Summer 2011

The hormone withdrawal hypothesis of postpartum depression: a translational approach

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THE HORMONE WITHDRAWAL HYPOTHESIS OF POSTPARTUM DEPRESSION: A TRANSLATIONAL APPROACH

by

Crystal Elizabeth Edler Schiller

An Abstract

Of a thesis submitted in partial fulfillment
of the requirements for the Doctor of
Philosophy degree in Psychology
(Clinical Psychology)
in the Graduate College of
The University of Iowa

July 2011

Thesis Supervisors: Professor Michael W. O'Hara
Professor Alan Kim Johnson

ABSTRACT

The *hormone withdrawal hypothesis* of postpartum depression (PPD) attributes the onset of depressive symptoms to the rapid postpartum withdrawal of the ovarian hormones estradiol and progesterone that occurs during the first five days following childbirth. Although a number of human and non-human animal studies have supported the hormone withdrawal hypothesis, several studies have failed to support this hypothesis. The current research was designed to test the hormone withdrawal hypothesis of PPD using a novel translational research design that includes a series of experimental animal studies and a longitudinal human study. It was hypothesized that estradiol and progesterone withdrawal would cause increased behavioral despair, anhedonia, and anxiety in the rodent studies. In the human study, it was hypothesized that 1) decreases in estradiol would be associated with increases in negative affect and decreases in positive affect; 2) decreases in progesterone would be associated with increases in anxiety; and 3) these associations would be stronger in women with a past episode of PPD compared to those without a history of PPD. In the animal studies, rats were ovariectomized and administered ovarian hormones or placebo (i.e., hormone administration), followed by placebo only (i.e., withdrawal). Animals in these experiments were given the forced-swim test to measure behavioral despair; lateral hypothalamic self- stimulation to measure anhedonia; or the elevated plus maze to measure anxiety. In the human study, women made mood ratings and collected saliva samples daily starting in the third trimester and continuing until 10 days postpartum. In the animal studies, withdrawal from estradiol alone was associated with behavioral despair (t=2.26, p=.02) and anhedonia (t=-3.2, p=.007). In the human study, there a significant prospective association between estradiol and negative affect in women who developed PPD (r=-0.34, p<.001). This association, when combined with group status (i.e., history of PPD versus no history of depression), was used to correctly identify 100% of women who developed PPD. The results of this project contribute to evidence of a neurobiological basis for PPD. Estradiol

withdrawal represents a promising candidate for further study, particularly with regard to individual differences in sensitivity to hormone withdrawal.

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CERTIFICATE OF APPROVAL

PH.D. THESIS

This is to certify that the Ph.D. thesis of

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ACKNOWLEDGMENTS

I would like to thank my mentors and dissertation advisors Professor Michael O'Hara and Professor Alan Kim Johnson for their guidance throughout my graduate career and in developing and executing this project. They have invested an incredible amount of time and resources in my training, and for that I am grateful. They showed me how to be an academic researcher, teacher, mentor, and leader, and I strive to follow their examples.

Many others have made significant contributions to this work. Jane Engeldinger generously assisted with recruitment and coordinated with the staff in the Department of Obstetrics and Gynecology. Donna Farley conducted the hormone assays for the human study. I am grateful to Terry Beltz for providing detailed instruction in animal research methods and for supervising all of the surgeries. Along with Terry, Connie Grobe, Ralph Johnson, Baojian Xue, and Michael Morris were kind enough to show me the ropes in the animal lab. Sarah Mott and Kevin Riley demonstrated uncanny organizational abilities and attention to detail as project coordinators. Thanks also to the team of undergraduate students and staff who contributed to this research, including Olivia Croskey, Emily Johnson, Cody Connor, and Amy Edler.

Thank you to Jim Marchman whose mentorship has had a profound impact on my life both inside and outside of the clinic. Thanks also to my classmates, Kelsie Forbush, Melissa Buttner, and Rachel Casas, who have provided friendship and inspiration on this journey.

To my family for their love and support. Most of all, thanks to my husband Drew for believing in me even when I doubted myself and for encouraging me to pursue my dreams.

Finally, I would like to thank all of the women who participated in this project. Their commitment made this research possible.

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LIST OF ABBREVIATIONS

5-HT: serotonin

BDI: Beck Depression Inventory

CAMKIID: Ca^{2+} calmodulin-dependent protein kinase IId

CDS: Cornell Dysthymia Scale

CPAS: Chapman Physical and Social Anhedonia Scales

DA: dopamine

DHEA-S: dehydroepiandrosterone sulfate

EB: estradiol benzoate

ECu_{50}: the electrical current required to motivate a rat to press the bar at half the maximum rate

ELISA: Enzyme-Linked ImmunoSorbent Assay

EPDS: Edinburgh Postnatal Depression Scale

EPM: Elevated Plus Maze

ERβ: estrogen receptor beta

FCPS: Fawcett-Clark Pleasure Capacity Scale

FST: forced-swim test

GABA: γ-aminobutyric acid

GAD: generalized anxiety disorder

GnRH: gonadotropin-releasing hormone

HSP: hormone-simulated pseudopregnancy

HSPP: hormone-simulated pseudopregnancy and postpartum

IDAS: Inventory of Depression and Anxiety Symptoms

IDCRC: Iowa Depression and Clinical Research Center

LH: lateral hypothalamus

LHSS: lateral hypothalamic self-stimulation

MASQ: Mood and Anxiety Symptoms Questionnaire

OVX: ovariectomized

PANAS: Positive and Negative Affect Schedule

PMDD: premenstrual dysphoric disorder

PPD: postpartum depression

RDC: Research Diagnostic Criteria

RPM: responses per minute

SCID: Structured Clinical Interview for DSM-IV Axis I Disorders

SNRI: serotonin-norepinephrine reuptake inhibitors

SSRI: selective serotonin reuptake inhibitors

INTRODUCTION

Postpartum depression (PPD) affects approximately one of every eight women following delivery (O'Hara & Swain, 1996). The diagnosis of PPD requires the presence of either depressed mood or anhedonia (i.e., the loss of interest or pleasure in nearly all daily activities), and PPD is often accompanied by anxiety (Wenzel, Haugen, Jackson, & Brendle, 2005). The *hormone withdrawal hypothesis* of postpartum depression (PPD) attributes the onset of depressive symptoms to the rapid postpartum withdrawal of the ovarian hormones estradiol and progesterone that occurs during the first five days following childbirth. Indeed, approximately 50-80% of women experience increased mood symptoms during the postpartum period (Bloch, Rotenberg, Koren, & Klein, 2005). Although a number of human and non-human animal studies have supported the hormone withdrawal hypothesis, several studies have failed to support this hypothesis because of a variety of methodological issues. Thus, the current research was designed to test the hormone withdrawal hypothesis of PPD using a novel translational research design that addresses certain methodological weaknesses of previous studies.

The first aim of this project was to test the effects of exogenous ovarian hormone administration on anhedonia, behavioral despair, and anxiety in a series of experimental animal studies. The concurrent withdrawal of estradiol and progesterone was hypothesized to result in a) decreased lateral hypothalamic self-stimulation (i.e., anhedonia), b) increased immobility in the forced swim test (i.e., behavioral despair), and c) decreased time spent in the open arms during the elevated plus maze procedure (i.e., anxiety).

The second aim of this project was to examine the prospective, longitudinal associations between perinatal ovarian hormone levels and depression and anxiety symptoms in women during pregnancy and the postpartum period. Decreased estradiol was hypothesized to be prospectively associated with a) increased negative affect (i.e., depressed mood) and b) decreased positive affect (i.e., increased anhedonia). A

secondary hypothesis was that decreased progesterone would be prospectively associated with increased anxiety.

The third aim of this project was to investigate differences in associations between perinatal ovarian hormone fluctuations and mood symptoms in women with a history of postpartum depression and women without a history of mood disorders (i.e., controls). It was hypothesized that women with a history of postpartum depression would demonstrate stronger associations between ovarian hormone levels and mood/anxiety symptoms during the postpartum than controls.

Developing a hormone withdrawal model of PPD that demonstrates consistent results across animal and human studies would provide strong evidence of a neurological basis for depression and anxiety in women and may lead to novel pharmacological treatments for PPD. In addition, increased knowledge of predictable increases in mood symptoms during the postpartum may be used to enhance psychotherapy for PPD by helping patients to identify biological as well as psychosocial triggers of depression and anxiety. Given that estrogen and progesterone fluctuations occur across a woman's lifetime from puberty to menopause, advances in our understanding of the influence of ovarian hormones on mood symptoms during the postpartum period may provide important clues for uncovering the neurobiological mechanisms that contribute to the etiology and recurrence of depression in women across the lifespan.

PART A

BACKGROUND LITERATURE

CHAPTER ONE

POSTPARTUM DEPRESSION

Definition and Prevalence

PPD is defined as a major depressive episode that occurs within four weeks following childbirth (American Psychiatric Association, 2000). PPD is therefore characterized by depressed mood, anhedonia, sleep and appetite disturbance, impaired concentration, psychomotor disturbance, lethargy, feelings of worthlessness or guilt, and suicidal ideation (American Psychiatric Association, 2000). Additional symptoms of PPD include mood lability and preoccupation with infant well-being. PPD also is frequently associated with anxiety and panic (American Psychiatric Association, 2000). In some cases, PPD is associated with psychotic symptoms and infanticide, although these cases are rare (American Psychiatric Association, 2000).

While some previous studies have strictly defined PPD according to the DSM-IV criteria, most have used more inclusive criteria, including episodes of depression that began before or during pregnancy and carried over into the postpartum and episodes with an onset several months following delivery. Recent estimates suggest that 7% of women experience an episode of major depression in the first three months following delivery, and the prevalence rate increases to 20% when episodes of minor depression are also included (Gavin et al., 2005). The majority of existing studies suggest that PPD is no more common than non-postpartum depression (O'Hara, 1995); however, the largest epidemiological study to date demonstrated an increased risk of depression during the postpartum period (Vesga-Lopez et al., 2008). Although it remains unclear whether it is more common than episodes of depression experienced throughout a woman's lifetime, the deleterious effects of PPD on mothers, infants, and families makes PPD a significant public health problem.

In contrast, the postpartum blues, which will simply be called "the blues" henceforth, is defined as "mild dysphoria occurring in the first week after delivery,"

(O'Hara, 1995). The most common symptoms of the blues include mood lability, crying, anxiety, and confusion (Kennerley & Gath, 1989). The blues affect 50-80% of women following delivery (Bloch et al., 2005), suggesting that the blues are a normative experience among childbearing women.

Consequences

Public Health Impact

The World Health Organization has identified major depression as the leading cause of disability and the fourth leading cause of burden among all diseases (World Health Organization, 2001). Depression is twice as common among women as men and most common among women of childbearing age (Weissman & Olfson, 1995). The economic impact of PPD is significant. The mean mother-infant dyad healthcare costs are 19% higher for women with postpartum depression, and the costs are highest among those with longer durations of PPD (Petrou, Cooper, Murray, & Davidson, 2002).

Effects on Maternal Health and Well-Being

Women who are depressed during pregnancy demonstrate poor health behaviors (Zuckerman, Amaro, Bauchner, & Cabral, 1989), including substance use and inadequate weight gain. Mothers with self-reported postpartum depressive symptoms have been shown to have more acute healthcare visits, worse general and mental health, and experience a greater impact of health problems on their regular activities (Gjerdingen, Crow, McGovern, Miner, & Center, 2009). Thus, maternal health consequences of PPD likely have direct implications for infant outcomes.

PPD also significantly impacts family dynamics and the quality of marital relationships. For example, PPD is associated with increased marital discord and family conflict, and men are more likely to become depressed when their wives/partners are depressed (Burke, 2003). Strained marital and family relationships likely have negative effects on both maternal and infant outcomes.

Effects on Child Outcomes

Maternal depression is related to a variety of adverse short- and long-term cognitive, emotional, and behavioral outcomes for children, including an increased prevalence of childhood psychiatric disorders (Connell & Goodman, 2002; Goodman & Gotlib, 1999). Children of mothers who experienced postpartum depression display more behavioral problems and lower cognitive function throughout childhood (Beck, 1998). The negative cognitive and emotional consequences of PPD are greatest among children with mothers who continue to experience depression beyond the postpartum period (Beck, 1998). The cognitive and emotional effects of PPD on children are likely mediated by parenting behaviors. For example, PPD is associated with less responsiveness in mothers and less reactivity in infants (Field, 2010). Women with PPD show less positive parenting interactions, including reading, singing, and telling stories (Paulson, Dauber, & Leiferman, 2006).

PPD also impacts physical health outcomes among children. Women with PPD are less likely to initiate breastfeeding and breastfeed for a shorter duration than non-depressed women (Dennis & McQueen, 2009; Ip et al., 2007). Lack of breastfeeding is associated with a variety of negative infant health outcomes, including asthma, obesity, diabetes, and sudden infant death syndrome (Ip et al., 2007). Mothers with PPD have also been shown to engage in less healthy sleep practices with their children, including putting the them to sleep on their backs and putting the them to bed with a bottle (Paulson et al., 2006). In addition to poor feeding and sleep practices, PPD is associated with fewer well-child visits, vaccinations, and safety practices (Field, 2010). Thus, the consequences of PPD on children are serious and extend well beyond infancy into childhood and beyond.

Causes

Demographic, Social, and Environmental Factors

Prevalence rates of PPD vary by income level, ethnicity, and culture. The prevalence of PPD is significantly higher among low-income women (Gress-Smith,

Luecken, Lemery-Chalfant, & Howe, in press; Segre, O'Hara, Arndt, & Stuart, 2007). The prevalence of PPD also varies by ethnicity, with ethnic minorities being more likely to meet the clinical cutoff for PPD (Le, Perry, & Ortiz, 2010). International studies have yielded estimates ranging from 0 to 60% globally (Halbreich & Karkun, 2006), indicating that socioeconomic and cultural influences play a significant role in the pathogenesis of PPD.

Decreased social support and poor marital satisfaction increase the risk of PPD (Beck, 2001; O'Hara & Swain, 1996). Women with higher quality social support experience less PPD (Collins, Dunkel-Schetter, Lobel, & Scrimshaw, 1993). Lower socioeconomic and employment status is also associated with increased risk for PPD (O'Hara & Swain, 1996), whereas longer maternity leave, both paid and unpaid, is associated with reduced depressive symptoms during the postpartum period (Chatterji & Markowitz, 2008). Other predictors of PPD include stressful life events (O'Hara & Swain, 1996), an unwanted or unplanned pregnancy (Beck, 2001), and a previous miscarriage or stillbirth (Blackmore et al., 2011). The effect sizes characterizing the association between social factors and postpartum depression range from small to moderate (Beck, 2001; O'Hara & Swain, 1996).

Both maternal and infant health problems increase the risk for PPD. Medical problems during pregnancy and delivery complications are associated with PPD (Josefsson et al., 2002; O'Hara & Swain, 1996; Vigod, Villegas, Dennis, & Ross, 2010). Infant health and temperament problems and low infant birth weight are also predictors of PPD (Bergant, Heim, Ulmer, & Illmensee, 1999; McMahon, Barnett, Kowalenko, Tennant, & Don, 2001; O'Hara, Neunaber, & Zekoski, 1984). Thus, cultural, social, and health factors play a significant role in the etiology of PPD.

Psychological Factors

Psychological variables have been shown to be one of the strongest predictors of PPD (O'Hara & Swain, 1996). The number of previous episodes of depression, a history

of postpartum depression, and depression during pregnancy are significant risk factors for PPD (Bloch, Rotenberg, Koren, & Klein, 2006; O'Hara et al., 1984; O'Hara & Swain, 1996). PPD is also more likely among women with a history of premenstrual dysphoric disorder and depression during past periods of oral contraceptive use (Bloch et al., 2005). Thus, a predisposition to depressive disorders significantly increases the risk for PPD.

Anxiety during pregnancy is also a strong predictor of PPD (Beck, 2001; O'Hara & Swain, 1996). In particular, generalized anxiety disorder during pregnancy is a significant predictor of PPD up to two years following delivery (Coelho, Murray, Royal-Lawson, & Cooper, 2011). A lifetime history of panic disorder and a diagnosis of panic disorder during pregnancy are also significant predictors of PPD (Rambelli et al., 2010).

In general, a history of any psychiatric illness, experienced before or during pregnancy, appears to increase risk for PPD. For example, both past and current eating disorder diagnoses increase risk for PPD (Micali, Simonoff, & Treasure, 2011). In addition, women with substance use disorders and those with a history of abuse are also more likely to experience PPD (Ross & Dennis, 2009).

Personality constructs associated with PPD include neuroticism (O'Hara & Swain, 1996) and perfectionism (Mazzeo et al., 2006). A negative cognitive attributional style, consistent with Beck's cognitive theory of depression, is also associated with PPD (O'Hara & Swain, 1996). Thus, an underlying predisposition to psychiatric illness and psychiatric diagnoses during pregnancy significantly increase the risk of major depression during the postpartum period.

Biological Factors

Aside from ovarian hormone withdrawal, biological models of PPD etiology have implicated genes, thyroid hormones, HPA axis function, and immune function. Evidence of a genetic vulnerability to PPD comes from family, twin, and gene studies. Episodes of depression appear to cluster in families, but only when PPD is narrowly defined by an onset within four weeks following delivery rather than the broader definition of onset

within six months (Forty et al., 2006). Among the group of women with a familial risk for PPD, 42% experienced PPD following their first delivery. In contrast, only 15% of women without a familial risk experienced PPD (Forty et al., 2006). An Australian twin study demonstrated a 38% heritability of postpartum depressive symptoms, which is similar to heritability estimates for nonpuerperal depression (Treloar, Martin, Bucholz, Madden, & Heath, 1999). Results also suggested that the genetic influences contributing to PPD are distinct from nonpuerperal depression (Treloar et al., 1999). Results of this study are similar to those of an earlier study by Cooper and Murray (1995), which demonstrated that the risk for PPD is distinct from nonpuerperal major depression.

A recent genetic study compared nine women with PPD and 10 postpartum non-depressed women (Segman et al., 2010). A distinct pattern of gene expression was observed among the women with PPD, and gene expression was correlated with the severity of depressive symptoms and clinical course of illness. Gene expression profiles correctly classified 84% of patients as depressed or non-depressed. Moreover, the women with PPD showed a global reduction of gene transcription after delivery, differential immune activation, and decreased transcriptional activation, cell proliferation, nucleotide binding, and DNA replication and repair (Segman et al., 2010). Results of this study may explain the differential vulnerability that predisposes some, but not all, women to PPD.

Genes also may make some women more susceptible to environmental stress during the postpartum. A recent study by Mitchell et al. (2011) demonstrated evidence of a significant gene-environment interaction between both polymorphisms of the serotonin transporter gene and socioeconomic status in PPD. Similarly, a study by Binder and colleagues demonstrated that the serotonin-transporter linked polymorphic region (5-HTTLPR) genotype predicted the onset of PPD in the first 8 weeks following delivery (Binder et al., 2010). Taken together, these studies suggest that PPD occurs among the most genetically reactive women in the context of environmental stress.

Endocrine abnormalities contributing to PPD have also been studied. Increased thyroid dysfunction is associated with pregnancy and may contribute to PPD in a small subset of women. However, previous studies have failed to detect a clear association between dysregulation of thyroid hormones and PPD in the majority of patients (Bloch, Daly, & Rubinow, 2003).

HPA axis dysfunction has also been implicated in the onset of PPD. Levels of corticotropin releasing hormone (CRH), ACTH, and cortisol increase substantially during pregnancy and drop four days following delivery (Mastorakos & Ilias, 2003). HPA axis function normalizes at approximately 12 weeks postpartum (Mastorakos & Ilias, 2003). Similar to the HPA axis dysregulation seen in nonpuerperal depression, basal concentrations of plasma cortisol are increased in women with PPD, and suppression of cortisol by dexamethasone is blunted (Bloch et al., 2003). In one recent study, for women with PPD there was no association between ACTH and cortisol levels in response to a stress test, whereas among non-depressed control women, there was a more regulated association with cortisol levels rising following the increase in ACTH (Jolley, Elmore, Barnard, & Carr, 2007). Some evidence suggests that higher cortisol levels at the end of pregnancy are associated with increased blues symptoms (Handley, Dunn, Waldron, & Baker, 1980). However, it remains unclear whether HPA dysregulation contributes to the onset of PPD or occurs as a consequence of PPD.

Immune dysregulation has been hypothesized to contribute to the development of PPD (Corwin & Pajer, 2008). During pregnancy, anti-inflammatory cytokines responsible for immunosuppression are elevated and promote pregnancy maintenance, whereas proinflammatory cytokines are downregulated. Delivery abruptly shifts the immune system into a proinflammatory state, which lasts for several weeks (Corwin, Brownstead, Barton, Heckard, & Morin, 2005). Patients with depression tend to have higher levels of proinflammatory cytokines, and administration of cytokines is associated with the onset of depression (Raison, Capuron, & Miller, 2006). Moreover, a recent study showed that

women with PPD versus those without have differential patterns of gene expression that are functionally related to differences in immunity (Segman et al., 2010). Thus, there is accumulating evidence that genes may increase the susceptibility to postpartum biological and environmental stress and account for differences in risk for PPD among women.

