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# ENVIRONMENTAL ENRICHMENT PREVENTS ANXIETY-LIKE BEHAVIOR INDUCED BY PROGESTERONE WITHDRAWAL IN TWO STRAINS OF RATS

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**Abstract—Stress vulnerability could influence the treatment response to anxiety associated with abrupt hormonal suppression. The present study explored the effects of different treatments on experimental anxiety induced by progesterone withdrawal (PW) in a stress-sensitive rat strain, Wistar Kyoto (WKY), in the burying behavior test (BBT). The following experimental series was conducted using independent groups of Wistar (control strain) and WKY ovariectomized rats: *Experiment 1:* Rats were treated for 5 days with oil, a constant dose of progesterone (0.5 mg/rat, s.c) or a combination of progesterone (0.5 mg/rat, s.c) plus fluoxetine (10 mg/kg, i.p); on day 6, all rats were subjected to BBT. *Experiment 2:* Rats received corn oil or decreasing doses of progesterone (0.84, 0.67, 0.5, 0.33 and 0.17 mg/rat; one dose daily); on day 6, the rats were subjected to BBT. *Experiment 3:* Rats were divided into two groups that were subjected to 30 days of standard conditions or environmental enrichment (EE); from days 25 to 30, all rats received a fixed dose of progesterone (0.5 mg/rat, s.c.) or vehicle. On day 31, the rats were tested with BBT. Results showed that PW increased anxiety in both strains, and fluoxetine prevented anxiety in WKY rats. In contrast, a gradual reduction of progesterone prevents the anxiety in Wistar but not in WKY. EE was preventive against the anxiety induced by PW in both strains of rats. Thus, the results suggest that anxiety induced by PW is prevented by EE while the anxiolytic effect of pharmacological treatments depends on stress vulnerability.** 

**Key words: Wistar Kyoto, progesterone withdrawal, gradual reduction of progesterone, environmental enrichment, fluoxetine, burying behavior test.**

## INTRODUCTION

Abrupt hormonal withdrawal has been considered a factor that may predispose vulnerable women to the development of depressive and anxiety disorders. Therefore, the abrupt decline of ovarian hormones during post-partum (Suda et al., 2008) or the late luteal phase of the menstrual cycle (Ströhle et al., 2000; Pearlstein and Steiner, 2008) may trigger symptoms of anxiety, irritability and depressive moods. Indeed, it is estimated that approximately 35% of women of reproductive age are moderately affected by Premenstrual Syndrome (PMS) and up to 5% meet the criteria for Premenstrual Dysphoric Disorder (PMDD) (Pearlstein and Steiner, 2008).

Experts of the American College of Obstetricians and Gynecologists suggest that the first line therapy for PMS is Selective Serotonin Reuptake Inhibitors (SSRIs), which significantly improve the psychological and physical premenstrual symptoms within the first few cycles of treatment (Eriksson et al., 2008; Shah et al., 2008). Although SSRIs are well tolerated and demonstrate efficacy in approximately 60% of women with PMDD (Halbreich, 2008), their efficacy is not stable after long-term treatment (Kleinstaüber et al., 2012). Furthermore, there are women who are not responsive, and typical medication may worsen their clinical condition (Halbreich et al., 2007). While there are several reports regarding the use of oral progestins for the treatment of PMS, there is not enough evidence to support it (Cunningham et al., 2009). For example, a pilot study reports that a five-day gradual reduction of chlormadinone acetate (progesterone derivative) reduces anxiety and depressive symptoms in a small sample of women with PMS (Contreras et al., 2006); however, this study has not been replicated. Additionally, the use of hormonal therapies to treat PMS may produce adverse events that limit their use, such as thrombosis, breast tenderness, nausea and irregular bleeding (Jarvis et al., 2008). Consequently, the exploration of other strategies to alleviate the symptoms of PMS or PMDD could be useful.

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*Abbreviations:* BBT, burying behavior test; CBT, Cognitive Behavior Therapy; EE, environmental enrichment; HPA, hypothalamus–pituitary-adrenal; PMDD, Premenstrual Dysphoric Disorder; PMS, Premenstrual Syndrome; PW, progesterone withdrawal; SSRIs, Selective Serotonin Reuptake Inhibitors; WKY, Wistar Kyoto.

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Another barely explored strategy to treat PMS or PMDD is lifestyle modification and exercise. Albeit controversial, it was reported that this approach could be useful to treat mild to moderate symptoms (Jarvis et al., 2008; Kleinstaüber et al., 2012). Cognitive Behavior Therapy (CBT) has been proposed as the first alternative for the treatment of mental disorders (Blake et al.,1998; Christensen and Oei, 1995; APA, 2001); however, in a meta-analysis study, it was reported that both CBT and serotonergic antidepressants were not enough for PMS treatments (Kleinstaüber et al., 2012). Therefore, results regarding the management of PMS/PMDD symptoms are still inconclusive.

