Neuropsychopharmacology (2005) 30, 1288–1301
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www.neuropsychopharmacology.org

Antianxiety and Antidepressive Behavior Produced by Physiological Estradiol Regimen may be Modulated by Hypothalamic–Pituitary–Adrenal Axis Activity

Alicia A Walf[1] and Cheryl A Frye*[,1,2,3]

[1]Department of Psychology, The University at Albany—SUNY, Albany, NY, USA; [2]Department of Biological Sciences, The University at Albany—SUNY, Albany, NY, USA; [3]The Center for Neuroscience Research, The University at Albany—SUNY, Albany, NY, USA

Variations in estradiol (E_2) may influence expression of stress-related anxiety and depression symptoms among women. Effects of E_2 and stress on anxiety and depressive behavior were investigated using an animal model. E_2 was administered subcutaneously (0, 2, 5, 10, 20, 50 μg/rat) to ovariectomized rats 2 days before testing. In experiment 1, open field (anxiety), elevated plus maze (anxiety), or forced swim test (depressive) behavior was evaluated following 20 min of restraint or no such stressor. Rats administered 5 or 10 μg E_2, which produced physiological plasma E_2 concentrations, showed significantly less anxiety and depressive behavior and lower corticosterone levels compared to vehicle, lower, or higher E_2 dosages. Restraint stress prior to behavioral testing attenuated the antianxiety and antidepressive effects of 5 or 10 μg E_2. In experiment 2, effects of adrenalectomy or sham surgery and vehicle or corticosterone replacement in their drinking water on behavior and neuroendocrine measures of rats administered 0, 10, or 50 μg E_2 were examined. E_2, 10 μg, compared to vehicle or 50 μg, reduced anxiety and depressive behavior of sham and adrenalectomized rats administered the low dosage of corticosterone, but not vehicle or the high dosage of corticosterone, suggesting that there may be an optimal level of corticosterone necessary for E_2 to exert these effects. Together, these data suggest that E_2 may have dose-dependent effects on anxiety and depressive behavior of female rodents, which may depend on the tone of the hypothalamic–pituitary–adrenal axis.
Neuropsychopharmacology (2005) **30,** 1288–1301, advance online publication, 9 March 2005; doi:10.1038/sj.npp.1300708

Keywords: estrogen; sex differences; hypothalamic–pituitary–adrenal axis; mood; restraint stress; porsolt

INTRODUCTION

Gender differences in anxiety and depressive disorders favor males. Women are more likely than men to develop anxiety disorders (Seeman, 1997). Women, compared to men, are twice as likely to experience depression and their depressive episodes are longer-lasting and recur more often (Earls, 1987; Nolen-Hoeksema, 1987). These gender differences suggest that sex-linked factors, such as gonadal steroids, may influence their etiology and/or expression (Young, 1998; Young and Korszun, 2002).

The ovarian hormone estradiol (E_2) may influence incidence of, or negative symptomology associated with, mood disorders. Gender differences in depression emerge at puberty and disappear after menopause (Kessler and Walters, 1998; Lewinsohn *et al*, 1998; Weissman and Olfson, 1995). In contrast, the incidence of generalized anxiety disorder is doubled in older, postmenopausal women (Bebbington, 1978; Jenkins, 1987; Weissman and Klerman, 1977; Wittchen and Hoyer, 2001). There are reports of negative symptomology associated with anxiety and depression being greater perimenstrually, postpartum, and after menopause or oophorectomy, when women's E_2 levels would be expected to be lower (Torizuka *et al*, 2000). Among depressed women, plasma E_2 levels are significantly lower than is observed in nondepressed women (Young *et al*, 2000). E_2-replacement therapy to perimenopausal women with depression may provide mood benefits (Cohen *et al*, 2003; Schmidt *et al*, 2000; Soares *et al*, 2001). As well, postmenopausal women taking E_2-replacement therapy had lower depression scores on the Profile of Mood States task than did age-matched women who were not on E_2-replacement therapy (Miller *et al*, 2002). However, some women with anxiety disorders report less anxiety when E_2 levels are low and/or stable (Schmidt *et al*, 1998). Although these data suggest that changes in E_2 levels may influence anxiety and/or depression, E_2's role is not completely understood.

*Correspondence: Dr CA Frye, Social Science 220, The University at Albany—SUNY, Albany, NY 12222, USA, Tel: +518 442 4836, Fax: +518 442 4247, E-mail: cafrye@albany.edu
Received 1 April 2004; revised 14 January 2005; accepted 20 January 2005
Online publication: 31 January 2005 at http://www.acnp.org/citations/NPP01310504150/default.pdf

Evidence from animal models also suggests that E_2 has variable effects on anxiety or depressive behavior of rodents. Rats show less anxiety and depressive behavior during the proestrous phase of the estrous cycle, when E_2 levels are high, compared to the diestrous phase, when E_2 levels are low (Diaz-Veliz *et al*, 1997; Frye *et al*, 2000; Marcondes *et al*, 2001; Mora *et al*, 1996). Ovariectomy (ovx), removal of the primary source of E_2, the ovaries, increases anxiety and depressive behavior, and acute, subchronic, or chronic physiological E_2-replacement reduces anxiety and depressive behavior of rodents (Bernardi *et al*, 1989; Bowman *et al*, 2002; Diaz-Veliz *et al*, 1997; Estrada-Camarena *et al*, 2003; Frye and Walf, 2004a; Hilakivi-Clarke, 1996; Luine *et al*, 1998; Marcondes *et al*, 2001; Mora *et al*, 1996; Nomikos and Spyraki, 1988; Rachman *et al*, 1998; Slater and Blizard, 1976). However, longer E_2-replacement regimen and/or a higher E_2 dosages may not reduce anxiety and/or depressive behavior (Diaz-Veliz *et al*, 1997, 2000; Galea *et al*, 2002; Martinez-Mota *et al*, 2000; Mora *et al*, 1996; Morgan and Pfaff, 2001, 2002; Stoffel and Craft, 2003). Thus, E_2's effects on anxiety and/or depressive behavior of rodents may depend upon the E_2 dosage and/or regimen.

Estradiol may have effects to alter the function of the hypothalamic–pituitary–adrenal axis (HPA). Proestrous rats have increased basal and stress-induced plasma corticosterone levels compared to rats in other stages of the estrous cycle (Carey *et al*, 1995; Figueiredo *et al*, 2002; Frye and Bayon, 1999; Raps *et al*, 1971; Viau and Meaney, 1991). Administration of E_2 to ovx rats may reduce basal and/or stress-induced corticosterone levels, but administration of higher E_2 dosages may increase corticosterone levels (Burgess and Handa, 1992; Carey *et al*, 1995; Dayas *et al*, 2000; Kitay, 1963; McCormick *et al*, 2002; Redei *et al*, 1994). Differences in HPA reactivity may underlie some affective responses of rodents (Conrad *et al*, 1997; Maccari *et al*, 2003; Welberg *et al*, 2000), but whether E_2's effects on anxiety and/or depressive behavior may require the HPA is not established.

Given E_2's influence on affective behavior and the HPA, whether E_2 may mitigate these behavioral processes in part through actions involving the HPA was of interest. We investigated this by testing the following hypotheses. First, if E_2 mediates anxiety (open field, elevated plus maze) and/or depressive (forced swim test) behavior, then E_2 should dose-dependently alter central entries in the open field, open arm time in the plus maze, and/or decrease immobility in the forced swim test. Second, if E_2's effects on anxiety or depressive behavior are mediated by the HPA, then increasing HPA activity with restraint stress before behavioral testing should alter effects of E_2 for anxiety or depressive behavior and corticosterone levels. Third, if E_2's effects on anxiety and depressive behavior require HPA feedback, then adrenalectomy (ADX) should attenuate E_2's dose-dependent effects on anxiety and depressive behavior. Fourth, if corticosterone mediates E_2's effects on anxiety and depressive behavior, then corticosterone replacement would be expected to shift effects of E_2 in ADX rats. To test these hypotheses, two experiments were performed. In experiment 1, ovx rats were administered different dosages of E_2 (0, 2, 5, 10, 20, or 50 μg) 48 h before testing in anxiety or depression tasks. Some rats were restraint stressed before testing to determine the effects of activating the HPA on anxiety and depressive behavior, plasma corticosterone and E_2 levels. In experiment 2, ovx rats were sham surgerized or ADX and replaced with vehicle, a low, or high dose of corticosterone in their drinking water and administered 0, 10, or 50 μg E_2 48 h before testing. Effects on anxiety and depressive behavior, plasma corticosterone and E_2 levels were examined.

