Windle et al., 2013). Thus, whether the mother was breastfeeding at the time is another important factor in the relationship between glucocorticoids and PPD to be included in statistical analyses. Additionally, due to issues defining and diagnosing PPD (see sections 1.2 and 1.3), inaccurate and under-sampling of women with PPD could contribute to variability in the relationship between PPD and glucocorticoids. Furthermore, history of early life stress or abuse can blunt HPA axis activity (Carlson and Earls, 1997) as well as increase risk for PPD (Kettunen and Hintikka, 2017); for these women with history of early life stress, HPA axis dysregulation in PPD may present differently from women developing de novo depression in the postpartum period. These issues highlight that additional research regarding HPA axis dysregulation in PPD are necessary to fully understand its contributing role to the etiology of PPD. Nonetheless, there is robust evidence that the higher levels of glucocorticoids and HPA axis dysregulation are observed in MDD (Stetler and Miller, 2011). Moreover, women in the postpartum exhibit these alterations in the glucocorticoid system (Brunton and Russell, 2008). Together, these issues highlight the importance stress and glucocorticoids in the etiology of PPD.

In addition to glucocorticoids, gonadal hormones have also been implicated in depression and antidepressant efficacy. Age-related decline in gonadal hormones is associated with increased risk for depression in women (Freeman et al., 2004; Cohen et al., 2006b) and men (Sachar et al., 1973; McIntyre et al., 2006). Conversely, increased gonadal hormone levels may facilitate or enhance antidepressant efficacy. Testosterone can bolster the effects of the tricyclic antidepressant imipramine on endophenotypes of MDD in adult male rats (Wainwright et al., 2016b), and testosterone can be a beneficial adjunct antidepressant to pharmacotherapy in men (Zarrouf et al., 2009). Ovarian hormones can moderate the effects of FLX on hippocampal neurogenesis and number of microglia in adult female rats (Mahmoud et al., 2016). In the postpartum period, there is a striking reduction in ovarian hormone levels, and this reduction may contribute to mood disturbances in mothers (Hendrick et al., 1998; Bloch et al., 2003). Furthermore, this transient state of hypogonadism in the postpartum period may partly explain why SSRI efficacy is reduced in mothers although additional research is necessary to address this possibility. Interestingly, transdermal application of estradiol to alleviate PPD in mothers has been investigated (Moses-Kolko et al., 2009), and future studies can investigate the effectiveness of estradiol as an adjunct therapy in mothers.

#### 6.2.2 Moderating factors of SSRI efficacy – genes and environment

This thesis found that FLX alleviated CORT-induced reductions in maternal care early in the postpartum period, but it did not prevent maternal depressive-like behaviour or reductions in hippocampal neurogenesis at the end of the postpartum period. This lack of SSRI efficacy in maternal depressive-like behaviour is in line with meta-analyses observing that antidepressant efficacy is limited in mothers. However, because FLX alleviated CORT-induced effects in early in the postpartum period, it is possible that FLX also prevented CORT-induced depressive-like

behaviour early in the postpartum period as well, and future studies may consider timing to be a crucial factor in observing antidepressant effects. Another important factor to note is genetic factors involved in antidepressant efficacy. In MDD, altered SSRI efficacy has been linked to allele variants of the serotonin transporter gene [*5HTTLPR*; (Arias et al., 2003)], genes associated with affecting hippocampal and amygdala volume [*rs1908557*; (Li et al., 2016)], and polymorphisms of *BDNF* (Niitsu et al., 2013). These genetic factors may also be influential in antidepressant efficacy in PPD although this has not yet been investigated and represents an interesting avenue of future research. Preclinical research using genetic manipulations of serotonin transporter, plasticity of limbic regions, and BDNF can begin to address these questions by studying their involvement in PPD-like endophenotypes. This can be informative in developing a precision medicine-based approach and identifying whether certain sub-populations of women with PPD respond better to particular antidepressants.

In addition to genetic factors, antidepressant efficacy may be influenced by experiential factors. For example, lower levels of parental stress and fewer behavioural problems in children predicted recovery from maternal depression 6 years after childbirth whereas pharmacotherapy was not significantly associated with recovery (Shankar et al., 2017). This thesis employed a model of PPD which induces the index episode of depression after parturition, which importantly accounts of 40% of cases of PPD (Wisner et al., 2013). However, previous history of depression is a strong risk factor for PPD (O'Hara and McCabe, 2013; Kettunen et al., 2014). Animal models of PPD may be useful in discerning windows of vulnerability and optimal antidepressant intervention. With regards to translational implications, this thesis may be more relevant to mothers with de novo depression in the postpartum period and beginning antidepressant intervention after parturition, pointing to limited efficacy under these conditions. Alternatively,

