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Parity and estrogen-administration alter affective behavior of ovariectomized rats

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Abstract

Evidence from clinical and basic research studies demonstrates that estradiol (E₂) reduces anxiety and/or depressive behavior; however, this effect is not observed in all studies. One factor that may mitigate differential responses to E₂ may be previous E₂ experience, i.e. parity. To investigate this, performance in tasks that are utilized to assess whether compounds, such as E₂, can alter anxiety-like behavior (elevated plus maze) and have anti-depressant-like effects (forced swim test) were determined. Performance of ovariectomized (ovx), young (3–6 months old) rats that had never had a litter (nulliparous) was compared to that of those that had several litters in their lifetime (multiparous) following 48 h of oil vehicle or E₂ (10 µg) administration. We predicted that E₂ would decrease anxiety-like behavior and increase anti-depressant-like effects of ovx rats and that this pattern may be influenced by parity. Multiparous rats, irrespective of E₂-priming, had increased open arm time compared to nulliparous rats. Administration of E₂ to ovx, nulliparous or multiparous rats decreased immobility in the forced swim test compared to vehicle-administration. Together, these data suggest that E₂ can alter affective behavior and rats with greater reproductive experience have decreased anxiety-like behavior in the elevated plus maze, irrespective of E₂-priming.

Keywords

Estrogen; Hippocampus; Neurosteroid; Affect; Reproductive experience

1. Introduction

Most studies have demonstrated that 17β-estradiol (E₂), a primary estrogen produced by the ovaries, decrease anxiety-like behavior and produce anti-depressant-like effects in animal models. In support, proestrous rats, which have high physiological E₂ levels, have decreased anxiety-like behavior across a variety of tasks and decreased immobility in the forced swim test compared to male rats or those in diestrus which have circulating E₂ levels at nadir [1,2]. Furthermore, when rats are ovariectomized (ovx) and replaced back with E₂ in a regimen that produces proestrous-like circulating E₂ levels, similar decreases in anxiety-like behavior and increased anti-depressant-like effects are observed [3]. However, there are some

inconsistencies reported for E₂'s effects for affective behavior [4], which are related to differences in E₂ exposure. For instance, there are biphasic effects of E₂ to alter affective behavior. Unlike E₂ regimen that produces proestrous-like E₂ levels (e.g. 10 µg SC 44–48 h before testing), E₂ regimen to ovx rats that produces very low (e.g. 2 µg SC 44–48 h before testing) or supraphysiological (e.g. 50 µg SC 44–48 h before testing) circulating E₂ levels do not alter anxiety or depressive behavior compared to vehicle-administration [3,5–12]. Additionally, the length of E₂ exposure may alter E₂'s effects in affective tasks. For example, defensive freezing is decreased in ovx rats administered E₂ via silastic implants for 28, but not 2, days, compared to those administered vehicle [3]. Together, these data suggest that E₂ dosing and length of exposure can alter responses to E₂ for these functional effects.

Another factor to consider when assessing the effects of E₂ for affective behavior is reproductive experience (i.e. parity). Reproductive experience has long-lasting effects on the neuroendocrine system that may be related to parturition, maternal experience and/or lactation. E₂ and prolactin levels are decreased in multiparous, compared to nulliparous, rats during proestrous [13,14]. Furthermore, sensitivity to E₂ is altered by parity. Multiparous rats have reduced sensitivity to E₂, as measured by dose-dependent effects of E₂ to stimulate prolactin surges 48 h after E₂ administration, compared to nulliparous rats [13]. Together, these data suggest that reproductive experience can have pervasive neuroendocrine effects.

