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Estrogen signaling in the medial amygdala decreases emotional stress responses and obesity in ovariectomized rats

Christina M. Estrada[a], Valentina Ghisays[a], Elizabeth T. Nguyen[b], Jody L. Caldwell[c], Joshua Streicher[c], Matia B. Solomon[a,b,c,*]

[a] Department of Psychology Experimental Psychology Program, University of Cincinnati, Cincinnati, OH 45237, United States
[b] Neuroscience Graduate Program, University of Cincinnati, Cincinnati, OH 45237, United States
[c] Department of Psychiatry and Behavioral Neuroscience, University of Cincinnati, Cincinnati, OH 45237, United States

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ABSTRACT

Declining estradiol (E2), as occurs during menopause, increases risk for obesity and psychopathology (i.e., depression, anxiety). E2 modulates mood and energy homeostasis via binding to estrogen receptors (ER) in the brain. The often comorbid and bidirectional relationship between mood and metabolic disorders suggests shared hormonal and/or brain networks. The medial amygdala (MeA) is abundant in ERs and regulates mood, endocrine, and metabolic stress responses; therefore we tested the hypothesis that E2 in the MeA mitigates emotional and metabolic dysfunction in a rodent model of surgical menopause. Adult female rats were ovariectomized (OVX) and received bilateral implants of E2 or cholesterol micropellets aimed at the MeA. E2-MeA decreased anxiety-like (center entries, center time) and depression-like (immobility) behaviors in the open field and forced swim tests (FST), respectively in ovariectomized rats. E2-MeA also prevented hyperphagia, body weight gain, increased visceral adiposity, and glucose intolerance in ovariectomized rats. E2-MeA decreased caloric efficiency, suggestive of increased energy expenditure. E2-MeA also modulated c-Fos neural activity in amygdalar (central and medial) and hypothalamic (paraventricular and arcuate) brain regions that regulate mood and energy homeostasis in response to the FST, a physically demanding task. Given the shared neural circuitry between mood and body weight regulation, c-Fos expression in discrete brain regions in response to the FST may be due to the psychologically stressful and/or metabolic demands of the task. Together, these findings suggest that the MeA is a critical node for mediating estrogenic effects on mood and energy homeostasis.

1. Introduction

Obesity is a primary national health concern, affecting over one-third of adults and one-sixth of children and adolescents (Flegal et al., 2012; Ogden et al., 2015). Obesity carries many well-known physical health risks (e.g., type 2 diabetes, heart disease, high blood pressure, and nonalcoholic fatty liver disease) and is also linked with psychopathology including depression and anxiety, which occur in one fourth of obesity cases in the United States (Simon et al., 2006; Strine et al., 2008; Zhao et al., 2009). Relative to men, women are disproportionately more likely to suffer from mood and anxiety disorders and are more vulnerable to the deleterious cardiometabolic consequences of obesity (Anderson et al., 2007; Boutelle et al., 2010; Breslau et al., 1995; Kasen et al., 2008; Lee et al., 2005).

Estrogens and especially estradiol (E2) have an important role in maintaining metabolic homeostasis and energy efficiency in both males and females, as well as in modulating mood in females (reviewed in Mauvais-Jarvis et al., 2013; Carr, 2003; Douma et al., 2005; Rogers et al., 2009). During the transition to menopause, as the ovaries reduce the production of estrogens, women tend to gain weight and report increased symptoms of anxiety and depression (Freeman et al., 2004; Sagsöz et al., 2001). These symptoms are also increased when women undergo bilateral oophorectomy (Rocca et al., 2008). Importantly, the weight gain in menopausal women is due to excess fat accumulation in the central-abdominal region (Douma et al., 2005; McClung et al., 2006). Abdominal fat, in particular, is closely linked with obesity-related health conditions (e.g., diabetes, heart attack and stroke) (Carmienke et al., 2013; Fujioka et al., 1987; Kanai et al., 1990; Pouliot et al., 1994) as well as depression and anxiety (Lee et al., 2005; Rivenes et al., 2009; Weber-Hamann et al., 2002; Zhao et al., 2009). Thus, developing effective treatment strategies to offset the increase in central adiposity as women reduce their estrogen output is a high priority.

In rodent models, administering E2 systemically prevents or reverses ovariectomy-induced hyperphagia, body weight gain, fat

* Corresponding author at: Reading Campus, 2170 E. Galbraith Rd./ML-0506, Bldg A Room 131, Reading, OH 45237-1625, United States.
E-mail address: matia.solomon@uc.edu (M.B. Solomon).

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accumulation and glucose intolerance (Asarian and Geary, 2007; McElroy and Wade, 1987; Stubbins et al., 2012). In addition, systemic E2 administration mitigates anxiety-like and depression-like behaviors in ovariectomized rodents (Bowman et al., 2002; Frye et al., 2007; Walf et al., 2009). As such, the clinical findings highlighting behavioral and metabolic phenotypes associated with menopause can be recapitulated at the bench in a rodent model of surgical menopause.

