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Estradiol Modulates Anhedonia and Behavioral Despair in Rats and Negative Affect in a Subgroup of Women at High Risk for Postpartum Depression

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Abstract

In an effort to address inconsistencies in the literature, we tested a cross-species estrogen withdrawal model of postpartum depression (PPD) with a series of rodent experiments and a prospective, naturalistic human study. All rats were ovariectomized prior to experimentation. The first rat experiment examined the effects of low- and high-dose estradiol administration and withdrawal on lateral-hypothalamic self-stimulation, a behavioral index of anhedonia, in experimental ($n=7$) and vehicle-only control animals ($n=7$). The second rat experiment examined the effects of high-dose estradiol withdrawal on activity and immobility during the forced swim test, an index of behavioral despair, in a separate group of experimental ($n=8$) and vehicle-only control animals ($n=8$). In the human study, women with ($n=8$) and without ($n=12$) a history of PPD completed mood ratings and collected saliva samples (to assess estradiol levels) daily during the third trimester of pregnancy through 10 days postpartum. The presence of PPD was assessed at one month postpartum. In the animal studies, rats in the estradiol withdrawal group demonstrated significantly greater immobility and less swimming than controls. Estradiol withdrawal resulted in reduced responding for electrical stimulation (multiple intensities) relative to estradiol administration. In the human study, there was no significant association between estradiol and negative affect among women with or without a history of PPD. However, there was a correlation between daily estradiol levels and negative affect in the women with incident PPD at one month postpartum. Despite important cross-species differences, both the rat and human studies provided evidence of the effects of estradiol on perinatal depressive symptoms.

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1. Introduction

Postpartum depression (PPD) affects seven to 13% of women following delivery [1,2] and is a leading cause of morbidity and mortality among mothers [3]. PPD is also related to adverse cognitive, emotional, and behavioral outcomes for offspring [4,5]. The *estrogen withdrawal hypothesis*, which attributes the onset of PPD to the precipitous drop in estradiol at delivery, is one of the most widely tested etiological models of PPD. Although a number of human and rodent studies support the estrogen withdrawal hypothesis [6-11], several studies have failed to support this hypothesis [12-15]. For example, experimental rat and human studies have demonstrated increased behavioral despair and depressive symptoms, respectively, following withdrawal from exogenous estradiol [6-9], and studies that administered estradiol to women with PPD have successfully reduced depressive symptoms [11,16]. In contrast, clinical studies have failed to demonstrate differences in estradiol levels between depressed and euthymic postpartum women [12-15], leading some to dismiss the potential deleterious effects of estradiol withdrawal on postpartum affective dysregulation altogether [17]. Indeed, there is no *naturalistic* evidence of a clear causal link between endogenous estradiol withdrawal and the onset of depressive symptoms in women. However, it remains unclear whether withdrawal from pregnancy levels of estradiol may interact with a pre-existing susceptibility to depression to yield PPD. In addition, although rat models have examined behavioral despair (i.e., a proxy for depressed mood) [18], none of the existing models has included both behavioral despair and anhedonia, analogs [19-21] of the two most prominent symptoms of major depression in humans (i.e., sadness/negative affect and anhedonia/low positive affect) [22].

The current study used a cross-species, multi-method approach to investigate whether estradiol withdrawal increases depressive symptoms and behaviors. The rat experiments examined the effects of low- and high-dose estradiol administration and withdrawal on lateral-hypothalamic self-stimulation, a behavioral index of anhedonia [19,20], and the effects of estradiol withdrawal on activity and immobility during the forced swim test, an index of behavioral despair [21]. In parallel, the human study examined prospective, within-subject associations between perinatal estradiol levels, the presence of negative affect, and the absence of positive affect (i.e., our operational definition of anhedonia) among women with and without a history of PPD. In the first animal experiment, we hypothesized that, compared to baseline, both low- and high-dose estradiol withdrawal would be associated with an increased threshold for lateral hypothalamic self-stimulation behavior, a behavioral indicator of anhedonia [19,20]. In the second animal experiment, we expected that estradiol withdrawal would be associated with increased immobility and reduced swimming, indicators of behavioral despair [21]. Based on prior experimental research [9], we hypothesized that human perinatal estradiol levels would be negatively correlated with self-reported negative affect and positively correlated with self-reported positive affect in individuals with a history of PPD but not in never-depressed control women.