as mentioned above, timing may be an important factor as it is possible an antidepressant effects of FLX was present earlier in the postpartum period (when FLX increased maternal care behaviour) but not by the end of the postpartum period when forced swim test was conducted. Given that 57% of individuals responding to SSRIs relapse and require a new prescription antidepressant (Byrne and Rothschild, 1998), it is possible that this cycle of relief and relapse is also present in the postpartum with antidepressant relief possibly occurring early and relapse occurring later. Indeed, a positive effect of FLX was observed early in the postpartum period when examining anhedonia-like behaviour related to time spent with pups. Others have similarly shown that maternal FLX reduced depressive-like behaviour in the forced swim test during the first postpartum week when dams are treated with FLX from gestation day 10 through the end of the postpartum period (Salari et al., 2016b). However, treating dams with another SSRI (paroxetine) exacerbated depressive-like behaviour when treatment began on postpartum day 2 through postpartum day 12 (Overgaard et al, under revision). Collectively, these studies underscore the importance of studying timing of maternal depression onset and antidepressant intervention to better understand treatment success for PPD as timing may be an important factor in determining whether SSRIs are efficacious. Importantly, it also should be noted that a combination of genetic and environmental factors could synergistically contribute to development of depression during pregnancy and/or in the postpartum, and these factors may interact to determine remission status.

# 6.3 Maternal postpartum FLX increased anxiety-like behaviour, impaired HPA axis negative feedback, and increased hippocampal neurogenesis in adult male offspring only

A major contributing factor in whether a mother chooses to utilize antidepressant treatment is concerns of neonatal SSRI exposure on child outcome. In the case of FLX, both FLX and its metabolite norfluoxetine remain active in breast milk, potentially directly impacting child outcome. In infants and children, neonatal SSRI exposure has been associated with some adverse outcomes, such as increased internalizing behaviour (which may predispose anxiety), pulmonary hypertension, lower levels of reelin, and delayed psychomotor development (Chambers et al., 2006; Brummelte et al., 2013; Santucci et al., 2014a; Hanley et al., 2015). Neonatal SSRI exposure has also been associated with some seemingly beneficial effects such as enhanced language acquisition as well as fewer behavioural problems (inattention and hyperactivity) in comparison to children of mothers with untreated PPD (Weikum et al., 2012a; Grzeskowiak et al., 2016) To date, clinical evidence is unavailable regarding the long term (i.e. in adulthood) effects of neonatal SSRI exposure on offspring outcome. For this reason, mothers and clinicians face a difficult decision: do the potential long-term effects of neonatal SSRI exposure outweigh the adverse effects of untreated PPD on child outcome? The data from chapter 3 indicate that maternal postpartum FLX exposure can indeed bear long-term effects on adult offspring outcome, predominantly affecting male offspring. Maternal postpartum FLX exposure increased anxiety-like behaviour, impaired HPA axis negative feedback, and increased density of DCX-expressing cells in dorsal hippocampus of adult male offspring. Maternal postpartum FLX had fewer effects in adult female offspring, selectively reducing density of DCX-expressing cells in dorsal hippocampus. Collectively, the data from this chapter indicate

that maternal postpartum FLX treatment can induce long-lasting effects on offspring outcome with males particularly more vulnerable to this maternal manipulation than females.

# 6.3.1 Methodological considerations impacting offspring outcome – route and timing of exposure

It should be noted that either maternal postpartum CORT or FLX could have impacted offspring outcome either indirectly (via alterations in maternal care), directly (via the presence of either drug in the milk), or by a combination of both routes. Maternal postpartum CORT reduced time spent nursing and increased time away from the nest (Workman et al., 2016; Chapter 2), and this treatment also increased HPA axis negative feedback in offspring of both sexes and increased density of DCX-expressing cells in adult male offspring. Similarly, maternal treatment with lower doses of CORT via drinking water (200 ug/ml) also reduced adult male offspring HPA axis activity without affect maternal care (females not studied; Catalani et al., 1993). This may suggest that presence of CORT in the milk may be particularly influential on reducing offspring HPA axis negative feedback. However, maternal care (high levels of licking and grooming) in reducing HPA axis activity in adult offspring (Caldji et al., 2000). Similarly, maternal postpartum FLX could have also influenced offspring outcome either indirectly or directly. Notably, maternal postpartum FLX prevented CORT-induced reductions in maternal care; however, FLX increased nursing and potentially allowed for the pups to ingest more FLX. Future studies employing radioactive tracers of CORT, or correlating milk concentrations of CORT and FLX with offspring outcome will be useful in disentangling the mechanism in how maternal postpartum CORT influences offspring HPA axis.

As in the case with the data presented in Chapter 2, the data presented in Chapter 3 may be particularly more translationally relevant in considering how a model of depression and FLX

treatment selectively arising in the postpartum affects adult offspring outcome. Interestingly, maternal postpartum CORT appeared to have fewer effects on offspring outcome than maternal postpartum FLX. Maternal postpartum FLX impaired HPA axis negative feedback in adult males but maternal postpartum CORT facilitated it in both adult males and females. However, exposure to either maternal postpartum CORT and FLX resulted in similar elevations of neurogenesis in adult males. It should be noted that others have found different effects of maternal FLX exposure using other types of maternal depression models. For example, prenatal stress in the final week of gestation followed by a lower dose of maternal postpartum FLX (5 mg/kg) has been associated with no anxiety-like behaviour in the elevated zero maze in adult offspring of either sex but increased depressive-like behaviour in adult female offspring (Boulle et al., 2016; Boulle et al., 2016). Alternatively, prenatal stress during the final two weeks of gestation and a higher dose of perinatal FLX exposure (25 mg/kg) from prenatal day 15 through postpartum day 12 reduced anxiety-like behaviour in adult male but not female mice (Kiryanova et al., 2016, 2017a). Thus, modelling maternal depression and its pharmacological treatments may have timing-specific implications for offspring development as well maternal outcome. This is important because as discussed in the introduction (section 1.1), one of the issues with the understanding PPD is that mothers are current diagnosed as "MDD with peripartum onset," (American Psychiatric Association, 2013). Without accurately incorporating timing of depression onset and antidepressant intervention into analysis and data interpretation, this can yield seemingly conflicting results of how maternal SSRI exposure influences offspring outcome and is a crucial point for both future preclinical and clinical research.