Another characteristic of reproductive experience is maternal, cognitive, and affective behavioral plasticity. Following parturition, responsiveness to pups and maternal aggression toward intruders is enhanced with parity in rats and mice [15,16]. Spatial, reference, and working memory are improved in multiparous compared to nulliparous rats [17–19]. Following restraint stress, rats with reproductive experience demonstrate more rearing, less freezing, and more total and central entries in an open field as well as decreased *c-fos* expression in limbic regions (i.e. the hippocampus and basolateral amygdala) compared to virgin, nulliparous rats [20]. A similar effect of parity is observed when estrous cycle is taken into account. In support, there is decreased anxiety-like behavior in proestrous primiparous, compared to nulliparous, rats in the elevated plus maze and open field; this effect was reversed in mid-aged constant estrous rats and abolished by ovariectomy in young and mid-aged rats [21]. Given that both parity and E₂ can alter affective behavior, a question is whether reproductive experience alters effects of E₂-replacement post-ovx for affective behavior of rats.

In a pilot study, we investigated the effects of ovx and E₂-administration to mid-aged (10–12 months old) retired breeder rats that had 5–8 litters before inclusion in the study and ovx 2 weeks before E₂- or vehicle-administration and behavioral testing. We found that despite ovx and E₂-replacement and aging (which can be associated with changes in response to E₂ [22]), these mid-aged multiparous rats demonstrated decreased anxiety-like behavior and increased anti-depressant-like effects compared to what is typically observed in younger, ovx rats administered vehicle in our laboratory. Multiparous rats had increased open arm entries (mean ±sem; vehicle: 2.9±0.6, E₂: 3.2±0.6), open arm time (mean±sem; vehicle: 53.4±18.4 secs, E₂: 46.8±12.8 secs), as well as increased duration struggling (mean±sem; vehicle: 108.4±23.3 secs, E₂: 120.4±17.9 secs), and swimming (vehicle: 219.6±32.4 secs, E₂: 246.4±34.5 secs), and less time immobile (vehicle: 272.0±31.8 secs, E₂: 233.2± 36.7 secs). Although these data were intriguing and suggest that there may be persistent effects of E₂ exposure/parity for these measures, it was not possible to compare these effects in age-matched nulliparous rats. As such, to investigate the role of parity for E₂'s anti-anxiety and anti-depressant-like effects, young adult rats that were nulliparous or multiparous were ovx, administered E₂ or vehicle, and then tested in the elevated plus maze and forced swim test. We hypothesized that ovx multiparous rats would have decreased anxiety-like behavior (i.e. more entries and/or time spent on the open arms) and increased anti-depressant-like effects (i.e. decreased immobility) than ovx

nulliparous rats, irrespective of E₂ levels, and this effect in nulliparous rats would be reversed by E₂ administration.

2. Methods

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

2.1. Subjects and housing

Experimental rats were female Long–Evans rats ($N=28$) that were obtained from our breeding colony at SUNY-Albany (original stock from Taconic Farms, Germantown, NY). Rats were singly-housed when nursing and group-housed (2–3 per cage) when included in the study in polycarbonate cages (45×24×21 cm) in a temperature-controlled room (21 ± 1 °C) in The Laboratory Animal Care Facility. The rats were maintained on a 12/12 h reversed light cycle (lights off 8:00 am) with continuous access to Purina Rat Chow and tap water.

Young (3–6 months old) rats were either virgin (nulliparous) or had 2–4 litters (multiparous). Multiparous rats were mated on day of parturition such that they were typically pregnant while lactating. Pups were weaned on postnatal day 21.

Given that age can be an important confound to consider, approximately half of each experimental group had rats that were 3–4 months old and half were 5 to 6 months of age. No differences were observed due to age.

2.2. Ovariectomy

Experimental rats were ovx under xylazine (12 mg/kg; Bayer Corp., Shawnee Mission, KS) and ketamine (60 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) anesthesia. In brief, an incision was made in the skin and then a small incision was made in the abdominal wall on each side of the rat. The ovary, oviduct, and top of the fallopian tube were ligated and removed, and the abdominal wall was sutured. The skin was closed with surgical adhesive.

