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Estrogen effects on the forced swim test differ in two outbred rat strains

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Abstract

Changes in reproductive hormones, such as estrogen, play a role in mood regulation. The present study examined strain differences (Long-Evans vs. Wistar-Hannover) in the behavioral and biochemical effects of estrogen manipulation. Adult ovariectomized female rats were treated with estradiol, vehicle, or withdrawn from estradiol. The two strains demonstrated differential behavioral responses to short-term estradiol administration in the forced swim test; estradiol induced an antidepressant-like effect in Long-Evans rats but not in Wistar rats. Conversely, withdrawal from estradiol resulted in a depressive-like state in the Wistar rats but not in the Long-Evans rats. Western blot analyses found no differences in estrogen receptors α and β within the hippocampus or the frontal cortex, two brain areas strongly implicated in affective disorders. These data demonstrate the importance of strain as a variable when interpreting behavioral effects of estrogen.

Keywords

estrogen; forced swim test; depression; strain differences; estrogen receptors

1. Introduction

Attempts to understand the role of gonadal steroids in the regulation of behavior are complicated by the fact that individuals respond differently to changes in gonadal steroids [1]. Additionally, women with reproductive endocrine-related mood disorders do not display abnormal endocrine function [2, 3], unlike the depression that can accompany endocrine disorders such as hypothyroidism and Cushing's syndrome. For many, this conceptual complication has led to a dismissal of the role of gonadal steroids in the regulation of mood in women. Nonetheless, a series of studies demonstrated that gonadal steroids clearly trigger the dysphoric mood states observed during the luteal phase of the menstrual cycle, but only in women with a history of premenstrual syndrome [1]. In these studies, cyclic depression in women with premenstrual syndrome was eliminated by ovarian suppression and then precipitated by exogenous administration of estradiol (E2) or progesterone. In contrast,

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women without mood changes during the menstrual cycle displayed no perturbation of mood when exposed to E2 or progesterone. Similarly, euthymic women with a history of postpartum depression experienced significant depression during high dose gonadal steroid addback and withdrawal, while women without a history of postpartum depression showed no mood changes under the same conditions [4]. These data suggest that women with a history of reproductive endocrine related mood disorders are differentially sensitive to gonadal steroids.

Genetic differences may be contributing to these differential sensitivities seen in women. Comparison studies of different animal strains have previously been used to investigate genetic differences in a variety of psychiatric disorders. For example, the Fischer 344 and the Lewis inbred rat strains show significant differences on a variety of measures, including susceptibility to psychoactive drugs and stress [5, 6]. Similarly, a number of mouse strains have been compared in an attempt to develop models for facets of mania [7, 8]. These strain differences have been used to explore the underlying biology of various diseases [5] and to develop possible new treatment approaches [9]. Aside from some studies showing that rodent strains display a differential E2 sensitivity in the periphery [10, 11], there have been few studies reporting differential behavioral responses to E2. One study did report an anxiogenic response to E2 in Swiss-Webster mice that was not seen in C57BL/6 mice [12]. The current study investigates behavioral responses, specifically depressive-like behavior, to estrogen administration and withdrawal in two outbred rat strains.

Effects of estrogen in animal models of depression are inconsistent, which may indicate strain differences. Most studies have found estrogen to have an antidepressant-like effect in rats [13-15], but some have not [16]. These differences have been attributed to dose and duration of E2 treatment [13, 14, 17], but strain may also be an important factor. Moreover, the activation of estrogen receptor β (ER β) has been found to be involved in the antidepressant effects of E2 in rodents [18-22], and alterations of the estrogen receptor α (ER α) have also been implicated in mood disorders in humans [23, 24]. Together these studies raise the possibility that ER subtype levels may be involved in the regulation of mood in women who suffer from endocrine-related mood disorders and in differential sensitivity in rodents.

The present study explores strain differences in behavior, which may help model in animals our prior findings in humans of differential sensitivities to E2 in women with a history of premenstrual syndrome or of postpartum depression compared with women without these disorders. For this purpose, we used Long Evans and Wistar Hannover rats. The Long Evans rat is a pigmented rat that is commonly used in reproductive studies. The Wistar rat is an albino rat and is one of the two original strains used to produce the Long Evans rat. The Wistar rat particularly was selected because it is frequently used in studies related to the underlying biology of mood disorders and has previously been shown to have differential behavioral responses to antidepressant treatment [25-27]. These strains were tested for different behavioral responses to the effects of E2 administration and withdrawal in the forced swim test. We also investigated alterations in ER subtype levels in the hippocampus and frontal cortex, two areas that are targets of estrogen [28, 29] and have been linked to mood disorders [30]. It was hypothesized that the behavioral responses in the two strains would be correlated with alterations in ER levels in the brain.