Assessment and Screening

Several self-report measures have been developed for the purpose of postpartum depression screening and symptom assessment. The most widely used and extensively studied self-report measure of PPD is the Edinburgh Postnatal Depression Scale (EPDS) (Boyd, Le, & Somberg, 2005). The EPDS is a 10- item self-report questionnaire that assesses symptoms of depression and anxiety experienced in the previous week. During the fourth week following delivery, an EPDS score greater than nine has been shown to identify 60% of women who experienced a recurrence of major depression within the first year postpartum (Peindl, Wisner, & Hanusa, 2004). However, EPDS cutoff scores have not been applied consistently across studies, few large-scale studies have examined specificity and sensitivity, and the validity for PPD screening in ethnic minority groups has been questioned (Gjerdingen & Yawn, 2007). Other widely used screening instruments include the Postpartum Depression Screening Scale (PDSS), Beck Depression Inventory (BDI) and BDI-II, and the Patient Health Questionnaire, 9-item depression module (PHQ-9).

Currently there is no federal mandate for either depression screening or follow-up services for postpartum women. Thus, screening for PPD remains at the discretion of individual hospitals, obstetrics clinics, and health care providers. Further research is needed to identify an ideal population-based screening instrument to identify at-risk women during postpartum medical visits or well-child visits. Concurrent with the movement toward improved screening, advances in the availability and delivery of empirically supported treatments are needed. Screening does not significantly improve

patient outcomes unless it is followed by collaborative care including diagnosis, treatment, and follow-up (Gjerdingen & Yawn, 2007).

Treatment

Postpartum women have traditionally been excluded from treatment research, in part, because many assumed that PPD was functionally the same as non-postpartum major depression (O'Hara, 2009). However, PPD differs from other depressive episodes because motherhood is a period of increased stress related to caring for a new infant, it is often accompanied by sleep deprivation, and the role transition of becoming a new mother can be fraught with changes in social support, interpersonal dynamics, and self-doubt (O'Hara, 2009). In the past 25 years, treatment research has begun to focus on PPD.

Although interpersonal psychotherapy (IPT) was developed as a specific treatment for depression (Klerman, Weissman, Rounsaville, & Chevron, 1984), it has been adapted for treatment of PPD (O'Hara, Stuart, Gorman, & Wenzel, 2000). IPT for PPD focuses specifically on the role transition to motherhood and the interpersonal conflicts that often strain marital relationships among new parents. A central goal of IPT is to enhance social support and strengthen interpersonal relationships, and thus, IPT has the advantage of bolstering support for new mothers at a time when it is most needed. Several studies support the efficacy of IPT for PPD. The first randomized controlled trial demonstrated that 12 weekly sessions of IPT were superior to no treatment among 120 women with PPD (O'Hara et al., 2000). Subsequent studies have supported the use of IPT for perinatal women (Clark, Tluczek, & Wenzel, 2003; Spinelli, 1997; Spinelli & Endicott, 2003), and others have adapted the treatment for a group setting (Klier, Muzik, Rosenblum, & Lenz, 2001; Mulcahy, Reay, Wilkinson, & Owen, 2010; Reay et al., 2006; Zlotnick, Johnson, Miller, Pearlstein, & Howard, 2001).

Cognitive-behavioral therapy (CBT) has also been used to treat PPD. CBT focuses on evaluating and modifying dysfunctional thoughts, enhancing problem-solving abilities, and promoting adaptive behavior (Beck, Rush, Shaw, & Emery, 1979). Several

studies suggest that CBT is superior to treatment as usual for PPD (Appleby, Warner, Whitton, & Faragher, 1997; Cooper, Murray, Wilson, & Romaniuk, 2003; Milgrom, Ericksen, Negri, & Gemmill, 2005). However, CBT does not appear to be more effective than medication or as part of a combination treatment for PPD (Appleby et al., 1997).

In-home client centered counseling sessions (i.e., "listening visits") have also been used to treat and prevent PPD (Holden, Sagovsky, & Cox, 1989). Listening visits were developed in the UK and are conducted by nurses during regularly scheduled in-home perinatal medical visits (Holden et al., 1989). Several studies support the effectiveness of listening visits for treating perinatal depression (Cooper et al., 2003; Holden et al., 1989; Morrell et al., 2009; Wickberg & Hwang, 1996).

In contrast to the psychotherapy literature, relatively few randomized controlled studies have examined the use of antidepressant medication for PPD. The selective serotonin reuptake inhibitor (SSRI) fluoxetine has been shown to be superior to placebo for treating PPD and as efficacious as psychotherapy (Appleby et al., 1997). Tricyclic antidepressants appear to be equally effective as SSRIs for treating PPD (Wisner et al., 2006). Despite data supporting the safety of antidepressant medication for breastfeeding infants (Fortinguerra, Clavenna, & Bonati, 2009; Wisner et al., 2006), many mothers remain reluctant to take antidepressant medications (Chabrol, Teissedre, Armitage, Danel, & Walburg, 2004).

A recent meta-analysis demonstrated that existing treatments for PPD result in clinically significant reductions in depressive symptoms (Sockol, Epperson, & Barber, 2011). IPT was found to be superior to CBT for treating PPD, and individual treatments produced greater symptom reduction than group treatments (Sockol et al., 2011). Despite the substantial evidence of symptom reduction following PPD treatment, evidence for improved child outcomes following effective treatment for PPD is lacking (Forman et al., 2007). Further research is needed to improve child outcomes as a result of treatment.

Prevention

Given the extensive literature regarding risk factors of PPD, prevention efforts have focused on early identification of women at risk for PPD followed by the provision of prophylactic treatment (Dennis & Creedy, 2004). Intensive postpartum support for at-risk women appears to effectively prevent PPD, particularly when the intervention is initiated during the postpartum rather than pregnancy (Dennis & Creedy, 2004). Psychotherapy, including IPT (Zlotnick et al., 2001) and CBT (Cho, Kwon, & Lee, 2008), and antidepressant medication (Wisner et al., 2004) have also been used to effectively prevent PPD. Prevention efforts are most effective for women with the greatest risk for PPD; however, identifying and treating at-risk women is costly and not always effective (Dennis & Creedy, 2004). Further research is needed to identify specific risk factors and deliver efficacious, cost-effective treatments. The current project has the potential to help identify a potential risk factor for PPD (i.e., sensitivity to hormone withdrawal) and provide clues about mechanisms and potential treatments.

Existing Etiological Models of PPD

Although many studies have examined specific risk factors for PPD, including genes, endocrine abnormalities, psychological vulnerabilities, and sociodemographic variables, few researchers have advanced and tested detailed etiological models. In addition to the hormone withdrawal hypothesis, existing theoretical models include the cognitive-behavioral model (O'Hara, Rehm, & Campbell, 1982), interpersonal model (Cutrona & Troutman, 1986), and the transactional conflict model (Hayes, Roberts, & Davare, 2000).

O'Hara et al. (1982) advanced a cognitive-behavioral model of PPD, which suggested that the onset of PPD following delivery and early childcare (i.e., a stressful live event) would be predicted by specific psychological vulnerabilities. Consistent with existing cognitive-behavioral models of nonpostpartum major depression, the psychological vulnerabilities included in the model were cognitive distortions, negative

attributional style, and poor social reinforcement. O'Hara et al. (1982) tested the applicability of the cognitive-behavioral model of PPD in a sample of 170 women. Results showed that cognitive-behavioral vulnerabilities assessed prenatally significantly predicted the onset of PPD, albeit to a small degree (i.e., the variables combined accounted for 4.2% of the variance in level of PPD). However, the model as a whole, which contained self-reported prenatal depressive symptoms, cognitive-behavioral vulnerabilities, social adjustment, and life stress, accounted for about 40% of the variance in level of PPD. A subsequent meta-analysis demonstrated that both a negative cognitive attributional style and a history of negative life events were risk factors for depression (O'Hara & Swain, 1996). The positive findings for the cognitive-behavioral model have significant treatment implications. For example, CBT has been used to address the underlying cognitive distortions and negative attributional style implicated in the onset of PPD as well as the stress associated with delivery and early childcare (Cooper et al., 2003).

The interpersonal model suggests that social support, including partner support, is significantly related to the onset of PPD following the role transition involved in becoming a mother (Beck, 2002; Cutrona & Troutman, 1986). Discrepancies between the mother's desired level of support and the level of support that she receives is hypothesized to influence postpartum depressive symptoms (Beck, 2002). A study by Cutrona & Troutman (1986) tested the interpersonal model in 55 married women during pregnancy and at 3 months postpartum. Results of their study suggest that the association between social support assessed during pregnancy and PPD was mediated by women's perceptions of their parenting efficacy. Of note, depressed mood during pregnancy and infant temperament were also significantly associated with PPD in this study. In total, the model accounted for 55% of the variance in postpartum depressive symptoms. Similar to the cognitive-behavioral model, the interpersonal model has direct treatment implications (Beck, 2002). IPT aims to improve a woman's interpersonal relationships and level of

social support while focusing on the difficulty transitioning to the role of motherhood (O'Hara, 2009; O'Hara et al., 2000).

A third etiological model of PPD, proposed more recently, is the transactional conflict model (Hayes et al., 2000). Hayes et al. (2000) propose that a transactional conflict between hard-wired psychobiological mechanisms and Western cultural practices related to childbirth and social isolation is implicated in the etiology of PPD. They identify a number of physiological and neurophysiological processes that accompany pregnancy, childbirth, and lactation and facilitate maternal responsiveness and moderate stress. In contrast, they suggest that Western medical practices and social norms (i.e., medicalization of childbirth, care for the infant exclusively provided by the mother, pressure to return to work following childbirth, and social isolation during the postpartum period) disrupt the traditional mechanisms of support necessary for postpartum psychological adjustment. Although the model was based on existing clinical research, the transactional nature of the psychobiological and sociocultural aspects of the model has not yet been tested. In addition, treatment implications of this model are unclear. Perhaps IPT could be used to bolster social support for women with PPD, but the model suggests that societal changes would have to occur in order to prevent PPD.

The various existing models of PPD account for a significant proportion of the PPD symptom variance. However, of the models that have been tested, the strongest predictor of PPD was depression during pregnancy, rather than cognitive vulnerabilities or lack of social support, as the models suggest. Moreover, each of the models have distinct treatment implications, and treatment selection for individual women based on the various models has not been tested. For example, it has yet to be determined whether women with a negative attributional style would benefit more from CBT, IPT, or pharmacotherapy. As such, additional research is needed in order to improve treatment selection and efficacy for women with PPD.

Statement of the Problem

The *hormone withdrawal hypothesis* attributes the onset of PPD to the rapid postpartum withdrawal of the ovarian hormones estradiol and progesterone and is one of the most widely tested etiological models of PPD. Although a number of human and non-human animal studies support the hormone withdrawal hypothesis (Bloch et al., 2000; Galea, Wide, & Barr, 2001; Gregoire, Kumar, Everitt, Henderson, & Studd, 1996; Sichel, Cohen, Robertson, Ruttenberg, & Rosenbaum, 1995; Stoffel & Craft, 2004; Suda, Segi-Nishida, Newton, & Duman, 2008), several studies have failed to support this hypothesis (Buckwalter et al., 1999; Heidrich et al., 1994; O'Hara, Schlechte, Lewis, & Varner, 1991; O'Hara, Schlechte, Lewis, & Wright, 1991). For example, human studies examining between-group differences in ovarian hormones levels and depressive symptoms during the postpartum period have failed to support the hormone withdrawal hypothesis (Buckwalter et al., 1999; Heidrich et al., 1994; O'Hara, Schlechte, Lewis, & Varner, 1991; O'Hara, Schlechte, Lewis, & Wright, 1991). In contrast, studies that have treated at-risk women with estradiol during the postpartum have successfully reduced depressive symptoms (Gregoire et al., 1996; Sichel et al., 1995), and animal studies have largely supported the hormone withdrawal hypothesis (Galea et al., 2001; Stoffel & Craft, 2004; Suda et al., 2008). Developing a hormone withdrawal model of PPD that demonstrates consistent results across animal and human studies would provide strong evidence of a neurological basis for depression and anxiety in women and would have direct treatment implications for PPD. An improved understanding of the neurological pathways implicated in PPD may lead to novel pharmacological treatments. In addition, increased knowledge of predictable increases in mood symptoms during the postpartum may be used to enhance psychotherapy for PPD by helping patients to identify biological as well as psychosocial triggers of depression and anxiety.

CHAPTER TWO

SEX STERIODS AND PPD

Animal Models of PPD

Existing animal studies largely support the hormone withdrawal hypothesis of PPD, yet they have demonstrated mixed results regarding anxiety symptoms during the postpartum. All of the existing studies have used the Forced Swim Test (FST) as a measure of depression-like behavior. The FST, developed by Porsolt and colleagues in 1977, is a well-validated measure of behavioral despair in which rodents are placed in a cylindrical container of water with dimensions that prevent the animals from touching the bottom and from escaping the container (Porsolt, Le Pichon, & Jalfre, 1977). During the FST, rodents display a period of behavioral activation characterized by vigorous swimming and diving in an attempt to escape; this behavior generally persists for 3-5 minutes after which the animal demonstrates a period of immobility.

The FST has been used extensively for screening the effects of antidepressant drugs, and immobility is significantly reduced by clinically effective antidepressants, such as desipramine, fluoxetine, and imipramine (Borsini & Meli, 1988). As such, immobility during the FST has been operationally defined as "depression-like behavior," and the FST has been widely accepted as a measure of behavioral despair.

Three existing studies (Galea et al., 2001; Stoffel & Craft, 2004; Suda et al., 2008) have demonstrated significant depression-like behavior following ovarian hormone withdrawal in rodents despite methodological differences in hormone administration and FST administration across studies. For example, Galea et al. (2001) induced a hormone-simulated pseudopregnancy (HSP) and administered the FST to examine the effects of hormone withdrawal on behavioral despair. All animals were ovariectomized (OVX), a procedure that involves removing the ovaries to effectively deplete animals of circulating ovarian hormones, prior to experimentation. HSP was induced over a 24-day period, which is consistent with the duration of rodent pregnancy. The HSP group received a

high dose (4 mg) of progesterone and a low dose (2.5 mg) of estradiol benzoate on days 1-16, and a high dose (50 mg) of estradiol only on days 17-24. On day 24, hormones were replaced with placebo injections to induce hormone withdrawal in the HSP animals. A second group, "HSP + EB," received the same treatment as the HSP animals on days 1-24, except that they continued to receive estradiol benzoate treatment during days 24-27 (the "postpartum period"). The control group received 0.1 ml safflower oil (i.e., vehicle) only for the entire duration of the study.

In this study, the FST involved one habituation trial and one test trial (Galea et al., 2001). The habituation trial was administered for 15 minutes on day 25 two hours following the last hormone injection. The test trial was administered 24 hours later for a period of 10 minutes and was scored for struggling, swimming, and immobility. On day 27 all of the rats were administered the open field test, a measure of locomotor activity in rodents.

As expected, the HSP group spent significantly more time immobile and less time swimming during the FST than HSP + EB and control group, demonstrating increased behavioral despair following hormone withdrawal. In the open field test, HSP animals had significantly more area crossings than the HSP + EB and control animals. According to the authors, this suggests that increased immobility in the HSP group could not be explained by a general decrease in motor activity and instead indicated a specific increase in behavioral despair (Galea et al., 2001). However, the open field test was administered 24 hours following the forced swim test, suggesting that decreased locomotor activity on the day of the FST cannot be completely ruled out. This was the first study to demonstrate depression-like symptoms following ovarian hormone withdrawal in a rodent model of PPD, and it was the first to show that exogenous estradiol administration attenuated these symptoms. However, both the influence of hormones on anxiety and the duration of the behavioral effects were unclear, making it difficult to determine if this animal model was appropriate analog for human postpartum depression. In addition, the

effects of the FST habituation trial on the results are unclear. A habituation trial was included in the original FST proposed by Porsolt et al. (1977) as an analog for environmental stress in order to induce behavioral despair. However, the use of a habituation trial has largely fallen out of practice because the experimental manipulation being tested (i.e., ovarian hormone withdrawal) is hypothesized to induce behavioral despair, making the habituation trial unnecessary. As such, it was unclear whether hormone withdrawal alone was sufficient to cause behavioral despair in this experiment or if both hormone withdrawal and environmental stress were necessary.

A subsequent study by Stoffel and Craft (2004) sought to address these issues by determining the duration of behavioral effects following hormone withdrawal, investigating the effects of hormone withdrawal on anxiety, and eliminating the FST habituation trial. In this study, OVX Sprague-Dawley rats in the hormone-simulated pseudopregnancy (HSP) group were administered 4 mg progesterone and 2.5 mg estradiol benzoate on days 1-16, and they were administered 50 mg estradiol on days 17-22, similar to the hormone administration protocol implemented by Galea et al. (2001). The control group received safflower oil (i.e., vehicle) only for the duration of the study. On day 23, injections were terminated to initiate hormone withdrawal. The FST was administered on withdrawal days 1, 2, 4, or 7, and each rat completed the FST only once in order to prevent the practice effects associated with repeated administrations. The first five minutes of the FST were scored for struggling, swimming, immobility, headshakes, and dives using a five-second time sampling technique. Separate rats were tested on the elevated plus-maze on hormone withdrawal days 1, 2, 4, or 7. The elevated plus maze is a plus-shaped maze with two open arms (i.e., unenclosed platforms) and two closed arms (i.e., platforms with enclosed sides). Fewer entries into the open arms, less time spent in the open arms, and fewer head dips are behavioral markers of anxiety in rats that respond to anxiolytic medication: rats administered a clinically effective anxiolytic enter the open arms more frequently, spend more time in the open arms, and demonstrate more head

dips than untreated rats. Spontaneous locomotor activity was also tested on hormone withdrawal days 1, 2, 4, and 7 in using the open field test.

As expected, the HSP group spent significantly more time immobile and less time struggling than the control group on withdrawal days 2 and 4. There were no significant differences between groups on the elevated plus maze or in locomotor activity. The authors concluded that the pseudopregnancy state induced in this study followed by rapid hormone withdrawal was sufficient to produce depression-like behavior. However, the applicability of this model to human PPD remained uncertain because the ovarian hormone profile associated with rodent pregnancy that was simulated in this study and in Galea et al. (2001) is significantly different from that associated with human pregnancy. During rodent pregnancy, estradiol increases steadily during the 24-day pregnancy, whereas progesterone increases until day 16 or 17, at which point it abruptly decreases until parturition on day 24 (as shown in Figure 1, p. 29). The primary difference between rodent and human pregnancy is that progesterone increases over the entire human pregnancy, reaching its peak just before term at approximately week 40 (see Figure 1, p. 29). Thus, during the rodent postpartum (days 24-27), animals experience withdrawal from estradiol but not progesterone, whereas humans experience withdrawal from both estradiol and progesterone.

Therefore, increased behavioral despair during the "postpartum" demonstrated in previous studies could be attributed to estradiol withdrawal alone. The effects of estradiol withdrawal on depression are well documented. Following bilateral ovariectomy, rats demonstrate increased immobility during the FST, and these effects are reversed by treatment with estradiol alone (Bekku & Yoshimura, 2005; Bernardi, Vergoni, Sandrini, Tagliavini, & Bertolini, 1989). In addition, reduced immobility following a single administration of estradiol lasts 2-3 days, and the behavioral effects are the same as those following fluoxetine treatment (Estrada-Camarena, Fernandez-Guasti, & Lopez-Rubalcava, 2003). The antidepressant effects of estradiol during the FST appear to

involve selective actions at intracellular estrogen receptor-β (ERβ) in the ventral tegmental area (Walf, Rhodes, & Frye, 2004), and in fact, may require ERβ (Walf & Frye, 2006). In addition, abrupt estradiol withdrawal following sustained high estradiol levels results in elevated brain cortical dehydroepiandrosterone sulfate (DHEA-S), a neuroactive steroid synthesized endogenously in the brain that attenuates GABA-ergic activity and may be relevant to postpartum depressive symptoms (Maayan, Strous, Abou-Kaoud, & Weizman, 2005). Chronic administration of estradiol leads to dopamine receptor up-regulation and increased presynaptic dopamine activity, which when followed by abrupt estradiol withdrawal, leads to dysregulation in brain dopaminergic pathways and depressive symptoms (Byrnes, Byrnes, & Bridges, 2001).

Thus, estradiol withdrawal is sufficient to cause behavioral despair in animals, and these effects are mediated by neurosteroid action in the brain. Taken together, these findings suggest that the rodent pregnancy model used by Galea et al. (2001) and Stoffel and Craft (2004) is sufficient to cause depression-like behavior in ovariectomized rats. However, a weakness of this model is the lack of anxiety symptoms following hormone withdrawal. This is particularly relevant to the applicability of the model to human PPD because depression and anxiety are highly comorbid in the postpartum period (Wenzel et al., 2005).

Interestingly, previous research suggests that progesterone withdrawal may exacerbate anxiety. Progesterone metabolites act on λ-aminobutyric acid (GABA) receptors in the brain, producing sedative-like effects by enhancing GABA neurotransmission (Beckley & Finn, 2007). Abrupt decreases in progesterone are associated with anxiety (Smith et al., 1998), and treatment with progesterone reduces anxiety (Dennerstein et al., 1985). In contrast, Stoffel and Craft's (2004) model had no effect on anxiety. This is likely attributable to the fact that "postpartum" animals in their study were experiencing withdrawal from estradiol but not from progesterone, which is

significantly different from the postpartum hormone levels experienced by human females.

