Pharmacology, Biochemistry and Behavior 93 (2009) 337–342

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Progestogens and estrogen influence impulsive burying and avoidant freezing behavior of naturally cycling and ovariectomized rats

Danielle C. Llaneza [a], Cheryl A. Frye [a,b,c,d,*]

[a] Dept. of Psychology, The University at Albany-SUNY, Albany, NY USA
[b] Dept. of Biological Sciences, The University at Albany-SUNY, Albany, NY USA
[c] The Center for Neuroscience Research, The University at Albany-SUNY, Albany, NY USA
[d] The Center for Life Sciences Research, The University at Albany-SUNY, Albany, NY USA

ARTICLE INFO

Available online 14 May 2009

Keywords:
Progestogen
Affect
Cognition
Estrogen
Steroid

ABSTRACT

Steroid hormones, progesterone and estradiol, may influence approach and/or anxiety behavior. Female rats in behavioral estrous, have elevated levels of these steroid hormones and demonstrate more approach and less anxiety behavior than do diestrous rats. Ovariectomy obviates these cyclic variations and systemic progesterone and/or estrogen replacement can enhance approach and anti-anxiety behavior. However, the role of progesterone and/or estrogen in mediating impulsive, avoidant and/or fear behaviors requires further investigation. We hypothesized that if progesterone and/or estrogen influences impulsivity and/or fear then rats in behavioral estrous would demonstrate less impulsive behavior in a burying task and freezing behavior in a conditioned fear task than will diestrous rats. Ovariectomized rats administered progesterone and/or estrogen would show less impulsive burying and freezing behaviors than will vehicle-administered rats. Experiment 1: Naturally cycling rats were tested in marble burying or conditioned fear when they were in behavioral estrous or diestrous. Experiment 2: Ovariectomized rats were administered progesterone, estrogen or vehicle, then tested in marble burying or conditioned fear. Results of Experiment 1 show rats in behavioral estrous demonstrate less impulsive burying and less freezing behavior than diestrous rats. Results of Experiment 2 show administration of progesterone or both estrogen and progesterone decreases impulsive burying and each decrease freezing behavior compared to vehicle. Thus, progesterone and/or estrogen may mediate impulsive and/or avoidant behavior.

Published by Elsevier Inc.

1. Introduction

Cyclical changes in estrogen and progestin levels may influence the integration of stimuli in the environment. Levels of estrogen and progestogens change over the estrous cycle, such that levels are high when female rodents are in behavioral estrous, and low when in diestrous. These hormonal changes can be associated with behavioral alterations, particularly approach, anxiety and cognitive behaviors. When rats are in behavioral estrous, approach behaviors are high while anxiety behavior is low, compared to diestrous and intact male rats (Frye et al., 2000). High progesterone levels during the luteal phase are positively correlated with motor coordination and improvement on visual, perceptual and verbal memory (Berman et al., 1997; Broverman et al., 1981; Hampson and Kimura, 1988; Hampson, 1990; Phillips and Sherwin, 1992). Progesterone improves attention, implicit memory and performance on frontal lobe tasks when levels are high (Maki et al., 2002; Solis-Ortiz et al., 2004). Rats in behavioral estrous demonstrate improved cognition in object recognition and object placement tasks (Frye et al., 2007; Walf et al., 2006). When endogenous hormone sources are removed by ovariectomy, estrogen and progestin levels are reduced and the cyclic increases in estrogen and progesterone are no longer observed (Walf et al., 2006). Administration of estrogen and/or progesterone can reinstate hormonal levels to those observed in behavioral estrous and can influence the expression of approach and avoidance behavior (Walf et al., 2006).

When rodents are ovariectomized, approach behaviors toward novelty are decreased, and anxiety is increased compared to that of rats in behavioral estrous (Frye et al., 2000; Frye and Walf, 2004; Walf et al., 2006). The increase in anxiety behaviors observed in ovariectomized rats also may be due to decline in estrogen and/or progestogens. Administration of estrogen and/or progesterone increases approach toward novel stimuli and decreases fear behaviors compared to vehicle (Frye and Walf, 2004; Walf et al., 2006). Furthermore, administration of estrogen and/or progesterone to ovariectomized rats decreases anxiety behaviors in the open field and elevated plus maze tasks (Frye and Walf, 2004). Administration of progesterone to ovariectomized rats post-training increases object memory (Frye and Lacey, 2000). However, administration of high, but

* Corresponding author. Department of Psychology, The University at Albany-SUNY, Life Sciences Research Building 01058, 1400 Washington Avenue, Albany, NY 12222, USA. Tel.: +1 518 591 8839; fax: +1 518 591 8848.
E-mail address: cafrye@albany.edu (C.A. Frye).

0091-3057/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.pbb.2009.05.003

not physiological, dosages of progesterone attenuates estrogen's effects to improve spatial memory consolidation, suggesting physiological levels of progesterone do not counter estrogen's mediation of learning and memory (Harburger et al., 2007). These results demonstrate that motivated behaviors, such as willingness to approach novel stimuli and/or explore novel environments, occur with physiological levels of progesterone and/or estrogen. However, it is important to examine not only effects of steroid hormones on mediating positive pro-approach and anti-anxiety behaviors, but also negative avoidant and/or impulsive behaviors.