Recently, it has been suggested that one of the factors that contributes to the surge of PMS/PMDD symptoms in some women is a functional alteration of the hypothalamus–pituitary-adrenal gland that could contribute to stress vulnerability (HPA; Rabin et al., 1990; Crowley and Girdler, 2014). Hence, it is possible to consider that stress vulnerability influences the treatment response to progesterone withdrawal (PW). Based on this assumption, the aim of present study was to explore the effect of different treatments on experimental anxiety induced by PW in a stress-vulnerable strain, Wistar Kyoto (WKY) rats. The SSRI fluoxetine, a hormonal treatment simulating a gradual reduction of progesterone and a non-pharmacological approach with environmental enrichment (EE) were tested. WKY is an animal model for the experimental study of anxiety and depression (Paré and Redei, 1993) that displays exacerbated physiological reactions and HPA axis function to stress stimuli (López-Rubalcava and Lucki, 2000). Wistar rats were used as a strain control in all experiments because most of the treatments have been previously tested in this strain (Ho et al., 2001; Estrada-Camarena et al., 2003; Saavedra et al., 2006). In addition, as an index of HPA activation, the plasmatic corticosterone levels were measured only in animals subjected to the EE protocol due to previous studies showed that EE decreases basal corticosterone levels in WKY but not in Wistar rats (Rosenfeld and Weller, 2012).

## EXPERIMENTAL PROCEDURES

### Subjects

Female Wistar and WKY rats (200–250 g) were housed in polycarbonate cages (5–6 animals per cage). All animals had free access to food and water throughout the experiment and were maintained in the local *vivarium* under a 12-h light–dark cycle (lights on at 10:00 PM) and controlled temperature (23 ± 2 °C). All procedures followed the guidance of the Mexican Official Norm of animal care and handling (NOM-062-ZOO-1999) and Local Institutional Ethics Committee.

### Ovariectomy

A ventral incision was made to remove and expose the ovaries in rats anesthetized with tribromoethanol (2%; 10 ml/kg i.p.). After surgery, the rats were returned to their home cages and remained there for a 3-week recovery period. Ovary elimination was confirmed by visual inspection.

### Burying behavior test (BBT)

The BBT consisted of placing the rodents in a cage ($34 \times 16 \times 24$ cm$^3$) with the floor covered with 2 cm of bedding material and with an electrified shock prod (7 cm long) emerging from the wall. When the rat made contact with the prod, a current up to 0.3 mA was delivered (Pinel and Treit, 1978). Test sessions lasted 10 min and were video-recorded with the objective of quantifying the latency to initiate burying after the first shock, cumulative burying behavior time (defined as pushing the bedding material with the snout or forelimbs forward to the prod), and freezing (cumulative time without any movement after receiving a shock). A reduction in burying behavior and freezing was interpreted as a reduction in anxiety (De Boer and Koolhaas, 2003; Rogel-Salazar and López-Rubalcava, 2011). Prior to the BBT sessions, the rats were habituated to the chamber-test for 10 min, once a day for 3 days in the absence of the shock prod (Rogel-Salazar and López-Rubalcava, 2011).

### Serum corticosterone levels

To quantify corticosterone in serum obtained from animals' trunk blood, specific commercial Radioimmunoassay reagents (TKRC1, Siemens Healthcare Diagnostics Inc., Los Angeles, CA, USA) were used following the manufacturer's instructions. All samples were assayed in duplicate. Intra and inter-assays variations were < 12.2% and < 14.9%, respectively; the sensitivity was 5.7 ng/ml.

### Experimental design

*Experiment 1: Effect of fluoxetine on anxiety-like behavior induced by PW in Wistar and WKY rats.* For this purpose, independent groups of animals were administered the following: (1) vehicle (corn oil, s.c.) for 5 days ($n$ = 7); (2) a constant dose of progesterone (0.5 mg/rat, s.c) for 5 days ($n$ = 7); and (3) a constant dose of progesterone (0.5 mg/rat, s.c) for 5 days, plus fluoxetine (10 mg/kg, i.p.) one dose per day from days 3 to 5 ($n$ = 8). On day 6, all animals were tested with the BBT (Fig. 1). The dose of fluoxetine was selected based on previous reports (Ho et al., 2001; Estrada-Camarena et al., 2003; Li et al., 2012), and the schedule of administration was selected to prevent anxiety induced by PW. The PW schedule was as described by Saavedra et al. (2006), who showed the effectiveness of this treatment in ovariectomized Wistar rats to induce anxiety-like behavior.

*Experiment 2: Effect of the gradual reduction of progesterone on anxiety-like behavior in Wistar and WKY rats.* To test whether the gradual reduction of progesterone is protective against anxiety development (Saavedra et al., 2006), groups of each strain were

