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Involvement of pregnane xenobiotic receptor in mating-induced allopregnanolone formation in the midbrain and hippocampus and brain-derived neurotrophic factor in the hippocampus among female rats

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Abstract

Rationale—Given that the pregnane neurosteroid, 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP), is increased following behavioral challenges (e.g. mating), and that there is behavioral-induced biosynthesis of 3 α ,5 α -THP in midbrain and mesocorticolimbic structures, 3 α ,5 α -THP likely has a role in homeostasis and motivated, reproduction and reproduction-related behaviors (e.g. affect, affiliation). The role of pregnane xenobiotic receptor (PXR), involved in cholesterol metabolism, for these effects is of continued interest.

Objectives—We hypothesized that there would be differences in brain levels of 3 α ,5 α -THP following varied behavioral experiences, an effect abrogated by knockdown of PXR in the midbrain.

Methods—Proestrous rats were infused with PXR antisense oligonucleotides (AS-ODNs) or vehicle to the VTA before different behavioral manipulations and assessments. Endpoints were expression levels of PXR in the midbrain, 3 α ,5 α -THP and ovarian steroids (estradiol, progesterone, dihydroprogesterone) in the midbrain, striatum, hippocampus, hypothalamus, prefrontal cortex and plasma.

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Results—Across experiments, knocking down PXR reduced PXR expression and 3 α ,5 α -THP levels in the midbrain and hippocampus. There were differences in terms of the behavioral manipulations, such that paced mating had the most robust effects to increase 3 α ,5 α -THP levels and reduce open field exploration and social interaction. An additional question that was addressed is whether brain derived neurotrophic factor (BDNF) is a downstream factor for regulating effects of behavioral-induced 3 α ,5 α -THP biosynthesis. Rats infused with PXR AS-ODNs had lower levels of BDNF in the hippocampus.

Conclusion—Thus, PXR may be a regulator of mating-induced 3 α ,5 α -THP formation and behavioral changes and neural plasticity, such as BDNF.

Keywords

PXR; brain derived neurotrophic factor- BDNF; midbrain; hippocampus; progesterone; neurosteroid; mating

Introduction

Neurosteroids have well-described effects for reproduction behaviors as well as those that are considered to be reproduction-relevant, such as motivation, affect, affiliation, and cognition, following production and paracrine effects in brain regions important for these behaviors. For example, one such neurosteroid, 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP; a.k.a. allopregnanolone), is increased in rat brain following exposure to acute challenges/stressors, such as swim stress (Barbaccia et al., 1996; Purdy et al., 1991; Vallée et al., 2000), but decreased with chronic stressors, such as social isolation (Agís-Balboa et al., 2007; Bortolato et al., 2011; Dong et al., 2001; Matsumoto et al., 1999; Nin et al., 2011; Pibiri et al., 2008; Pinna and Rasmusson, 2012; Serra et al., 2004). These and other data suggest that neurosteroids, such as 3 α ,5 α -THP, may have an important role for regulating the stress response.

Mating has been utilized as an ecologically-relevant laboratory challenge to further ascertain this potential role of 3 α ,5 α -THP as a homeostatic regulator of the stress response and behavioral consequences. For example, with the paced mating task, female rats can “pace,” or control the timing of, their sexual contacts with males by leaving the vicinity of the male following a contact and then returning; this pacing increases fertility and fecundity of female rats, compared to standard mating, which occurs in a small chamber that precludes the female pacing (Frye and Erskine, 1990). Rapidly after paced mating, 3 α ,5 α -THP is increased in the midbrain ventral tegmental area (VTA), one site of the novel, paracrine effects of 3 α ,5 α -THP for reproductive behaviors of female rodents (reviewed in Frye, 2011). The rapidity of the rise in midbrain 3 α ,5 α -THP levels, and independence of ovaries and/or adrenals as sources of steroids, suggests that biosynthesis underlies mating-induced increases that we have observed for female rats and other rodents (reviewed in Frye, 2011). Beyond paced mating increasing 3 α ,5 α -THP in the VTA, which is also a well-known region of the reward pathway, this experience produces behavioral-induced biosynthesis of 3 α ,5 α -THP in other structures of the mesocorticolimbic circuit, including the striatum, hippocampus, and cortex (reviewed in Frye, 2011) and changes in other motivated behavior (e.g. conditioned place preference; Frye et al., 1998). Thus, there is rapid behavioral-induced

biosynthesis of neurosteroids, such as 3 α ,5 α -THP, and likewise 3 α ,5 α -THP can have rapid actions in reward and limbic regions to modulate these behaviors. The role of behavior-induced biosynthesis and plasticity mediated by 3 α ,5 α -THP in these circuits is of continued interest.

One question about paced mating-induced biosynthesis of 3 α ,5 α -THP is the mechanisms for these effects. Recent studies have begun to address the role of pregnane xenobiotic receptor (PXR) in biosynthesis of 3 α ,5 α -THP from cholesterol in the brain. PXR has well-described effects for cholesterol metabolism and xenobiotic clearance in peripheral targets, such as the liver (Dussault and Forman, 2002; Francis et al., 2003; Geick et al., 2001; Kliewer et al., 2002). The role of PXR for these effects on metabolism and clearance in the brain have been garnering greater attention, especially in considering passage of compounds through the blood-brain-barrier and drug and steroid metabolism in the brain involving P450 enzymes that PXR regulates (Bauer et al., 2004, 2006; Ma et al., 2008; Ott et al., 2009; Xu et al., 2005; Zhang et al., 2008). There is further evidence to suggest that the PXR, which is ubiquitous in the liver, may have a defined action in the brain. PXR has been identified in the brain across many species (e.g. humans, pigs, rabbits, and rodents; Bauer et al., 2004; Frye, 2011; Lamba et al., 2004; Marini et al., 2007; Mellon et al. 2008). Additional evidence that is may be involved in a homeostatic mechanism involving neurosteroids is that knocking down its expression specifically in the midbrain reduces formation of 3 α ,5 α -THP in this region over the estrous cycle as well as attenuates the response to 3 α ,5 α -THP administration to the midbrain for reproductive behavior of female rats (Frye et al., 2013a; 2014). These experiments suggested the role of PXR in the midbrain VTA that is required for 3 α ,5 α -THP's effects on reproductive and reproduction-relevant behaviors among female rats, but they did not address whether engaging in these behaviors may alter 3 α ,5 α -THP levels in the brain due to actions involving PXR. In considering behavioral plasticity, in naturalistic mating situations, mating for female rodents requires suppressed anxiety and aggressive responses to allow social interactions that may result in mating opportunities. 3 α , 5 α -THP, from peripheral sources, may reduce anxiety and/or aggression so that exploration and social interactions can be initiated and maintained. Then central 3 α ,5 α -THP, from biosynthesis, may buffer stress in response to behavioral/environmental stimuli, so that further interactions can occur.

How these changes may be related to downstream mechanisms involving factors involved in neural plasticity, such as brain derived neurotrophic factor (BDNF), is of interest. As with the case of PXR, BDNF is highly conserved, being first identified in pig, and subsequently characterized in many species, including rodents and humans (Barde et al., 1982; Klein et al., 2011). BDNF is produced in glia and neurons (Causing et al., 1997; Pruginin-Bluger et al., 1997), and has well-described effects for neurogenesis and cell survival, synaptic plasticity, cognition as well as stress-related affective disorders and their treatment (reviewed in: Pluchino et al., 2013; Singh & Su, 2013) as does 3 α ,5 α -THP (e.g. panic disorder, Brambilla et al., 2005). A question is whether BDNF may be downstream of 3 α , 5 α -THP for its effects for behavioral plasticity, such as those observed in reproductive, affective, and cognitive responses of rodents.