Impulsive burying and freezing behaviors in response to aversive stimuli may represent attempts to avoid novel and/or aversive stimuli. These behaviors can be assessed utilizing the marble burying and conditioned fear tasks, respectively. For example, Wistar rats in proestrus and ovariectomized rats bury fewer marbles when exposed to red light and white noise than those in metestrus do (Schneider and Popik, 2007). These findings suggest that steroids may influence the expression of burying behavior. Ovariectomized rats administered systemic progesterone and/or estrogen spend less time freezing after they touch a shock-associated prod compared to vehicle-administered controls (Frye and Walf, 2004). Effects of progesterone in the conditioned fear paradigm can be utilized to assess avoidant behaviors related to contextual aversive stimuli. Progesterone and/or estrogen's ability to mediate freezing behaviors of female rats in response to re-exposure to stimuli associated with aversive stimuli, such as a tone associated with a shock in the conditioned fear paradigm, has not been systematically investigated. Because steroid hormones have been implicated in mediating sex differences in affective processes, it is important to elucidate their effects on avoidance of aversive stimuli. Thus, we conducted experiments to examine the mediating effects of progesterone and/or estrogen on impulsive and avoidant behaviors of intact and ovariectomized rats.

Experiments tested the hypothesis that physiological levels of progesterone and/or estrogen will mediate impulsive burying behavior in the marble burying task and avoidance behavior in the conditioned fear paradigm. It was predicted that during behavioral estrous, when progestogen and estrogen levels are elevated, impulsive burying behavior and fear-related freezing behavior would be less than that observed among diestrous rats. As well, similar effects were expected when progesterone and/or estrogen were systemically administered to ovariectomized rats, compared to rats administered vehicle.

2. Methods

Animal care was in accordance with the Guide for the Care and Uses of Laboratory Animals (National Institute of Health, publication 865-23, Bethesda, MD). These experiments were approved by the Institutional Animal Care and Use Committee.

2.1. Animals and housing

Adult, female Long–Evans rats ~50 days old and between 150 and 200 g were used ($n=88$) from our breeding colony at SUNY-Albany (original stock from Taconic Farms, Germantown, NY). Rats were group housed (3–5 per cage) throughout the study in polycarbonate cages (45×24×21 cm) in a temperature-controlled room (21±1 °C) in the Laboratory Animal Care Facility. The rats were on a 12/12 h reversed light cycle (lights off at 8:00 am) and had *ad libitum* access to food and tap water in their home cages.

2.2. Hormone condition

2.2.1. Surgery

Rats were anesthetized with xylazine (12 mg/kg; Bayer Corp., Shawnee Mission, KS) and ketamine (60 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA). Ventral incisions were made between the ribs and hip, so that the ovaries could be isolated. Some rats had the ovaries ligated and removed, whereas others that were to remain intact, neither had the ovaries ligated, nor removed. After a 1 week recovery period, rats were behaviorally tested in the tasks described below.

2.2.2. Evaluation of estrous cycle phase

Rats that were sham ovariectomized had vaginal epithelium collected daily by lavage between 0800 and 1000 h. Rats that had vaginal cytology characterized by nucleated cells, and that exhibited a pronounced lordosis posture in response to sexually-relevant stimuli (palpation) were considered in behavioral estrous. Diestrous rats had heterogeneous cell types in their vaginal epithelium, were not sexually-responsive to palpation, and had been in behavioral estrous two days prior (Long and Evans, 1922).

2.2.3. Hormone administration

Rats that were ovariectomized (ovx) were administered progesterone (P_4; 4 mg/kg, SC), 17β-estradiol (E_2; 10 μg) or sesame oil vehicle (0.2 cm^3). Results of a dose–response curve study, in which ovx rats were administered vehicle or E_2 (2, 5, 10, 20, and 50 μg/0.2 cm) 44–48 h before behavioral testing demonstrated that 5–10 μg E_2 produces proestrous-like circulating E_2 levels when rats were tested (Walf and Frye, 2005). Within 1 h (and sustained for up to 6 h), this P_4 regimen produces circulating and central progestogen levels within the range that typically occurs during behavioral estrous. Within 24 h of administration of this progesterone regimen, plasma levels reach nadir (Frye and Lacey 2000; Frye et al., 2007).

2.3. Procedure

In Experiment 1, effects of estrous cycle (diestrous vs. behavioral estrous) on marble burying behavior ($n=10$/group) and conditioned fear behavior ($n=12$/group) were evaluated in intact rats. In Experiment 2, effects of P_4 and/or E_2 administration on marble burying ($n=12$/group) and conditioned fear ($n=10$/group) behaviors were assessed in ovx rats. E_2, or vehicle, was administered to ovx rats 44–48 h prior to testing and training. P_4, or vehicle, was administered to ovx rats 3 h before behavioral testing in marble burying or immediately after training in the conditioned fear task. Rats were randomly-assigned to experiments, endogenous and/or exogenous hormone conditions.

2.4. Behavioral testing

One week prior to behavioral testing, rats were handled daily and habituated to different novel environments for 5–10 min. Rats were tested either in marble burying or conditioned fear. During behavioral testing, rats were singly housed, with neither access to food nor water for up to 6 h, during which time their temporary cages were in a darkened, quiet environment. At the beginning of each task, rats were habituated to the apparatus for 5 to 10 min. All data was collected by an observer and/or with a video-tracking system (Any-Maze, Stoelting, Wood Dale, IL), which were 95% concordant.