#### 6.3.2 Methodological considerations impacting offspring outcome – sex differences

Maternal postpartum FLX exposure predominantly affect adult male offspring, increasing anxiety-like behaviour, HPA axis activity, and dorsal hippocampal neurogenesis. Interestingly, there are sex differences in maternal care, with male pups eliciting more maternal care than female pups (Moore and Morelli, 1979). Furthermore, in adult mice, males and females are differentially sensitive to doses of FLX with males exhibiting greater hippocampal cell proliferation at lower doses of FLX and females exhibiting such increases at higher doses of FLX (Hodes et al., 2010). These sex differences in indirect routes (maternal care) and direct routes (administration of FLX) of FLX exposure may have contributed to the preponderance of maternal postpartum FLX effects on male offspring. This sex-specific vulnerability is generally in line with broad findings that males are more vulnerable to early life adversity than females in terms of neurodevelopmental and behavioural outcome (Gobinath et al., 2015). It is important to note that serotonin can impact development of the HPG axis. Treating mice pups with the SSRI citalopram during the postnatal period reduced the number of androgen receptor-expressing cells in the preoptic area of adult male mice (Soga et al., 2012). Additionally, a low dose of maternal postpartum FLX treatment reduced volume of the preoptic area in adult male rats (Rayen et al., 2013). Early FLX exposure has also been associated with delayed maturation of the testosterone in adult male rodents (de Oliveira et al., 2013). Thus, early manipulation of the serotonin system may alter development of areas relevant to sexual differentiation, potentially explaining in part why males were mostly affected by maternal postpartum FLX exposure.

Although maternal postpartum FLX exposure did not affect anxiety-like behaviour or HPA axis negative feedback in adult female offspring, it selectively reduced dorsal hippocampal neurogenesis. Maternal postpartum FLX has also been linked to reductions in density of DCX- expressing cells in pre-adolescent females (Gobinath, Workman, Chow, Lieblich, & Galea, 2017) as well as reductions in reelin in infant girls (Brummelte et al., 2013). Thus, females may also be vulnerable to the effects of maternal FLX exposure but these effects may be apparent earlier in life. This highlights the importance of studying sex differences because males and females were differentially sensitive to the effects of maternal postpartum FLX exposure such that females were specifically vulnerable in terms of hippocampal neurogenesis whereas males were affected in several measures (anxiety-like behaviour, HPA axis negative feedback, and hippocampal neurogenesis). Methodological considerations impacting offspring outcome – human vs. rat brain development

With regards to the translational implications of these data, it should be noted that rat brain development is different from infant brain development with rats being born at the equivalent to third trimester in humans (Clancy et al., 2007). Because the pups were exposed to FLX via its presence in the milk or FLX-induced alterations in maternal care, the methods of the data in this chapter are relevant for treating PPD with FLX after parturition. Maternal postpartum FLX treatment could have either indirectly affected offspring via its effects on maternal care or directly via its presence (along with its active metabolite norfluoxetine) in the milk. However, in understanding the translational implication, the neurodevelopmental outcome may be more comparable to fetuses exposed to FLX during the third trimester. This is an important consideration in using rodent models to assess neurodevelopment. Although there are neurodevelopmental distinctions between rodents and humans, rodent models also offer many advantages with regards to addressing the effects of maternal SSRI treatment on offspring outcome, such as modelling timing specificity, route of administration (via the dam), and effects on adult male and female offspring neurodevelopment.

# 6.4 In dams, maternal exercise reduced immobility in the forced swim test, and the combination of both FLX and exercise increased hippocampal neurogenesis

Because SSRIs may have limited efficacy in the postpartum period and are associated with adverse effects for offspring outcome, mothers may be more inclined to seek nonpharmacological interventions, such as exercise, for PPD. However, while exercise has been extensively studied as an antidepressant in males and non-parous women, it is unclear whether exercise functions as an antidepressant in the postpartum period. Because of the increased circulating glucocorticoids, reduced ovarian hormone levels, and neural plasticity in the maternal hippocampus, it is plausible that the capacity for exercise to exert its antidepressant effects are also compromised (as has also been the case for SSRIs). The data from Chapter 4 indicate that maternal exercise over the course of pre-conception, gestation, and postpartum reduced CORTinduced maternal depressive-like behaviour and elevated neurogenesis with concurrent FLX exposure. However, maternal exercise was not able to prevent CORT-induced disturbances in maternal care while maternal postpartum FLX did prevent CORT-induced disruptions in maternal care regardless of sedentary or exercise conditions.