The present experiments compared effects of PXR manipulations in the midbrain VTA of proestrous rats exposed to different behavioral manipulations, in the complete or partial battery of tasks that has been previously utilized to assess the role of 3 α ,5 α -THP and PXR (Frye et al., 2013a; 2014). Mating is characterized by exploration and affiliations, social behaviors that bring individuals together, as well as inhibition of anxiety and aggressive behaviors that keep individuals apart. Paced and standard mating may diverge in these parameters. To address how midbrain 3 α ,5 α -THP is altered by paced versus standard mating, and the role of PXR in these effects, paced and standard mating, as well as exploratory, anxiety, and social interaction, were assessed in the present series of experiments. Experiments tested the following hypotheses. Experiment 1 tested the hypothesis that PXR AS-ODN would inhibit open field exploration, time on the open arms of the elevated plus maze, social interaction, and lordosis in a paced mating context. Additionally, Experiment 1 tested the hypothesis that mating elevates levels of 3 α ,5 α -THP, an effect mediated by the PXR. Experiments 2 and 3 tested the hypothesis that paced mating would induce greater lordosis quotients and proceptivity, less aggression, and higher 3 α ,5 α -THP than does standard mating, effects mediated by the PXR. Experiment 3 further tested the hypothesis that when compared to standard mating, paced mating would result in more open field activity, more time on the open arms of the elevated plus maze, increased social interaction, and greater levels of 3 α ,5 α -THP. Moreover, it was predicted that PXR manipulations in the midbrain VTA would reduce BDNF levels (measure of neural plasticity) in the hippocampus.

Materials and Methods

All methods utilized were pre-approved by the Institutional Animal Care and Use Committee at the institution where the experiment was conducted (The University at Albany-SUNY). United States laws governing use of animal subjects and the Principles of laboratory animal care ("Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research"; National Research Council 2003), were followed in completing the experiments described herein. Figure 1 depicts the manipulations and measures collected across the three experiments.

Experimental Animals and Housing

Adult (55–60 day old), Long-Evans female rats (N=132), were obtained from breeding colony in the Life Sciences Laboratory Animal Core Facility at The University at Albany-SUNY (original stock obtained from Taconic, Germantown, NY, USA) or purchased from Taconic, to be utilized as the experimental rats in this study. Experimental rats were group-housed in polycarbonate cages (45 cm long $\times$ 24 cm wide $\times$ 21 cm high) with autoclaved woodchip bedding. Rats had continuous access to Purina Rat Chow and tap water, both of which were suspended in their cage tops, when they were housed in a dedicated room in the Animal Core Facility. This housing room was maintained at 21 ± 1 °C on a 12:12 h reversed light cycle (lights off at 08:00 AM), such that rats were behaviorally assessed during their dark/active phase (between 12:00 PM and 3:00 PM).

Surgical Protocol

Experimental rats underwent stereotaxic surgery under general anesthesia produced by systemic administration of a xylazine (12 mg/kg) and ketamine (80 mg/kg) cocktail. Stereotaxic surgery was performed on each subject to implant bilateral guide cannulae aimed at the VTA, with coordinates from bregma (-5.3 mm posterior, ± 0.4 mm lateral, -7.0 mm ventral; Paxinos and Watson, 1986; Frye et al 2013). Rats were allowed to recover immediately after surgery in a warm room (27° C), with constant monitoring. When they regained consciousness, rats were administered a loading dose of analgesic (ibuprofen). Post-operative analgesic (ibuprofen) was provided in water bottles *ad libitum* for 5 days post-surgery and recovery from surgery (neurological exams and body weight gain) was assessed daily for subjects (Frye et al., 2014). All rats passed neurological evaluations and gained weight following surgery and were included in the experiment.

Estrous Cycle

Rats had daily assessments of their estrous cycle phase by examining with light microscopy prominent cell types in vaginal smears obtained (Frye et al., 2013). Rats were cycled through two normal estrous cycles (4–5 day cycle) prior to testing. In all experiments, rats were infused with PXR AS-ODNs or saline control beginning in the metestrous phase so that they were assessed two days later when in proestrus.

Somatic manipulations- antisense oligonucleotide infusions

Rats were infused once a day for three days (0, 24 and 44 hours before behavioral manipulations). Rats were infused with 1 μ l control (sterile saline vehicle) or PXR AS-ODN (purchased from Invitrogen Life Technologies, Carlsbad, CA; as described in Frye et al., 2014).

Behavioral manipulations- exposure to behavioral tests

In Experiment 1 (n=66), comparisons were made between rats that were not-tested (home cage controls), tested in the full battery (consecutive testing in the open field, elevated plus maze, social interaction, and paced mating task), the battery without paced mating (consecutive testing in the open field, elevated plus maze, and social interaction), or paced mating alone. In Experiment 2 (n=40), rats were not tested, standard mated, or paced mated. In Experiment 3 (n=67), rats were not tested, standard mated, or paced mated and then tested in the battery without paced mating (consecutive testing in the open field, elevated plus maze, and social interaction).

Standard methods were utilized for open field, elevated plus maze, social interaction task, and paced mating (described in more detail in Frye et al., 2014). For standard mating, testing was conducted in clear glass chamber (37.5×75×30cm), containing bedding with a sexually-experienced male that had been acclimated to the testing chamber before the experimental subject is introduced. Sexual behavior of rats was assessed for 10 minutes or 10 mounts, whichever occurred first. The same measures of reproductive responding as recorded in the paced mating tasks were assessed in this task: lordosis, proceptivity and aggression/rejection

quotients (the incidence of these responses as a percentage of the number of total mounts by the male during testing).

During these behavioral manipulations, data were collected in each task. Behavioral data were simultaneously collected by using the Any-maze behavioral assessment computer program (Stoelting Inc., Wood Lawn, IL; for open field, elevated plus maze, and social interaction) or a digital video camera (for paced mating) and trained experimenters. Immediately after the last behavioral task that they were exposed to, rats were euthanized by rapid decapitation and had brains and trunk blood collected.

Tissue Collection and preparation for qPCR, radioimmunoassay, and ELISAs

Immediately after testing, rats were euthanized by rapid decapitation. Whole brains, immediately placed on dry ice, and trunk blood, rapidly placed in chilled glass test tubes in an ice bath, were collected from each subject. Trunk blood was spun and plasma stored in microcentrifuge tubes at -20°C until radioimmunoassay. Brains were stored at -80°C until dissections.

Determining PXR expression- qPCR

For qPCR, punches from the midbrain, around the VTA, were taken from frozen coronal slices of this region. Punches were stored in chilled RNALater and then stored at -80°C until qPCR was performed. Standard qPCR methods were utilized for determining PXR expression in the VTA punches, as has been described recently (Frye et al., 2013a; 2014). Placement of cannulae/infusion tracks aimed at the VTA was noted for each subject when brains were sliced and punches taken for qPCR (as described in Frye et al., 2013a; 2014, with accompanying figure). There were 26 rats that had placement outside of the VTA; their data was excluded from analyses. The number of observations of “missed sites” varied greatly across conditions ($n=0-5$), precluding systematic and valid analyses of effects of these vehicle or PXR AS-ODN infusions to regions nearby the VTA (substantia nigra, central gray); the responses of these rats with cannulae placement outside of the VTA were similar to the control group compared to those with PXR manipulations to the VTA.

Determining PXR expression- PXR ELISA

Following this punching out of the VTA, the rest of the midbrain that was remaining, and the hippocampus, prefrontal cortex, hypothalamus, and striatum, were dissected out, and placed into separate microcentrifuge tubes. These brain regions were homogenized in 1ml of ddH₂O to be used for radioimmunoassay and ELISAs. PXR protein levels in midbrain homogenates were determined from rats in each experimental condition of Experiments 1 ($n=34$) and 2 ($n=25$) with standard methods from commercially-available kit (USCN Life Science, E93950Ra). The range of the protein concentration of the midbrain homogenates was Experiment 1 (0.11–2.47 mg/dl) and Experiment 2 (0.13–3.04 mg/ml), using a Nanodrop Spectrometer.

Measurement of Steroid Hormones

Levels of steroids were determined with standard radioimmunoassay methods employed by our lab (Frye et al., 2013a; 2014), utilizing tritiated estradiol (specific activity 51.3 Ci/

mmol), progesterone (specific activity 47.5 Ci/mmol), and 3 α ,5 α -THP (specific activity 65.0 Ci/mmol) were from Perkin Elmer (Boston, MA) and antibodies to estradiol (Dr. G.D. Niswender, Colorado State University, Fort Collins, CO), progesterone (Dr. G.D. Niswender, Colorado State University), dihydroprogesterone, and 3 α ,5 α -THP (Dr. Robert Purdy, Veterans Medical Affairs, La Jolla, CA, our long-time collaborator, who is dearly missed).