2.4.1. Marble burying

Impulsive behaviors were assessed per previous methods (Schneider and Popik, 2007) with a marble burying task in which repetitive marble burying was observed. Rats were taken from their home cages and placed individually in a polypropylene, experimental cage (45×24×21 cm), that neither contained food nor water. Nine clear glass marbles (1.5-cm diameter) were evenly spaced in two lines along the short wall of the cage. Marbles were placed on top of 5-cm-deep wood chip bedding (Betachip, Charles River). Time spent actively burying and the number of marbles that were buried, defined as completely covered, was recorded for 10 min.

2.4.2. Conditioned fear paradigm

This task was utilized per previous methods (Edinger et al., 2004; Sanders et al., 2003).

2.4.2.1. Training/aquisition. Prior to training/acquisition, ovx rats received a subcutaneous injection of E_2 or vehicle. Rats were allowed a 5 min habituation period to the apparatus. A 10-second tone was administered, with a shock (0.5 mA) delivered during the last 2 s of the tone. Immediately following discontinuation of shock, a 1-min inter-trial interval began. This sequence was repeated twice more, for a total of 3 acquisition trials. All ovx rats received subcutaneous injection of P_4 or vehicle immediately following the 3 acquisition trials. Flinch and jump responses to shock were recorded for all rats (Edinger et al., 2004).

2.4.2.2. Testing/extinction test. Rats were tested for extinction 4 h following training to allow ample time for progestogen levels to rise following injection. A habituation period of 5 min to the apparatus was allowed again. A 10-second tone was administered (without shock), followed by a 1-min inter-trial interval. This sequence was repeated twice more. Freezing duration (in seconds) was calculated during each 10-second tone. Mean freezing was calculated across trials.

2.5. Radioimmunoassay for steroid hormones

E_2, P_4, and 3α,5α-THP concentrations were measured as described below, using previously reported methods (Frye and Bayon, 1999; Frye et al., 1996).

2.5.1. Radioactive probes

[^{3}H] E_2 (NET-317: specific activity = 51.3 Ci/mmol), P_4 (NET-208: specific activity = 47.5 Ci/mmol), and 3α,5α-THP (NET-1047: specific activity = 65.0 Ci/mmol), were purchased from Perkin-Elmer (Boston, MA).

2.5.2. Extraction of steroids from serum

E_2, P_4, and 3α,5α-THP were extracted from serum with ether following incubation with water and 800 cpms of ^{3}H steroid (Frye and Bayon 1999). After snap-freezing twice, test tubes containing steroid and ether were evaporated to dryness in a Savant. Dried down tubes were reconstituted with phosphate assay buffer to the original serum volume.

2.5.3. Extraction of steroid from brain tissues

E_2, P_4, and 3α,5α-THP were extracted from brain tissues following homogenization with a glass/glass homogenizer in 50% MeOH, 1% acetic acid. Tissues were centrifuged at 3000 ×g and the supernatant was chromatographed on Sepak-cartridges equilibrated with 50% MeOH:1% acetic acid. Steroids were eluted with increasing concentrations of MeOH (50% MeOH followed by 100% MeOH). Solvents were removed using a speed drier. Samples were reconstituted in 300 µl assay buffer.

2.5.4. Set-up and incubation of radioimmunoassay

The range of the standard curves was 0–1000 pg for E_2, and 0–8000 pg for P_4, and 3α,5α-THP. Standards were added to assay buffer followed by addition of the appropriate antibody (described below) and ^{3}H steroid. Total assay volumes were 800 µl for E_2 and P_4 and 1250 µl for 3α,5α-THP. All assays were incubated overnight at 4 °C.

2.5.5. Antibodies

The E_2 antibody (E#244, Dr. G.D. Niswender, Colorado State University, Fort Collins, CO) was used in a 1:40,000 dilution, which generally binds between 40% and 60% of [^{3}H] E_2 (Frye and Bayon, 1999) and bound 48% in the present study. This E_2 antibody has negligible (<1%) cross-reactivity with other steroid hormones including, esterone, 17α-estradiol, P_4, 17-hydroxyprogesterone (Frye et al., 2000). The P_4 antibody (P#337 from Dr. G.D. Niswender, Colorado State University), used in a 1:30,000 dilution, typically binds between 30% and 50% of [^{3}H] P_4 (Frye and Bayon, 1999), and bound 43% in the present study. The P_4 antibody has very low levels (<4%) of cross-reactivity with DHP and 3α,5α-THP (Frye et al., 1996). The 3α,5α-THP antibody (#921412-5, purchased from Dr. Robert Purdy, Veterans Medical Affairs, La Jolla, CA), were used in a 1:5000 dilution, typically bind between 40% and 60% of [^{3}H] 3α,5α-THP (Frye and Bayon, 1999), and bound 51% in the present study. The 3α,5α-THP antibody cross-reacts with 3α-hydroxypregn-4en-20-one (84%) and DHP (11%) and its β isomer (7%), P_4 (6%), and pregnenolone (<2%) (Purdy et al., 1990; Finn and Gee, 1994).

