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Estradiol or diarylpropionitrile decrease anxiety-like behavior of wildtype, but not estrogen receptor beta knockout, mice

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Abstract

Clinical and basic studies demonstrate that estrogen (E₂)-based therapies influence anxiety and mood, but the receptor targets (e.g. α or β isoform of the estrogen receptor, ER) for these effects requires further investigation. To address the specificity of E₂'s anxiolytic-like effects through ER β , anxiety, motor, and nociceptive behavior of ovariectomized, wildtype (WT) and ER β knockout (β ERKO) mice was examined. Mice were administered oil vehicle or ER agonists, 17 β -E₂ (E₂; 0.1 mg/kg; similar affinity for ER α and ER β), and a selective estrogen receptor modulator, diarylpropionitrile (DPN; 0.1 mg/kg; greater affinity for ER β than ER α). Performance of mice in anxiety (open field, elevated plus maze, elevated zero maze, social interaction), motor activity (activity monitor) and nociception (tailflick, pawlick) measures was compared. Results supported our hypothesis that ER β is important in modulation of anxiety-like behavior by E₂ in some tasks. Administration of E₂ or DPN to WT, but not β ERKO, mice increased open field central entries, plus maze open arm time, zero maze open quadrant time, social interaction. This pattern was neither seen in motor activity nor pain threshold measures. Thus, actions of ER β may be important for modulating anxiety-like behavior of mice.

Keywords

estradiol; estrous; depression; SERM; hippocampus

Among adults, anxiety-related disorders are diagnosed in at least twice the number of women than men (Seeman, 1997). This trend continues into aging, when it becomes even more marked following decline in estradiol (E₂) secretion from the ovaries around menopause (Jenkins, 1987; Wittchen & Hoyer, 2001). As such, the role of E₂ in both the etiology and treatment of

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anxiety disorders is of interest. An increased incidence of anxiety symptoms coincident with a decline and/or rapid change in E_2 levels as occurs in both young (premenstrually, postpartum) and older (menopausal, post-menopausal) women suggests a role of E_2 in the etiology of anxiety disorders (Bebbington, Hurry, Tennant, Sturt & Wing, 1981; Bloch, Daly & Rubinow, 2003; Jenkins, 1987; Rubinow & Schmidt, 1995). E_2 -based therapies have been used for many years to treat physiological symptoms associated with menopause, such as hot flashes, night sweats, genital dryness, as well as mental symptoms, such as cognition, anxiety, and mood. Although there are some disparities in results (mainly attributed to E_2 regimen utilized, age, and/or length of ovarian cessation before initiation of treatment), several studies in perimenopausal and/or postmenopausal women demonstrate that E_2 therapies can reduce anxiety or improve mood (Schmidt, Nieman, Danaceau, Tobin, Roca, et al., 2000; Sherwin, 1991; Sherwin & Gelfand, 1985; reviewed in Walf & Frye, 2006). However, limitations of E_2 -based therapies due to their proliferative effects in breast and/or uterus have dampened enthusiasm for their use as treatments for anxiety/mood disturbances.

Some of these limitations in the clinical studies of E_2 for affective processes have been explored using animal models. A post-pubertal sex difference, favoring males, in anxiety-like behaviors of rodents is less apparent among adult females during proestrus, coincident with an increase in E_2 levels (Frye, Petralia & Rhodes, 2000; Palanza, 2001). Female mice spend twice as long on the open arms of the elevated plus maze during proestrus compared to diestrus (Galeeva & Tuohimaa, 2001). Compared to male rats or female rats in diestrus, proestrous rats spend more time on the open arms of the elevated plus maze, more time in social interaction with a conspecific, and less time freezing in response to footshock in a defensive freezing paradigm (Frye, Petralia & Rhodes, 2000; Mora, Dussaubat & Diaz-Veliz, 1996). Cessation of ovarian secretion of E_2 , achieved via ovariectomy (ovx), abolishes this effect among young rodents and administration of E_2 decreases anxiety-like behavior across a variety of tasks in both rats and mice (Frye, Petralia & Rhodes, 2000; Nomikos & Spyraiki, 1988; reviewed in Walf & Frye, 2006). However, these effects are dose-dependent, such that only moderate increases in E_2 levels, which produce proestrous-like concentrations, decrease anxiety-like behavior (Walf & Frye, 2005a). In these studies, rodents were young adults that were acutely administered E_2 within a few weeks of ovx because the length of E_2 treatment may influence the anxiolytic-like effects of E_2 (Walf & Frye, 2006). Fewer studies have investigated the age and/or time-dependent effects of E_2 for anxiety-like behavior, but there is some data in support of this using cognitive function as a measure. E_2 improved cognitive behavior of younger vs. older rats more effectively following long-term ovx; however, older rats responded more favorably than did younger rats to E_2 treatment that produced cyclical fluctuations in E_2 rather than steady chronic E_2 levels (Markowska & Savonenko, 2002). Thus, E_2 treatment parameters may determine the magnitude of E_2 's anxiety-reducing effects.