Thus, to examine the effects of concurrent estradiol and progesterone withdrawal, which is more consistent with the human postpartum period, Suda et al. (2008) created a novel rodent model of postpartum depression by administering hormone levels more consistent with human pregnancy than rat pregnancy. In addition, the researchers aimed to examine the effects of pregnancy- and postpartum-level hormones on hippocampal gene expression. This study included three groups: controls that were not given any hormones over the duration of the study (OVX); hormone simulated pseudopregnancy (HSP); and hormone simulated pseudopregnancy and postpartum (HSPP). All rats were ovariectomized prior to experimentation. The HSP and HSPP groups underwent continuous pellet implantation (.5 mg estradiol and 50 mg progesterone) for a period of 21 days before the pellets were surgically removed. The HSP group was tested on day 21 (the final day of hormone administration). The HSPP group was tested on withdrawal days 1 through 7.

A variety of behavioral tests were administered in this study. The learned helplessness test, which involved foot shock administered in a shuttlebox, was used as a measure of behavioral despair. The FST was used to measure depression-like behavior; the open field test was used to measure locomotor activity and anxiety; the elevated plus maze was used to measure anxiety; and the resident intruder test was used to measure behavioral aggression. Animals that underwent the FST did not undergo any of the other behavioral tests, whereas the rats that underwent the open field test were administered other behavioral tests. DNA microarray analysis was performed to identify candidate genes regulated by HSP and HSPP.

In the learned helplessness test, HSPP animals tested on withdrawal day 7 showed increased failure to escape and latency to escape compared with OVX animals, indicating increased behavioral despair in the HSPP group. Contrary to expectations, HSPP animals

showed *decreased* FST immobility on days 4 and 7 and increased struggling and climbing on day 7 compared to OVX animals, a result that will be interpreted below. In the open field test, there was a trend for HSPP rats to spend less time in the center field than the other groups, indicating increased locomotor activity in the HSPP group. In the elevated plus maze, HSPP animals spent less time in the open arms than HSP animals, and the HSP group had an increased number of open arm entries compared to the OVX group, which indicates increased anxiety in the HSPP group. On the resident intruder test, HSP animals engaged in offensive behaviors toward the intruder for a longer duration than OVX and HSPP animals, and both HSP and HSPP animals showed significantly lower attack latencies than OVX animals, indicating higher behavioral aggression among the HSP and HSPP groups.

Finally, the HSP and HSPP animals showed several significant differences in gene expression. Ca^{2+} calmodulin-dependent protein kinase IId (CAMKIID) was up-regulated during pseudopregnancy and returned to OVX levels after 4 days of hormone withdrawal. CAMKIIA expression was lower in the postpartum than during pseudopregnancy or in OVX. Serotonin transporter expression was significantly increased during pregnancy and returned to OVX levels after four days of withdrawal. GABA type A receptor α4 and aquaporin 4 expressions were down-regulated during pregnancy and returned to OVX levels after 4 days of withdrawal.

Taken together, results of Suda et al. (2008) suggest that the hormone withdrawal model proposed in this study may be a valid model of postpartum depression. Results of the learned helplessness test suggest increased depression-like behavior in the postpartum compared to pregnancy. However, the learned helplessness results were somewhat inconsistent with results of the FST, which showed decreased immobility in the postpartum compared to pregnancy. Animals in this study also showed increased anxiety during the postpartum, and increased anxiety has been associated with decreased immobility on the FST (Cannizzaro, Flugy, Cannizzaro, Gagliano, & Sabatino, 1993;

Consoli, Fedotova, Micale, Sapronov, & Drago, 2005; Nishimura, Ida, Tsuda, & Tanaka, 1989). These results may also be a function of the FST habituation trial, whereby rats were exposed to the FST the day before the test was conducted, and data were collected. This was the first animal model to demonstrate increased anxiety following hormone withdrawal, which may be related to the increased level of progesterone administered to animals during the "pseudopregnancy" portion of the trial (in contrast to previous studies that stopped administering progesterone midway through the pseudopregnancy to more closely mimic rodent pregnancy). Finally, gene expression in the hippocampus was altered during the stimulated pregnancy and the postpartum period, providing important insight into brain mechanisms associated with the onset of postpartum depression.

Taken together, results of the extant animal literature provide support for the hormone-withdrawal hypothesis of PPD. However, studies have yielded inconsistent results in the FST following hormone withdrawal, with two studies demonstrating increased immobility and one reporting decreased immobility. Discordant findings may have resulted from the different methodologies employed in the various studies (see Table 1, p. 28).

Stoffel and Craft (2004) showed increased immobility during the FST and no change in anxiety during the simulated postpartum period. Of note, they did not include a FST habituation treatment, and they administered hormone levels consistent with rodent pregnancy, resulting in estradiol depletion without the accompanying progesterone depletion during the postpartum period. In contrast, Suda et al. (2008) showed decreased immobility during the FST and increased anxiety. They included a habituation phase in their FST, and they also administered hormone levels consistent with human pregnancy (high levels of estradiol and progesterone throughout the pseudopregnancy). Thus, animals in this study experienced estradiol and progesterone withdrawal, which is more consistent with the human postpartum than the rodent postpartum simulated in previous studies. Therefore, it is difficult to ascertain whether immobility results were a function

of the differing hormone profiles of the rats or inclusion of an exposure phase in the FST. Given that anxiety and depression often co-occur in women during the postpartum, it is essential that animal models address this issue. Finally, none of the animal studies of PPD have examined anhedonia, a core symptom of major depression in humans. Behavioral tests of anhedonia, including lateral hypothalamic self-stimulation, may provide a better test of depression-like behavior than the FST because they aren't influenced by anxiety (Borisenko, Meng, Rauhala, & Mannisto, 1996).

Ovarian Hormones and Anhedonia

The lateral hypothalamus (LH) is an area through which the medial forebrain bundle passes that is especially sensitive to rewarding electrical stimulation (Olds & Milner, 1954). Stimulation of the LH activates descending fibers that innervate dopaminergic neurons in the ventral tegmental area and increases extracellular dopamine (DA) concentrations among several forebrain regions, including the nucleus accumbens. Activity in the mesolimbic DA system, originating in the ventral tegmental area and terminating in the nucleus accumbens, is related to reward processes in the brain, and rewarding stimuli activate the mesolimbic DA system. Mesolimbic activity is responsible for reinforcing various behaviors, including eating, drug taking, and brain self-stimulation. Reduced availability of DA and serotonin (5-HT) in the mesolimbic system is associated with anhedonia (Willner, Golembiowska, Klimek, & Muscat, 1991). The ovarian hormones estradiol and progesterone exert effects in the mesolimbic system and alter the reinforcing effects of lateral-hypothalamic self-stimulation (LHSS).

In 1966, Prescott demonstrated that LHSS bar-pressing rates were significantly higher in rodents during the estrous phase compared to other phases of the estrous cycle (Prescott, 1966). Estrous is the phase during which ovulation occurs, and it is characterized by the highest levels of estradiol of any of the estrous cycle phases. Thus, high levels of estradiol were associated with an increase in the reinforcing effect of LHSS. Subsequent studies found similar results examining the number of response

"bursts" rather than rates of bar-pressing (Steiner, Katz, & Carroll, 1982). However, both of these measures are subject to changes in motor activity. More recent studies have examined the animals' bar-pressing rates on a number of descending currents at a constant frequency, which generates a rate-current curve. Two measures that can be generated using this method are the electrical current required to reward or motivate a rat to press the bar at half the maximum rate (ECu_{50}) and the upper asymptote, the maximum rate of bar pressing. A decrease in ECu_{50} indicates an increase in the reinforcing effect of the stimulation, whereas an increase in ECu_{50} indicates a decrease in the reinforcing effect of the stimulation. If the upper asymptote remains constant, then the change in ECu_{50} cannot be attributed to changes in motor activity (Bless, McGinnis, Mitchell, Hartwell, & Mitchell, 1997). During estrous, a time during the estrous cycle when estradiol is highest and progesterone is lowest, there is a significant decrease in ECu_{50} (Bless et al., 1997). In OVX animals, ECu_{50} also decreases following estradiol and progesterone treatment (Bless et al., 1997). Thus, it was hypothesized that the concurrent withdrawal of estradiol and progesterone would increase ECu_{50}, indicating a decrease in the rewarding effect of self-stimulation (i.e., anhedonia).

Table 1. Inconsistencies in the methods and results of existing animal models of PPD.

Study	Simulated Pseudopregnancy Hormone Profile	FST Habituation Trial?	FST Immobility Result[1]	EPM Anxiety Result[1]
Galea et al. (2001)	Rodent	Yes	Increased	Not measured
Stoffel & Craft (2004)	Rodent	No	Increased	No difference
Suda et al. (2008)	Human	Yes	Decreased	Increased

Note: FST: forced-swim test; EPM: elevated plus maze

[1]Result comparing "postpartum" animals to OVX control animals

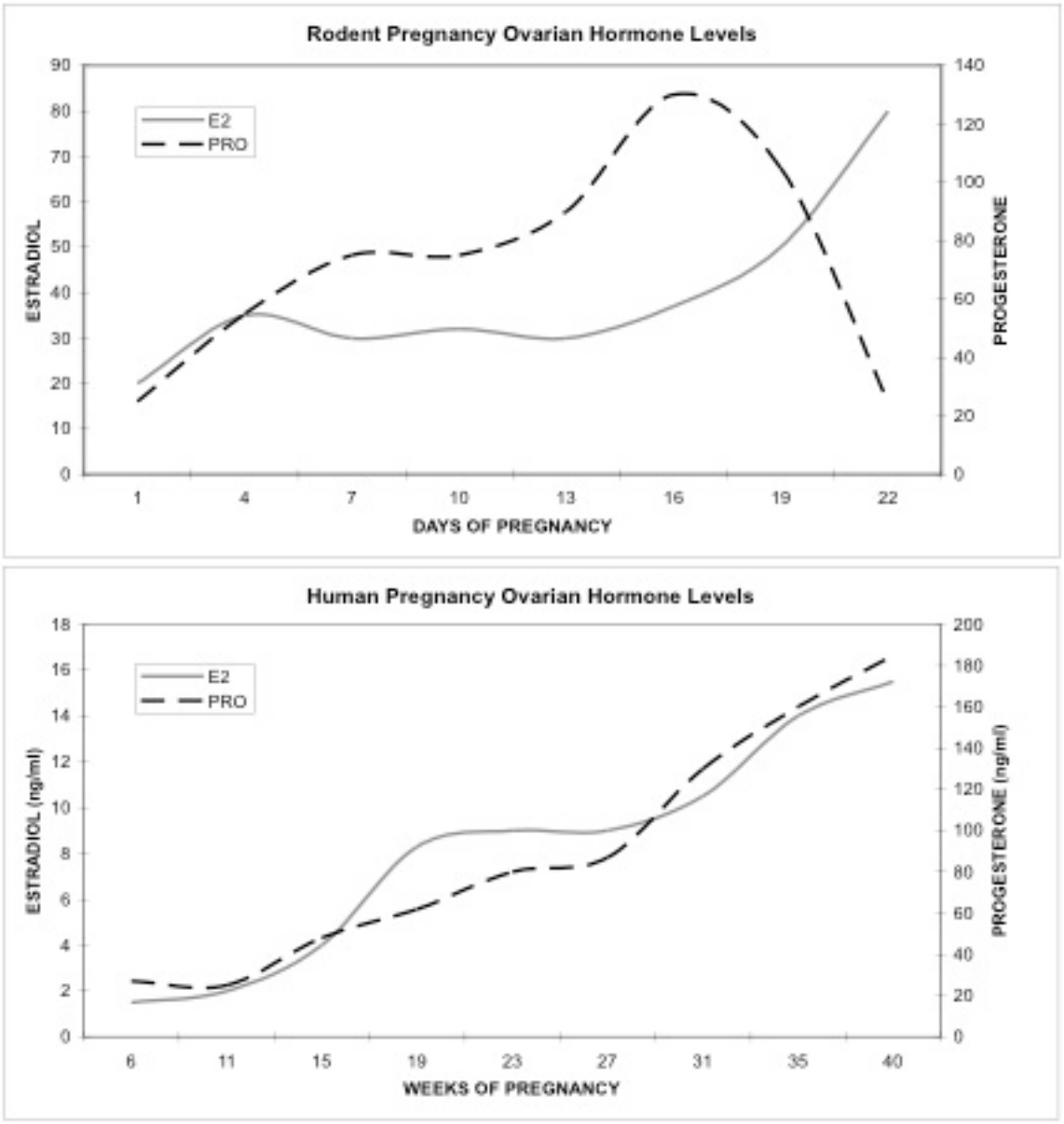


Figure 1. Estradiol and progesterone levels during pregnancy in rodents and humans.

CHAPTER THREE

OVARIAN HORMONES, DEPRESSION, AND ANXIETY IN POSTPARTUM WOMEN

Introduction and Methodological Issues

Although ovarian hormone withdrawal has been widely implicated in the pathogenesis of the blues, a common set of criteria for the blues has not been used across studies, and the blues construct has not been well defined in the literature. Similarly, differences in methods and inconsistencies in diagnostic criteria used in previous studies have yielded mixed results regarding associations between ovarian hormones and depressive symptoms. The majority of these studies have examined between-subject associations to explain why some women experience depression following ovarian hormone withdrawal, while others do not. As such, researchers have hypothesized that women who develop PPD experience more rapid hormone withdrawal, have lower levels of ovarian hormones during the postpartum period, and demonstrate greater reductions in ovarian hormone levels from pregnancy to the postpartum than women without PPD. However, these hypotheses have generated only weak support.

Evidence of the Hormone Withdrawal Hypothesis

A study by O'Hara et al. (1991) examined the blues during the first ten days postpartum in 182 women. Blues symptoms were assessed retrospectively at postpartum week nine using the Handley Blues Index, which assesses dysphoric mood, mood lability, crying, anxiety, insomnia, loss of appetite, and mood instability (O'Hara, Schlechte, Lewis, & Wright, 1991). Participants met criteria for the blues if they endorsed four of the seven blues symptoms. Depressive symptoms were assessed using the Beck Depression Inventory, the Premenstrual Assessment Form, the Research Diagnostic Criteria for depression, and the Schedule of Affective Disorders and Schizophrenia. Prolactin, progesterone, and estradiol were measured in serum. Free and total estriol and cortisol levels were also obtained. Subjects completed self-report measures and

interviews during the second trimester and at nine weeks postpartum. Blood and urine samples and visual analog ratings of blues symptoms were obtained during the third trimester and on days 1, 2, 3, 4, 6, and 8 postpartum. Compared to women without the blues, women who met the Handley Blues criteria were more likely to meet criteria for PPD nine weeks after delivery. Women who met the Handley Blues criteria had higher levels of free estriol during pregnancy and higher levels of total estriol during pregnancy and on days 2 and 3 postpartum. There were no significant differences between groups in estradiol, progesterone, prolactin, or cortisol levels on any of the days measured. There were also no differences between groups on a dexamethasone suppression test administered on day 3 postpartum. However, separate t-tests were used to compare groups on each day, and therefore, analyses did not capitalize on the longitudinal nature of the design. In addition, this study focused on mood symptoms and blues diagnoses rather than PPD.

A subsequent study by O'Hara et al. (1991) of the same 182 postpartum women showed that those with PPD had significantly lower estradiol levels on Day 2 postpartum compared to non-depressed postpartum women. However, there were no differences in estradiol levels between groups on postpartum days 1, 3, 4, 6, or 8, nor were there differences in estriol, progesterone, or prolactin on any postpartum day (O'Hara, Schlechte, Lewis, & Varner, 1991). The depressed group included women who met current Research Diagnostic Criteria (RDC) (Spitzer, Endicott, & Robins, 1978) for major or minor depression during an assessment that occurred approximately nine weeks following delivery. Of the 19 women in the postpartum depressed group, 11 had an onset during the first four weeks following delivery, three had an onset during pregnancy, and five had an onset more than four weeks following delivery. This study examined total estradiol and progesterone levels in serum samples. A later study by Heidrich et al. (1994) yielded similar results. Total plasma estradiol concentrations were higher among postpartum women with depressive symptoms than those without depressive symptoms

on the fifth day following delivery but not on postpartum days 0 or 3. Free plasma estradiol concentrations were also higher among women with depressive symptoms on days 3 and 5 postpartum but not on day 0. In addition, there were no differences in total or free concentrations of progesterone or prolactin on any of the postpartum days measured (Heidrich et al., 1994). Both O'Hara et al. (1991) and Heidrich et al. (1994) examined between-subject associations of ovarian hormones and depressive symptoms and analyses did not capitalize on the repeated assessment occasions included in these studies. Instead, separate between-group comparisons were made for each assessment day.

A later study by Buckwalter and colleagues (1999) used repeated measures ANOVA to examine whether ovarian hormone levels were associated with mood measured once during and once after pregnancy (Buckwalter et al., 1999). Progesterone levels were positively correlated with psychotic symptoms during pregnancy, and testosterone was associated with depressive symptoms, hostility, tension, and anger. Estradiol was not associated with symptoms at either time point alone; however, changes in estradiol over time demonstrated stronger associations with mood symptoms than any other hormone measured. As estradiol decreased, phobic anxiety and fatigue also decreased (Buckwalter et al., 1999). Although this association was not in the expected direction, measurements were taken only at two time points and the timing of each measurement varied across subjects as much as two months. Given that ovarian hormones vary widely between subjects and across days during and after pregnancy, these results must be interpreted with caution.

Thus, although naturalistic studies of ovarian hormones and mood during the postpartum have not as of yet demonstrated strong support for the hormone withdrawal hypothesis, these studies did not examine daily changes in estradiol, progesterone, and mood symptoms. Recent advances in hormone assay techniques allow for ovarian hormone detection in saliva rather than serum. Existing research suggests that saliva

sampling methods are not only more acceptable to women, but they also detect the unbound or "biologically available" portion of ovarian hormones, allowing for more powerful tests of associations between hormones and mood symptoms (Edler, Lipson, & Keel, 2007; Shirtcliff et al., 2000). Previous studies also used varying definitions of "postpartum" depression and often included women with depressive episodes that began during pregnancy or more than four weeks after delivery. Further, advances in statistical techniques allow for analyses that account for within-subject associations over time, which increase the power to detect between-subject differences. Thus, previous naturalistic studies may have failed to provide support for the hormone withdrawal hypothesis either because humans are not vulnerable to changes in estradiol and progesterone in the same ways that non-human animals are vulnerable to these effects, or the diagnostic and hormone assessment methods employed by past human studies did not provide powerful enough tests of the effects of hormone withdrawal on mood symptoms.

Evidence that human females are vulnerable to postpartum changes in estradiol and progesterone comes from treatment studies examining the effects of administering exogenous estradiol to postpartum women. In a pilot study of 11 women with a history of PPD (onset occurring within the first two weeks postpartum) and no other history of affective disorder, participants were prophylactically administered oral Premarin, a conjugated estrogen tablet, immediately following delivery to prevent the onset of depressive symptoms (Sichel et al., 1995). Ten of the 11 women remained well during the postpartum and for the first year following delivery (Sichel et al., 1995). A later double-blind, placebo-controlled study of 61 women with PPD that began within three months following delivery showed that women treated with estradiol (n=34) delivered via a transdermal patch improved significantly more than women who received placebo (n=27) (Gregoire et al., 1996). On average, women in the treatment group showed a 4.38-point decrease in EPDS scores (Gregoire et al., 1996), which represents a clinically significant reduction in symptoms. A subsequent study examined the effects of estradiol

treatment on a group of 23 women with severe postpartum depression, many of whom had attempted treatment with antidepressant medication or psychotherapy, which yielded no notable effect (Ahokas, Kaukoranta, Wahlbeck, & Aito, 2001). At baseline, 16 of the 23 patients had serum estradiol concentrations consistent with gonadal failure. All women in the study received sublingual estradiol treatment for 8 weeks. After the first week, depressive symptoms significantly decreased, and by the end of the eight weeks all patients had achieved depressive symptom scores consistent with clinical recovery (i.e., a Montgomery Asberg Depression Rating Scale score less than or equal to seven).

Taken together, results of studies examining ovarian hormones' influence on postpartum depressive symptoms have yielded support for the hormone withdrawal hypothesis; however, majority of this support has been generated by treatment studies that have administered estradiol to postpartum women with positive effects. None of the previous studies have directly examined the effects of hormone withdrawal on anxiety or specific symptoms of depression. In addition, previous studies have not distinguished between high-risk and low-risk women when examining the effects of hormone withdrawal on mood.

Anhedonia: Definition and Assessment

Anhedonia was first identified as a loss of the capacity to experience pleasure by Ribot in 1894, and it is currently regarded a core symptom of major depression in humans (American Psychiatric Association, 2000). In the animal literature, anhedonia is defined behaviorally as a deficit in hedonic behavior, including reduced intake in palatable solutions, reduced preference for palatable solutions, and reduced brain self-stimulation, which have been shown to respond to a variety of medications that treat depression in humans, including tricyclic antidepressants (e.g., desipramine) (Moreau, Jenck, Martin, Mortas, & Haefely, 1992), selective serotonin reuptake inhibitors (SSRI) (e.g., citalopram) (Strekalova, Gorenkova, Schunk, Dolgov, & Bartsch, 2006), and atypical antidepressants (e.g., mianserin) (Moreau, Bourson, Jenck, Martin, & Mortas, 1994). The

mesolimbic DA system is implicated in anhedonic behavior and involves a neural pathway originating in the ventral tegmental area and terminating in the nucleus accumbens.