All rats were ovx within a few days of weaning their last litter and recovered for 2 weeks post-ovx so that endogenous factors were similarly at nadir. Given that E₂ withdrawal can alter ER expression, rats in each group were ovx for this similar length of time.

2.3. Subcutaneous hormone-priming

Rats were administered vegetable oil vehicle (0.2 cm³) or 17 β -E₂ (10 μ g/0.2 cm³) subcutaneously 44 h before behavioral testing. Results of a dose–response curve study, in which ovx rats were administered vehicle or E₂ (2, 5, 10, 20, and 50 μ g/0.2 cm³) 44–48 h before behavioral testing demonstrated that 5–10 μ g E₂ produces proestrous-like circulating E₂ levels when rats were tested [3]. 17 β -E₂ was obtained from Steraloids, Inc. (Newport, RI).

2.4. Behavioral testing

Rats were tested in the elevated plus maze immediately followed by the forced swim test. Order was not counterbalanced because behavior in the elevated plus maze would likely be altered if rats were tested in this while wet from prior forced swim testing. Data was collected simultaneously by an observer blind to the condition of the rat and a video-tracking system (Anymaze; Stoelting, Wood Dale, IL). All testing was done between hours 10:00 am and 2:00 pm.

2.4.1. Elevated plus maze—The elevated plus maze consisted of four arms, 49 cm long and 10 cm wide, elevated 50 cm off the ground. Two arms are enclosed by walls 30 cm high

and the other two arms are exposed. Rats were placed at the junction of the open and closed arms and total arm entries made, and entries made, and time spent, in the open arms during a 5-min test were recorded [23]. Time spent on the open arms, or entries made to the open arms, are utilized as an indices of decreased anxiety-like behavior.

2.4.2. Forced swim test—Rats were placed in a cylindrical chamber (45 cm high, 20 cm diameter; Stoelting, Wood Dale, IL) containing 30 cm deep 30 °C tap water. This temperature of water was utilized because lower temperature water (e.g. 25 °C) reduces body and brain temperature and/or produces hypothermia simultaneously with increasing immobility behavior of rodents [24–26]. We have found that ovx rats administered E₂ or an anti-depressant, desipramine, reliably show similar levels of anti-depressive-like behavior utilizing this paradigm [27]. The time spent struggling, swimming, and immobile was recorded during the 10-min test. Time spent immobile is utilized when determining antidepressant-like effects of compounds.

2.5. Statistical analyses

Two-way analyses of variance, with parity and hormone condition as the independent measures, were utilized. Main effects for behavioral measures in the elevated plus maze (open arm duration, open arm entries, total arm entries) and forced swim test (duration swimming, struggling, and immobile) were considered when $p < 0.05$. Main effects were followed with Fisher's *post hoc* tests to determine group differences.

3. Results

Analyses performed on elevated plus maze behavior revealed significant main effects of parity, but not hormone condition, for open arm time, $F(1,24)=17.57, p < 0.01$, open arm entries, $F(1,24)=5.79, p < 0.02$, total arm entries, $F(1,24)=6.38, p < 0.02$, and percent of open arm entries/total arm entries, $F(1,24)=8.39, p < 0.01$. Multiparous rats spent more time on the open arms, entered the open and total arms more than did the nulliparous rats (see Fig. 1 and Table 1).

As for forced swim test behavior, there was no main effect of parity, but E₂-administered rats spent less time immobile than did rats administered vehicle $F(1, 24)=5.76, p < 0.02$ (see Fig. 2 and Table 1). No differences due to parity or hormone condition were observed for time spent swimming or struggling.