MATERIALS AND METHODS

These methods were preapproved by the Institutional Animal Care and Use Committee at SUNY, Albany.

Animals and Housing

Female Long–Evans rats ($N = 284$), approximately 55 days old, were obtained from the breeding colony at SUNY, Albany (original stock from Taconic Farms, Germantown, NY). Rats were group housed (4–5 per cage) in polycarbonate cages ($45 \times 24 \times 21$ cm) in a temperature-controlled room ($21 \pm 1^{\circ}$C) in The Laboratory Animal Care Facility. The rats were maintained on a 12/12 h reversed light cycle (lights off 0800 hours) with continuous access to Purina Rat Chow and tap water in their cages. To prevent wasting, ADX and sham ADX rats also had continuous access to 0.09% saline in their cages.

Surgery

All rats were ovariectomized (ovx) under Rompun (12 mg/kg; Bayer Corp., Shawnee Mission, KS) and Ketaset (80 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) anesthesia 1 week prior to testing. Some rats ($n = 118$) were also bilaterally ADX, 7–10 days post ovx and 1 week before testing.

Estradiol-Priming

In experiment 1, 48 h before testing, ovx rats were subcutaneously administered the following dosages of 17β-E_2 (Sigma Chemical Co., St Louis, MO) in sesame oil vehicle: 0, 2, 5, 10, 20, or 50 μg E_2. In experiment 2, ovx rats were subcutaneously administered 0, 10, or 50 μg E_2 48 h before testing. This regimen of E_2 48 h prior to testing was used because it mimics the gradual increase in plasma E_2 levels during the estrous cycle and is commonly employed to induce sexual receptivity in rats (Frye *et al*, 1998). We have also used this regimen with success in the past to examine E_2's modulation of affective behavior of ovx rats (Frye and Walf, 2004a; Walf and Frye, 2003; Walf, Rhodes, and Frye, 2004)

Restraint Stress

In experiment 1, immediately before behavioral testing in the open field, elevated plus maze, or forced swim task, some rats from each E_2 condition were placed in a plexiglas animal restrainer under a 60-Watt light for 20 min or were not stressed before testing (Frye and Orecki, 2002a, b).

ADX Conditions and Corticosterone Replacement

To determine completeness of ADX surgery, rats were restrained for 20 min and tail blood was collected from rats 24 h following ADX. Plasma corticosterone levels were determined for each rat. Rats with plasma corticosterone levels above typical basal levels (greater than 2.0 μg/dl) were considered to have incomplete ADX and comprised the sham surgerized condition. Rats were considered to be completely ADX if their plasma corticosterone levels were less than 2.0 μg/dl. These ADX rats were randomly assigned to receive vehicle (0.09 % sodium chloride in water and $<0.01\%$ ethyl alcohol), 25 μg/ml, or 250 μg/ml corticosterone in the same vehicle in their drinking water. These dosages were based on previous findings that replacement of 25 μg/ml corticosterone in drinking water produces basal levels of plasma corticosterone and 250 μg/ml corticosterone produces stress-like plasma corticosterone levels (Glavas *et al*, 2001; McCormick *et al*, 2001). Daily average intake of corticosterone were determined for each cage of rats, and basal plasma corticosterone levels were determined in rats 4 days after replacement began, to determine effectiveness of replacement regimen used. ADX rats administered vehicle (ADX + veh group) had corticosterone levels (mean ± SD) below basal levels typically observed in intact rats (0.7 ± 1.1 μg/dl). ADX rats administered 25 μg/ml corticosterone (ADX + 25 CORT) had corticosterone levels similar to the sham ADX group (4.3 ± 9.3 and 5.2 ± 9.4 μg/dl, respectively) and ADX rats administered 250 μg/ml corticosterone (ADX + 250 CORT) had plasma corticosterone levels of 20.5 ± 16.8 μg/dl.

Behavioral Testing

Most rats in experiment 1 were tested in all three of the following tasks in the same order once a week for 3 weeks. There were no observable differences in behavior of rats that were only tested in one task, compared to those that were tested in all three tasks. In experiment 2, all rats were tested once a week for 3 weeks so that each rat was tested in the open field, elevated plus maze, and forced swim task.

Open field. Rats were placed in a brightly lit open field (76 × 57 × 35 cm) and observed for 5 min, while the number of central and peripheral squares entered was recorded (Frye *et al*, 2000; McCarthy *et al*, 1995). The percentage of central entries compared to total entries made were determined for each rat (# of central entries/# of total entries × 100). Central entries are considered an index of antianxiety behavior.

Elevated plus maze. Rats were placed in a brightly lit elevated plus maze and the time spent in the closed and open arms of this maze was recorded for 5 min (Frye *et al*, 2000; Pellow and File, 1986). The plus maze consisted of four arms (50 × 10 cm) that are elevated 50 cm above the floor; two of these arms are enclosed by 37 cm high walls. Open arm duration is considered an index of antianxiety behavior.

Forced swim task. Rats were tested in a modified version of the forced swim test (Frye and Walf, 2002; Porsolt *et al*, 1977). Rats were placed in a cylindrical container filled with 30 cm of 30°C water. The amount of time the rats spent swimming, struggling, and immobile was recorded for 10 min. Struggling was characterized as the rats' movement in the water, which was typically quick movement of the front paws that break the surface of the water and appear to be attempts to escape the chamber. Swimming was recorded when the rats were engaged in movement of the front and back paws without the front paws breaking the surface of the water. Immobility was defined as an absence of any movement other than those necessary to keep the head and nose above the surface of the water (eg floating). Immobility is considered an index of depressive behavior.

Tissue Collection and Radioimmunoassay

Tissue collection. In experiment 1, blood was collected from all rats once—either immediately after removal from home cage (no restraint stress and not tested), or after behavioral testing with restraint, or testing with no prior restraint. In experiment 2, blood was collected from all rats on three occasions—24 h after ADX, 4 days after corticosterone-replacement, and immediately after elevated plus maze testing. Blood, collected from rats via a small nick in the tip of the tail, was placed in EDTA-containing chilled eppendorfs, centrifuged (3000*g* for 20 min), and plasma was stored at −20°C until radioimmunoassay (Tomie *et al*, 2002; Walf and Frye, 2003).

Estradiol radioimmunoassay. In experiment 1, to determine plasma E_2 levels, plasma samples were pooled from some rats in the same E_2 dosage and stress (no stress and not tested, no stress and tested, and restraint stress and tested), which produced 3–8 data points per group. From these samples, there was at least 1 data point for each of the three behavioral tasks so that we could analyze if testing itself altered plasma E_2 levels of rats. In experiment 2, rats were not stressed, which resulted in larger plasma samples such that E_2 could be measured in each rat, yielding 7–12 data points per group. E_2 was extracted twice with ether, by snap freezing. Following chromatographic separation, pellets were reconstituted in PBS (pH = 7.4). The standard curve was prepared in duplicate (12.5–1000 pg/0.1 ml). Standards were added to PBS, with E_2 antibody (Dr Niswender, #244, Colorado State University, Fort Collins, CO), and [^{3}H]E_2 (NET-317, 51.3 ci/mmol; New England Nuclear, Boston, MA, 8000 dpm/100 ml; Frye and Bayon, 1999; Frye *et al*, 1996). Assay tubes are incubated at room temperature for 50 min. Dextran-coated charcoal was used to separate bound and free following a 10-min incubation on ice and centrifugation at 3000*g* for 10 min. Supernatant was pipetted into a glass scintillation vial with scintillation cocktail and counted using a Tri-Carb 2000CA Liquid Scintillation Analyzer. Unknowns were interpolated from the standard curve using Assay Zap, a program for radioimmunoassay analyses. The inter- and intra-assay coefficients of variance were 0.08 and 0.10, respectively.

Corticosterone radioimmunoassay. Corticosterone was measured in each sample collected. Corticosterone was extracted from plasma by heating at 60°C for 30 min. Samples were incubated for 60 min at room temperature

with [^{3}H] corticosterone (NET 182: specific activity = 48.2 ci/mmol; New England Nuclear) and a 1 : 20 000 dilution of antibody (Endocrine Sciences:#B3-163; Frye and Bayon, 1999; Frye *et al*, 1996). Dextran-coated charcoal was used to separate bound and free following a 15-min incubation on ice and centrifugation at 3000 *g* for 10 min. Supernatant was decanted into a glass scintillation vial with scintillation cocktail and then counted using a Tri-Carb 2000CA Liquid Scintillation Analyzer. Unknowns were interpolated from the standard curve using Assay Zap. The inter- and intra-assay reliability coefficients were 0.05 and 0.08, respectively.