Measurement of BDNF Levels

Hippocampus homogenates from rats in each experimental condition in Experiments 1 (n=44) and 2 (n=35) were analyzed using standard methods for BDNF using the Emax Immunoassay system (Promega; Frye et al. 2013; Frye and Rhodes, 2005).

Statistical Analyses

Two way analyses of variances (ANOVAs) were used to examine effects of PXR AS-ODN condition (control, PXR AS-ODN) and behavioral manipulations in each Experiment. When the α level for statistical significance was reached ($p \leq 0.05$), or a trend was observed ($p < 0.10$), for main effects and interactions, Fisher's Least Significant Differences *post hoc* tests were utilized to ascertain group differences.

Results

Experiment 1: Engaging in mating, but not other behavioral tasks, enhances midbrain and striatum 3 α ,5 α -THP levels; PXR AS-ODNs to the VTA reduce lordosis, dihydroprogesterone and 3 α ,5 α -THP levels in the midbrain and striatum and 3 α ,5 α -THP and BDNF levels in the hippocampus

PXR Expression—PXR AS-ODNs reduced PXR expression in the midbrain. PXR expression, as measured by mRNA, was reduced by 50% compared to control infusions [$F_{1, 58} = 5.9, P < 0.01$]. PXR AS-ODN infusions tended to reduce PXR protein expression, with a reduction of expression by 45% compared to vehicle infusions [$F_{1, 26} = 2.8, P < 0.10$].

Reproductive Measures—There was a significant main effect of PXR AS-ODN condition [$F_{1, 14} = 6.6, P < 0.02$] for lordosis quotients. Rats infused with PXR AS-ODN who were placed in the pacing chamber with the male had lower lordosis quotients compared to control infused rats (see Table 1).

Progesterone levels- hypothalamus, striatum, prefrontal cortex, midbrain and hippocampus—There were no significant differences between groups for progesterone levels in the brain regions assessed in this experiment (Table 4).

Dihydroprogesterone levels- hypothalamus, striatum, prefrontal cortex, midbrain and hippocampus—There was a main effect of PXR AS-ODN condition [$F_{1, 58} = 5.4, P < 0.02$] for dihydroprogesterone levels in the midbrain. Rats infused with PXR AS-ODN had lower levels of dihydroprogesterone in the midbrain compared to rats infused with saline. In addition, there was a main effect of behavioral manipulation [$F_{2, 58} =$

5.4, $P < 0.02$] for dihydroprogesterone levels in the striatum. Rats that were paced mated had higher levels of dihydroprogesterone in the striatum compared to all other testing conditions (Table 4).

3 α ,5 α -THP levels- hypothalamus, striatum, prefrontal cortex—There was a significant main effect of PXR AS-ODN condition [$F_{1, 58} = 11.2$, $P < 0.02$], and tendency for an interaction of PXR AS-ODN condition and behavioral manipulation [$F_{2, 58} = 2.5$, $P < 0.06$], for 3 α ,5 α -THP levels in the striatum. Rats infused with PXR AS-ODN had lower levels of 3 α ,5 α -THP in the striatum compared to rats infused with saline, particularly those that were mated (Table 4).

3 α ,5 α -THP levels- midbrain—There was significant main effect of PXR AS-ODN condition [$F_{1, 58} = 6.0$, $P < 0.01$; Figure A] for midbrain 3 α ,5 α -THP levels. Rats infused with PXR AS-ODN had lower levels of midbrain 3 α ,5 α -THP compared to rats infused with saline.

3 α ,5 α -THP levels- hippocampus—There was a significant main effect of PXR AS-ODN condition [$F_{1, 58} = 5.8$, $P < 0.02$; Figure 3A] for hippocampal 3 α ,5 α -THP levels. Rats infused with PXR AS-ODN had lower levels of 3 α ,5 α -THP in the hippocampus compared to rats infused with saline.

BDNF levels- hippocampus—There was a significant main effect for PXR AS-ODN condition [$F_{1, 34} = 5.8$, $P = 0.021$] for BDNF levels in the hippocampus. Rats infused with PXR-AS-ODN had lower levels of BDNF in the hippocampus than did rats infused with saline (see Figure 4A).

Behavioral measures—In Experiments 1, few differences were noted in the open field, elevated plus maze, or social interaction task; data from all behavioral measures assessed in these experiments are indicated in Tables 1. There was a significant effect for PXR AS-ODNs to reduce inner 8 entries made in the open field [$F_{1, 13} = 14.9$, $P < 0.01$] (Table 1).

Experiment 2: Engaging in paced mating versus standard mating or no mating enhances 3 α ,5 α -THP levels; PXR AS-ODNs to the VTA reduce lordosis, 3 α ,5 α -THP levels in the midbrain and hippocampus and BDNF levels in the hippocampus

PXR Expression—PXR AS-ODNs reduced PXR expression in the midbrain. PXR expression, as measured by mRNA, was reduced by 35% compared to control infusions [$F_{1, 34} = 26.7$, $P < 0.01$]. Although it was not statistically significant, PXR AS-ODN infusions reduced PXR protein expression by 33% compared to vehicle infusions. There were no significant differences based upon behavioral manipulations.

Reproductive measures—There was a main effect for PXR AS-ODN condition (but not testing condition) for lordosis quotients, such that rats infused with PXR AS-ODN had lower lordosis quotients compared to saline-infused rats [$F_{1, 23} = 4.7$, $P < 0.03$] (see Table 2). There was a tendency for rats infused with PXR AS-ODNs to have lower proceptivity quotients, compared to those infused with saline, irrespective of testing condition, but this

did not reach statistical significance [$F_{1, 23} = 3.7, P < 0.06$] (see Table 2). Rats infused with PXR AS-ODN had higher aggression quotients compared to saline-infused rats [$F_{1, 23} = 14.1, P < 0.01$], and standard mated rats had higher aggression quotients than did paced mated rats [$F_{1, 23} = 4.7, P < 0.03$] (see Table 2).

Progesterone levels- hypothalamus, striatum, prefrontal cortex, midbrain and hippocampus—There was a significant main effect of PXR AS-ODN condition for progesterone levels in the hypothalamus [$F_{1, 34} = 6.1, P < 0.01$] and prefrontal cortex [$F_{1, 34} = 4.1, P < 0.05$]. Rats infused with PXR AS-ODN had higher levels of progesterone levels in the hypothalamus and prefrontal cortex, compared to rats infused with saline (Table 5).

Dihydroprogesterone levels- hypothalamus, striatum, prefrontal cortex, midbrain and hippocampus—There were no significant differences between groups for dihydroprogesterone levels in the brain regions assessed in this experiment (Table 5).

3 α ,5 α -THP levels- hypothalamus, striatum, and prefrontal cortex—There were no significant differences between groups for 3 α ,5 α -THP levels in the hypothalamus, striatum, or prefrontal cortex in this experiment (Table 5)

3 α ,5 α -THP levels- midbrain—There was a significant main effect of PXR AS-ODN condition [$F_{1, 34} = 8.4, P < 0.01$; Figure 2B] for midbrain 3 α ,5 α -THP levels. Rats infused with PXR AS-ODN had lower levels of midbrain 3 α ,5 α -THP compared to rats infused with saline.

3 α ,5 α -THP levels- hippocampus—There were significant main effects of PXR AS-ODN condition [$F_{1, 34} = 8.2, P < 0.01$] and behavioral manipulation [$F_{1, 34} = 3.3, P < 0.04$] for hippocampal 3 α ,5 α -THP levels (see Figure 3B). Rats infused with PXR AS-ODN had lower levels of 3 α ,5 α -THP in the hippocampus compared to rats infused with saline. In addition, rats that were paced mated had higher hippocampal 3 α ,5 α -THP compared to all other testing conditions.

Hippocampus BDNF levels—There was a tendency for an effect for BDNF levels in the hippocampus in this experiment [$F_{1, 31} = 2.9, P = 0.09$]. Rats infused with PXR-AS-ODN had lower levels of BDNF in the hippocampus than did rats infused with saline (see Figure 4B).

Experiment 3: Engaging in paced mating enhances 3 α ,5 α -THP levels in the midbrain, striatum and hypothalamus and reduced open field activity and social interaction; PXR AS-ODNs to the VTA reduce lordosis and attenuate effects of paced mating for 3 α ,5 α -THP levels and behavior

PXR Expression—PXR AS-ODNs reduced PXR mRNA expression in the midbrain by 73% compared to control infusions [$F_{1, 62} = 13.8, P < 0.01$]. There were no significant differences based upon behavioral manipulations.