2.5.6. Termination of binding

Separation of bound and free steroid was accomplished by the rapid addition of dextran-coated charcoal. Following incubation with charcoal, samples were centrifuged at 3000 ×g and the supernatant was pipetted into a glass scintillation vial with 5 ml scintillation cocktail. Sample tube concentrations were calculated using the logit–log method of Rodbard and Hutt (Rodbard and Hutt, 1974), interpolation of the standards, and correction for recovery with Assay Zap. The inter- and intra-assay reliability coefficients were: E_2 0.09 and 0.10, P_4 0.12 and 0.13, and 3α,5α-THP 0.13 and 0.15.

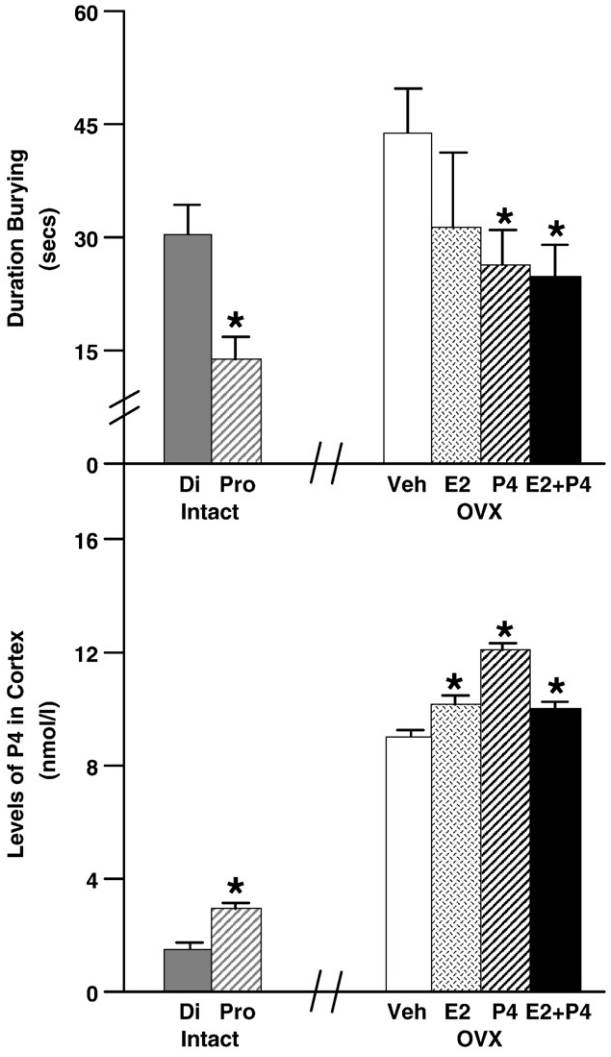

Fig. 1. Top panel indicates that intact rats in behavioral estrous ($n = 10$; pro) and ovariectomized rats ($n = 12$) administered progesterone (P_4) spend less time burying than diestrous (di) or vehicle rats, respectively. Bottom panel depicts mean progesterone levels are higher in the cortex of rats in proestrous and those administered progesterone and/or estradiol. *Indicates $p < 0.05$.

2.6. Statistical analyses

One-way analyses of variance (ANOVAs) were used to examine effects of hormone condition, which in Experiment 1 compared behavioral responses of rats in behavioral estrous and diestrous, and in Experiment 2 evaluated behavioral effects of P_4, E_2 or vehicle administration to ovx rats. The alpha level for statistical significance was $P<0.05$.

3. Results

3.1. Experiment 1a: rats in behavioral estrous exhibit less burying than do diestrous rats

There was a main effect of estrous cycle phase on duration of time spent burying marbles [$F(1,18)=5.77$, $P<0.01$]. As Fig. 1 (top left) shows, rats in behavioral estrous spent significantly less time burying marbles than did diestrous rats. Diestrous rats buried on average 1.5 marbles (SEM = 0.7) in 10 min, whereas rats in behavioral estrous buried on average 1.2 marbles (SEM = 0.5) in the same time.

Rats in behavioral estrous had significantly higher P_4 and E_2 levels in cortex compared to diestrous rats [(Fig. 1) P_4: $F(1,16)=16.41$, $P<0.01$; E_2: $F(1,16)=30.94$, $P<0.01$]. Plasma E_2, P_4 and 3α,5α-THP levels were higher in rats in behavioral estrous compared to diestrous [E_2:$F(1,16)=3.45$, $P<0.10$; P_4:$F(1,16)=3.13$, $P<0.10$; 3α,5α-THP: $F(1,16)=17.93$, $P<0.01$].