The assessment of anhedonia in humans has been clouded by inconsistencies in the operational definition of anhedonia (Leventhal, Chasson, Tapia, Miller, & Pettit, 2006) and the absence of a unitary anhedonia construct (Watson et al., 2007). Previous studies have examined anhedonia using a variety of laboratory assessments and questionnaires. Laboratory assessments include behavioral measures and assessments of subjective responses to pleasant stimuli. Self-report questionnaires have focused on physical manifestations of anhedonia, subjective experiences of pleasure, interest in activities, or some combination of these variables.

A study by Berlin and colleagues (1998) examined the hedonic response to sucrose solutions and self-report measures of anhedonia among depressed patients (n=20) and healthy controls (n=20) in a randomized, double-blind design (Berlin, Givry-Steiner, Lecrubier, & Puech, 1998). The sucrose test required subjects to taste five concentrations of sucrose: 0, 5, 10, 20, and 40% wt/wt in a volume of 15mL at three-minute intervals. Subjects rinsed their mouths with tap water before and between tasting each sucrose solution. They were told to sip one mouthful of solution and hold it in their mouth (without swallowing) for a period of five seconds before spitting it out. Immediately afterward, they rated the pleasantness of each solution on a 9-point Likert scale. This test was used to determine the *maximum hedonic response*, defined as the difference between the highest score and the neutral score (i.e., neither pleasant nor unpleasant). A second test was conducted to determine the perception threshold of sweet taste, which involved administering increasing concentration of sucrose (0,0.5,1, 2, 3, 4, 5, 10, 20, and 40%) following the same tasting and rinsing procedure discussed above. The *sweet perception threshold* was defined as the sucrose concentration at which subjects' first perceived sweet taste. Subjects also completed the Chapman Physical and Social Anhedonia Scales

(CPAS) (Chapman, Chapman, & Raulin, 1976) and the Fawcett-Clark Pleasure Capacity Scale (FCPS) (Fawcett, Clark, Scheftner, & Gibbons, 1983). The CPAS is a 61-item questionnaire that asks participants to respond to statements about their typical response to pleasurable stimuli and activities (e.g., "The taste of food has always been important to me") using a true-false response format. The FCPS is a 36-item questionnaire that asks participants to rate imagined hedonic reactions to hypothetical situations (e.g., "I would enjoy my favorite television or radio program") and rate the extent to which they agree or disagree with each statement on a 5-point Likert scale. Both the CPAS and FCPS were created using the *theoretical-rational approach* to scale development (see Clark & Watson (1995) for a discussion of the problems associated with this approach when used by itself), yet both measures were related to outcomes on the sucrose tests. Among depressed patients, the maximum hedonic response was negatively correlated with the CPAS Physical Anhedonia score, and the sweet perception threshold was negatively correlated with the FCPS Pleasure Scale score. In addition, the depressed patients had higher scores on both the CPAS and FCPS compared to controls, and the mean sweet perception threshold was higher among depressed patients than controls. Thus, self-report measures of anhedonia and the hedonic response to sucrose test distinguish depressed patients from healthy controls, and self-report measures of anhedonia are correlated with the sucrose test, a behavioral measure of anhedonia.

The construct validity of anhedonia as measured by the CPAS and FCPS has been widely disputed. Some studies show that the CPAS and FCPS do not correlate consistently with behavioral measures of anhedonia (e.g., participants' response to pleasant pictures), and other studies show that they do not distinguish between diagnostic groups (e.g., depression, schizophrenia, and personality disorders) (Leventhal et al., 2006). Further, in a recent paper describing the development of a new measure of depression and anxiety symptoms using the factor-analytic approach, anhedonia did not emerge as a unitary factor (Watson et al., 2007). Instead, items designed to tap anhedonia

split across the dysphoria and well-being factors. Taken together, these results suggest that anhedonia is not a unitary construct circumscribed by the diagnosis of depression. Rather, anhedonia may be better defined by a broader mood factor that cuts across diagnostic categories.

The absence of positive affect is a promising candidate for an operational definition of anhedonia. Positive affect includes a range of positive mood states, including joy, energy, enthusiasm, interest, alertness, and self-confidence, that are diminished in patients with depression (Watson et al., 1995), schizophrenia (Blanchard, Mueser, & Bellack, 1998), and certain personality disorders (Clark, 2005). In Clark and Watson's (1991) seminal paper on the tripartite model of anxiety and depression, they describe the absence of positive affect as being similar to anhedonia, a notion that is more consistent with Ribot's original conceptualization of anhedonia than any definition since. In the development of the Mood and Anxiety Symptoms Questionnaire (MASQ) (Watson & Clark, 1991), questions related to positive affectivity and anhedonia clustered into a single factor (Watson et al., 1995). Low positive affect in combination with high negative affect is uniquely related to depression (Watson et al., 1995), and measures of positive and negative affect are sensitive to daily mood changes, making these affective states ideal for capturing fluctuations in mood during the postpartum. Moreover, positive affect increases following treatment with SSRIs (e.g., paroxetine) and serotonin-norepinephrine reuptake inhibitors (SNRI) (e.g., venlafaxine) (Dichter, Tomarken, Freid, Addington, & Shelton, 2005). This pattern of results is similar to the animal literature on anhedonia and response to antidepressant treatment discussed above.

Anhedonia, PPD, and Ovarian Hormones

In order to diagnose PPD, depressed mood, anhedonia, or both must be present nearly every day for at least two weeks and have an onset within four weeks following delivery (American Psychiatric Association, 2000). Thus, although anhedonia is a core feature of PPD, its presence is not necessary for a diagnosis. Specific symptoms of

depression have not received a lot of attention in the PPD literature, except to the extent that researchers have examined how individual symptoms may distinguish PPD from depressive disorders not experienced during the postpartum. For example, postpartum women experience more somatic depressive symptoms than non-childbearing women; however, these groups show no significant differences in the cognitive symptoms associated with depression (e.g., feelings of worthlessness and guilt, impaired concentration, and suicidal ideation) (O'Hara, Zekoski, Philipps, & Wright, 1990). Several studies have replicated this finding, suggesting that the cognitive symptoms of depression, including anhedonia, are similar to depression occurring at other times in a woman's life.

Further support that anhedonia is an important symptom during the postpartum comes from a recent study that aimed to examine the co-occurrence of depressed mood, anhedonia, and anxiety among postpartum women by conducting a factor analysis of the Edinburgh Postnatal Depression Scale items (Tuohy & McVey, 2008). Principal axis factor extraction and oblique rotation resulted in three factors that the authors labeled as "non-specific depressive symptoms," "anhedonia," and "anxietal symptoms." Two items that had significant loadings on the anhedonia factor were "I have been able to laugh and see the funny side of things," and "I have looked forward with enjoyment to things." The non-specific depression factor included items related to depressed mood and self-harm (e.g., "I have felt sad or miserable," "I have been so unhappy that I have had difficulty sleeping," "I have been so unhappy that I have been crying," and "The thought of harming myself has occurred to me."). The third factor included the three items most closely related to anxiety (i.e., "I have been anxious or worried for no good reason", "I have felt scared or panicky for no very good reason," and "I have blamed myself unnecessarily when things went wrong."). Thus, the emergence of anhedonia as a factor independent of depressed mood may be related to the positive valence of the items or the distinctiveness of anhedonia from depressed mood among postpartum women. The

authors conclude that the three-factor solution provides support for the tripartite model of depression and anxiety in postpartum women and that anhedonia is a core symptom of PPD.

Taken together, these results suggest that anhedonia characterizes postpartum mood disorders and that anhedonia is at least as important during the postpartum as it is during non-postpartum mood episodes. Previous human studies have not examined the relationship between anhedonia and ovarian hormone levels. However, the animal literature discussed in the previous chapter suggests that anhedonia likely increases as a result of estradiol withdrawal during the postpartum (Bless et al., 1997).

Anxiety During the Postpartum Period

Given the prevalence of PPD, the disability caused by depression in women, and the effects of PPD on children and families, majority of the research conducted with postpartum women in the United States has focused on the causes and correlates of depression. However, recent research has challenged the privileged status afforded to PPD and suggests that anxiety may be more prevalent during the postpartum than depression (Wenzel et al., 2005). Postpartum prevalence rates range from 1.4% for panic disorder to 8.2% for generalized anxiety disorder (Wenzel et al., 2005). Moreover, generalized anxiety disorder may be more common among postpartum women than non-pregnant women (Wenzel et al., 2005). Previous research also suggests that a significant proportion of women with pre-existing anxiety disorders experience postpartum exacerbations, including 29% of those with obsessive compulsive disorder (Williams & Koran, 1997) and 35% of those with panic disorder (Cohen, Sichel, Dimmock, & Rosenbaum, 1994).

Anxiety also is highly comorbid with depression during the postpartum. In one study, 31% of postpartum women with generalized anxiety disorder or subsyndromal anxiety reported experiencing concurrent depressive symptoms (Wenzel, Haugen, Jackson, & Robinson, 2003). In a subsequent study of postpartum women, 25% of those

with GAD or OCD, 33% of those with social phobia, and 50% of those with panic disorder met diagnostic criteria for a comorbid mood disorder (Wenzel et al., 2005). Women who present for PPD treatment are more likely to report comorbid anxious features than women seeking treatment for non-postpartum depression (Hendrick, Altshuler, Strouse, & Grosser, 2000). This comorbidity may contribute to the delayed response to treatment that occurs among postpartum women compared to depressed non-postpartum women (Hendrick et al., 2000).

Symptoms of anxiety that emerge during the postpartum may result from the rapid withdrawal of ovarian hormones. Progesterone metabolites act on GABA receptors in the brain, producing sedative-like effects by enhancing the function of GABA. Abrupt decreases in progesterone are associated with anxiety (Smith et al., 1998), and treatment with progesterone reduces anxiety (Dennerstein et al., 1985). Thus, the onset of anxiety symptoms may be precipitated by the rapid withdrawal of progesterone that occurs during the first three days postpartum.

At-Risk Women

Several studies have demonstrated that a subgroup of women may be at increased risk for the development of PPD. For example, a study by Cooper and Murray (1995) demonstrated that primiparous women who had experienced postpartum depression but no past episodes of major depression outside the postpartum period were at increased risk for developing a future episode of postpartum depression but not non-postpartum depression. In contrast, primiparous women who experienced postpartum depression and had at least one past episode of major depression outside the postpartum period were at increased risk for developing a future episode of non-postpartum depression but not PPD (Cooper & Murray, 1995).

Similarly, an innovative study by Bloch et al. (2000) examined differences in women's vulnerability to depressive symptoms following changes in estrogen and progesterone. This study included two groups of women. Inclusion criteria for the

postpartum depression group were a) the absence of a psychiatric diagnosis within the last year, b) at least one past episode of DSM-IV PPD without suicidal ideation or psychotic features, and c) no history of depression occurring outside the puerperium. The inclusion criterion for the control group was the absence of any past or present psychiatric disorder. Participants provided daily mood ratings for two months prior to receiving any medications. After this baseline period, participants received the first of five monthly injections of leuprolide, a gonadotropin-releasing hormone (GnRH) agonist that suppresses gonadal steroid levels. After one month of leuprolide and placebo tablets, estradiol and progesterone were administered daily for eight weeks. Active hormones were replaced with placebo during the withdrawal phase, and hypogonadal levels were maintained for the 4-week withdrawal phase with leuprolide, then women were followed for eight additional weeks. Participants made daily self-ratings of their mood, behavioral symptoms, and hot flashes for the duration of the study. They also completed the Beck Depression Inventory (BDI), Edinburgh Postnatal Depression Scale (EPDS), and the Cornell Dysthymia Scale (CDS) at bimonthly appointments. Hormone levels were measured periodically as a fidelity check. During the withdrawal phase, women with a history of postpartum depression demonstrated a significant increase in sadness, anxiety, mood lability, and irritability self-ratings, and EPDS, CDS, and BDI scores, compared to baseline. These effects were not seen in the control group. In addition, 63% of those with a history of PPD and none of the controls developed clinically significant depressive symptoms (i.e., an increase of at least 12 points on the CDS) during the withdrawal phase. Results demonstrate a differential sensitivity to ovarian hormone withdrawal among women with a history of PPD, compared to those without. This was the first study to provide direct evidence that estrogen and progesterone are involved in the development of PPD.

Subsequent studies by Bloch and colleagues (2005, 2006) also have supported the hormone withdrawal hypothesis of PPD in at-risk women. Women with a history of

premenstrual dysphoric disorder (PMDD), mood symptoms during the first 2-4 days postpartum, and a history of depressive symptoms during past oral contraceptive use were more likely to experience PPD. Women were considered "high-risk" if they met criteria for at least one of the following: a) a history of a major depressive episode; b) a history of a PPD episode; c) a history of PMDD; d) mood symptoms during the third trimester of the current pregnancy; or e) an EPDS score greater than 10 assessed 2-4 days after childbirth. Women categorized as low-risk did not meet any of these criteria. Based on these criteria, 209 women were considered high-risk and 1,591 were low-risk. High-risk women had a 65% likelihood of meeting diagnostic criteria for major depression, minor depression, adjustment disorder, or the blues occurring during the first 6-8 weeks postpartum, whereas low-risk women had a 35% risk. A subsequent paper by Bloch and colleagues (Bloch et al., 2006) provides further support for this notion. Specifically, they found that risk factors associated with postpartum depressive symptoms in the days following delivery included a) a personal history of past PPD, major depression, or other psychiatric disorder, b) a family history of an affective disorder, c) a history of premenstrual dysphoric disorder, and d) mood symptoms during the third trimester of pregnancy. However, income level, marital status, ethnic/racial background, number of children, planned vs. unplanned pregnancy, mood symptoms associated with oral contraceptive use, and mood instability at puberty were not associated with mood symptoms during the postpartum. Thus, women who have demonstrated mood symptoms associated with ovarian hormone changes in the past are at greater risk for postpartum mood symptoms than women who have not experienced hormone-dependent mood changes.

The pattern of PPD risk demonstrated in these studies is consistent with the diathesis-stress model wherein some women have a genetic or biological sensitivity or diathesis for postpartum depression, which may be related to a differential sensitivity to the withdrawal of ovarian hormones. This biological diathesis combines with the stress of

having a baby and the transition to being a new mother in an additive fashion. Women with a low vulnerability to changes in ovarian hormones may or may not go on to develop PPD, and this determination is made by the amount of environmental stress associated with having a new baby. This explains why some women develop PPD after one pregnancy and not subsequent pregnancies. Thus, women with a history of major depression exclusively occurring in the postpartum period seem to have a differential sensitivity to changes in ovarian hormones compared to women who experience episodes of major depression that are not related to the puerperium. Several researchers have speculated about the source of this differential sensitivity, which may include a homeostatic regulatory dysfunction or abnormal intracellular steroid signaling (Bloch et al., 2000).

PART B

ANIMAL MODEL OF POSTPARTUM DEPRESSION: METHODS AND RESULTS

CHAPTER FOUR

EXPERIMENT 1A: ANHEDONIA IN AN ANIMAL MODEL OF POSTPARTUM DEPRESSION

Methods

Animals

Ten-week old female Sprague Dawley rats (*N*=14) were maintained on a 12-hour light/12-hour dark cycle at a room temperature of 22.0 ± 0.2° C. Rat chow (Harlan Teklad Global Rodent Diet) and tap water were available *ad libitum*.

Experimental Protocol

Rats were ovariectomized and instrumented with a single bipolar stimulating electrode directed into the lateral hypothalamus. Self-stimulation training was initiated, and two baseline measures of operant responding were recorded. During the baseline period, animals received daily placebo (i.e., vehicle only) injections. After this baseline period, all animals received either hormone injections or vehicle only injections daily for five days (i.e., the hormone administration period). After the hormone administration period, all animals received vehicle only injections daily for five days (i.e., the withdrawal period). Responding to electrical stimulation was assessed daily two hours following injections in both the hormone administration and withdrawal periods.

Surgical Procedures

All survival surgeries were performed using an aseptic tip technique, sterile instruments, surgeon's mask, and lab gloves.

LHSS electrode placement. Under Equithesin anesthesia (3ml/kg i.p.: University of Iowa Hospital Pharmacy) bipolar stimulating electrodes were chronically implanted into the medial forebrain bundle at the level of the lateral hypothalamus (LH). The LH was chosen based on its reliability in supporting self-stimulation behavior (Olds & Olds, 1962). Rats were placed in a Kopf stereotaxic instrument and the head was leveled between bregma and lambda. The electrode was implanted in the LH at 3.0 mm posterior

to bregma, 1.7 mm lateral to the midline, and 8.5 mm ventral to the surface of the skull. Three jeweler's screws and dental acrylic were used to fix the electrode to the skull. LHSS placement was immediately followed by bilateral ovariectomy.

*Ovariectomy***.** Bilateral ovariectomy using aseptic technique was performed on all animals immediately following LHSS electrode placement while still under anasthesia. One small (0.6 cm) medial dorsal incision was made, through the skin, connective tissue, and underlying muscle layer. The ovaries were isolated and exteriorized with the associated fat pad, fallopian tube and upper uterine horn. A sterile suture knot was tied snugly around the blood supply to the ovary, and the ovary was removed. The muscle wall was sutured on each side, and the single cutaneous incision was sutured. Animals were allowed to recover for at least seven days prior to the first operant conditioning training session.

LHSS Behavioral Training and Baseline Measurement

Following recovery from electrode implantation surgery, rats were trained in a Plexiglas operant chamber equipped with a lever that delivers a negative-going, square pulse train of approximately 300 ms at 60 Hz through the electrode. The behavioral training procedure consisted of two days of adaptation to the chamber and learning the association between lever pressing and current-pulse delivery. The electrical parameters were set to predetermined values (frequency = 60 Hz; current intensity = 250μA), and systematically varied with "free" pulses given until the rat began to respond by pressing the lever. Once the optimum parameters were determined for each rat, they were held constant for the duration of the study (with the exception of current intensity, as described below). Rats that did not respond to electrical stimulation, displayed marked motor effects in response to the stimulation that interfered with responding, or did not achieve at least 50 responses per minute (RPM) at 250μA by the second day of training were eliminated from the study (i.e., this is a functional assessment of successful

electrode placement). Of note, rats were dropped from the study because of poor electrode placement prior to randomization.

After establishing consistent response rates, current-response curves were determined for each rat by using a curve-shift paradigm. Baseline LHSS current-response functions were determined for each rat immediately following the operant training period. Current was delivered in a descending series in ten discrete presentations of 25μA decrements, and the animals were allowed to respond for one minute at each current intensity. An optimal current-response curve was generated for each rat according the following criteria outlined by Grippo, Francis, Weiss, Felder, and Johnson (2003): 1) the range of current intensities to which the rat responded was between 50 and 250 μA; 2) the response rate was minimal for low levels of current (e.g., ~50-100 μA) and increased monotonically, eventually reaching a stable plateau during 10 consecutive presentations of 25-μA increment current intensities, so that there was a sigmoid relation between current intensity and behavioral responses; and 3) the maximum current intensity for which the rat would respond did not also produce a motor effect. Baseline current-response functions (RPM at each of the 10 current intensities) were generated over a two-day period.

Data points were plotted using Sigma Plot (Jandel Scientific, Chicago, IL) and fitted to a 3-parameter sigmoidal function from which three parameters were calculated: 1) maximum rate of responding, 2) current intensity that supported 50% of the maximum response rate or "effective current 50" (ECu_{50}), and 3) minimum rate of responding.

LHSS Behavioral Measurements During Hormone Withdrawal

For the five days of placebo only injections (i.e., withdrawal days 1-5), anhedonia was assessed by generating current-response curves in OVX and HSPP groups in the same manner as the baseline measurements. Current is delivered in a descending series of 10 discrete presentations of 25-μA decrements, and the animal is allowed to respond for 1 min at each intensity.

Anhedonia was operationally defined as an increase in ECu_{50} relative to baseline, with no significant reduction in the maximum RPM. A consistent maximum RPM provides evidence that the motor ability to lever press was not compromised by treatment (i.e., the animals are not motorically impaired), but rather reflects a specific hedonic deficit. The validity of LHSS as a measure of anhedonia has been shown in multiple studies where administration of psychotropic medication increased responding to self-stimulation (Lin, Bruijnzeel, Schmidt, & Markou, 2002; Moreau et al., 1992).

Hormone Administration

Prior to experimentation, animals were randomly assigned to undergo one of two hormone treatments. The two hormone treatments were vehicle only (OVX) and hormone-simulated pseudopregnancy/postpartum (HSPP). Hormones were delivered by subcutaneous injection. Animals were administered vehicle only and tested on two consecutive days to establish a stable baseline ECu_{50}. Animals in the HSPP condition (n=7) were subsequently administered a combination of estradiol (25μg) and progesterone (4mg) daily for five days followed by vehicle only for five days. Animals in the OVX condition (n=7) were administered vehicle only for all ten days. LHSS was examined daily during baseline, hormone administration, and withdrawal. Animals in both groups were euthanized following the last behavioral test.

Data Analytic Approach

In order to create stable measures of LHSS during baseline, hormone administration, and withdrawal conditions, ECu_{50} results from the two baseline days were averaged; results from hormone administration days 2-5 were averaged; and results from vehicle only administration days 2-5 were averaged within animals. To examine the influence of ovarian hormones on anhedonia, t-tests were used to compare ECu_{50} between HSPP and OVX groups during baseline, hormone administration, and withdrawal. In addition, paired t-tests were used to examine differences in ECu_{50} between baseline, hormone administration, and withdrawal conditions within the HSPP group. A

significance level of $p<.05$ was used. Statistical testing was conducted using SPSS and Sigma Plot statistical packages. A power analysis suggested that seven animals per group were needed to have 80% power to detect a large effect with a one-tailed significance level of $p=.05$.

