INVESTIGATING AN ANIMAL MODEL OF POSTPARTUM DEPRESSION: THE ROLE OF ESTRADIOL WITHDRAWAL IN CHANGES TO ADULT HIPPOCAMPAL NEUROGENESIS AND SUCROSE CONSUMPTION

by

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B.Sc., Queen's University, 2005

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Neuroscience)

THE UNIVERSITY OF BRITISH COLUMBIA

July 2007

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Abstract

‘Estradiol withdrawal’ after pregnancy is hypothesized to precipitate depressive symptoms in vulnerable women. In this study, a hormonally-simulated ‘pregnancy’ was induced in ovariectomized female rats and the effect of a ‘postpartum’ drop in estradiol was examined on hippocampal cell proliferation (Experiment 1) and sucrose consumption, a model of anhedonia (Experiment 2). All non-sham groups were ovariectomized prior to treatment. Rats were randomly assigned to “pregnant”, “pregnant + EB (estradiol benzoate)”, “pregnant + IMI (imipramine; a tricyclic antidepressant)”, “pregnant + DPN (diarylpropionitrile; an ERβ agonist)”, “sham” or “ovariectomized (OVX)” controls and “sham/ovx + IMI” treatments. All “pregnant” groups received daily hormone injections (estradiol and progesterone) over 23 days to simulate pregnancy, while IMI groups also received daily injections of imipramine. After Day 23 of the “hormone simulated pregnancy”, “pregnant” rats were withdrawn from the hormone regime (mimicking the postpartum drop in gonadal hormones), while other “pregnancy” groups received daily injections of compounds indicated (DPN, EB, IMI). In Experiment 1, on ‘Postpartum’ Day 3, all rats were injected with bromodeoxyuridine and perfused 24 hours later to assess cell proliferation and cell death, in the dentate gyrus. In Experiment 2 groups of rats were tested for consumption of, and preference for, sucrose weekly throughout ‘pregnancy’ and on ‘Postpartum’ Days 2-4. The “pregnant” group had significantly less cell proliferation in the dentate gyrus compared to all other groups except “pregnant +EB”. Estradiol withdrawal after ‘pregnancy’ decreased cell proliferation and this decrease was reversed/prevented by imipramine or DPN treatment. Chronic imipramine increased cell proliferation in sham, but not ovariectomized, rats, suggesting that the imipramine-induced increase in hippocampal cell proliferation is related to reproductive status. In Experiment 2, all groups except “OVX” showed a significant decrease in body weight. Furthermore, there were trends for “pregnant” and “pregnant + EB” groups to have lower sucrose consumption and preference in the postpartum
period compared to pregnancy. “OVX + IMI” had decreased sucrose consumption relative to OVX controls, suggesting a negative effect of imipramine on consumption. Together, these results suggest an important, though complex, role for gonadal hormones in the behavioural and cellular changes accompanying this depression model.
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Acknowledgments

I offer my appreciation to the members of the Behavioural Neuroendocrinology Laboratory for their assistance and support, both moral and technical, in all stages of this project. I owe particular thanks to my supervisor, Dr. Liisa Galea, for her continued guidance, encouragement and helpful analyses. Thanks to Stephanie Lieblich for teaching me to ask questions, and to my parents and partner for providing me with moral support when I struggled to find the answers. I would like to thank the members of my committee for their availability, advice, and assistance in the planning and development of this venture. This work was supported by NARSAD and HELP operating grants. I also gratefully acknowledge research funding from the BC Ministry of Children and Family Development through the Human Early Learning Partnership (HELP). The views presented within are solely those of the author and do not represent the policy of HELP or the Province.


**Introduction**

Postpartum depression (PPD) is a serious medical condition that affects more than 11% of new mothers. The effects of PPD are debilitating to both a mother and her children. Longitudinal studies have revealed that women with untreated PPD have impaired cognitive ability, experience increased marital difficulties, are more likely to abuse their children, and to commit infanticide. In addition, children of mothers with PPD have an increased risk of developing depression or anxiety disorders in adulthood (Nomura et al 2002), present with impaired cognitive, motor and social development (Murray and Cooper 1997; Nomura et al 2002) and are less attached to their mothers during infancy (Barr 2006; Righetti et al 2005).

According to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, text revised (DSM-IV-TR; American Psychological Association, 2000), postpartum depression is defined as an episode of clinical depression occurring within the first four weeks after childbirth - though many researchers and clinicians suggest this window should be widened, as women often experience symptoms of postnatal depression up to six months or a year after parturition (Gaynes et al 2005; Hendrick et al 1998). To qualify for a diagnosis of PPD, a patient must present with five or more of the following symptoms for a minimum of two weeks: insomnia or hypersomnia, psychomotor agitation or retardation, fatigue, changes in appetite, feelings of worthlessness or guilt, decreased concentration, and/or suicidality. Patients must also have at least one of the two “hallmark” symptoms: depressed mood or anhedonia (loss of interest or pleasure in activities). Interestingly, aside from events surrounding onset of the disorder, symptoms of PPD do not seem to differ in their presentation from other forms of depression (Cooper et al 2007).

It is important to distinguish postpartum depression from the postpartum ‘blues’, which may be a common reaction to the dramatic changes associated with delivery (Beck 2006). The ‘blues’ are very common, occurring in between 50-85% of new mothers, and lasting up to 10
days after delivery (Beck 2006). Postpartum depression is a more severe, and more long-lasting, disruption of the mother’s mood state that requires treatment intervention, and occurs in a smaller portion of new mothers. Many factors may contribute to a woman’s vulnerability to PPD. A recent meta-analysis determined that the most pertinent risk factors for PPD included prenatal anxiety and depressive disorders, recent stressful life events, a lack of social support and a previous history of depressive disorders (Robertson et al 2004). Other moderate contributing factors included neuroticism, marital difficulties, obstetric complications, and low socioeconomic status (Robertson et al 2004).

The Role of Gonadal Hormones in PPD

Despite recent interest in the prevalence and risk factors of PPD, the underlying etiology of postpartum depression remains unknown. Depressive disorders are often heterogeneous, and many factors, including environmental, hormonal, and neurotransmitter differences, may be involved. One likely factor in PPD is the dramatic changes in gonadal hormone levels that occur during pregnancy, at parturition, and postpartum. Women are twice as likely as men to suffer from major depression (Lehtinen and Joukamaa 1994; Pearlstein et al 1997), and this difference first appears in adolescence, which suggests the involvement of gonadal hormones (Payne 2003). Studies also indicate that women report more depressive symptoms during times of large hormonal fluctuations; including menopause, the premenstrual period, and the postpartum, which further points to the involvement of hormonal changes in mood disorders (Parry et al 2003; Steiner et al 2003; Studd and Panay 2004). Indeed, the ovarian hormones estradiol and progesterone, which are involved in the Hypothalamic-Pituitary-Gonadal axis (HPG) axis, are often associated with depression (Steiner et al 2003), and are believed to be important modulators of mood and cognition, affecting numerous aspects of central nervous system functioning (McEwen 2002).
Estradiol and progesterone levels change dramatically during pregnancy and the postpartum in significant ways which may contribute to depression in vulnerable women (Figure 1). During pregnancy, levels of estradiol and progesterone increase dramatically, and then abruptly drop off in the postpartum. Levels of estradiol and progesterone climb steadily throughout pregnancy as the result of placental production, with estradiol levels increasing 100-fold, and progesterone levels increasing by 10-fold, compared to normal menstrual cycle levels (Hendrick et al 1998). At delivery, the placenta is expelled, causing a sudden and dramatic drop in both estradiol and progesterone levels (Hendrick et al 1998). This is followed by a prolonged postpartum hypogonadal state, which occurs shortly after parturition and lasts until the restoration of ovulation and the menstrual cycle. Estradiol concentrations reach early follicular levels by days 1 to 3, progesterone by days 3 to 7, of the postpartum (Bloch et al 2003) and they remain at these low follicular levels for at least 40 days after parturition in non-lactating women and 160 days after parturition in lactating women (McNeilly 2001a; McNeilly 2001b).

![Figure 1. Hormonal fluctuations during pregnancy and the postpartum period.](image)

Levels of several hormones, including progesterone and estradiol increase throughout pregnancy then drop off dramatically during labour and the early days of the postpartum period. This early hypogonadal state corresponds to a “period of vulnerability”, designated by the dark bar, in which many women who are vulnerable to depression begin to manifest symptoms of PPD. CRH = corticotrophin releasing hormone. ACTH = adrenocorticotropic hormone. Reproduced with permission (Chrousos et al 1998)
This protracted rise and abrupt withdrawal of estradiol and progesterone, followed by sustained low levels during the postpartum period, may precipitate depressive symptoms in vulnerable women (Douma et al 2005; Hendrick et al 1998; Studd and Panay 2004). This is known as the "estradiol withdrawal" hypothesis of PPD. This hypothesis has garnered growing support from both clinical studies (Ahokas et al 2001; Gregoire et al 1996; Sichel et al 1995) and more recently an animal model of PPD (Galea et al 2001; Stoffel and Craft 2004). Evidence suggests an important role for estrogens in depression (Parry et al 2003; Pearlstein et al 1997; Steiner et al 2003) and a recent meta-analysis suggests that estrogens have a significant beneficial effect on mood in perimenopausal and postmenopausal women taking hormone replacement therapies (HRTs; Zweifel and O’Brien 1997). Some studies have also found a significant improvement in mood in menopausal women with major depressive disorders (MDDs) given estradiol treatment alone (Schmidt 2005), or given estradiol as a supplement to antidepressant therapies (Morgan et al 2005). In PPD, estradiol replacement appears to significantly alleviate symptoms in a subset of depressed women (Ahokas et al 2001; Gregoire et al 1996; Sichel et al 1995), though broader clinical trials are required to confirm its therapeutic applications (Karuppaswamy and Vlies 2003).