2. Materials and Methods

2.1 Animals

Groups of female Wistar Hannover (Wistar) and Long-Evans rats (Taconic Labs, Germantown, NY) were used. All rats were ovariectomized at Taconic Labs at

approximately 65 days of age. Animals were housed three per cage with food and water *ad libitum* at the National Institutes of Health animal facilities, where all experiments were conducted. A 12-hour light-dark cycle (lights on at 07:00 and off at 19:00) and a constant (23°C) temperature were maintained. Animals had a 10-day acclimatization period before the start of experimental procedures, which included four days of handling. Each strain was behaviorally tested in three different cohorts at approximately 80-90 days of age. Each cohort contained 5-7 animals per group, tested a maximum of 6 months apart and did not differ significantly in behavioral performance. All treatment and experimental procedures were conducted during the light phase of the light-dark cycle. All experimental procedures were approved by the Animal Use Committee of the National Institute of Mental Health and were conducted according to NIH guidelines.

2.2 The Forced Swim Test

The forced swim test consisted of two exposures to a water tank (25 cm in diameter and 50 cm high) in which rats could swim, struggle, or float. The first exposure was a training phase, and the second was the test phase. Rats usually try to escape the cylinder-like apparatus during their first exposure either by swimming or by struggling (climbing walls or diving). However, later within the trial, they switch to a floating behavior, which is considered to reflect a depression-like or “despair” state. This behavior is more prominent during the test phase (second phase) [31]. The standard FST includes a 10-15 minute pre-trial followed a day later by a 5 minute test-trial [32]. The present study followed the general protocol except that the time lag between the training and testing sessions was six days instead of 24 hours. This protocol permitted multiple hormone injections and adequate time for withdrawal between tests. Longer time lags between pre-test and test in the FST procedure were previously demonstrated to have similar validity as the standard 24 hour lag [33, 34]. Three separate cohorts of animals were trained without E2 and tested 4 hours after last injection of E2 or vehicle. Tests were videotaped and later scored by an observer uninformed about the experimental expectations and/or outcomes for immobility time (floating with only minimal movements needed to keep the head above water), swimming time, and struggle time. Whereas the initially validated measures in the forced swim test are immobility versus active behaviors [32], the additional separation between swimming and struggling can add information about possible mechanisms involved in the tests [35, 36].

2.3 Hormone Administration

Hormones were administered after the first exposure to the forced swim test. E2 groups received three daily subcutaneous injection of sesame oil prior to three daily injections of E2 (Sigma, St. Louis, MO; 2.5µg/rat/day; equivalent to ~10µg/kg/day) administered by subcutaneous injection daily using 0.1ml sesame oil as vehicle. Withdrawal groups received the same dose of E2 except in reverse order (i.e., three days of E2 followed by three days of vehicle). Control animals received six daily injections of the same volume of sesame oil. The injection schedule was designed in order to administer the same number of injections across groups. This dose and duration were chosen because they produce levels similar to peak physiological levels in intact rats (Koss, unpublished data) and because radioimmunoassay measurements demonstrated a complete withdrawal of E2 after three days (see Results). Administration of E2 or vehicle began approximately 2 weeks post ovariectomy surgery.

2.4 17β-Estradiol Measurements

Plasma E2 levels were measured to ensure that E2 treatment resulted in levels within the physiological range and to verify that animals were fully withdrawn from E2 after three days. All samples were collected via cardiac puncture approximately four hours after the last injection of E2 or oil, which was approximately the equivalent time between last injection

and behavioral testing. E2 was measured in a separate group of animals using a radioimmunoassay kit (DSL, Inc., Webster, TX) with a lower sensitivity limit of 2.2 pg/ml. This assay has been previously used and has detected low levels of estrogen in ovariectomized animals [37]. The intra-assay variation was less than 8.9% and the inter-assay variation was less than 12.2%.

2.5 Western Blot Analysis

Protein measurements were obtained using standard western blot techniques. Hippocampus and frontal cortex were dissected quickly after decapitation immediately after behavioral testing in one cohort of animals. Samples were rapidly placed on dry ice and subsequently stored at -80°C until the samples were utilized. To analyze changes in levels of cytosolic and nuclear receptors (ER α and ER β) a fractionation was performed by means of the Chemicon 2145 Compartment Protein Extraction kit (Chemicon, Temecula, CA) as per manufacturer's instructions. This was done to investigate the site of possible strain differences in ER compartmentalization and trafficking. Protein measurements were then taken from all samples, using the standard Bradford method (Pierce Biotechnologies, Rockford, IL). All cytosol samples were standardized to equal 30 μ g of protein per well, and nuclear samples were standardized to equal 15 μ g. However, some samples, after fractioning, lacked the optimum amount of protein and therefore were not analyzed. A molecular weight ladder (Biorad, Hercules, CA), samples from individual animals, and pooled samples were run on 4-12% tris glycine gels (Invitrogen, Carlsbad, CA) and were transferred to nitrocellulose membranes (Invitrogen, Carlsbad, CA). Multiple gels were required for this study. Each gel contained one animal per group counterbalanced to lane. Each lane contained tissue from one animal. Additionally, the following 3 pools of tissue from all animals were added as controls: a nuclear sample, a cytosolic sample, and one containing both nuclear and cytosolic samples. After transfer, a ponceau stain (Sigma, St. Louis, MO) was used to ensure equal loading across and between membranes. All membranes were washed with 0.1% tris buffered saline (TBS) 3 times for 10 minutes. Blocking was performed by immersing all membranes in 5% milk TBS for 1 hour. After blocking, the primary antibody was added to the blocking solution for an overnight incubation at 4 degrees in the following dilutions: Anti-ER α antibody (Santa Cruz Technologies, Santa Cruz, CA; 1:2000); anti-ER β antibody (Affinity Bioreagents, Golden, CO; 1:500). Secondary antibody (Cell Signaling, Beverly, MA; anti-rabbit 1:2000) was added after three 10 minute washes with TBS and was visualized with horseradish peroxidase (Amersham, Piscataway, NJ). Films were then developed and analyzed on the Kodak imaging system. Blocking peptides were used to ensure the specificity of the ER β antibody (Affinity Bioreagents, Golden, CO), and pure subcellular fractionation was confirmed by the nuclear protein histone-3 (anti-histone-3, Chemicon, Temecula, CA; 1:500) accompanied by anti-rabbit secondary antibody (Cell Signaling, Beverly, MA; 1:2000) and by the cytosolic protein LDH (anti-LDH, Santa Cruz Technologies, Santa Cruz, CA; 1:2000) accompanied by anti-goat secondary antibody (Santa Cruz Technologies, Santa Cruz, CA; 1:3000). Results are expressed in percent relative to nuclear or cytosolic pool.

