Available online at www.sciencedirect.com
SCIENCE DIRECT
Pharmacology, Biochemistry and Behavior 83 (2006) 130–135
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Differential progesterone effects on defensive burying and forced swimming tests depending upon a gradual decrease or an abrupt suppression schedules

Margarita Saavedra [a], Carlos M. Contreras [a,*], Georgina Azamar-Arizmendi [a,b], Minerva Hernández-Lozano [a]

[a] *Unidad Periférica, Xalapa. Laboratorio de Neurofarmacología, Instituto de Investigaciones Biomédicas, UNAM e Instituto de Neuroetología, Universidad Veracruzana, Av Castelazo Ayala s/n Col. Industrial, 91192 Xalapa, Veracruz, México*
[b] *Unidad de Medicina Familiar No 10. Delegación Veracruz Norte del Instituto Mexicano del Seguro Social, Xalapa, Veracruz., México*

Received 5 August 2005; received in revised form 8 December 2005; accepted 29 December 2005
Available online 17 February 2006

Abstract

A single dose of progesterone reduces the cumulative time in the defensive burying test and the immobility in the forced swim test, whereas the abrupt suppression of repeated doses increases the anxiety indicators. Whether anxiety and despair indicators reduce by a gradually decreased schedule of progesterone is unknown. Therefore, we subjected adult ovariectomized Wistar rats to open field, defensive burying and forced swim tests. One group received a constant schedule of progesterone (0.50 mg, daily), abruptly suppressed (AS) after five days. Another group received a gradual reduction schedule of progesterone (GR: 0.84, 0.67, 0.50, 0.33, 0.17 mg, each day). Control group received vehicle (VEH). The GR group displayed similar crossing in the open field test as the VEH group ($F_{2,19}=8.78$, $p<0.002$), but also the shortest cumulative time in defensive burying ($F_{2,28}=13.3$, $p<0.0001$) and the shortest time in freezing ($F_{2,24}=6.39$, $p<0.006$). In the forced swim test, the GR group displayed the shortest immobility time ($F_{2,19}=12.1$, $p<0.0005$), the lowest number of immobility periods ($F_{2,19}=4.26$, $p<0.03$) and the longest latency to the first period of immobility ($F_{2,1}=4.06$, $p<0.03$). It is concluded that a gradually reduced schedule of progesterone reduces anxiety and despair in the Wistar rat.

Keywords: Anxiety; Depression; Defensive burying test; Forced swim test; GABA receptor; Premenstrual syndrome; Progesterone; Withdrawal syndrome

1. Introduction

Several behavioral observations support the assumption that progesterone exerts antidepressant-like and anxiolytic actions. 1) During the estrous cycle of the rat, the highest plasma level of estradiol and progesterone occurs during the Proestrus–estrus phases (Freeman, 1994; Frye et al., 2000). Precisely during these phases the total time of immobility in the forced swim test of Porsolt et al. (1979) is shorter as compared with the diestrus–metaestrus phases (Contreras et al., 1998). 2) In consistence, systemically injected progesterone reduces immobility (Martínez-Mota et al., 1999). 3) From a physiological point of view, by the 14th day of gestation, when the plasma level of progesterone peaks, the number of reinforcers received in the low rate 72s task reaches its maximum (Molina et al., 2000). In addition, the neuronal activity of the lateral septal nucleus (LSN) is related to motivational and hedonic behavior. For instance: a) the firing rate of the lateral septal neurons decreases during the process of experimental despair in rats (Contreras et al., 2004); b) but increases during the proestrus–estrus phases, showing a similar effect as some antidepressant treatments (Contreras et al., 2000).

In regard to anxiety, the defensive burying test is widely utilized and validated. The behavioral and physiological responses displayed in this paradigm are expressions of normal and functionally adaptive coping patterns; the animals go from active burying to passive freezing (De Boer and Koolhaas, 2003). The measure of the burying latency represents the rat's reactivity and the anxiety level is represented by the cumulative time of burying (Pinel and Treit, 1978). Along the estrous cycle, cumulative burying decreases during Proestrus–estrus (Fernandez-Guasti and Picazo, 1992), while by the 14th day of pregnancy the cumulative time spent in burying is the shortest

* Corresponding author. Tel.: +52 228 8 41 89 00x13613; fax: +52 228 8 41 89 20.
E-mail address: ccontreras@uv.mx (C.M. Contreras).

