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Chronic Estradiol Replacement to Aged Female Rats Reduces Anxiety-like and Depression-like Behavior and Enhances Cognitive Performance

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Summary

Decline in the ovarian steroid, estradiol (E₂), with the menopause transition may influence cognitive and affective processing of older women and there is evidence that hormone replacement therapies (HRTs) with E₂-mimetics may provide benefit in some, but not all, women. The parameters that play a role in determining whether the response to HRTs is positive are of interest. It may be that the likelihood for positive responses is related to the timing of E₂-replacement following E₂ decline. As such, in the present study an animal model was utilized to investigate this. We investigated the effects of long- vs. short-term E₂ replacement by examining cognitive (object placement task), anxiety (open field, mirror maze, light-dark transition task), and depression (forced swim task) behavior of female rats that were ovariectomized (OVX) at middle-age (14 months) or older (19 months) and implanted with E₂-filled implants at the time of surgery or after a delay of 5 months, or OVX at 14 months of age and never replaced with E₂. Rats were tested at 20 months of age. The hypothesis that was tested was that rats would have reduced anxiety and depression behavior and improved cognitive performance with E₂-replacement at ovarian cessation, compared to with a delay in E₂-replacement. Performance in the object placement task was improved in rats that were OVX and then received continuous E₂-replacement, compared to those that were OVX and continuously administered placebo vehicle. In the open field and forced swim task, there was an increase in anti-anxiety and anti-depressive behavior, respectively, among rats that were OVX and then received continuous E₂-replacement, compared to OVX rats administered vehicle or those that experienced a delay in E₂ replacement. In the mirror maze and light-dark transition task, E₂-replacement at OVX, or after a delay, reduced anxiety-like behavior. Thus, E₂ replacement reduced anxiety and depression behavior and improved cognitive performance of aged female rats; however, delay in E₂ treatment influenced whether there were favorable effects of E₂ in some tasks.

Keywords

estrogen; aging; object placement; spatial learning; mood; anxiety; menopause

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1. Introduction

Steroid hormones secreted by the ovaries, such as 17β -estradiol (E_2), can have profound effects on women's physiological and/or psychological function. Physical and psychological effects of E_2 are most evident with ovarian decline in E_2 secretion at menopause. About 75% of women experience, and seek treatment for, negative physical (hot flashes, night sweats, drying of eyes and vaginal mucosa) and/or psychological/quality-of-life (forgetfulness, anxiety, depression, sleeplessness, reduced sex drive) symptoms (Henriques and Dickson, 1992). Some women choose hormone replacement therapies (HRTs) with compounds that act similar to E_2 (E_2 -mimetics) to manage these effects. Furthermore, treatment of postmenopausal women with E_2 -mimetics HRTs improves performance in verbal, visual, and semantic memory tasks (Asthana et al., 2001; Janowsky, 2002; Linzmayer et al., 2001; Nappi et al., 1999; Sherwin, 2003). Despite some support from clinical studies for E_2 to ameliorate negative physical and psychological effects of E_2 decline, the large Women's Health Initiative (WHI) trials were ended early because some women on the most commonly-prescribed HRTs, conjugated equine estrogen and/or progesterone, experienced negative pro-thrombotic (stroke, cardiovascular complications) and/or oncogenic (increased risk of breast, uterine cancer) effects (Anderson et al., 2003; Cuna et al., 2004). Additionally, not all studies find a clear benefit of HRTs in women (Sherwin, 2007), but this may be due to the nature of the therapy utilized, age, and delay between menopause and the onset of HRT.

Little evidence for beneficial effects of E_2 -mimetic therapies on mood or cognitive processes of elderly, post-menopausal women found in the WHI trials suggest that, in addition to age, differences in length of time in an E_2 -deficient state before initiation of E_2 therapy may be an important factor to consider. The average age of women in the WHI trials was 73 years old at recruitment, and few had previously utilized HRT, which is typically initiated at the onset of menopausal symptoms (Gleason et al., 2005; Pinkerton and Henderson, 2005). It may be that the aging system may be more responsive to E_2 if E_2 treatment is initiated with, not after, ovarian decline (i.e. "critical period" hypothesis reviewed in Sherwin, 2007). Less cognitive decline with aging was found among women who initiated E_2 -based treatments at menopause compared to those who were older, had more recent exposure to E_2 and/or longer delay in initiation of E_2 following menopause (MacLennan et al., 2006; Matthews et al., 1999). HRT initiated at menopause reduced risk of Alzheimer's Disease (Zandi et al., 2002). Among women who had received E_2 -based therapies for 2–3 years, better cognitive performance was reported 15 years later, compared to placebo (Bagger et al., 2005). Although these data support the notion of a critical period for beneficial effects of E_2 -replacement, inherent limitations in clinical studies warrant further investigation in animal models to begin to ascertain the parameters of E_2 -therapy that may be most favorable.