A barrier in the use of exercise as an antidepressant intervention is that it requires high behavioural investment and motivation from the individual. Because MDD is characterized by a loss of motivation as well as other symptoms such as lethargy and fatigue (American Psychiatric Association, 2013), it may be difficult for individuals with MDD to engage with exercise regimens given the disruptive nature of MDD itself on physical activity. For this reason, the exercise has been investigated as adjunct intervention to pharmacotherapy (Trivedi et al., 2011). In Chapter 4, the data indicate that the combination of both exercise and FLX increased hippocampal neurogenesis relative to either antidepressant alone. Interestingly, this combination effect was present even though maternal postpartum FLX treatment reduced amount of running in the postpartum period. This possibly suggests that exercise prior to FLX exposure (during preconception and gestation) followed by postpartum FLX treatment together elevated neurogenesis in dams. Although exercise did not have an additive effect on FLX in either maternal care or depressive-like behaviour, it is possible that the functional implications of this combination may be related to other neurogenesis-related behaviour, such as pattern separation (Braun and Jessberger, 2014). Importantly, exercise alone and SSRI exposure alone have both been shown to increase hippocampal neurogenesis in male rodents (Malberg et al., 2000; Tanti et al., 2013; Grégoire et al., 2014). However, the observation that these treatments independently did not stimulate neurogenesis in dams underscores the importance of studying antidepressant interventions in pregnancy and postpartum periods.

#### 6.4.1 Moderating factors of exercise efficacy – timing of intervention

It should be noted that the effects of exercise to impact CORT-induced endophenotypes of PPD may be related to timing of the intervention. In Chapter 4, dams voluntarily ran prior to pregnancy, during pregnancy, and during the postpartum period. In this way, this chapter evaluated whether history of exercise coupled with concurrent exercise during exposure to high CORT levels affected CORT-induced endophenotypes of PPD. With regards to the translational implications, the methods would be comparable to evaluating whether exercise is an efficacious antidepressant in women regularly engaging in an active lifestyle and developed de novo depression in the postpartum. However, some women may view pregnancy as an opportunity to begin a more active life and may begin to incorporate regular exercise into their lives during

pregnancy and/or after childbirth. It should be noted that women are encourage to be active during pregnancy, but moderate to high intensity exercise is recommended only for pregnant women already regularly engaging in such exercise regimens (Artal, 2016). In Chapter 4, high amounts of running, but not low amounts of running, were associated with the greatest benefits on neurogenesis in the ventral hippocampus in dams that had been begun exercising prior to conception. In this regard, it appears that high levels of exercise are beneficial for upregulating hippocampal neurogenesis in the dam but with the note that the bulk of exercise intensity begun prior to conception. Interestingly, in male rats, voluntary running mitigated the effects of CORT administration on endophenotypes of MDD (immobility in the forced swim test and hippocampal neurogenesis) only in rats running prior to CORT exposure and continuing to run during CORT exposure (Yau et al., 2014). Voluntary running that begun at the same time as CORT treatment failed to prevent CORT-induced depressive-like behaviour and reductions in neurogenesis (Yau et al., 2014). Thus, in the context of MDD and PPD related to high levels of CORT, regular exercise is an important protective agent against development of depressive-like symptoms. Furthermore, women report feeling fatigued and loss of motivation to exercise in the postpartum (Saligheh et al., 2016). PPD may further exacerbate this issue, which may become a barrier in initiating exercise intervention in the postpartum. Collectively, these data encourage regular exercise even prior to pregnancy to reduce risk of developing depression.

#### 6.4.2 Moderating factors of exercise efficacy – individual differences

Notably, the data in Chapter 4 found that amount of running was highly variable within exercising dams and was relevant to neurogenesis outcomes. Specifically, while exercise did not significantly increase neurogenesis in comparison to no exercise, high running dams had

significantly greater density of DCX-expressing cells in ventral hippocampus in comparison to no running or low running dams. Furthermore, of control dams, amount of running in the postpartum period positively correlated with density of DCX-expressing cells in ventral hippocampus. This indicates that for dams, amount of voluntary running was a significant moderator in whether exercise affected maternal neurogenesis. Some rodent studies circumvent individual differences in exercise by using forced running paradigms which control time and intensity via forced running on a treadmill (e.g. Griesbach, Tio, Vincelli, McArthur, & Taylor, 2012). An important consideration in using this method is that forced running is a more stressful intervention than voluntary running. For example, in male rats, voluntary running is associated with transiently increased basal serum CORT and increased hippocampal BDNF protein levels; however, forced running sustained basal serum CORT at high levels and did not increase hippocampal BDNF protein levels (Ke et al., 2011; Griesbach et al., 2012). To prevent this additional stress, voluntary running was selected as the intervention in this study. Future studies can further explore how timing and amount of exercise intervention affect mood-related outcomes in mothers but may need to account for individual differences in voluntary running or account for additional stress if using forced running.