0091-3057/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.12.016

(Picazo and Fernández-Guasti, 1993), which is related with the highest plasma level of progesterone, but not of estradiol, which in turn peaks by the end of gestation (Tolmacheva et al., 2004). The injection of progesterone (Picazo and Fernández-Guasti, 1995; Martínez-Mota et al., 1999, 2000; Reddy et al., 2005) or allopregnanolone (Bitran et al., 1999; Reddy and Kulkarni, 1999; Gulinello et al., 2003; Shen et al., 2005) reduces the total cumulative burying through actions on the $GABA_A$ receptor since bicuculline or picrotoxin blocks these anxiolytic actions (Reddy and Kulkarni, 1997; Laconi et al., 2001). However, these actions are seemingly independent of the benzodiazepine allosteric site in the GABA receptor, since the injection of flumazenil did not modify the anxiolytic effect of progesterone or allopregnanolone (Gulinello et al., 2002).

Some evidence supports the existence of a withdrawal syndrome for progesterone. Indeed, the abrupt suppression of progesterone diminishes the sedative action of lorazepam as a result of the direct action upon the α-4 subunit of the $GABA_A$ receptor (Moran et al., 1998), and progesterone withdrawal diminishes the threshold for seizures induced by picrotoxin or beta-carbolines in rats (Moran and Smith, 1998). Besides, the withdrawal of long-term progesterone treatment decreases the latency to burying and increases the cumulative time in burying test (Gallo and Smith, 1993), through actions on the $GABA_A$ receptor mediated by the main metabolite of progesterone, i.e., allopregnanolone (Costa et al., 1995).

These observations may be applied to the management of the premenstrual dysphoric disorder (Smith, 2001), which can be taken as a progesterone withdrawal syndrome (Gallo and Smith, 1993). The present study tests the hypothesis that the behavioral effects of a gradually reduced schedule of progesterone (GR) are less than those associated with an abruptly suppressed schedule of progesterone (AS) in animal models of anxiety and depression.

2. Materials and methods

We included 67 female Wistar rats, each weighing 300 g at the beginning of our study. All the animals lived in housing facilities with a light–darkness cycle of 12/12 h (lights ON at 7:00 a.m.), temperature of 24 °C, and ad libitum access to water and food. Ventral bilateral ovariectomy was practiced under ethylic ether anesthesia. After recovery from the anesthesia and once amikacine (Labs Zafiro S.A. de C.V., México) had been applied to the surgical wounds, the animals were returned to their housing facilities. At least two weeks elapsed between surgery and treatments. The experiments were carried out in compliance with the National Institute of Health's Guide for Care and Use of Laboratory Animals (1996).

2.1. Groups

In our study, we distributed the animals in three groups. All treatments were administered daily at 7:00 a.m., in a volume of 0.12 ml/rat. The rats from AS group ($N=29$) received a daily (s.c.), fixed dose of progesterone during 5 consecutive days. From AS group 22 rats underwent the burying test and 7 were forced to swim. The GR group ($N=18$) was also injected daily (s.c.) with progesterone during 5 days, but in a gradually decreased schedule. From GR group 12 rats were submitted to the burying defensive tests and 6 to forced swim test. The remaining group ($N=20$: 13 to burying test and 7 to forced swim test) received five injections (s.c.) of corn oil as vehicle (VEH) during five days.

2.2. Drug treatment

We selected a progesterone dose of 1.5 mg/kg, because it reduces the immobility in the forced swim test and the cumulative time in the defensive burying test (Martínez-Mota et al., 1999, 2000). This dose is similar to that reported by Gallo and Smith (1993) to produce a withdrawal syndrome. In the AS group, we used a fixed dose of 0.5 mg/rat = 1.6 mg/kg (Sigma Chemicals Co. USA), once per day, during 5 consecutive days; thus, each rat received a total amount of 2.5 mg. The GR group received the same total amount of progesterone, but in a gradually decreasing schedule, by reducing one fifth of the initial dose each day, during five days (0.84, 0.67, 0.50, 0.33, 0.17 mg). Behavioral tests began 24 h after the last injection.