There is some evidence from animal studies that supports the critical period hypothesis. An approach that is often utilized to begin to determine functional effects of ovarian cessation (e.g. postmenopausal osteoporosis, psychological changes) is surgical removal of the ovaries (ovariectomy; OVX; Daniel, 2006; Gurkan et al., 1986; Kalu et al., 1989; Kalu, 1991; Walf and Frye, 2006). Chronic E_2 -replacement to middle-aged rats that have been OVX for 6 months was effective in improving cognitive performance when it was paired with injections of E_2 (Markowska and Savonenko, 2002). Aged rats' performance was enhanced when chronic E_2 was administered 3, but not 10, months post-OVX (Gibbs, 2000). Working memory improvement are only observed among 12 or 17 month old rats that were administered chronic E_2 immediately following OVX, and not when E_2 administration was delayed 5 months (Daniel et al., 2006). Furthermore, choline acetyltransferase (ChAT; as a measure of cholinergic function) was increased in the hippocampus, a region important for cognitive and affective behavior, of 10 and 15 month old rats if E_2 was administered at time of OVX, but not 5 months

later (Bohacek et al., 2008). Thus, the duration of E₂ deprivation may be an important factor that influences the nature of the beneficial effects of E₂-replacement.

In the present study, an animal model was utilized to investigate the hypothesis that rats would have improved cognitive performance and affective behavior with E₂-replacement at ovarian cessation, compared to with a delay in E₂-replacement. Effects of E₂-replacement was examined for cognitive, anxiety-like, and depression-like behavior of female rats that were OVX at middle-age (14 months) or older (19 months) and implanted with E₂-filled implants at the time of surgery, or after a delay of 5 months, or rats OVX at 14 months and administered a vehicle/placebo implant.

2. Methods

The methods utilized were pre-approved by the Institutional Animal Care and Use Committee at the University of Albany- SUNY.

2.1. Animals and Housing

Adult (14 months old) female Long-Evans rats (N=23) were obtained from in-house breeding from rats originally obtained from Taconic Farms (Germantown, NY). Rats were experimentally-naïve, and had all been breeders from our colony until they began to show acyclicity and reduced fertility and fecundity (at approximately 12–14 months of age). Rats were group-housed (3–4 per cage) in polycarbonate cages (45 × 24 × 21 cm) in a temperature-controlled room (21 ± 1 °C) in the core Laboratory Animal Care Facility in The Life Sciences Building at The University at Albany-SUNY. Rats lived on a 12/12-hour reversed-light cycle (with lights off at 8:00 am) and continuous access to rodent chow and tap water in their home cages.

2.2. Surgery and E₂-replacement

At 14 months of age, rats were OVX under xylazine (12 mg/kg IP) and ketaset (80 mg/kg IP) anesthesia. Because there can be great variability in levels of ovarian steroids that are circulating among older rats as they progress through reproductive senescence, rats were OVX and replaced back with E₂ in a regimen that produces known circulating E₂ levels, or vehicle, to obviate this potential for ambiguity in interpreting the results based upon variations in ovarian function. Rats were implanted with a silastic capsule (0.062 i.d., 0.125 o.d.; 10 mm/100 g body weight; left blank or filled with 17β-E₂; Steraloids, Inc., Newport, RI) between the muscle wall and skin. These types of silastic capsules that are filled with E₂ produce high physiological plasma levels of E₂ (~48.9±8.0 pg/ml; Vongher and Frye, 1999). There were three experimental groups. Some rats were OVX at 14 months and administered an empty implant and then sham surgerized at 19 months and administered an empty implant (OVX + blank/sham + blank; n=7). Another group of rats was OVX at 14 months and administered an E₂ implant and sham surgerized at 19 months and administered an E₂ implant (OVX + E₂/sham + E₂; n=6). Other rats were OVX at 14 months and administered an empty implant and sham surgerized at 19 months and administered an E₂ implant (OVX + blank/sham + E₂; n=9).