Statistical Analyses

In experiment 1, two-way analyses of variance (ANOVAs) were utilized to examine effects of E_2 dosage and stress condition (no stress *vs* restraint stress) on open field, elevated plus maze, and forced swim test behavior. Two-way ANOVAs were used to examine effects of E_2 dosage and stress condition (no stress and not tested, no stress and tested, or restraint stressed and tested) on plasma E_2 and corticosterone levels. In experiment 2, two-way ANOVAs were also used to examine effects of E_2 dosage and ADX condition (sham, ADX + veh, ADX + 25 corticosterone, and ADX + 250 corticosterone) on open field, elevated plus maze, and forced swim test behavior, plasma corticosterone and E_2 levels. Where appropriate, Fisher's *post hoc* tests were utilized to determine group differences. The α level for statistical significance was $p \leqslant 0.05$, and for a trend was $p \leqslant 0.10$. In all figures and text, data are expressed as the mean and standard deviation (SD).

RESULTS

Plasma Estradiol Levels

E_2-primed ovx rats had significantly higher plasma E_2 concentrations, such that 0,2 < 5 < 10 < 20 < 50 μg E_2 produced higher plasma E_2 levels (see Table 1). There were no observable differences in E_2 levels based upon which tasks the rats were tested in. There were no effects of restraint stress on plasma E_2 levels.

As in the first experiment, administration of 0, 10, and 50 μg E_2 produced statistically different plasma concentrations of E_2 in these rats ($F(2,106) = 90.23$, $p < 0.01$; see Table 2). There were no differences produced by ADX condition on these plasma E_2 levels.

Effects of E_2 Dosage and Stress on Anxiety Behavior

Dosages of E_2 (5 or 10 μg) that produced moderate physiological plasma levels of E_2 decreased anxiety behavior in the open field compared to vehicle and all other E_2 dosages administered. There was a significant effect of E_2 dosage on percentage of central entries in the open field ($F(5,115) = 17.12$, $p < 0.01$; see Figure 1, left) and duration spent by rats on the open arms of the plus maze ($F(5,107) = 5.28$, $p < 0.01$; see Figure 2, left). *Post hoc* tests revealed that 5 or 10 μg E_2, which produced plasma E_2 levels within a moderate physiological range akin to levels observed during behavioral estrus (Frye and Bayon, 1999), significantly increased central entries in the open field compared to vehicle (0 μg), lower (2 μg), and higher (20 and 50 μg) E_2 dosages (all *post hoc* comparisons were $p < 0.01$). There was a similar pattern in the plus maze, such that 5 or

Table 1 Experiment 1: Plasma Estradiol Levels (±SD)

E_2 dosage (μg/rat)	Stress condition	*n* =	Plasma E_2 levels (pg/ml)
0	No stress and no testing	3	0.8 ± 0.7
	No stress and testing	5	1.6 ± 0.6
	Stress and testing	8	1.4 ± 0.5
2	No stress and no testing	3	3.7 ± 1.7
	No stress and testing	7	2.8 ± 1.4
	Stress and testing	8	3.8 ± 2.0
5	No stress and no testing	3	27.7 ± 3.8*
	No stress and testing	6	15.0 ± 4.8*
	Stress and testing	5	14.0 ± 4.4*
10	No stress and no testing	3	38.1 ± 18.9*
	No stress and testing	5	42.8 ± 16.8*
	Stress and testing	8	40.0 ± 20.8*
20	No stress and no testing	3	72.6 ± 16.2*
	No stress and testing	6	68.0 ± 15.0*
	Stress and testing	7	63.6 ± 14.7*
50	No stress and no testing	3	78.1 ± 33.2*
	No stress and testing	7	92.6 ± 34.3*
	Stress and testing	7	78.1 ± 35.4*

*Indicates a significant difference from all other dosages of E_2 ($p < 0.05$).

Table 2 Experiment 2: Plasma Estradiol Levels (±SD)

E_2 dosage (μg/rat)	Stress condition	*n* =	Plasma E_2 levels (pg/ml)
0	Sham	10	1.0 ± 0.4
	ADX+veh	8	1.4 ± 0.5
	ADX+25 CORT	9	1.4 ± 0.8
	ADX+250 CORT	8	1.6 ± 0.7
10	Sham	11	38.9 ± 25.3*
	ADX+veh	9	40.2 ± 20.8*
	ADX+25 CORT	12	50.4 ± 24.7*
	ADX+250 CORT	12	47.4 ± 26.8*
50	Sham	10	81.9 ± 44.8*
	ADX+veh	9	93.0 ± 49.8*
	ADX+25 CORT	9	80.9 ± 35.1*
	ADX+250 CORT	11	92.7 ± 26.1*

*Indicates a significant difference from all other dosages of E_2 ($p < 0.05$).

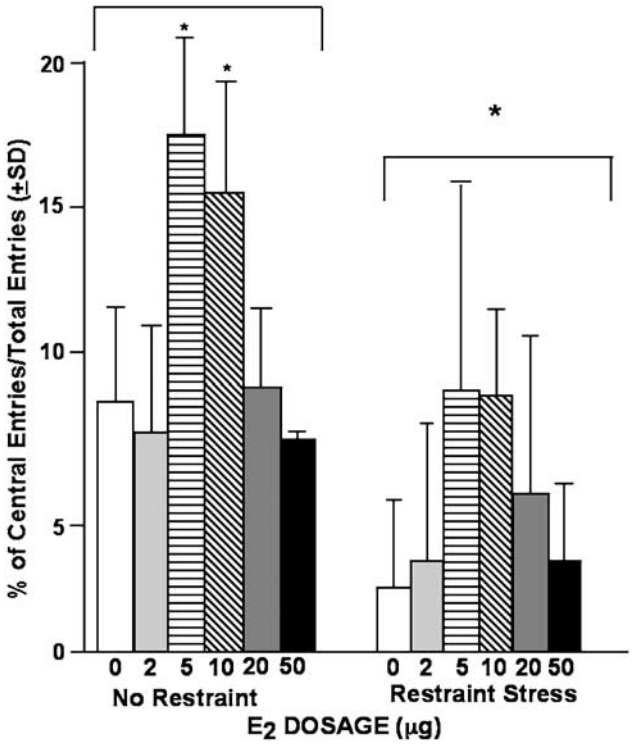

Figure 1 Effects of E_2 dosage and restraint stress on open field behavior. Rats were not stressed and administered 0 ($n=9$), 2 ($n=11$), 5 ($n=9$), 10 ($n=12$), 20 ($n=9$), or 50 ($n=10$) μg E_2 or stressed and administered 0 ($n=11$), 2 ($n=11$), 5 ($n=12$), 10 ($n=11$), 20 ($n=11$), or 50 ($n=11$) μg E_2 before testing. *Above a single bar indicates that E_2 dosage is significantly different from all other E_2 dosages. *Above grouped bars indicates that restraint stress is significantly different from no stress condition.

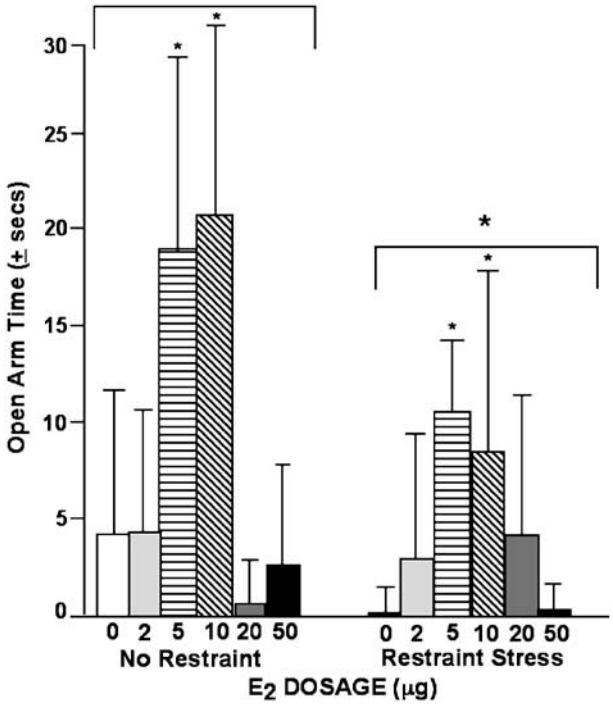

Figure 2 Effects of E_2 dosage and restraint stress on elevated plus maze behavior. Rats were not stressed and administered 0 ($n=9$), 2 ($n=9$), 5 ($n=8$), 10 ($n=7$), 20 ($n=11$), or 50 ($n=10$) μg E_2 or stressed and administered 0 ($n=10$), 2 ($n=12$), 5 ($n=12$), 10 ($n=12$), 20 ($n=9$), or 50 ($n=11$) μg E_2 before testing. *Above a single bar indicates that E_2 dosage is significantly different from all other E_2 dosages. *Above grouped bars indicates that restraint stress is significantly different from no stress condition.