2.3. Defensive burying test

At first, each rat was placed in an individual Plexiglas cage (27 × 17 × 15.5 cm) for 72 h before the test session. For the defensive burying test, we used a similar Plexiglas cage. An electrified probe (90 mm, length; 8 mm, ∅) protruded from one of the walls about 2 cm above the bed of sawdust. A stimulator (Grass S-44) coupled in series to an isolation unit (Grass SIU5) and a constant current unit (Grass, CCU1A) delivered direct

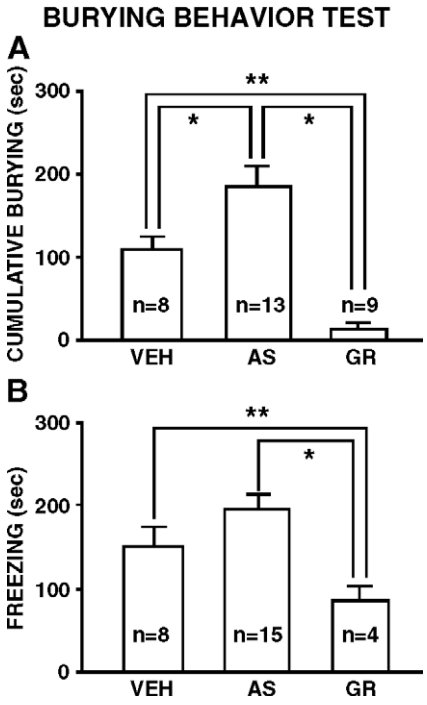

Fig. 1. Burying behavior test. In the gradually reduced progesterone group (GR), the cumulative burying (A) proved to be significantly lesser ($F_{2,28}=13.3$, $p<0.0001$) than the abruptly suppressed (AS) or vehicle (VEH) groups. The lowest time of freezing (B) corresponded to GR group ($F_{2,24}=6.39$, $p<0.006$).

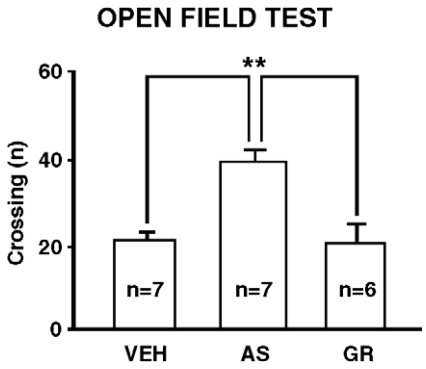

Fig. 2. Open field test. Crossing was significantly ($F_{2,19}=8.78$, $p<0.002$) higher in the AS group. (*$p<0.05$, Student–Newman–Keuls test. Abbrev. as in Fig. 1).

current (0.3 mA) through the probe during the 10-min test. When a rat incidentally touched the electrode, it received an electric shock and after some time (burying latency) it began to displace the sawdust vigorously in order to hide the electrode. Some rats remained quiet, in an expectant attitude (freezing). All sessions were videotaped for subsequent analysis and to measure burying latency and total cumulative burying time, as well as total freezing time.

2.4. Open field test

In order to discard any influence of locomotor activity on swimming, a 5-min open field test preceded the forced swim test. We used an acrylic box (33 × 44 × 20 cm) with the floor divided into squares (11 × 11 cm) to count the number of times each rat crossed a square completely (crossing) with its four paws.

2.5. Forced swim test

In a first 15-min habituation session, not included in the data analysis, each rat was gently placed in a pool (40 × 20 × 60 cm) of water (25 ± 1 °C) at a level that just permitted the animals to touch the bottom with their forepaws. After a period of vigorous swimming, all rats reduced their movements to only those necessary to maintain their head above the water level, without any other displacement (immobility). The 5-min test session began 24 h later. All sessions were videotaped to measure the total time, number of periods and latency to immobility.

2.6. Statistical analysis

The data were grouped according to progesterone schedule and analyzed by one-way ANOVA, using the post hoc Student–Newman–Keuls test, when statistical differences reached $p \leq 0.05$. Results are presented as mean ± standard error.

3. Results

3.1. Defensive burying test

The GR group spent ($F_{2,28}=13.3$, $p<0.0001$) the shortest time in cumulative burying (17.6 ± 6.76 s), significantly different from the vehicle group (111.5 ± 17.44 s) and the AS group (184.8 ± 29.03 s), which displayed the longest time. The longest burying latency occurred in the GR group (84.3 ± 27.24 s) with respect to the other two groups, which displayed similar and shorter latencies (AS: 40.3 ± 22.96 s and vehicle: 35.2 ± 6.9 s, Fig. 1A), although not statistically significant.

The percentage of animals who displayed freezing was also different among the groups. The highest percentage occurred in the AS group (68%), followed by the vehicle (62%), and lastly the GR group which showed the lowest percentage of freezing animals (33%). Consistently, the GR group displayed ($F_{2,24}=6.39$, $p<0.006$) the shortest time in freezing (88 ± 11.3 s), significantly different from the vehicle (153.5 ± 14.9 s), and AS groups, which showed the longest freezing time (197.2 ± 17.1 s, see Fig. 1B).