Beyond the therapeutic evidence for the involvement of estradiol withdrawal in PPD, some research points to a more direct correlation, suggesting that large changes in levels of estradiol and progesterone, or particularly low levels of estradiol after delivery, may be correlated with the development of depression postpartum (Bloch et al 2003). However, these results are controversial, and many others have failed to replicate this relationship (Buckwalter et al 1999; Harris et al 1996; Heidrich et al 1994; Klier et al 2007; O’Hara et al 1991). Some women may be more vulnerable than others to these fluctuations in gonadal hormones. Bloch et al (2000) investigated depressive symptoms in women with a history of postpartum depression using an experimentally induced model of postpartum hormone withdrawal. High doses of
estradiol and progesterone were administered to a group of women who had previously experienced PPD, and a group of reproductively similar controls who had not developed a mood disorder. The hormone regime was administered for 8 weeks and then rapidly discontinued, to mimic the three main hormonal phases of pregnancy: elevated estradiol and progesterone during pregnancy, the sharp decline in hormone levels at parturition, and the postpartum hypogonadal state (Bloch et al 2000). The study found that women with a history of PPD experienced greater depressive symptoms than controls following the drop in hormone levels (Bloch et al 2000). This suggests that women who experience PPD may be more sensitive to changes in levels of estradiol and progesterone, and it is fluctuations in hormone levels, rather than the absolute hormone levels themselves, which contribute to mood disorders. This may explain, in part, the conflicting results found in studies of absolute hormonal changes at parturition, though further research is required to confirm this possibility. Together, these studies provide a clinical basis for the proposed connection between estrogen as a regulator of mood, the ‘estradiol withdrawal’ hypothesis, and the development of postpartum mood disorders. It is important to note that a number of other gonadal and adrenal hormones, including estriol, oxytocin, corticotrophin releasing hormone, and cortisol, also fluctuate over pregnancy and the postpartum period, and may be involved in PPD. However, this study will concentrate on the role of estradiol and progesterone.

Gonadal Hormone Involvement in ‘Depression’ in Animal Models

Support for the involvement of estrogens in depression can also be found in the animal literature, primarily from studies examining the effects of ovariectomy (the surgical removal of the ovaries) and estradiol replacement on behavioural tests for ‘depression’ (Bekku and Yoshimura 2005; Rachman et al 1998). The Porsolt Forced Swim Test (FST), a common behavioural test used in predicting the efficacy of antidepressant drugs (Cryan et al 2005; Lucki 1997; Posolt et al 1977), is also used as a measure of ‘behavioural despair’ in rats (Dalla et al
2004; Galea et al 2001). The test involves placing a rodent in a cylindrical chamber filled with water (to a height such that the animals legs and tail cannot reach the bottom) and measuring the duration of swimming, struggling, and immobility behaviours (Posolt et al 1977). Several models of depression increase immobility and decrease active behaviours in this paradigm, while clinically effective antidepressants reduce immobility and increase active behaviours (swimming, struggling), supporting the potential role of immobility on the forced swim test as a model of ‘depressive’ behaviours (reviewed in Cryan et al 2005). Rodent models of “estradiol withdrawal” have demonstrated significant increases in immobility after ovariectomy compared to sham controls (Dalla et al 2004; Frye and Wawrzycki 2003; Okada et al 1997; Rachman et al 1998), and this effect can be attenuated with acute or chronic estradiol replacement, as well as by administering antidepressants (Estrada-Camarena et al 2003; Estrada-Camarena et al 2004; Frye and Wawrzycki 2003; Rachman et al 1998; Rocha et al 2005; Walf et al 2004). Recent research has begun to suggest that the antidepressant actions of estradiol in the forced swim test are mediated by its activation of the estrogen receptor (ER) β, rather than ERα (Rocha et al 2005; Walf et al 2004). Using various doses of diarylpropionitrile (DPN; an ERβ agonist) as well as propyl pyrazole triol (PPT; an ERα agonist) in the forced swim test, it has been demonstrated that ERβ, but not ERα, activation is required for the antidepressant activity of estradiol (Estrada-Camarena et al 2003; Walf et al 2004). These antidepressant effects may be acting in the hippocampus, as direct infusion of DPN into the hippocampal formation, but not into the ventral tegmental area, decreases immobility in the FST (Walf and Frye 2007).

An important symptom of depression is anhedonia, or the loss of interest or pleasure in daily activities. A rodent model of this behaviour involves examining the consumption of, and preference for, a 1-2% sucrose solution, relative to consumption of water in a two-bottle choice. Rodent consumption of sucrose appears to be significantly decreased by chronic stress, and this is believed to be a measure of a decreased ‘pleasure’ in consuming the sweet solution (Jayatissa...
et al 2006). Importantly, treatment with antidepressants rescues this behaviour, or prevent its induction, in chronically stressed ‘anhedonic’ rats (Strekalova et al 2006). Further, changes in adult hippocampal cell proliferation may correlate with antidepressant-induced recovery from this stress-induced anhedonia (Jayatissa et al 2006). However, no studies to date have used sucrose consumption to model gonadal hormone-induced ‘depression’, and few sucrose consumption models have used female subjects (Baker et al 2006; Grippo et al 2007), thus the importance of sucrose consumption in this ‘postpartum’ depression model remains to be characterized.

Creating animal models of ‘postpartum’ hormonal changes allows for a controlled manipulation of factors such as hormone levels, genetic background, and environmental experiences, and presents an important opportunity to investigate the role of gonadal hormones on symptoms of PPD. Galea and colleagues (2001) recently employed a hormone-simulated pregnancy (HSP; (Rosenblatt et al 1988) model, which mimics levels of estradiol and progesterone observed during rodent pregnancy, in order to observe ‘postpartum’ depressive behaviours in these rats. In this model, ovariectomized rats undergo an injection regime of increasing doses of estradiol and progesterone over 23 days (the duration of normal pregnancy), after which hormones are abruptly withdrawn to mimic the postpartum period. Studies have shown that three and four days after the abrupt hormone cessation (‘postpartum’) rats demonstrate increased levels of immobility in the forced swim test, a behaviour which can be prevented by continuing treatment with estradiol (Galea et al 2001; Stoffel and Craft 2004). Despite these increased ‘depressive’ symptoms in the ‘postpartum’ period, rats in this paradigm show no significant alterations in anxiety behaviours (as measured using the elevated plus maze) or general locomotion (Galea et al 2001; Stoffel and Craft 2004). These findings complement the results obtained in the studies by Bloch and colleagues, where a hormonally-induced “estradiol withdrawal” precipitated depressive symptoms in vulnerable women (Bloch et al 2000). Further,
the finding that continued estradiol treatment prevented ‘depressive’ behaviours in the forced swim test (Galea et al 2001), is consistent with clinical research demonstrating the therapeutic effect of estradiol on postpartum depressive symptoms (Ahokas et al 2001; Gregoire et al 1996; Sichel et al 1995).

Neuroplasticity in the Hippocampus and Depression

Beyond mimicking behavioural symptoms, and providing a paradigm in which to test for new treatments, animal models often contribute to our understanding by providing a chance to parse out some of the underlying neurobiology associated with disease (Matthews et al 2005). The hippocampus is an area of the brain that has recently come into focus, in both animal and human research, as a structure with possible mechanistic links to depression. The hippocampus may be a key structure in depressive disorders, as it is involved with many functions known to be altered in depressed individuals, including cognitive difficulties, altered response to stress, and mood (McEwen 2005). A 10-18% reduction in hippocampal volume appears to be a common feature in many MRI studies of major depressive disorders (Campbell et al 2004; Geuze et al 2005; Sheline et al 1999; Videbech and Ravnkilde 2004). This decrease appears to correlate with the duration of the illness, not the age of the subject (Sheline et al 1999) and antidepressant treatment may reverse or prevent these volume changes (Sheline 2003; Sheline et al 2003). Smaller hippocampal volumes also appear to correlate with the degree of cognitive difficulties accompanying depression (Frodl et al 2006) and may be predictive of a poor antidepressant response (Hsieh et al 2002; Vakili et al 2000). It should be noted here that the direct relationship between hippocampal volume and depression is not known – whether depression causes a decrease in hippocampal volume, or if a smaller hippocampus increases vulnerability to depression (or, indeed, if both processes are involved) remains unclear (Gage 2000), though some twin studies suggest a pre-existing, environmentally driven, reduced volume in twins discordant for depression risk (de Geus et al 2007).
The cellular changes that underlie this decrease in hippocampal volume have yet to be determined, and further studies, particularly in post-mortem tissue from depressed individuals, are needed to elucidate its precise mechanisms (Czeh and Lucassen 2007; Warner-Schmidt and Duman 2006). Many studies have begun to focus on the possible role of adult hippocampal neurogenesis in depression. Neurogenesis involves at least two processes; cell proliferation (the birth of new cells) and cell survival (new cells that survive to maturity). It is well established that progenitor cells, residing within the dentate gyrus of the hippocampus, continually produce new neurons in the adult brain of all mammals studied to date, including humans (Eriksson et al 1998; Taupin 2005). Progenitor cells located in the subgranular zone (SGZ) of the dentate gyrus undergo cell division, differentiation, migration, and maturation, and the majority of progeny become functioning granule neurons. The timeline for the development and maturation of these new neurons is shown in Figure 2. Briefly, dividing progenitor cells proliferate into 2 daughter cells within 24 hours (Cameron and McKay 2001). New cells destined to become neurons begin to express the protein markers of immature neurons by 48 hours (Brown et al 2003), extend axons within 4-10 days after production (Hastings and Gould 1999), and begin to develop spines and dendritic protrusions by day 15-17 (Zhao et al 2006). These new neurons are considered electrophysiologically mature at approximately 4 weeks, and morphologically new neurons resemble mature granule neurons by 4 months (Lledo et al 2006; van Praag et al 2002).
Figure 2. Generation of new granular neurons in the dentate gyrus of the hippocampus.
New neurons in the dentate gyrus are produced through a multi-stage developmental process, some of which are illustrated above. 1) Proliferation: progenitor cells in the subgranular zone in the dentate gyrus can divide and give rise to daughter cells. 2) Differentiation: some of these daughter cells will begin to express protein markers for immature neurons shortly after production. 3) Migration: immature neurons migrate into the granule cell layer. 4) Axon/dendrite extension: these immature neurons begin to extend their axonal projections towards the CA3 pyramidal cell layer within 4-10 days after production, and extend their dendrites into the molecular layer and begin to express more mature neuronal proteins. 5) Synaptic integration: granule neurons begin to receive inputs from the entorhinal cortex and send outputs to the CA3 region and reach electrophysiological maturity.