2.6 Statistical Analysis

All statistical analyses were performed on Graph Pad Prism 4.0 or Systat 10.0. Statistical comparisons were performed using two-way ANOVAs (with strain and hormone condition as main factors) followed (where justified by the ANOVA) by Bonferroni corrected *t*-tests. A *p*-value of 0.05 was selected to reflect statistical significance.

3. Results

3.1 Behavioral Response to E2

Statistical analyses revealed a strain by treatment interaction in immobility measures ($F(2,103) = 8.71$ $p=0.003$). As shown in Figure 1, post hoc analyses indicated a significant decrease of immobility in the Long-Evans rats treated with E2 when compared to the Long-Evans vehicle control group ($p<0.05$), whereas Wistar rats showed no difference in immobility between treatment groups. In contrast, Wistar rats displayed a significant increase in immobility in the withdrawal group ($p<0.01$), whereas no effect of withdrawal was demonstrated in the Long-Evans rats. There were no differences in immobility time between the Long-Evans control group and the Wistar control group.

Significant strain-dependent differences were observed in struggle behavior (Table 2) in the forced swim test ($F(1,103) = 30.81$ $p<0.0001$) (Long-Evans > Wistar). A significant treatment effect was also seen ($F(2,103) = 4.43$ $p=0.027$). Post hoc tests revealed no significant differences between the treatments; however there was a trend for E2 treated groups to show more struggling behavior than the vehicle control ($p=0.09$) and withdrawal groups ($p=0.06$). There was no significant strain by treatment interaction found, which constrained permissible subsequent post-hoc testing. Nonetheless, within the Long Evans animals, E2 treated animals struggled more than the oil ($p<0.01$) and withdrawal groups ($p=0.02$). Additionally, the Wistar rats during withdrawal struggled less compared to oil ($p<0.01$) and E2 treated rats ($p=0.03$) (Table 2). No significant differences were found in swim behavior (Table 2).

3.2 Western Blot Analysis

No strain or treatment differences were found in either fractions of the frontal cortex or the hippocampus in ER α protein measurements (Figure 2). Similarly, no strain or treatment differences were found in the amount of ER β (Figure 3), although a different display of bands was found in the nucleus than the cytosol. The cytosol had two clear bands at 53 and 49 kd, whereas the nucleus appeared to have a triple band at 53, 49 and 42 kd (Figure 3c). All measurements were taken from the 53 kd band equivalent to the proper molecular weight of the ER β protein. This band, in addition to the 49kd band, disappeared when blocking peptide was added (data not shown); the 42 kd band faded but did not completely disappear. Measures for nuclear versus cytosol content (Histone-3 and LDH, respectively) indicated pure nuclear and cytosol fractionation (data not shown). No differences across samples were seen in Histone -3 or LDH, suggesting equivalent protein concentrations within and across strains. Equivalent protein staining was observed as well by ponceau stain (data not shown).

3.3 E2 Measurements

There were no significant strain differences in E2 measurements. As expected, there was an overall treatment effect ($F(2,30) = 129.13$ $p<0.001$), with E2 treatment significantly increasing plasma E2 levels, which within 72 hours of withdrawal returned to control levels in both the Long-Evans ($F(2,15) = 61.53$ $p<0.001$; E2 versus oil or E2 withdrawal $p<0.001$) and Wistar rats ($F(2,15) = 72.71$ $p<0.001$; E2 versus oil or E2 withdrawal $p<0.001$; Table 1).

4. Discussion

Our study demonstrated a differential behavioral response to E2 in two outbred strains of the rat. However, contrary to our hypothesis, the observed behavioral responses were not associated with strain-related differences or changes in ER levels in the brain. Long-Evans rats had an antidepressant-like response to E2, whereas the Wistar rats did not; conversely

the Wistar rats had a depressive-like response to the withdrawal of E2, whereas the Long-Evans rats did not. To the authors' knowledge, the present study is the first to report strain differences in response to E2 treatment and withdrawal in a model of depression.