3.2. Open field test

The crossing in the GR group (21.0 ± 5.25) and the vehicle group (21.0 ± 2.6) was similar, whereas the AS group (39.3 ±

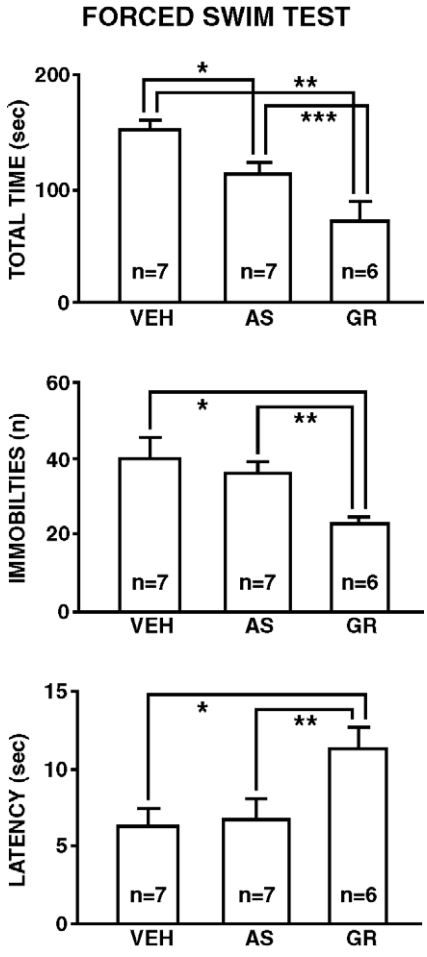

Fig. 3. Forced swim test. The gradual reduction of progesterone schedule (GR) produced: (A) less immobility ($F_{2,19}=12.1$, $p<0.0005$); (B) fewer periods of immobility ($F_{2,19}=12.1$, $p<0.0005$); and (C) longer latency to the first period of immobility ($F_{2,1}=4.06$, $p<0.03$), as compared to other groups. (*$p<0.05$, Student–Newman–Keuls test. Abbrev. as in Fig. 1).

3.34) showed significantly greater crossing ($F_{2,19}=8.78$, $p<0.002$, see Fig. 2).

3.3. Forced swim test

The GR group displayed the shortest total time of immobility (72.3±15.99 s), followed by the AS group (113.7±10.60 s) and then by the vehicle group (153.1±7.53 s). These differences among groups reached the statistical criterion of significance ($F_{2,19}=12.1$, $p<0.0005$). Likewise, the number of immobilities was significantly ($F_{2,19}=4.26$, $p<0.03$) smaller in the GR group (23.7±0.67) than in the AS group (36.4±3.4) and in the vehicle group (40.1±5.6). Lastly, the latency to the first period of immobility was significantly ($F_{2,1}=4.06$, $p<0.03$) longer in the GR group (11.19±1.51 s) than in the AS group (6.63±1.40 s) and in the vehicle group (6.27±1.08 s, Fig. 3).

4. Discussion

The aim of the present study was to determine whether progesterone administered in a schedule of gradual reduction produces different behavioral actions compared to progesterone administration in a schedule of abrupt suppression. We found fewer indicators of anxiety and despair with the gradual reduction of progesterone than with the abrupt suppression schedule.

Majewska (1992) reported that the binding of progesterone to the $GABA_A$ (Weiland and Orchinik, 1995; Follesa et al., 2000) receptor increases the opening frequency of the chloride channel leading to an increased neuronal hyperpolarization. Therefore, the abrupt descent of the plasma level of progesterone possibly associates with hyperexcitability. For instance, barbiturates, benzodiazepines and alcohol are other $GABA_A$ receptor ligands (Faingold et al., 2000; Reilly et al., 2000) that produce physical dependence. A rapid descent in their plasma level is followed by a withdrawal syndrome which is dominated by neuronal hyperexcitability (Watson and Little, 2002; Cagetti et al., 2003; Casasola et al., 2004). During withdrawal, convulsive seizures are commonly observed, but in a less extreme situation the main symptoms are anxiety and tremors. These behavioral changes may be attenuated by a gradual decrease in the plasma levels of addictive drugs to avoid or at least attenuate the withdrawal syndrome (Pinna et al., 1997; Jimenez Ruiz et al., 1999).