Results

Daily LHSS results are shown in Figure 2 (p. 50). Contrary to the *a priori* hypotheses, there were no significant differences between the treatment and control groups during hormone administration ($t=0.86$, $p=.85$) or withdrawal ($t=0.88$, $p=.53$). Within the treatment group, there was no difference between the hormone administration and withdrawal conditions ($t=0.59$, $p=.58$).

Given the lack of significant findings, a subsequent study (i.e., Experiment 1B) was undertaken to examine varying concentrations of estradiol only on LHSS, given that previous studies demonstrated significant depression-like behavior following estradiol withdrawal only (Bekku & Yoshimura, 2005).

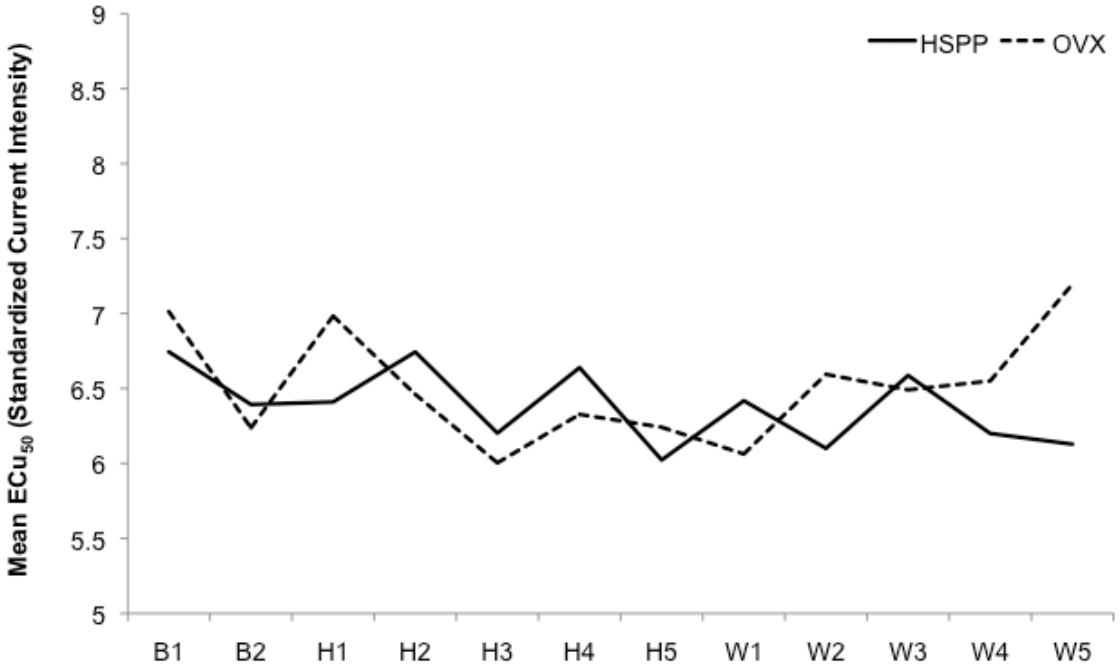


Figure 2. ECu_{50} data for animals in the HSPP and OVX groups in experiment 1A. B1-B2: baseline days 1-2; H1-H5: hormone administration days 1-5; W1-W5: withdrawal days 1-5.

CHAPTER FIVE

EXPERIMENT 1B: THE EFFECTS OF ESTRADIOL WITHDRAWAL ON ANHEDONIA

Methods

Animals

Ten-week old female Sprague Dawley rats (*N*=14) were maintained on a 12-hour light/12-hour dark cycle at a room temperature of 22.0 ± 0.2° C. Rat chow (Harlan Teklad Global Rodent Diet) and tap water were available *ad libitum*.

Experimental Protocol

Rats were ovariectomized and instrumented with a single bipolar stimulating electrode directed into the lateral hypothalamus. Self-stimulation training was initiated, and five baseline measures of operant responding were recorded. During baseline, animals received daily vehicle only injections. Following baseline, animals received daily injections of 25μg of 17-β estradiol or vehicle only for five days. Both groups then received vehicle only injections for five days. Then animals received daily injections of 50μg of 17-β estradiol or vehicle only for five days. Both groups then received vehicle only injections for five days. Responding to electrical stimulation was assessed two hours following injections each day for a total of 25 days. At the conclusion of the protocol, histological procedures were performed on a subset of the animals to verify the presence of proper electrode placement in the brain.

Surgical Procedures

All survival surgeries were performed using an aseptic tip technique, sterile instruments, surgeon's mask, and lab gloves.

LHSS electrode placement. Under Equithesin anesthesia (3ml/kg i.p.: University of Iowa Hospital Pharmacy) bipolar stimulating electrodes were chronically implanted in the medial forebrain bundle of the lateral hypothalamus (LH). LHSS electrode placement

procedures replicated those used in Experiment 1A. This surgery was immediately followed by bilateral ovariectomy.

Ovariectomy. Bilateral ovariectomy procedures replicated those used in Experiment 1A. Animals were allowed to recover for seven days prior to experimentation.

LHSS Behavioral Training, Baseline Measurement, and Testing

LHSS procedures replicate those used in Experiment 1A.

Hormone Administration

Animals randomly assigned to undergo one of two groups: treatment (n=7) or control (n=7). To examine the behavioral effects estradiol administration and withdrawal at varying levels, animals in the treatment condition were administered vehicle only for five days (i.e., baseline), then 25μg of 17-β estradiol for five days (i.e., low dose estradiol), vehicle only for five days (i.e., withdrawal), 50μg of 17-β estradiol for five days (i.e., high dose estradiol), and finally, vehicle only for five days (i.e., withdrawal). Of note, both 25μg and 50μg of estradiol represent superphysiologic levels. Control animals were administered vehicle only according to the same schedule as the treatment group. LHSS was examined every day two hours following hormone/placebo administration. Animals in both groups were euthanized following the last day of testing.

Histology

At the conclusion of the protocol, a subset of animals were administered nembutal followed by transcardial perfusion with saline and later with 4% formalin solution. The brains were removed and fixed in 10% buffered formalin. Brain sections were taken at 50-μm intervals throughout the hypothalamus. The sections were mounted on slides, stained with cresyl violet solution, and examined by light microscopy. As shown in Figure 3 (p. 56), the slices were evaluated for proper electrode placement in the lateral hypothalamus based on Paxinos and Watson (1998).

Data Analytic Approach

ECu_{50} results from days 2-5 were averaged within each of the five conditions: baseline, low-dose estradiol, low-dose withdrawal, high-dose estradiol, and high-dose withdrawal. Results from the first day of each condition were excluded because previous research suggests that the behavioral effects of ovarian hormone administration are seen 18-36 hours following a single dose (Clark & Roy, 1987). As shown in Figure 4 (p. 57), the same effect was observed in the current study. Testing on the first day of the high-dose estradiol administration yielded no significant difference from the day before (t=-0.55, p=.61). However, ECu_{50} declined significantly on the second day of hormone administration (t=2.54, p=.04).

To examine the influence of estradiol administration and withdrawal on anhedonia, a repeated measures ANOVA was used to examine main effects of dose (low- versus high-dose estradiol) and treatment condition (baseline, hormone administration, and withdrawal) and the dose x treatment interaction. Significant effects were followed by t-tests when warranted. A significance level of p<.05 was used. Statistical testing was conducted using SPSS and Sigma Plot statistical packages. A power analysis showed that seven animals per group were needed to have 80% power to detect a large effect with a one-tailed significance level of p=.05.

Results

There was a sigmoidal current-response relationship between current intensity and response rate for rewarding electrical brain stimulation. As current intensity increased, response rates increased until reaching an upper asymptote (i.e., the maximum response rate). Figure 5 (p. 58) shows raw data points and the three-parameter fit curves from a representative treatment (*A*) and control (*B*) animal.

Treatment group data for each condition are shown in Figure 6 (p. 59). Within the treatment group, a repeated measures ANOVA revealed a significant main effect for condition (i.e., baseline, hormone administration, and withdrawal) (F=8.0, p=.007) but

not significant dose (F=0.02, p=.88) or interaction (F=0.23, p=.80) effects. Given that there was not a significant dose effect, data from each hormone level were combined to provide increased power to detect differences between hormone administration and withdrawal within the treatment group. Results demonstrated that estradiol withdrawal resulted in reduced responding for electrical stimulation across a range of current intensities, relative to estradiol administration (i.e., the current-response function generated during estradiol administration) (Figure 7a, p. 60). Table 2 (p. 55) displays the curve parameters for the current-response curves shown in Figure 7a (p. 60). During withdrawal, a parallel rightward shift was observed in the current-response function of the treatment group compared with estradiol administration. Figure 7b (p. 60) presents the mean ECu_{50} responses during estradiol administration and withdrawal in the treatment group. A paired t-test revealed a significant difference between estradiol administration and withdrawal conditions. Neither the maximum nor minimum responses per minute differed between conditions.

A repeated measures ANOVA was used to examine differences between the treatment and control groups across conditions (i.e., baseline, hormone administration, and withdrawal). Although there was not a significant main effect for group (F=0.0, p=1.0), there was a significant condition effect (F=3.7, p=.04) and a significant group x condition interaction (F=4.79, p=.02). Means for each condition are displayed in Figure 8 (p. 61).

Given the significant influence of estradiol on anhedonia, subsequent studies (i.e., experiments 2 and 3) examined the influence of estradiol withdrawal rather than the withdrawal of both estradiol and progesterone.

Table 2. Curve parameters defining current-response functions in animals in the treatment group (*n*=7) during estradiol administration and withdrawal.

	Estradiol Administration		**Withdrawal**		
	M	**(*SD*)**	***M***	**(*SD*)**	***t***
Minimum, responses per minute	0.5	(*0.2*)	0.5	(*0.2*)	0.0
Midpoint, standardized current intensity	5.9	(*1.6*)	6.3	(*1.6*)	-3.2**
Maximum, responses per minute	91.5	(*13.7*)	94.1	(*12.7*)	-1.0

**p=.007

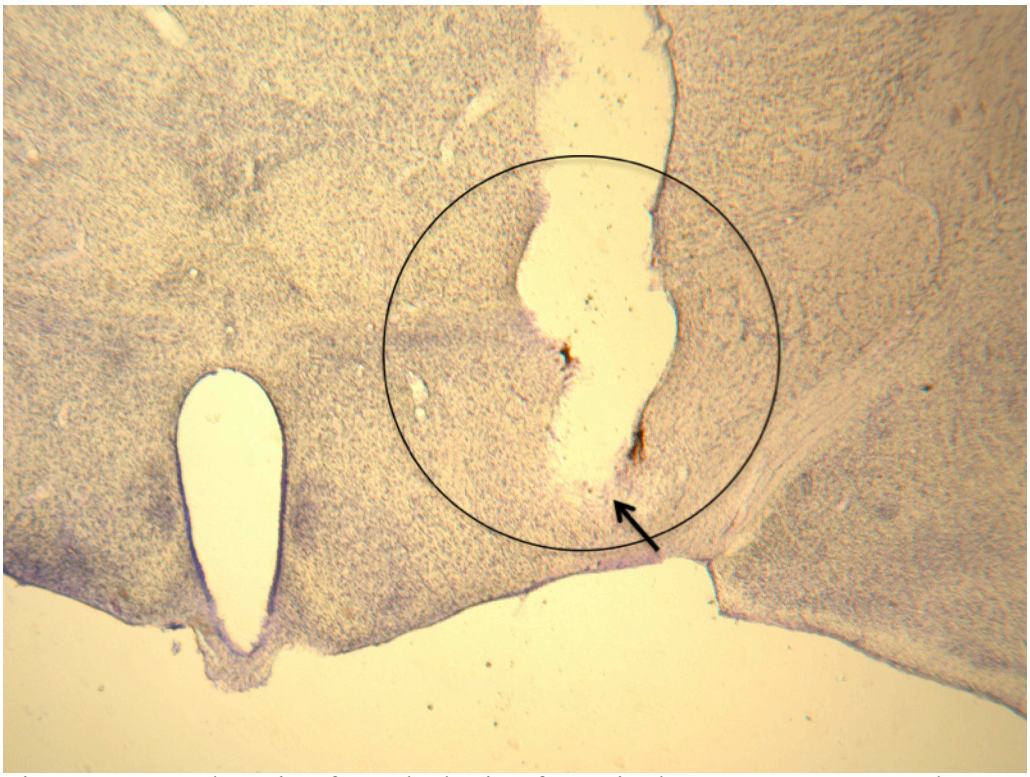
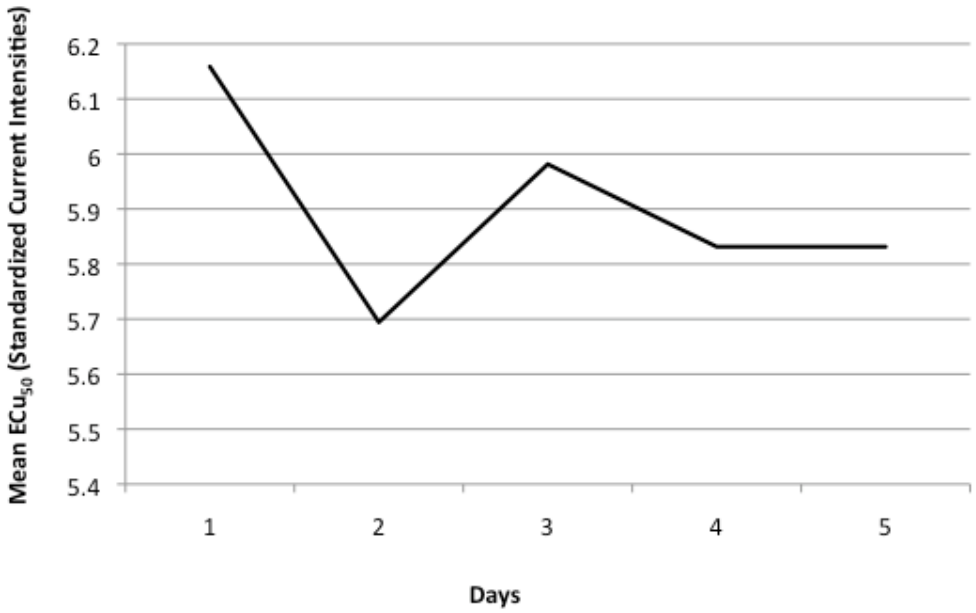
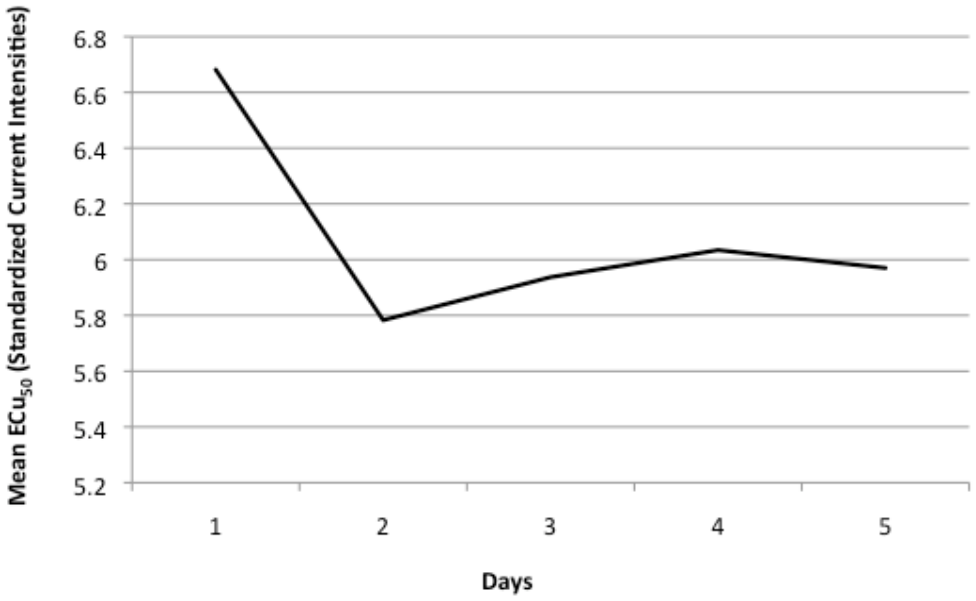


Figure 3. Frontal section from the brain of a rat in the treatment group. The arrow points to the lesion made by the electrode, the tip of which is centered the lateral hypothalamus (i.e., the area denoted by the circle).

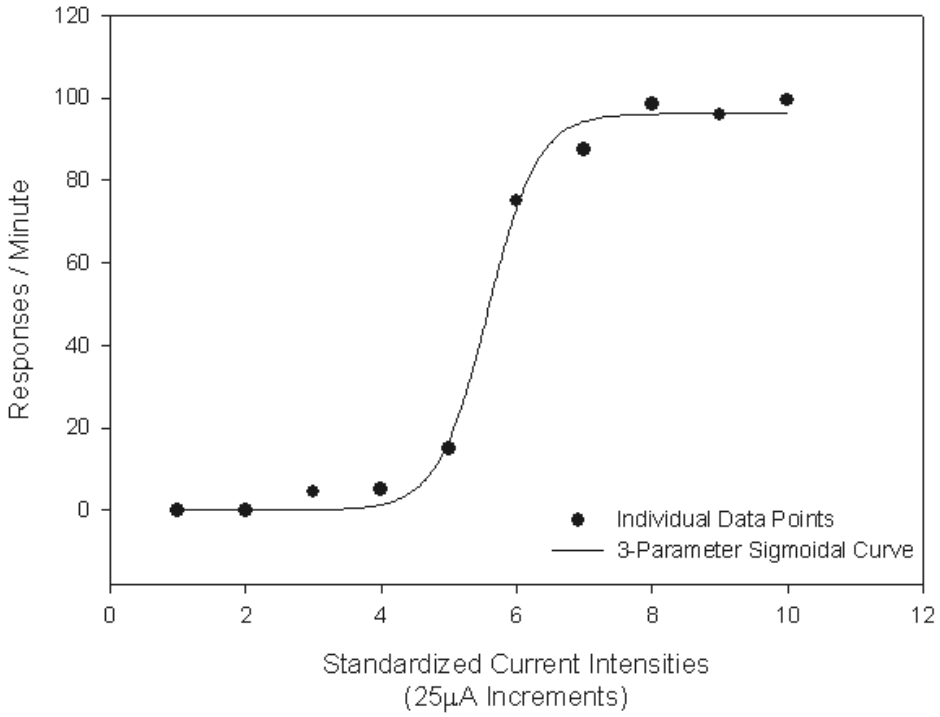


a. Low dose estradiol administration.

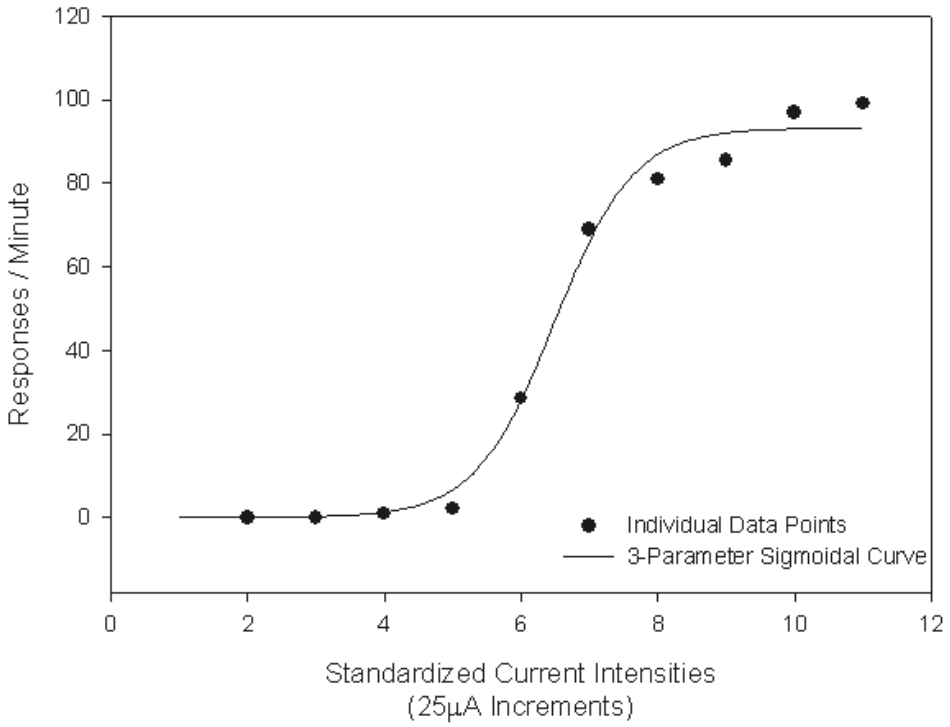


b. High dose estradiol administration.

Figure 4. Standardized ECu_{50} during estradiol administration each day during a five-day administration period.



a. Animal in the treatment condition.



b. Animal in the control condition.

Figure 5. Current-response curves for a representative treatment and control animal. Circles represent raw data points, and the line represents the 3-parameter fit curve.

