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The timing and duration of estradiol treatment in a rat model of the perimenopause: Influences on social behavior and the neuromolecular phenotype☆

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ABSTRACT

This study tested the effects of timing and duration of estradiol (E_2) treatment, factors that are clinically relevant to hormone replacement in perimenopausal women, on social behavior and expression of genes in brain regions that regulate these behaviors. Female rats were ovariectomized (OVX) at 1 year of age, roughly equivalent to middle-age in women, and given E_2 or vehicle for different durations (3 or 6 months) and timing (immediately or after a 3-month delay) relative to OVX. Social and ultrasonic vocalization (USV) behaviors were assessed at the 3 and 6 month timepoints, and the rats' brains were then used for gene expression profiling in hypothalamus (supraoptic nucleus, paraventricular nucleus), bed nucleus of the stria terminalis, medial amygdala, and prefrontal cortex using a 48-gene qPCR platform. At the 3-month post-OVX testing period, E_2 treatment significantly decreased the number of frequency-modulated USVs emitted. No effects of hormone were found at the 6-month testing period. There were few effects of timing and duration of E_2 in a test of social preference of a rat given a choice between her same-sex cagemate and a novel conspecific. For gene expression, effects of timing and duration of E_2 were region-specific, with the majority of changes found for genes involved in regulating social behavior such as neuropeptides (*Oxt*, *Oxtr* & *Avp*), neurotransmitters (*Drd1*, *Drd2*, *Htr2a*, *Grin2d* & *Gabbr1),* and steroid hormone receptors (*Esr2*, *Ar*, *Pgr*). These data suggest that the mode of E_2 treatment has specific effects on social behavior and expression of target genes involved in the regulation of these behaviors.

1. Introduction

All women who live long enough will experience menopause during their lifetime, either natural or surgical. The loss of ovarian hormones during menopause is associated with a variety of physiological changes that sometimes impair the quality of life. Hormone replacement therapy (HRT) with estradiol (E_2) is the single most effective treatment for vasomotor symptoms (e.g. hot flashes) and urogenital atrophy, among other health problems (Baber et al., 2016). However, the early termination of the Women's Health Initiative (WHI) due to a small but significant increase in certain adverse incidents in the hormone treatment group led to a dramatic decline in the use of HRT in symptomatic women (Rossouw et al., 2002). Subsequent re-evaluation of the WHI suggested that there may be a critical window of opportunity during which HRT is beneficial (Klaiber et al., 2005; Bhupathiraju and Manson, 2014; Manson et al., 2013; Baber et al., 2016). However, empirical evidence for the critical window hypothesis has been provided by only a few preclinical studies (Gibbs, 2000; Daniel et al., 2006; Garcia et al., 2016, 2017; Yin et al., 2015).

Some of the most problematic symptoms experienced by perimenopausal women are neurobiological, including hot flashes and sleep disturbances. A smaller subset of women experience neurobehavioral changes such as anxiety and depression, sometimes leading to problems in interpersonal relationships and a loss of desire for social interactions (Uguz et al., 2011; Deeks and McCabe, 2004; Lanza di Scalea et al., 2012; Schmidt et al., 2000). Studies in female rodents show that the neural circuits and neurotransmitters involved in the control of social behavior, and the behaviors themselves, are subject to age- and hormone (especially E_2) regulation. With aging, rodents show increased anxiety and decreased social interaction (Salchner et al., 2004; Guan & Dluzen, 1994; Boguszewski & Zagrodzka, 2002). The importance of hormones is provided by evidence that ovariectomy in female rats leads to decreased social interaction independent of age, and E_2 treatment improves social memory and increases social interaction (Hlinák, 1993;

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Tang et al., 2005). Mice lacking either of the nuclear estrogen receptors show deficits in social interaction and social memory (Choleris et al., 2003, 2006; Kavaliers et al., 2004). The neurotransmitter and neuropeptide systems involved in regulating these behaviors are highly sensitive to E_2, including serotoninergic and dopaminergic neurons (Morissette & Di Paolo, 1993; Van De Kar et al., 2002; Bazzett & Becker, 1994), and the nonapeptides oxytocin, vasopressin, and their receptors (Winslow and Insel, 2004; Hrabovszky et al., 1998; Quiñones-Jenab et al., 1997; Axelson and van Leeuwen, 1990; Garcia et al., 2016).

The current study sought to test the critical window hypothesis on the social behavioral phenotype, and to determine how underlying neurotransmitter systems in a defined neural network are altered by differential treatment regimes, using middle-aged rats. Social behaviors were tested using a test of ultrasonic vocalizations (USVs) between cagemates as an index of communicative and affective state (Garcia et al., 2017), followed by a test of social preference (Tang et al., 2005; D'Amato & Moles, 2001; Moles et al., 2007; Hlinák, 1993). The neuromolecular phenotype of these animals was assessed using low-density qPCR arrays of relevant brain regions.