Alterations to hippocampal neurogenesis have been suggested as a potential causative pathway to depressive disorders, as well as a critical step in effective treatments (Duman 2004a; Duman 2004b; Feldmann et al 2007; Kempermann and Kronenberg 2003; Vollmayr et al 2007). The “neurogenic theory of depression” (Jacobs et al 2000) has been forwarded primarily on evidence from histological correlates of animal ‘depressive’ behaviours and antidepressant treatments. One line of evidence is based on the observation that hippocampal cell proliferation in the dentate gyrus is increased by chronic (but not acute) treatment with a variety of antidepressants (Malberg et al 2000). This upregulation of hippocampal neurogenesis is seen
with several types of antidepressant treatments, including both serotonin (5-HT) and norepinephrine (NE) reuptake inhibitors (Duman et al 2001), atypical antipsychotics (Kodama et al 2004) and electroconvulsive therapy (Madsen et al 2000). The necessity of chronic, rather than acute, treatment to increase neurogenesis is potentially important for the argument of a functional role of neurogenesis in depression. Despite the fact that most antidepressant drugs induce rapid changes in brain monoamine levels, they often require a few weeks to become therapeutically effective, which overlaps with the delay required before antidepressants begin to induce cell proliferation (Malberg et al 2000). However, it should be noted here that the “delayed antidepressant response theory”, while common clinical doctrine, is itself somewhat controversial, with studies suggesting earlier treatment benefits of antidepressants, and recent metaanalyses suggesting that antidepressant effects may already be present within the first week of treatment (Feldmann et al 2007).

The second line of evidence underlying the neurogenic theory of depression is based on findings that adult hippocampal cell proliferation is reduced in animal models of depression, using chronic mild stress or olfactory bulbectomy (Jaako-Movits and Zharkovsky 2005). Antidepressants may prevent or reverse the decrease in cell proliferation seen with chronic stress paradigms, while also preventing the behavioural changes that accompany chronic stress (Malberg and Schechter 2005; Warner-Schmidt and Duman 2006). Despite these positive findings, evidence on the impact of hippocampal neurogenesis on symptoms of depression remain correlational and conclusions about this relationship are controversial (Dranovsky and Hen 2006; Duman 2004a; Kempermann and Kronenberg 2003). One recent study has directly investigated this possible causal link between neurogenesis and antidepressant action (Santerelli et al., 2003). The authors demonstrated that low-level irradiation of the hippocampus, which reduced cell proliferation rates in the dentate gyrus by 85%, prevented normal antidepressant-induced neurogenesis, and, importantly, prevented a normal behavioural response on the novelty-
suppressed feeding (NSF) task after chronic antidepressant treatments (Santarelli et al 2003).
This suggests that increased cell proliferation may be necessary for the behavioural actions of antidepressants in novelty-suppressed feeding. An effect of radiation alone on the NSF task was not found, suggesting that the decrease in neurogenesis alone was not sufficient to induce ‘depressive’ behaviours (Santarelli et al 2003). However, critics have suggested that even at low doses, the irradiation regime may have altered more than just hippocampal cell proliferation and further, the paradigm used in this study (NSF) is considered by many to be a test of anxiety and not depression, suggesting further research is required to clarify the study’s conclusions (Sapolsky 2004). Virtually all of the studies examining the influence of antidepressants, and depressive behaviours, on adult hippocampal neurogenesis have used male rodents (Lagace et al 2007), which highlights a need for more research on the ability of antidepressants to produce these same neurogenic effects in female, as well as male, animals.

**Estrogen and Adult Hippocampal Neurogenesis**

As reviewed previously, fluctuations in levels of the gonadal hormones, progesterone and estradiol, are associated with a greater vulnerability to depression in women, particularly during times of dramatic change, including menopause and the postpartum period. Estradiol is also a potent regulator of hippocampal neurogenesis, mediating both cell proliferation and survival in a complex and dose-dependent manner (Galea et al 2006). Thus, it seems plausible that fluctuations in estradiol levels may be contributing to depressive disorders in part through their effects on neurogenesis, though further study into this possible mechanistic link is required. Fluctuations in estradiol levels are particularly dramatic during pregnancy and the postpartum period, and estradiol’s effects on hippocampal neurogenesis could provide a mechanistic link between the “estradiol withdrawal hypothesis”, the “neurogenic theory of depression” and the development of postpartum mood disorders. The goal of this project was to further investigate
this possibility using the “hormonally simulated pregnancy” rodent model developed by Galea et al (2001), described previously.

Experiment 1 examined ‘postpartum’ changes in adult hippocampal cell proliferation using the animal model of PPD described in Galea et al (2001), and the impact of treatment with estradiol benzoate (EB), diarylpropionitrile (DPN; an ERβ specific agonist), and a tricyclic antidepressant (imipramine) to counteract any potential decrease in cell proliferation. Following the HSP, on the 3rd day ‘postpartum’, all rats were injected with 5’bromo-2-deoxyuridine (BrdU), a thymidine analog which is taken up into cells actively dividing during the s-phase of mitosis (Miller and Nowakowski 1988). Rats were perfused 24 hours after BrdU injection, and the number of BrdU-immunoreactive (ir) (newly proliferated) cells was assessed using immunohistochemistry. Based on previous pilot data (Galea, unpublished observations) it was expected that “pregnant” animals would have a significant decrease in their levels of cell proliferation and that continued treatment with EB would not return proliferation to normal levels. As other studies have demonstrated an increase in cell proliferation following chronic treatment with imipramine (Keilhoff et al 2006; Sairanen et al 2005) it was predicted that cell proliferation would be significantly increased in all antidepressant-treated groups.

Experiment 2 examined sucrose consumption (a paradigm which is believed to measure ‘anhedonia’ or the loss of pleasure in daily activities) in this model of PPD. Briefly, in the second experiment, groups of rats in the same hormone-simulated pregnancy paradigm described above were measured for their sucrose intake and preference throughout an initial baseline period, once a week during ‘pregnancy’, and on days 2-4 of the ‘postpartum’ period. This allowed for analyses of both the effects of both ‘pregnancy’ and ‘postpartum’ hormone levels on anhedonia. As previous research has suggested that performance on the FST in response to chronic stress may also correlate with sucrose consumption (Strekalova et al 2004), it was
hypothesized that rats with postpartum ‘depression’ would exhibit decreased sucrose consumption.
Methods

All experiments were conducted in accordance with the policies established by the University of British Columbia, the Canadian Council on Animal Care and National Institutes of Health Guide for the Care and Use of Laboratory Animals regarding the ethical treatment of animals used for research. All efforts were made to reduce the number and the suffering of animals. Procedures were approved by the Animal Care Committee at the University of British Columbia.

Animals

Female Long-Evans rats (Charles River, Quebec), age 70-86 days, were used in this experiment. All rats were singly housed in a standard 45 x 25 x 15 opaque polycarbonate cages filled with bedding (Absorption Corporation, Bellingham, WA). Purina rat chow and tap water were available ad libitum. The rats were kept in a temperature-controlled colony room (21±2°C) with a 12-h light-dark cycle (lights on at 7:00am). Rats were either ovariectomized bilaterally or sham ovariectomized while under halothane or isoflurane anaesthesia (5% flow rate for induction and 2-3%, as required, for maintenance of anaesthesia) and aseptic techniques. Animals were given 7 days to recover after surgery before protocol commenced.

Procedure

Drug Treatment

Rats were randomly assigned to one of 8 treatment groups (n = 6 per group); ovariectomized (OVX) control, sham control, OVX + imipramine (IMI), sham + IMI, “pregnant”, “pregnant + EB”, “pregnant + DPN”, or “pregnant + IMI”, where each day either hormone or vehicle was injected subcutaneously (s.c.), and either saline or the tricyclic antidepressant imipramine (IMI) was injected intra-peritoneally (i.p.), between 12:00-14:00, for 27 days (regime described below, see Figure 3 for a timeline). Both sham and ovariectomized control rats received daily injections of 0.1ml sesame oil (s.c.) and 0.2ml 0.9% saline (i.p.). Rats
in the OVX + IMI and sham + IMI groups received daily injections of 0.1ml sesame oil (s.c.) and 15mg/kg of imipramine (i.p.) on each of the 27 days. Rats in the “pregnant”, “pregnant + EB”, and “pregnant + DPN” groups received a low dose of estradiol benzoate (EB; 2.5µg) and a high dose of progesterone (P; 4mg), dissolved in 0.1ml sesame oil (s.c.), and 0.2ml saline (i.p.), on days 1-16. On days 17-23, rats in these groups received a high dose of EB (50µg) dissolved in sesame oil (s.c.), and 0.2ml saline (i.p.). On days 24-27, “pregnant” rats were withdrawn from the hormones, and received injections of 0.1ml sesame oil, and 0.2ml saline. Days 24-27 were considered the “postpartum” period. Rats in the “pregnant + EB” group continued to receive the high dose of EB (50µg), and 0.2ml saline during these “postpartum” days (days 24-27), while rats in the “pregnant + DPN” group received 10µg of diarylpropionitrile (DPN) dissolved in sesame oil (s.c.) on these ‘postpartum’ days. The “pregnant + IMI” group followed the same hormone regime as the “pregnant” rats, except they received 15mg/kg imipramine (i.p.) on each of the 27 days instead of saline. The “pregnancy” hormone doses were based on previous studies demonstrating this regime could induce maternal behaviour in nulliparous ovariectomized rats (Rosenblatt et al 1998), and “postpartum” depressive behaviours on the forced swim test, which were rescued by the continued high EB (Galea et al 2001). Doses of DPN and imipramine were selected based on previous studies of their effectiveness in alleviating “depressive” symptoms in other depression models (Sun and Alkon 2006; Walf et al 2004). On Day 27 (Postpartum Day 3), immediately after treatment, all rats received an i.p. injection of bromodeoxyuridine (BrdU), a thymidine analog and DNA synthesis marker, in a volume of 1.0 ml/100 g body weight (200 mg BrdU/kg). Rats were perfused 24 hours after BrdU administration to examine cell proliferation.