2. Methods and Materials

2.1. Rat Experiment 1: Influence of Estradiol Administration on Anhedonia

2.1.1. Subjects—Ten-week-old¹ female Sprague-Dawley rats (Harlan, Indianapolis) were maintained on a 12-hour light/12-hour dark cycle at a room temperature of $22.0 \pm 0.2^\circ \text{C}$. Rat chow (Harlan Teklad Global Rodent Diet) and tap water were available *ad libitum*.

¹Although there is no standard definition of rat adolescence or adulthood, female sexual maturation is defined by vaginal opening, first ovulation (which marks the initiation of regular estrus cycling), and mating, which occurs at 5, 6, and 7 weeks, respectively.

2.1.2. Surgical Procedures—All surgeries were performed using an aseptic tip technique, sterile instruments, surgeon's mask, and lab gloves. Bipolar stimulating electrodes were chronically implanted into the medial forebrain bundle at the level of the lateral hypothalamus while the animals were under an Equithesin®-like anesthetic (composed of 0.97 g pentobarbital sodium and 4.25 g chloral hydrate/100 ml distilled water; 0.33 ml/100 g body wt; University of Iowa Hospital Pharmacy, Iowa City, IA). The lateral hypothalamus was chosen based on its reliability in supporting self-stimulation behavior [23]. Rats were placed in a Kopf stereotaxic instrument and the head was leveled between bregma and lambda. The electrode was implanted in the lateral hypothalamus 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. Electrode placement was immediately followed by bilateral ovariectomy while animals were still under anesthesia.

Bilateral ovariectomy was performed on all animals. 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 10 days prior to the first operant conditioning training session.

At the conclusion of the study, two randomly selected rats from each group 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- μ m intervals throughout the hypothalamus. The sections were mounted on slides, stained with cresyl violet solution, and examined by light microscopy. Slices were evaluated for proper electrode placement in the lateral hypothalamus based on Paxinos and Watson [24]. Electrode placement was within the lateral hypothalamus for all animals evaluated.

2.1.3. Hormone Manipulation—Following bilateral ovariectomy, electrode implantation, and operant conditioning, rats were randomized to the experimental or control group. The experimental group received daily injections of vehicle only on days 1-5, 25 μ g of 17 β -estradiol (low-dose) on days 6-10, vehicle only on days 11-15, 50 μ g of 17 β -estradiol (high dose) on days 16-20, and vehicle only on days 21-25. Control rats received daily vehicle-only injections on days 1-25. The first day of injections occurred on the first day of behavioral testing. Estradiol was obtained from Sigma-Aldrich, St. Louis.

2.1.3. Lateral Hypothalamic Self Stimulation—All apparatus and procedures were identical to those described by Grippio et al. [25]. Following a 10-day recovery from surgery, rats ($N=25$) 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 association between lever pressing and current-pulse delivery was trained on two consecutive days. Rats that did not achieve at least 50 responses per minute (RPM) at 250 μ A by the second day of training ($n=9$), either because of a lack of response or marked motor effects in response to the stimulation, were eliminated from the study prior to randomization. For one rat in the experimental group, the dental cement holding the electrode in place came free from the skull after randomization, and the animal was promptly euthanized. Thus, 15 rats were assigned to one of the two groups, and 14 (i.e., 7 in each group) completed the data collection.

Testing was initiated on the first day following training and was conducted daily during the hormone administration protocol. After establishing consistent response rates, current-response curves were determined for each rat by using a curve-shift paradigm. Baseline lateral-hypothalamic self-stimulation 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 25 μ A decrements, and the rats were allowed to respond for one minute at each current intensity.