10 μg E_2 increased duration spent by rats on the open arms compared to 0, 2, 20, and 50 μg E_2 (all *post hoc* comparisons were $p<0.01$). There was a main effect of E_2 dosage on total entries in the open field ($F(5,115)=5.32$, $p<0.01$). *Post hoc* tests revealed that 2 (124.4 ± 54.8), 5 (142.0 ± 55.6), or 10 (138.3 ± 50.2) μg E_2 increased total entries compared to 0 (85.0 ± 39.0; *vs* 2 μg $p<0.01$; *vs* 5 μg $p<0.01$; *vs* 10 μg $p<0.01$), 20 (81.6 ± 35.2; *vs* 2 μg $p=0.04$; *vs* 5 μg $p=0.01$; *vs* 10 μg $p<0.01$), and 50 (90.7 ± 47.1; *vs* 2 μg $p<0.01$, *vs* 5 μg $p<0.01$; *vs* 10 μg $p<0.01$) μg E_2. However, there were no differences among groups for total entries in the plus maze (data not shown).

Restraint stress increased anxiety behavior of ovx rats compared to no restraint stress before testing. There was a significant effect of stress on total ($F(1,115)=33.96$, $p<0.01$) and percentage of central ($F(1,115)=61.91$, $p<0.01$; see Figure 1, right) entries in the open field and time spent in the open arms of the elevated plus maze ($F(1,107)=5.79$, $p<0.01$; see Figure 2, right). *Post hoc* tests revealed that 20 min of restraint stress immediately before behavioral testing significantly reduced central entries in the open field ($p<0.01$), and tended to decrease open arm time ($p=0.07$), compared to no restraint stress before testing. Restraint stress significantly reduced the total number of entries in the open field (87.1 ± 11.8) compared to no restraint stress before testing (133.7 ± 16.3; $p<0.01$). Restraint stress did not alter total entries in the plus maze (data not shown).

There was an interaction of E_2 dosage and restraint stress on behavior in the open field. In the open field, restraint stress significantly decreased total ($F(5,115)=2.28$, $p<0.05$) and central ($F(5,115)=6.91$, $p<0.01$) entries of rats administered 5 or 10 μg E_2, but not other E_2 dosages, compared to no prior restraint stress. Rats that were restraint stressed prior to testing and administered 5 (90.1 ± 44.2), 10 (103.2 ± 45.7), but not 0 (72.9 ± 28.0), 2 (102.7 ± 58.4), 20 (95.4 ± 25.5), or 50 (72.8 ± 28.0) μg E_2, made significantly fewer total entries in the open field compared to rats that were not restraint stressed prior to testing and administered 5 (198 ± 67.0), or 10 (173.3 ± 54.6) μg E_2, but not 0 (97.1 ± 49.9), 2 (146.0 ± 51.2), 20 (117.7 ± 45.4), and 50 (108.6 ± 66.2) μg E_2 (all *post hoc* comparisons were $p<0.01$).

In experiment 2, effects of E_2 dosages on anxiety behavior replicated findings from experiment 1. There were significant effects of E_2 dosage on percentage of central entries ($F(2,106)=10.43$, $p<0.01$; see Figure 3) and the duration of time spent on the open arms of the plus maze ($F(2,106)=16.42$, $p<0.01$; see Figure 4). *Post hoc* tests revealed that 10 μg E_2, which produced moderate physiological plasma E_2 concentrations, significantly increased central entries in the open field and duration spent by rats on the open arms of the plus maze compared to vehicle (0 μg) or 50 μg E_2 (all *post hoc* comparisons were $p<0.01$). There was a main effect of E_2 dosage on total entries in the

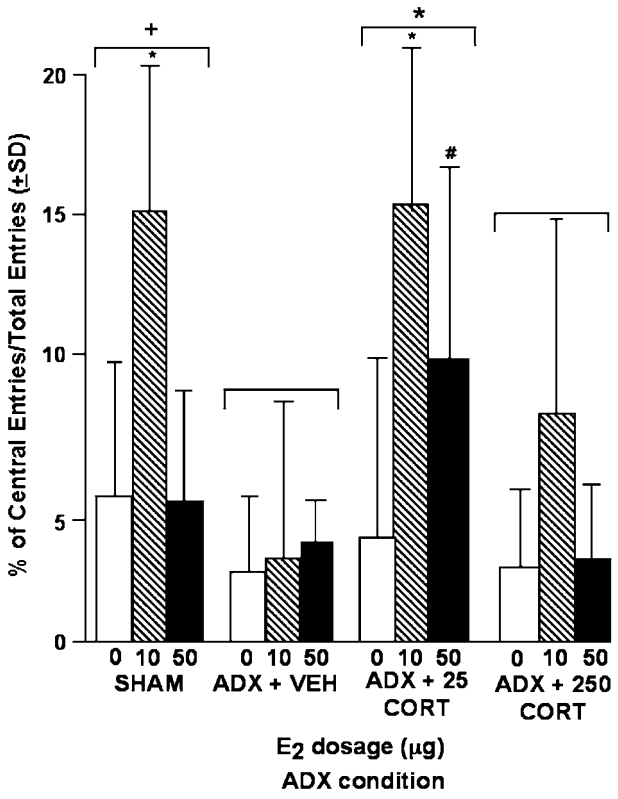

Figure 3 Effects of E_2 dosage and ADX on open field behavior. Rats were sham surgerized and administered 0 ($n=10$), 10 ($n=11$), or 50 ($n=10$) μg E_2 , ADX + veh and administered 0 ($n=8$), 10 ($n=9$), or 50 ($n=9$) μg E_2, ADX + 25 CORT and administered 0 ($n=10$), 10 ($n=9$), or 50 ($n=11$) μg E_2, or ADX + 250 CORT and administered 0 ($n=10$), 10 ($n=11$), or 50 ($n=10$) μg E_2 before testing. *Above a single bar indicates that E_2 dosage is significantly different from all other E_2 dosages. # above a single bar indicates that there is a tendency for 50 μg E_2 to be different from 0 μg E_2 condition. + Above grouped bars indicates that ADX condition is significantly different than ADX + veh condition. *Above grouped bars indicates that ADX condition is significantly different than ADX + veh and ADX + 250 CORT condition.

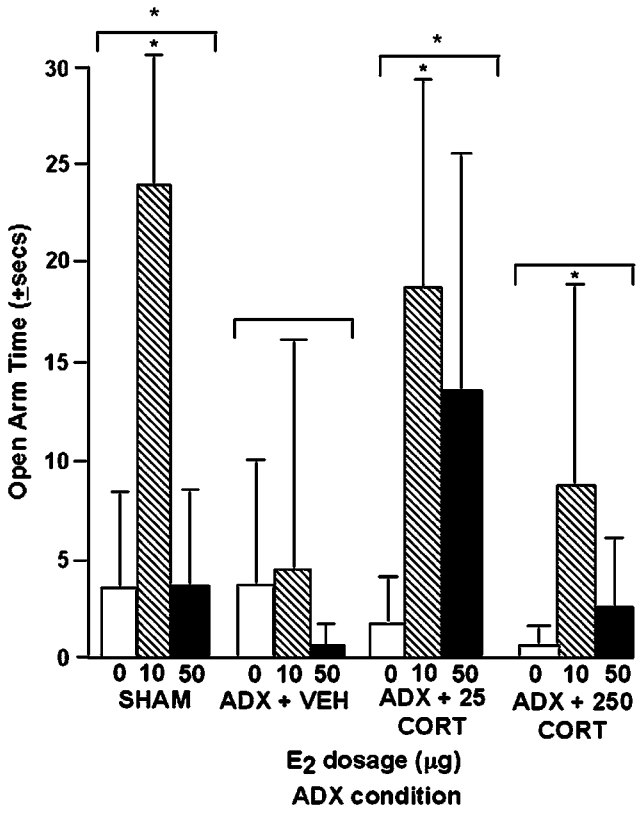

Figure 4 Effects of E_2 dosage and ADX on elevated plus maze behavior. Rats were sham surgerized and administered 0 ($n=10$), 10 ($n=11$), or 50 ($n=10$) μg E_2 , ADX + veh and administered 0 ($n=8$), 10 ($n=9$), or 50 ($n=9$) μg E_2, ADX + 25 CORT and administered 0 ($n=10$), 10 ($n=9$), or 50 ($n=11$) μg E_2 , or ADX + 250 CORT and administered 0 ($n=10$), 10 ($n=11$), or 50 ($n=10$) μg E_2 before testing. *Above a single bar indicates that E_2 dosage is significantly different from all other E_2 dosages. *Above grouped bars indicates that ADX condition is significantly different than ADX + veh and ADX + 250 CORT condition.

open field ($F(2,106)=7.70$, $p<0.01$) and in the elevated plus maze ($F(2,106)=6.16$, $p<0.01$). In the open field, 10 (79.0 ± 31.8) or 50 (66.0 ± 40.4) μg E_2 significantly increased total entries compared to vehicle (56.3 ± 25.4; all *post hoc* comparisons were $p<0.01$). In the plus maze, 10 (5.8 ± 3.4) μg E_2 significantly increased total entries in the elevated plus maze compared to 0 (3.8 ± 1.6) and 50 (4.5 ± 2.0) μg E_2 (all *post hoc* comparisons were $p<0.01$).