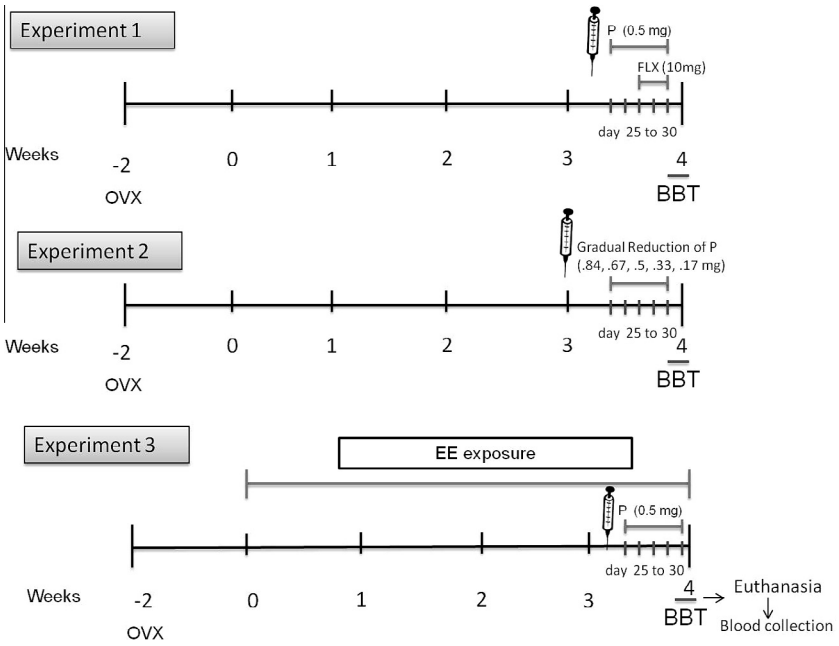


**Fig. 1.** Schematic representation of the experimental design used to evaluate the effect of Fluoxetine (experiment 1), the gradual reduction of progesterone (experiment 2) and the environmental enrichment (experiment 3) on anxiety-like behavior induced by progesterone withdrawal in burying behavior test.

formed as follows: group 1 received vehicle (corn oil, s.c.) for 5 days ($n = 6$), and group 2 received decreasing doses of progesterone (0.84, 0.67, 0.5, 0.33 and 0.17 mg/rat, s.c) one dose per day ($n = 6$). On day 6, all animals were tested with the BBT (Fig. 1). The gradual reduction schedule was as described by Saavedra et al. (2006).

*Experiment 3: Effect of environmental enrichment on anxiety-like behavior induced by PW in Wistar and WKY rats.* To test the effect of a non-pharmacological intervention on anxiety induced by PW, independent groups of rats of each strain ($n = 7$–9 per group) were exposed to EE for 30 days. This procedure consisted of placing the animals in a large black cage (110 × 60 × 50 cm) for 4 h daily with running wheels, wood and plastic objects, ropes of different textures, ramps and balls, all of which were changed daily to ensure novelty (Fox et al., 2006). The control group remained in the *vivarium* (standard condition) for 30 days. From days 25 to 30, all rats received the same treatment of a fixed dose of progesterone (0.5 mg/rat, s.c.) or vehicle as described above. On day 31, all groups were tested with the BBT (Fig. 1).

*Effect of environmental enrichment on corticosterone levels in Wistar and WKY rats.* The plasmatic levels of corticosterone were measured in rats with or without PW treatment that were subjected to EE or the standard condition. For this aim, four animals per group were euthanized by decapitation 60 min after BBT, the trunk blood was collected and centrifuged at 30,000 rpm for 30 min, and serum samples were stored at −20 °C until were processed.

### Statistical analyses

Data were presented as the mean ± S.E.M of time involved in the burying behavior, freezing behavior and latency to show burying behavior; when necessary, the data were normalized to perform parametric statistical analyses. The two-Way ANOVA test was used for all experiments. For experiments one and two, factor A was treatment, and factor B was strain. For experiment three, factor A was exposure to EE, and factor B was exposure to PW. Holm–Sidak test was used as the method of paired comparison. In all cases, $p < 0.05$ was accepted as the significance value.

## RESULTS

### Effect of fluoxetine on anxiety-like behavior induced by PW in Wistar and WKY rats

Fig. 2 shows the effect of PW and PW in combination with fluoxetine on the BBT. As shown in panel A, PW increased the burying behavior time in Wistar ($p < 0.05$) and WKY rats ($p < 0.03$) compared to the respective vehicle group. Fluoxetine prevented the increase of anxiety-like behavior induced by PW only in WKY rats ($p = 0.01$). The two-Way ANOVA test values were as follows: for strain, $F_{1,42} = 5.74$, $p = 0.02$; for treatment, $F_{2,42} = 5.82$, $p = 0.006$; and for the factor interaction, $F_{2,42} = 1.93$, ns.

Fig. 2 (panel B) shows the effect of fluoxetine on freezing behavior. As it can be observed, none of the treatments (PW or PW + FLX) induced significant