The presence of anxiety as a component of the progesterone withdrawal syndrome has been demonstrated before (Gallo and Smith, 1993; Costa et al., 1995; Gulinello et al., 2003). Our present contribution consists in the comparison of an abruptly suppressed schedule of progesterone with a gradual reduction schedule. We confirmed that the abrupt suppression of progesterone increased anxiety indicators, including freezing, which is a passive behavior reflecting extreme anxiety (De Boer and Koolhaas, 2003). On the contrary, the gradual reduction schedule lowered the anxiety indicators (freezing and burying), reaching roughly one-sixth of the values found in the vehicle-treated group. Whereas the rats of the gradual reduction group displayed the lowest freezing values, the abrupt suppression group displayed the highest. These results suggest that the schedule of gradual progesterone reduction effectively decreases the anxiety indicators as compared with the schedule of abrupt suppression.

A similarly encouraging conclusion arises from the forced swim test analysis. The gradual progesterone reduction schedule decreased the total time of immobility, and the number of immobilities by about one-half, and increased the latency to the first period of immobility by two-fold, as compared with the vehicle. The anti-despair action of antidepressants includes a decrease in the time of immobility (Consoli et al., 2005), but they also enlarge the occurrence of the first period of immobility, which represents the strength of the first effort to escape from the stressful situation generated by the forced swim (Contreras et al., 1998, 2000, 2001; Espejo and Miñano, 1999). The abrupt suppression of progesterone also produced a lesser but significant reduction in immobility; in agreement with the anti-despair action exerted by a single dose of progesterone (Contreras et al., 2002) and its antidepressive action in humans (Mortola et al., 1991).

Withdrawal from progesterone induces anxiety (Gallo and Smith, 1993), and during the rat postpartum increases the immobility in the forced swim test (Stoffel and Craft, 2004). A similar change may occur in the premenstrual dysphoric disorder in women (Cronje and Studd, 2002) since there also occurs an abrupt decrease of plasma levels of progesterone and allopregnanolone (Freeman et al., 2002; Smith, 2002; Uziel-Miller and Dresner, 2002; Gulinello et al., 2003). On the contrary, the allopregnanolone content in hippocampus and amygdaline nuclei is higher in animals with lower anxiety and depression indicators in open field, social interaction and forced swim tests (Zimmerberg et al., 2005). Thus, an abrupt decrease of plasma progesterone levels may bear some relationship with the premenstrual syndrome in some susceptible women (Smith, 2002; Uziel-Miller and Dresner, 2002; Gulinello et al., 2003).

Certainly, progesterone is not an addictive drug, but it is noteworthy that the cerebral pathways and neurotransmitter systems involved in drug dependence, withdrawal and craving seem to be different (Lingford-Hughes et al., 2003; Kreek et al., 2005). However, progesterone may behave like many other therapeutic drugs which produce a withdrawal syndrome, in the absence of craving (McGrath et al., 2005). The adaptive changes of the $GABA_A$ receptor are associated with decreased reactivity and coupling towards its ligands, leading to the development of tolerance (Listos and Fidecka, 2005) and probably to withdrawal symptoms. Hence, the withdrawal syndrome to benzodiazepines (Tsuda et al., 1998), alike the abrupt cessation of GABA cortical infusion, is characterized by cortical hyperexcitability (Fukuda et al., 1987; Silva-Barrat et al., 1989; Calixto et al., 1995, 2000; Casasola et al., 2001, 2004), i.e., the opposite to normal actions. Noticeably, flumazenil by itself is unable to promote defensive burying changes (Saldívar-González et al., 2000), however, flumazenil during progesterone withdrawal exerts an anxiolytic action (Smith et al., 1998). Hence, the action of flumazenil depends on the previous state of the receptor.

Progesterone has been assayed in different regimens for premenstrual syndrome management with varying success (Dennerstein et al., 1985; Rapkin et al., 1997; Johnson, 1998). In view of our present results, we conclude that a schedule of

gradual progesterone reduction has anxiolytic and anti-despair effects and may be used in clinic for the management of the premenstrual dysphoric disorder.

Acknowledgements

The authors are grateful to Warren Haid, Irene Marquina and Isabel Pérez Montfort for revising the manuscript. During this investigation M.H.L. and G.A.A. received fellowships from the Consejo Nacional de Ciencia y Tecnología (CONACYT, México, Reg.170667) and the Instituto Mexicano del Seguro Social, (IMSS, México, Reg. 2005/0340), respectively.

References

Bitran D, Dugan M, Renda P, Ellis R, Foley M. Anxiolytic effects of the neuroactive steroid pregnanolone 3a-OH-5b-pregnan-20-one after microinjection in the dorsal hippocampus and lateral septum. Brain Res 1999;850:217–24.