In experiment 2, ADX altered anxiety behavior of rats. There were significant effects of ADX on percentage of central entries ($F(3,107)=5.37$, $p<0.01$; see Figure 3) and the duration of time spent on the open arms of the plus maze ($F(3,107)=6.24$, $p<0.01$; see Figure 4). *Post hoc* tests revealed that ADX rats administered 25 μg/ml corticosterone in their drinking water had significantly increased percentage of central entries in the open field compared to ADX rats administered vehicle or 250 μg/ml corticosterone in their drinking water (all *post hoc* comparisons were $p<0.01$). In the plus maze, *post hoc* tests revealed that sham ADX rats and ADX rats administered 25 μg/ml corticosterone in their drinking water spent significantly more time on the open arms of the plus maze compared to rats administered vehicle or 250 μg/ml corticosterone (all *post hoc* comparisons were $p<0.01$). Although there was no main effect of ADX on total entries in the open field (data not shown), there was a difference among groups for total entries in the plus maze ($F(3,106)=3.67$, $p<0.01$). *Post hoc* tests revealed that ADX rats administered 25 μg/ml corticosterone in their drinking water made more total entries (6.0 ± 2.7) in the plus maze than did ADX rats administered vehicle (3.8 ± 1.6; $p=0.01$) or 250 μg/ml corticosterone (4.2 ± 2.3; $p<0.01$) in their drinking water.

In the open field, there was a tendency for an interaction between E_2 dosage and ADX condition. Although these data did not reach statistical significance, results suggest that 10 μg E_2, compared to vehicle or 50 μg E_2, increased percentage of central entries in sham and ADX rats administered 25 μg/ml corticosterone, but not ADX rats administered vehicle or 250 μg/ml corticosterone. Furthermore, there was a tendency for 50 μg E_2 to increase central entries compared to rats administered 0 μg E_2 in the group of ADX rats administered 25 μg/ml corticosterone only.

A similar pattern of effects was observed in the elevated plus maze. There was a significant interaction between E_2 dosage and ADX condition ($F(6,106)=3.16$, $p<0.01$). *Post hoc* tests revealed that, compared to vehicle or 50 μg E_2,

10 μg E_2 increased time spent on the open arms of the plus maze in sham and ADX rats administered 25 μg/ml corticosterone, but not ADX rats administered vehicle or 250 μg/ml corticosterone (see Figure 4; all *post hoc* comparisons were $p<0.01$).

Depressive Behavior

Dosages of E_2 (5 or 10 μg) that produced moderate physiological plasma levels of E_2 decreased depressive behavior in the forced swim test compared to vehicle and all other E_2 dosages. There was a significant effect of E_2 dosage on immobility ($F(5,154)=19.92$, $p<0.01$), struggling ($F(5,154)=6.56$, $p<0.01$), and swimming ($F(5,154)=9.10$, $p<0.01$) in the forced swim test. *Post hoc* tests revealed that 5 or 10 μg E_2 significantly decreased duration of immobility in the forced swim test compared to vehicle or all other E_2 dosages (see Figure 5, left; all *post hoc* comparisons were $p<0.01$). Struggling and swimming were increased by administration of 5 (155.2 ± 43.7 and 223.5 ± 54.8) or 10 (138.3 ± 35.5 and 236.0 ± 62.5) μg compared to 0 (110.0 ± 40.7 and 159.3 ± 45.0), 2 (117.0 ± 32.6 and 187.8 ± 58.1), 20 (131.5 ± 40.7 and 159.1 ± 65.0), or 50 (103.5 ± 29.9 and 170.7 ± 57.1) μg E_2 (all *post hoc* comparisons were $p<0.01$).

Restraint stress increased depressive behavior of ovx rats compared to no restraint stress before testing. There was a significant effect of restraint stress on immobility ($F(1,154)=67.63$, $p<0.01$), struggling ($F(1,154)=5.67$, $p<0.01$), and swimming ($F(1,154)=45.99$, $p<0.01$). *Post hoc* tests revealed that restraint stress significantly increased immobility in the forced swim test compared to no restraint stress (see Figure 5, right; $p<0.01$). Duration of struggling and swimming were also decreased in rats that were restraint stressed (118.6 ± 33.9 and 158.8 ± 59.2) compared to nonstressed rats (133.1 ± 40.4 and 219.8 ± 54.9; all *post hoc* comparisons were $p<0.01$).

There was an interaction of E_2 dosage and restraint stress on immobility and swimming behavior in the forced swim test. Restraint stressed rats administered 5 or 10 μg E_2 had significantly reduced duration of immobility ($F(5,154)=2.31$, $p<0.04$; see Figure 5) and increased swimming ($F(5,154)=1.05$, $p<0.04$) compared to rats administered vehicle or 2, 20, or 50 μg E_2. For swimming behavior, rats that were restraint stressed and administered 5 (181.1 ± 59.3) or 10 (183.4 ± 72.8) μg E_2, but not 0 (119.4 ± 46.8), 2 (163.7 ± 45.7), 20 (144.6 ± 74.7), or 50 (160.8 ± 56.2) μg E_2, had decreased swimming compared to nonstressed rats administered 5 (265.9 ± 50.4) or 10 (288.5 ± 52.1) μg E_2, but not 0 (199.1 ± 43.2), 2 (211.3 ± 70.4), 20 (173.5 ± 55.3), or 50 (180.5 ± 58.0) μg E_2 (all *post hoc* comparisons were $p<0.01$).

In experiment 2, E_2 altered forced swim test behavior and replicated the pattern of results observed in experiment 1. There was a significant effect of E_2 dosage on immobility ($F(2,106)=38.14$, $p<0.01$), struggling ($F(2,106)=4.48$, $p<0.01$), and swimming ($F(2,106)=7.65$, $p<0.01$) in the forced swim test. *Post hoc* tests revealed that 10 μg E_2 significantly decreased duration of immobility in the forced swim test compared to vehicle or 50 μg E_2 (see Figure 6; $p<0.01$). E_2 (50 μg) significantly reduced duration of immobility compared to vehicle administration ($p<0.01$),

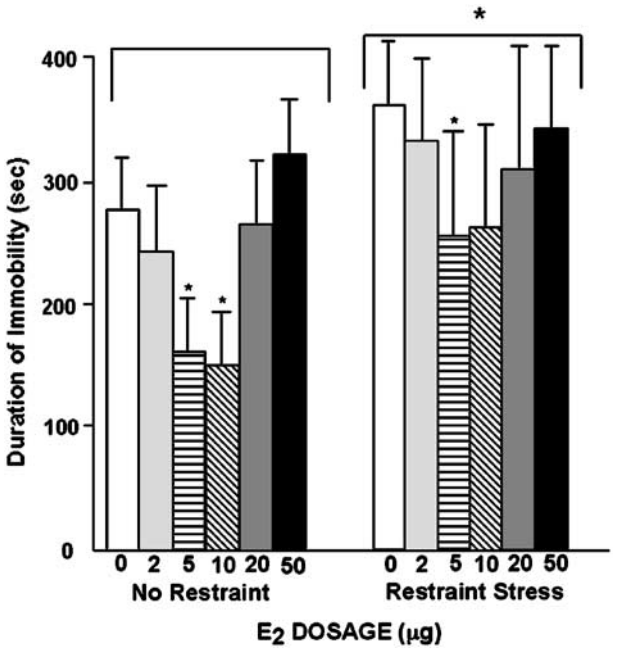

Figure 5 Effects of E_2 dosage and restraint stress on forced swim test behavior. Rats were not stressed and administered 0 ($n=15$), 2 ($n=14$), 5 ($n=16$), 10 ($n=17$), 20 ($n=17$), or 50 ($n=13$) μg E_2 or stressed and administered 0 ($n=13$), 2 ($n=12$), 5 ($n=13$), 10 ($n=11$), 20 ($n=13$), or 50 ($n=12$) μg E_2 before testing. *Above a single bar indicates that E_2 dosage is significantly different from all other E_2 dosages. *Above grouped bars indicates that restraint stress is significantly different from no stress condition.