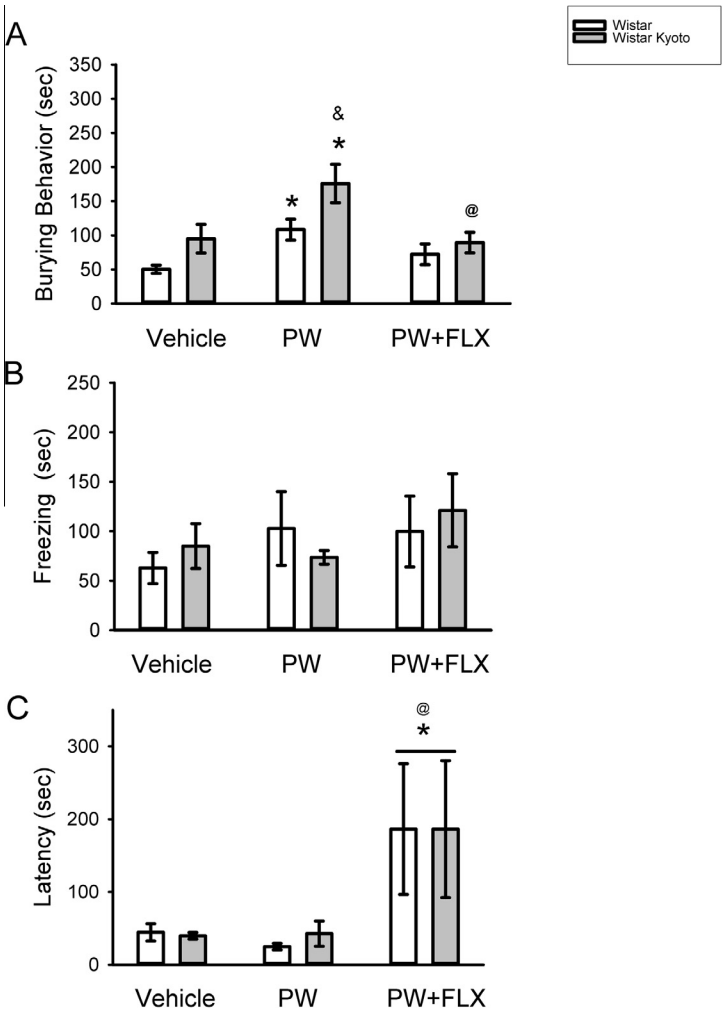


**Fig. 2.** Effect of Fluoxetine on anxiety-like behavior induced by progesterone withdrawal in the burying behavior test. Data are presented as the mean ± S.E.M of cumulative burying behavior (panel A), freezing behavior (panel B) and latency to buying behavior (panel C). Fluoxetine prevents the burying behavior increase induced by progesterone withdrawal (PW) and the latency to show the burying behavior without change freezing. Two-way ANOVA followed by Holm–Sidack test $^{*}p < 0.05$ *versus* vehicle-treated group; $^{\&}p < 0.05$ *versus* Wistar; $^{@}p < .0.05$ *versus* PW-treated group; FLX = fluoxetine.

changes in this variable. The two-Way ANOVA test showed the following values: for strain, $F_{1,42} = 0.46$, ns; for treatment, $F_{2,42} = 0.85$, ns; and for the interaction, $F_{2,42} = 0.74$, ns.

Finally, Fig. 2(panel C) shows the effect of fluoxetine on the latency to display the burying behavior. Fluoxetine significantly increased the latency in comparison to the respective control group in both strains ($p < 0.02$). The two-Way ANOVA test yielded the following values: for strain, $F_{1,42} = 0.90$, ns; for treatment, $F_{2,42} = 4.90$, $p = 0.01$; and for the interaction, $F_{2,42} = 0.96$, ns.

### Effect of the gradual reduction of progesterone on anxiety-like behavior in Wistar and WKY rats

Fig. 3 shows the effect of the gradual reduction of progesterone on developing anxiety-like behavior in Wistar and WKY rats. As can be observed, panel A shows that the gradual reduction of progesterone did not induce changes in the burying behavior of Wistar rats. In contrast, WKY rats subjected to a gradual reduction of progesterone increased burying behavior in comparison to vehicle ($p < 0.05$) or the gradual reduction Wistar groups ($p = 0.01$). The two-Way ANOVA values were as follows: for strain, $F_{1,20} = 6.41$, $p = 0.02$; for treatment, $F_{1,20} = 0.18$, ns; for the interaction, $F_{1,20} = 0.79$, ns.

For freezing behavior (panel B), the gradual reduction of progesterone did not induce changes in the Wistar or the WKY rats. The two-Way ANOVA values were as follows: for strain, $F_{1,20} = 0.04$, ns; for treatment, $F_{1,20} = 1.34$, ns; for the interaction, $F_{1,20} = 2.57$, ns.

In panel C, it is observed that the gradual reduction of progesterone decreased the latency to display burying behavior significantly in WKY rats compared to the control group ($p < p < 0.05$). The two-Way ANOVA values were as follows: for strain, $F_{1,20} = 2.15$, ns; for treatment, $F_{1,20} = 6.38$, $p = 0.02$; for the interaction, $F_{1,20} = 3.43$, ns.