Cagetti E, Liang J, Spigelman I, Olsen RW. Withdrawal from chronic intermittent ethanol treatment changes subunit composition, reduces synaptic function, and decreases behavioral responses to positive allosteric modulators of $GABA_A$ receptors. Mol Pharmacol 2003;63:53–64.

Calixto E, Montiel T, Lemini C, Brailowsky S. Allopregnanolone potentiates a GABA-withdrawal syndrome in the rat cerebral cortex. Neurosci Lett 1995;195(2):73–86.

Calixto E, Lopez-Colome AM, Casasola C, Montiel T, Bargas J, Brailowsky S. Neocortical hyperexcitability after GABA withdrawal in vitro. Epilepsy Res 2000;39(1):13–26.

Casasola C, Bargas J, Arias-Montano JA, Calixto E, Montiel T, Galarraga E, et al. Hippocampal hyperexcitability induced by GABA withdrawal is due to down-regulation of GABA(A) receptors. Epilepsy Res 2001;47 (3):257–71.

Casasola C, Montiel T, Calixto E, Brailowsky S. Hyperexcitability induced by GABA withdrawal facilitates hippocampal long-term potentiation. Neuroscience 2004;126(1):163–71.

Consoli D, Fedotova J, Micale V, Sapronov NS, Drago F. Stressors affect the response of male and female rats to clomipramine in a model of behavioral despair (forced swim test). Eur J Pharmacol 2005;520(1–3):100–7.

Contreras CM, Martínez-Mota L, Saavedra M, Molina M. Desipramine restricts estral cycle oscillations in swimming. Prog Neuro-Psychopharmacol Biol Psychiatry 1998;22:1121–8.

Contreras CM, Molina M, Saavedra M, Martínez Mota L. Lateral septal neuronal firing increases during proestrus–estrus in the rat. Physiol Behav 2000;68:279–84.

Contreras CM, Rodríguez-Landa JF, Gutiérrez-García AG, Bernal-Morales B. The lowest effective dose of fluoxetine in the forced swim test significantly affects the firing rate of lateral septal nucleus neurons in the rat. J Psychopharmacol 2001;15:231–6.

Contreras CM, Saavedra M, Rodríguez-Landa JF, Bernal-Morales B, Gutiérrez-García AG. Neuroquímica de la motivación y la emoción. In: Hernández-González M, editor. Motivación Animal y Humana. México, D.F: Manual Moderno; 2002. p. 39–64.

Contreras CM, Chacón L, Rodríguez-Landa JF, Bernal-Morales B, Gutiérrez-García AG, Saavedra M. Spontaneous firing rate of lateral septal neurons decreases after forced swimming test in Wistar rat. Prog Neuro-Psychopharmacol Biol Psychiatry 2004;28(2):343–8.

Costa AM, Spence KT, Smith SS, French-Mullen JM. Withdrawal from the endogenous steroid progesterone results in $GABA_A$ currents insensitive to benzodiazepine modulation in rat CA1 hippocampus. J Neurophysiol 1995;74:464–9.

Cronje WH, Studd JW. Premenstrual syndrome and premenstrual dysphoric disorder. Prim Care 2002;29(1):1-12 [Clinic in Office Practice].

De Boer SF, Koolhaas JM. Defensive burying in rodents: ethology, neurobiology and psychopharmacology. Eur J Pharmacol 2003;463(1–3):145–61.

Dennerstein L, Spencer-Garner C, Gotts G, Brown JB, Smith MA, Burrows GD. Progesterone and premenstrual syndrome: a double blind crossover trial. Br Med J 1985;290:1617–21.

Espejo EF, Miñano FJ. Prefrontocortical dopamine depletion induces antidepressant-like effects in rats and alters the profile of desipramine during Porsolt's test. Neuroscience 1999;88(2):609–15.

Faingold C, Li Y, Evans MS. Decreased GABA and increased glutamate receptor-mediated activity on inferior colliculus neurons in vitro are associated with susceptibility to ethanol withdrawal seizures. Brain Res 2000;868(2):287–95.

Fernandez-Guasti A, Picazo O. Changes in burying behavior during the estrous cycle: effect of estrogen and progesterone. Psychoneuroendocrinology 1992;17(6):681–9.

Follesa P, Serra M, Cagetti E, Pisu MG, Porta S, Floris S, et al. Allopregnanolone synthesis in cerebellar granule cells: roles in regulation of $GABA_A$ receptor expression and function during progesterone treatment and withdrawal. Mol Pharmacol 2000;57:1262–70.