2. Materials and methods

2.1. Animals and husbandry

All animal procedures were conducted under protocols approved by The University of Texas at Austin institutional animal care and use committee and in accordance with The Guide for the Care and Use of Experimental Animals. Middle-aged adult (~12 months, retired breeders) female Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN). Animals were given water and food ad libitum and kept on a 12-h dark cycle (lights on at 0700). Upon arrival animals were pair-housed and allowed to acclimate for 2 weeks prior to surgery, during which time estrous cyclicity was monitored daily by vaginal lavage. Only animals that had a regular 4–5 day cycles were used for the experiment. Then, animals underwent bilateral ovariectomy (OVX) surgery as described in our previous studies (Garcia et al., 2016, 2017). During the surgery, each rat was implanted subcutaneously between the shoulder blades with a Silastic capsule containing either 100% cholesterol (VEH) or 5% 17β-estradiol/95% cholesterol. This replacement regime maintains serum E_2 concentrations at physiological levels for at least 6 months (Garcia et al., 2017; Yin et al., 2015). Three months after the OVX surgery, animals were subjected to a second surgery. Half of the animals had their Silastic capsules checked to verify that they were intact. The other half of the rats had their capsules removed and switched to the opposite treatment (E_2 to vehicle, or vehicle to E_2). This resulted in four groups (Fig. 1), each with an n of 14, subsequently referred to as V6 (6 months vehicle), E6 (6 months E_2), V3/E3 (3 months vehicle, switched to 3 months E_2) and E3/V3 (3 months E_2, switched to 3 months vehicle) to evaluate effects of hormone timing and duration. Each rat was pair-housed with a cagemate of the same treatment group.

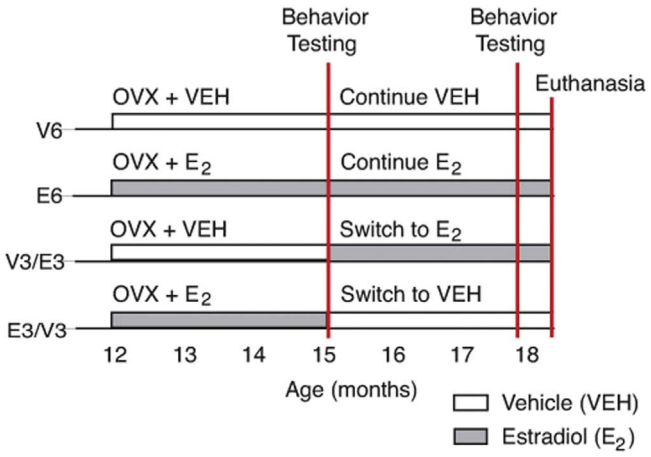

Fig. 1. The rat model is shown. Rat were ~1 year of age at the time of OVX, and received either vehicle (cholesterol, VEH) or 5% 17β-estradiol (E_2) capsule implantation at the time of surgery. Groups 1 & 2 received treatment for a duration of 6-months. Groups 3 & 4 were the "switch" groups that received either VEH or E_2 initially at the time of OVX and were switched to the opposite treatment 3 months later. Behavioral testing occurred twice, once at the end of the 3-month period, and again at the 6-month period. Rats were euthanized one week after the second round of behaviors was completed.

2.2. Behavioral testing

Prior to the start of behavioral testing each rat was separated from her cagemate for one week and the two rats were singly housed. Two rounds of behavioral testing were performed for this study, the first at the end of the first 3-month period, and the second round 3 months later (Fig. 1). This allowed us to examine the effects of timing and duration of hormone replacement therapy on the same animals' behavior.

2.2.1. Ultrasonic vocalization test (USV)

Following a week of separation of the cagemates, USV testing was conducted over 2 consecutive days in four 5-min trials, during which USVs were recorded. Details of this test have been published (Garcia et al., 2017). In brief, on day 1 each rat was habituated to a rectangular Plexiglas apparatus (23 L × 29 W × 40 H cm) for 5 min (Trial 1). On day 2, rats were given three sequential 5-min trials, referred to as Trials 2, 3, and 4. In Trial 2, the apparatus was fitted with a removable plastic perforated grid that bisected the apparatus. The cagemates were placed into the apparatus separated by the grid, allowing them to engage in limited interactions across the grid but no physical contact. For Trial 3, the grid was removed and the cagemates were allowed to freely interact with one another. Video recording of this trial was performed, and recordings used to quantify activity, time interacting, and anogenital investigation. For Trial 4 the cagemates were separated into 2 identical apparati and recorded separately. Upon completion of Trial 4, all animals were housed separately from their cagemates until they completed the social preference test.

UltraSoundGate hardware and software was used to record USVs during all four trials. Saslab Pro (Avisoft, Germany) was optimized so that we were able to use it to automatically detect and quantify calls. We found that all calls fell in the range between 30 and 70 kHz. We used these data to further differentiate calls into frequency modulated (FM), defined as > 9 kHz change in frequency, or non-frequency modulated (NFM; < 9 kHz change). Because we were unable to distinguish which rat was calling when the cagemates were together in the apparatus during Trials 2 and 3, we treated each set of cagemates, both of which were in the same treatment group, as a unit for analysis. Therefore, calls from the separated cagemates during Trials 1 and 4 were summed for analysis to be comparable to results in Trials 2 and 3. Video recordings of USV behavior during Trial 3 were scored by an observer who was blind to the treatment of the animals for time interacting, activity (number of times each animal crossed the center line of the chamber with all four limbs) and anogenital investigation, as published (Garcia et al., 2017).

2.2.2. Social preference test

The day after USV testing, a 2-day test was performed using a 3-chambered apparatus, as published (Garcia et al., 2017). On day 1, one of the cagemates was randomly chosen to be the experimental rat. She was allowed to habituate for 5 min to a Stoelting Plexiglas three-chamber apparatus (100 L × 100 W × 34.5 H cm total), which contained two holding cages in each corner of the side chambers. Once habituation was completed the experimental rat was returned to her home cage, during which time, her cagemate was placed into one of the holding cages, and a novel rat of the same sex, age, and treatment was