Reproductive measures—There was a significant main effect of PXR AS-ODN condition for lordosis quotients [$F_{1, 33} = 25.1, P < 0.01$] (see Table 3). Rats infused with

PXR AS-ODN had lower lordosis responding in the pacing or standard mating tasks, compared to control infused rats. No differences were observed based upon testing condition for lordosis. There was a significant main effect of PXR AS-ODN condition for aggression quotients [$F_{1, 33} = 9.2, P < 0.01$] (see Table 3). No differences were observed based upon testing condition for aggression. There was a tendency for an effect of PXR AS-ODN condition for proceptivity quotients [$F_{1, 33} = 3.2, P < 0.07$] and a significant main effect of testing condition quotients [$F_{1, 33} = 5.1, P < 0.03$] (see Table 3). Rats infused with PXR AS-ODN had lower proceptivity and greater aggression in the pacing or standard mating tasks, compared to control infused rats; standard mated rats had higher proceptivity quotients than did paced mated rats.

Progesterone levels- hypothalamus, striatum, prefrontal cortex, midbrain and hippocampus—There was a significant main effect of PXR AS-ODN condition [$F_{1, 62} = 15.2, P < 0.01$] for progesterone levels in the midbrain (Table 6). Rats infused with PXR AS-ODN had lower levels of progesterone in the midbrain compared to rats infused with saline. No differences were observed based upon testing condition.

Dihydroprogesterone levels- hypothalamus, striatum, prefrontal cortex, midbrain and hippocampus—There was a significant main effect of PXR AS-ODN infusions to reduce plasma [$F_{1, 62} = 4.3, p < 0.04$] and midbrain levels of dihydroprogesterone, but no clear differences in other regions assessed.

3 α ,5 α -THP levels- hypothalamus, striatum, and prefrontal cortex—There were significant main effects of PXR AS-ODN condition to reduce 3 α ,5 α -THP levels, compared to control, in the hypothalamus [$F_{1, 62} = 4.5, P < 0.03$] striatum [$F_{1, 62} = 5.8, P < 0.02$] prefrontal cortex [$F_{1, 62} = 6.5, P < 0.01$], and plasma [$F_{1, 62} = 11.7, P < 0.01$]. There were main effects of behavioral manipulations for 3 α ,5 α -THP levels in the hypothalamus [$F_{2, 62} = 5.1, P < 0.01$] and striatum [$F_{2, 62} = 4.0, P < 0.02$], with paced mated rats having the highest 3 α ,5 α -THP levels in the hypothalamus and striatum compared to controls (Table 6).

3 α ,5 α -THP levels- midbrain—There was a significant main effect of PXR AS-ODN condition [$F_{1, 62} = 27.9, P < 0.01$] for midbrain 3 α ,5 α -THP levels (see Figure 5). Rats infused with PXR AS-ODN had lower levels of midbrain 3 α ,5 α -THP compared to rats infused with saline. There were no statistically significance differences for testing condition, but an apparent effect in that paced mated rats had the highest midbrain 3 α ,5 α -THP levels.

3 α ,5 α -THP levels- hippocampus—There was a significant effect of PXR AS-ODN condition [$F_{1, 62} = 5.8, P < 0.01$] for hippocampal 3 α ,5 α -THP levels (Figure 6). Rats infused with PXR AS-ODN had lower levels of 3 α ,5 α -THP in the hippocampus compared to rats infused with saline. There were no statistically significance differences for testing condition for 3 α ,5 α -THP levels in the hippocampus.

Behavioral measures—There was a significant effect for PXR AS-ODNs [$F_{1, 62} = 15.4, P < 0.01$] and a tendency for testing condition [$F_{2, 62} = 2.7, P = 0.07$] to alter total entries in the open field. Rats infused with PXR-AS-ODN made fewer total entries than did rats infused with saline; paced mated rats made fewer total entries compared to all other

behaviorally-manipulated conditions. There was a main effect of PXR AS-ODN [$F_{1, 62} = 15.4$, $P < 0.01$] for the number of inner 8 made in the open field. Rats infused with PXR-AS-ODN made fewer inner 8 entries than did rats infused with saline (see Table 3). There was a significant main effect of testing condition [$F_{2, 62} = 3.0$, $P = 0.05$] for the time spent interacting with a conspecific. Rats that were paced mated before behavioral testing spent less time interacting with a conspecific compared to non-mated or standard-mated rats (see Table 3).

Discussion

The present results partly supported our *a priori* hypotheses. Experiment 1 demonstrated that engaging in mating (versus other tasks of exploration, anxiety and social interaction) enhanced midbrain 3 α ,5 α -THP levels and this effect was attenuated in rats administered PXR AS-ODNs to the VTA. In Experiment 2, the finding that more robust increases in 3 α ,5 α -THP in the midbrain among paced compared to standard mated rats, and attenuation with PXR AS-ODNs suggests that greater exploration and/or social contact are essential to elevate brain 3 α ,5 α -THP levels in a PXR-dependent fashion. In Experiment 3, mating-induced enhancement of midbrain 3 α ,5 α -THP levels mediated subsequent responding in the open field and social interaction task, but this did not occur in rats with PXR knocked down. However, the direction of these effects was not entirely as predicted, with paced mating reducing exploration (i.e. total entries made) in the open field and time spent interacting with an ovariectomized female in the social interaction task. In all experiments, manipulations of PXR and behavior altered 3 α ,5 α -THP levels in the midbrain VTA as well as the hippocampus. PXR knockdown had more variable effects across experiments in the striatum and hypothalamus compared to what was observed in the midbrain or hippocampus. Indeed, in Experiments 1 and 2, PXR knockdown reduced levels of BDNF in the hippocampus. Given that 3 α ,5 α -THP is increased following challenges, such as acute stressors (Barbaccia et al., 1996; Purdy et al., 1991; Vallée et al., 2000) and mating, and that there is behavioral-induced biosynthesis of 3 α ,5 α -THP in midbrain and mesocorticolimbic structures, which are attenuated with knockdown of PXR, 3 α ,5 α -THP likely has a role in motivated, homeostatic responses involving PXR. Moreover, BDNF may be one downstream factor important for regulating behavioral-induced 3 α ,5 α -THP biosynthesis and indices of behavioral and neural plasticity (e.g. changes in behavior and BDNF expression). Another interpretation that must be considered is that changes in BDNF may regulate these differences noted in 3 α ,5 α -THP synthesis. The present study indirectly supports an association of 3 α ,5 α -THP and of PXR receptors with BDNF expression by using PXR AS-ODNs, which results in decreased allopregnanolone levels as well as hippocampus BDNF expression. The question is whether pharmacological interventions of PXR would result in upregulation or downregulation of 3 α ,5 α -THP biosynthesis and BDNF expression, and the direction of these effects. For example, Nin and colleagues have previously suggested the involvement of allopregnanolone as well as steroidogenic antidepressants in stimulating BDNF expression associated with improvement of post-traumatic stress disorder-like behavior among rodents (Nin et al., 2011).

The present study confirms and extends previous work on the role of 3 α ,5 α -THP for reproductive processes involving PXR. We have recently described that deficits in

reproductive responding can be reversed with direct administration of 3 α ,5 α -THP to the VTA, but not when PXR expression is attenuated in the midbrain (Frye et al., 2014). Effects of these manipulations of PXR in the VTA and behavioral experience suggest other brain regions beyond the midbrain that may be important for these behaviors, such as the hippocampus. In the present study, knocking down PXR in the midbrain VTA reduced 3 α ,5 α -THP content in the midbrain as well as the hippocampus, and central entries made in the open field, suggesting an important role of PXR in the VTA as a regulator of behavioral-induced 3 α ,5 α -THP biosynthesis. For example, administration of 3 α ,5 α -THP, or its prohormone, progesterone, to the hippocampus, or other limbic structures, such as the amygdala, increases anti-anxiety behavior (Akwa et al., 1999; Frye and Walf, 2004; Mòdol et al., 2011). A question is the role of social challenges (e.g. differences in maternal exposure/care, social interactions and social isolation) or context for the observed effects.