In experiment 2, a second set of rats (n=7/8 per group; all non-sham treatments) were investigated for their behavioural responses to a test of anhedonia (sucrose consumption task). All groups received hormone treatments on the same schedule as described above (OVX, OVX + IMI, “pregnant”, “pregnant + EB”, “pregnant + DPN”, pregnant + IMI”; sham groups were not
tested) and were tested throughout the hormone-simulated pregnancy and the ‘postpartum’ period for sucrose consumption. These rats were perfused 5 days after BrdU injection to phenotype BrdU-ir cells.

**Figure 3. Timeline of hormone injection regimes.**
All rats were ovariectomized and allowed 7 days to recover, prior to starting treatment (Day 1). OVX and Sham control groups received vehicle (sesame oil) and saline control injections throughout the treatment protocol, while “OVX + IMI” and “Sham + IMI” controls received daily vehicle and (i.p.) imipramine injections. “Pregnant” rats received daily hormone injections (daily EB and P) throughout the hormone simulated pregnancy (Day 1-Day 23), then switched to vehicle during the ‘postpartum’ period. “Pregnant + EB” and “pregnant + DPN” groups received the hormone simulated pregnancy, and on day 23 were given daily EB or DPN, respectively, in the ‘postpartum’ period. All of these groups also received i.p. saline injections. “Pregnant + IMI” rats received hormones throughout ‘pregnancy’ and a ‘postpartum’ vehicle, but also received daily i.p. imipramine injections throughout the protocol. EB = estradiol benzoate, P = progesterone, DPN = diarylpropionitrile, BrdU = bromodeoxyuridine.

**Histology**
Rats were anaesthetized with sodium pentobarbital and perfused with 4% paraformaldehyde solution within 24 hours after injection of BrdU (to assess cell proliferation) or 5 days after injection (to phenotype cells). Following extractions, brains were stored in 4% paraformaldehyde for 48 hours, and then transferred into a solution of 30% sucrose dissolved in phosphate buffer and stored for a minimum of 48 hours. Brains were sliced throughout the entire hippocampus in a bath of TBS (0.1 M tris-phosphate buffer in 0.9% saline; pH 7.4), using a vibratome (Leica VT1000S). The 40μm sections were stored at -20°C in sterile culture plates filled with a 50% TBS and 50% antifreeze (ethylene glycol and glycerol) solution prior to immunohistochemical processing.

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Experiment 1: Cellular Markers of Estradiol Withdrawal

BrdU Immunohistochemistry

Free floating tissue sections were processed to reveal BrdU labelling. Sections were first placed in a solution of TBS overnight to wash out antifreeze. Sections were rinsed repeatedly prior to the start of processing, and were rinsed between steps in TBS unless otherwise indicated. Tissue was first incubated in 0.6% H$_2$O$_2$ for 30 minutes at room temperature. To denature the DNA, tissue was incubated in 2N HCl at 37°C for 30 minutes. Sections were then immediately placed in a 0.1M borate buffer for 10 minutes. Sections were blocked with TBS+ (3% normal horse serum (NHS) Jackson Immunoresearch Laboratories, West Grove, PA, USA and 0.1% Triton-X dissolved in TBS) for 30 minutes and then immediately placed in a mouse monoclonal antibody against BrdU (1:200, 3% NHS and TBS+) and left at 4°C for 48 hours. Sections were incubated in mouse secondary antisera (1:100, 3% NHS and TBS+) for 4 hours at room temperature. Sections were incubated in avidin–biotin horseradish peroxidase complex (ABC Elite Kit; 1:50; Vector Laboratories) for 30 minutes. Sections were placed in 0.02% diaminobenzidine (DAB; Sigma Aldrich) and 0.0003% H$_2$O$_2$ to react for 10 minutes. Sections were mounted on slides and left to dry overnight. Sections were counterstained with Cresyl Violet acetate, dehydrated and cover slipped with Permount (Fisher Scientific).

Fluorescent Probe Microscopy

Immunofluorescent labelling was performed to verify the phenotype of BrdU-ir cells. Sections from a sample of free floating tissue from subjects injected with BrdU 5 days before perfusion (to assess phenotype). Each tissue sample set was double-stained with fluorescent probes to assess the percentage of BrdU-ir that were co-labelled with DCX-ir. Sections were rinsed repeatedly prior to the start of processing, and were rinsed between steps in TBS unless otherwise indicated. Sections were blocked in a TBS+ solution (3% normal donkey serum; Jackson Immunoresearch Laboratories, West Grove, PA, USA), 3% Triton-X 10%) for 30
minutes and then placed in a solution containing TBS+ (3% normal donkey serum, 1% Triton-X 10%) and goat polyclonal anti-DCX (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4 °C overnight. Sections were incubated in donkey anti-goat Cy3 (1:200; Jackson Immunoresearch Laboratories) to visualize DCX, for 4 h. Sections were fixed in 4% paraformaldehyde for 10 min. Sections were rinsed twice with 0.9% saline. DNA was denatured by applying 2 N HCl for 30 min at 37 °C. Sections were blocked in a TBS plus solution (3% normal donkey serum, 0.3% Triton-X) for 20 min. Sections were incubated in rat anti-BrdU (1:250, Oxford Biotechnology, Kidlington, Oxfordshire, UK) overnight and in donkey anti-rat fluorescence (1:200; Jackson Immunoresearch Laboratories) to visualize anti-BrdU. Sections were then rinsed and cover slipped with an anti-fading agent (Invitrogen Canada) and stored at 4°C.

**Data Analysis**

All cells were coded prior to examination, so that the experimenter was blind to the condition at the time of counting.

**BrdU-ir Cell Counts**

Figure 4 illustrates representative BrdU-ir cells, pyknotic cells and BrdU-ir cells co-labelled with DCX. The number of BrdU-ir cells in the granular cell layer and subgranular zone of the dentate gyrus were counted on every 10th section throughout the dentate gyrus using a Nikon Ellipse (E600) microscope at 100x magnification on peroxidase-treated tissue. The subgranular zone (SGZ) was defined as approximately the 50 μm band between the granule cell layer and the hilus. Cells were considered BrdU-ir if they exhibited oval or medium round cell bodies, as in previous studies (Cameron et al 1993; Ormerod and Galea 2001) (Figure 4A). The total numbers of BrdU-ir cells were estimated stereologically by taking the total number of cells and multiplying by 10. Area of the granule cell layer and hilus were measured using the ImageJ software program and dentate volumes were estimated using Cavalieri’s principle (\( V = \text{sum of} \))
area x section thickness x distance between measured sections). BrdU-ir cell densities were calculated by dividing total BrdU-labelled cell by the granule cell layer volume.

**BrdU-ir Cell phenotyping**

Phenotype of BrdU-ir cells was analyzed for all non-sham treatment groups on fluorescent probe-treated tissue. Twenty-five BrdU-ir cells on 10th sections per brain (n = 4 per group) were counted in the SGZ of the dentate gyrus using a Nikon Ellipse (E600) fluorescent microscope at 40x magnification. Phenotype was confirmed on a subset of cells using a confocal microscope (Zeiss Axiovert 200M) using a 63× objective. The percentage of BrdU-ir cells that expressed an immature neuronal (DCX-ir) phenotype was determined. Images were created using Z-sections at intervals of 0.4μm and optical stacks of images were taken with NIH Image for PC (Scion Corporation, Frederick, MD, USA). Images were imported to Image J software system for channel merging, and digital manipulations were restricted to contrast enhancements and color level adjustments (Figure 4C).

**Pyknotic Cell Counts**

The total number of pyknotic cells in the granule cell layer and SGZ was stereologically estimated on a separate series of slides stained with cresyl violet. Total pyknotic cells were counted on every 20th section throughout the dentate gyrus of each rat. Pyknotic cells were defined as lacking a nuclear membrane, having pale or absent cytoplasm and darkly stained spherical chromatin, according to criterion set forth by Gould et al (1991) (Figure 4 B).
Figure 4. Photomicrographs of various types of cells under 100x (A and B) and 63x magnification (C). Arrows indicate cells of interest. (A) Photomicrograph of a cluster of BrdU-ir cells located in the subgranular zone of an OVX female rat perfused 24 hours after BrdU injection (B) Photomicrograph of a representative pyknotic (dying) cell in the subgranular zone between the granule cell layer (GCL) and the hilus. (C) Photomicrograph of BrdU-ir (red) and Doublecortin-ir (green) cells. The white arrow indicates a double labeled cell in the GCL of the dentate gyrus. Scale bar in A represents 10 μm in A and B and in 17μm in C.

Experiment 2: Sucrose Consumption and Estradiol Withdrawal

Prior to being placed into treatment groups, all rats in experiment 2 were exposed to a single bottle of a 2% sucrose solution for 48 h in their home cage to allow acclimatization to the solution (Baker et al 2006). Subjects were then presented with a two-bottle choice; one bottle filled with normal tap water and one with the 2% sucrose solution for 48 hours. The 2% sucrose
solution was selected based on previous data suggesting estradiol levels may increase thresholds to detect dilute sucrose solutions (Curtis et al 2005). Intake and preference data, as well as body weight, were collected in two-bottle choice paradigms of 1 hour each, six times over two weeks to establish a baseline rate of consumption. Using baseline rates of consumption, rats were counterbalanced between groups to control for possible individual differences in preference. The hormone treatments for each group is described previously; OVX, OVX + IMI, “pregnant”, “pregnant + EB” “pregnant + DPN”, “pregnant + IMI”, n=7/8 per group. The two-bottle choice was performed on Day 2, Day 9, Day 16, and Day 23 of the hormone simulated pregnancy, as well days ‘Postpartum’ Day 2, ‘Postpartum’ Day 3, and ‘Postpartum’ Day 4. All two-bottle choice exposures took place in the first hour of the dark cycle (19:00-20:00h; (D’Aquila et al 1997) and bottle position was alternated with each trial to control for side preference. Bottle weight was recorded prior to, and immediately after, each one hour exposure, as a measure of fluid intake. After each exposure, test bottles were removed and replaced with regular water bottles. Sucrose intake was calculated as a function of body weight, with the amount (g) of fluid consumed per 100g of weight. Preference for sucrose, determined by the ratio of sucrose solution to total fluid intake, was calculated for each one hour measure and converted to a percent score.