The medial amygdala (MeA) is an important brain area that integrates endocrine, emotional and metabolic responses to stress (Bryzgalova et al., 2008; Ebner et al., 2004; Frye and Walf, 2004; Herman et al., 2004; Solomon et al., 2010). In particular, the posterodorsal region of the medial amygdala (MePD) regulates body weight, energy efficiency, and anxiety-like behaviors (King et al., 1993a; Xu et al., 2015; Spiteri et al., 2010). Following lesions targeted to the MePD in rats, both sexes gain body weight, but the increase is significantly higher in females compared to males (King et al., 1993a, 1999). Sex differences in weight gain are likely due to differential sensitivity to gonadal steroids in the MeA since both androgen and estrogen receptors are expressed in this region (Rasia-Filho et al., 2004). The goal of the present study was to determine whether the MeA is a critical node for estradiol mediated effects on emotionality and energy homeostasis. Female rats were concurrently ovariectomized and received micropellets containing either E2 or cholesterol bilaterally implanted into the brain just dorsal to the MeA. This approach allowed determination as to whether E2 signaling in the MeA prevents the increased emotionality and obesity resulting from ovariectomy. Body weight, food intake, adiposity, caloric efficiency, and glucose levels in response to an intragastric glucose challenge were determined. In addition, open field and forced-swim tests were used to gauge anxiety-like and depression-like behaviors, respectively. Finally, we assessed c-Fos activity in brain regions regulating emotionality and energy homeostasis, including several amygdalar and hypothalamic subnuclei, in response to the forced-swim test. Collectively, the findings demonstrate that within the MeA, E2 decreases emotionality and obesity-related sequelae in ovariectomized rats.

2. Materials and methods

2.1. Subjects

Adult female Long-Evans rats weighing 225–250 g (Harlan, Indianapolis, IN) were singly housed in standard tub cages for at least one wk. before the initiation of the experiments. Rats were maintained in a temperature and humidity-controlled room with a 12:12-h light-dark cycle with Purina rodent lab chow and tap water available ad libitum. The Institutional Animal Care and Use Committee of the University of Cincinnati approved all procedures.

2.2. Stereotaxic surgery and ovariectomy

Prior to surgery, rats were assigned to one of two groups, matched for body weight, anesthetized with intraperitoneal ketamine (87 mg/kg) and xylazine (13 mg/kg) and treated with buprenorphine and meloxicam to reduce pain and gentamicin to prevent infection. They were secured in a David Kopf small animal stereotaxic apparatus, the skull was bared and two small holes were drilled at anteroposterior − 3.0 mm and mediolateral ± 4.0 mm based on coordinates from the Paxinos et al. (2009) Atlas, 6th ed. Construction of the steroid micropellets and surgical procedures were based on previously published methods (Ghosal et al., 2014; Myers and Greenwood-Van Meerveld, 2010). Briefly, undiluted 17β-estradiol (~15μg) (E2) (Sigma-Aldrich, St. Louis, MO) or cholesterol (~15μg) (CHOL) was tamped into a 25-gauge stainless steel cannula that was lowered to 8.9 mm below dura targeting the dorsal margin of the posterior medial amygdala, and the resulting micropellet was than expelled using a stylet. After micropellet deposition, the stylet and cannula were removed. In order to eliminate any confound due to ovarian E2, during the same surgical procedure the rats also underwent bilateral ovariectomy (OVX) where the ovaries were ligated and removed as described previously (Zhu et al., 2013). Animals were allowed at least one wk. recovery from surgical procedures and body weight and overall health (i.e., fur appearance, incision healing, movement, aggressive behavior toward handling) were closely monitored.

3. Experiment 1

3.1. Open field

The open field (1 m × 1 m × 0.5 m, opaque box) was conducted on post-surgical Day 10 and used to assess locomotor, exploratory and anxiety-like behaviors (Hall and Ballachey, 1932). A camera was mounted above the field and testing was conducted under infrared lighting. Each animal was placed into a corner of the field allowed to freely explore the arena for 5 min; for analytical purposes, the field was divided into two domains, center and periphery. The following behaviors were scored: Activity: total distance traveled (in) in the arena and velocity (in/s); Location: time spent in center and number of entries into the center; Exploratory behavior: rearing (i.e., up on hind limbs), Risk-assessment: stretch-attend postures, and 'other behaviors,' including immobility and grooming. One or more of the following observations are typically characterized as an anxiety-like phenotype: reduced exploration (e.g., rearing), hypoactivity, increased risk-assessment, increased freezing, and/or excessive grooming. Increased time spent in the center and increased center entries are purported to reflect a low anxiety-like state. Activity measures (distance traveled and velocity) were recorded and quantified with the Ethovision tracking software system v. 9 (Noldus Information Technology, Inc., Leesburg, VA). All other behaviors were scored by two independent observers, blind to the experimental condition of each animal using the AnnoStar behavioral software system (CleverSys, Inc., Reston, VA).

3.2. Forced-swim test

The modified FST was conducted on the final day of the experiment (Day 16) and adapted from previously published methods from our group (Nguyen et al., 2017; Solomon et al., 2014; Wulsin et al., 2010) and was used to assess depression-like behavior and as a potent stressor to induce c-Fos neural activation. A semi-clear Plexiglas cylinder (45 cm high and 20 cm in diameter) was filled with 31 cm of water (23 ± 2 °C) to prevent the rat from touching the bottom or from escaping; a camera recorded all activity. Each animal was placed in the cylinder for 6 min and then removed, dried and returned to its home cage. Two independent observers (blind to experimental condition) scored FST behavior with the AnnoStar behavioral software system. A depressive-like phenotype in the FST is characterized by increased immobility and decreased latency to immobility, both of which are attenuated with antidepressants (Cryan et al., 2005) or systemic E2 treatment (Rachman et al., 1998). All behavioral testing occurred during the first 4 h of the dark phase. In order to remove chemosensory cues, all behavioral equipment was cleaned between each testing session with 30% ethanol dissolved in water. The time interval between surgery and behavioral testing was the same for all animals to ensure consistent treatment duration on the day of testing.

3.3. Body weight and terminal measures

Body weight and food intake were recorded weekly. 90 min following FST onset, rats were deeply anesthetized with an overdose of sodium pentobarbital and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde (PFA) in 0.1 M PBS pH 7.6. Following perfusion, brains were removed, post-fixed in 4% paraformaldehyde overnight at 4 °C and then placed in 30% sucrose