### 6.5 Maternal exercise increased offspring neurogenesis unless offspring were also exposed to maternal postpartum FLX

Given that maternal SSRI use has been controversially linked to adverse outcomes in offspring, mothers may be inclined to pursue non-pharmacological antidepressants such as exercise which are generally associated with beneficial effects on maternal and child health. However, given that it was unclear how exercise would function as an antidepressant in the

postpartum period, it was also unclear how exercise would influence offspring development within this form of early life adversity. The data from chapter 5 indicate that maternal exercise increased neurogenesis (particularly in the dorsal hippocampus) in adult offspring even though the offspring themselves did not exercise. Furthermore, concurrent exposure to maternal postpartum FLX prevented this neurogenic effect of maternal exercise. These effects on neurogenesis were present in both sexes. However, there were opposing effects of maternal exercise on adult offspring HPA axis based on sex: maternal exercise facilitated HPA axis negative feedback in adult males but impaired it in adult females. Contrary to the effects of maternal exercise, maternal postpartum FLX treatment reduced neurogenesis in the dorsal hippocampus of offspring. Furthermore, maternal postpartum FLX facilitated HPA axis negative feedback in adult males whereas it had no significant effect on adult females. Maternal postpartum CORT also facilitated HPA axis negative feedback in adult males and females but had no significant effect on offspring neurogenesis. Collectively, these data indicate that maternal exercise and FLX can bear long-term effects on offspring neurodevelopment in a model of PPD.

#### 6.5.1 Consistent effects of maternal FLX on adult female offspring

Although the methodology of maternal postpartum FLX treatment remained the same between Chapters 3 and 5, there were noteworthy replications of maternal postpartum FLX in adult female outcome but more discrepancies in adult male outcomes. Namely, maternal postpartum FLX consistently did not affect adult female outcome in terms of anxiety-like behaviour in the novelty suppressed feeding test (regardless of using rat chow or Froot loops as the motivation) or HPA axis negative feedback (regardless of dexamethasone dose). Maternal postpartum FLX also consistently reduced dorsal hippocampal neurogenesis in adult females. Although the functional implications of this reduction in hippocampal neurogenesis is unclear, the dorsal hippocampus has been linked to spatial memory and cognition (Fanselow and Dong, 2010a). Thus, while maternal postpartum FLX treatment consistently did not affect measures of affective behaviour or HPA axis negative feedback in female offspring, it is possible that it could alter behavioural outcomes relevant to spatial memory or navigation. Indeed, other studies examining effects of maternal FLX have found that it enhanced spatial memory in the Morris water maze in adult female mice (exposure from gestation day 15 – postpartum day 12; Kiryanova et al., 2017). Furthermore, we have also observed that this reductive effect of maternal postpartum FLX on density of dorsal hippocampal DCX-expressing cells in preadolescent female offspring but not adolescent or adult female offspring under basal conditions (Gobinath et al., 2017). Given that the reduction in hippocampal neurogenesis was not observed under cage control conditions in adult females, this indicates that this reductive effect emerges after some amount of stress and/or novelty of behavioural testing. Collectively, this points to postpartum FLX exposure affecting dorsal hippocampus and dorsal hippocampus-related functions in females but this may depend on amount of concurrent challenges present especially when examining adult female outcomes.

#### 6.5.2 Variable effects of maternal FLX on adult male offspring

With regards to adult male outcomes, discrepancies in the influence of maternal FLX on adult male offspring outcome between in Chapters 3 and 5 were present and may point to an important role for context in evaluating maternal postpartum FLX effects on male offspring outcome. Specifically, these discrepancies were related to the effects of maternal postpartum FLX on anxiety-like behaviour (Chapter 3: increased anxiety-like behaviour; Chapter 5: no

significant effect on anxiety-like behaviour), HPA axis negative feedback (Chapter 3: impaired HPA axis negative feedback; Chapter 5: facilitated HPA axis negative feedback), and density of DCX-expressing cells in dorsal hippocampus (chapter 3: increased density of DCX-expressing cells; chapter 5: decreased density of DCX-expressing cells). However, it should be noted that the route, dose, and administration of maternal postpartum FLX is the only consistency between Chapters 3 and 5. There were many methodological differences relevant to the offspring between these chapters: slightly older age studied (chapter 3: ~ 65 d old; chapter 5: ~125 d old), differences in amount of behavioural testing (chapter 3: 4 behavioural tests and dexamethasone suppression test; chapter 5: 1 behavioural test and dexamethasone suppression test), and facility differences (chapter 3: barrier facility; chapter 5: conventional facility).

In this thesis as well as other published findings, maternal postpartum FLX resulted in many possible effects on adult male offspring neurogenesis: increased dorsal hippocampal neurogenesis (Chapter 3), reduced dorsal hippocampal neurogenesis (Chapter 5), and no significant effect on hippocampal neurogenesis under cage control conditions (Gobinath et al., 2017). These dynamic effects may be partly explained by the fact that FLX and its metabolite norfluoxetine remain active in milk, directly reaching the developing offspring. It is possible that pup exposure to FLX throughout the postnatal period may altered the long-term plasticity potential of hippocampal neurogenesis in males. Indeed, the postnatal period is the time of peak neurogenesis in the dentate gyrus (Altman and Bayer, 1990). In adult male rodents, chronic FLX exposure can increase elements of neuroplasticity such as BDNF (Hodes et al., 2010), dendritic spine density (McAvoy et al., 2015), and neurogenesis (Malberg et al., 2000; Hodes et al., 2010). During development, it is possible that FLX had programming effect on male offspring

neurogenesis, rendering this process sensitive to environmental conditions (i.e. amount of behavioural testing) and yielding different results depending on the amount of concurrent challenges. To further support this point, maternal postpartum FLX increased the proportion of proliferative DCX-expressing cells in the dorsal hippocampus under conditions of several behavioural tests (Chapter 3) but not under conditions of few behavioural tests (Chapter 5).