Another important consideration to make regarding the efficacy of E_2 for its anxiolytic-like effects is the mechanism of E_2 for these effects. E_2 has actions at two distinct ERs isoforms, which are regionally distributed throughout the body and brain, in addition to having distinct N-terminal regions and being encoded by different genes (reviewed in Zhao, Dahlman-Wright, & Gustafsson, 2008). It is likely that some of the regional differences in ER α vs. ER β distribution in the brain may confer specific functional effects of E_2 acting via these receptor isoforms. Although ER α is predominant in the ventromedial hypothalamus, which is related to E_2 's facilitatory actions for female rodent sexual responding, ER β is predominant in limbic and/or stress-responsive regions of the brain, such as the hippocampus, bed nucleus of the stria terminalis, and paraventricular nucleus of the hypothalamus (Shughrue, Lane & Merchenthaler, 1997; Shughrue, Scrimo & Merchenthaler, 1998). Thus, it may be that E_2 has actions to alter affective behavior of rodents in part through ER β .

In our laboratory and others, the functional effects of E₂ via actions at ER β for anxiety behavior have been investigated using both ER β knockdown and ER β activation approaches. First, ER β knockdown occurring over a long period of time, such as is observed in mice with an ER β knockout mutation (β ERKO mice), increases anxiety-like and depression-like behavior, compared to that observed in wildtype (WT) mice. This effect was not reversed with subcutaneous E₂ treatment (Imwalle, Gustafsson & Rissman, 2005; Krezel, Dupont, Krust, Chambon & Chapman, 2001; Rocha, Fleischer, Schaeffer, Rohrer & Hickey, 2005; Walf & Frye, 2006; Walf, Koonce, & Frye, 2008a). Second, anxiety-like and depression-like behavior of ovx rats intracerebroventricularly administered ER β antisense oligonucleotides coincident with acute E₂-priming was increased, compared to administration of scrambled or ER α antisense oligonucleotides (Walf, Ciriza, Garcia-Segura & Frye, 2008). Third, behavioral effects of selective estrogen receptor modulators (SERMs) that have greater affinity for ER β support the notion that ER β may be important for E₂'s anxiety-reducing effects. Lifelong administration of phytoestrogens with a greater affinity for ER β than ER α , such as genistein and daidzein, in the diet of male and female rats decreases anxiety-like behavior in the elevated plus maze (Lund & Lephart, 2001). As well, acute (44–48 hour) subcutaneous administration of coumestrol and diarylpropionitrile (DPN), which are SERMs with greater affinity for ER β than ER α , decrease anxiety-like and depression-like behavior of ovx and male rats compared to administration of vehicle or ER α -selective SERMs (Lund, Rovis, Chung & Handa, 2005; Walf & Frye, 2005b; Walf, Rhodes & Frye, 2004). To investigate further the specificity of E₂ and DPN's effects for anxiety behavior, WT and β ERKO mice were administered vehicle, E₂, or DPN and, 44 hours later, performance effects were compared. We hypothesized that WT, but not β ERKO, mice would be responsive to the effects of E₂ and DPN to decrease anxiety-like behavior.

METHOD

All methods utilized were pre-approved by the Institutional Animal Care and Use Committee at University at Albany- SUNY. All experimentation was conducted in accordance with the accepted standards of humane animal use.

Animals & Housing

Subjects (N= 90) were adult (8–10 weeks old) female mice. Mice were group-housed (4–5 per cage) in polycarbonate cages (26 X 16 X 12.5 cm), containing woodchip shavings for bedding, in a temperature-controlled room (21 $\pm$ 1 °C) in the Laboratory Animal Care Facility of The Life Sciences Research Building at The University at Albany-SUNY. Mice were maintained on a 12/12-hour reversed light cycle (lights off at 8:00 am) with Purina Mice Chow and tap water in their home cages *ad libitum*.