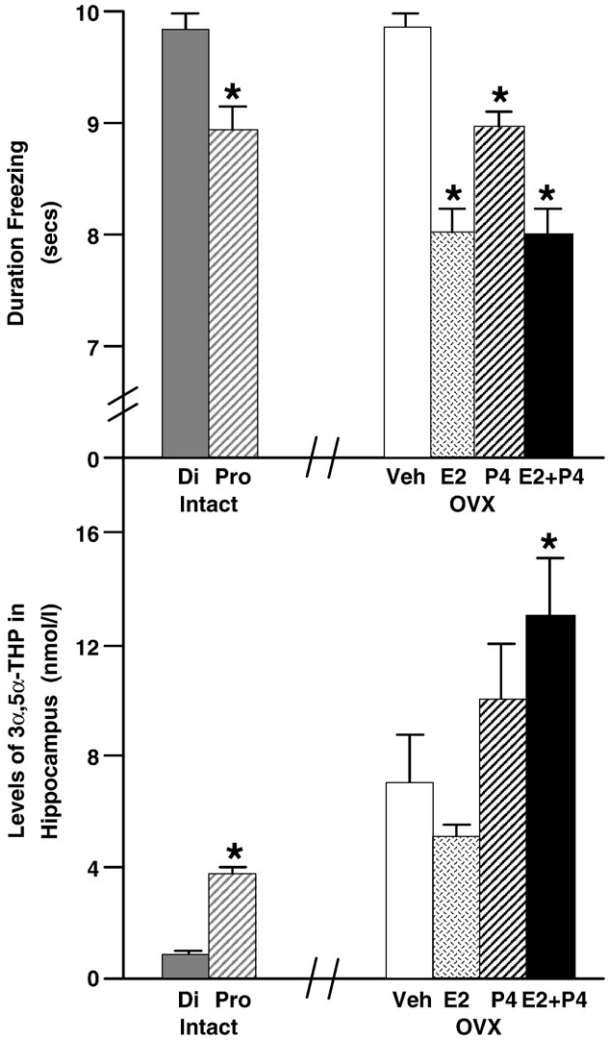

Fig. 2. Top panel shows that intact rats in behavioral estrous ($n=12$) and ovariectomized rats ($n=10$) administered progesterone show less freezing than diestrous or vehicle rats, respectively. Bottom panel depicts that 3α,5α-THP levels are higher in the hippocampus of rats in behavioral estrous (pro) and those administered estradiol and progesterone. *Indicates $p<0.05$.

3.2. Experiment 1b: rats in behavioral estrous spent less time freezing than did diestrous rats

Estrous cycle phase influenced duration of time spent freezing [$F(1,45)=6.68$, $P<0.01$]. As Fig. 2 (top left) shows, rats in behavioral estrous spent significantly less time freezing than did diestrous rats. There was no significant effect of estrous phase on the flinch nor jump thresholds.

Rats in behavioral estrous had significantly higher 3α,5α-THP levels in hippocampus compared to diestrous rats [(Fig. 2) $F(1,16)=47.75$, $P<0.01$]. Furthermore, rats in behavioral estrous had elevated levels of E_2 in hippocampus compared to diestrous rats.

3.3. Experiment 2a: rats administered progesterone and estrogen spend less time burying than do rats administered vehicle

As Fig. 1 (top right) shows, ovariectomized rats administered P_4 or E_2+P_4 spent significantly less time burying marbles than did ovariectomized rats administered vehicle [$F(3,40)=2.23$, $P>0.05$]. Administration of E_2 did not improve burying behavior compared to vehicle. Rats administered P_4, E_2 or both had a significant increase in P_4 levels in cortex compared to rats administered vehicle [$F(3,36)=5.63$, $P<0.01$].

3.4. Experiment 2b: rats administered progesterone and/or estrogen showed less time freezing than did rats administered vehicle

P_4 influenced duration of time spent freezing [$F(3,42)=2.83$, $P<0.05$]. As Fig. 2 (top right) shows, rats administered P_4 and/or E_2 spent significantly less time freezing than did rats administered vehicle. There was no effect of P_4 or E_2 on the flinch nor jump thresholds.

Ovx rats administered E_2+P_4 had higher levels of 3α,5α-THP in hippocampus [$F(3,36)=4.23$, $P<0.01$], and rats administered P_4 or E_2+P_4 had higher levels of P_4 and 3α,5α-THP in plasma compared to rats administered vehicle [P_4: $F(3,36)=65.48$, $P<0.01$; 3α,5α-THP: $F(3,36)=7.70$, $P<0.01$]. Rats administered E_2 or E_2+P_4 had higher levels of E_2 in hippocampus as well [$F(3,36)=2.91$, $P<0.05$].

4. Discussion

Results of the present study supported our hypothesis that estrous cycle and P_4 and/or E_2 administration to ovx rats would influence impulsivity and freezing behavior. Rats in behavioral estrous spent significantly less time burying marbles than did diestrous rats. As well, rats in behavioral estrous spent less time freezing when in a contextual situation associated with shock than did diestrous rats. Rats in behavioral estrous had elevated P_4, E_2 and 3α,5α-THP levels in plasma, and higher P_4 and E_2 levels in cortex, compared to diestrous rats. Furthermore, rats in behavioral estrous had elevated 3α,5α-THP and E_2 levels in hippocampus compared to diestrous rats. Ovx rats administered P_4 or E_2+P_4 spent less time burying marbles than did rats administered vehicle or E_2. As well, P_4 administration decreased freezing when there was contextual association with a shock. Rats administered P_4, E_2 or both had a significant increase in P_4 levels in cortex compared to rats administered vehicle. As well, rats administered E_2+P_4 had higher levels of 3α,5α-THP in hippocampus and rats administered P_4 or E_2+P_4 had higher levels of P_4 and 3α,5α-THP in plasma compared to rats administered vehicle. Rats administered E_2 or E_2+P_4 had higher levels of E_2 in hippocampus as well. There were behavioral differences during diestrous and behavioral estrous, when E_2 and P_4 levels are low and high, respectively. Moreover, administration of P_4 to ovx rats produced similar

patterns of behavior, as was observed in rats in behavioral estrous. This implies that progestogens and/or estrogens may be important hormonal factors that contribute to marble burying, an index of impulsivity, and freezing, a measure of conditioned fear behaviors.