Freeman ME. The neuroendocrine control of the ovarian cycle of the rat. In: Knobil E, Neill JD, editors. The physiology of reproduction. USA: Raven Press. Ltd; 1994. p. 613–21.

Freeman EW, Frye CA, Rickels K, Martin PA, Smith SS. Allopregnanolone levels and symptom improvement in severe premenstrual syndrome. J Clin Psychopharmacol 2002;22(5):516–20.

Frye CA, Petralia SM, Rhodes ME. Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3alpha,5alpha-THP. Pharmacol Biochem Behav 2000;67 (3):587–96.

Fukuda H, Brailowsky S, Menini C, Silva-Barrat C, Riche D, Naquet R. Anticonvulsant effect of intracortical, chronic infusion of GABA in kindled rats: focal seizures upon withdrawal. Exp Neurol 1987;98 (1):120–9.

Gallo MA, Smith SS. Progesterone withdrawal decreases latency to and increases duration of electrified prod burial: a possible rat model of PMS anxiety. Pharmacol Biochem Behav 1993;46(4):897–904.

Gulinello M, Gong QH, Smith SS. Progesterone withdrawal increases the α4 subunit of the $GABA_A$ receptor in male rats in association with anxiety and altered pharmacology: a comparison with female rats. Neuropharmacology 2002;43(4):701–14.

Gulinello M, Orman R, Smith SS. Sex differences in anxiety, sensorimotor gating and expression of the alpha4 subunit of the $GABA_A$ receptor in the amygdala after progesterone withdrawal. Eur J Neurosci 2003;17(3):641–8.

Jimenez Ruiz CA, Florez Martin S, Ramos Pineda A, Lorza JJ, Hernandez-Mezquita MA, Solano Reina S, et al. Nasal nicotine spray in smoking cessation. Results of a multicenter study. Arch Bronconeumol 1999;35 (11):535–8.

Johnson S. Premenstrual syndrome therapy. Clin Obstet Gynecol 1998;41 (2):405–21.

Kreek MJ, Bart G, Lilly C, Laforge KS, Nielsen DA. Pharmacogenetics and human molecular genetics of opiate and cocaine addictions and their treatments. Pharmacol Rev 2005;57:1-26.

Laconi MR, Casteller G, Gargiulo PA, Bregonzio C. The anxiolytic effect of allopregnanolone is associated with gonadal hormonal status in female rats. Cabrera RJ. Eur J Pharmacol 2001;417:111–6.

Lingford-Hughes AR, Davies SJC, McIver S, Williams TM, Daglish MRC, Nutt DJ. Addiction: imaging in clinical neuroscience. Br Med Bull 2003; 65:209–22.

Listos D, Fidecka MS. Influence of adenosine receptor agonists on benzodiazepine withdrawal signs in mice. Eur J Pharmacol 2005;523:71–8.

Majewska MD. Neuroesteroids: endogenous bimodal modulators of the $GABA_A$ receptor. Mechanism of action and physiological significance. Prog Neurobiol 1992;38:379–95.

Martínez-Mota L, Contreras C, Saavedra M. Progesterone reduces immobility in rats forced to swim. Arch Med Res 1999;30(4):286–9.

Martínez-Mota L, Estrada Camarena E, López Ruvalcaba C, Contreras CM, Fernández-Guasti A. Interaction of desipramine with steroid hormones on experimental anxiety. Psychoneuroendocrinology 2000;25:109–20.

McGrath A, Crome P, Crome IB. Substance misuse in the older population. Postgrad Med J 2005;81:228–31.

Molina M, Contreras CM, Tellez-Alcantara P. Antidepressant-like effects of pregnancy and progesterone in Wistar rats as measured in the differential reinforcement of the low-rate 72s task. Psychopharmacology 2000;151:306–11.

Moran MH, Smith SS. Progesterone withdrawal I: pro-convulsant effects. Brain Res 1998;807(1–2):84–90.

Moran MH, Goldberg M, Smith SS. Progesterone withdrawal II: insensitivity to the sedative effects of benzodiazepine. Brain Res 1998;807:91-100.

Mortola JF, Girton L, Fischer U. Successful treatment of severe premenstrual syndrome by combined use of gonadotropin-releasing hormone agonist and estrogen/progestin. J Clin Endocrinol Metab 1991;72:252A–F.

National Institute of Health's Guide for Care and Use of Laboratory Animals. Washington DC, National Academy Press, 1996.

Picazo O, Fernández-Guasti A. Changes in experimental anxiety during pregnancy and lactation. Physiol Behav 1993;54(2):295–9.