Mouse Strain and Genotyping

All experimental mice were bred in our colony in the Laboratory Animal Care Facility (original breeder pairs on a C57BL/6 background were from Jackson Laboratories, Bar Harbor, ME). Given that the genetic background of this strain of mice cannot be determined by phenotypic characteristics, genotypes of experimental mice were determined with a typical polymerase chain reaction (PCR) analyses. The detailed methods used to genotype these mice have been published (Walf, Koonce, Frye, 2008b).

Ovariectomy

Under sodium pentobarbital anesthesia (80 mg/kg), experimental mice were ovariectomized via bilateral incisions on their flanks. Mice recovered from surgery for one week before initiation in experiment.

Drug administration

Mice were randomly-assigned to be administered vegetable oil vehicle (0.2 cc), E₂ (0.1 mg/kg; Steraloids, Newport, RI), or DPN (0.1 mg/kg; Tocris, Ellisville, MO). Compounds were readily suspended in vegetable oil vehicle following brief vortexing and spinning on low heat for approximately an hour. Treatments were done by subcutaneous (SC) injections in the dorsal neck region 44 hours before behavioral testing in all tasks. Dosing of drugs is based upon dose-response pilot studies done in the laboratory. As well, this dosing of E₂ was utilized because it produces physiological E₂ levels in the hippocampus in WT (10.7 + 2.1 ng/g) and β ERKO (11.0 + 3.3 ng/g) mice (pilot data, and Frye, Rhodes, & Dudek, 2005; Walf, Koonce, & Frye, 2008b). We verified that E₂ dosing utilized produced similar proestrous-like E₂ levels in plasma as has been previously reported (See Table 1; Edwards, 1970; Frye & Walf, 2008).

Screening procedure

Before behavioral testing, mice were evaluated for their general health and normative responses to stimuli, using methods modified from (Crawley, Chen, Puri, Washburn, Sullivan, et al., 2007) and those previously reported by our laboratory (Walf, Koonce, & Frye, 2008a; Walf, Koonce, & Frye, 2008b). In the present study, no differences in general health and normative responses were noted due to genotype and all mice were included in the study.

Handling procedure

To habituate mice to steroid injections, handling by the investigator, and behavioral testing, mice were first subjected to a 5-day, habituation/handling protocol, as per published methods (Frye, Sumida, Dudek, Harney, Lydon, et al., 2006; Walf, Koonce, & Frye, 2008a; Walf, Koonce, & Frye, 2008b). Behavioral testing commenced the following week after this habituation/handling protocol was completed.

Behavioral Testing

Estrogens may have a wide-array of effects via ERs, which are widely distributed throughout the brain (albeit there are regional differences in ER isoform predominance), for anxiety-like and other behaviors. As such, the effects of E₂ and DPN for anxiety-like behavior across a variety of tasks was determined in the open field, elevated plus maze, elevated zero maze, and social interaction task. Given that performance in these tasks can be modulated by other factors, such as activity and/or responsiveness to aversive stimuli, and E₂ may alter these responses, control measures of general motor behavior (activity monitor, total entries in the open field, elevated plus maze, and elevated zero maze), and nociception (tailflick, pawlick) were also evaluated.

Mice were tested once per week in a single task, for approximately 8 weeks, to assess behavior in the tasks described below. The order in which mice were tested through behavioral tasks was the same for all animals and is the same as described below. Behavioral data were collected by trained observers, blind to the hypothesized outcome of the study and condition of the mice, and simultaneously video-recorded with a video-camera and/or video-tracking system (Anymaze- Stoelting, Inc., Wood Dale, IL).

Open Field/Activity Monitor—Anxiety-like and motor behavior of mice was assessed in an activity monitor, with a grid floor (AccuScan Instruments, Inc., Columbus, OH), as per previous methods (Frye, Walf, Rhodes & Harney, 2004). In this apparatus, horizontal movements of mice are automatically recorded (i.e. beams breaks), and total square and central square entries are recorded. To assess their initial reaction to placement in this novel chamber, and to have comparable durations of observation across behavioral tasks, the behavior of mice was assessed for five minutes in this task. Total square entries made are considered a measure

of general motor behavior and increased central square entries are indicative of reduced anxiety-like behavior.