Social challenges among rodents, such as perinatal stressors (e.g. prenatal stress, maternal separation, or differences in maternal care) as well as social isolation in adulthood, reduce 3 α ,5 α -THP levels, measures of neural plasticity such as neurogenesis, and have negative behavioral consequences (Agís-Balboa et al., 2007; Brunton and Russell, 2011; Lieberwirth et al., 2012; Lieberwirth and Wang, 2012; Llidó A et al., 2013; Paris et al., 2011; Zimmerberg and Blaskey, 1998). For example, social isolation has been described as a model of major depression disorder as well as post-traumatic stress disorder among rodents, characterized by lower 3 α ,5 α -THP content in corticolimbic circuits, greater stress responding, increased depressive, fear, and anti-social/aggressive behaviors (Agís-Balboa et al., 2007; Nelson and Pinna, 2011; Nin et al., 2011; Pibiri et al., 2008; Pinna et al., 2008; 2012). A complementary question is to investigate the role of social engagement for 3 α ,5 α -THP and subsequent behavioral changes. We have noted increased 3 α ,5 α -THP levels in the brains of rats that had been exposed to a battery of tasks to assess exploration (open field), anxiety (elevated plus maze), affiliation (social interaction) immediately preceding the paced mated task (reviewed in Frye, 2011). An important aspect of this response is that it seems to be driven by paced mating, not any mating experience, as has been demonstrated for behavioral plasticity/changes in motivated processes (e.g. conditioned place preference; Camacho et al., 2009; Frye et al., 1998; García-Horsman et al., 2008; González-Flores et al., 2004; Nyuyki et al., 2011), well as neural plasticity (Arzate et al., 2012; Corona et al., 2011; Holder and Mong, 2010), involving endogenous opioids and oxytocin. It may be that PXR is necessary for behavioral-induced 3 α ,5 α -THP biosynthesis in reward and limbic pathways mediating the behaviors assessed, as well as effects on neural plasticity, which may serve to facilitate consolidation about mating experience or challenge.

A novel finding in the present study concerns the role of PXR manipulations in the VTA for modulating BDNF expression in the hippocampus. The role of BDNF for behavioral plasticity measures, such as changes in affective or cognitive responding, has been demonstrated in recent reports. For example, in an study of anxiety behaviors among female mice over the estrous cycle, genetically manipulated to overexpress a human single nucleotide polymorphism of the BDNF gene associated with anxiety and depression disorders among people, increased anxiety responding over the estrous cycle (Bath et al., 2012). The roles of ovarian steroids, such as estradiol and progesterone, across endogenous cycles, aging, and in animal models of neurodegeneration, for BDNF expression have been

described (Bimonte-Nelson et al., 2008; Gibbs, 1998; Meyer et al., 2012); albeit, there is some controversy as to whether progesterone promotes or inhibits estradiol-induced BDNF expression (Aguirre et al., 2010; Aguirre and Baudry, 2009; Bimonte-Nelson et al., 2004; Lorenz et al., 2009; Nilsen and Brinton, 2002; Yao et al., 2011).

One factor that may be important to further understanding the interactions between estradiol and progesterone for BDNF may be $3\alpha,5\alpha$ -THP, and the regulation of its synthesis in the brain. In the present study, we have demonstrated in separate subjects that knocking down PXR in the midbrain VTA reduces BDNF levels and behavioral changes following paced mating. These results extend reports showing acute actions of $3\alpha,5\alpha$ -THP for BDNF expression in the hippocampus, amygdala, and hypothalamus (Naert et al., 2007). Moreover, administration of synthetic progestins, such as medroxyprogesterone acetate, is not associated with an increase in $3\alpha,5\alpha$ -THP or BDNF (Frye et al., 2013b; González et al., 2004; 2007; Jodhka et al., 2009); whereas, administration of another hormone replacement therapy, conjugated equine estrogen, is (Engler-Chiurazzi et al., 2001; Frye et al., 2010; Genazzani et al., 2004). Indeed, these differences in capacity for synthetic hormones (for menopause therapy or contraceptives) to regulate neurosteroidogenesis of $3\alpha,5\alpha$ -THP and affective and reproductive behavior is of great interest (e.g. Porcu et al., 2012; Santoru et al., 2014 this issue). In the present study, the focus was to ascertain the extent to which PXR and behavioral manipulations altered BDNF in the hippocampus. The focus was on BDNF expression in the hippocampus as this is an area of the brain that is important for the behaviors assessed as well as being well-established as a site of neural plasticity (e.g. long-term potentiation, changes in growth factors, neurogenesis, dendritic branching and spine formation). Given the dynamic actions of behavioral experience to alter $3\alpha,5\alpha$ -THP levels in the midbrain VTA, investigations are ongoing to measure markers of neural plasticity, such as BDNF, in this region. As such, it is of interest to further understand the role of $3\alpha,5\alpha$ -THP, produced via regulatory actions of PXR, for BDNF as one index of neural plasticity.

Some of the limitations in the interpretation of the results of this study need to be considered. First, although strengths of this manuscript may be that naturally-cycling rats, with somatic and behavioral manipulations, as well as several converging measures utilized, there were limits on the sample sizes and great variability for some of these measures. For example, responses in the elevated plus maze in Experiment 3 were uncharacteristically low. Although this may similar to the unexpected pattern observed for reduced open field activity and social interaction following paced mating, and reflect differences in stress responding or behavioral inhibition, this could not be directly assessed here. Second, further exploration of the role of BDNF for observed effects is warranted. Here a majority, but not all, rats in each group in Experiments 1 and 2 were assessed for BDNF levels in the hippocampus alone. It is of interest, but not possible to adequately assess herein, whether such variability in behavioral responses is due to variability in BDNF expression in the hippocampus. As well, studies investigating whether there are differences following behavioral manipulations, or coinciding with different behavioral responses, for BDNF levels in other regions where there was some indication of behavior-induced $3\alpha,5\alpha$ -THP synthesis (e.g. striatum, hypothalamus, prefrontal cortex, midbrain) are substantiated.

In conclusion, the present study supports the notion that PXR is a regulator of $3\alpha,5\alpha$ -THP's role in homeostatic and motivated processes (e.g. reproduction, affect, affiliation). In Experiments 1–3, knocking down PXR in the VTA reduced PXR expression and $3\alpha,5\alpha$ -THP levels in the midbrain as well as the hippocampus. Paced mating had the most robust effects to increase $3\alpha,5\alpha$ -THP levels in the midbrain and the hippocampus, and reduce open field exploration and social interaction, but this was attenuated with PXR knock down in the VTA. Moreover, rats infused with PXR AS-ODNs had lower levels of BDNF in the hippocampus. An important consideration is that a challenge to maintaining health and wellbeing may be related to impairments in individuals' adaptation to their (changing) environment, such as stress responding, which is likely to be regulated by interactions of $3\alpha,5\alpha$ -THP, other steroids, and BDNF (Angelucci et al., 2005; Duman and Monteggia, 2006; Franklin and Perrot-Sinal, 2006). Further investigation of the novel role of PXR as a regulator of mating-induced $3\alpha,5\alpha$ -THP formation and behavioral and neural changes supporting homeostatic and motivated processes is substantiated.

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Figure 1.

The general procedure that was utilized for Experiments 1, 2, and 3 is depicted. Briefly, experimental rats were tested in separate cohorts for each experiment. Rats had stereotaxic surgery and recovered from surgery for 7 days. Rats had estrous cyclicity assessed and were administered control (saline) or PXR AS-ODN infusions (during metestrus), which were initiated 44 hours before behavioral testing. Twenty-four hours before endpoints were collected, rats received a second dosing of saline or PXR AS-ODNs to the VTA. A third infusion of saline or PXR AS-ODNs was administered one half-hour before endpoints

collected (when all rats were in proestrus). At hour 44, behavioral manipulations occurred and endpoints (behavioral testing and/or brains and plasma) were collected. In Experiment 1 (n=66), comparisons were made between rats that were not-tested (home cage controls), tested in the full battery (consecutive testing in the open field, elevated plus maze, social interaction, and paced mating task), the battery without paced mating (consecutive testing in the open field, elevated plus maze, and social interaction), or paced mating alone. In Experiment 2 (n=40), rats were not tested, standard mated, or paced mated. In Experiment 3 (n=67), rats were not tested, standard mated, or paced mated and then tested in the battery without paced mating (consecutive testing in the open field, elevated plus maze, and social interaction). Levels of progesterone, dihydroprogesterone, and 3 α ,5 α -THP were measured by radioimmunoassay in dissected brain regions (midbrain, hypothalamus, striatum, prefrontal cortex, and hippocampus) in Experiments 1, 2, and 3. Levels of BDNF were determined in the hippocampus in Experiment 1 and 2.