10 μg (141.0 ± 50.3 and 200.1 ± 66.9) similarly increased duration of struggling and swimming in the forced swim test compared to that observed in rats administered vehicle (112.6 ± 51.4 and 143.9 ± 54.5) or 50 (125.6 ± 43.3 and 165.2 ± 69.8) μg E_2 (all *post hoc* comparisons were $p<0.01$).

ADX condition altered forced swim test behavior of rats. There was a significant effect of ADX on immobility ($F(3,106)=19.12$, $p<0.01$), struggling ($F(3,106)=2.85$, $p<0.04$), and swimming ($F(3,106)=4.42$, $p<0.01$) in the forced swim test. *Post hoc* tests revealed that sham ADX rats and ADX rats administered 25 μg/ml corticosterone had significantly decreased duration of immobility in the forced swim test compared to vehicle or 250 μg/ml corticosterone-administered ADX rats (see Figure 6; all *post hoc* comparisons were $p<0.01$). Struggling behavior was significantly decreased by vehicle (106.4 ± 37.6) administration to ADX rats compared to 25 (137.8 ± 55.3) or 250 (134.4 ± 55.3) μg/ml corticosterone in their drinking water (all *post hoc* comparisons were $p<0.01$). *Post hoc* tests revealed the same pattern of effects for swimming behavior, as were observed for immobility. Sham (184.5 ± 62.8) and ADX rats administered 25 (188.7 ± 57.6) μg/ml corticosterone had significantly increased swimming behavior compared to ADX rats administered vehicle (141.7 ± 59.1) or 250 (164.0 ± 75.3) μg/ml corticosterone in their drinking water (all *post hoc* comparisons were $p<0.01$).

There were significant interactions between E_2 dosage and ADX condition for behavior in the forced swim test. There were significant interactions between E_2 dosage and ADX condition on immobility ($F(6,106)=8.53$, $p<0.01$),

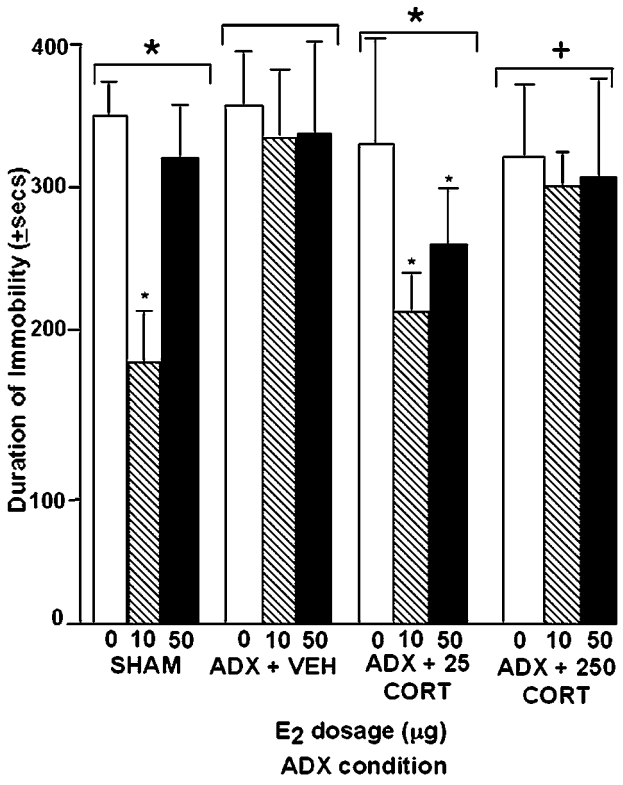

Figure 6 Effects of E_2 dosage and ADX on forced swim test behavior. Rats were sham surgerized and administered 0 ($n=10$), 10 ($n=11$), or 50 ($n=10$) μg E_2 , ADX + veh and administered 0 ($n=8$), 10 ($n=9$), or 50 ($n=9$) μg E_2, ADX + 25 CORT and administered 0 ($n=10$), 10 ($n=9$), or 50 ($n=11$) μg E_2 , or ADX + 250 CORT and administered 0 ($n=10$), 10 ($n=11$), or 50 ($n=10$) μg E_2 before testing. *Above a single bar indicates that E_2 dosage is significantly different from all other E_2 dosages. *Above grouped bars indicates that ADX condition is significantly different than ADX + veh and ADX + 250 CORT condition.

Table 3 Experiment 1: Plasma Corticosterone Levels (±SD)

E_2 dosage (μg/rat)	Stress Condition	$n=$	Plasma corticosterone levels (μg/dl)
0	No stress and no testing	16	5.0±0.9
	No stress and testing	20	5.3±0.7
	Stress and testing	25	5.1±0.6
2	No stress and no testing	18	5.9±1.2
	No stress and testing	21	5.6±0.8
	Stress and testing	24	4.7±0.6
5	No stress and no testing	16	2.3±0.3*
	No stress and testing	21	2.2±0.3*
	Stress and testing	26	3.9±0.4*
10	No stress and no testing	14	2.1±0.4*
	No stress and testing	21	2.3±0.5*
	Stress and testing	22	3.1±0.5*
20	No stress and no testing	16	8.1±2.0*
	No stress and testing	18	12.9±1.7***
	Stress and testing	25	10.7±1.3***
50	No stress and no testing	17	8.8±1.7*
	No stress and testing	17	17.0±2.1***
	Stress and testing	29	14.9±1.4***

*Indicates a significant difference from all other dosages of E_2 ($p<0.05$).
**Indicates a significant effect of stress condition compared to other conditions ($p<0.05$).

struggling ($F(6,106)=3.10$, $p<0.01$), and swimming ($F(6,106)=2.35$, $p<0.04$) in the forced swim test. In all, 10 μg decreased duration spent immobile in sham and ADX rats administered 25 μg/ml corticosterone, but not ADX rats administered vehicle or 250 μg/ml corticosterone, compared to vehicle or 50 μg E_2 (see Figure 6; all *post hoc* comparisons were $p<0.01$). Rats administered 50 μg E_2 had significantly decreased immobility, compared to rats administered 0 μg E_2, in the group of ADX rats administered 25 μg/ml corticosterone only (all *post hoc* comparisons were $p<0.01$). For swimming behavior, 10 μg, but not vehicle or 50 μg, E_2 increased duration spent swimming in sham (240.7±7.35) and ADX rats administered 25 (228.4±71.7) μg/ml corticosterone, but not ADX rats administered vehicle (112.5±57.9) or 250 (170.3±46.5) μg/ml corticosterone (all *post hoc* comparisons were $p<0.01$).

Plasma Corticosterone Levels

Dosages of E_2 that produced moderate physiological plasma levels of E_2 decreased plasma corticosterone levels. There was a significant main effect of E_2 dosage on corticosterone levels ($F(5,348)=46.77$, $p<0.01$). E_2 (5 or 10 μg) significantly decreased plasma corticosterone levels compared to vehicle (0 μg), lower (2 μg), and higher (20 and 50 μg) E_2 dosages (see Table 3); 20 and 50 μg E_2 increased corticosterone levels compared to all other dosages, as revealed by *post hoc* tests (all *post hoc* comparisons were $p<0.01$).

Restraint stress, compared to the no stress and no testing condition, increased plasma corticosterone, but not E_2, levels of rats. There was a significant effect of stress condition on plasma corticosterone levels ($F(2,348)=2.67$, $p<0.01$; see Table 3), such that rats that were exposed to testing alone or with restraint had significantly higher plasma corticosterone levels than did rats that were not exposed to restraint stress or tested ($p<0.01$).

Dosages of E_2 (5 or 10 μg) that produced moderate physiological plasma levels of E_2 decreased basal, post-testing, and post-testing and restraint plasma corticosterone levels ($F(10, 348)=2.57$, $p<0.01$; see Table 3). E_2 (5 or 10 μg) significantly decreased corticosterone levels of rats that were not stressed or tested, as well as corticosterone levels after testing without prior restraint stress compared to vehicle (0 μg), lower (2 μg), and higher (20 and 50 μg) E_2 dosages (all *post hoc* comparisons were $p<0.01$). Compared to vehicle or lower E_2 dosages, 20 or 50 μg E_2 significantly increased basal, post-testing, and post-testing and restraint stress corticosterone levels (all *post hoc* comparisons were $p<0.01$).