Elevated Plus Maze—Anxiety-like and motor behavior of mice was assessed in the elevated plus maze (Columbus Instruments, Inc., Columbus, OH), using previously described methods (Walf & Frye, 2007b), for five minutes. Increases in open arm time reflect reduced anxiety-like behavior. Total arm entries made are indicative of general motor behavior in this task.

Elevated Zero Maze—The elevated zero maze was utilized as another measure to assess anxiety-like and motor behavior of mice. The task and methods utilized in this five-minute task have been described (Frye, Sumida, Dudek, Harney, Lydon, et al., 2006). An increased time spent on the open quadrants of the zero maze indicate reduced anxiety-like behavior. Total quadrant entries made can be used as an index of general motor behavior in this task.

Social Interaction—The social interaction test was done as previously reported in rats, with methods modified for mice (Frye, Petralia & Rhodes, 2000). Briefly, experimental mice were placed in the opposite corner of the activity chamber (described above) from a stimulus female mouse. This task was novel to the experimental mice, but they had been exposed to the chamber. The stimulus mouse was an intact retired breeder of the same strain that had been habituated to the chamber and trained in the task on several occasions, such that its movements during the task were minimal, compared to that of the experimental mice. The time spent by the experimental mice interacting with the stimulus mouse (i.e. sniffing, grooming, licking, touching, following with contact), which was only considered when the experimental mouse initiated the contact, during the five-minute task was utilized as a measure of reduced anxiety-like behavior.

Tailflick—Pain thresholds of mice were determined using methods previously described in rats (Frye, Petralia, & Rhodes, 2000). The latency of mice to move their tail from a heat source (10 secs maximum; 50°C; San Diego Instruments, San Diego, CA) was utilized as an index of pain threshold. A longer mean latency (averaged over three consecutive trials) for mice to move their tail from a heat source indicates a higher pain threshold.

Pawlick—Pain thresholds of mice were determined using the latencies to lick front and back paws following placement on a heated surface (60 secs maximum; 50°C; Fisher Scientific Culture Tube Warmer) as per previously described methods (Frye, Walf, Rhodes & Harney, 2004). A longer latency to lick paws indicates a higher pain threshold.

Tissue Collection

Forty-four hours after administration of vehicle, E₂, or DPN, blood was collected from mice so that levels of steroids in the timeframe associated with testing could be assessed. Cardiac and/or trunk blood was collected from experimental mice following cervical dislocation and decapitation.

Radioimmunoassay of Steroid Hormones

To address whether mice exhibited normal increases in E₂, as well as stress and other ovarian hormones, corticosterone, E₂, progesterone (P₄), and its metabolite allopregnanolone were measured in plasma. The radioactive probes utilized, ³H corticosterone (NET 182: specific activity = 48.2 ci/mmol), E₂ (NET-317, 51.3 Ci/mmol), P₄ (NET-208: specific activity = 47.5 Ci/mmol), and allopregnanolone (NET-1047: specific activity = 65.0 Ci/mmol), were purchased from Perkin Elmer (Boston, MA). The corticosterone antibody (#B3-163), obtained from Esoterix Endocrinology (Calabasas Hills, CA), which typically binds 40–60% of [³H] corticosterone was used in a 1:20,000 dilution and bound 45% in the present study. The E₂

antibody (E#244, Dr. G.D. Niswender, Colorado State University, Fort Collins, CO), which generally binds between 40% and 60% of [^3H]E₂, was used in a 1:40,000 dilution and bound 54% in the present study. It is not clear if this antibody binds DPN. As such, the E₂ levels data from mice treated with DPN are not included. The P₄ antibody (P#337), obtained from Dr. G.D. Niswender (Colorado State University), when used in a 1:30,000 dilution typically binds between 30% and 50% of [^3H]P₄, and bound 48% in the present study. The allopregnanolone antibody (#921412-5), obtained from Dr. Robert Purdy (Veterans Medical Affairs, La Jolla, CA), when used in a 1:5000 dilution binds between 40–60% of [^3H] allopregnanolone and bound 47% in the present study. Standard steroid extraction and radioimmunoassay techniques used by our laboratory for measurement of these steroids in plasma were utilized (Frye, Sumida, Dudek, Harney, Lydon et al., 2006; Walf & Frye, 2005a).

Statistical Analyses

Two-way analyses of variance tests were utilized to determine effects of genotype (wildtype vs. βERKO) and treatment condition (vehicle vs. E₂ vs. DPN) on behavioral endpoints. A p -value of ≤ 0.05 was considered significant for main effects and the interaction of these variables. If there were significant main effects, group differences were determined by Fisher's PLSD *post hoc* tests. In the case of significant interactions, we ran interaction contrasts at each level of the treatment condition. In this case, significant differences were supported when the p -value was ≤ 0.017 to control for family-wise error.