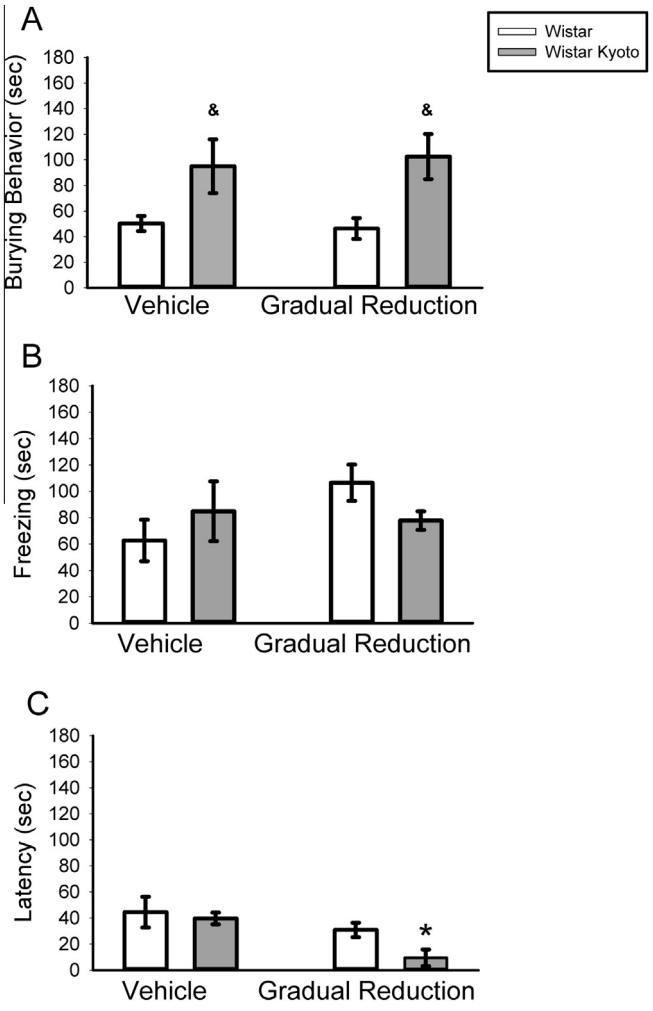


**Fig. 3.** Effect of gradual reduction of progesterone on anxiety-like behavior in the burying behavior test. Data are presented as the mean ± S.E.M of cumulative burying behavior (panel A), freezing behavior (panel B) and latency to buying behavior (panel C). Gradual reduction of progesterone increases the burying behavior and decrease latency in WKY rats without changing freezing. Two-way ANOVA followed by Holm–Sidack test $^{*}p < 0.05$ *versus* vehicle-treated groups; $^{\&}p < 0.05$ *versus* Wistar.

### Effect of environmental enrichment on anxiety-like behavior induced by PW in Wistar and WKY rats

Fig. 4 shows the effect of housing in standard conditions versus with EE on burying behavior (panel A) induced by PW in Wistar rats. In standard conditions, PW tended to increase burying behavior. In contrast, PW did not modify the burying behavior time in EE housed rats. The two-Way ANOVA test values were as follows: for experimental condition, $F_{1,23} = 0.64$, ns; for treatment, $F_{1,23} = 1.34$, ns; for the interaction, $F_{1,23} = 1.04$, ns.

For the freezing behavior (panel B), it was observed that PW increased this effect in the standard condition group ($p < 0.05$) while EE prevents this effect ($p < 0.05$). The two-Way ANOVA test yielded the following values: for experimental condition, $F_{1,23} = 1.22$, ns; for treatment, $F_{1,23} = 0.93$, ns; for the interaction, $F_{1,23} = 5.05$, $p = 0.03$.

Finally, in both the standard and EE conditions, the PW exposure did not modify the latency to burying behavior in Wistar rats. The two-Way ANOVA test values were as follows: for experimental condition, $F_{1,23} = 3.12$, ns; for treatment, $F_{1,23} = 0.25$, ns; for the interaction, $F_{1,23} = 0.55$, ns.

Fig. 5 shows the effects of the standard condition versus EE exposure on burying behavior (panel A) induced by PW in WKY rats. As evident, EE exposure *per se* increased the burying behavior in relation to the standard condition group ($p < 0.01$). PW increased burying behavior in the standard conditions ($p < 0.005$), and this effect was prevented by EE exposure ($p < 0.05$). The two-Way ANOVA test values were as follows: for experimental condition, $F_{1,23} = 1.62$, ns; for treatment, $F_{1,23} = 0.03$, ns; for the interaction, $F_{1,23} = 11.25$, $p = 0.003$.

In contrast, PW decreased freezing (panel B) in both the standard conditions and EE relative to the respective control groups ($p < 0.05$). The two-Way ANOVA test values were as follows: for experimental condition, $F_{1,23} = 6.19$, $p = 0.02$; for treatment, $F_{1,23} = 5.28$, $p = 0.03$; for the interaction, $F_{1,23} = 6.12$, $p = 0.02$.

Finally, in panel C, it is evident that EE, independent of the treatment, increased the latency to display the burying behavior; however, this change was significant only in the