2.3. Behavioral Testing

At 20 months of age, rats were tested in the following behavioral tasks. Rats were tested in one task per day by an experimenter blind to the hypothesized outcome of the study. Data were simultaneously collected by the experimenter and a video-tracking system (Any-maze; Stoelting, Inc).

2.3.1. Object Placement—The object placement task was done in accordance with previously published methods (Frye et al., 2007). Briefly, rats were placed in the open field

(76 × 57 × 35 cm), which had two identical objects in opposite corners on the same side of the chamber (NW and NE position). Rats explored these objects during 3 min training trials. Rats that did not explore objects during training were excluded from data analyses. Rats equally explored (mean secs ± sem) the object in the NW corner (on training trial 1 and 2, respectively: OVX + E₂/sham + E₂: 5.1 ± 1.9 and 3.5 ± 0.9; OVX + blank/sham + E₂: 2.4 ± 0.4 and 2.4 ± 0.8; OVX + blank/sham + blank: 2.3 ± 0.9 and 2.2 ± 0.3) and the NE corner (on training trial 1 and 2, respectively: OVX + E₂/sham + E₂: 2.5 ± 0.9 and 2.5 ± 0.9; OVX + blank/sham + E₂: 2.7 ± 0.5 and 3.1 ± 0.9; OVX + blank/sham + blank: 2.5 ± 0.7 and 1.3 ± 0.4). Four hours later, during the testing trials, one of the objects was moved to the opposite corner of the chamber (SE or SW, counterbalanced across groups). The percentage of time rats spent exploring the displaced object, as a function of the total time spent by the rat exploring both objects, was utilized as an index of performance. Data from rats' performance in this task on two test sessions with different target objects is analyzed, with little evidence for test decay effects (see Frye et al., 2007; Luine et al., 2003; Paris & Frye, 2008).

2.3.2. Open Field—The open field (76 × 57 × 35 cm) has a 48-square grid floor (6 × 8 squares, 9.5 cm/side), and an overhead light illuminating the central squares (all but the 24 perimeter squares are considered central). The number of central squares entered during a 5 min period was recorded and used as an index of anti-anxiety behavior (Frye et al., 2000).

2.3.3. Mirror Chamber Task—Rats were placed inside a non-mirrored alleyway (57 × 12.5 × 35 cm) connected to an open field chamber (76 × 57 × 35 cm) with four mirrored walls using modified methods (Frye et al., 2007b). The time spent in the mirrored part of the chamber in this 5-minute test is utilized as a measure of anti-anxiety behavior.

2.3.4. Light Dark Transition Task—As previously described, rats were placed on the side of a two-chambered box (30 × 40 × 40 cm) with white walls and floor and illuminated by a 40-watt light from above; the other side of the box was painted black and had a lid so it was not illuminated (Walf and Frye, 2005). The time spent on the light side of this chamber during five minutes compared to the dark side was recorded and used as an index of anti-anxiety behavior.

2.3.5. Forced Swim Test—Rats were tested in the forced swim test as previously described (Frye and Walf, 2002). For this test, rats were placed in cylindrical container (50 × 20 cm, Stoelting, Inc) filled with 30 cm of 30°C water. The amount of time the rats spend struggling, swimming, and immobile are recorded in a ten minute test. The time spent immobile is considered an index of depression-like behavior in rodents.

2.3.6. Lordosis testing—Sexual behavior, characterized by the lordosis posture, of female rats was utilized as a positive control, E₂-sensitive measure in the present study. A subset of rats (n=8) were tested for lordosis frequency (lordosis quotient; LQ) in a Plexiglas chamber (50 × 25 × 30 cm) using previously described methods (Frye et al., 1998; Walf and Frye 2005).

2.4. Statistical Analyses

Data were analyzed with repeated measures one-way Analyses of Variance (ANOVAs) for performance in the object recognition task, in which rats were tested twice, or between-subjects one-way ANOVAs for all other tasks, which rats were tested on a single occasion. Where appropriate, group differences were ascertained with Fisher's LSD *post hoc* tests. A p value of ≤0.05 was considered statistically significant. Logistical problems with data collection precluded the inclusion of results from three experimental rats in the forced swim test (1 per group).