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” (ECu50), and 3) minimum rate of responding. Anhedonia was operationally defined as an increase in ECu50 relative to baseline, with no significant reduction in the maximum RPM.

2.1.4. Data Analysis—ECu50 results from the second to the fifth day 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 [26].

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 $\times$ treatment interaction. Significant effects were followed by *t* tests. A family-wise significance level of $p < .05$ was used. Statistical testing was conducted using SPSS and Sigma Plot statistical packages.

2.2. Rat Experiment 2: Influence of Estradiol on Behavioral Despair

2.2.1. Subjects—A separate group of ten-week-old female Sprague-Dawley rats (Harlan, Indianapolis) were maintained on a 12-hour light/12-hour dark cycle at a room temperature of $22.0 \pm 0.2^\circ$ C. Rat chow (Harlan Teklad Global Rodent Diet) and tap water were available *ad libitum*.

2.2.2. Ovariectomy—All surgeries were performed using an aseptic tip technique, sterile instruments, surgeon’s mask, and lab gloves. As in Experiment 1, bilateral ovariectomy was performed on all animals under an Equithesin®-like anesthetic (composed of 0.97 g pentobarbital sodium and 4.25 g chloral hydrate/100 ml distilled water; 0.33 ml/100 g body wt; University of Iowa Hospital Pharmacy, Iowa City, IA). 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.

2.1.3. Hormone Manipulation—Following bilateral ovariectomy, rats were randomized to the experimental or control group. Ten days after ovariectomy, rats were randomized to the experimental or control group. The experimental group ($n=8$) received daily injections of 50 μ g of 17 β -estradiol (Sigma-Aldrich, St. Louis) on days 1-5 and vehicle only (i.e., safflower oil) on days 6-8. The control group ($n=8$) received daily vehicle-only injections on days 1-8.

2.1.3. Forced Swim Test—The forced swim test [21] was administered on day 8 (i.e., the third day of hormone withdrawal). During the test, rats were placed in a cylindrical container of 24-degree-celcius water at least 30-cm deep that rose to a height no less than 15-cm from the top of the container, which prevented the rats from touching the bottom or escaping. Rats were closely monitored and videotaped during the test.

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 rat was immobile, immobility was coded “1” whereas other behaviors (i.e., swimming and climbing) were coded “0.” The same convention was followed for coding swimming and climbing behaviors. Thus, three separate variables (i.e., swimming, climbing, and immobility) were coded and analyzed during the test, and the score for all three variables combined was 60. For example, an animal that spent half the time immobile, half the time swimming, and none of the time climbing would have a score of 30 for immobility, 30 for swimming, and 0 for climbing, whereas an animal that spent all of the time immobile would have a score of 60 for immobility, 0 for swimming, and 0 for climbing. Behavioral despair was operationally defined as greater immobility and less swimming and climbing.

2.1.4. Data Analysis—Determination of group differences in swimming, immobility, and climbing behaviors was made by *t*-tests.

2.3. Human Study: Estradiol and Affect in Perinatal Women with and without a History of PPD

2.3.1. Subjects—Twenty-two euthymic, multiparous women, ages 18-40, in the third trimester of a singleton pregnancy, either with ($n=10$) or without ($n=12$) a history of PPD following a prior pregnancy, were recruited from the University of Iowa Hospitals and Clinics obstetrics and gynecology clinic. Women in the “high-risk” group had a history of DSM-IV major depressive disorder with postpartum onset (i.e., occurring within one month postpartum) following a past pregnancy. Women in the control group had no current or past history of DSM-IV mood disorders, despite having given birth before. Exclusion criteria for both groups were current mood disorder, hormone therapy, and use of medications. Two women in the high-risk group were excluded from the study following enrollment: the first woman dropped out of the study after the baseline assessment and did not collect any saliva samples; the second was depressed at the baseline session and therefore did not meet inclusion criteria for the current study. On average, participants were young (mean age = 30.2 years, SD = 3.3 years), married (85%), white (95%), and non-Hispanic (100%), and there were no demographic differences between groups. Baseline psychiatric diagnoses and mood symptoms are summarized in Supplementary Appendix B.