Our findings confirm and extend previous observations regarding endogenous and exogenous effects of progestogens and/or estrogens on impulsive burying in this model. Previous findings demonstrate Wistar rats in proestrus buried fewer marbles (~2.7 marbles) than did those in metestrus (~3.5 marbles), and ovx rats demonstrated burying behavior (~2.6 marbles) similar to proestrous rats (Schneider and Popik, 2007). Our findings extend this work to demonstrate the sensitivity of other measures in the marble burying task. Long–Evans rats in behavioral estrous spent less time burying marbles than did diestrous rats, and ovx rats administered P_4 or both P_4 and E_2 spent less time burying marbles than did rats administered E_2 or vehicle. Although there were differences in the duration of time rats spent burying marbles, there was no significant differences in the number of marbles buried by diestrous, behavioral estrous, ovx, P_4 and/or E_2,or vehicle-administered rats. In our hands, the lack of effect on the number of marbles buried may be attributable to the more stringent burying criteria we utilized (the entire marble, rather than half of it, had to be covered to be considered buried) and/or potential differences between rat strains (we used Long–Evans rats but the earlier investigation was in Wistar rats) in sensitivity to progestogens and/or estrogens.

These findings further extend our knowledge of how P_4 and/or E_2 can mediate anxiety and fear responses. Female rats in behavioral estrous and ovx rats administered systemic P_4 and/or E_2 demonstrated significantly less anxiety in the open field and/or elevated plus maze, compared to diestrous rats, and those administered vehicle, respectively (Frye et al., 2000; Frye and Walf, 2004; Mora et al., 1996; Walf and Frye, 2005). Previous work demonstrated that rats in behavioral estrous, or ovx rats administered P_4, show significantly less burying and freezing after touching a electrified prod, than do diestrous rats or ovx rats administered vehicle (Frye et al., 2000; Frye and Walf, 2004). The present findings extend these results to the marble burying and conditioned fear paradigms, in which rats in behavioral estrous or ovx rats administered P_4 and/or E_2, exhibited significantly less freezing than did those in diestrous or vehicle-administered rats, respectively. These findings suggest high progestogens and/or estrogen levels among intact and/or ovx rats may contribute to immediate responses to novel (marbles) and/or later response to contextual settings that had been paired with aversive (shock) stimuli.

The present findings provide insight into how progestogens and/or estrogens may influence response to novel and/or aversive stimuli. Rats in behavioral estrous spent less time burying than did diestrous rats. Typically, rats in behavioral estrous exhibit more motor, and less anxiety, behavior than do diestrous rats (Frye et al., 2000). Administration of P_4 or E_2 alone to ovx rats does not increase motor activity in the open field, but co-administration does increase motor activity (Frye and Walf, 2004). Whether endogenous increases in progestogens and/or estrogens, or other hormones, that are elevated during behavioral estrous, may have contributed to differences in burying behavior were unclear. Given that ovx rats administered P_4 or both P_4 and E_2 spent less time burying than did their E_2-only or vehicle-administered counterparts, this implies that increases in progestogens and/or estrogens may underlie differences in burying responses to novel stimuli (marbles). However, in the conditioned fear task, progestogen- and estrogen-attributable differences in the flinch/jump responses of intact rats were not observed. Exposure to marbles and shock are very different. Shock is more aversive and cannot be avoided or ignored. As such, differences in aversive response to shock during training likely did not contribute to the freezing that was later observed during testing. Progestogen- or estrogen-related decreases in freezing associated with re-experiencing the contextual setting associated with aversive shock, may be in part due to effects of progestogens and/or estrogens to attenuate anxiety and/or fear. As well, pre-and/or post-training exposure to progestogens or estrogens did not produce amnestic effects in the conditioned fear paradigm. Indeed, progestogens and estrogens can enhance learning and/or memory when present after training in cognitive tasks (Frye et al., 2007; Walf et al., 2006). However, most cognitive tasks examined have assessed willingness to approach novel stimuli, rather than assessing avoidance of aversive stimuli. Performance in the conditioned fear task does not dissociate cognitive effects, from avoidance and/or coping response to aversive stimuli. Lower anxiety behavior generally observed when progestogen and estrogen levels are high may positively influence consolidation of aversive stimuli and later responses. Thus, physiological increases in progestogens and estrogens may enhance approach to novel stimuli, as well as decrease avoidance of aversive stimuli, while consolidating and/or engendering adaptive coping responses.