4. Discussion

Data from the present study partially supported our hypotheses. Rats that had multiple litters had decreased anxiety-like behavior compared to nulliparous rats in the elevated plus maze, irrespective of E₂ condition. Furthermore, anti-depressant-like effects of E₂ were observed in both nulliparous and multiparous rats, compared to vehicle-administration. Although parity increased total entries in the plus maze, there was little evidence for parity or E₂ condition to alter motor indices in the forced swim test, suggesting that behavioral effects observed were not due only to changes in general motor behavior. Indeed, in comparing these effects with our pilot data in mid-aged multiparous rats, a similar behavioral pattern to younger multiparous rats was apparent. Together, these data suggest that reproductive experience decreases anxiety-like behavior and E₂ increases anti-depressant-like effects.

The present data, that rats with reproductive experience had increased anti-anxiety-like behavior in the elevated plus maze, compared to nulliparous rats, confirm and extend previous findings. It has been reported that multiparous rats showed decreased anxiety-like behavior (i.e. more rearing, less freezing, and more total and central entries in an open field) following restraint stress exposure than did similarly stressed nulliparous rats [20]; however, these rats

were intact and endogenous hormonal milieu was not taken into account. Another report demonstrated that young adult proestrous, primiparous rats have increased open arm activity in the elevated plus maze and increased time spent in the center of the open field compared to age-matched, multiparous rats [21]. In this study, ovx eliminated any clear effects of parity for responses in these tasks, which is similar to what was observed in the forced swim test, but not the elevated plus maze, in the present study. These differences in effects may be due to changes in neuroendocrine feedback and/or substrate availability, rather than E₂ levels, which were congruous in ovx, E₂-replaced rats in the present study. Indeed, further evidence for this is that there is reduced circulating E₂ levels among intact primiparous vs. nulliparous rats [21], suggesting that effects for anxiety-like behavior cannot be solely due to changes in circulating E₂ levels with reproductive experience. Although the receptor mechanisms for these effects were not investigated in the present study, it has been proposed that the shift in the dose–response curve of E₂ to initiate prolactin secretion of reproductively-experienced rats may reflect changes in expression and/or activity at estrogen receptor (ER) α and/or β [13]. Indeed, E₂'s effects for affective behavior in the elevated plus maze and forced swim test of young, virgin, ovx rats are dose-dependent [3] and may reflect different actions at ERs. It is likely that activity of E₂ at these ER subtypes may have specific behavioral effects, such that ER α is important for socio-sexual behavior and ER β is important for affective responses in rats and transgenic mice models [4,27–31]. As such, future investigation of these putative ER-specific and dose-dependent effects, and other neuroendocrine changes associated with pregnancy/parturition (i.e. prolactin, progestins) are of interest.

Data from the present study, that E₂ decreased immobility in the forced swim test compared to vehicle-administration, confirm and extend previous studies investigating endocrine modulation of anti-depressive-like behavior in the forced swim test. Several reports have confirmed that E₂ to ovx rats reduces immobility in the forced swim test [3,7,8,11,32]; albeit, these effects are dose-dependent, and attenuated by acute or chronic gestational stressor exposure [3,8,33]. As such, whether task demand and/or ovx (as discussed above) masked effects of parity in the forced swim test must be considered. Another consideration to make in interpreting the present data is the potential for E₂ withdrawal to mitigate behavior in this task. Indeed, other reports have shown increased depressive-like behavior during post-partum or when post-partum decline in E₂ levels is mimicked in ovx rats [34–36]. However, rats in the present study were ovx for 2 weeks before receiving a single injection of vehicle or E₂ before behavioral testing, so effects of E₂ withdrawal in observed responses is unlikely. Furthermore, the present study demonstrates that effects of parity are persistent. A similar pattern of behavior in the elevated plus maze and forced swim test was revealed in multiparous young (3–6 months old) and mid-aged (10–12 months old) rats; however, an interesting question that was not addressed in the present study was whether differences would have been observed in primiparous vs. multiparous rats over the lifespan. Indeed, other studies have shown that, when comparing these groups for cognitive performance and hippocampal morphology, a distinctive pattern emerges in primiparous rats [18,19,37]. Although investigating effects of primiparous vs. multiparous rats would be important, this was beyond the scope of the current experiment. It would be important for future studies to investigate this as well as brain regions and mechanisms for observed effects.