2.3.2. Procedures—A baseline assessment was conducted during the third trimester to confirm inclusion/exclusion criteria and assess depressive symptoms. Participants collected 1-mL saliva samples and made mood ratings each morning within 30 minutes of waking starting 15 days before the expected date of delivery and continuing until 10 days following delivery. Saliva samples were stored at or below -20°C, and Enzyme-Linked ImmunoSorbent Assay (ELISA) was used to determine estradiol levels. A follow-up assessment was conducted approximately one month following delivery to assess depressive symptoms.

2.3.3. Psychiatric and Behavioral Measures—The Structured Clinical Interview for DSM-IV-TR Axis-I Disorders (SCID) [27] was administered at baseline to examine past and present psychopathology. To assess for the onset of PPD within the first postpartum month,

the SCID mood disorders module was administered approximately four weeks following delivery. Self-reported mood symptoms were assessed at baseline Inventory of Depression and Anxiety Symptoms (IDAS) [28]. Daily mood symptoms were assessed with the Positive and Negative Affect Schedule (PANAS) [29] each morning for the duration of the study.

2.3.4. Salivary Estradiol Assay—Human salivary estradiol was measured by the use of a high-sensitivity immunoassay kit (Salimetrics, State College, PA), which employed a microtitre plate coated with rabbit antibodies to estradiol. Specificity of the antiserum was tested with numerous steroids and only estriol and estrone exhibited any crossreaction (0.234% and 1.276%, respectively). The lower limit of sensitivity for estradiol detection was determined to be 0.1 pg/ml. Intra- and inter-assay coefficients of variation averaged 7.1% and 7.5%, respectively. Frozen saliva samples were thawed and centrifuged at $1500 \times g$ for 15 minutes on the day of assay to remove mucins and particulate matter prior to assay.

2.3.5. Human Data Analysis—Group differences in the presence of psychiatric diagnoses were examined using chi square analyses. Differences in IDAS scores were examined using t tests. Meta-analytic techniques based on the Stouffer method [30] were used to examine the association between hormones and mood symptoms. This statistical approach is similar to that described in previous studies of daily hormone and mood assessments [31–33] and has the advantage of providing readily interpretable effect sizes for associations among variables of interest. 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 [34]. 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 [34]. Thus, the results represent combined correlation effect sizes and significance levels from within-subject analyses. A significance level of $p < .05$ was used. Finally, 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.

3. Results

3.1. Rat Studies

3.1.1. Experiment 1: Influence of Estradiol Administration on Anhedonia—As shown in Supplementary Appendix A, ECu50 declined significantly on the second day of hormone administration ($t = -2.54$, $p = .04$), whereas testing on the first day of the high-dose estradiol administration yielded no significant difference from the day before ($t = -0.55$, $p = .61$). Thus, the results supported our *a priori* decision to exclude the first day of each hormone phase from subsequent analyses.

When rats receiving estradiol treatment and withdrawal (i.e., the experimental group) were compared to ovariectomized control rats that received vehicle only injections, there was a significant group $\times$ condition interaction ($F(2,25) = 4.79$, $p = .02$). Means for each condition are displayed in Figure 1. Within the experimental group, 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 2A). Table 1 displays the curve parameters for the current-response curves shown in Figure 2A. During withdrawal, a parallel rightward shift was observed in the current-response function of the experimental group compared with estradiol

administration. Figure 2B presents the mean ECu50 responses during estradiol administration and withdrawal. Although there was a significant difference in ECu50 between the estradiol administration and withdrawal conditions, neither the maximum nor minimum responses per minute differed between conditions.