RESULTS

E₂ and DPN decrease corticosterone levels, and E₂ increases E₂ levels, in plasma

Treatment condition influenced concentrations in plasma of corticosterone ($(F(2,93)=9.19, P < 0.01)$ and E₂ ($F(2,93)=14.51, P < 0.01$), but progesterone levels were not significantly different (Table 1). *Post hoc* tests revealed that circulating concentrations of corticosterone were reduced by E₂ and/or DPN, compared to vehicle, and E₂ levels were increased by E₂ administration compared to vehicle.

E₂ and DPN decrease anxiety-like behavior of WT, but not βERKO , mice, in some measures

In the open field, there were significant main effects of genotype ($F(1,84)=15.56, P < 0.01$) and treatment condition ($F(2,84)=3.09, P \leq 0.05$), but no interaction between these variables, to alter the number of central squares entered by mice (Table 2). *Post hoc* tests revealed that WT mice entered more central squares than did βERKO mice and administration of E₂ or DPN, compared to vehicle, increased central entries. A similar pattern was observed in the elevated plus maze. There were significant main effects of genotype ($F(1,84)=10.64, P < 0.01$) and treatment condition ($F(2,84)=3.22, P \leq 0.04$), but no interaction between these variables, for time spent on the open arms of the plus maze. *Post hoc* tests revealed that WT mice spent more time on the open arms of the plus maze did βERKO mice and administration of E₂ or DPN increased central entries, compared to vehicle-administration (Table 2). In the elevated zero maze, there were significant main effects of genotype ($F(1,84)=28.2, P \leq 0.01$) and treatment condition ($F(2,84)=3.4, P \leq 0.03$) for time spent on the open quadrants of the maze. *Post hoc* tests revealed that WT mice spent longer on the open quadrants than did βERKO mice and E₂ and DPN, compared to vehicle, increased time spent on the open quadrants of the maze. Additionally, there was a significant interaction between genotype and treatment condition ($F(2,84)=3.43, P < 0.04$), such that E₂ ($F(1,28)=15.06, P \leq 0.01$) or DPN ($F(1,28)=13.20, P < 0.01$) increased time spent on the open quadrants in WT, but not βERKO , mice compared to vehicle (Figure 1). In the social interaction task, there was a significant main effect of genotype ($F(1,84)=10.64, P < 0.01$), but not treatment condition, for time spent interacting with a conspecific. There was a significant interaction between genotype and treatment condition ($F(2,84)=3.22, P \leq 0.04$),

such that, compared to vehicle or DPN, E_2 ($F(1,28)=6.41$, $P<0.01$) to WT, but not β ERKO, mice increased time spent interacting with a conspecific (Table 2).

Effects of treatment condition and genotype for motor activity and pain thresholds

There were differences due to genotype for motor measures in the open field (total square entries made ($F(1,84)=43.61$, $P<0.01$), elevated plus maze (total arm entries made ($F(1,84)=12.70$, $P<0.01$), and elevated zero maze (total quadrant entries made ($F(1,84)=18.44$, $P<0.01$)). β ERKO mice made fewer entries compared to WT mice in these tasks (Table 2). Although there was some indication that genotype decreased general motor behavior in the anxiety tasks, there were no differences due to genotype or treatment condition, or an interaction, on the number of beam breaks made in the activity chamber (Table 2). There were no effects of genotype or treatment condition, or an interaction, for latencies to lick front or back paws or tailflick latencies (Table 2).

DISCUSSION

The results of the present study generally supported the *a priori* hypothesis that mice that do not express ER β do not show the same patterns of behavior as do WT mice administered E_2 or an ER β selective SERM. Despite similar effects of E_2 to produce proestrous-like plasma E_2 levels in WT and β ERKO mice, WT mice administered E_2 , compared to vehicle, had less anxiety-like behavior in the zero maze and social interaction task than did β ERKO mice. DPN similarly reduced anxiety-like behavior in the zero maze. In the open field and elevated plus maze, there were significant effects for β ERKO mice to have increased anxiety-like behavior than WT mice and for E_2 and DPN to reduce anxiety-like behavior, compared to vehicle-administration. There were some differences between WT and β ERKO mice for total activity in these measures, but not in the activity monitor. Additionally, there was no evidence for effects of genotype or treatment condition in response to aversive heat stimuli. Together, these data suggest that ER β may be an important receptor target for E_2 's effects to reduce anxiety-like behavior of mice in some tasks.