Progesterone may play a role in mediating anxiety, impulsivity, fear and motor responses, and can produce other non-behavioral effects that are important when considering treatment options in a clinical population. As well, estrogen may be mediating effects on burying and aversive responses to novel and/or aversive stimuli. In intact naturally cycling female rats, progestogen and estrogen levels are high in behavioral estrous, and low in diestrous. Our lab has demonstrated systemic administration of estrogen to ovariectomized rats reduces anxiety in the open field and freezing in response to shock in the defensive freezing task compared to vehicle counterparts (Frye and Walf, 2004). In addition, administration of E_2 at 10 μg or coumestrol at 10 μg, an ERβ selective estrogen receptor modulator (SERM), and post-training in the inhibitory avoidance task result in increased latencies to cross-over to the shock-associated side of the chamber (Rhodes and Frye, 2006). Co-administration of E_2 and P_4 to ovx rats decreases anxiety in the open field and freezing in response to shock in the defensive freezing task (Frye and Walf, 2004). Results from this research extend this to progestogen and/or estrogen's effects on impulsive burying and fear responses, such that administration of P_4 alone or in combination with E_2 to ovx rats reduces impulsive burying in the marble burying task, but estrogen alone does not. Additionally, administration of P_4 and/or E_2 reduces fear responding in the conditioned fear task. This suggests high levels of P_4 and/or E_2 may have actions to reduce impulsivity and fear responding. However, administration of RU38486, a progesterone receptor antagonist, to ovx female rats prior to P_4 injection does not alter P_4 elicited anxiolytic behavior in the elevated plus maze task (Bitran et al., 1995). This suggests P_4 may not be altering burying behaviors or fear responding directly through progesterone receptors.

Progesterone's metabolite, 3α,5α-THP, may also be mediating burying and freezing responses. Results show female Long–Evans rats in behavioral estrous have higher P_4 levels in serum, cortex and hippocampus compared to diestrous female Long–Evans rats. Additionally, ovx female Long–Evans rat administered P_4 at 4 mg/kg have higher P_4 levels in serum, cortex and hippocampus compared to ovx females administered vehicle (Walf et al., 2006). Levels of 3α,5α-THP in plasma, cortex and hippocampus are high when P_4 levels are also high, either in behavioral estrous or by subcutaneous injection of P_4 (Frye et al., 2000; Walf et al., 2006). These levels have been shown to coincide with reductions in anxiety behaviors (Frye et al., 2000). Results indicate high levels of 3α,5α-THP coincide with reductions in impulsive burying and fear responding. High levels of progestogens (P_4 and/or 3α,5α-THP) may mediate burying behavior and/or avoidant behavior as a result of exposure to novel and/or contextually aversive stimuli. Furthermore, E_2 alone can enhance 3α,5α-THP biosynthesis, which may contribute to progestogen and/or estrogen's effects to mediate impulsivity, anxiety, and/or fear responding (Cheng and Karavolas, 1973; Pluchino et al., 2006; Vongher and Frye, 1999). When P_4 is high, performance on tasks that involve the frontal lobe is improved (Solis-Ortiz et al., 2004). The frontal lobe has been

implicated in sustained attention, which can include recognition, alertness and working memory (Bearden et al., 2004; Posner and Raichle 1994; Riccio et al., 2001). Progestogens may improve attention and memory, and influence impulsive and avoidant behaviors. While progestogens may produce positive behavioral effects, physiological effects need to be considered when exploring treatment options. P_4 can attenuate trophic effects of E_2 on the uterus, which in turn reduce the risk for uterine cancer (Beresford et al., 1997). However, high levels of P_4 can produce negative effects as well, such as increasing risk for breast cancer, blood clots, stroke, and heart attack (Thomas et al., 2003). P_4 has been implicated as an agonist of blood platelets, which can contribute to cardiovascular complications associated with P_4 treatments by increasing coagulation and clotting (Blackmore, 2008). Further investigation is needed to parse out P_4's beneficial and harmful effects to better understand risks associated with treatments in clinical populations. Thus, progesterone may improve anxiety, impulsivity, and/or fear behaviors through actions by estrogen and/or 3α,5α-THP. These possibilities need to be further explored to determine possible outcomes of hormonal treatments in clinical populations.

Acknowledgments

This research was supported by grants from the National Science Foundation and The National Institute of Mental Health.