Figure 2.

Depicts mean ($\pm$ ng/g) 3 α ,5 α -THP levels in the midbrain (Experiment 1 panel A; nontested (n=11 control, n=8 pregnane xenobiotic receptor (PXR) antisense oligonucleotides (AS-ODN)), Full Battery (n=7 control, n=5 AS-ODN), Battery no paced mated (n=9 control, n=10 AS-ODN and Paced mated (n=8 control, n=8 AS-ODN) and Experiment 2 (Panel B; non-tested (n=12 control, n=10 AS-ODN, Standard mated (n=9 control, n=10 AS-ODN) and Paced Mated (n=9 control, n=11 AS-ODN). * above bar indicates a significant effect of PXR ASODN infusions ($P < 0.05$).

Figure 3.

Depicts mean ($\pm$ ng/g) 3α,5α-THP levels in the hippocampus. (Experiment 1 panel A; non-tested (n=11 control, n=8 pregnane xenobiotic receptor (PXR) antisense oligonucleotides (AS-ODN)), Full Battery (n=7 control, n=5 AS-ODN), Battery no paced mated (n=9 control, n=10 AS-ODN and Paced mated (n=8 control, n=8 AS-ODN) and Experiment 2 panel B; non-tested (n=12 control, n=10 AS-ODN, Standard mated (n=9 control, n=10 AS-ODN) and Paced Mated (n=9 control, n=11 AS-ODN). * above bar indicates a significant effect of

PXR AS-ODN infusions ($P < 0.05$) * above line indicated significant effect of testing condition.

Figure 4.

Depicts mean ($\pm$ ng/g) BDNF levels. (Experiment 1 panel A; non-tested (n=7 control, n=4 pregnane xenobiotic receptor (PXR) antisense oligonucleotides (AS-ODN)), Full Battery (n=6 control, n=4 AS-ODN), Battery no paced mated (n=7 control, n=5 AS-ODN and Paced mated (n=6 control, n=5 AS-ODN) and Experiment 2 panel B; non-tested (n=5 control, n=8 AS-ODN), Standard mated (n=5 control, n=7 AS-ODN) and Paced Mated (n=5 control, n=6 AS-ODN). * above bar indicates a significant effect of PXR ASODN # above line indicates a tendency of an effect of PXR AS-ODN

Figure 5.

Depicts mean ($\pm$ ng/g) 3 α ,5 α -THP levels in the midbrain (Experiment 3 non-tested (n=5 control, n=9 pregnane xenobiotic receptor (PXR) antisense oligonucleotides (AS-ODN)), Standard mated (n=7 control, n=8 AS-ODN) and Paced Mated (n=6 control, n=6 AS-ODN).

* above bar indicates a significant effect of PXR AS-ODN infusions ($P < 0.05$).

Figure 6.

Depicts mean ($\pm$ ng/g) 3 α ,5 α -THP levels in the hippocampus. (Experiment 3 non-tested (n=5 control, n=9 pregnane xenobiotic receptor (PXR) antisense oligonucleotides (AS-ODN)), Standard mated (n=7 control, n=8 AS-ODN) and Paced Mated (n=6 control, n=6 ASODN).

* above bar indicates a significant effect of PXR AS-ODN infusions ($P < 0.05$).

Table 1

Depicts the mean ($\pm$ S.E.M.) of behavioral testing measures in Experiment 1.

Conditions	Non tested		Full Battery		Battery, not paced mated		Paced Mated	
	Control	ODN	Control	ODN	Control	ODN	Control	ODN
Open Field-Total entries	N/A	N/A	271.9 $\pm$ 39.8	162.0 [^] $\pm$ 25.6	184.8 $\pm$ 25.9	192.9 $\pm$ 25.6	N/A	N/A
Open Field-Inner 8 entries	N/A	N/A	15.1 $\pm$ 2.7	2.2 [*] $\pm$ 1.7	7.1 $\pm$ 2.5	5.0 [*] $\pm$ 1.7	N/A	N/A
Plus maze-open arm time (secs)	N/A	N/A	30.6 $\pm$ 21.0	7.6 $\pm$ 5.0	7.7 $\pm$ 3.7	13.4 $\pm$ 6.6	N/A	N/A
Social interaction (secs)	N/A	N/A	63.0 $\pm$ 9.1	73.9 $\pm$ 17.4	79.7 $\pm$ 10.9	92.0 $\pm$ 8.6	N/A	N/A
Paced Mating-Lordosis quotients	N/A	N/A	46.9 $\pm$ 9.7	23.3 $\pm$ 11.3	N/A	N/A	50.3 $\pm$ 10.2	13.7 [*] $\pm$ 7.0
Paced Mating-Proceptivity quotients	N/A	N/A	13.8 $\pm$ 7.1	17.5 $\pm$ 10.8	N/A	N/A	8.0 $\pm$ 4.8	6.6 $\pm$ 5.1
Paced Mating-Aggression quotients	N/A	N/A	25.9 $\pm$ 7.1	32.9 $\pm$ 8.4	N/A	N/A	23.3 $\pm$ 9.2	33.4 $\pm$ 11.5

* indicates a significant effect of PXR AS-ODN infusions ($P < 0.05$).

[^] indicates a tendency for PXR AS-ODN infusions to have an effect ($P < 0.10$).

Table 2
Depicts the mean ($\pm$ S.E.M.) lordosis, proceptivity quotients, and aggression quotients in Experiment 2.

Conditions	Non tested		Standard Mated		Paced Mated	
	Control	ODN	Control	ODN	Control	ODN
Lordosis quotients	N/A	N/A	42.5 $\pm$ 16.1	25.0* $\pm$ 10.4	49.3 $\pm$ 17.8	14.2* $\pm$ 9.8
Proceptivity quotients	N/A	N/A	25.0 $\pm$ 17.1	25.0^ $\pm$ 15.4	34.0 $\pm$ 16.4	10.0^ $\pm$ 10.0
Aggression quotients	N/A	N/A	51.7 $\pm$ 19.4	70.8* $\pm$ 14.9	0.0 $\pm$ 0.0	31.7* $\pm$ 12.1

* indicates a significant effect of PXR AS-ODN infusions ($P<0.05$).
^ indicates a tendency for PXR AS-ODN infusions to have an effect ($P<0.10$).
+ indicates a significant effect of testing condition ($P<0.05$).

Table 3

Depicts the mean ($\pm$ S.E.M.) of behavioral testing measures in Experiment 3.

Conditions	Non mated		Standard Mated		Paced Mated	
	Control	ODN	Control	ODN	Control	ODN
Lordosis quotients	N/A	N/A	56.1 $\pm$ 11.4	26.8* $\pm$ 7.3	58.9 $\pm$ 18.3	18.3* $\pm$ 6.3
Proceptivity quotients	N/A	N/A	58.9+ $\pm$ 14.7	28.1^+ $\pm$ 8.8	33.3 $\pm$ 14.4	10.2^ $\pm$ 5.0
Aggression quotients	N/A	N/A	13.3 $\pm$ 1.1	59.4* $\pm$ 9.8	1.8 $\pm$ 1.5	47.4* $\pm$ 10.5
Open Field-Total entries	248.1 $\pm$ 19.7	224.6* $\pm$ 23.2	242.1# $\pm$ 22.3	160.9*# $\pm$ 15.5	235.6# $\pm$ 25.5	149.8*# $\pm$ 12.2
Open Field-Inner 8 entries	25.4 $\pm$ 10.5	10.9* $\pm$ 2.4	15.6 $\pm$ 2.7	9.1* $\pm$ 2.3	18.2 $\pm$ 3.8	7.8* $\pm$ 1.5
Plus maze-open arm time (secs)	4.1 $\pm$ 1.6	2.8 $\pm$ 8.4	6.6 $\pm$ 2.0	4.6 $\pm$ 1.4	6.6 $\pm$ 1.8	4.3 $\pm$ 1.6
Social Interaction (secs)	85.9 $\pm$ 13.2	91.1 $\pm$ 9.9	102.9 $\pm$ 19.5	72.5 $\pm$ 6.8	69.3 $\pm$ 8.3	59.7 $\pm$ 6.8

* indicates a significant effect of pregnane xenobiotic receptor (PXR) antisense oligonucleotides (AS-ODN) infusions ($P < 0.05$).