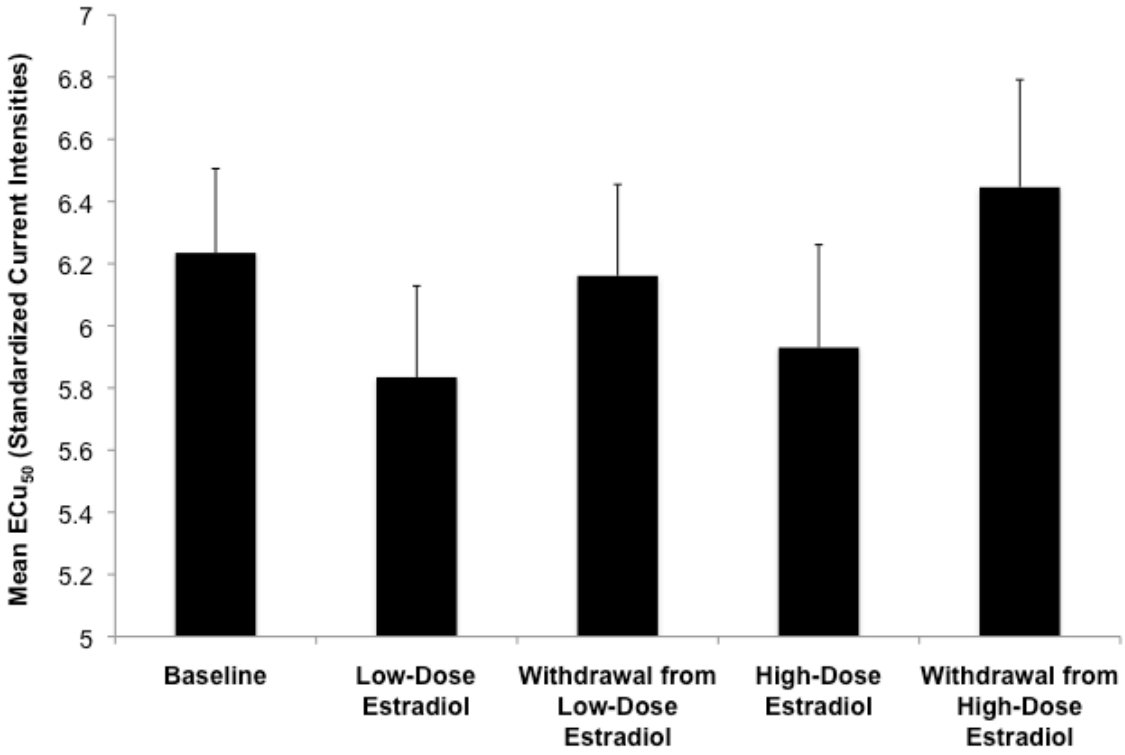
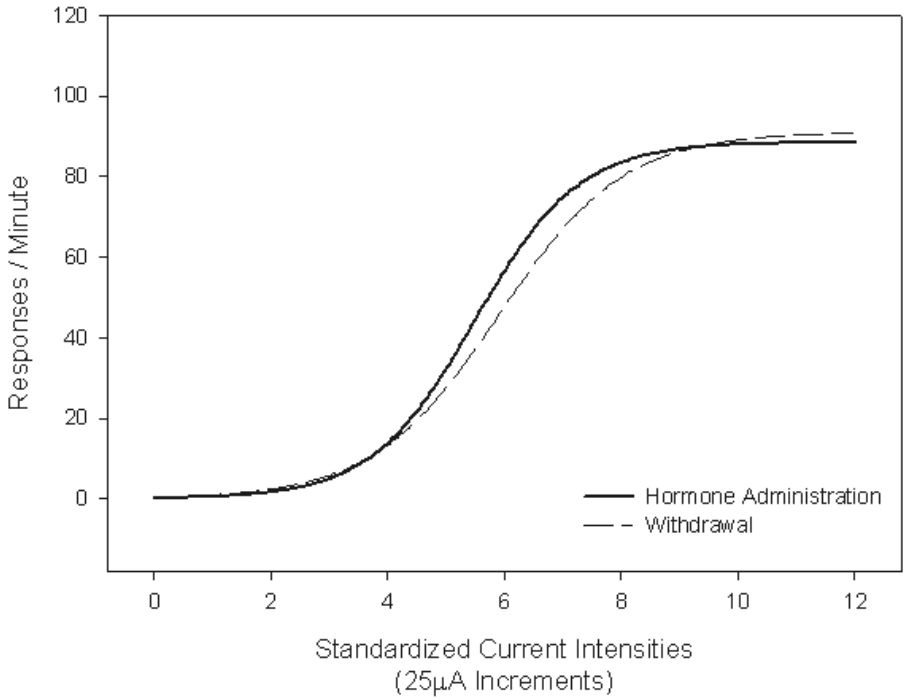
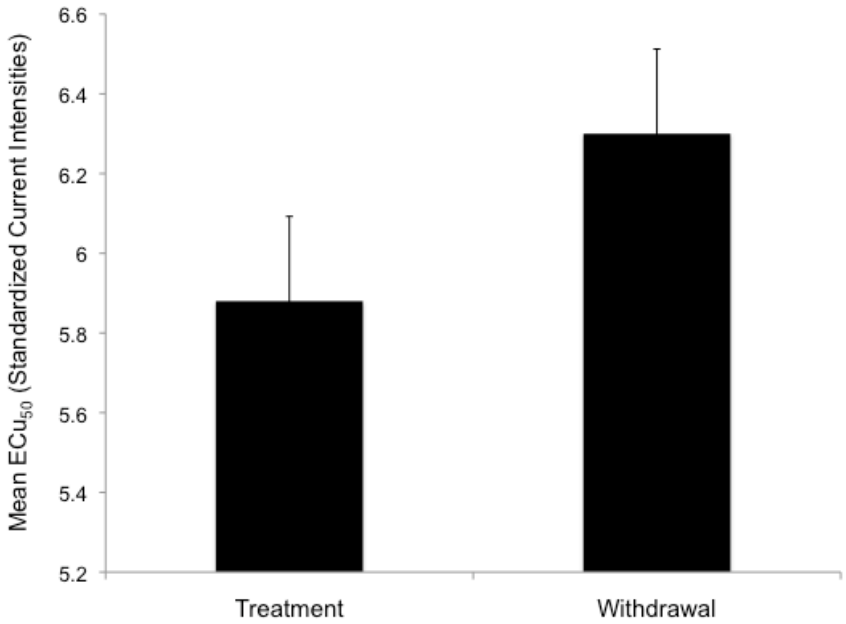


Figure 6. Mean + SE effective current (ECu_{50}) values during baseline, low-dose estradiol administration, withdrawal, high-dose estradiol administration, and withdrawal in the treatment group.



a. Mean current-response curves showing a rightward shift in the current-response function during the withdrawal period. Data are displayed with a sigmoid curve fit to all of the values generated during testing across animals.



b. Mean + SE effective current (ECu_{50}) values. There was an elevated ECu_{50} during withdrawal versus estradiol administration (t=-3.2, p=.007).

Figure 7. Mean current-response curves and ECu_{50} values for animals in the treatment group (*n*=7) during estradiol administration and withdrawal.

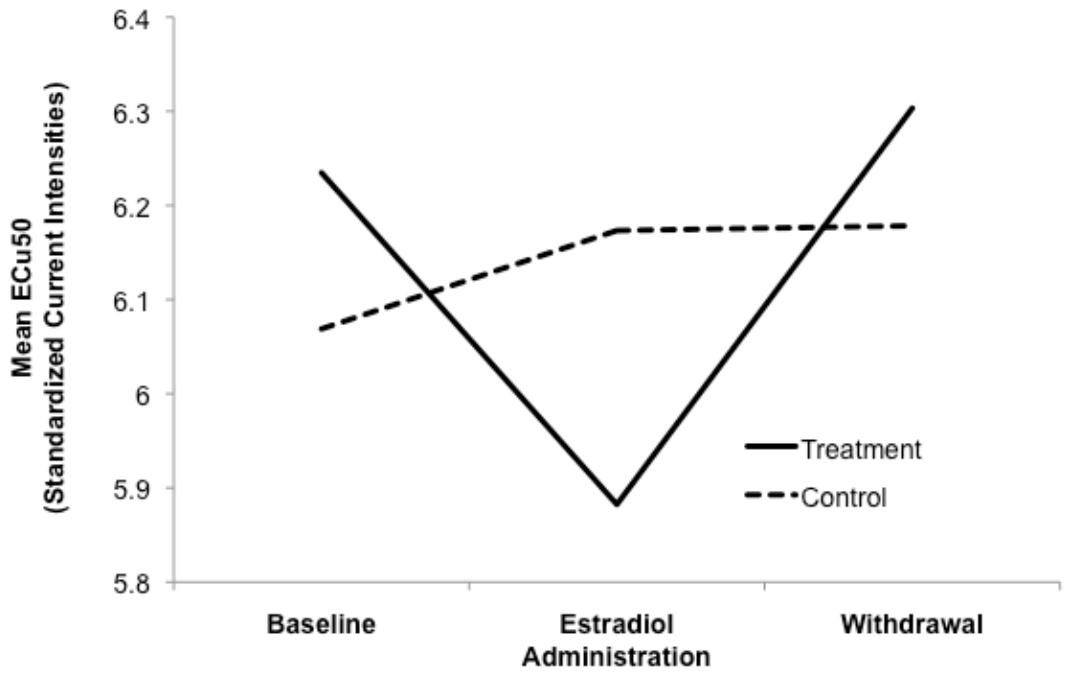


Figure 8. ECu_{50} data for animals in the treatment and control groups during baseline, estradiol administration, and withdrawal.

CHAPTER SIX

EXPERIMENT 2: THE EFFECTS OF ESTRADIOL WITHDRAWAL ON BEHAVIORAL DESPAIR

Methods

Animals

Ten-week old female Sprague Dawley rats (*N*=15) were maintained on a 12-hour light/12-hour dark cycle at a room temperature of 22.0 ± 0.2° C. Rat chow (Harlan Teklad Global Rodent Diet) and tap water were available *ad libitum*.

Ovariectomy

Under Equithesin anesthesia (3ml/kg i.p.: University of Iowa Hospital Pharmacy) bilateral ovariectomy using aseptic technique was performed on all animals included in this study. Ovariectomies were performed in the same manner as in Experiment 1A. The animals were allowed to recover for at least seven days prior to injections and testing.

Hormone Administration

Animals in the treatment group (*n*=8) were administered vehicle only for five days, high levels of estradiol (50μg 17-β estradiol) for five days, then vehicle only for five days. Control animals (*n*=7) were administered vehicle only for all 15 days. The forced swim test was administered on withdrawal day three.

Forced Swim Test Procedures

In the Forced Swim Test (FST; Porsolt, Le Pichon, & Jalfre, 1977), rodents were placed in a cylindrical container of water at least 30 cm deep that rose to a height no less than 15 cm from the top of the container. These dimensions prevented the animals from touching the bottom and escaping from the container. The temperature of the water was maintained at approximately 24° C. The rats displayed a period of behavioral activation characterized by vigorous swimming and diving; this behavior persisted for several minutes after which the animals displayed periods of immobility. The rats were videotaped for later coding. All animals were closely monitored during the test, and none

were at risk of drowning at any point. At the end of the test, the animals are towel dried, and then placed on a heating pad set at low heat for at least 20 minutes prior to returning to their home cages.

Data Analytic Approach

Swimming, climbing, and immobility were coded by an independent rater using five-second behavior sampling during the last five minutes of the 15-minute test. Behaviors were dummy coded according to the following convention: when the animal was swimming, swimming was coded "1" whereas both climbing and immobility were coded "0." Thus, three separate variables were coded and analyzed during the test, and the maximum score for each variable was 60. Independent t-tests were used to examine differences in swimming, immobility, and climbing between groups. A power analysis showed that seven animals per group were needed to have 80% power to detect a large effect with a one-tailed significance level of p=.05.

Results

Results are shown in Figure 9 (p. 64). Animals in the estradiol withdrawal group showed significantly greater immobility (t=2.26, p=.02) and less swimming (t=-2.26, p=.02) than animals in the control group. Animals exhibited very little climbing in both the treatment M (SD) = 0.13 (.35) and control groups M (SD) = 0.14 (.38), and there was no difference between groups (t=-0.10, p=.93).

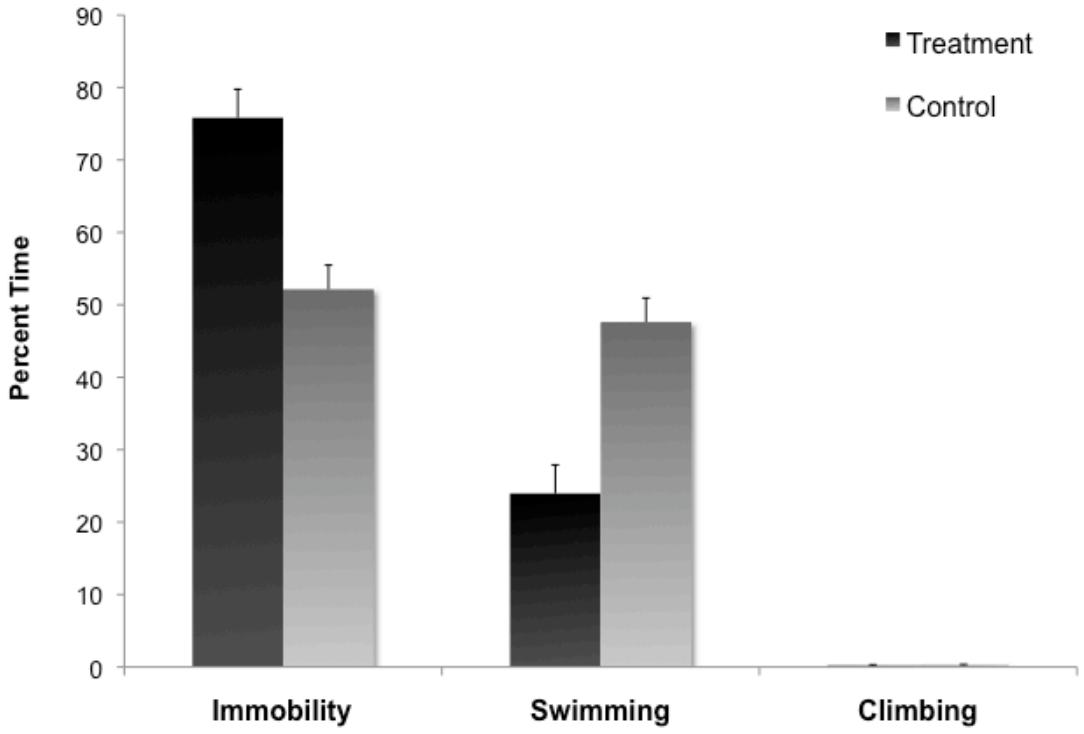


Figure 9. Mean + SE percent time spent immobile, swimming, and climbing during the forced swim test in the treatment and control groups. The treatment group showed significantly greater immobility and less swimming than the control group ($p<.05$).

CHAPTER SEVEN

EXPERIMENT 3: THE EFFECTS OF ESTRADIOL WITHDRAWAL ON ANXIETY

Methods

Animals

Ten-week old female Sprague Dawley rats (*N*=16) were maintained on a 12-hour light/12-hour dark cycle at a room temperature of 22.0 ± 0.2° C. Rat chow (Harlan Teklad Global Rodent Diet) and tap water were available *ad libitum*.

Ovariectomy

Under Equithesin anesthesia (3ml/kg i.p.: University of Iowa Hospital Pharmacy) bilateral ovariectomy using aseptic technique was performed on all animals included in this study. Ovariectomies were performed in the same manner as in Experiment 1A. The animals were allowed to recover for at least seven days prior to injections and testing.

Hormone Administration

Animals in the treatment group (*n*=8) were administered vehicle only for five days, high levels of estradiol (50μg 17-β estradiol) for five days, then vehicle only for five days. Control animals (*n*=8) were administered vehicle only for all 15 days. The forced swim test was administered on withdrawal day three.

Elevated Plus Maze

During the elevated plus maze (EPM; Pellow, Chopin, File, & Briley, 1985) procedure as modified by Rodgers and Dalvi (1997), rats were placed in an elevated, plus-shaped Plexiglas maze with two opposite enclosed arms and two open arms (each 50x10 cm) in a dimly lit room. The open arms had 1-cm edges to prevent rats from slipping off the sides of the maze. The enclosed arms had 40-cm high walls, and the maze was elevated 50 cm above the ground. The maze also had a central square (10 cm^2) that provided access to each arm. Animals were placed in the center of the maze facing an open arm and allowed to freely explore the maze for 5 minutes. Testing was videotaped for later scoring by an independent rater unaware of each animal's treatment group. The

cumulative time spent in the open/closed arms, the number of open-arm and closed-arm entries, and the number of head dips (i.e., rat's nose extending below the edge of an open arm) were recorded during the five-minute session. From these data, the total number of open and closed arm entries, time spent in the open arms time spent in the closed arms, and the total number of head dips were calculated.

Sucrose Preference Test

During the sucrose preference tests (Willner, Towell, Sampson, Sophokleous, & Muscat, 1987), rats had *ad libitum* access to food and water for the duration of the study. For one hour on withdrawal day four, a second bottle containing a 1% sucrose solution was presented to the rat's cage, and the rat was allowed to drink freely from either bottle during this time. The amount of sucrose solution consumed each day was measured. Anhedonia was operationally defined as decreased sucrose solution consumption.

Data Analytic Approach

Time spent in the center, open arms, and closed arms of the elevated plus maze were coded. The number of times each animal entered the open and closed arms and the number of times each animal's head crossed over the boundary of the open arms were also calculated. Independent samples t-tests were used to examine differences in between groups. A power analysis showed that seven animals per group were needed to have 80% power to detect a large effect with a one-tailed significance level of p=.05.

Results

As shown in Table 3 (p. 67), there were no statistically significant differences between groups on time spent in the open and closed arms of the elevated plus maze, although there was a trend-level result suggesting that control animals spent greater time in the closed arms than animals experiencing estradiol withdrawal. There were no group differences in the number of open arm entries, closed arm entries, or head dips. On withdrawal day four, there was no significant difference in sucrose consumption between groups (see Table 3, p. 67).

Table 3. Elevated plus maze results.

	Treatment (*n*=8)		Control (*n*=8)			
Behavioral Measure	***M***	***SD***	***M***	***SD***	***t***	***p***
Time in open arms (sec)	86.38	53.32	59.88	22.71	1.29	.21
Time in closed arms (sec)	149.00	52.77	194.38	46.77	-1.82	.09
Open arm entries	4.13	1.81	3.63	1.30	0.64	.54
Closed arm entries	7.00	2.45	7.50	1.07	-0.53	.61
Head dips	12.25	6.67	11.00	4.28	0.45	.66
Sucrose consumed (mL)	3.49	2.06	2.84	1.17	0.78	.45

PART C

PERINATAL OVARIAN HORMONES AND MOOD SYMPTOMS IN WOMEN: METHODS AND RESULTS

CHAPTER EIGHT

HUMAN STUDY METHODS

Participants

Participants included 10 women with a history of postpartum depression (PPD) and 12 control women, ages 18-40 recruited from the University of Iowa Hospitals and Clinics maternity unit using flyers and brochures. Inclusion criteria for the PPD group included a history of DSM-IV major depressive disorder with postpartum onset (i.e., occurring within one month postpartum) following a past pregnancy. Inclusion criteria for the control group included the absence of DSM-IV mood disorders. Exclusion criteria for both groups included current hormone therapy and current use of psychotropic medications. Eligibility screening was conducted by telephone or online questionnaire.

Procedures

Overview

This study included the following assessment periods: 1) an intake session conducted in the Iowa Depression and Clinical Research Center (IDCRC) or in participants' homes; 2) daily behavioral and hormone assessments were completed by participants in their homes; and 3) a final assessment conducted in the IDCRC or in participants' homes. The measures used for each session are summarized in Table 4 (p. 75) and described in more detail below.

Intake Session

The intake session took place 15-20 days before participants' due date. At the intake session, participants completed the SCID, IDAS, PANAS, and sucrose preference test (described below). Participants were also provided with materials and training for hormone sample collection and a logbook for recording daily mood and anxiety symptoms.

Daily Hormone and Mood Assessment

Participants collected saliva samples and made mood ratings each morning starting 20 days before the expected date of delivery and continuing until 10 days following delivery. Although previous research has not demonstrated a reliable diurnal variation in either estradiol or progesterone (Choe, Khan-Dawood, & Dawood, 1983), the morning assessment schedule was used to prevent contaminated salivary samples and to provide consistency among participants.

Saliva collection for the purpose of daily estradiol and progesterone assessment had several distinct advantages over serum or blood spot collection. First, saliva collection has been shown to be more acceptable to research participants and less invasive than blood collection procedures, and therefore conferred less risk to both participants and researchers. Second, saliva samples contain the unbound or biologically active portion of the total serum concentration of both estradiol and progesterone, making it more relevant for biobehavioral research and yielding larger effect sizes for associations between ovarian hormones and behavior (Edler et al., 2007; Shirtcliff et al., 2000). Participants collected 4mL of saliva (indicated by a line on collection vials) each morning within 30 minutes of waking and before brushing their teeth, eating, drinking, chewing gum, or smoking. Enzyme-Linked ImmunoSorbent Assay (ELISA) was used to determine estradiol and progesterone levels in the daily saliva samples.