Prior literature has described contradictory effects for E2 in the forced swim test. These differences are most likely due to differing doses and durations of E2 treatment (for review see [17]), as well as strain differences, as highlighted by the present study. Rachman and colleagues observed that 7-day E2 administration in Sprague-Dawley rats induced antidepressant-like effects [15], but others found no effect after 24-day E2 treatment (with progesterone) in Long-Evans rats [16]. Compatible with our findings, Estrada-Camarena and colleagues found no antidepressant-like effect with a 2.5µg injection of E2 on the forced swim test in Wistar rats, but did observe an antidepressant-like effect when using a higher dose of 5µg-10µg/rat [14]. Previous studies have also observed depressive-like effects in the forced swim test in response to E2 withdrawal after 24-day E2 administration (with progesterone) in Long-Evans and Sprague-Dawley rats [16, 38] as well as in intact Wistar rats when ovarian hormone levels are low [39, 40].

Plasma levels of E2 did not differ significantly between the strains (Table 1). E2 levels also confirmed that peak physiological levels were obtained during E2 administration, and that E2 levels returned to those seen in the vehicle-treated animals (at the time of behavioral testing) following withdrawal. These observations suggest the absence of metabolic/pharmacokinetic differences between the strains as an explanation for differing responses to E2 administration or withdrawal. It is important to note that even though plasma levels were in the physiological range, the conditions of the normal estrous cycle were not replicated in this study.

Strain-dependent differential behavioral responses to E2 could be consequent to differences in E2 receptor levels. This hypothesis was tested in the current study by measuring ERα and ERβ protein in the hippocampus and frontal cortex. No differences between strains were found in protein levels in either type of ER within the cytosol or nucleus regardless of hormone treatment. Reported effects of E2 on ER have been inconsistent. Four weeks of OVX without E2 treatment led to upregulation of ERα and ERβ protein in the hypothalamus [50], and an acute supraphysiological dose of E2, administered 4 weeks or 7 days after OVX, decreased ERα immunoreactivity [51, 52]. In contrast, in the hippocampus and frontal cortex, immediate 10-day administration of E2 in young adult OVX females upregulated ERα, but not ERβ levels [53]. It is possible that the different outcomes of the studies described above could result from the differences in timing after ovariectomy, dose, or regimen. In our study, E2 was given for 3 days, 2 weeks after ovariectomy, and we measured ER proteins in the cytosol and the nucleus only within the hippocampus and the frontal cortex in young OVX females. It is possible, therefore, that ERs were upregulated from OVX, as previously shown [50], but our failure to observe a decrease in ER following E2 would suggest that 3 days of physiological levels of E2 may not be long enough or at a high enough dose to produce an effect. Further, our study design did not allow us to detect possible strain-dependent differences in membrane ER subtypes, which would be included in whole cell measurements, or in additional brain regions, such as the dorsal raphe, which has been demonstrated to be important for antidepressant effects [18]. Future studies are needed to determine if membrane ERs or if ER in other brain regions might contribute to the observed strain differences in behavior.

Our findings should be viewed with some caution. First, we used relatively small samples. Nonetheless, an approximately 50% change in ERα protein levels has been reported previously after E2 administration in both the hippocampus and frontal cortex [53]; thus the number of animals in the present study should have been sufficient to detect such a large

change. A second caveat involves the specificity of the ER β antibody utilized. Although this antibody has been used in prior western blot studies [53, 54], Snyder et al recently reported that this antibody, along with other commercially available ER β antibodies, produced the appearance of a band in ER β knockout tissue at a similar molecular weight to the ER β receptor [55]. We also found non-specific binding from this antibody, especially in the nuclear fractions (see figure 3). As a consequent precaution, we tested our blots with a blocking peptide, and we carefully measured only the ~53KD band that disappeared completely with the blocking peptide. Furthermore, it should be noted that the publication by Snyder and colleagues reported that the ER β antibody used in the current experiment performed as well as or better than any other currently available antibody [55]. In comparison, the ER α antibody used in this study (as well as many others [50, 51, 52, 53, 56]) was very specific and produced findings similar to the ER β results; i.e., neither strain differences nor effects of E2 were observed. These observations and caveats notwithstanding, it is unlikely that the strain difference we observed in the forced swim test was due to substantial differences in concentrations of classical ERs.

Other means by which E2 can alter behavior in the FST can be inferred by comparisons to antidepressants. For example, E2 and antidepressants share many mechanisms involving the serotonergic system. Both *in vivo* and *in vitro* studies have shown that a single injection of E2 can promote a functional desensitization of serotonin receptors (5HT1A and 5HT1B) [41, 42], inhibition of serotonergic reuptake sites [43], and down-regulation of the serotonin transporter [44], all of which are described effects of antidepressants. In addition, E2 modulates the serotonergic 5HT2A receptor [45], which has been well-correlated with antidepressant-like effects in the forced swim test [31, 41, 46-48]. Yet, clearly the behavioral effects of E2 may be mediated by non-serotonergic systems [49]. Alterations in noradrenergic function, for example, might be implicated given the increased (albeit non-significant) struggling - a presumed noradrenergic effect [35, 36] - seen in the forced swim test after E2 administration in the Long Evans rats. Of note, the Long-Evans rats struggled more in all groups when compared with the Wistar rats (Table 2). Hence, it is possible that strain differences in the effects of E2 could reflect strain-related differences in the impact of noradrenergic or serotonergic functioning on escape behaviors.