In the present experiment, different patterns of effects of parity and/or E₂-administration were observed in the elevated plus maze and forced swim test. Among nulliparous, ovx rats administered E₂, similar patterns are typically observed in these two tasks in our laboratory [3]. The present data may suggest that prior E₂ exposure alters brain targets underlying these behaviors. It is clear that reproductive experience alters brain plasticity, particularly in limbic/stress responsive brain regions. For instance, oxytocin-induced long-term potentiation in the hippocampus is enhanced in multiparous compared to nulliparous mice [38]. In addition to morphological changes described in hippocampus (i.e. increased spine density in CA1 of

multiparous vs. nulliparous rats; [37]), there are some functional changes observed in this and other limbic regions. For instance, following restraint stress, rats with reproductive experience have decreased *c-fos* expression in the hippocampus and basolateral amygdala compared to nulliparous rats as well as fewer indices of anxiety/stress behavior [20]. Furthermore, using RT-PCR, GABA $\alpha 2$ subunit mRNA expression is reduced in the medial amygdala, increased in the periaqueductal gray, and not changed in the median preoptic area, in proestrous primiparous compared to nulliparous rats [39]. Differences in neurotransmitter substrates are also observed with reproductive experience. Multiparous rats had increased striatal and hypothalamic dopamine levels during pregnancy or when not pregnant compared to primiparous rats [40]. Compared to nulliparous rats, multiparous rats have reduced sensitivity to dopaminergic agents [4], opiates 354 A.A. Walf, [41], and benzodiazepines [42]. Together, these data suggest putative brain sites and neurotransmitter mechanisms for parity and/or E₂ to alter affective behavior and potential targets to explore in the future.

In summary, these data show that, compared to nulliparous rats, multiparous rats, spent more time and made more entries in the open arms of the plus maze and made more total arm entries in the plus maze. E₂ administered, compared to vehicle-administered, ovx rats, decreased time spent immobile, but not struggling, nor swimming, in the forced swim test. Similar effects occurred in mid-aged multiparous rats. Together, these data suggest differences in some of E₂'s effects for affective behavior in that parity decreases anxiety-like behavior, irrespective of E₂ levels, and E₂ produces anti-depressant-like effects, irrespective of reproductive experience.

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Fig. 1. The mean ($\pm$ sem) time spent on the open arms of young nulliparous and multiparous rats administered vehicle or E₂. A line over the bars indicates a difference compared to young, nulliparous group. $p \leq 0.05$.

Fig. 2.
The mean ($\pm$ sem) time spent immobile in the forced swim test of young nulliparous and multiparous rats administered vehicle or E₂. An * indicates a significant difference from vehicle-administered controls. $p < 0.05$.

Table 1
Open arm entries, total arm entries, and % of open arm entries/total arm entries, and time spent struggling and swimming (s) in the forced swim test

Parity condition	E ₂ condition	n	Open arm entries	Total entries	% Open arm entries	Struggling duration	Swimming duration
Young nulliparous	Vehicle	7	0.7±0.4	3.1±1.0	17.3±8.4	122.1±25.5	177.6±36.7
	E ₂	7	2.4±1.1	6.0±2.1	27.7±7.8	179.9±26.7	239.7±36.2
Young multiparous	Vehicle	7	4.0±0.8 [^]	8.1±0.8 [^]	46.7±5.3 [^]	129.4±34.9	163.4±24.9
	E ₂	7	2.9±0.5 [^]	7.1±0.3 [^]	40.3±7.3 [^]	130.3±31.6	217.9±41.9

[^] Indicates difference in multiparous vs. nulliparous. There were no main effects of E₂ condition, nor an interaction between E₂ condition and parity condition, for these measures.