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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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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.

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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.

1. Introduction

Postpartum depression (PPD) affects 7 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

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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/high 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, 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-old[1] female Sprague–Dawley rats (Harlen, Indianapolis) 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.

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-μm 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 μg of 17-β estradiol (low-dose) on days 6–10, vehicle only on days 11–15, 50 μg 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.

2.1.4. Lateral hypothalamic self stimulation

All apparatus and procedures were identical to those described by Grippo 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 μA 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 μA decrements, and the rats were allowed to respond for 1 min 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.

[1] Although 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.