3.1.2. Rat Experiment 2: Influence of Estradiol on Behavioral Despair—

Behavioral despair was measured using the forced swim test in a group of rats following estradiol withdrawal and a group of ovariectomized controls. Rats in the estradiol withdrawal group showed significantly greater immobility ($t=2.26$, $p=.02$) and less swimming ($t=-2.26$, $p=.02$) than rats in the control group (Figure 3), indicative of behavioral despair. Rats exhibited very little climbing in both the experimental $M(SD) = 0.13 (0.35)$ and control groups $M(SD) = 0.14 (0.38)$, and there was no difference between groups ($t=-0.10$, $p=.93$).

3.2. Human Study

Estradiol levels and mood symptoms were measured daily starting in the third trimester of pregnancy through day 10 postpartum in two groups: 1) women with a history of PPD following a prior delivery (at “high-risk” for recurrence), and 2) women without a history of depression despite having given birth in the past. Figure 4 shows the association between mood and estradiol levels in the high-risk women who developed PPD, the high-risk women who did not develop PPD, and the controls. Contrary to our hypothesis, there was no significant association between estradiol and negative affect in the women with a history of PPD ($r=-0.06$, $p=.19$) or the controls ($r=-0.05$, $p=.24$). However, the four high-risk women who were diagnosed with PPD at 4-weeks postpartum showed a negative correlation between estradiol and negative affect ($r=-0.34$, $p<.001$). Also contrary to our 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 women with a history of PPD ($r=-0.07$, $p=.18$) or within the subset of women who developed PPD ($r=-0.01$, $p=.73$).

4. Discussion

The current research provided an integrative, cross-species examination of the possible endocrine mechanisms of PPD; the translational nature of the results supports the role of estradiol withdrawal in postpartum mood symptomatology. In rodents, we showed that 1) both low- and high-dose estradiol withdrawal precipitates anhedonia and 2) high-dose estradiol withdrawal following short-term exogenous administration results in behavioral despair. In a parallel, albeit naturalistic, study of human females, we showed that estradiol levels on the days surrounding delivery are negatively correlated with mood symptoms in women who developed PPD by one month postpartum. Despite inherent differences in the study designs, the rat and human studies help clarify the role of estradiol in the pathophysiology of PPD.

4.1. Discussion of Rat Results

As hypothesized, results of experiment 1 suggest that supraphysiologic levels of estradiol administration over a period of five days followed by five days of hormone withdrawal (precipitated by vehicle administration) is sufficient to produce “anhedonia” in rats. The absence of differences in motor behavior between the hormone administration and withdrawal conditions suggests that the change in ECu50 reflects a change in hedonic drive specifically rather than a change in motor behavior in general [35]. Prior research suggests that estrogen receptor-mediated effects on dopaminergic neurons in the ventral tegmental area impact affective and motivational functions [36] and thus could be a potential

mechanism by which estradiol withdrawal influenced lateral-hypothalamic self-stimulation in the current study. Results of this study also supplement findings of Bless et al. [35], which suggested that estradiol exerts the strongest effects on behavior 24 hours after administration. In experiment 1, significant behavioral effects were seen 24 hours following the administration of estradiol and did not appear to diminish during the five days rats were tested (as shown in Appendix A).

As hypothesized, estradiol withdrawal was associated with increased immobility and decreased swimming during the forced swim test. These results were consistent with previous PPD rat models that showed withdrawal from high levels of estradiol and that relatively low levels of progesterone (consistent with the rat peripartum hormonal milieu) resulted in *increased* behavioral despair [6,7]. In addition, prior research demonstrated that postpartum rats showed greater immobility than pregnant rats [37], an affect that was mediated by hippocampal progesterin and 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP). Additional rat studies have implicated adrenal and brain derived neurosteroids in perinatal depression-like behavior [38-41]. In contrast, a recent PPD rat model that involved withdrawal from high levels of both estradiol and progesterone (consistent with the human peripartum hormonal milieu) demonstrated *reduced* behavioral despair during the forced swim test [8]. Although estradiol withdrawal does not fully model the complex hormonal milieu of human parturition, the presence of both behavioral despair and anhedonia following estradiol withdrawal in the current study supports its utility as a model of reproductive-related affective dysfunction.