Maternal postpartum FLX was also had variable effects on adult male HPA axis negative feedback, either impairing it (Chapter 3) or enhancing it (Chapter 5). Interestingly, treating dams with a lower dose of FLX (5 mg/kg) blunted stress-induced c-Fos activation expression in the basolateral amygdala and medial amygdala in adult male rat offspring but not adult female rat offspring (Francis-Oliveira et al., 2013). This dose of maternal postpartum FLX also reduced density of glucocorticoid receptors in the CA3 region of the hippocampus selectively in adolescent male but not female rat offspring (Pawluski et al., 2012c). Together, this suggests that maternal FLX exposure can impact areas involved in HPA axis negative feedback. Coupled with the varied results observed in the dentate gyrus, it is possible that maternal postpartum FLX altered intrahippocampal activity as well as hippocampal connections with areas like the amygdala to perturb HPA axis activity. Interestingly, adult treatment with the same dose of FLX used in this thesis exaggerated HPA axis activity in male mice bred for high reactivity to novelty. However, it had the opposite effect to reduce HPA axis activity in mice bred for low reactivity to novelty (Surget et al., 2016). This suggests that FLX can influence HPA axis sensitivity to novelty, which may partly explain why developmental exposure to FLX had variable effects between Chapters 3 and 5 on adult male HPA axis negative feedback as the amount of novelty/behavioural testing differed.

In NSF, maternal postpartum FLX increased anxiety-like behaviour in adult male offspring in Chapter 3 but had no significant effect on anxiety-like behaviour in chapter 5. Notably, different incentives were used in NSF (chapter 3: standard rat chow, chapter 5: Froot Loops), pointing to the importance of NSF methodologies in evaluating the effects of developmental FLX on anxiety-like phenotypes. First, the use of the more palatable incentive in chapter 5 may have driven a floor effect in male offspring. In chapter 3, control male offspring had a latency of approximately 400s (FLX offspring had a significantly higher latency to feed of approximately 500s). However, the control male offspring in Chapter 5 had a much lower latency of approximately 250 s (FLX offspring had similar latency of approximately 220 s). Thus, the difference in palatability of the incentive used in NSF may have altered anxiety-like behaviour in both the control and FLX-exposed male offspring. Second, given the differences in amount of behavioural testing, it is possible that conditions of higher stress and/or novelty exposure may predispose anxiety in FLX-exposed offspring that was not apparent under baseline conditions. Finally, adult male rat offspring were slightly older in chapter 5 (~125 d old) than in chapter 3 (~75 d old). These methodological considerations may be particularly influential on the dynamic set of outcomes. This may be an important consideration when evaluating maternal SSRI exposure on child outcome in the clinical setting. Currently, clinical evidence is inconclusive but generally points to a minimal or transient disturbance on offspring motor development and behavioural outcomes such as speech perception and social behaviour (28495514). However, clinical studies have not yet been able to ascertain the long term (i.e. in adulthood) effects of maternal SSRI exposure. The preclinical research in this thesis as well as the other studies discussed in this thesis emphasize the importance of analyzing sex of the offspring, age of the offspring, and timing of maternal FLX treatment. Indeed, variable outcomes

were observed in adult male offspring even when the maternal treatment with FLX is consistent (chapter 3 vs. chapter 5). Thus, timing of SSRI exposure as well as context of testing environment may be important to consider as a factor in interpreting clinical studies examining the effects of perinatal SSRI exposure on child outcome.

#### 6.5.3 Variable effects of maternal FLX on adult male offspring – influence of facility

Another noteworthy difference that may contribute to replication issues is that data from Chapters 2 and 3 were collected in a clean barrier facility (Centre for Disease Modeling) whereas data from Chapters 4 and 5 were collected in a conventional facility. These facility differences may have contributed to different environmental factors involved in neurodevelopment. For example, exposure to the environmental microorganism Mycobacterium vaccae during adolescence can promote stress resilience via immune system regulation in male mice and prevent expression of depressive-like and anxiety-like behaviour in adulthood (Reber et al., 2016). This may suggest that facilities with microbes present in natural environments influence stress susceptibility. On the other hands, sterile environments promote stress vulnerability as mice born in germ-free environments (thus sterile environments) exhibit increased stress susceptibility with regards to HPA axis negative feedback dysregulation and vulnerability to anxiety-like behaviour and depression-like behaviour (Sudo et al., 2004). Thus, differences in the expression of anxiety-like behaviour following maternal postpartum FLX in adult male offspring in Chapters 3 and 5 may be related to the developmental effects of barrier and conventional facilities on immune system regulation and subsequently stress resilience. Although the extent or exact mechanism underlying facility differences in this thesis are unclear, this environmental

factor may have been influential in the variable effects of maternal postpartum FLX exposure on offspring outcome. Additional research investigating how sterility of the facility influences neurodevelopment is necessary to understand how this aspect of laboratory studies affects observed differences in affective-related behaviour in rodents.