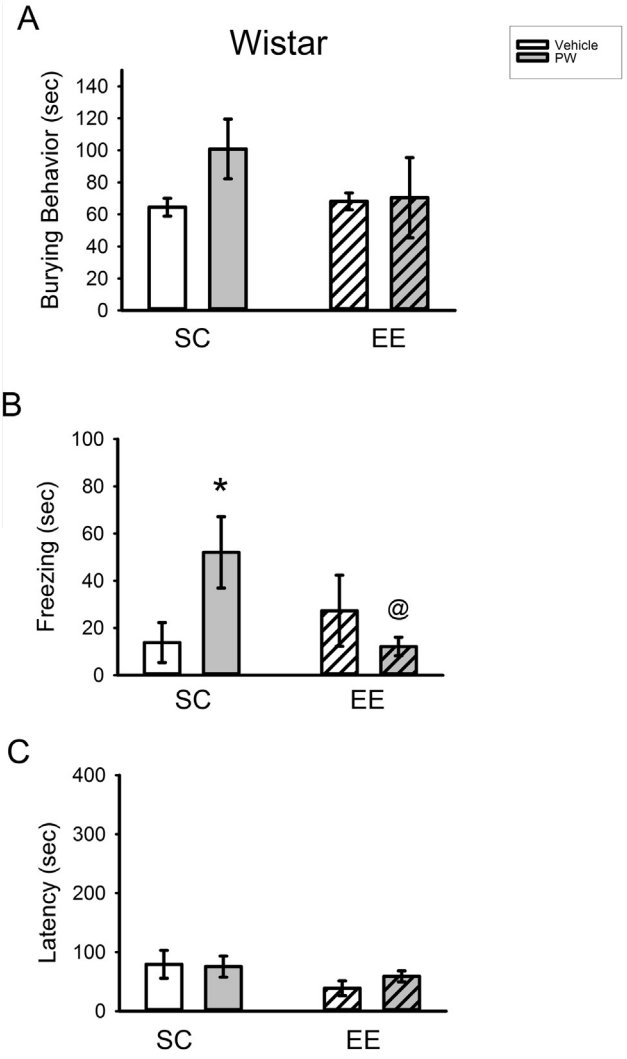


**Fig. 4.** Effect of environmental enrichment on anxiety induced by progesterone withdrawal in burying behavior test in Wistar strain. Data are presented as the mean ± S.E.M of cumulative burying behavior (panel A), freezing behavior (panel B) and latency to buying behavior (panel C). Environmental Enrichment prevents the increase of burying and freezing behavior induced by progesterone withdrawal (PW) without changing latency. Two-way ANOVA followed by Holm–Sidack test $^{*}p < 0.05$ *versus* vehicle; $^{@}p < .0.05$ *versus* PW-treatment in SC. SC = standard condition; EE = environmental enrichment; PW = progesterone withdrawal.

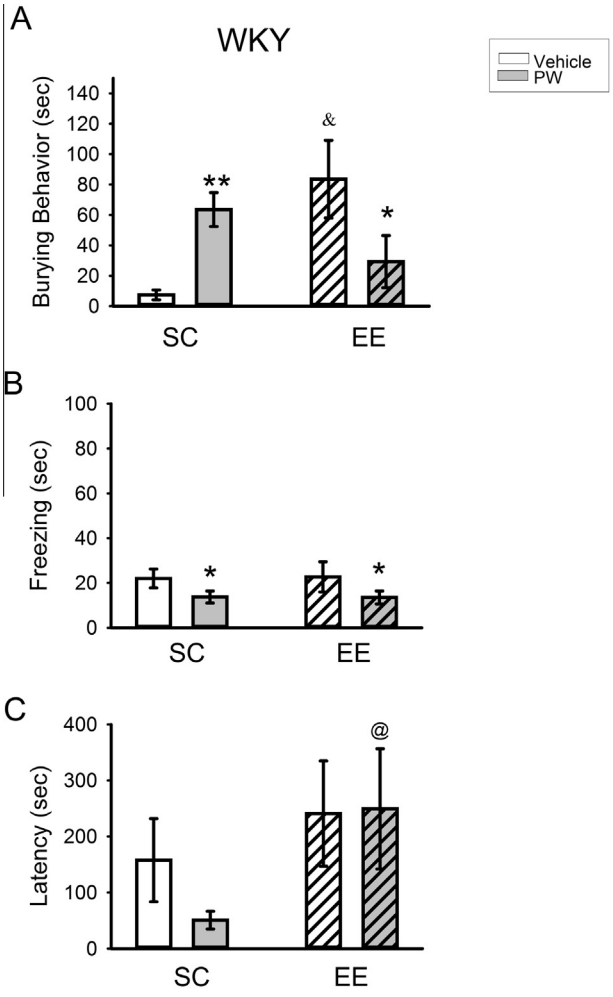


**Fig. 5.** Effect of environmental enrichment on anxiety induced by progesterone withdrawal in burying behavior test in Wistar-Kyoto strain. Data are presented as the mean ± S.E.M of cumulative burying behavior (panel A), freezing behavior (panel B) and latency to buying behavior (panel C). Environmental enrichment prevents the increase of burying behavior without modifying freezing behavior. Environmental enrichment increases the latency to show burying behavior. Two-way ANOVA followed by Holm–Sidack test $^{*}p < 0.05$ and $^{**}p < 0.005$ *versus* vehicle; $^{\&}p < 0.05$ *versus* vehicle in SC; $^{@}p < .0.05$ *versus* PW-treatment in SC. SC = standard condition; EE = environmental enrichment; PW = progesterone withdrawal.

group treated with PW and subjected to EE compared to the standard condition group ($p < 0.05$). The two-Way ANOVA test values were as follows: for experimental condition, $F_{1,23} = 0.133$, ns; for treatment, $F_{1,23} = 7.06$, $p = 0.01$; for the interaction, $F_{1,23} = 0.91$, ns.

### Effect of environmental enrichment on corticosterone levels in Wistar and WKY rats

Table 1 shows the effect of EE on corticosterone levels in Wistar rats exposed to PW. No differences were observed for PW or housing conditions. The two-Way ANOVA test values were as follows: for experimental condition, $F_{1,15} = 0.036$, ns; for treatment, $F_{1,15} = 4.80$, ns; for the interaction, $F_{1,15} = 0.007$, ns.