4.2. Discussion of Human Results

Results of our study are consistent with previous studies demonstrating that those with a history of PPD are at increased risk for subsequent episodes [42]. Indeed, 50% of the high-risk women developed PPD during this study, whereas none of the control women developed PPD. Contrary to our hypothesis, estradiol withdrawal was not associated with negative affect in either the high-risk or control group. However, there was a significant negative association between estradiol and negative affect in the high-risk women who developed PPD. Notably, Figure 4A shows that negative mood was relative low at the end of pregnancy and began increasing before the drop in estradiol at delivery, which suggests that estradiol withdrawal did not necessarily precipitate the onset of negative mood symptoms. The correlation between estradiol and negative affect may have been spurious given that there was a net decrease in estradiol for all women in the study, and negative mood increased in the subgroup that developed PPD. Alternatively, this subgroup may be differentially sensitive to the effects of estradiol, both increases and decreases. Indeed, perinatal estradiol trajectories were highly consistent across those who did and did not develop PPD, which suggests that women who demonstrate an association between negative affect and estradiol may be psychologically vulnerable to the effects of normative hormone changes. Previous studies have identified similar “sensitive” subgroups of women who experience mood symptoms in response to changes in ovarian hormone levels [9,43,44]. Although the naturalistic, and therefore correlational, nature of the current study precludes conclusive evidence of causality, the results are strikingly consistent with the experimental results of Bloch et al. [9], which demonstrated that both increases and decreases in ovarian hormone levels precipitated negative mood symptoms in sensitive individuals. Whether the current association was spurious or indicative of an underlying vulnerability in the women who developed PPD, the coupling of estradiol and negative mood levels predicted the onset of PPD and therefore warrants further investigation.

Also contrary to our hypothesis, controls showed a negative association between estradiol levels and positive affect. Although most studies examine negative mood during the postpartum, a previous study of positive mood states demonstrated that healthy women

report high levels of happiness following childbirth [45]. Interestingly, the high-risk women did not experience the same increase in positive affect. Low positive affect is associated with depression, and thus, the lack of increased positive affect following delivery may have contributed to depression vulnerability in the high-risk group.

4.3. Integration and Future Directions

The rodent and human studies provided a cross-species examination of the role of estradiol in PPD. Estradiol withdrawal was associated with behavioral despair in the rats, and the women who had developed PPD by one month postpartum showed a prospective, negative association between perinatal estradiol levels and negative affect. However, estradiol withdrawal was not associated with increased negative mood symptoms in all women, or even all of the high-risk women, suggesting that the complex reproductive hormonal milieu may not be well modeled by estradiol withdrawal alone. Perhaps a more important problem in reconciling the rodent and human data is the diagnostic heterogeneity within PPD. The PPD diagnosis captures depressive episodes that result from either the psychosocial stressors of childbirth, the changing ovarian hormone levels that occur during pregnancy and delivery, or a combination of the two. Moreover, PPD susceptibility in humans, wherein only some develop the disorder, is not well modeled by the relative uniformity of the response to estradiol withdrawal alone in rats. Thus, to serve as a true PPD analog, future rat models should account for both the psychosocial stressors and changing hormonal milieu attendant to childbirth, including changes in both estradiol and progesterone. Alternatively, to isolate the role of changing ovarian hormone in PPD, future human studies should build on the results of Bloch et al.'s experimental hormone challenge paradigm [9] by examining neurobiological mechanisms by which hormones influence depressive symptoms.