#### 6.6 Future directions

#### 6.6.1 Mechanisms beyond hippocampal neurogenesis – implications for mothers

During the postpartum period, neurogenesis levels are reduced (Leuner et al., 2007; Pawluski and Galea, 2007), and postpartum CORT treatment exacerbated this reduction (Chapter 3: Workman et al., 2016; Chapter 5: Gobinath et al., under review). It has been posited that one of the underlying mechanisms of antidepressant efficacy is increased neurogenesis (Wainwright and Galea, 2013). Thus, it is possible that high levels of CORT coupled with the naturally low levels of neurogenesis typical of the postpartum period rendered FLX unable to increase neurogenesis and contributed to the limited behavioural efficacy. Because of the robust evidence that hippocampal neurogenesis is involved in depression and antidepressant effects, this thesis predominantly focused on hippocampal neurogenesis. However, several other neural processes and regions are associated with MDD as well as PPD.

The prefrontal cortex dysfunction is implicated in the neurobiology of MDD (Drevets, 2001). High levels of CORT can impact the dendritic architecture of the prefrontal cortex (Wellman, 2001). Interestingly, dendritic complexity in the prefrontal cortex is enhanced in the postpartum (Leuner and Gould, 2010) but chronic restraint stress during gestation prevented this dendritic enhancement and reduced cognitive flexibility in rat dams (Leuner et al., 2014). This
method of gestational stress also reduced quality of maternal care and increased maternal depressive-like behaviour in the forced swim test (Leuner et al., 2014), implicating the prefrontal cortex in endophenotypes of PPD. Antidepressants such as SSRIs and exercise also affect the prefrontal cortex. Chronic FLX treatment prevented chronic unpredictable stress-induced reductions in BDNF protein levels in the prefrontal cortex of adult female mice (Filho et al., 2015). Additionally, chronic voluntary running increased prefrontal cortex angiogenesis in adult male rats (Ekstrand et al., 2008), and aerobic exercise increased oxygenated blood flow even just after a singular acute bout in humans (Giles et al., 2014). This suggests that the prefrontal cortex is another important site in antidepressant efficacy and may be involved in the PPD.

While neurogenesis levels are low in the postpartum hippocampus, neurogenesis levels are elevated in the subventricular zone of the olfactory bulb during late pregnancy and early postpartum in rodents (rats: Furuta & Bridges, 2005; mice: Shingo et al., 2003). During pregnancy and postpartum, the olfactory bulb and its projections to areas such as the amygdala are critical in promoting maternal behaviour in rodents (Lévy et al., 2004; Numan, 2007). Thus, it is possible that high levels of CORT diminished neurogenesis in the olfactory bulb and contributed to CORT-induced disruptions in maternal care. Interestingly, olfactory bulbectomy is also used as a model of MDD in rodents (Song and Leonard, 2005), and it is possible that high levels of CORT also impacted olfactory bulb neurogenesis which consequently contributed to maternal depressive-like behaviour. SSRIs have also been shown to increase olfactory bulb neurogenesis (Hitoshi et al., 2007), but whether this neurogenic effect of SSRIs in the olfactory bulb is present during the postpartum period is unknown. Given the pivotal role of the olfactory bulb in expression of maternal caregiving behaviour, it is possible that FLX prevented CORT-induced disruptions in maternal caregiving behaviour, in this region.

Increased neuroinflammation is also hypothesized to mechanistically contribute to the development of depression (Furtado and Katzman, 2015). Furthermore, both central and peripheral levels of neuroinflammation change over the course of pregnancy and postpartum with transient immune suppression occurring during late gestation and early postpartum (Sherer et al., 2017). Dysregulation of these immune changes, particularly interleukin-6, are posited to contribute to postpartum mood disturbances such as anxiety and depression (Anderson and Maes, 2013). Antidepressants may in part exert their therapeutic effects by reducing neuroinflammation. Pharmacological antidepressants can reduce peripheral inflammation (Eller et al., 2008). Chronic FLX treatment reduced number of microglia in ventral dentate gyrus but not dorsal dentate gyrus in adult female rats (Mahmoud et al., 2016). Reducing neuroinflammation both centrally and peripherally is also proposed mechanism underlying the positive effects of exercise on mood, perhaps by modulating the relationship between proinflammatory cytokines and trophic factors (Cotman et al., 2007). Although it is unclear how antidepressant exposure to either SSRIs or exercise affect neuroinflammation in the context of PPD, future studies can address the extent to which neuroinflammation mediates the effects of antidepressant efficacy in the postpartum.

Finally, it should be noted that while SSRIs are commonly prescribed for PPD (Smolina et al., 2015), there are several other available pharmacological antidepressants targeting other neurotransmitter and hormone systems that may yield different results than presented in this thesis. For example, transdermal estradiol treatment has been investigated in PPD to mitigate ovarian hormone withdrawal (Moses-Kolko et al., 2009). However, it should be noted that this can lead to reduced breast milk production and cessation of breastfeeding, which has been separately linked to PPD (Kim et al., 2014b; Moura et al., 2016). Several other antidepressants

targeting corticotropin release hormone, glutamate, GABA, and neuroinflammation have also been investigated albeit with limited efficacy (Dale et al., 2015). Future studies can explore whether these antidepressants or a combination of these therapies are efficacious for PPD.