The strain-dependent responses that we describe following short-term exposure to (or withdrawal from) E2 may be helpful in identifying potential mediating mechanisms or molecular targets underlying the differential sensitivity to reproductive hormones observed in humans. Strain-dependent responses were due to differences in neither E2 levels nor ER α or ER β levels; nonetheless, other ER subtypes, membrane ERs, or other E2 targets (e.g., serotonergic or noradrenergic systems) may contribute to these findings. Subsequent replications should employ additional behavioral and physiological measures as well as array technology in an effort to explain and model a critical behavioral question, namely why do different individuals respond differently to the same endocrine stimulus. In conclusion, this study demonstrates that strain is an important variable that along with dose and duration must be considered when interpreting behavioral responses to E2.

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References

1. Schmidt PJ, Nieman LK, Danaceau MA, Adams LF, Rubinow DR. Differential behavioral effects of gonadal steroids in women with and in those without premenstrual syndrome. *N Engl J Med.* 1998; 338:209–16. [PubMed: 9435325]

2. Backstrom T, Sanders D, Leask R, Davidson D, Warner P, Bancroft J. Mood, sexuality, hormones, and the menstrual cycle. II. hormone levels and their relationship to the premenstrual syndrome. *Psychosom Med.* 1983; 45:503–7. [PubMed: 6686333]
3. Rubinow DR, Hoban MC, Grover GN, Galloway DS, Roy-Byrne P, Andersen R, Merriam GR. Changes in plasma hormones across the menstrual cycle in patients with menstrually related mood disorder and in control subjects. *Am J Obstet Gynecol.* 1988; 158:5–11. [PubMed: 2962499]
4. Bloch M, Schmidt PJ, Danaceau M, Murphy J, Nieman L, Rubinow DR. Effects of gonadal steroids in women with a history of postpartum depression. *Am J Psychiatry.* 2000; 157:924–30. [PubMed: 10831472]
5. Cohen H, Zohar J, Gidron Y, Matar MA, Belkind D, Loewenthal U, Kozlovsky N, Kaplan Z. Blunted HPA axis response to stress influences susceptibility to posttraumatic stress response in rats. *Biol Psychiatry.* 2006; 59:1208–18. [PubMed: 16458266]
6. Suzuki T, George FR, Meisch RA. Differential establishment and maintenance of oral ethanol reinforced behavior in lewis and fischer 344 inbred rat strains. *J Pharmacol Exp Ther.* 1988; 245:164–70. [PubMed: 3258911]
7. Gould TD, O'Donnell KC, Picchini AM, Manji HK. Strain differences in lithium attenuation of d-amphetamine-induced hyperlocomotion: A mouse model for the genetics of clinical response to lithium. *Neuropsychopharmacology.* 2007; 32:1321–33. [PubMed: 17151598]
8. Hiscock K, Linde J, Einat H. Black swiss mice as a new animal model for mania: A preliminary study. *J of Med and Bio Sci.* 2007; 1
9. Vit JP, Clauw DJ, Moallem T, Boudah A, Ohara PT, Jasmin L. Analgesia and hyperalgesia from CRF receptor modulation in the central nervous system of fischer and lewis rats. *Pain.* 2006; 121:241–60. [PubMed: 16495007]
10. Harvell DM, Strecker TE, Tochacek M, Xie B, Pennington KL, McComb RD, Roy SK, Shull JD. Rat strain-specific actions of 17beta-estradiol in the mammary gland: Correlation between estrogen-induced lobuloalveolar hyperplasia and susceptibility to estrogen-induced mammary cancers. *Proc Natl Acad Sci U S A.* 2000; 97:2779–84. [PubMed: 10688907]
11. Bailey JA, Nephew KP. Strain differences in tamoxifen sensitivity of sprague-dawley and fischer 344 rats. *Anticancer Drugs.* 2002; 13:939–47. [PubMed: 12394257]
12. Morgan MA, Pfaff DW. Estrogen's effects on activity, anxiety, and fear in two mouse strains. *Behav Brain Res.* 2002; 132:85–93. [PubMed: 11853861]
13. Walf AA, Frye CA. Effects of two estradiol regimens on anxiety and depressive behaviors and trophic effects in peripheral tissues in a rodent model. *Gend Med.* 2009; 6:300–11. [PubMed: 19467526]
14. Estrada-Camarena E, Fernandez-Guasti A, Lopez-Rubalcava C. Antidepressant-like effect of different estrogenic compounds in the forced swimming test. *Neuropsychopharmacology.* 2003; 28:830–8. [PubMed: 12637949]
15. Rachman IM, Unnerstall JR, Pfaff DW, Cohen RS. Estrogen alters behavior and forebrain c-fos expression in ovariectomized rats subjected to the forced swim test. *Proc Natl Acad Sci U S A.* 1998; 95:13941–6. [PubMed: 9811905]
16. Galea LA, Wide JK, Barr AM. Estradiol alleviates depressive-like symptoms in a novel animal model of post-partum depression. *Behav Brain Res.* 2001; 122:1–9. [PubMed: 11287071]
17. Walf AA, Frye CA. A review and update of mechanisms of estrogen in the hippocampus and amygdala for anxiety and depression behavior. *Neuropsychopharmacology.* 2006; 31:1097–111. [PubMed: 16554740]
18. Donner N, Handa RJ. Estrogen receptor beta regulates the expression of tryptophan-hydroxylase 2 mRNA within serotonergic neurons of the rat dorsal raphe nuclei. *Neuroscience.* 2009
19. Hughes ZA, Liu F, Platt BJ, Dwyer JM, Pulicicchio CM, Zhang G, Schechter LE, Rosenzweig-Lipson S, Day M. WAY-200070, a selective agonist of estrogen receptor beta as a potential novel anxiolytic/antidepressant agent. *Neuropharmacology.* 2008; 54:1136–42. [PubMed: 18423777]
20. Walf AA, Frye CA. Administration of estrogen receptor beta-specific selective estrogen receptor modulators to the hippocampus decrease anxiety and depressive behavior of ovariectomized rats. *Pharmacol Biochem Behav.* 2007; 86:407–14. [PubMed: 16916539]