Statistics

For Experiment 1, the dependent variables (total BrdU-ir cells, volume, and total pyknotic cells) were each analyzed using a repeated-measures analysis of variance (ANOVA) with group (OVX, sham, OVX + IMI, sham + IMI, “pregnant”, “pregnant + EB”, “pregnant + DPN”, or “pregnant + IMI”) as the between-subject factor and region (granule cell layer, hilus) as the within-subject factor. The dependent variable, percentage BrdU/DCX-ir cells was analyzed using an analysis of variance (ANOVA) with group (OVX, sham, “OVX + IMI”, “sham + IMI”, “pregnant”, “pregnant + EB”, “pregnant + DPN”, or “pregnant + IMI”), as the between-subject factor.
For Experiment 2, repeated-measures ANOVAs were performed on each of the dependent variables of sucrose consumption, and sucrose preference, respectively, with test days (Baseline, Day 2, Day 9, Day 16, Day 23, ‘Postpartum’ Day 2, ‘Postpartum’ Day 3, ‘Postpartum’ Day 4) as the within-subjects factor and group (OVX, “OVX + IMI”, “pregnant”, “pregnant + EB”, “pregnant + DPN”, or “pregnant + IMI”) as the between-subject factor. Post-hoc comparisons used Neuman-Keuls’s method unless otherwise specified. All statistical procedures were set at $\alpha=0.05$.

**Results**

**Experiment 1**

Treatment Group had no significant impact on the volume of the dentate gyrus

There were no significant group or interaction effects on the volume of the granule cell layer or the hilus of the dentate ($p>.4$). As expected, there was a significant main effect of region, $F(1, 36) = 202.8$, $p<.001$, with the hilus having a larger volume than the granule cell layer.

Estradiol-withdrawal significantly reduced the density of BrdU-ir cells in “pregnant” rats, and this decrease was prevented by treatment with antidepressants or the ERβ agonist, DPN

There was a significant interaction between group by area on the density of BrdU-ir cells, ($F(7, 36) = 6.3$, $p<.0001$; Fig. 5). Post-hoc tests revealed no significant differences in BrdU-ir cell density in the hilus (all $p's>4$). In the granule cell layer, “pregnant” rats had significantly fewer BrdU-ir cells than all other groups except for “pregnant + EB” (all $p's<.02$). Rats in the “pregnant + EB” group also had significantly fewer BrdU-ir cells compared to all other groups (all other $p's<.02$) except “pregnant” ($p<.32$) and the “pregnant + DPN” groups, ($p<0.08$). There were no significant differences in density of BrdU-ir cells between the two control groups, sham and OVX ($p<.85$) “Pregnant + IMI” rats were not significantly different from OVX ($p<.54$) or sham ($p<.73$) controls. “Sham + IMI” rats had a greater density of BrdU-ir cells relative to sham controls ($p<.01$). However, the “OVX + IMI” group did not have a greater density of BrdU-ir
There were also significant main effects of both group (p<.0001) and region (granular and subgranular zone compared to hilus; p<.00001).

**Figure 5.** Mean (±S.E.M.) density of BrdU-ir cells in the dentate gyrus (DG).
Density of BrdU-ir cells in the DG of adult female rats given BrdU on day 3 'postpartum' and perfused 24 hours later. Postpartum estradiol withdrawal ('pregnant' group) significantly decreased the density of BrdU-ir cells found within the granule cell layer (GCL) of the dentate gyrus relative to all other groups but 'pregnant + EB' (p<.02). 'Pregnant + EB' rats also had significantly less BrdU-ir cells compared to all other groups but 'pregnant' and 'Pregnant + DPN' (p<.04). Treatment of Sham rats with chronic antidepressants (Imipramine; IMI), significantly increased cell proliferation over control levels (p<.05). * Significantly different from control group (OVX or Sham) (p<.05).

Estradiol-withdrawal significantly reduced pyknotic cells in "pregnant" rats compared to control and antidepressant-treated groups.

There was a weak trend towards an interaction effect between group and region on the density of pyknotic cells (F (7, 35) = 1.94, p<.09; Figure 6). A priori we expected a difference
between groups in the density of pyknotic cells in the granule cell layer. No significant differences were found between groups in hilus pyknotic cell density (all p's ≥ 0.3). "Pregnant" animals had significantly decreased density of pyknotic cells compared to OVX controls, "pregnant + IMI", "OVX + IMI" and "sham + IMI" groups (all p's ≤ 0.003). There was also a trend towards a main effect of group (F(7, 35)=1.94, p<.09,) and a significant main effect of region, indicating that the granule cell layer had a greater density of pyknotic cells compared to hilus (p≤.00001).

![Figure 6](image)

**Figure 6.** Mean (±S.E.M.) density of pyknotic cells in the dentate gyrus (DG).
Density of pyknotic cells in the granular cell layer (GCL) of the dentate gyrus of adult female rats on Day 4 "postpartum". Postpartum estradiol withdrawal ("pregnant" rats) decreased the number of pyknotic cells within the dentate gyrus relative to the OVX control group, (p<0.009). "Pregnant" rats also had significantly decreased levels of cell death (p≤0.003) compared to all groups treated with chronic antidepressants (imipramine; IMI). * Significantly different from OVX control, $ Significantly different from IMI treated animals (all p’s ≤ 0.001).
Cell phenotyping

The majority of 5-day-old BrdU-labelled cells expressed the immature neuronal protein marker DCX (approximately 60%), and this percentage was not significantly different between treatment groups (F(5, 18)=.73, p>.61; Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>% DCX-ir/BrdU-ir cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVX (n=4)</td>
<td>57.3 ± 5.7</td>
</tr>
<tr>
<td>OVX + IMI (n=4)</td>
<td>56.5 ± 2</td>
</tr>
<tr>
<td>'Pregnant’ (n=4)</td>
<td>62.5 ± 3.8</td>
</tr>
<tr>
<td>'Pregnant + EB’ (n=4)</td>
<td>53.1 ± 4.3</td>
</tr>
<tr>
<td>'Pregnant + DPN’ (n=4)</td>
<td>62.5 ± 4.5</td>
</tr>
<tr>
<td>'Pregnant + IMI’ (n=4)</td>
<td>60.4 ± 4.3</td>
</tr>
</tbody>
</table>

Table 1. Mean (±S.E.M.) percentage of 5-day old BrdU-ir cells co-expressing DCX-ir.
The percentage of cells co-expressing BrdU and the immature neuronal protein doublecortin (DCX) based on a sample of 25 cells per rat in the subgranular zone (SGZ) of the dentate gyrus. No significant differences were found between treatment groups (p<.61).

Experiment 2

OVX rats gained body weight over time while treatment with antidepressants significantly decreased this weight gain.

There was a significant interaction between test day and group for body weight (F(35, 273) = 27.87, p<.0001; Figure 7). There were also main effects of both day and group (day: F (7, 273) = 174.70, p<.0001; group: F (5, 39) = 16.31, p<.0001). Post-hoc tests revealed no significant group differences in baseline weights (all p’s ≥ .5), but OVX animals gained significantly more weight than all other groups by Day 9 (all p’s ≤.0001). “OVX + IMI” animals also gained significantly more weight than “pregnant + IMI” after D9, and “pregnant”, “pregnant + DPN” and “pregnant + EB” groups by Day 23. This suggests that the ovariectomy-induced weight gain was diminished by commitment treatment with imipramine.
Figure 7. Mean (±S.E.M.) Body Weight (g) of female rats on all sucrose consumption test days.

Body weights of female rats (g) prior to a 1 hour choice between 2% sucrose solution and tap water on each of the days of ‘pregnancy’ and the ‘postpartum’ indicated. A significant interaction existed between body weight and day of treatment (F (35, 273) = 27.87, p<.0001). There were no group differences in baseline weights, but OVX animals gained significantly more weight than all other groups by Day 9 of treatment (all p’s<.0001). OVX + IMI” animals also gained more weight than “pregnant + IMI” group by D9, and all other “pregnant” animals on and after Day 23.

Sucrose intake increased across all time points compared to baseline, and showed a trend towards a decrease in sucrose intake during the ‘postpartum’ in ‘pregnancy’ groups.

There was no main effect of group, nor any interaction effect, on sucrose intake (measured as g sucrose consumed per 100g body weight; main effect of group: F (5, 37) = 1.72,
p \geq 0.16; \text{ interaction effect: } F (35, 259) = 1.0, p \geq .48; \text{ Figure 8), while test day was significant (main effect of day: } F (7, 259) = 7.769, p \leq .00001). \text{ Post-hoc tests revealed that, compared to baseline intake levels, sucrose consumption increased on all days (all } p' \text{s} \leq .04) \text{ except Day 2 } (p \geq .20) \text{ and 'Postpartum' Day 4 } (p \leq .06). \text{ During the 'postpartum' period, there was a trend towards an overall decrease in sucrose intake relative to Day 23 (the last day of 'pregnancy') only on 'Postpartum' Day 4 } (p \leq .07), \text{ regardless of group membership. However, } a \ priori \text{ it was expected that sucrose consumption would decrease in the 'postpartum' in the pregnancy groups, and the 'pregnant' group had a trend for lower levels of sucrose consumption from Day 16 of 'pregnancy' compared to 'Postpartum' Day 3 } (p \leq .06). \text{ Similarly the 'pregnant + EB' group showed a trend towards a decrease in consumption on 'Postpartum' Day 3 compared to Day 16 } (p \leq .09), \text{ and the 'pregnant + DPN' group also showed lower sucrose consumption on 'Postpartum' Day 4 compared to 'pregnancy' Day 16 } (p \leq .06) \text{ or 'pregnancy' Day 23 } (p \leq .052). \text{ This trend was also seen for the 'pregnant + IMI' group with lower sucrose consumption levels see from 'pregnancy' Day 16 to all postpartum days } (p' \text{s} \leq .03). \text{ Sucrose consumption in the OVX +IMI group and OVX group did not significantly differ across the treatment days } (all p' \text{s} \leq .3), \text{ though overall sucrose consumption appeared lower in the 'OVX + IMI' } (p \leq .02) \text{ and 'Pregnant + IMI' } (p \leq .05) \text{ groups compared to OVX controls.}
Figure 8. Mean (±S.E.M.) intake of sucrose (g) per 100g body weight in female rats on sucrose consumption test. Amount of sucrose consumed by female rats given a 1 hour choice between 2% sucrose solution and tap water on each of the days of ‘pregnancy’ and the ‘postpartum’ indicated. No overall main effect of treatment group was apparent (F (5, 37) = 1.715, p<.16), while a main effect of treatment day was observed (F (7, 259) = 7.769, p = .00001). Overall, a significant increase in sucrose intake was seen on Day 9, 16, 23 of pregnancy and ‘Postpartum’ Day 2 and 3 (p < 0.05) compared to baseline levels, while ‘Postpartum’ Day 4 maintained a trend towards an increase in sucrose consumption p ≤ 0.06). A priori tests revealed “pregnant” rats had a trend towards a decrease in consumption between Day 16 and ‘Postpartum’ Day 3 (p≤.06), as did the “Pregnant + EB” group. “Pregnant + DPN” rats also showed decreased consumption on ‘Postpartum’ Day 4 compared to Day 16 (p≤.06) or Day 23 (p≤.052), as did “Pregnant + IMI” rats, with lower sucrose consumption between Day 16 to all ‘postpartum’ days (p’s ≤.03).