Table 4 Experiment 2: Plasma Corticosterone Levels (±SD)

E_2 dosage (μg/rat)	Stress condition	n =	Plasma corticosterone levels (ng/dl)
0	Sham	10	6.7±5.9*
	ADX+veh	8	0.8±0.6
	ADX+25 CORT	10	6.1±1.6*
	ADX+250 CORT	10	17.0±11.2**
10	Sham	11	3.3±3.9*
	ADX+veh	9	0.8±0.5
	ADX+25 CORT	11	6.4±3.7*
	ADX+250 CORT	11	19.9±3.8**
50	Sham	10	16.1±13.4*
	ADX+veh	9	0.9±0.5
	ADX+25 CORT	9	7.3±1.4*
	ADX+250 CORT	10	17.3±9.9**

*Indicates that ADX condition is significantly different than ADX+veh and ADX+250 CORT condition ($p<0.05$).
**Indicates that ADX condition is significantly different than ADX+veh condition ($p<0.05$).

Although there were no main effects of E_2 dosage on plasma corticosterone levels of sham and ADX rats, there were effects of ADX condition on plasma corticosterone levels ($F(3,106)=24.73$, $p<0.01$; see Table 4). *Post hoc* tests revealed that sham surgerized rats and ADX rats replaced with 25 μg/ml corticosterone had significantly higher corticosterone levels than did ADX rats administered vehicle, and significantly lower corticosterone levels compared to ADX rats administered 250 μg/ml corticosterone (all *post hoc* comparisons were $p<0.01$).

DISCUSSION

The present results support the hypotheses that E_2 may have antianxiety and antidepressive effects, which may depend on E_2 dosage, and be modulated by activity of the HPA. Ovx rats administered E_2 dosages (5 or 10 μg) that produced moderate physiological levels of E_2 entered significantly more central entries in the open field, spent significantly more time on the open arms of the plus maze, spent significantly less time immobile in the forced swim test, and had lower plasma corticosterone levels than did rats with lower or higher plasma E_2 levels. Restraint stress before testing attenuated the antianxiety and antidepressive behavior of rats in these tasks and increased plasma corticosterone levels. In rats without an intact HPA, the effects of 10 μg E_2 to reduce anxiety and depressive behavior were not observed, unless ADX rats were replaced with corticosterone that produced low plasma levels of corticosterone. Together, these data suggest that moderate E_2 levels, like that produced by 5 or 10 μg E_2 administration, may decrease anxiety and depressive behavior and corticosterone levels and that these effects may be modulated by the tone of the HPA.

The present results, that dosages of E_2 that produced physiological levels of plasma E_2 reduced anxiety behavior, confirm previous reports of E_2's effects for affective behavior of rodents. Similar E_2 regimen (5–10 μg; SC), which likely also produced physiological plasma E_2 levels, increased open field activity and time spent on the open arms of the plus maze, and decreased freezing in response to a footshock (Slater and Blizard, 1976; Frye and Walf, 2004a). However, some E_2 regimen of lower or higher E_2 dosages, or a longer duration of E_2 exposure, produce no effect on anxiety behavior, decrease anxiety behavior, or may indeed increase anxiety behavior of female rodents in these tasks (Diaz-Veliz *et al*, 1997; Luine *et al*, 1998; Martinez-Mota *et al*, 2000; Mora *et al*, 1996; Morgan and Pfaff, 2001, 2002; Nomikos and Spyraki, 1988; Stoffel and Craft, 2003). The present results that low dosages of E_2 (<5 μg) were without effect, and that high dosages of E_2 (>10 μg) also did not have antianxiety effects, are consistent with this previous research. Together, these data from the present report suggest that E_2's effects on anxiety behavior may be sensitive to the E_2 dosages and regimen employed, such that E_2 dosages that produce physiological E_2 levels (5 or 10 μg) produce antianxiety behavior in the open field and elevated plus maze tasks compared to lower (0 or 2 μg) or higher (20 or 50 μg) E_2.

Our present results, that 5 or 10 μg E_2, but not lower or higher E_2 dosages, decreases immobility in the forced swim test support previous findings that E_2 reduces depressive behavior of rodents. Similar to effects of E_2 on anxiety behavior, there is evidence for dose-dependent effects of E_2 on depressive behavior in the forced swim test. E_2 regimen similar to the effective one utilized in this study (5–10 μg) reduced immobility and increased swimming in the forced swim test of ovx rodents (Bernardi *et al*, 1989; Estrada-Camarena *et al*, 2003; Hilakivi-Clarke, 1996; Rachman *et al*, 1998). However, E_2 to ovx voles, in a high dosage (0.75 μg) that is sufficient to induce a lordosis response, but is not considered a supraphysiological dosage, did not reduce depressive behavior in the forced swim test (Galea *et al*, 2002). Moreover, administration of high dosages of E_2 (50 μg) to rats for 4 days in an experimentally induced postpartum state (ie, withdrawal from high E_2 and progesterone levels akin to pregnancy) reduces immobility compared to vehicle administration in one, but not another, study (Galea *et al*, 2001; Stoffel and Craft, 2003). Although a low dosage of E_2 (2 μg) administered 48 h before testing did not significantly increase E_2 levels of ovx rats at the time of testing and did not reduce immobility in the present study and another study, which utilized a similar regimen (2.5 μg/rat 1 or 48 h before testing; Estrada-Camarena *et al*, 2003), a longer exposure (2 weeks) to similar low dosages of E_2 (0.3–3.0 μg/rat/day) to ovx rats decreased immobility in the forced swim test (Okada *et al*, 1997). These data, and the present results, suggest that E_2's effects to reduce depressive behavior in the forced swim test of female rodents may be mitigated by the dosage and/or duration of E_2 regimen utilized. Thus, E_2 regimen that produced physiological E_2 levels (5 or 10 μg, 48 h before testing), but not regimen that produced lower (0, 2 μg) or higher (20, 50 μg) E_2 levels, reduced depressive behavior in the forced swim.

The results from the present study extend previous research of E_2's effects for anxiety and depressive behavior by suggesting that these effects of E_2 may be modulated by HPA responding. Restraint stress attenuated the antianxiety and antidepressive effects of 5 and 10 μg E_2 to ovx rats. Moreover, plasma corticosterone levels of rats were lowest in ovx rats administered 5 or 10 μg E_2, irrespective of stressors, such as testing and/or restraint. These data support previous findings that 10 μg E_2 for 7 days reduced immobility in the forced swim task and reduced stress responses (*c-fos* immunoreactivity in the hippocampus and amygdala, brain areas integral for affective behavior; Rachman *et al*, 1998). However, it must be noted that in the present study, the dosages of E_2 that reduced immobility were administered in a more acute regimen of 48 h before testing. Furthermore, stress (or HPA activation) may alter rodents' response to gonadal hormones. This has been examined in an animal model of gestational stress. In addition to having a different pattern of corticosterone secretion compared to nongestationally stressed rats (Weinstock, 2001), gestationally stressed female rats may have reduced responsiveness to E_2. For instance, female rats that are gestationally stressed and then tested as adults have decreased sexual behavior towards a male when naturally receptive or when E_2-primed (Frye and Orecki, 2002a, b). Effects of E_2 replacement to reduce depressive behavior are attenuated in female gestationally stressed rats compared to controls (Frye and Wawrzycki, 2003). As well, gestationally stressed female, but not gestationally stressed male, rats have a reduced number of hippocampal granule cells, compared to effects observed in nongestationally stressed controls (Schmitz *et al*, 2002). Thus, the present findings that E_2's effects to reduce anxiety and depressive behavior and corticosterone levels are attenuated by an acute stressor (restraint) substantiate these effects of gestational stress on behavioral and hormonal responses in female rodents.

The effects observed in this study for physiological E_2 concentrations to reduce anxiety and depressive behavior, which may be influenced by a prior stressor, are analogous to recent studies of E_2's biphasic effects on cognitive behavior among female rodents. Moderate, but not low or high, levels of E_2 may improve cognitive performance (Shors and Leuner, 2003). Stressor exposure produces performance deficits among female rodents and this may be due to stress-induced elevations in plasma E_2 levels (Shors *et al*, 1998, 1999; Shors and Leuner, 2003; Wood and Shors, 1998; Wood *et al*, 2001). However, chronic exposure of female rats, but not males, to a stressor has enhancing effects on cognitive performance (Bowman *et al*, 2001, 2002). Although the stress paradigm that we used in the present study did not increase E_2 levels, effects of E_2 were sensitive to the dosage administered and restraint stress reduced the antianxiety and antidepressive effects of moderate, physiological E_2 levels.