A specific comparison between W and WKY in the standard condition was made, and WKY rats showed the highest levels of corticosterone ($p < 0.04$). Also in WKY, PW increased corticosterone levels in rats exposed to standard conditions ($p < 0.01$). EE prevented the increase in corticosterone levels in animals subjected to PW. The two-Way ANOVA test values were as follows: for experimental condition, $F_{1,15} = 6.64$, $p < 0.05$; for treatment, $F_{1,15} = 1.01$, ns; for the interaction, $F_{1,15} = 3.63$, $p < 0.05$.

## DISCUSSION

The results can be summarized as follows: PW increased anxiety-like behavior in BBT in both strains, an effect that was prevented by fluoxetine in WKY rats and by EE

**Table 1.** Effect of environmental enrichment on corticosterone levels in Wistar and Wistar-Kyoto rats subjected to PW

| Strain/ treatment | Standard condition | Environmental enrichment |
|---|---|---|
| *Wistar* | | |
| Vehicle | 724.8 ± 13.55 | 717.1 ± 67.14 |
| PW | 569.35 ± 116.73 | 548.77 ± 60.17 |
| *Wistar Kyoto* | | |
| Vehicle | 872.06 ± 58.60 | 729.71 ± 157.64 |
| PW | 1486.95 ± 451.77* | 538.18 ± 145.51@ |

Data are expressed as medium ± SEM of four animals per group. Two way Anova test followed of Holm–Sidack as paired comparison method.
PW = progesterone withdrawal, SC = standard condition.
* $p = 0.05$ versus vehicle group.
@ $p = 0.01$ versus PW-SC group.

exposure in both strains. The gradual reduction of progesterone induced anxiety in WKY rats whereas it prevents in Wistar rats. The data support that anxiety-like behavior induced by PW is prevented by EE while the anxiolytic-like effect of pharmacological treatments depends on stress vulnerability.

PW induced an anxiogenic-like effect reflected by the increase of burying or freezing behavior in Wistar and WKY rats. The present results are in agreement with previous studies in ovariectomized (Saavedra et al., 2006) and intact cycling rats (Gallo and Smith, 1993). BBT offers the opportunity to evaluate different behaviors that resemble an anxiety-like condition in rodents. In this case, after receiving a shock, rats can exhibit active responses, such as burying and compulsive grooming, or passive responses, such as freezing (De Boer and Koolhaas, 2003; Rogel-Salazar and López-Rubalcava, 2011). In fact, WKY expresses anxiety-like behavior by means of passive responses instead of active responses (Pare, 1989, 1992; Paré et al., 2001; Rogel-Salazar and López-Rubalcava, 2011); for this reason, PW effects on freezing were also evaluated. In addition, latency to display burying behavior was evaluated because Gallo and Smith (1993) also considered latency decrease reflexes to be an anxiogenic-like effect. The present results showed that both freezing and latency were not modified by the abrupt hormonal decline. Therefore, it could be suggested that the anxiety trait responsive to the PW challenge was the burying behavior in both strains.

Fluoxetine prevented the anxiety induced by PW in both strains, but the response was conspicuous in WKY strain. The present result is in agreement with a previous report using clomipramine treatment, a serotonergic antidepressant, to reduce anxiety in WKY (Effect of Environmental Enrichment). However, this is in contrast with previous results indicating that WKY rats were unresponsive to serotonergic agents, such as fluoxetine and 8-OHDPAT, in the forced swimming test, which is an animal model for the screening of antidepressants (López-Rubalcava and Lucki, 2000). Hormonal withdrawal models that resemble the premenstrual condition induce depressive-, aggressive- and anxiety-like behaviors (Gallo and Smith, 1993; Ho et al., 2001; Saavedra et al., 2006; Doornbos et al., 2009; Li et al., 2012), and, in several protocols, drugs, such as SSRIs, are able to reverse or prevent some behaviors but are unable to reduce others. Therefore, it is possible that fluoxetine acts on anxiety and irritability but not on depressive-like behavior induced by PW. Furthermore, a previous report showed that fluoxetine, at same dose used in the present report, had no effect on the immobility behavior induced by PW in the forced swimming test (Li et al., 2012) but reduced the aggressive behavior in a premenstrual irritability animal model (Ho et al., 2001). Finally, the fact that the fluoxetine response was more conspicuous in the WKY strain could be due to stress vulnerability, which is a trait of this strain (Redei et al., 1994; Rogel-Salazar and López-Rubalcava, 2011).

Conversely, it has been described that fluoxetine at very low doses (in the micromolar range) acts on enzymes that participate in the synthesis of neurosteroids in animal models of anxiety and depression (Pinna et al., 2009). However, in the present study, the dose of fluoxetine used to prevent the anxiety induced by PW was 10 mg/kg, which makes it unlikely that its anxiolytic-like effect could be related to this mechanism of action.