Sucrose preference increased significantly across all time points compared to baseline, with a trend towards decreased sucrose preference during the ‘postpartum’ compared to ‘pregnancy’.
There was no significant interaction effect of treatment group and day ($F(35, 252) = .99, p < .49$). There was a weak trend for a main effect of treatment group on sucrose preference ($F(5, 36) = 2.01, p < .10$; Figure 9). "OVX + IMI" treated rats had an overall lower level of sucrose preference compared to OVX ($p < .014$) and "Pregnant + DPN" ($p < .016$) rats, and a trend towards a lower preference compared to "pregnant" ($p < .08$) and "Pregnant + EB" ($p < .07$) groups. "Pregnant + IMI" rats also showed a trend towards a lower overall sucrose preference compared to OVX controls ($p < .08$). Compared to baseline, there was an overall increase in sucrose preference on all days of 'pregnancy' and the 'postpartum' period (all $p$'s $< .00001$) regardless of group (main effect of day $F(7, 252) = 15.102, p < .00001$). During the 'postpartum' period, there was a significant overall decrease in sucrose preference relative to Day 16 on all 'Postpartum' Days (all $p$'s $< .04$), and compared to Day 23 (the last day of 'pregnancy') only on 'Postpartum' Day 4 ($p < .02$). On closer inspection the effect is primarily driven by the "pregnant", "pregnant + EB" and "pregnant + IMI" groups, as trends for a significant decrease in sucrose preference on 'Postpartum' Day 3 and Day 4 compared to 'pregnancy' Day 16 were found in these groups. "Pregnant" rats demonstrated a trend towards a decrease in preference on 'Postpartum' Day 3 compared to Day 16 ($p < .11$), "Pregnant + EB" rats had decreased preference compared to Day 16 on 'Postpartum' Day 3 ($p < .052$) and 'Postpartum' Day 4 ($p < .051$), while "Pregnant + IMI" rats had decreased sucrose preference from Day 16 to Day 23 ($p < .01$) and 'Postpartum' Day 4 ($p < .0003$).
Figure 9. Mean (+S.E.M.) preference for sucrose (g) as a percentage (%) of total fluid intake (g) on sucrose consumption test.

Intake of 2% sucrose solution (g) in female rats given a 1 hour choice between 2% sucrose solution and tap water on each of the days of 'pregnancy' and the 'postpartum' indicated. A trend for the effects of treatment group was apparent (F (5, 36) =2.01, p≤10), with OVX rats given the antidepressant imipramine (IMI; OVX + IMI) showing a significant overall decrease in sucrose preference compared to OVX controls (p≤01), while "pregnant + IMI" rats showed a trend towards a decrease in preference compared to OVX controls (p≤08). A main effect of treatment day was observed (F (7, 252) =15.102, p≤00000). Overall, a significant increase in sucrose preference was intake was seen on all days of pregnancy (Day 2, 9, 16, 23) and the 'postpartum' ('Postpartum' Day 2, 3, 4) compared to baseline levels (p ≤ 0.001). A priori tests revealed that this main effect was driven by trends in the "pregnant + EB", "pregnant + IMI" and "pregnant" groups (with a decrease in sucrose preference on 'Postpartum' Day 3 and 'Postpartum' Day 4 compared to Day 16 (p≤.051 to .06 for "pregnant + EB", p≤0003 and p≤.01 for "pregnant + IMI", and p≤.11 for "pregnant").

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Discussion

In Experiment 1, "pregnant" rats were found to have significantly lower levels of cell proliferation in the dentate gyrus compared to all other groups except "pregnant + EB". Chronic treatment with the antidepressant imipramine significantly increased hippocampal cell proliferation compared to controls in the sham, but not the ovariectomized, groups, partially consistent with previous literature using fluoxetine (Lagace et al., 2007). Cell death as measured by the number of pyknotic cells, was significantly decreased in 'pregnant' rats relative to OVX controls and all imipramine-treated groups.

In Experiment 2, contrary to our expectations, there was no significant effect of treatment group on sucrose consumption. There were, however, trends for both sucrose consumption and preference to decrease in the “pregnant”, “pregnant + EB” and “pregnant + IMI” groups from Day 16 of ‘pregnancy’ to ‘Postpartum’ Day 4. Unlike OVX controls, sucrose intake did not increase (relative to baseline) in the “OVX + IMI” group, and overall preference for sucrose was slightly decreased relative to the OVX group, suggesting a possible negative role of imipramine in sucrose consumption and preference. While body weight increased with ovariectomy, this increase appeared to be prevented by chronic imipramine treatment, suggesting a possible role for imipramine in food and sucrose intake. Furthermore, relative to Day 2 of pregnancy, body weight decreased in all groups except the OVX across days.

Estradiol Withdrawal and the Neurogenic Theory of Depression

The results of the present study partially supports the idea that ‘depressive’ behaviours may be accompanied by suppression of adult hippocampal neurogenesis as there was a suppression in hippocampal cell proliferation in the PPD group (“pregnant”) which parallels the timeline of ‘depressive’ behaviours seen previously in this model (Galea et al 2001; Stoffel and Craft 2004). These results are in agreement with findings in other animal models of depression, including olfactory bulbectomy and chronic stress, which demonstrate a decrease in hippocampal
cell proliferation in adult male rats accompanying behavioural deficits (Jaako-Movits and Zharkovsky 2005; Rygula et al 2006). To our knowledge, this is the first study to demonstrate a decrease in hippocampal cell proliferation in a model of ‘depression’ in female rodents, an important proof of principle. Though speculative, these results also suggest that the increased vulnerability to depressive symptoms in the postpartum period could be, in part, a result of decreased hippocampal cell proliferation, brought on by fluctuations in hormone levels.

However, “pregnant + EB” rats also had a decrease in hippocampal cell proliferation relative to controls, despite a lack of behavioural ‘depression’ in the forced swim test (Galea et al 2001; Stoffel and Craft 2004), suggesting an uncoupling of cell proliferation rates and measures of depression. Recent research has suggested that estradiol’s antidepressant effects on immobility in the FST occurs through its effects on the hippocampus (Walf and Frye 2007). Together with the present findings, this suggests that the recovery of greater immobility in the FST in “pregnant + EB” rats does not require a concurrent upregulation of hippocampal cell proliferation, suggesting a possible uncoupling of hippocampal cell proliferation and behavioural ‘recovery’ in rodent PPD.

This dissociation between adult hippocampal neurogenesis and the appearance of ‘depressive’ symptoms is not unprecedented in the literature (Vollmayr et al 2007). Decreases in hippocampal neurogenesis do not always correlate with whether rats will develop learned helplessness (a model of depressive behaviour) in the face of inescapable stress (Vollmayr et al 2003). Further, escapable stress (which does not induce learned helplessness) causes a similar decrease in cell proliferation to that caused by inescapable stress (Malberg and Duman 2003), suggesting that decreased hippocampal cell proliferation may not be a causal requirement of depressive behaviours, but perhaps a source/marker of stress-induced vulnerability to depression (Vollmayr et al 2007). Generalizations from these paradigms are somewhat controversial, as both the learned helplessness test and the forced swim test are responsive to acute, as well as chronic,
antidepressant treatments (Kusmider et al 2006). Thus, some have suggested that these contradictory findings may be a reflection of different mechanistic pathways of acute antidepressant actions, unrelated to the delayed therapeutic effects of antidepressants in human depression (Dranovsky and Hen 2006).

Interestingly, estradiol does have acute effects on FST immobility, decreasing ‘depressive’ behaviours within an hour of a single injection of estradiol benzoate, with effects lasting for 2-3 days (Estrada-Camarena et al 2003). Therefore, it is possible that this ‘uncoupling’ of hippocampal cell proliferation and depressive behaviours in our model may be a reflection of sensitivity in the FST to acute changes that do not mimic the long-term alterations in hippocampal cell proliferation caused by chronic antidepressant treatments. However, the FST itself is considered a good predictor of viability in the development of new antidepressant drugs, even acutely, suggesting some commonalities exist between the mechanisms involved in acute FST response and later clinical recovery (Borsini and Meli 1988). Thus, while an acute positive response in the FST may not require increases in hippocampal cell proliferation, it may reflect other neural changes that are early indicators of antidepressant effectiveness. Importantly, while acute physiological doses of estradiol are known to increase hippocampal cell proliferation (Galea et al 2006), neither acute antidepressant treatment (Malberg et al 2000), nor the repeated high doses of estradiol benzoate used in this paradigm, increase cell proliferation, yet all three treatments have effects on acute FST behaviour (Estrada-Camarena et al 2003; Galea et al 2001; Kusmider et al 2006), which again suggests a possible uncoupling between behavioural ‘depression’ and cell proliferation.

As described previously, Santerelli et al (2003) demonstrated that by blocking hippocampal cell proliferation (using irradiation of progenitor cells) normal behavioural response to two types of antidepressants could be prevented. This study has been the cornerstone of many arguments about the causal role of cell proliferation in antidepressant action. However, a recent
study by the same group (Holick et al 2007) has demonstrated that a different mouse strain (BALB/cJ) demonstrates a robust response to chronic antidepressants in the forced swim test and the novelty-induced hypophagia test that does not require increases in hippocampal neurogenesis. Ablation of hippocampal progenitor cells using low dose radiation also had no impact on behavioural response to the antidepressants, in contrast to previous results by this group (Santerelli et al, 2003). Thus, there may be strain-dependent differences underlying the mechanisms of antidepressant action. Further, the causal role of hippocampal neurogenesis in rodent depressive-like behaviours that were demonstrated previously may not be generalizable (Holick et al 2007).