The present results characterize some of the effects of different dosages of E_2 to ovx rats and manipulation of the HPA with pretesting restraint stress and ADX for anxiety and depressive behavior. We found an effect of moderate dosages (5–10 μg), but not lower (0–2 μg) or higher (20–50 μg) dosages, of E_2 to reduce basal corticosterone levels and levels following behavioral testing with or without prior restraint. When the HPA was disrupted in rats with ADX, the ability of the moderate dose of E_2 (10 μg) to reduce anxiety and depressive behavior was abrogated; however, E_2's antianxiety and antidepressive effects were reinstated with replacement of low (25 μg/ml), but not high (250 μg/ml), dosages of corticosterone. Unlike in experiment 1, the dose-dependent effects of E_2 to reduce anxiety and depressive behavior of rats did not have the same pattern of effects on plasma corticosterone levels. In experiment 2, 10 μg E_2 reduces anxiety and depressive behavior of ADX rats replaced with 25 μg/ml corticosterone in their drinking water, but corticosterone levels of rats administered 0, 10, or 50 μg E_2 were not different. This suggests that E_2's effects on affective behavior cannot completely be explained by corticosterone levels.

There are complex effects of E_2 and HPA interactions that may involve more than interactions of E_2 and corticosterone. In our study, we grossly manipulated the HPA and replaced back corticosterone to determine whether E_2's effects on antianxiety and antidepressive behavioral patterns require corticosterone. That E_2's effects were absent in ADX rats suggest that E_2's effects may be modulated by HPA feedback, but the nature of these effects were not completely revealed. That E_2's antianxiety and antidepressive effects were reinstated in ADX rats with low dosage corticosterone-replacement suggests that some corticosterone may be necessary for E_2's effects for these behaviors. Interestingly, there were differences in how much corticosterone-replacement was necessary for E_2's behavioral effects. There were different effects of low *vs* high dose corticosterone replacement on E_2's behavioral effects, which is consistent with variable reports of E_2's effects on corticosterone secretion in intact female rodents. For instance, during behavioral estrus, when E_2 levels are typically high in female rodents, there is evidence for increased basal and stress-induced corticosterone secretion (Carey *et al*, 1995; Figueiredo *et al*, 2002; Frye and Bayon, 1999; Raps *et al*, 1971; Viau and Meaney, 1991), albeit there is variability within and between reports. Despite some evidence of higher corticosterone levels, proestrous rats also have reduced anxiety and depressive behavior compared to rats in other stages of estrous (Diaz-Veliz *et al*, 1997; Frye *et al*, 2000; Marcondes *et al*, 2001; Mora *et al*, 1996). Some of these variable effects may be due to differences in the stress reactivity of these animals and the type of stressor paradigm utilized and how stressful the behavioral task utilized is (reviewed in Herman *et al*, 2003). Interestingly, the dose-dependent effects of E_2 that we observed in sham surgerized rats were less robust in ADX, corticosterone-replaced rats. E_2 (50 μg) produced similar effects as 10 μg E_2 to reduce anxiety and depressive behavior in ADX rats that were replaced with a low dosage of corticosterone. Indeed, previous studies have demonstrated a complex relationship between E_2 and the HPA. As such, it would be important to examine effects of different dosages of E_2 on other indices of HPA activity, such as corticosteroid-binding globulin (CBG) and/or adrenocorticotropin (ACTH) levels, which also can be altered by gonadal hormones (Carey *et al*, 1995; Dayas *et al*, 2000; Figueiredo *et al*, 2003; McCormick *et al*, 2002; Viau and Meaney, 1991; Young *et al*, 2001), for these effects on anxiety and depressive behavior. Indeed, it has been suggested that E_2 may sensitize adrenals to corticosterone secretion and have effects on HPA feedback via changing

CBG and/or ACTH levels (Figueiredo *et al*, 2002, 2003). Thus, these effects further support that reductions in anxiety and depressive behavior may not be completely accounted for by changes in corticosterone levels *per se*.

E_2 has been reported to enhance arousal of rodents, which may underlie some of the effects observed in the present study. For instance, E_2 increases motor behavior of rodents (Colvin and Sawyer, 1969; Frye and Walf, 2004a; Morgan and Pfaff, 2001, 2002). However, profound effects of E_2 on gross motor behavior of rats cannot completely explain our present results of decreased anxiety and depressive behavior. A consistent effect of E_2 on total entries in the open field or elevated plus maze or struggling and swimming in the forced swim test was not observed, but the changes observed in these indices suggests that some of the effects observed may be due to changes in arousal/motor behavior following E_2. Indeed, these alterations in arousal may be related to the effects of E_2 on HPA activity, but the extent to which E_2's modulation of arousal is related to effects on HPA processes needs further attention.

The present data provide justification for further examining the nature and mechanisms by which E_2 and HPA activity interact for effects on anxiety and depressive behavior. First, although these data suggest involvement of the HPA at least for some of E_2's effects on anxiety and depressive behavior, direct manipulation of this putative mechanism by pharmacological blockade of mineralocorticoid or glucocorticoid receptors would be of interest. Notably, E_2 may have effects on distribution of glucocorticoid receptors in the brain (Patchev *et al*, 1995). Second, as discussed, it would also be of interest to examine the effects of E_2 for altering other indices of HPA activity besides corticosterone. Further examination may reveal a more direct target of E_2 on HPA activity, which would underlie the effects observed for anxiety and depressive behavior. For instance, the effects of E_2 for the observed behavioral differences may be mediated by CBG, corticotropin releasing factor, and/or ACTH, which are also involved in modulating affective behavior (Almeida *et al*, 1997; Bale and Vale, 2003; Bale *et al*, 2000, 2002; Landgraf *et al*, 1999). Another possibility to consider is E_2's effects on monoamines, such as serotonin, which also mitigate anxiety and depressive behavior and HPA activity, and may underlie the behavioral effects observed poststress and/or E_2 administration (Bowman *et al*, 2003). Fourth, it is also important to consider the role that E_2 has on progestins, which may underlie some of the effects observed. Progestins, particularly 5α-reduced progestins, such as 5α-pregnan-3α-ol-20-one (3α, 5α-THP), modulate antianxiety and antidepressive behavior of female rodents (Frye *et al*, 2000, 2004; Frye and Walf, 2002, 2004b; Picazo and Fernandez-Guasti, 1995; Martinez-Mota *et al*, 2000; Rhodes and Frye, 2001). E_2 enhances 3α,5α-THP formation by increasing 5α-reductase activity and can enhance progestins' effects on behavior (Cheng and Karavolas, 1973; Frye and Duncan, 1996; Vongher and Frye, 1999). Notably, 5α-reduced progestins may dampen HPA activity (Patchev and Almeida, 1996). Thus, although the present data suggest that E_2's antianxiety and antidepressive behavioral effects may be mitigated by the HPA tone of the animal, and not corticosterone levels alone, the other modulating factors are not presently known.

In summary, rats administered E_2 dosages (5 or 10 μg), which produced physiological plasma E_2 concentrations, had significantly reduced anxiety (open field central entries, open arm duration in the plus maze) and depressive (immobility in the forced swim test) behavior and corticosterone levels compared to rats administered vehicle, lower (2 μg), or higher (20 or 50 μg) E_2 dosages. Restraint stress attenuated the antianxiety and antidepressive effects of E_2. ADX abrogated the effects of E_2 to reduce anxiety and depressive behavior, but these effects could be partially reversed by administration of 10 or 50 μg E_2 in ADX rats replaced with a low, but not high, dosage of corticosterone. Thus, these data suggest that E_2 may have dose-dependent effects on anxiety and depressive behavior of female rodents, in part through actions involving HPA responsiveness. Given that hormone-replacement therapy for women has recently come under fire (Rapp *et al*, 2003; Rossouw *et al*, 2002; Shumaker *et al*, 1998), these data that response to E_2 may be different depending on stress/HPA are important to consider. There is evidence that E_2 can enhance mood of some women; however, there are differences in reports (Bjorn *et al*, 2003; Cohen *et al*, 2003; Halbreich, 1997; Morrison *et al*, 2004; Sherwin 1991, 1996; Schmidt *et al*, 1998, 2000; Soares *et al*, 2001). Indeed, the variable reports on the efficacy of E_2 replacement to influence anxiety or depression of women may in part involve individual differences in stress responsiveness related to past and/or current experiences.

ACKNOWLEDGEMENTS

This research was supported by grants from the National Science Foundation (98-96263; 03-16083). The technical assistance provided by Marie Rafalski is greatly appreciated.

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