21. Rocha BA, Fleischer R, Schaeffer JM, Rohrer SP, Hickey GJ. 17 beta-estradiol-induced antidepressant-like effect in the forced swim test is absent in estrogen receptor-beta knockout (BERKO) mice. *Psychopharmacology (Berl)*. 2005; 179:637–43. [PubMed: 15645223]
22. Walf AA, Rhodes ME, Frye CA. Antidepressant effects of ERbeta-selective estrogen receptor modulators in the forced swim test. *Pharmacol Biochem Behav*. 2004; 78:523–9. [PubMed: 15251261]
23. Mill J, Kiss E, Baji I, Kapornai K, Daroczy G, Vetro A, Kennedy J, Kovacs M, Barr C. International Consortium for Childhood-Onset Mood Disorders. Association study of the estrogen receptor alpha gene (ESR1) and childhood-onset mood disorders. *Am J Med Genet B Neuropsychiatr Genet*. 2008; 147B:1323–6. [PubMed: 18449864]
24. Perlman WR, Tomaskovic-Crook E, Montague DM, Webster MJ, Rubinow DR, Kleinman JE, Weickert CS. Alteration in estrogen receptor alpha mRNA levels in frontal cortex and hippocampus of patients with major mental illness. *Biol Psychiatry*. 2005; 58:812–24. [PubMed: 16112656]
25. Tejani-Butt S, Kluczynski J, Pare WP. Strain-dependent modification of behavior following antidepressant treatment. *Prog Neuropsychopharmacol Biol Psychiatry*. 2003; 27:7–14. [PubMed: 12551720]
26. Lopez-Rubalcava C, Lucki I. Strain differences in the behavioral effects of antidepressant drugs in the rat forced swimming test. *Neuropsychopharmacology*. 2000; 22:191–9. [PubMed: 10649831]
27. Porsolt RD, Bertin A, Jalfre M. “Behavioural despair” in rats and mice: Strain differences and the effects of imipramine. *Eur J Pharmacol*. 1978; 51:291–4. [PubMed: 568552]
28. Dohanich GP. Gonadal steroids, learning, and memory. *Hormones, Brain and Behavior*. 2002; 2:265–327.
29. Keenan PA, Ezzat WH, Ginsburg K, Moore GJ. Prefrontal cortex as the site of estrogen’s effect on cognition. *Psychoneuroendocrinology*. 2001; 26:577–90. [PubMed: 11403979]
30. Krishnan V, Nestler EJ. Linking molecules to mood: New insight into the biology of depression. *Am J Psychiatry*. 2010; 167:1305–20. [PubMed: 20843874]
31. Einat H, Clenet F, Shaldubina A, Belmaker RH, Bourin M. The antidepressant activity of inositol in the forced swim test involves 5-HT(2) receptors. *Behav Brain Res*. 2001; 118:77–83. [PubMed: 11163636]
32. Porsolt RD, Le Pichon M, Jalfre M. Depression: A new animal model sensitive to antidepressant treatments. *Nature*. 1977; 266:730–2. [PubMed: 559941]
33. Detke MJ, Johnson J, Lucki I. Acute and chronic antidepressant drug treatment in the rat forced swimming test model of depression. *Exp Clin Psychopharmacol*. 1997; 5:107–12. [PubMed: 9234045]
34. Drugan RC, Skolnick P, Paul SM, Crawley JN. A pretest procedure reliably predicts performance in two animal models of inescapable stress. *Pharmacol Biochem Behav*. 1989; 33:649–54. [PubMed: 2587607]
35. Cryan JF, Page ME, Lucki I. Differential behavioral effects of the antidepressants reboxetine, fluoxetine, and moclobemide in a modified forced swim test following chronic treatment. *Psychopharmacology (Berl)*. 2005; 182:335–44. [PubMed: 16001105]
36. Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. *Psychopharmacology (Berl)*. 1995; 121:66–72. [PubMed: 8539342]
37. Koss WA, Gehlert DR, Shekhar A. Different effects of subchronic doses of 17-beta estradiol in two ethologically based models of anxiety utilizing female rats. *Horm Behav*. 2004; 46:158–64. [PubMed: 15256305]
38. Stoffel EC, Craft RM. Ovarian hormone withdrawal-induced “depression” in female rats. *Physiol Behav*. 2004; 83:505–13. [PubMed: 15581673]
39. Schneider T, Popik P. Increased depressive-like traits in an animal model of premenstrual irritability. *Horm Behav*. 2007; 51:142–8. [PubMed: 17049520]
40. Marvan ML, Santana S, Chavez Chavez L, Bertran M. Inescapable shocks accentuate fluctuations of forced swimming immobility in different phases of the rat estrous cycle. *Arch Med Res*. 1997; 28:369–72. [PubMed: 9291632]