The production of new cells is only one of the processes involved in hippocampal plasticity, and, as suggested by Holick et al (2007), changes in hippocampal cell proliferation are likely just one possible mechanism by which depression and antidepressant treatments alter cellular and molecular dynamics in the hippocampus. Antidepressant treatments alter several pathways associated with synaptic connectivity, dendritic branching, gene expression, cell death, and cell survival, along with the described changes to cell proliferation in the hippocampus (Chen and Manji 2006; D'Sa and Duman 2002; Fricker et al 2005; Malberg et al 2000; Manev et al 2003; Nakagawa et al 2002; Sairanen et al 2005), and any of these changes could alter ‘normal’ hippocampal functions in the brain (Kempermann et al 2004). In this study, for example, the number of pyknotic (dying) cells was deceased in the “pregnant” animals relative to all other groups, including “pregnant +EB”, suggesting a role for cell death or turnover in animal models of depression, as well as behavioural outcome. This is consistent with recent research demonstrating that antidepressants increase both cell proliferation and cell death in the dentate gyrus, which may play a role in the plasticity and flexibility of hippocampal circuits (Sairanen et al 2005). It is interesting to speculate on the relative role of these different cellular changes in different symptom profiles, and in vulnerability to depressive disorders.
A reduction in hippocampal cell proliferation in this model of PPD appeared associated with, though not causally linked to, increased immobility in the FST. This suppression of hippocampal cell proliferation may be contributing to the manifestation of other depressive ‘symptoms’ in this model. Alternatively, decreases in cell proliferation could be a marker of vulnerability to depression (in the presence of stress, for example), but are not sufficient to induce ‘depression’ alone. One of the strongest predictors of postpartum depression is antenatal depression, suggesting a common vulnerability in both groups (Lee and Chung 2007). A better understanding of both how ‘pregnancy’ and ‘postpartum’ hormone profiles alter hippocampal plasticity, and how these changes relate to ‘depressive’ symptoms would greatly improve our understanding of this vulnerability, and perhaps further improve the chances of implementing successful treatments.

Actions of Estradiol Withdrawal on Cell Proliferation

This study demonstrates a significant impact of estradiol withdrawal on hippocampal cell proliferation, which can be altered by a variety of treatments. The decrease in cell proliferation following withdrawal of a hormone simulated pregnancy is consistent with previous work suggesting that the impact of estrogens on the brain and behaviour are extremely sensitive to both dose and timing (Galea et al 2006; Walf and Frye 2005). Acute estradiol initially increases (within 4 hours) and subsequently decreases (within 48 hours) cell proliferation in the adult female rodent (Ormerod et al 2003). This initial increase in cell proliferation is mediated in part by changes in serotonin levels (Banasr et al 2001), as well as by activation of the estrogen receptors (Mazzucco et al 2006), while the subsequent suppression in cell proliferation is mediated by adrenal steroids (Ormerod et al 2003), suggesting possible links between this system and the monoamine and HPA axis changes known to be associated with depression (Shelton 2007).
These acute changes induced by estradiol may not necessarily reflect the mechanisms involved in the present model, as both dose and exposure duration have significant impacts on behavioural and cellular responses to estrogens (Galea et al 2006; Walf and Frye 2005). Behaviourally, for example, acute physiological doses of estradiol decrease depressive behaviours of ovariectomized rats on the FST (Estrada-Camarena et al 2003), whereas acute high doses of estradiol, equivalent to those used in our study, do not effect FST immobility (Walf and Frye 2005). Recent cell proliferation data has similarly demonstrated that an acute 50μg dose of estradiol (similar to that used in our study) does not increase hippocampal cell proliferation relative to OVX controls, where a more moderate dose of 10μg estradiol does (Tanapat et al 2005). The decrease in ‘postpartum’ cell proliferation following hormone-simulated pregnancy may also be due to the effects of progesterone on the system. The role of progesterone in adult hippocampal cell proliferation has not been studied extensively to date, but existing research suggests progesterone modulates cellular responses to estradiol (Galea et al 2006; Tanapat et al 2005).

Duration of exposure is also likely to play a key role in response to hormone treatment. Subchronic doses of physiological levels of estradiol (but not higher doses), may decrease anxiety behaviour in OVX rats (Koss et al 2004). Length of exposure to estradiol (5 days vs. 35 days) can also dramatically alter its anxiolytic effects on the open field task (Luine et al 1998). Tanapat et al (2005) found that chronic estradiol exposure resulted in no change in cell proliferation compared to OVX controls, while in contrast, recent research by Barker and Galea (submitted) found an increase in cell proliferation following chronic injections of estradiol. These differences may be attributed in part to method of administration (pellets vs. injections) or dose (Barker and Galea, submitted). In this study, continued estradiol treatment resulted in a suppression of cell proliferation, again suggesting a possible role for dose, timing of BrdU, and/or co-administration of progesterone in our findings.
“Pregnant + DPN” rats had higher levels of cell proliferation than “pregnant” animals, and a trend towards an increase relative to “pregnant + EB” rats - suggesting that the ERβ agonist, DPN, restores some of the drop in cell proliferation ‘postpartum’, where continued estradiol treatment does not. Recent research indicates that the antidepressant effects of estradiol infused into the hippocampus are the result of its activation of ERβ, and not ERα, receptors (Estrada-Camarena et al 2004; Walf and Frye 2007). Thus, the increase in cell proliferation following DPN appears to correlate with its behavioural antidepressant effects, a pattern consistent with findings in most traditional antidepressants (Malberg and Schechter 2005). Thus the increase in cell proliferation rates seen with DPN, but not estradiol, may be due to different activation patterns of the ERβ and ERα receptors. As changes in ‘dose’, described previously, may also play a vital role in response to estrogens, binding capacities of these two compounds, or the disparate doses used in this paradigm, may also have contributed to these different effects of continued estradiol and DPN (Carlstedt-Duke 2001; Meyers et al 2001; Pettersson et al 2000).

In this model, reproductive status also appears to have an impact on hippocampal cell proliferation in antidepressant-treated animals. Consistent with a previous study using fluoxetine in female mice (Lagace et al 2007), sham rats treated with chronic imipramine had significantly higher levels of cell proliferation than untreated shams. Intriguingly, however, gonadal hormones appeared to modulate this relationship as the same pattern was not seen in ovariectomized rats (“OVX + IMI” rats did not show an increase in proliferation relative to OVX controls). This suggests that, in rats, normal cycling levels of ovarian hormones may be important to the cell proliferation response to antidepressant drugs. Fluctuations in levels of gonadal hormones are known to be involved in the normal diurnal rhythm of the HPA axis, and release of corticosterone (Seale et al 2004). These pulsatile changes in corticosterone are necessary for fluoxetine-induced increase in hippocampal cell proliferation in male rats (Huang and Herbert 2006). Thus, alterations of normal HPA activity following ovariectomy could explain these
differences in the sham and OVX response to imipramine. Few studies to date have characterized
the cellular effects of antidepressants in females, and should this finding prove generalizable, it
may have important implications for depression research. Most models of antidepressant action
do not include female subjects (except Lagace et al, 2007), and the impact of gonadal hormones
on antidepressant effectiveness could prove an important consideration in our interpretation of
data from animal models.

Sucrose Preference and Estradiol Withdrawal

Both sucrose consumption and preference appeared to be marginally affected in this
rodent model of PPD. When body weight was controlled for, an overall main effect of day was
found, that was driven by fluctuations in the “pregnancy” groups; suggesting an important role
for the gonadal hormones in sucrose intake. Indeed, female rats respond more to a dilute sucrose
solution during proestrus (high estrogen levels), than during estrus (low estrogen levels) (Clarke
and Ossenkopp 1998). This finding is consistent with the finding in the present study showing an
increase in sucrose consumption/100g body weight, compared to baseline, over the first 16 days
of ‘pregnancy’ in hormone-treated groups (given high physiological levels of estradiol).

Compared to Day 16 of ‘pregnancy’, all ‘pregnancy’ groups had decreased sucrose intake on
either ‘Postpartum’ Day 3 or 4, regardless of treatment condition. These results were contrary to
initial expectations that, while the “pregnant” rats would show a ‘postpartum’ decrease in
sucrose consumption, continued treatment with imipramine or hormones would minimize this
decrease.

Sucrose preference, rather than sucrose intake, may be a more sensitive measure of
anhedonia as it controls for changes in overall fluid intake (Kioukia et al 2000), thus differences
in preference and consumption may provide important clues to the underlying deficits in these
groups. There was a trend for a ‘postpartum’ decrease in sucrose preference in “pregnant + EB”,
“pregnant” and “pregnant + IMI” rats, but not the “pregnant + DPN” group. The decrease in

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‘postpartum’ sucrose preference across several different ‘pregnancy’ conditions is somewhat counterintuitive, particularly since “pregnant” animals experience a drop in hormone levels while “pregnant + EB” animals experience no change in their ‘postpartum’ hormone levels - yet both groups experience a drop in sucrose consumption and preference. As described previously, hormone dose is an important consideration in these analyses. It is possible that, as with the findings in cell proliferation, the impact of estradiol on sucrose consumption is dose dependent. That is, there may be an optimal ‘intermediate’ level of estradiol at which sucrose preference is maintained, whereas both repeated high doses of estradiol and the rapid ‘postpartum’ decrease in hormones will decrease sucrose consumption and preference. Indeed, ovariectomy can significantly decrease sucrose consumption relative to sham controls, suggesting a minimal level of estradiol may be necessary for normal consumption and preference (Duncko et al 2001a; Duncko et al 2001b) while estradiol valerate can also decrease sucrose consumption (Boswell et al 2005). Furthermore, estradiol can produce a dose-dependent decrease in short term saccharin consumption, which has been suggested to be the result of an initial weak taste aversion due to “malaise” inducing side effects of the hormone (de Beun et al 1991; Merwin and Doty 1994). Thus estradiol-induced aversion to sucrose may be contributing to the ‘postpartum’ decrease in consumption found in “pregnant + EB” rats, while an optimal level of estradiol could also explain why “pregnant + DPN” rats did not experience the same drop in sucrose preference as “pregnant + EB”, as this group received a lower dose of an estrogenic compound (10µg) than the estradiol benzoate group (50µg). It may also be that, like behaviours in the forced swim test (Walf and Frye 2007), the antidepressant effects of estradiol on sucrose preference are dependent on ERβ activation, and these results reflect a greater ‘antidepressant’ effect of DPN on sucrose preference, though further research is required to examine this possibility.