3. Results

3.1. Continuous E₂-replacement to rats OVX at 14 months improves object placement performance

In the object placement task, hormone condition ($F_{(2,16)}=5.26$, $p=0.02$; Figure 1), but not test session ($p=0.29$), had a significant effect on the percentage of time spent exploring the displaced object. *Post hoc* tests revealed that rats that were OVX and received continuous E₂ (E₂ implants at 14 months and 19 months) had significantly better performance in this task, than did rats from any other hormone condition.

3.2. E₂-replacement to OVX rats at 14 and/or 19 months reduces anxiety behavior

In the open field, hormone condition had a significant effect on the number of central entries made ($F_{(2,19)}=3.70$, $p=0.02$; Figure 2). *Post hoc* tests revealed that rats that were OVX and administered E₂ at 14 months entered significantly more central squares than did rats that were OVX at 14 months and never administered E₂ or were administered E₂ at 19 months. Rats administered E₂ at 19 months entered more central squares than did rats that were never administered E₂.

In the mirror maze, hormone condition had a significant effect on the duration of time spent in the mirrored chamber ($F_{(2,19)}=5.94$, $p<0.01$; Figure 3). *Post hoc* tests revealed that rats that were OVX and administered E₂ at 14 or 19 months spent more time in the mirrored chamber than did rats that were OVX at 14 months and did not receive any E₂-replacement.

In the light-dark transition task, hormone condition tended to influence the duration of time spent on light side of chamber ($F_{(2,19)}=3.44$, $p=0.05$; Figure 4). Compared to rats that were OVX and not E₂-replaced, rats administered E₂ spent more time on the light side of the chamber, irrespective of the timing of replacement.

3.3. E₂-replacement to OVX rats at 14 and/or 19 months reduces depression behavior

In the forced swim task, there were main effects of condition for time spent swimming ($F_{(2,16)}=5.47$, $p=0.02$) and struggling ($F_{(2,16)}=14.57$, $p<0.01$), and immobile ($F_{(2,16)}=7.06$, $p<0.01$; Figure 5). *Post hoc* tests revealed that rats that were OVX at 14 months and administered E₂ spent less time swimming and more time struggling than did rats that were OVX at 14 months and administered E₂ at 19 months. Rats that were OVX at 14 months and administered E₂ at 19 months spent less time swimming than did rats that were OVX and not E₂-replaced.

3.4. E₂-replacement to OVX rats at 14 and/or 19 months increases lordosis

Hormone condition influenced lordosis quotients of rats ($F_{(2,5)}=101.25$, $p<0.01$; Figure 6). Rats administered E₂ at OVX or 5 months later had higher lordosis quotients than did rats that were OVX at 14 months of age and not E₂-replaced.

4. Discussion

The hypothesis that, a delay in E₂-replacement would influence cognitive and affective behavior, was partially supported. In the object placement task, E₂ was effective at enhancing performance of rats when it was administered at the time of OVX, and not after a 5 month delay. However, a slightly different pattern emerged for affective behavior. In the open field, continuous E₂-replacement that was initiated at OVX at 14 months of age increased central entries. In the mirror maze and light-dark transition task, E₂-replacement, irrespective of when it was initiated, increased time spent in the mirror and in the light, respectively. In the forced swim test, time spent immobile and struggling, were significantly decreased and increased,

respectively, among rats that were administered E₂ at 14 months of age, compared to rats that had a delay in E₂-replacement or were not administered E₂. All rats responded to E₂ similarly with increased lordosis quotients, verifying that the E₂ regimen utilized was producing physiologically-relevant behavioral effects. These data support the idea that timing of E₂-replacement following its decline may alter functional responses to E₂; albeit, this may depend upon task.

The present results confirm and extend previously published work supporting the notion that beneficial effects of E₂ in cognitive tasks may be most evident when E₂ is initiated at E₂ decline. In the present study, rats that had improved performance above what is typically considered chance (50%) in the object placement task were those with continuous E₂ from 14 to 20 months of age. Rats administered chronic E₂ immediately after OVX or 3, but not 10, months later have improved performance in a delayed-matching-to-sample task (Gibbs, 2000). Enhancements in working memory are only observed among 12 or 17 month old OVX rats that were administered chronic E₂ at surgery, but not 5 months later (Daniel et al., 2006). Similarly, ChAT levels were increased in the hippocampus of 10 and 15 month old rats that were administered E₂ at OVX, but not 5 months later (Bohacek et al., 2008). As such, the present data extend these previous reports to include results using another cognitive task that relies heavily on hippocampal function, the object placement task, in aged female rats. Systemic and/or intra-hippocampal E₂ to young OVX rodents enhances hippocampal structure and function, as well as performance in many hippocampally-mediated cognitive tasks (Daniel et al., 1997; Daniel and Dohanich, 2001; Frye, 2001; Frye and Rhodes, 2002; Gibbs, 1999; Gould et al., 1990; Packard and Teather, 1997; Woolley et al., 1997). Thus, the hippocampus may be sensitive to E₂ decline and replacement in the young and aged.