41. Raap DK, Van de Kar LD. Selective serotonin reuptake inhibitors and neuroendocrine function. *Life Sci.* 1999; 65:1217–35. [PubMed: 10503938]
42. Osterlund MK, Hurd YL. Acute 17 beta-estradiol treatment down-regulates serotonin 5HT1A receptor mRNA expression in the limbic system of female rats. *Brain Res Mol Brain Res.* 1998; 55:169–72. [PubMed: 9645972]
43. Chang AS, Chang SM. Nongenomic steroidal modulation of high-affinity serotonin transport. *Biochim Biophys Acta.* 1999; 1417:157–66. [PubMed: 10076044]
44. Bethea CL, Gundlah C, Mirkes SJ. Ovarian steroid action in the serotonin neural system of macaques. *Novartis Found Symp.* 2000; 230:112–30. discussion 130-3. [PubMed: 10965505]
45. Sumner BE, Fink G. Testosterone as well as estrogen increases serotonin2A receptor mRNA and binding site densities in the male rat brain. *Brain Res Mol Brain Res.* 1998; 59:205–14. [PubMed: 9729388]
46. Barnes NM, Sharp T. A review of central 5-HT receptors and their function. *Neuropharmacology.* 1999; 38:1083–152. [PubMed: 10462127]
47. Massou JM, Trichard C, Attar-Levy D, Feline A, Corruble E, Beaufils B, Martinot JL. Frontal 5-HT2A receptors studied in depressive patients during chronic treatment by selective serotonin reuptake inhibitors. *Psychopharmacology (Berl).* 1997; 133:99–101. [PubMed: 9335087]
48. Lucki I, Singh A, Kreiss DS. Antidepressant-like behavioral effects of serotonin receptor agonists. *Neurosci Biobehav Rev.* 1994; 18:85–95. [PubMed: 8170624]
49. Pinna G, Costa E, Guidotti A. Changes in brain testosterone and allopregnanolone biosynthesis elicit aggressive behavior. *Proc Natl Acad Sci U S A.* 2005; 102:2135–40. [PubMed: 15677716]
50. Ma S, Wu J, Feng Y, Chen B. Elevated estrogen receptor expression in hypothalamic preoptic area decreased by electroacupuncture in ovariectomized rats. *Neurosci Lett.* 2011; 494:109–13. [PubMed: 21376784]
51. Yamada S, Noguchi D, Ito H, Yamanouchi K. Sex and regional differences in decrease of estrogen receptor alpha-immunoreactive cells by estrogen in rat hypothalamus and midbrain. *Neurosci Lett.* 2009; 463:135–9. [PubMed: 19646508]
52. Yuri K, Kawata M. The effect of estrogen on the estrogen receptor-immunoreactive cells in the rat medial preoptic nucleus. *Brain Res.* 1991; 548:50–4. [PubMed: 1868347]
53. Bohacek J, Daniel JM. The ability of oestradiol administration to regulate protein levels of oestrogen receptor alpha in the hippocampus and prefrontal cortex of middle-aged rats is altered following long-term ovarian hormone deprivation. *J Neuroendocrinol.* 2009; 21:640–7. [PubMed: 19453823]
54. Li X, Schwartz PE, Rissman EF. Distribution of estrogen receptor-beta-like immunoreactivity in rat forebrain. *Neuroendocrinology.* 1997; 66:63–7. [PubMed: 9263202]
55. Snyder MA, Smejkalova T, Forlano PM, Woolley CS. Multiple ERbeta antisera label in ERbeta knockout and null mouse tissues. *J Neurosci Methods.* 2010; 188:226–34. [PubMed: 20170675]
56. Pawluski JL, Barakauskas VE, Galea LA. Pregnancy decreases oestrogen receptor alpha expression and pyknosis, but not cell proliferation or survival, in the hippocampus. *J Neuroendocrinol.* 2010; 22:248–57. [PubMed: 20136685]
57. Scharfman HE, MacLusky NJ. Estrogen and brain-derived neurotrophic factor (BDNF) in hippocampus: Complexity of steroid hormone-growth factor interactions in the adult CNS. *Front Neuroendocrinol.* 2006; 27:415–35. [PubMed: 17055560]
58. Wehling M, Schultz A, Losel R. Nongenomic actions of estrogens: Exciting opportunities for pharmacology. *Maturitas.* 2006; 54:321–6. [PubMed: 16730139]
59. Simoncini T, Genazzani AR. Non-genomic actions of sex steroid hormones. *Eur J Endocrinol.* 2003; 148:281–92. [PubMed: 12611608]
60. Karege F, Perroud N, Burkhardt S, Schwald M, Ballmann E, La Harpe R, Malafosse A. Alteration in kinase activity but not in protein levels of protein kinase B and glycogen synthase kinase-3beta in ventral prefrontal cortex of depressed suicide victims. *Biol Psychiatry.* 2007; 61:240–5. [PubMed: 16876135]
61. Einat H, Yuan P, Gould TD, Li J, Du J, Zhang L, Manji HK, Chen G. The role of the extracellular signal-regulated kinase signaling pathway in mood modulation. *J Neurosci.* 2003; 23:7311–6. [PubMed: 12917364]