Contrary to expectations, chronic treatment with imipramine did not prevent the ‘postpartum’ decrease in sucrose preference and consumption. Indeed, treatment with
imipramine resulted in no change in overall sucrose consumption in “OVX + IMI” rats and decreased sucrose preference, on Day 23 of ‘pregnancy’ and ‘Postpartum’ Day 4, in the “pregnant + IMI” group. These results are particularly surprising, as antidepressants, including imipramine, prevent/recover ‘anhedonic’ changes in sucrose consumption in male rats exposed to chronic mild stress (Bekris et al 2005; Jayatissa et al 2006; Rygula et al 2006). There are several possibly explanations for these findings. First, as mentioned, the majority of studies to date use models of chronic mild stress, which may be disrupting sucrose consumption via different mechanistic pathways than gonadal hormone withdrawal, and thus responding differently to the actions of antidepressant treatment. Importantly, while antidepressants do counteract the negative effects of chronic stress on sucrose consumption, administration of imipramine alone, without stress, may decrease sucrose consumption slightly in male rats (Benelli et al 1999), as in our study, suggesting possible side effects of the drug on sucrose consumption. Indeed, studies have demonstrated that imipramine treatment arrested normal weight gain, similar to the effects in this study, as well as decreasing food and water intake in other models (Benelli et al 1999; Mogensen et al 1994). Thus, the lower overall sucrose consumption and preference in the imipramine-treated groups in this study may be the result of negative side effects of the drug that are more apparent in the absence of chronic stress. Alternatively, however, it could be argued that antidepressants, as a method of ‘stabilizing’ or recovering from depressive changes induced by chronic stress, may be preventing an ‘abnormal’ increase in sucrose consumption and/or preference brought on by ovariectomy itself. Little research to date has focused on increased consumption as a model of behavioural depression, however, and further study is required to examine this possibility.

The majority of studies that demonstrate a role for imipramine in preventing stress-induced anhedonia have used male rodents. Pharmacologically, sex differences, and changes in hormone levels, can significantly impact absorption, binding, metabolism, and elimination of an
active drug, which may have important influences on its therapeutic effectiveness (Bukulmez et al 2001; Godfroid 1999; Weizman et al 1988). For example, pre-menopausal women treated with imipramine appear to have greater tolerability issues, including side effects such as nausea and dizziness, than men given the same treatment regime (Kornstein et al 2000). It is possible that the side effects of imipramine in these rats are amplified by sex differences and/or fluctuations in the gonadal hormones, which would correspond with the decreased weight gain observed in these groups. Thus, as with high estradiol treatment alone, it may be that these groups are experiencing a “malaise” and/or aversion to sucrose that occurs as a result of the negative side effects of the imipramine treatment. Co-administration of high doses of estradiol and imipramine have been shown to have paradoxical effects on FST behaviour in female rats, increasing immobility by a mechanism which appears dependent on prolactin levels (Ribeiro et al 2000) providing another possible mechanism by which estradiol and imipramine may interact to alter sucrose consumption in a counterintuitive direction. Clearly, further studies in females are needed to better characterize these potential sex and hormone related differences in response to antidepressants.

Contrary to expectations, these results did not appear to correlate with the cell proliferation changes found in Experiment 1. Both “pregnant + DPN” and “pregnant + IMI” rats had decreased ‘postpartum’ sucrose consumption, while “pregnant + IMI” also had decreased sucrose preference, similar to that in “pregnant” and “pregnant + EB” rats, despite significantly different cell proliferation levels in these groups. This initially suggests that cell proliferation does not correlate with sucrose consumption or preference in our model. While results in the “pregnant + IMI” group indicate that levels of hippocampal cell proliferation can be dissociated from both sucrose consumption, and sucrose preference, it could be argued these results are due to the interference of drug-induced negative side effects or ‘stabilizing’ effects of the drugs (as discussed previously) which limits the interpretation of these findings. Alternatively, ignoring the
imipramine paradox, sucrose preference, but not consumption, could be demonstrating a
correlation between hippocampal cell proliferation and ‘anhedonia’, as “pregnant + DPN” rats
did not demonstrate the same decrease in sucrose preference, or cell proliferation levels, as
“pregnant” and “pregnant + EB” groups. Thus, decreased cell proliferation could be a marker, if
not a causal necessity, of a mild anhedonic deficit in sucrose preference. However, as the
possible role of aversive conditioning in these changes is difficult to parse out, further research
examining different drug combinations, and other (non-gustatory) measures of decreased
motivation is required. To date, the correlation between chronic mild stress, cell proliferation,
and sucrose consumption has only been examined in male rats (Jayatissa et al 2006), and it is
possible that sex differences may exist in both manifestation of ‘anhedonia’, and the underlying
cellular changes. Indeed, recent research suggests that female rats may not respond to chronic
mild stress in the sucrose consumption paradigm with an ‘anhedonic’ decrease in sucrose
preference, but instead with a gradual decrease in total fluid intake, which only manifests in a 24
hour, and not a 1 hour, test paradigm (Baker et al, 2006). As many studies have not separated out
the effects of chronic mild stress on overall fluid consumption from those on sucrose preference,
conclusions about the relationship between chronic mild stress, hippocampal cell proliferation,
and ‘depression’ are difficult to make, and further research on both males and females is
required.

Possible Mechanisms of Action: Estrogen, Cellular Plasticity and Depression

Together, these results suggest an important, if complex, role for changes in levels of
gonadal hormones and the behavioural and cellular markers of depression. There are several
possible mechanisms, including serotonin and brain derived neurotrophic factor (BDNF), by
which estradiol may be acting to influence ‘mood’. Serotonin levels are believed to play an
important role in both antidepressant therapy and vulnerability to depression. Serotonin is also
known interact with changing levels of estradiol in the brain (Rubinow et al 1998). Chronic
estradiol treatment in ovariectomized rats appears to increase serotonin synthesis, release and turnover, while also down-regulating serotonin transporter activity in the hippocampus (Amin et al 2005; Bertrand et al 2005). Changes in levels of brain derived neurotrophic factor (BDNF) may also provide an important mechanism by which estrogen influences depressive symptoms and vulnerability to the disorder. Antidepressants increase levels of both hippocampal cell proliferation and cell turnover, but work with knockout mice has demonstrated that BDNF is necessary for the increased rate of survival of these new neurons, as well as for antidepressant effects in the forced swim test (Saarelainen et al 2003; Sairanen et al 2005). Levels of BDNF appear to fluctuate across the estrous cycle and are increased by chronic treatment with estradiol (Gibbs 1998; Gibbs 1999; Scharfman et al 2003; Solum and Handa 2002) and many of the structural and physiological changes associated with estradiol appear to be mediated downstream by interactions with BDNF (Bloch et al 2000; Scharfman and Maclusky 2005). A recent study has suggested that serum levels of BDNF and serotonin tended to be lower, during gestation and in the early postnatal period, in mothers with depressive symptoms (Lommatzsch et al 2006), providing further evidence that these links between PPD, serotonin, and BDNF, may prove clinically important to the development of depression, though further study is required to determine precise interactions (Scharfman and Maclusky 2005).

Limitations

Depression is a complex, multifactorial illness, and it is unlikely that any one model will reproduce all the behavioural deficits of the disorder, particularly as different clusters of symptoms are present within individual patients, and no common etiology is currently known. While many facets of the disorder can be investigated, it is unlikely that any one animal model can reproduce, or test for, the entirety of the clinical syndrome of 'depression' (Matthews et al 2005). Further, the postpartum withdrawal state mimicked in this study does not occur in isolation. Pregnancy and the postpartum period are accompanied by an array of hormonal and
physiological changes; including alterations to other hormones such as progesterone, oxytocin, and cortisol (Driscoll 2006; Hendrick et al 1998; Parry et al 2003; Spinelli 2005) which likely add another layer of complexity to the estrogen-depression relationship. As discussed, while estrogen is an important possible treatment for PPD, it only alleviates depressive symptoms in a subset of patients (Karuppaswamy and Vlies 2003), suggesting that while estrogen withdrawal may precipitate depressive symptoms in predisposed women, a collection of other genetic and environmental factors likely contribute to underlying vulnerability (Payne 2003; Strous et al 2006; Studd and Panay 2004).

Conclusions

The results of the present study highlight the likely complexity of the relationship between depression, estrogen, and hippocampal neurogenesis. Neurogenesis is a complex process, involving the proliferation, survival, differentiation, and functional integration of new cells into the hippocampus; and at which stage, and by which mechanisms, antidepressants may be involved remains unclear (Warner-Schmidt and Duman 2006). Further, it remains controversial as to whether direct changes in neurogenesis are even required for the behavioural effects of antidepressants (or the development of mood disorders), and changes to existing populations of neurons, alterations in synaptic connectivity, or changes in the neurochemical milieu may be equally relevant to the observed changes in hippocampal volume in depression (Czeh and Lucassen 2007). While our results do suggest some links between hippocampal neurogenesis, ‘depressive’ behaviours, and gonadal hormones, more work is required to help determine the mechanisms by which changes in neurogenesis may impact depressive symptoms, as well as the role of antidepressants, sex, and other environmental or genetic factors, in contributing to these changes.

The results of this study point to a potential contribution of hippocampal neurogenesis to some of the behavioural ‘symptoms’ of PPD, however, they also highlight the paucity of
research to date on the role of sex, and gonadal hormones, in existing models of depression. Despite limitations, the “estrogen withdrawal” model of PPD allows for an important framework in which to examine some of these underlying mechanisms and predispositions and, through convergence with clinical trials, may greatly improve our understanding of the mechanisms and treatment of postpartum depression, and perhaps even the greater vulnerability for depression in women.
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