Over the same time period, participants recorded mood and anxiety symptoms in a daily logbook each morning. The daily logbook included the full PANAS and the MASQ anxious arousal subscale. Based on the current ELISA kit specifications, saliva samples were stored at or below -20°C. In order to ensure compliance with procedures, to answer questions, and to assess continued eligibility (i.e., absence of suicidal intent), a member of the research team spoke with participants on a weekly basis for the duration of the study.

Final Session

The final study visit occurred approximately one month following delivery. At the final study visit, participants completed a SCID Mood Disorders Module Interview to assess new onset (i.e., postpartum) depressive symptoms, a second IDAS, PANAS, sucrose test, and the EPDS.

Measures

Edinburgh Postnatal Depression Scale (EPDS; Cox et al., 1987). This 10-item self-report questionnaire assesses symptoms of depression and anxiety experienced in the previous week and was designed to identify mothers at risk for developing postpartum depression. The EPDS is the most widely used self-report measure of postpartum depressive symptoms, and it has been translated for use around the world. The EPDS has been used extensively as an early screening measure for PPD. An EPDS score greater than nine at week four postpartum has been shown to identify 60% of women who experienced a recurrence of major depression within the first year postpartum (Peindl et al., 2004). The Reliable Change Index for the EPDS is four points, which suggests that a four-point change in EPDS scores represents a clinically significant difference (Matthey, 2004). In addition, there is a strong correlation between EPDS scores completed at 2-3 days postpartum and 4-6 weeks postpartum (r=0.61, p<.01). The EPDS has adequate internal validity (α=0.87), and it is adequately sensitive to changes over time (Cox et al., 1987).

Hedonic Response to Sucrose Test (Moskowitz, Kluter, Westerling, & Jacobs, 1974). Subjects were asked to taste five concentrations of sucrose: 0, 5, 10, 20, and 40% wt/wt in a volume of 15mL at 3-minute intervals. Solutions were administered in a randomized order in a double-blind manner. Before and between each sucrose tasting subjects rinsed their mouths with tap water. Each participant was asked to sip 15mL and hold it in her mouth without swallowing for 5 seconds before spitting into a discard beaker. Immediately after spitting the solution, participants rated the pleasantness elicited

by the solution on a nine-point Likert scale with responses ranging from "extremely pleasant" to "extremely unpleasant." Sucrose pleasantness ratings have been shown to be correlated with measures of physical anhedonia in patients with depression and schizophrenia (Berlin et al., 1998). Test-retest reliability was r=1.0 (Berlin et al., 1998).

Inventory of Depression and Anxiety Symptoms (IDAS; Watson et al., 2007). This 64-item self-report questionnaire assesses anxiety and depression and includes the following subscales: dysphoria, panic, social anxiety, appetite change, lassitude, well-being, suicidality, traumatic intrusions, insomnia, and ill temper. The IDAS also contains items that assess each of the DSM-IV diagnostic criteria for a major depressive episode (MDE), including anhedonia. Internal consistency for each of the subscales is adequate, with Cronbach's alpha ranging from .75 to .92, and nearly all of the subscales have alpha levels of .80 or higher. The IDAS also demonstrates excellent convergent validity: the general depression scale was highly correlated with the Beck Depression Inventory II (r=.83) across two independent samples, and the panic scales was highly correlated with the Beck Anxiety Inventory (r=.78-.79) (Watson et al., 2007). In addition, the subscales related to depressive symptoms were more highly correlated with the BDI-II than the BAI and vice versa (Watson et al., 2007). The one-week test-retest reliability of the IDAS is .72 or greater for each of the subscales (Watson et al., 2007). Thus, the IDAS has excellent psychometric properties and has been validated for use with postpartum women.

Mood and Anxiety Symptom Questionnaire – Anxious Arousal Subscale (MASQ; Watson & Clark, 1991). This 22-item subscale assesses physiological symptoms of anxiety. The initial item pool from which items on the anxious arousal subscale were selected included the symptom criteria for the anxiety disorders, including generalized anxiety disorder, panic disorder, and posttraumatic stress disorder. The items included in the anxious arousal subscale tap manifestations of anxiety related to somatic tension and arousal that are distinct from the general distress associated with depression. Items

include: “felt tense, high strung,” “felt nervous,” felt afraid,” “lump in my throat,” “felt nauseous.” The anxious arousal subscale is highly correlated with subscales tapping general distress, general anxiety, and general depression and only moderately correlated with the anhedonia/positive affect subscale. The anxious arousal scale also has excellent convergent and discriminant validity (Watson et al., 1991).

Positive and Negative Affect Schedule (PANAS; Watson, Clark, & Tellegen, 1988). This 20-item questionnaire measures positive affect (PA) and negative affect (NA). Items used to assess PA include enthusiastic, interested, excited, inspired, and active. Items used to assess NA include afraid, upset, ashamed, guilty and irritable. The PANAS has been validated to assess mood symptoms during various time frames, including in the moment, today, the past few days, the past few weeks, the past year, and in general. Normative populations tend to endorse more PA than NA. The PANAS has acceptable levels of internal consistency: Cronbach’s alpha values range from .86 to .90 for the 10-item PA scale and from .84 o.87 for the 10-item NA scale, and higher values are associated with shorter time frames (e.g., today versus over the past year). The correlation between the PA and NA scales range from -.12 to -.23, depending on the time frame being assessed, which suggests that they are quasi-independent (Watson et al., 1988). Test-retest reliability ratings, which were made on two occasions separated by one week, ranged from .47 for PA and .39 for NA when participants rated their affect that day to .68 for PA and .71 for NA when participants rated their affect “in general.” This demonstrates that while PANAS scores are stable across days, they are sensitive to changes in affect over time. The PANAS has also demonstrated excellent factor validity and external validity (Watson et al., 1988).

The Structured Clinical Interview for DSM-IV-TR Axis-I Disorders (SCID; First, Spitzer, Gibbon, & Williams, 2002). This semi-structured clinical interview assesses current and past psychopathology, including mood, anxiety, eating, and substance use disorders. Previous research has demonstrated interrater reliability estimates ranging from

kappa = 0.93 for substance use disorders to kappa = 1.0 for mood and anxiety disorders (Schneider et al., 2004). Test-retest reliability over a period of approximately 78.5 days (SD=53) ranges from kappa = 0 .84 for adjustment disorders to kappa = 1.0 for other Axis I disorders (Schneider et al., 2004). Demographic information was also assessed during this interview.

Data Analytic Approach

Group differences in the presence of psychiatric diagnoses were examined using chi square analyses. Differences in IDAS, PANAS, EPDS, and the maximum hedonic response were examined using *t* tests. Meta-analytic techniques based on the Stouffer method were used to examine the association between hormones and mood symptoms (Stouffer, Suchman, DeVinney, Star, & Williams, 1949). This statistical approach is similar to that described by Edler et al. (2007). Correlations between daily hormone levels and mood symptom scores were calculated separately for each participant using Pearson's *r* over the entire collection period. Within-subject Pearson's *r* values were converted to Fisher z scores, then averaged across subjects. The average Fisher *z* score was then converted back to a Pearson's *r* value to yield an overall effect size (Rosenthal & Rosnow, 1991). One-tailed significance levels were converted to *Z* scores, and then assigned negative values if the direction of effect was opposite to the predicted direction. Adjusted *Z* scores were then summed and divided by the square root of the total number of observations. The resulting *Z* score was then converted to a *p* value to yield an overall significance level (Rosenthal & Rosnow, 1991). Thus, the results represent combined correlation effect sizes and significance levels from within-subject analyses. This approach has the advantage of providing readily interpretable effect sizes for associations among variables of interest. A significance level of $p<.05$ was used.

Table 4. Measures employed at each assessment session.

Measure	Variable Assessed
Intake session:	
SCID (full)	Current and lifetime Axis I disorders
IDAS (full)	Baseline mood and anxiety symptoms
PANAS	Positive and negative affect
Sucrose test	Physical anhedonia
Daily assessments:	
Saliva sample	Estradiol and progesterone
PANAS	Positive and negative affect
MASQ	Anxiety
IDAS (Anhedonia items only)	Anhedonia
Final session:	
SCID (MDE only)	Postpartum onset mood disorders
IDAS (full)	Postpartum mood and anxiety symptoms
PANAS	Positive and negative affect
EPDS	Postpartum depressive symptoms
Sucrose test	Physical anhedonia

CHAPTER NINE

HUMAN STUDY RESULTS

Demographics and Psychological Assessment

Twenty-two women agreed to participate in this study. One woman in the at-risk group withdrew from the study. Of the 21 women who completed in this study, 9 were in the at-risk group, and 12 were in the control group. On average, women in this study were 30 years old (SD=3.2), had been pregnant a total of three times (SD=1.2), and had 1.5 children (SD=0.6). Annual household incomes ranged from less than $9,999 to over $100,000, and the median household income was between $50,000 and $59,999. The majority of participants were Caucasian (95%), not Latino (95%), and married (86%). None of the participants were currently taking psychotropic medications or receiving psychotherapy.

At the intake session, there were significant differences between the at-risk and control groups on several psychological variables. Current and lifetime Axis-I psychiatric diagnoses are shown in Table 5 (p. 79). As a result of the inclusion and exclusion criteria for each group, none of the control women had a history of major depression, and all of the at-risk women had at least one past episode of major depression that began within four weeks after giving birth (i.e., PPD). One at-risk woman met criteria for current major depression at the first study visit. Not surprisingly, women in the at-risk group were more likely to have both current and lifetime anxiety disorder diagnoses. The majority of women in the at-risk group met diagnostic criteria for an anxiety disorder in their lifetime, and five women had a current anxiety disorder.

As shown in Table 6 (p. 80), at-risk participants reported significantly greater dysphoria and less well being and positive affect than the control group. There were no significant differences between groups in the maximum hedonic response or in the anhedonia subscale created using IDAS items.

At the second study visit, five of the at-risk participants and none of the controls met diagnostic criteria for major depression. Four of the five at-risk participants with major depression had a postpartum onset; one of the five participants developed depression during pregnancy, and thus, her onset was not consistent with the postpartum diagnostic specification. In response to the IDAS items, at-risk participants reported significantly greater dysphoria and panic and less well being than control participants. Of note, there were no longer differences in positive affect, and there were no significant differences in EPDS scores. There were no significant group x time interactions.

Ovarian Hormones and Mood Symptoms

Estradiol and Negative Affect

Although hypothesized that there would be a negative correlation between estradiol levels and negative affect over time, there was no significant association between estradiol and negative affect in the at-risk group (r=-0.06, p=.19) or the control group (r=-0.05, p=.24). However, among the four women in the at-risk group who developed PPD during the study, there was a significant, negative correlation between estradiol and negative affect (r=-0.34, p<.001). The graphs presented in Figure 10 (p. 81) show the association between negative mood and estradiol levels the women who developed PPD and the control group. In order to provide a graphical depiction of changes in hormones and mood over time in each group, 5-day rolling averages were calculated to smooth the pattern of hormone and mood variability, making the graphs more readily interpretable. Five-day rolling averages were converted to Z scores based on each participant's overall mean and standard deviation to reduce the between-subject variability within groups, which allows each participant's scores to contribute equally to the averages represented in the graph. Interestingly, the peak in negative mood in the control group following delivery reflects the postpartum blues, wherein negative mood peaks within the first five days following delivery (Kendell, McGuire, Connor, & Cox, 1981).

Estradiol and Positive Affect

It was hypothesized that there would be a positive correlation between estradiol levels and positive affect over time. Contrary to this hypothesis, estradiol was *negatively* correlated with positive affect in the control group (r=-0.29, p<.001). There was no significant association between estradiol and positive affect in the at-risk group (r=-0.07, p=.18) or within the subset of women who developed PPD (r=-0.01, p=.73).

Progesterone and Anxiety

It was hypothesized that there would be a negative correlation between progesterone levels and anxiety over time. Contrary to this hypothesis, progesterone was *positively* correlated with anxiety in the control group (r=0.33, p<.001), the at-risk group (r=0.28, p<.001), and the subset of women who developed PPD (r=0.18, p=.04).

Exploratory Analyses

Previous research suggests that one of the strongest predictors of postpartum depression is the presence of a past episode of PPD (Bloch et al., 2006). Therefore, a logistic regression analysis was conducted to determine the incremental validity of adding the correlation between estradiol and negative mood to the predictive model. The presence of a past episode of PPD was entered into the model as a binary variable (1=past episode of PPD; 0=no past episode of PPD), and the within subject correlation between estradiol and negative affect was entered as a dimensional variable. Using the presence of a past episode of PPD alone to predict future PPD correctly classifies 80% of participants. When the correlation between estradiol and negative mood is added to the model, 100% of participants are correctly classified.

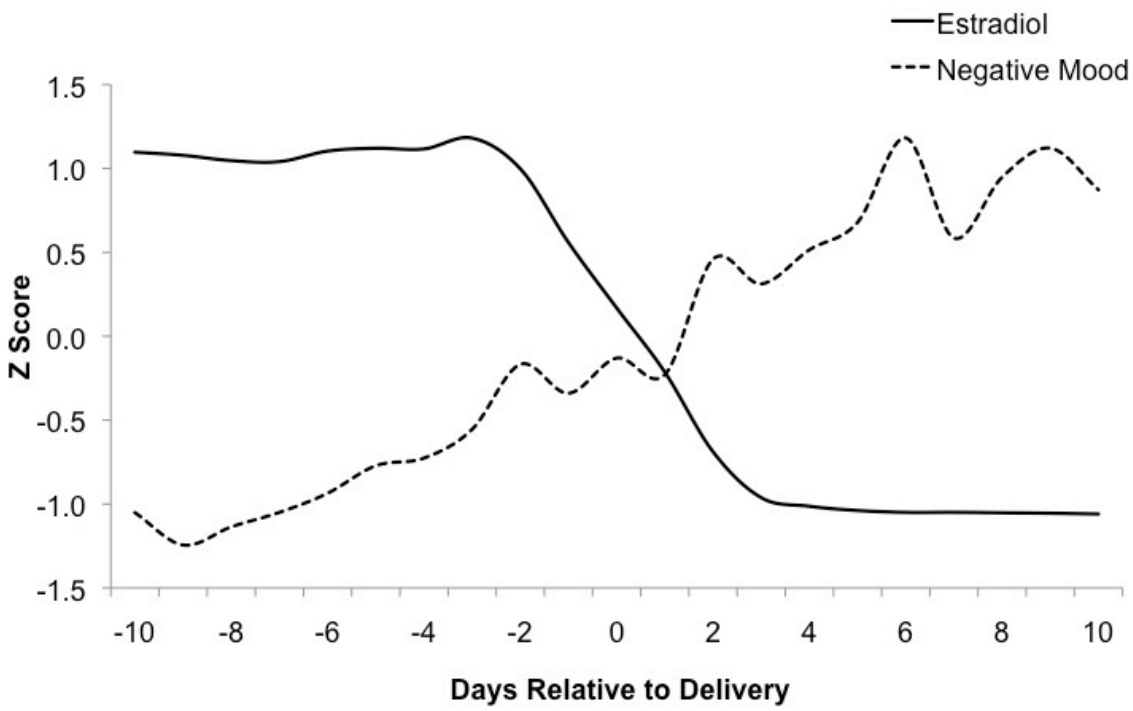
Table 5. Psychiatric diagnoses at intake.

	At-Risk		Control		
	n	***%***	***n***	***%***	χ^2
Current Diagnosis					
Mood Disorder	1	*11*	0	*0*	1.4
Anxiety Disorder	5	*56*	0	*0*	8.8**
Alcohol Use Disorder	0	*0*	0	*0*	--
Eating Disorder	0	*0*	0	*0*	--
Lifetime Diagnosis					
Mood Disorder	9	*100*	0	*0*	21.0***
Anxiety Disorder	7	*78*	0	*0*	14.0***
Substance Use Disorder	1	*11*	2	*17*	1.5
Eating Disorder	1	*11*	0	*0*	1.4

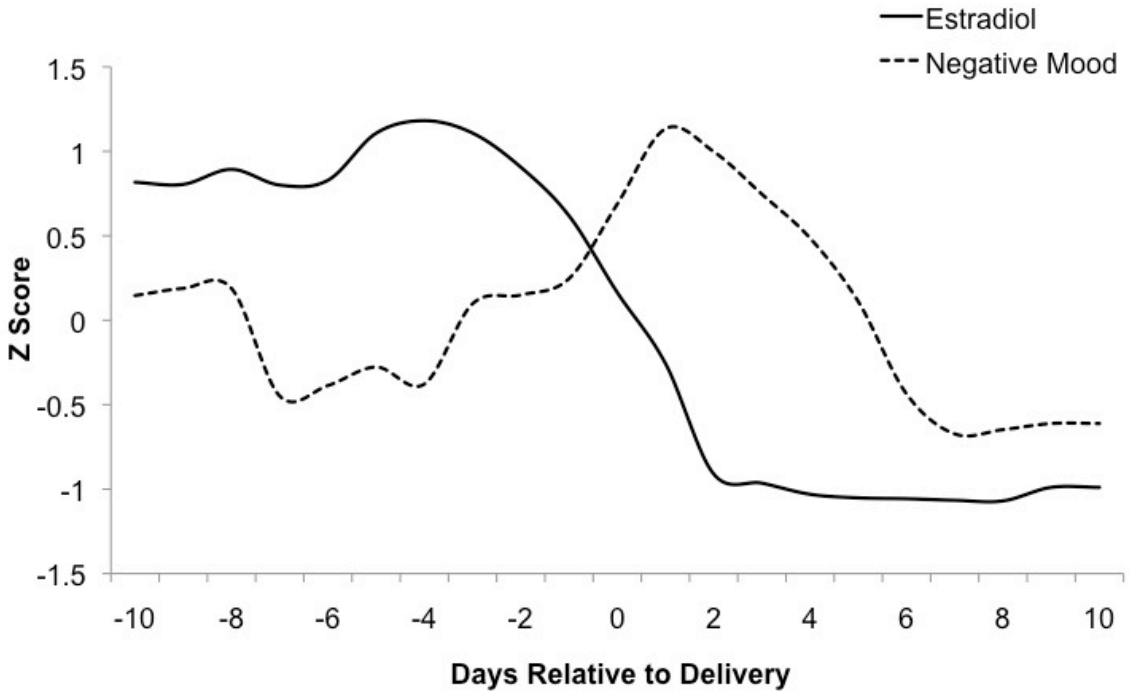
**p<.01, ***p<.001

Table 6. Self-reported psychological data and the first and second study visits.

	Study Visit 1					Study Visit 2				
	At-Risk		Control			At-Risk		Control		
Measure	***M***	**(*SD*)**	***M***	**(*SD*)**	***t***	***M***	**(*SD*)**	***M***	**(*SD*)**	***t***
IDAS										
Depression										
General Depression	39.9	(*9.3*)	32.3	(*5.8*)	-2.3*	37.0	(*5.7*)	30.5	(*6.9*)	-2.3*
Dysphoria	17.4	(*4.3*)	13.4	(*3.1*)	-2.5*	16.1	(*2.8*)	12.8	(*4.0*)	-2.1*
Ill Temper	8.8	(*4.0*)	6.7	(*2.1*)	-1.6	7.4	(*3.0*)	6.8	(*2.3*)	-0.6
Insomnia	14.7	(*6.4*)	12.1	(*4.9*)	-1.1	12.7	(*4.1*)	11.8	(*4.1*)	-0.5
Lassitude	11.8	(*3.2*)	10.1	(*2.5*)	-1.4	13.3	(*4.0*)	10.9	(*3.9*)	-1.4
Appetite Gain	3.7	(*0.9*)	4.6	(*1.2*)	2.0	3.9	(*1.7*)	4.6	(*3.1*)	0.6
Appetite Loss	5.2	(*2.4*)	4.0	(*1.3*)	-1.5	4.9	(*2.8*)	3.5	(*0.8*)	-1.0
Suicidality	6.1	(*0.3*)	6.2	(*0.4*)	0.3	6.2	(*0.4*)	6.0	(*0.0*)	-1.5
Well-Being	21.4	(*5.4*)	26.3	(*4.6*)	2.2*	22.1	(*5.6*)	27.1	(*4.4*)	2.3*
Anhedonia	8.4	(*2.8*)	6.3	(*0.7*)	-2.5	7.4	(*2.2*)	6.3	(*2.3*)	-1.1
Anxiety										
Panic	11.1	(*2.7*)	9.3	(*2.6*)	-1.5	10.7	(*2.2*)	8.6	(*1.2*)	-2.8*
Social Anxiety	6.3	(*1.9*)	5.8	(*1.3*)	-0.7	6.6	(*2.5*)	5.9	(*2.0*)	-0.7
Traumatic Intrusions	4.4	(*1.0*)	4.1	(*0.3*)	-1.0	6.1	(*2.6*)	4.2	(*0.4*)	-2.2
PANAS										
Negative Affect	13.2	(*3.7*)	11.1	(*1.2*)	-1.9	11.1	(*1.9*)	10.7	(*1.2*)	-0.7
Positive Affect	20.3	(*4.5*)	28.8	(*5.9*)	3.6**	23.3	(*7.7*)	28.8	(*6.2*)	0.7
Maximum Hedonic Response	2.3	(*1.0*)	1.9	(*0.9*)	-1.0	2.3	(*1.2*)	2.4	(*0.7*)	0.3
EPDS						7.3	(*4.1*)	4.2	(*3.3*)	-2.0



a. women who developed PPD (*n*=4)



b. control women (*n*=12)

Figure 10. Ovarian hormones and mood.