The present results, that administration of E_2 or an ER β -selective SERM to ovx WT, but not β ERKO, mice reduces anxiety-like behavior in some measures confirms and extends previous findings that ER β is important for anxiety-related behavior of rodents. Although the role of ER β for anxiety-like responses has been characterized in male and female rats (Lund & Lephart, 2001; Lund, Rovis Chung & Handa, 2005; Walf & Frye, 2005a; Walf & Frye, 2006; Walf & Frye, 2007b), few studies have looked at the effects in mice. One study found that administration of coumestrol, which is a less specific ER β agonist than DPN, decreased freezing in a fear conditioning task. In this study, coumestrol produced less robust effects to alter general motor activity, or anxiety-like behavior in the open field, dark-light transition, or elevated plus maze task (Garey, Morgan, Frohlich, McEwen & Pfaff, 2001). We found a similar pattern of results in the present study. Although apparent, there were neither significant main effects of treatment, nor an interaction between treatment and genotype, for behavior in the open field or elevated plus maze. In these tasks, the effect of genotype appeared more robust than that produced by treatment with E_2 or DPN. However, this same pattern was not observed in the elevated zero maze or social interaction task. Although we typically see similar patterns of behavior across these standard measures of anxiety-like behavior, there is some indication in the literature that differences can be observed across tasks. For example, sex differences in the effects of food deprivation are observed in the elevated plus maze, but not the social interaction task (Genn, Tucci, Thomas, Edwards, & File, 2003). Another consideration is that this strain of mice had the ER β gene knocked out throughout development. As such, some of the effects observed may be attributed to differences in the capacity for estrogen(s) to exert organizing effects at critical periods during development (i.e. pre/peri-natal, puberty). Another

factor to consider is the effects on general motor behavior, particularly given E_2 's well-known effects to alter motor behavior/arousal (Becker, Snyder, Miller, Westgate & Jenuwine, 1987; Morgan & Pfaff, 2001). We did not find a main effect of treatment for motor activity in the tasks utilized, or differences in the ratio between center and total entries made in the open field. However, there was some indication that β ERKO mice made fewer total entries in the open field, plus maze, and zero maze, but not the activity monitor. No differences in, or a trend towards increased, open field activity has been reported for β ERKO mice (Ogawa, Chan, Gustafsson, Korach & Pfaff, 2003). E_2 's effects on motor behavior have generally been deemed an ER α -dependent effect; however, in ER α knockout mice, this effect may be task-dependent (Ogawa, Chan, Gustafsson, Korach & Pfaff, 2003). Although these findings suggest that the effects of E_2 or DPN were not due solely to non-specific motor effects, it is important to continue to consider the effects of treatment and genotype for both anxiety-like and motor-related behaviors in different tasks.

The present study extends reported effects on the role of ER β for anxiety-like behavior to demonstrate that E_2 and/or an ER β -selective SERM to ovx WT, but not β ERKO, mice decreases anxiety-like behavior in tasks that are similar (i.e. elevated zero maze) and dissimilar (i.e. social interaction) to typically-used anxiety tasks, the open field and elevated plus maze. These effects in a socially-relevant anxiety task are intriguing given that the anxiolytic-like effects at ER β have focused on typical measures of anxiety behavior that involve assessment of approach/avoidance of precarious situations in the experimental rodents' environment, such as open arms of the elevated plus maze. Although ER α is typically considered the receptor target of E_2 for sexual behavior of rodents (Ogawa, Chan, Chester, Gustafsson, Korach, et al., 1999; Ogawa, Eng, Taylor, Lubahn, Korach, et al., 1998), ER β may be important for other social behaviors, such as aggression and social discrimination in mice (Choleris, Gustafsson, Korach, Muglia, Pfaff, et al., 2003; Choleris, Ogawa, Kavaliers, Gustafsson, Korach, et al., 2006; Nomura, Andersson, Korach, Gustafsson, Pfaff, et al., 2006). However, given that a greater effect of E_2 , which binds both ER α and ER β , than vehicle or DPN was found in the social interaction task, the possible involvement of ER α needs to be considered. These data suggest that the functional effects of E_2 and SERMs for anxiety behavior in some measures may involve ER β , but the effects may be dependent upon SERM-specificity and task-utilized.