Picazo O, Fernández-Guasti A. Anti-anxiety effects of progesterone and some of its reduced metabolites: an evaluation using the burying behavior test. Brain Res 1995;680(1–2):135–41.

Pinel J, Treit D. Burying as a defensive response in rats. J Comp Physiol Psychol 1978;92(4):708–12.

Pinna G, Galici R, Schneider HH, Stephens DN, Turski L. Alprazolam dependence prevented by substituting with the β-carboline abecarnil. PNAS 1997;94:2719–23.

Porsolt RD, Bertin A, Blavet N, Deniel M, Jalfre M. Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. Eur J Pharmacol 1979;57:201–10.

Rapkin AJ, Morgan M, Goldman L, Brann DW, Simone D, Mahesh VB. Progesterone metabolite allopregnanolone in women with premenstrual syndrome. Obstet Gynecol 1997;90:709–14.

Reddy DS, Kulkarni SK. Differential anxiolytic effects of neurosteroids in the mirrored chamber behavior test in mice. Brain Res 1997;752(1–2):61–71.

Reddy DS, Kulkarni SK. Sex and estrous cycle-dependent changes in neurosteroid and benzodiazepine effects on food consumption and plus-maze learning behaviors in rats. Pharmacol Biochem Behav 1999;62 (1):53–60.

Reddy DS, O'Malley BW, Rogawski MA. Anxiolytic activity of progesterone in progesterone receptor knockout mice. Neuropharmacology 2005;48 (1):14–24.

Reilly MT, Crabbe JC, Rustay NR, Finn DA. Acute neuroactive steroid withdrawal in withdrawal seizure-prone and withdrawal seizure-resistant mice. Pharmacol Biochem Behav 2000;67(4):709–17.

Saldívar-González A, Gómez C, Martínez-Lomelí I, Arias C. Effect of flumazenil and diazepam on transient actions in defensive burying elicited by the social interaction experience in rats. Pharmacol Biochem Behav 2000;66(2):265–73.

Shen H, Gong QH, Yuan M, Smith SS. Short-term steroid treatment increases delta $GABA_A$ receptor subunit expression in rat CA1 hippocampus: pharmacological and behavioral effects. Neuropharmacology 2005;49 (5):573–86.

Silva-Barrat C, Champagnat J, Brailowsky S, Menini C, Naquet R. Relationship between tolerance to $GABA_A$ agonist and bursting properties in neocortical neurons during GABA-withdrawal syndrome. Brain Res 1989;498 (2):289–98.

Smith SS. Premenstrual steroids. Cell Mol Life Sci 2001;58(9):1263–75.

Smith SS. Withdrawal properties of a neuroactive steroid: implications for GABA(A) receptor gene regulation in the brain and anxiety behavior. Steroids 2002;67(6):519–28.

Smith SS, Gong QH, Hsu FC, Markowitz RS, ffrench-Mullen JM, Li X. GABA (A) receptor alpha4 subunit suppression prevents withdrawal properties of an endogenous steroid. Nature 1998;392(6679):926–30.

Stoffel EC, Craft RM. Ovarian hormone withdrawal-induced "depression" in female rats. Physiol Behav 2004;83(3):505–13.

Tolmacheva EA, Chepurnov SA, Chepurnova NE, Kochetkov YA, van Luijtelaar G. Absence seizures during pregnancy in WAG/Rij rats. Physiol Behav 2004;81(4):623–7.

Tsuda M, Shimizu N, Yajima Y, Suzuki T, Misawa M. Hypersusceptibility to DMCM-induced seizures during diazepam withdrawal in mice: evidence for upregulation of NMDA receptors. Naunyn-Schmiedeberg's Arch Pharmacol 1998;357(3):309–15.

Uziel-Miller N, Dresner N. Addressing substance abuse in obstetrics and gynecology. Prim care OB/GYNS 2002;9(3):98-104.

Watson WP, Little HJ. Selectivity of the protective effects of dihydropyridine calcium channel antagonists against the ethanol withdrawal syndrome. Brain Res 2002;930(1–2):111–22.

Weiland NG, Orchinik M. Specific subunit mRNAs of the $GABA_A$ receptor are regulated by progesterone in subfields of the hippocampus. Brain Res Mol 1995;32(2):271–8.

Zimmerberg B, Brunelli SA, Fluty AJ, Frye CA. Differences in affective behaviors and hippocampal allopregnanolone levels in adult rats of lines selectively bred for infantile vocalizations. Behav Brain Res 2005;159 (2):301–11.