In agreement with previous reports, the gradual reduction of progesterone did not increase burying and freezing behavior in Wistar rats (Saavedra et al., 2006), supporting that the slow decline of progesterone protects against the development of anxiety-like behavior (Saavedra et al., 2006; Doornbos et al., 2009). Indeed, some reports indicate that rapid changes in gonadal hormones rather than constant levels facilitate mood swings in women (Halbreich, 1997; Contreras et al., 2006; Doornbos et al., 2009). In contrast, in WKY rats, an increase in burying behavior and a reduction in latency behavior were observed, suggesting that, in this strain, anxiety increased with this treatment. The exact mechanism by which the gradual reduction of progesterone exerts its anxiolytic-like effects in one strain but not in another is unknown; however, it was reported that a gradual reduction of progesterone prevents changes in the expression of $GABA_A$/benzodiazepine receptor subunits associated with hormone withdrawal (Smith et al., 1998) and anxiety (Contreras et al., 2006; Saavedra et al., 2006). A trait of WKY rats is the hyper-reactivity to stressful events (Redei et al., 1994) showing high levels of corticosterone (Rittenhouse et al., 2002, and present data), which were further increased by the PW challenge (present data). It has been reported that high corticosterone levels affect the sensitivity of $GABA_A$ receptors (López-Rubalcava et al., 2013), and differences in the binding of $GABA_A$ receptors between WKY and other strain of rats were reported (Lei et al., 2009). Therefore, it is possible that the different response to the gradual reduction of progesterone between the WKY and Wistar rats is related to changes in $GABA_A$ receptor sensitivity that could be facilitated by corticosterone levels. Nevertheless, specific experiments are necessary to confirm this hypothesis.

Conversely, basal differences in the levels of burying behavior were observed in the groups housed in standard conditions, and WKY rats showed lower burying than Wistar rats. Additionally, the differences in basal burying levels in WKY rats suggest that the daily handling routine could affect the basal levels of active behaviors displayed in BBT (Paré and Kluczynski, 1997; Gulinello and Smith, 2003; Li et al., 2012), which is conducive to develop passive strategies to cope with stress. Noteworthy, EE increased burying behavior in WKY rats, suggesting that this housing condition may contribute to the development of an active coping strategy instead of a passive one. In addition, some reports suggest that adopting an active-coping skill is an adaptative response (Roth et al., 2012), which could be considered a component of resilience (Feder et al., 2009), and EE may induce this skill (Lehmann and Herkenham, 2011). Furthermore, the anxiety—like behavior induced by PW in the standard condition was prevented and also decreased by EE. A similar response was observed with corticosterone levels in WKY rats; therefore, it is possible that the reduction in corticosterone levels and the adaption of active behavior is related to the resilience process. However, specific experiments, including rats with no manipulation, are necessary to support this hypothesis. In addition, the present data support the stress vulnerability trait of the WKY strain and provide evidence that this strain is also sensitive to hormonal challenge and EE manipulation.

The effects of EE were less evident in Wistar rats because only freezing behavior was modified, and this variable was not changed by fluoxetine or the gradual reduction of progesterone. Although freezing behavior is not a trait of the Wistar strain under basal conditions, if handling the rats affects coping strategies, it is possible that after manipulation, Wistar rats show anxiety-like behavior using a passive strategy that was then reversed by EE.

The latency to display burying behavior was increased by fluoxetine and EE in both strains. This variable is considered by some authors an index of rodents' reactivity (Rodríguez-Manzo et al., 1999) and reflects an anxiety state (Gallo and Smith, 1993). The increase in latency to display burying behavior induced by fluoxetine and EE could then reflect a decrease in anxiety-like behavior (Gallo and Smith, 1993).

Finally, a weakness of present report is that we used ovariectomized rats to model PW without estradiol restitution, and we observed an increase in anxiety-like behavior in both strains, which is in line with previous reports. From the present results, it is not possible to discard that the lack of estradiol in the milieu could increase the vulnerability to develop anxiety-like behavior or alter the effect of the different treatments. However, a previous report showed that when progesterone is combined with estradiol, the abrupt decline in the combination lacks of anxiogenic effect (Gallo and Smith, 1993). Further, estradiol withdrawal increases dehydroepiandrosterone sulfate in the brain, and it has been suggested that this could decrease the GABAergic tone, precipitating an anxiogenic state (Maayan et al., 2005); however, this behavioral effect did not occur (Gallo and Smith, 1993). In contrast, PW alone at doses of 0.5 mg/rat is able to induce anxiety-like behavior in intact (Gallo and Smith, 1993) and ovariectomized rats without estradiol priming (Saavedra et al., 2006). Furthermore, PW decreased the 5α-reduced metabolite of progesterone, allopregnanolone (Smith et al., 1998), which has been linked with the anxiogenic effect of hormonal withdrawal (Gallo and Smith, 1993; Gulinello and Smith, 2003). Also, in a pilot study comparing intact versus ovariectomized rats, PW induced higher anxiety-like behavior levels in the latter group than in the intact group (data no show). Therefore, albeit the only manipulation of progesterone levels in ovariectomized rats did not completely resemble the PMS endocrine condition, the PW in the absence of estrogens was able to induce an anxiogenic condition that was sensitive to different treatments.

## CONCLUSION

Stress vulnerability influences the pharmacological treatment of anxiety induced by PW. The non-pharmacological approach, EE, was particularly effective in WKY rats, which are hyper-reactive to stress, and could, therefore, represent an alternative to pharmacological approaches for anxiety control.

## AUTHOR CONTRIBUTIONS

D. I.-P. and E. E.-C. conceived and designed the experiments; A. G.-T. and D. I.-P. performed the experiments; D. I.-P., E. E.-C. and A. G.-T. analyzed the data; C. L.-R., J. G.-O., D. I.-P. and E. E.-C. discussed data and D. I.-P. and E. E.-C. wrote the paper.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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