The brain targets for the observed effects need to be investigated further. Studies using SERMs, or antisense oligonucleotides targeted against ER β , suggest that the hippocampus, and not other ER β -rich sites (i.e. hypothalamus or midbrain ventral tegmental area), is a targets for anxiolytic-like effects of E_2 (Walf, Ciriza, Garcia-Segura & Frye, 2008; Walf & Frye, 2007a). The specificity of E_2 signaling at ER β (and/or ER α) and within and between these brain sites for anxiety/fear responses are of interest. In the medial amygdala (Frye & Walf, 2004) and the bed nucleus of the stria terminalis, there is high co-expression of ER α and ER β (Greco, Lubbers & Blaustein, 2003; Shughrue, Lane & Merchenthaler, 1997; Shughrue, Scrimo & Merchenthaler, 1998) and how E_2 's functional effects may be related to an interaction of ER α and ER β needs to be addressed further (Turgeon, McDonnell, Martin, & Wise, 2004). The present results leave open the possibility that effects were ER β -specific, as there were similar effects in WT mice administered E_2 , which binds ER α and ER β , and the ER β -specific SERM, DPN, and no effects in β ERKO mice, for some anxiety measures. Investigation of E_2 's actions in other limbic and stress-integration brain sites that predominantly express ER β , such as the hippocampus and paraventricular nucleus of the hypothalamus, are warranted (Shughrue, Lane & Merchenthaler, 1997). We found that E_2 or DPN, compared to vehicle, administration decreased plasma corticosterone levels, irrespective of genotype. However, all mice demonstrated typical basal corticosterone levels, which may, in part, account for this lack of genotype effect and modest effects of treatment. It may be important to investigate these effects in stressed animals to determine contributions of genotype and treatment for HPA responsiveness.

Determining the effects, targets, and mechanisms of E₂ for its actions to alter anxiety-like behavior has great clinical significance. Increasing life expectancy of women, together with a relatively constant age of onset for menopause, has resulted in women now spending about one-third of their lives with levels of endogenous E₂ that are low or at nadir (Wise, 2003). It is likely that more women will use E₂-based therapies to relieve some symptoms, including those neuropsychiatric in nature, associated with E₂ decline (Osterlund, Witt & Gustafsson, 2005). An important topic in investigation of the therapeutic efficacy of E₂ is the differential distribution of ER α and ER β . A recent study has shown that shorter microsatellites in the ER β gene may be associated with first onset major depressive disorder among adolescent females (Geng, Su, Su, Chen, Ren, Shen, Yu, & Xia, 2007). It is clear that E₂ can have trophic effects to increase cell proliferation, which may involve interactions with other steroids, and this is most evident in ER α containing cells in mammary and uterine tissue (as reviewed in Gustafsson, 2003; Pawlak & Wiebe, 2007). It is critical to discern the receptor mechanisms that are important for E₂'s beneficial psychological effects compared to unwanted proliferative effects.

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Figure 1.

Mean ($\pm$ sem) time spent on the open quadrants of the elevated zero maze by vehicle-administered WT (n=12) or estrogen receptor β knockout (β ERKO; n=18) mice, E₂-administered WT (n=16) or β ERKO (n=14) mice, or DPN-administered WT (n=18) or β ERKO (n=12) mice. ** indicates a significant ($P \leq 0.05$) interaction between genotype and treatment condition.

Mean ($\pm$ standard error of the mean) plasma concentrations of corticosterone (B), estradiol (E₂), progesterone (P₄) and allopregnanolone (3 α ,5 α -THP) of ovariectomized wildtype (WT) or estrogen receptor β knockout (β ERKO) mice administered subcutaneous vehicle (far left), E₂ (middle), and DPN (far right). NB: Because it is not clear if the E₂ antibody utilized binds DPN, analyses of E₂ levels from mice treated with DPN are not included.