## 6.6.2 Mechanisms beyond hippocampal neurogenesis – implications for offspring

Neurogenesis in the dentate gyrus reaches peak levels on postnatal day 6 in rats (Altman and Bayer, 1990); thus, exposure to maternal CORT and/or FLX in the postnatal period could be highly influential on offspring hippocampal neurogenesis. This thesis focused primarily on how these maternal treatments would influence offspring hippocampal neurogenesis. However, it is possible that these maternal treatments influenced other systems to affect the observed behavioural and neuroendocrine outcomes. For example, SSRIs primarily target the serotonin transporter to increase extracellular serotonin, and exercise can also increase serotonin levels in the brain (Wang et al., 2013; Liu et al., 2017). Developmental exposure to FLX has been linked to altered expression of serotonin and its metabolites in offspring at weaning (Gemmel et al., 2016). These effects can be long-lasting on the serotonin system as postnatal exposure to the SSRI citalopram reduced density of serotonin transporter expression in the prefrontal cortex in adult male rats (Maciag et al., 2005). Importantly, serotonin plays a pivotal role in postnatal brain development including synaptogenesis and neural circuitry refinement (Kepser and Homberg, 2015). Thus, maternal antidepressant exposure could have altered development of the serotonin system as well as impacted the downstream effects of serotonin as a trophic factor and regulator of neurodevelopment.

Besides the hippocampus, serotonin transmission is also critical in the development of the barrel cortex, a region important for somatosensory information processing (Feldmeyer et al.,

2013) Increasing serotonin activity by treating male and female rat pups with a serotonin agonist from late pregnancy through the end of the postnatal period resulted in over-reactive responses to sensory and motor stimuli as well as social deficits, which interestingly approximating autism-related phenotypes in adult rats (Kahne et al., 2002). Maternal SSRI exposure has also been controversially linked to autism in the clinical population (Brown et al., 2017). Part of the controversy is due to whether this association is moderated by neonatal SSRI exposure or by concurrent maternal mental mood disturbances (Viktorin et al., 2017). To this end, future studies can utilize rodent models and compare how inducing MDD at different timepoints over the course of pregnancy and postpartum as well as timing of SSRI interventions influence autism-like endophenotypes. Because exercise can increase serotonin as well as circulating glucocorticoid levels, it may also be of interest to examine whether maternal exercise counters risk of PPD on adverse behavioural outcomes in children or has any effects itself on behavioural outcomes relevant to autism in children.

## 6.7 Conclusions

The data presented in this thesis demonstrates that treating dams with either a pharmacological antidepressant (FLX) or non-pharmacological antidepressant (exercise) can differentially and synergistically affect CORT-induced PPD-like endophenotypes. Specifically, postpartum FLX alleviated CORT-induced disruptions in maternal care (Chapter 2, Workman et al., 2016; Chapter 4, Gobinath et al., under review) whereas exercise during pre-conception, gestation, and postpartum period prevented CORT-induced depressive-like behaviour in the forced swim test (Chapter 4, Gobinath et al., under review). The combination of both

antidepressants increased hippocampal neurogenesis (Chapter 4, Gobinath et al., under review). These antidepressant interventions also affected adult male and female offspring outcome. Maternal postpartum FLX exposure increased anxiety-like behaviour, dysregulated HPA axis negative feedback, and elevated hippocampal neurogenesis in dorsal hippocampus selectively in adult male but not female offspring (Chapter 3; Gobinath et al., 2016). Alternatively, maternal exercise enhanced offspring hippocampal neurogenesis regardless of sex but had opposing effects on HPA axis activity with males exhibiting enhanced HPA axis negative feedback and females exhibiting impaired HPA axis negative feedback. However, these effects were observed when assessing FLX treatment beginning in the postpartum period and the protective effects of exercise beginning prior to conception. Furthermore, the model of PPD used tested the relationship between hypercortisolemia and PPD as well as de novo depression arising after parturition. Notable caveats include that these antidepressants may function differently in other models of MDD (ex: allele variants of serotonin transporter gene) or timing of PPD (such as history of MDD and antidepressant use). Future directions can address these caveats as well as explore how other mechanisms besides hippocampal neurogenesis, such as altered serotonin transmission and neuroinflammation, are affected by these treatments.

Collectively, these data emphasize the importance of studying maternal mental health and sex differences in neurodevelopment. FLX and exercise affected endophenotypes of PPD differently than how the literature has robustly observed these antidepressants to affect endophenotypes of MDD in male rodents. Furthermore, maternal antidepressant exposure differentially affected offspring outcome based on sex. This underscores the importance of evaluating both males and females in both preclinical and clinical research. The data in this thesis highlight the importance of studying antidepressant interventions specifically in the

context of motherhood as well as shed light on how these antidepressants impact offspring outcome. It is imperative for future studies to evaluate optimal antidepressant interventions in mother as well as evaluate the neurodevelopmental outcomes of males and females to benefit the mental health of mothers and children.

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