A second inconsistency between the rodent and human findings was related to anhedonia. Estradiol withdrawal precipitated anhedonia in rats but not in humans. One possible explanation is that self-reported anhedonia is not a unitary construct and may not be related to the behavioral reward paradigm captured by lateral-hypothalamic self-stimulation. Another explanation is that the combination of estradiol and progesterone withdrawal that characterizes human postpartum period may not result in anhedonia. Future studies could address this question by examining the effects of estradiol on anhedonic behaviors rather than self-reported anhedonia in women.

Finally, the simulation of pregnancy through hormone manipulation in the rat experiments may have also been a source of inconsistencies across the rodent and human results. A more face valid, albeit less rigorously controlled, model would include pregnant rats and a normally cycling, intact control group. In both naturalistic and experimental designs, future studies should directly assess circulating hormone levels in the rats to determine the extent to which behavior change is actually dependent on changing hormone levels.

Despite certain weaknesses, the current study also had several notable strengths. First, the current study is the first to provide *naturalistic* evidence of a within subject association between endogenous estradiol levels and depressive symptoms in susceptible women. In addition, this is the first study to examine the role of ovarian hormones in PPD-like behavior across species. Additional strengths include the daily sampling of hormone levels and depressive symptoms over an extended period of time spanning the end of pregnancy, delivery, and the immediate postpartum period; establishment of the high-risk group by limiting the definition of past PPD to a major depressive episode with an onset within four weeks postpartum; the use of well-established psychiatric assessments to examine depressive symptoms; the abbreviated time course of hormone administration in the rat model; and the use of gold-standard animal models of behavioral despair and anhedonia.

Future studies using the proposed estradiol withdrawal rat model of PPD should examine the factors that modify the influence of ovarian hormones on behavior. Whether estradiol administration, antidepressant treatment, or a combination treatment provides the largest reduction in hormone-precipitated anhedonic behavior should be examined. 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.

In conclusion, the results of the animal and human studies were complementary and provided cross-species evidence of the role of estradiol in postpartum depressive symptoms. Rapid estradiol withdrawal was associated with increased anhedonia and behavioral despair in rats. In contrast, a subgroup of high risk women exhibited increasing depressive symptoms prior to ovarian hormone withdrawal following delivery, suggesting that both increases and decreases in ovarian hormones surrounding delivery may contribute to PPD in susceptible women. 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 ovarian hormones.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Estradiol withdrawal was associated with behavioral despair and anhedonia in rodents.
- Perinatal estradiol was not associated with negative affect in all women.
- Estradiol and negative affect were positively correlated in women with incident PPD.
- Rodent and human data suggest estradiol affects perinatal depressive symptoms.

Figure 1. ECu50 data for animals in the treatment and control groups during baseline, estradiol administration, and withdrawal.

Figure 2.

(A) Mean current-response curves showing a rightward shift in the current-response function during the estradiol withdrawal phase. Data are displayed with a sigmoid curve fit to all of the values generated during testing across animals in the experimental group ($n=7$). (B) Mean + SE effective current (ECu₅₀) values. There was an elevated ECu₅₀ during estradiol withdrawal versus estradiol administration ($t=-3.2$, $p=.007$).

Figure 3. Mean + SE percent time spent immobile, swimming, and climbing during the forced swim test in the treatment and control groups. The experimental group showed significantly greater immobility and less swimming than the control group ($p < .05$).

Figure 4.

Scatterplots depicting salivary estradiol and PANAS Negative Affect scores on the days surrounding delivery (i.e., day 0) in (A) high-risk women who developed PPD ($n=4$), (B) high-risk women who did not develop PPD ($n=4$), and (C) never-depressed control women ($n=12$). Scatterplots depicting salivary estradiol and PANAS Negative Affect scores in the same (D) high-risk women who developed PPD, (E) high risk women who did not develop PPD, and (F) never-depressed control women.

Table 1

Curve parameters defining current-response functions in animals in the experimental group ($n=7$) during estradiol administration and withdrawal.

	Estradiol Administration		Withdrawal		<i>t</i>
	<i>M</i>	(<i>SD</i>)	<i>M</i>	(<i>SD</i>)	
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

**
 $p=0.007$