^ indicates a tendency for PXR AS-ODN infusions ($P < 0.10$).

indicates a tendency for testing condition to have an effect ($P < 0.10$).

+ indicates a significant effect of PXR AS-ODN infusions ($P < 0.05$).

Table 4

Depicts the mean ($\pm$ SEM) progesterone levels in Experiment 1.

Conditions	Non tested		Full Battery		Battery not paced mated		Paced Mated	
	Control	ODN	Control	ODN	Control	ODN	Control	ODN
	3 α ,5 α -THP							
Plasma ($\pm$ ng/ml)	12.1 $\pm$ 0.5	13.8 $\pm$ 0.8	13.0 $\pm$ 1.5	13.9 $\pm$ 1.4	12.3 $\pm$ 1.0	12.1 $\pm$ 0.8	11.1 $\pm$ 0.4	14.0 $\pm$ 1.5
Hypothalamus ($\pm$ ng/g)	4.1 $\pm$ 3.7	4.6 $\pm$ 2.7	9.1 $\pm$ 3.4	6.1 $\pm$ 1.1	8.0 $\pm$ 4.6	6.7 $\pm$ 1.2	8.9 $\pm$ 2.2	6.0 $\pm$ 1.2
Prefrontal Cortex ($\pm$ ng/g)	5.5 $\pm$ 2.3	5.5 $\pm$ 2.1	10.3 $\pm$ 5.2	7.8 $\pm$ 3.2	4.3 $\pm$ 2.5	3.0 $\pm$ 1.3	7.2 $\pm$ 3.2	5.1 $\pm$ 3.2
Striatum ($\pm$ ng/g)	7.4 $\pm$ 1.3	3.2 $\pm$ 0.7	5.7 $\pm$ 1.4	3.5 $\pm$ 1.5	4.2 $\pm$ 1.8	3.5 $\pm$ 0.8	13.2 $\pm$ 4.2	2.8 $\pm$ 0.6
Midbrain ($\pm$ ng/g)								
Hippocampus ($\pm$ ng/g)								
	Please see Figure							
	Please see Figure							
	Dihydroprogesterone							
Plasma ($\pm$ ng/ml)	21.3 $\pm$ 2.8	19.4 $\pm$ 4.4	22.7 $\pm$ 2.8	21.3 $\pm$ 5.5	16.9 $\pm$ 4.1	15.2 $\pm$ 3.5	19.2 $\pm$ 3.4	26.0 $\pm$ 2.7
Hypothalamus ($\pm$ ng/g)	6.9 $\pm$ 2.3	6.4 $\pm$ 1.5	5.5 $\pm$ 3.2	5.7 $\pm$ 2.8	8.5 $\pm$ 3.4	5.3 $\pm$ 4.4	5.5 $\pm$ 4.2	6.4 $\pm$ 3.6
Prefrontal Cortex ($\pm$ ng/g)	7.4 $\pm$ 1.3	3.2 $\pm$ 0.7	5.7 $\pm$ 1.4	3.5 $\pm$ 1.5	4.2 $\pm$ 1.8	3.5 $\pm$ 0.8	13.2 $\pm$ 4.2	2.8 $\pm$ 0.6
Striatum ($\pm$ ng/g)	7.4 $\pm$ 1.3	3.2 $\pm$ 0.7	5.7 $\pm$ 1.4	3.5 $\pm$ 1.5	4.2 $\pm$ 1.8	3.5 $\pm$ 0.8	13.2 $\pm$ 4.2	2.8 $\pm$ 0.6
Midbrain ($\pm$ ng/g)	5.9 $\pm$ 0.9	5.2 $\pm$ 1.2	8.1 $\pm$ 1.8	4.4 $\pm$ 1.8	5.2 $\pm$ 1.0	3.7 $\pm$ 1.3	10.7 $\pm$ 4.5	3.4 $\pm$ 1.0
Hippocampus ($\pm$ ng/g)	8.9 $\pm$ 4.2	7.6 $\pm$ 2.2	7.5 $\pm$ 4.4	8.8 $\pm$ 3.5	11.0 $\pm$ 4.5	7.1 $\pm$ 4.2	11.4 $\pm$ 5.7	8.9 $\pm$ 3.7
	Progesterone							
Plasma ($\pm$ ng/ml)	10.3 $\pm$ 1.3	11.2 $\pm$ 0.7	11.5 $\pm$ 1.4	12.6 $\pm$ 1.5	13.2 $\pm$ 1.8	14.6 $\pm$ 0.8	15.6 $\pm$ 4.2	12.4 $\pm$ 0.6
Hypothalamus ($\pm$ ng/g)	4.5 $\pm$ 1.1	8.1 $\pm$ 1.9	4.8 $\pm$ 1.3	8.6 $\pm$ 2.9	8.3 $\pm$ 1.9	6.5 $\pm$ 2.3	3.6 $\pm$ 1.4	4.6 $\pm$ 0.9
Prefrontal Cortex ($\pm$ ng/g)	4.7 $\pm$ 1.3	5.3 $\pm$ 1.0	6.2 $\pm$ 1.9	3.0 $\pm$ 0.8	6.9 $\pm$ 2.4	3.9 $\pm$ 0.9	4.1 $\pm$ 2.3	2.6 $\pm$ 0.8
Striatum ($\pm$ ng/g)	8.1 $\pm$ 2.1	8.9 $\pm$ 2.4	7.7 $\pm$ 1.9	6.6 $\pm$ 1.6	9.0 $\pm$ 1.4	9.5 $\pm$ 2.7	7.1 $\pm$ 1.9	7.8 $\pm$ 1.6
Midbrain ($\pm$ ng/g)	3.0 $\pm$ 0.6	5.3 $\pm$ 1.3	5.6 $\pm$ 2.3	8.8 $\pm$ 1.6	9.7 $\pm$ 3.9	4.1 $\pm$ 0.8	3.6 $\pm$ 1.1	6.6 $\pm$ 2.9
Hippocampus ($\pm$ ng/g)	7.4 $\pm$ 1.3	3.9 $\pm$ 1.1	9.7 $\pm$ 2.8	5.5 $\pm$ 1.4	11.2 $\pm$ 2.8	7.3 $\pm$ 2.2	8.6 $\pm$ 3.5	6.4 $\pm$ 2.2

* indicates a significant effect of pregnane xenobiotic receptor (PXR) antisense oligonucleotides (AS-ODN) infusions (P<0.05).

Table 5

Depicts the mean ($\pm$ SEM) progesterone levels In Experiment 2.