62. Eisch AJ, Bolanos CA, de Wit J, Simonak RD, Pudiak CM, Barrot M, Verhaagen J, Nestler EJ. Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: A role in depression. *Biol Psychiatry*. 2003; 54:994–1005. [PubMed: 14625141]
63. Manji HK, Duman RS. Impairments of neuroplasticity and cellular resilience in severe mood disorders: Implications for the development of novel therapeutics. *Psychopharmacol Bull*. 2001; 35:5–49. [PubMed: 12397885]

Research Highlights

- Data suggest strain-dependent differential behavioral sensitivity to estradiol.
- Estradiol produced an anti-depressant behavior in Long Evans rats but not in Wistar rats.
- Estradiol withdrawal induced depressant-like behavior in Wistar rats but not in Long Evans rats.
- No differences in estradiol levels, ER alpha or ER beta protein levels were found between the strains.

Figure 1. Immobility measurements in the forced swim test indicating strain-dependent differential responses to administration or withdrawal of estradiol as well as withdrawal. VEH=vehicle, EST=estradiol, WDL=withdrawal. * = significantly different when compared to corresponding vehicle control group.

Figure 2.

Western Blot results for ER α in cytosol cell fractions (a) from Wistar VEH (frontal cortex n=5/ hippocampus n=5); EST (n=5/5); WDL (n=5/5) and Long Evans VEH (n=4/5); EST (n=4/5); WDL (n=5/5) animals. Nuclear cell fractions (b) from Wistar VEH (n=5/5); EST (n=5/5); WDL (n=5/5) and Long Evans VEH (n=5/4); EST (n=5/5); WDL (n=5/4) animals. Photographic representations of ER α western blots (c). L=Long Evans, W=Wistar, N=nuclear pool, C=cytosolic pool, P=combined pool.

Figure 3.

Western Blot results for ER β in cytosol cell fractions (a) from Wistar VEH (frontal cortex n=5/ hippocampus n=5); EST (n=4/5); WDL (n=5/5) and Long Evans VEH (n=5/4); EST (n=4/5); WDL (n=5/5) animals. Nuclear cell fractions (b) from Wistar VEH (n=5/5); EST (n=5/5); WDL (n=5/5) and Long Evans VEH (n=5/5); EST (n=5/5); WDL (n=3/5) animals. Photographic representations of ER β western blots (c). L=Long Evans, W=Wistar, N=nuclear pool, C=cystolic pool, P=combined pool.

Table I

17 β -Estradiol plasma levels from Wistar and Long Evans ovariectomized female rats treated with oil vehicle, E2, and E2 withdrawal (n = 6 per group).

Treatment	Wistar (E2 pg/ml)	Long Evans (E2)
Vehicle	3.61 $\pm$ 2.97	10.44 $\pm$ 4.65
Estradiol	100.10 $\pm$ 26.48 *	125.80 $\pm$ 36.80 *
Withdrawal	9.32 $\pm$ 3.87	7.00 $\pm$ 3.87

* = p<0.001 significantly different from vehicle and withdrawal groups within each strain.

Table II

Swimming and struggling measurements in the forced swim test. There was an overall strain dependent effect in struggling indicating Long Evans rats struggled more than Wistar rats in all treatments.

	n	Swimming (mean sec $\pm$ SD)	Struggling (mean sec $\pm$ SD)
Wistar			
Vehicle	18	123 $\pm$ 47	57 $\pm$ 35
Estradiol	18	113 $\pm$ 52	53 $\pm$ 28
Withdrawal	17	89 $\pm$ 52	34 $\pm$ 26
Long Evans			
Vehicle	18	92 $\pm$ 32	84 $\pm$ 36
Estradiol	19	100 $\pm$ 25	120 $\pm$ 40
Withdrawal	19	101 $\pm$ 43	92 $\pm$ 50