Table 1

	CONDITION					
	vehicle		E ₂		DPN	
	WT	β ERKO	WT	β ERKO	WT	β ERKO
n=	17	15	15	12	23	17
B (μ g/dl)	2.7 $\pm$ 0.2	2.5 $\pm$ 0.3	1.4 $\pm$ 0.4 [*]	1.5 $\pm$ 0.3 [*]	1.7 $\pm$ 0.3 [*]	1.1 $\pm$ 0.3 [*]
E ₂ (pg/ml)	6.2 $\pm$ 1.3	6.7 $\pm$ 1.8	48.1 $\pm$ 15.9 [*]	47.8 $\pm$ 15.7 [*]	-	-
P ₄ (ng/ml)	3.9 $\pm$ 0.7	4.2 $\pm$ 0.8	3.8 $\pm$ 1.0	4.4 $\pm$ 0.5	3.8 $\pm$ 0.6	3.7 $\pm$ 0.8
3 α ,5 α -THP (ng/ml)	2.4 $\pm$ 0.3	2.4 $\pm$ 0.1	3.4 $\pm$ 0.9	2.8 $\pm$ 0.5	2.5 $\pm$ 0.3	2.4 $\pm$ 0.2

* indicates a significant ($P \leq 0.05$) effect of treatment, which is attributable to higher levels vs. vehicle.

Table 2
Behavior (mean $\pm$ sem) of vehicle-administered WT (n=12) or estrogen receptor β knockout (β ERKO; n=18) mice, E₂-administered WT (n=16) or β ERKO (n=14) mice, or DPN-administered WT (n=18) or β ERKO (n=12) mice.

	CONDITION					
	vehicle		E ₂		DPN	
	WT	β ERKO	WT	β ERKO	WT	β ERKO
Open Field-central square entries	35.2 $\pm$ 3.0	26.6 $\pm$ 3.2 [^]	53.3 $\pm$ 9.2 [*]	34.7 $\pm$ 3.9 [^]	49.3 $\pm$ 3.4 [*]	25.3 $\pm$ 4.9 [^]
Open Field-total square entries	160.3 $\pm$ 9.4	108.9 $\pm$ 8.0 [^]	157.0 $\pm$ 9.3	121.0 $\pm$ 10.2 [^]	157.4 $\pm$ 9.2	93.6 $\pm$ 8.7 [^]
Activity Monitor-# of beam breaks	220.2 $\pm$ 43.9	234.8 $\pm$ 49.5	221.5 $\pm$ 32.4	163.2 $\pm$ 49.4	170.6 $\pm$ 25.2	173.9 $\pm$ 90.8 [^]
Elevated Plus Maze-open arm duration (secs)	6.8 $\pm$ 2.7	4.2 $\pm$ 1.5 [^]	15.7 $\pm$ 3.5 [*]	7.0 $\pm$ 1.8 [^]	14.6 $\pm$ 2.5 [*]	6.1 $\pm$ 1.8 [^]
Elevated Plus Maze-total arm entries	10.9 $\pm$ 1.7	9.5 $\pm$ 1.0 [^]	16.3 $\pm$ 2.0	9.3 $\pm$ 1.2 [^]	14.6 $\pm$ 1.7	9.3 $\pm$ 1.4 [^]
Elevated Zero Maze-total arm entries	9.9 $\pm$ 2.6	5.2 $\pm$ 1.0 [^]	14.8 $\pm$ 2.1	7.3 $\pm$ 2.3 [^]	13.6 $\pm$ 1.8	6.0 $\pm$ 1.2 [^]
Social Interaction-total duration (secs)	21.7 $\pm$ 4.0	25.7 $\pm$ 5.0	38.9 $\pm$ 6.8 [^]	18.5 $\pm$ 3.8	28.4 $\pm$ 4.3 [^]	14.9 $\pm$ 3.3
Front Paw Lick- latency (secs)	29.5 $\pm$ 9.0	26.2 $\pm$ 2.7	23.8 $\pm$ 4.8	18.6 $\pm$ 3.5	22.2 $\pm$ 5.7	22.7 $\pm$ 2.6
Back Paw Lick- latency (secs)	24.7 $\pm$ 7.5	25.4 $\pm$ 3.0	22.0 $\pm$ 3.0	21.7 $\pm$ 3.0	18.4 $\pm$ 3.2	22.0 $\pm$ 2.1
Tailflick - latency (secs)	6.9 $\pm$ 0.6	7.4 $\pm$ 0.6	6.9 $\pm$ 0.4	6.8 $\pm$ 0.6	7.2 $\pm$ 0.5	6.9 $\pm$ 0.8

* indicates a significant ($P \leq 0.05$) effect of treatment, which is attributable to higher levels vs. vehicle.

[^] indicates a significant ($P \leq 0.05$) effect of genotype.

** indicates a significant ($P \leq 0.05$) interaction.