PART D

DISCUSSION AND INTEGRATION

CHAPTER TEN

DISCUSSION AND INTEGRATION OF ANIMAL RESULTS

Results of Experiment 1A suggest that administering both estradiol and progesterone at high levels, then withdrawing them simultaneously, similar to human pregnancy does not produce anhedonic effects. However, when estradiol alone was administered and withdrawn, animals showed anhedonic behavior. During the withdrawal condition, there was no significant change in motor behavior, which suggests that the change in ECu_{50} reflects a change in hedonic drive specifically rather than a change in motor behavior in general. Taken together, results of Experiment 1B suggest that superphysiologic levels of estradiol administration over a period of five days, followed by five days of hormone withdrawal precipitated by vehicle administration alone may be a valuable model of PPD.

Anhedonia may result from estradiol withdrawal based on action in the ventral tegmental area. ERβ -immunoreactive nuclei are present in dopaminergic neurons of the ventral tegmental with mesocortical and mesolimbic projections (Creutz & Kritzer, 2002). The presence of ERβ in this region strongly suggests that ER-mediated effects impact affective and motivational functions associated with the mesolimbic dompaminergic system (Creutz & Kritzer, 2002), including anhedonia. ERβ in neurons located in the ventral tegmental area modulate the release of dopamine to the nucleus accumbens (see Figure 11, p. 88), and this pathway is likely responsible for the effects of estradiol withdrawal on anhedonia. However, this hypothesis has not been systematically tested, and the model advanced in this study could be used in future studies to test this and other hypothesized mechanisms by which estradiol withdrawal causes depression-like behavior in rodents.

Although this was the first set of experiments to directly compare the results of estradiol withdrawal alone and in combination with progesterone withdrawal, results are consistent with previous animals models of PPD. Two previous animal models of PPD

involved administration of ovarian hormones consistent with rodent pregnancy, and therefore, produced as state of estradiol withdrawal alone during the simulated postpartum period (Galea et al., 2001; Stoffel & Craft, 2004). Both studies demonstrated *increased* behavioral despair during estradiol withdrawal. In contrast, one previous model of PPD involved administration of ovarian hormones consistent with human pregnancy, and as a result, produced a state of estradiol and progesterone withdrawal during the simulated postpartum (Suda et al., 2008). Animals in this study showed *decreased* behavioral despair during hormone withdrawal.

The mechanisms by which estradiol withdrawal alone may increase vulnerability to anhedonia, whereas a combination of estradiol plus progesterone withdrawal may be protective are unclear. Previous research suggests that progesterone withdrawal is associated with behavioral excitability (Smith & Woolley, 2004). Further analysis of the data from Experiment 1A revealed that, compared to combined estradiol and progesterone administration, withdrawal was associated with increased motor activity (t=-2.65, p=.04). Perhaps the increased motor activity associated with progesterone withdrawal offset the anhedonic effects of estradiol withdrawal. Subsequent studies should examine the effects of progesterone administration and withdrawal on LHSS.

As hypothesized, estradiol withdrawal was associated with increased immobility and decreased swimming during the forced swim test. Results of the current study were consistent with two previous studies, which demonstrated behavioral despair following estradiol withdrawal (Galea et al., 2001; Stoffel & Craft, 2004). The presence of both behavioral despair and anhedonia following estradiol withdrawal lends further support to the utility of estradiol withdrawal as a model of PPD.

Estradiol withdrawal alone was not associated with increased anxiety in this study, and results were consistent with those of previous studies (Stoffel & Craft, 2004; Suda et al., 2008). The only previous animal model of postpartum anxiety that involved estradiol withdrawal alone failed to produce anxious behavior (Stoffel & Craft, 2004),

whereas the previous model that involved estradiol and progesterone withdrawal showed increased anxiety (Suda et al., 2008), although it was not tested in the current study. Progesterone metabolites have been shown to act as a sedative, decreasing anxiety by enhancing GABA function (Smith et al., 1998). Progesterone withdrawal has also been shown to result in enhanced transcription of the gene encoding the a4 subunit of the $GABA_A$ receptor, which was both necessary and sufficient to induce anxiety in rodents (Smith et al., 1998). Intra-amygdala administration of progesterone reduces anxiety in rodents as demonstrated by increased central entries in the open field trial, increased time spent in the open arms in the elevated plus maze, and decreased time spent freezing after foot shock, compared to placebo (Frye & Walf, 2004). Taken together, these results suggest that increased anxiety during the postpartum period is the result of progesterone withdrawal (Frye & Walf, 2004). Thus, it is not surprising that the exclusion of progesterone from the current study resulted in no difference in anxiety between the hormone withdrawal and control groups.

Results of this study supplement findings of Bless et al (1997) regarding the time course of the behavioral effects of estradiol. In Experiment 1B, significant behavioral effects were seen 24 hours following the administration of estradiol. The behavioral effect of estradiol did not appear to diminish during the five days animals were tested, and future studies should examine whether animals habituate to constant levels of estradiol administration over time.

The current set of experiments had several strengths. This was the first study to specifically examine the influence of estradiol withdrawal on anhedonia. This was also the first study to demonstrate that hormone administration for five days in ovariectomized animals followed by withdrawal may serve as an effective model of PPD. Previous animal models of PPD used a hormone administration period of 21 days, consistent with rodent pregnancy. A 21-day hormone administration period prior to experimental testing presents methodological difficulties when conducting LHSS experiments because the

cement holding the electrode in place loosens over time, allowing the electrode tip to shift in the brain. Moreover, reducing the hormone administration period from 21 to five days shortens the duration of the entire experiment by over 50%, which reduces unnecessary subject burden, per diem expenses associated with housing animals, and the latency to conduct follow-up experiments. Finally, a notable advantage of testing the hormone withdrawal hypothesis using an animal model is the improved experimental control and ability to address unexpected findings with follow-up studies.

The current set of experiments also had certain weaknesses. One weakness was the simulation of pregnancy through hormone manipulation. A more face valid, albeit less rigorously controlled, model would include pregnant animals. It is therefore more accurate to interpret the results of the current experiments as a model of estradiol administration and withdrawal on behavior rather than a model of human PPD. Another weakness was the use of ovariectomized animals as the control group. The closest human analog to ovariectomized rats is menopausal women. Comparing the postpartum period to menopause is less meaningful than comparing it to a non-pregnant, premenopausal state.

Future studies using the proposed model could be used to examine the effects of antidepressant administration. In animals experiencing estradiol withdrawal, it would be valuable to know whether estradiol administration, antidepressant treatment, or a combination treatment provides the largest reduction in anhedonic behavior. Additional research is needed to ascertain the time course of the effects of estradiol administration and withdrawal on anhedonia and behavioral despair. For example, it remains unclear whether longer administration or higher doses yield a longer period of behavioral change. The mechanisms by which estradiol withdrawal influences anhedonia should also be explored. Specifically, the use of selective estrogen receptor modulators (SERMs) could be used to examine whether ERα, ERβ, or both receptors mediate the effects of estradiol on anhedonia. Moreover, specific brain areas (e.g., the ventral tegmental area) could be targeted to isolate the areas in which estradiol administration has an effect on anhedonia.

In conclusion, these results suggest that estradiol withdrawal alone rather than the combination of estradiol and progesterone withdrawal influences anhedonia. Given that estradiol withdrawal was associated with both anhedonia and behavioral despair suggests that it may serve as a model of PPD in future research. The animal model of estradiol withdrawal developed in the current study has several advantages over previous models, including reduced subject burden.

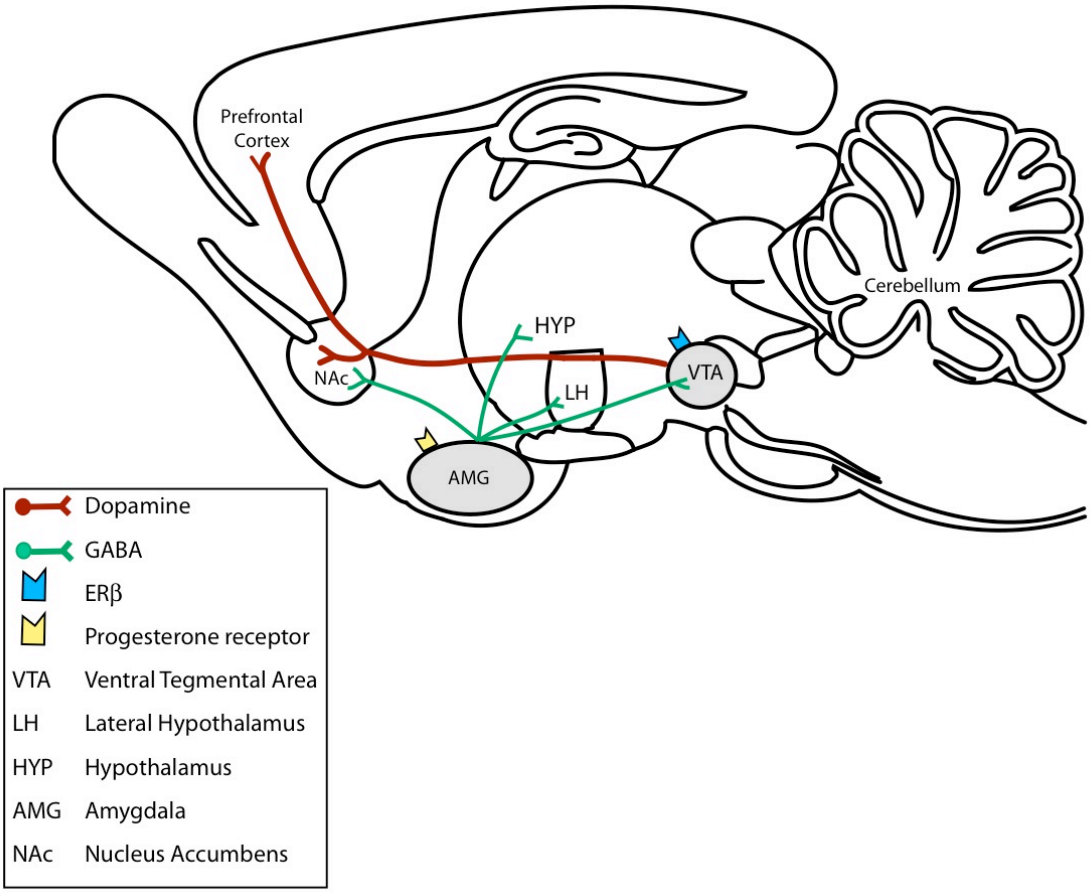


Figure 11. Brain regions associated with the effects of estrogen and progesterone on behavior.

CHAPTER ELEVEN

DISCUSSION OF HUMAN RESULTS

Contrary to the hypothesized results, estradiol withdrawal was not associated with negative affect in either the at-risk or control group. However, there was a significant negative association between estradiol and negative affect among the women who developed PPD. Moreover, all of the at-risk women who were not depressed during pregnancy and showed a negative correlation between estradiol and negative affect later developed PPD. This subgroup may be differentially sensitive to the effects of ovarian hormone withdrawal. Previous studies have identified similar "sensitive" subgroups of women who experience mood symptoms in response to changes in ovarian hormones (Bloch et al., 2005, 2006; Bloch et al., 2000). This appears to be the first study to directly examine the association between ovarian hormones and mood symptoms daily during the days immediately before and after delivery. The results are consistent with previous experimental animal and human studies, which have shown that depressive symptoms occur during periods of estradiol withdrawal (Bloch et al., 2000; Galea et al., 2001; Stoffel & Craft, 2004).

An alternative explanation is that the correlation between estradiol and negative affect may have been artifactual given that estradiol decreased following delivery for all women in the study and negative mood increased in the subgroup that developed PPD. However, this alternative explanation is limited for several reasons. First, it would have been just as plausible for the women with PPD to have been experiencing increased mood symptoms prior to delivery and have maintained a stable symptom level in the days following delivery. Depressed mood during pregnancy has been shown to be one of the strongest predictors of PPD (O'Hara & Swain, 1996), and women with mood symptoms during pregnancy were not excluded from participating in the current study. Thus, it is noteworthy that among the women with PPD, depressive symptoms increased in the days before and following childbirth. Second, it is notable that among the women with PPD,

the increase in negative mood was concurrent with the drop in estradiol. Thus, a differential sensitivity to the effects of ovarian hormone withdrawal may have precipitated the onset of mood symptoms. Third, although the current study was correlational, it is consistent with the experimental results of Bloch et al. (2000), which suggest that estradiol withdrawal may cause increased mood symptoms. Fourth, whether the association was artifactual or indicative of an underlying vulnerability in the PPD group, it was used to predict the onset of PPD with a high degree of accuracy and therefore warrants further investigation.

Estradiol may influence mood symptoms by several mechanisms, including serotonin and dopamine. Reduced serotonergic function is associated with major depressive disorder as well as a number of basic biological functions, including sleep and appetite (Mann, 1999). Estrogen has been shown to modulates serotonergic function (Rubinow, Schmidt, & Roca, 1998), and women with PPD have lower plasma serotonin levels than non-depressed controls (Gu et al., 2003). Experimental animal studies demonstrate increased serotonin neurotransmission in the dorsal raphe nucleus during pregnancy (Klink, Robichaud, & Debonnel, 2002). In contrast, serotonin firing in the dorsal raphe nucleus is diminished during the postpartum period (Klink et al., 2002). Estradiol has also been shown to modulate serotonin receptor expression and activity (Rubinow et al., 1998). Thus, estradiol withdrawal may increase depressive symptoms through its modulation of the serotonin system. Diminished dopaminergic function may also play a role in PPD (Dailly, Chenu, Renard, & Bourin, 2004). Experimental animal studies suggest that chronic administration of estradiol leads to dopamine receptor up-regulation and increased presynaptic dopamine activity (Byrnes et al., 2001). Abrupt estradiol withdrawal leads to dysregulation in brain dopaminergic pathways and depressive symptoms (Byrnes et al., 2001). Thus, the effects of estradiol withdrawal on mood symptoms during the postpartum may be mediated, in part, by dopaminergic

function. Future studies should examine the complex interplay of estradiol, serotonin, and dopamine in the pathogenesis of PPD.

Contrary to the hypothesized results, estradiol levels were *negatively* associated with positive affect in the control group, and there was no association between estradiol and positive affect in either the at-risk group as a whole or in the women who developed PPD. The mechanism by which estradiol withdrawal may cause increased positive affect is unclear. For example, a third variable may influence both estradiol and positive affect following childbirth. Although most studies examine negative mood during the postpartum, a previous study of positive mood states demonstrated that women report high levels of happiness following childbirth (Kendell et al., 1981). As such, women in the control group may have experienced increased positive affect as a result of childbirth rather than estradiol withdrawal per se. Interestingly, the at-risk women did not experience the same increase in positive affect. Low positive affect is associated with depression, and thus, the lack of an increase in positive mood following delivery may have contributed to depression vulnerability in the at-risk group. Notably, five of the nine at-risk women met criteria for major depression, and four of them had a postpartum onset.

Similarly, progesterone levels were positively associated with anxiety symptoms, which suggests that that anxiety decreased over the course of the study. This may reflect two features of the study design. First, the study was designed to detect PPD rather than postpartum anxiety. At-risk women were recruited based on their history of depressive symptoms, not anxiety symptoms. Future studies should examine the association between progesterone and anxiety in a group of women with a history of postpartum exacerbation of anxiety. Second, the MASQ anxious arousal subscale taps somatic symptoms of anxiety generally associated with panic, and it is possible that progesterone withdrawal is more highly associated with cognitive symptoms of anxiety (i.e., neuroticism).

This study had several strengths. It appears that this was the first study to examine within subject association between changes in ovarian hormones and mood symptoms during days surrounding delivery. This allowed for an examination of whether human females are sensitive to naturalistic changes in ovarian hormones as suggested by previous experimental studies involving exogenous hormone administration. This study involved daily assessments of hormones and mood over time, which provided for increased power to detect associations between variables. Moreover, the use of salivary rather than plasma assessment of ovarian hormones reduced the risk to participants, allowed for frequent hormone assessment, and provided information about the unbound, biologically active portion of each circulating hormone. Finally, this study had a high retention rate; 95% of participants completed the study.

This study also had several limitations. First, the sample size was smaller than anticipated. A much larger sample size is required for more sophisticated data analytic approaches designed to detect associations between non-linear variables over time. As such, results should be interpreted with caution until they can be confirmed in a larger sample. Second, as a result of slower than expected recruitment, inclusion criteria for the at-risk group were relaxed to include women with a history of PPD and major depression outside the postpartum period, rather than restricting inclusion to women with a history of PPD alone, as was originally intended. As a result, one woman included in the study met criteria for major depression at the first study visit. Third, although the study was longitudinal, it was not experimental, and thus, causal inferences regarding the effects of hormones and mood symptoms on PPD are limited.

In conclusion, postpartum depression is related at least in part to the hormonal milieu of pregnancy and childbirth. Experimental studies have demonstrated that estradiol withdrawal is associated with depression-like behavior in animals and increased negative mood in at-risk women. Results of the current study further suggest that ovarian hormones represent a promising candidate for unraveling the neurobiological

mechanisms of PPD. The experimental study by Bloch et al. (2000) suggests that at-risk women are sensitive to both increases and decreases in ovarian hormones. This represents an important area for future exploration. Future studies could examine hormone levels and mood symptoms beginning in the second trimester to capture periods of increasing and decreasing hormones. Finally, an improved understanding of the influence of estradiol on mood symptoms may lead to novel pharmacological treatments for PPD and allow for better treatment selection for hormone-mediated depressive symptoms in women.

CHAPTER TWELVE

INTEGRATION OF ANIMAL AND HUMAN RESULTS

The animal and human studies were designed to be complementary and provide an integrative examination neurobiological mechanisms underlying PPD. Although neither the animal nor human studies yielded the anticipated pattern of results, both implicate estradiol withdrawal in the onset of depression. Estradiol withdrawal was associated with anhedonia and behavioral despair in the animal model of PPD, and the negative association between estradiol and mood symptoms significantly predicted the onset of PPD in the women. The translational nature of these results provides strong evidence for estradiol withdrawal as a neurobiological mechanism of PPD. Moreover, estradiol function is implicated in depressive disorders occurring across the lifespan in women. Previous research has demonstrated that estradiol withdrawal has a causal role in premenstrual dysphoric disorder and postmenopausal depression (Rubinow et al., 1998), and hormone therapies that stabilize estradiol levels are efficacious for treating both disorders (Rubinow et al., 1998). Thus, estradiol withdrawal represents a promising candidate for understanding depressive disorders across the lifespan in women.

Perhaps the more pertinent question is why some women experience mood symptoms in response to changing estradiol levels while others do not. Previous genetic studies have identified specific patterns of gene expression during pregnancy that predict the onset of PPD and are correlated with symptom severity (Segman et al., 2010). The nature of the interaction between genetic expression, rapid hormone changes that occur during pregnancy and childbirth, and environmental stress remains unclear. This line of research could provide important clues for understanding the differential sensitivity to changes in estradiol that increase the risk for postpartum depression. Neuroimaging also represents a promising technology for examining the increased sensitivity to ovarian hormones. For example, patterns of brain activation in response to a stressor may change following estradiol treatment and withdrawal. Women with a history of hormone-

mediated depression (i.e., PMDD, PPD, or postmenopausal depression) may show greater changes in brain activation following hormone administration and withdrawal than women without a history of a mood disorder.

The translational nature of this project had several distinct advantages. First, the similarities between the animal and human study provide strong evidence of a biological basis for PPD. Second, previous animal models and human studies of PPD had such divergent designs and patterns of results that it was difficult to draw conclusions about the influence of hormones on mood. This project was designed to be translational, and the results support an integrative model of estradiol withdrawal. Third, the animal studies allowed for stringent experimental control, whereas the human studies provided a more complex uncontrolled context to validate the experimental animal results.

This series of translational studies also had important shortcomings. The animal model demonstrated increased anhedonia following estradiol withdrawal. However, estradiol withdrawal was not associated with decreased positive affect in the human study. There are several possible explanations for this inconsistency. First, LHSS may not be an appropriate analog for anhedonia in humans. Second, low positive affect may not be an adequate operational definition anhedonia. Previous factor-analytic studies have not identified anhedonia as a unified construct (Watson et al., 2007). Instead, items assessing anhedonic symptoms tent to split across a number of factors (Watson et al., 2007), suggesting that self-reported anhedonia may not be a unitary construct in humans. Third, the combination of estradiol and progesterone withdrawal that characterizes human postpartum period does not result in anhedonia, which is consistent with the results of Experiments 1A. Future studies could address this question by examining the effects of estradiol on anhedonic behaviors rather than self-reported anhedonia in women. Finally, the use of estradiol withdrawal alone in the animal model is inconsistent with the postpartum withdrawal of both estradiol and progesterone experienced by humans. Although the current study yielded null findings in the context of estradiol and

progesterone withdrawal, future studies may examine depression-like behavior following estradiol and progesterone withdrawal in the context of a stress. The addition of a stressor to the model may more closely mimic the stress associated with childbirth and may provide a more face-valid animal model of PPD.

In conclusion, the translational results of this project contribute to evidence of a neurobiological basis for PPD. Estradiol withdrawal represents a promising candidate for further study, particularly with regard to individual differences in sensitivity to hormone withdrawal. An improved understanding of the influence of estradiol on mood symptoms may lead to novel treatments for PPD and allow for better treatment selection among postpartum women sensitive to effects of hormone withdrawal. For example, transdermal estradiol administration may be the most efficacious treatment for women with PPD who have demonstrated a strong association between estradiol levels and negative mood during delivery.

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