Condition	Non tested		Standard Mated		Paced Mated	
	Control	ODN	Control	ODN	Control	ODN
	3 α ,5 α -THP					
Plasma ($\pm$ ng/ml)	10.5 $\pm$ 1.2	9.6 $\pm$ 0.8	12.3 $\pm$ 4.7	8.3 $\pm$ 0.2	16.5 $\pm$ 5.5	12.5 $\pm$ 0.3
Hypothalamus ($\pm$ ng/g)	10.5 $\pm$ 3.4	6.8 $\pm$ 1.5	11.4 $\pm$ 5.9	6.6 $\pm$ 2.5	4.6 $\pm$ 1.3	14.7 $\pm$ 7.4
Prefrontal Cortex ($\pm$ ng/g)	7.8 $\pm$ 0.4	4.5 $\pm$ 0.8	3.5 $\pm$ 1.5	4.1 $\pm$ 0.8	6.3 $\pm$ 1.2	16.7 $\pm$ 8.9
Striatum ($\pm$ ng/g)	6.5 $\pm$ 1.4	4.8 $\pm$ 0.5	6.2 $\pm$ 3.3	5.3 $\pm$ 1.9	3.8 $\pm$ 1.3	3.9 $\pm$ 0.4
Midbrain ($\pm$ ng/g)	Please see Figure					
Hippocampus ($\pm$ ng/g)	Please see Figure					
	Dihydroprogesterone					
Plasma ($\pm$ ng/ml)	13.2 $\pm$ 1.4	12.7 $\pm$ 1.9	11.4 $\pm$ 4.3	13.5 $\pm$ 8.1	12.1 $\pm$ 3.6	13.1 $\pm$ 6.8
Hypothalamus ($\pm$ ng/g)	6.3 $\pm$ 1.5	7.9 $\pm$ 2.5	8.5 $\pm$ 1.6	16.7 $\pm$ 5.9	4.8 $\pm$ 2.4	12.4 $\pm$ 5.9
Prefrontal Cortex ($\pm$ ng/g)	9.3 $\pm$ 2.1	5.5 $\pm$ 2.3	8.8 $\pm$ 2.1	10.5 $\pm$ 4.1	9.8 $\pm$ 3.1	9.5 $\pm$ 2.2
Striatum ($\pm$ ng/g)	8.1 $\pm$ 2.1	5.8 $\pm$ 1.2	6.4 $\pm$ 2.8	8.8 $\pm$ 2.3	9.6 $\pm$ 1.8	9.5 $\pm$ 2.2
Midbrain ($\pm$ ng/g)	4.4 $\pm$ 0.9	7.7 $\pm$ 1.6	5.1 $\pm$ 3.2	8.2 $\pm$ 1.4	7.5 $\pm$ 3.4	9.7 $\pm$ 1.9
Hippocampus ($\pm$ ng/g)	7.8 $\pm$ 3.7	6.5 $\pm$ 2.5	5.5 $\pm$ 1.6	8.0 $\pm$ 2.8	10.2 $\pm$ 7.5	9.4 $\pm$ 5.9
	Progesterone					
Plasma ($\pm$ ng/ml)	10.4 $\pm$ 1.7	11.7 $\pm$ 4.2	17.6 $\pm$ 9.0	9.4 $\pm$ 2.8	11.3 $\pm$ 3.1	9.5 $\pm$ 3.3
Hypothalamus ($\pm$ ng/g)	6.3 $\pm$ 0.8	12.4* $\pm$ 2.8	8.7 $\pm$ 3.1	7.2 $\pm$ 5.9	6.0 $\pm$ 1.1	13.2* $\pm$ 2.8
Prefrontal Cortex ($\pm$ ng/g)	6.5 $\pm$ 2.5	7.4* $\pm$ 1.9	10.6 $\pm$ 4.7	12.5* $\pm$ 4.1	5.6 $\pm$ 1.7	11.4* $\pm$ 2.1
Striatum ($\pm$ ng/g)	2.2 $\pm$ 0.6	4.7 $\pm$ 0.6	10.7 $\pm$ 3.4	9.1 $\pm$ 1.9	5.2 $\pm$ 1.4	7.1 $\pm$ 1.3
Midbrain ($\pm$ ng/g)	7.2 $\pm$ 1.9	8.8 $\pm$ 1.4	10.8 $\pm$ 3.8	10.2 $\pm$ 1.7	9.2 $\pm$ 2.7	10.2 $\pm$ 1.2
Hippocampus ($\pm$ ng/g)	5.9 $\pm$ 1.7	4.6 $\pm$ 1.1	8.0 $\pm$ 2.5	12.3 $\pm$ 0.9	11.9 $\pm$ 4.2	10.4 $\pm$ 2.9

* indicates a significant effect of pregnane xenobiotic receptor (PXR) antisense oligonucleotides (AS-ODN) infusions ($P < 0.05$).

Table 6

Depicts the mean ($\pm$ SEM) progesterone levels in Experiment 3.

Conditions	Non tested		Standard Mated		Paced Mated	
	Control	ODN	Control	ODN	Control	ODN
	3 α ,5 α -THP					
Plasma ($\pm$ ng/ml)	15.7 $\pm$ 2.7	7.8* $\pm$ 2.6	19.6 ⁺ $\pm$ 3.6	10.0* $\pm$ 2.5	23.1 ⁺ $\pm$ 7.7	8.9* $\pm$ 2.6
Hypothalamus ($\pm$ ng/g)	5.6 $\pm$ 1.2	1.8* $\pm$ 0.6	11.6 $\pm$ 3.0	7.2* $\pm$ 2.4	12.8 $\pm$ 3.2	8.8* $\pm$ 2.1
Prefrontal Cortex ($\pm$ ng/g)	7.5 $\pm$ 2.2	6.6* $\pm$ 1.8	13.4 $\pm$ 3.8	8.3* $\pm$ 1.4	14.5 $\pm$ 3.1	6.2* $\pm$ 1.5
Striatum ($\pm$ ng/g)	6.2 $\pm$ 1.3	4.9* $\pm$ 0.5	12.4 $\pm$ 2.1	8.5* $\pm$ 1.7	12.8 $\pm$ 3.1	6.7* $\pm$ 1.8
Midbrain ($\pm$ ng/g)	Please see Figure					
Hippocampus ($\pm$ ng/g)	Please see Figure					
	Dihydroprogesterone					
Plasma ($\pm$ ng/ml)	22.8 $\pm$ 4.2	9.3 $\pm$ 3.2	17.4 $\pm$ 5.8	12.1 $\pm$ 3.5	16.2 $\pm$ 5.3	12.8 $\pm$ 4.1
Hypothalamus ($\pm$ ng/g)	5.6 $\pm$ 1.6	3.9 $\pm$ 1.3	4.7 $\pm$ 1.7	4.2 $\pm$ 1.1	6.4 $\pm$ 1.7	3.9 $\pm$ 0.9
Prefrontal Cortex ($\pm$ ng/g)	2.7 $\pm$ 1.0	3.3 $\pm$ 1.0	3.4 $\pm$ 2.6	2.9 $\pm$ 0.8	4.8 $\pm$ 1.5	3.4 $\pm$ 0.8
Striatum ($\pm$ ng/g)	2.5 $\pm$ 0.8	3.1 $\pm$ 1.2	4.3 $\pm$ 1.6	2.8 $\pm$ 0.6	4.2 $\pm$ 1.2	2.2 $\pm$ 0.7
Midbrain ($\pm$ ng/g)	4.0 $\pm$ 0.7	3.2* $\pm$ 0.9	8.1 $\pm$ 3.4	3.1* $\pm$ 0.6	6.6 $\pm$ 1.3	6.2* $\pm$ 1.3
Hippocampus ($\pm$ ng/g)	2.2 $\pm$ 0.8	1.7 $\pm$ 0.5	1.2 $\pm$ 0.5	1.7 $\pm$ 0.3	4.1 $\pm$ 1.8	1.9 $\pm$ 0.6
	Progesterone					
Plasma ($\pm$ ng/ml)	11.1 $\pm$ 6.1	9.8 $\pm$ 3.6	18.3 $\pm$ 2.1	16.9 $\pm$ 4.2	17.6 $\pm$ 2.8	19.9 $\pm$ 4.7
Hypothalamus ($\pm$ ng/g)	2.7 $\pm$ 0.6	3.7 $\pm$ 0.9	9.2 $\pm$ 4.8	5.1 $\pm$ 1.6	7.0 $\pm$ 2.4	3.9 $\pm$ 0.9
Prefrontal Cortex ($\pm$ ng/g)	7.6 $\pm$ 1.6	9.9 $\pm$ 2.5	6.3 $\pm$ 2.3	8.8 $\pm$ 2.6	9.8 $\pm$ 2.6	6.4 $\pm$ 2.5
Striatum ($\pm$ ng/g)	5.2 $\pm$ 1.2	6.9 $\pm$ 1.6	3.5 $\pm$ 1.1	5.1 $\pm$ 1.1	4.8 $\pm$ 1.2	5.1 $\pm$ 1.1
Midbrain ($\pm$ ng/g)	3.3 $\pm$ 0.9	2.7* $\pm$ 0.6	5.4 $\pm$ 1.3	2.0* $\pm$ 0.4	5.9 $\pm$ 1.2	2.0* $\pm$ 0.4
Hippocampus ($\pm$ ng/g)	7.3 $\pm$ 2.3	7.6 $\pm$ 2.1	7.1 $\pm$ 3.1	8.8 $\pm$ 2.1	11.5 $\pm$ 2.9	6.9 $\pm$ 1.7

* indicates a significant effect of pregnane xenobiotic receptor (PXR) antisense oligonucleotides (AS-ODN) infusions (P<0.05)

+ indicates a significant effect of testing condition (P<0.05).