The present study confirms previous reports on the role of E₂ for hippocampus-mediated affective behavior. Young, proestrous rats have less anxiety-like and depression-like behavior than do their diestrous counterparts coincident with higher circulating ovarian steroids (Frye et al., 2000; Frye and Walf, 2002). OVX of young rodents increases anxiety-like and depression-like behavior, and this effect can be reversed with replacement of E₂ in a regimen that mimics proestrous E₂ levels (Estrada-Camarena et al., 2003; Marcondes et al., 2001; Mora et al., 1996; Rachman et al., 1998; reviewed in Walf and Frye, 2006). Withdrawal from chronically-sustained E₂ levels in young OVX rats increases depression-like behavior in the forced swim test (Galea et al., 2001). The long-term consequences of OVX on affective behavior among rats is less well-studied, but, in one study, an increase in anxiety-like behavior was found in rats that were OVX for 12, versus 3, weeks (Picazo et al., 2006). In the present study, rats that were OVX for 5 months and then replaced with E₂ did not demonstrate reduced anxiety-like behavior in the open field as did rats that were OVX and immediately replaced back with E₂. Although a similar pattern was observed for immobility and struggling in the forced swim test, the mirror maze and light-dark transition task were less sensitive to the effects of delay in E₂. Rats were treated with E₂ for 1 or 6 months and this may have altered the responses in these tasks, in addition to the effects of delay in treatment, and this should be investigated more closely in future studies. This is a topic of interest because women are typically prescribed a chronic, daily regimen of HRT for menopausal symptoms, but some preclinical studies suggest that this may not be the optimal regimen to employ. For example, E₂ for 5, compared to 35 days, to young OVX rats increased open field activity (Luine et al., 1998). Chronic E₂ regimen increases depression-like behavior of young OVX rats and voles (Galea et al., 2002; Okada et al., 1997). E₂ dosing may influence these effects of chronic E₂. E₂ for 3–7 days in a proestrous-like regimen, but not one that should produce higher circulating E₂, decreases anxiety-like behavior of young OVX rats or mice (Koss et al., 2004; McCarthy et al., 1995; Morgan and Pfaff, 2001; 2002; Nomikos and Spyraiki, 1988; Rodriguez-Sierra et al., 1984). Proestrous-like E₂ dosing via subcutaneous E₂ injections reduces time spent freezing post-footshock; however, silastic capsules of E₂ for 2 days increased, and, for 28 days,

decreased, freezing compared to vehicle to OVX rats (Walf and Frye, 2006). In conclusion, the results of the present study suggest that delay in E₂ treatment following OVX can alter responses of aged rats in some measures of cognitive and affective behavior.

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Figure 1.

The mean ($\pm$ sem) percentage of time spent exploring the displaced object in the object placement task. ** above bar indicates a difference from all other conditions ($p < 0.05$).

Figure 2.

The mean ($\pm$ sem) central entries made in the open field. ** above bar indicates a difference from all other conditions ($p < 0.05$). * above bar indicates a difference from OVX + blank/sham + blank condition ($p < 0.05$).

Figure 3.

The mean ($\pm$ sem) time spent in the mirrored chamber in the mirror maze. * above bar indicates a difference from OVX + blank/sham + blank condition ($p < 0.05$).

Figure 4.

The mean (± sem) time spent on the light side of the chamber. * above bar indicates a difference from OVX + blank/sham + blank condition (p < 0.05).

Figure 5.

The mean ($\pm$ sem) time spent swimming (top), struggling (middle), and immobile (bottom) in the forced swim task. * above bar indicates a difference from OVX + blank/sham + blank condition ($p < 0.05$). ** above bar indicates a difference from all other conditions ($p < 0.05$).

Figure 6.

The mean ($\pm$ sem) lordosis quotients of rats. * above bar indicates a difference from OVX + blank/sham + blank condition ($p < 0.05$).