References

Bearden TS, Cassisi JE, White JN. Electrophysiological correlates of vigilance during continuous performance test in healthy adults. Appl Psychophysiol Biofeedback 2004;29:175–88.
Beresford SA, Weiss NS, Voigt LF, McKnight B. Risk of endometrial cancer in relation to use of oestrogen combined with cyclic progestagen therapy in postmenopausal women. Lancet 1997;349:458–61.
Berman KF, Schmidt PJ, Rubinow DR, Danaceau MA, Van Horn JD, Esposito G, et al. Modulation of cognition-specific cortical activity by gonadal steroids: a positron-emission tomography study in women. PNAS 1997;94:8836–41.
Bitran D, Shiekh M, McLeod M. Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain GABAA receptors. J Neuroendocrinol 1995;7:171–7.
Blackmore PF. Progesterone metabolites rapidly stimulate calcium influx in human platelets by a src-dependent pathway. Steroids 2008;73:738–50.
Broverman DM, Vogel W, Klaiber EL, Majcher D, Shea D, Paul V. Changes in cognitive task performance across the menstrual cycle. J Comp Physiol Psychol 1981;95:646–54.
Cheng YJ, Karavolas HJ. Conversion of progesterone to 5α-pregnane-3,20-dione and 3α-hydroxy-5α-pregnan-20-one by rat medical basal hypothalami and the effects of estradiol and stage of estrous cycle on the conversion. Endocrinology 1973;93:1157–62.
Edinger KL, Lee B, Frye CA. Mnemonic effects of testosterone and its 5alpha-reduced metabolites in the conditioned fear and inhibitory avoidance tasks. Pharmacol Biochem Behav 2004;78:559–68.
Finn DA, Gee KW. The estrus cycle, sensitivity to convulsants and the anticonvulsant effect of a neuroactive steroid. J Pharmacol Exp Ther 1994;271:164–70.
Frye CA, Bayon LE. Mating stimuli influence endogenous variations in the neurosteroids 3α,5α-THP and 3α-diol. J Neuroendocrinol 1999;11:839–47.
Frye CA, Lacey EH. Progestins influence performance on cognitive tasks independent of changes in affective behavior. Psychobiology 2000;28:550–63.
Frye CA, Walf AA. Estrogen and/or progesterone administered systemically or to the amygdala can have anxiety-, fear-, and pain-reducing effects in ovariectomized rats. Behav Neurosci 2004;118:306–13.
Frye CA, McCormick CM, Coopersmith C, Erskine MS. Effects of paced and non-paced mating stimulation on plasma progesterone, 3α-diol and corticosterone. Psychoneuroendocrinology 1996;21:431–9.
Frye CA, Petralia SM, Rhodes ME. Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3a,5a-THP. Pharmacol Biochem Behav 2000;67:587–96.
Frye CA, Duffy CK, Walf AA. Estrogens and progestins enhance spatial learning of intact and ovariectomized rats in the object placement task. Neurobiol Learn Mem 2007;88:208–16.
Hampson E. Estrogen-related variations in human spatial and articulatory–motor skills. Psychoneuroendocrinology 1990;15:97-111.
Hampson E, Kimura D. Reciprocal effects of hormonal fluctuations on human motor and perceptual–spatial skills. Behav Neurosci 1988;102:456–9.
Harburger LL, Bennett JC, Frick KM. Effects of estrogen and progesterone on spatial memory consolidation in aged females. Neurobiol Aging 2007;28:602–10.
Long JA, Evans HM. Oestrus cycle in the rat and its associated phenomena. Mem Univ Calif 1922;6:1-146.
Maki PM, Rich JB, Rosenbaum RS. Implicit memory varies across the menstrual cycle: estrogen effects in young women. Neuropsychologia 2002;40:518–29.
Mora S, Dussaubat N, Diaz-Veliz G. Effects of the estrous cycle and ovarian hormones on behavioral indices of anxiety in female rats. Psychoneuroendocrinology 1996;21:609–20.
Phillips SM, Sherwin BB. Variations in memory function and sex steroid hormones across the menstrual cycle. Psychoneuroendocrinology 1992;17:497–506.
Pluchino N, Luisi M, Lenzi E, Centofanti M, Begliuomini S, Freschi L, et al. Progesterone and progestins: effects on brain, allopregnanolone and β-endorphin. J Steroid Biochem Mol Biol 2006;102:205–13.
Posner MI, Raichle ME. Images of mind. New York: Freeman; 1994.
Purdy RH, Moore Jr PH, Rao PN, Hagino N, Yamaguchi T, Schmidt P, et al. Radioimmunoassay of 3α-hydroxy-5α-pregnan-20-one in rat and human plasma. Steroids 1990;55:290–6.
Rhodes ME, Frye CA. ERβ-selective SERMs produce mnemonic-enhancing effects in the inhibitory avoidance and water maze tasks. Neurobiol Learn Mem 2006;85:183–91.
Riccio CA, Reynolds CR, Lowe PA. Clinical applications of continuous performance tests. New York: Wiley; 2001.
Rodbard D, Hutt DM. Statistical analysis of radioimmunoassay and immunoradiometric assays: a generalized, weighted iterative, least squares method for logistic curve fitting. In: International Atomic Energy Agency, editor. Symposium on radioimmunoassay and related procedures in medicine. New York: Uniput; 1974. p. 209–23.
Sanders MJ, Wiltgen BJ, Fanselow MS. The place of the hippocampus in fear conditioning. Eur J Pharmacol 2003;463:217–23.
Schneider T, Popik P. Attenuation of estrous cycle-dependent marble burying in female rats by acute treatment with progesterone and antidepressants. Psychoneuroendocrinology 2007;32:651–9.
Solis-Ortiz S, Guevara MA, Corsi-Cabrera M. Performance in a test demanding prefrontal functions is favored by early luteal phase progesterone: an electroencephalographic study. Psychoneuroendocrinology 2004;29:1047–57.
Thomas T, Rhodin J, Clark L, Garces A. Progestins initiate adverse events of menopausal estrogen therapy. Climacteric 2003;6:293–301.
Vongher JM, Frye CA. Progesterone in conjunction with estradiol has neuroprotective effects in an animal model of neurodegeneration. Pharmacol Biochem Behav 1999;64:777–85.
Walf AA, Frye CA. Antianxiety and antidepressive behavior produced by physiological estradiol regimen may be modulated by hypothalamic-pituitary-adrenal axis activity. Neuropsychopharmacology 2005;30:1288–301.
Walf AA, Rhodes ME, Frye CA. Ovarian steroids enhance object recognition in naturally cycling and ovariectomized, hormone-primed rats. Neurobiol Learn Mem 2006;86:35–46.