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Review Article

Pregnane xenobiotic receptor knockout rats show decrements in exploratory, anti-anxiety, social, & sexual behaviors

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Abstract

Progestogens are well known to mediate reproductive cycles. However, the effects and mechanisms by which these processes occur are not completely understood. There are variations in progestogens over the estrous cycle of rodents, such that increased levels of progesterone and metabolites coincide with periods of increased exploration, activity, social engagement, and a greater likelihood of mating. Progesterone is secreted by ovaries and in brain areas where the necessary enzymes are localized and where there is *de novo* production of progestogens. The midbrain ventral tegmental area is an important brain region for mediating sexual receptivity of female rodents. Progestogens have actions in this brain area, the enzymes necessary to metabolize progesterone are localized to this region, and *de novo* production occurs here. In a genebank analyses of tissue from midbrain, the prenane xenobiotic receptor (PXR) was identified. Here, we utilize PXR knock out rats (PXR KO) and Sprague Dawley wild type controls to test the hypothesis that PXR is involved in natural estrous cycle variations in anxiety behavior, exploration, social, and/or sexual behavior. These behaviors were compared between PXR KO rats and their wildtype counterparts in behavioral estrous. We hypothesized that if the natural rise in progestogen-sensitive anxiety, exploration, social, and sexual behavioral estrous requires action through PXR then there would be decrements in these behaviors among PXR KO compared, to wildtype, rats. Further, we expected that these behavioral decrements would be amplified when a primary source of progestogens, the ovaries, were removed. We predicted that decrements in cycling effects of progestogens would be even more salient in ovariectomized PXR KO and wild type rats that would be more reliant on central PXR for production of progestogens to mediate behavioral responses. A similar pattern of results was revealed among proestrous and ovariectomized PXR rats to have less exploration in the open field, make fewer entries to the open arms in the plus maze, and to have fewer social and sexual interaction compared to their wild type counterparts.

Introduction

Progestogens mediate exploration, anxiety, social and sexual behavior in rodents, in part through non-classical actions in the midbrain ventral tegmental area (VTA).[1, 2] Previous work has demonstrated a role of the pregnane xenobiotic receptor (PXR) in mediating formation of progestogens and their non-traditional actions in the midbrain VTA.[1, 3]

Pregnane xenobiotic receptor also known as the steroid and xenobiotic sensing nuclear receptor (SXR) or nuclear receptor subfamily 1, group I, member 2 (NR1I2). PXR is a nuclear receptor, which is a transcription factor, characterized by a ligand-binding domain and a DNA-bind domain, with a primary function to sense foreign substances and upregulate proteins to detoxify and clear them. Pregnane xenobiotic receptor is a transcription regulator of the cytochrome P450 gene, CYP3A4, binding to the response element of the CYP3A4 promoter, as a heterodimer with the 9-cis retinoic acid receptor (RXR). It is activated by many broadly acting and therapeutic compounds that induce CYP3A4, such as steroids and dexamethasone. See reviews of this topic[4]

There are several experimental approaches that are used to ascertain whether a steroid's action is necessary and sufficient for a behavioral function. One is to examine how behaviors change across well-established natural variations in hormone-based cycles and how they those changes are modified by cessation of cyclicity. Another is to remove a target involved in its underlying mechanism and examine how behavior changes.

To test the hypothesis that PXR is involved in natural estrous cycle variations in anxiety behavior, exploration, social and/or sexual behavior, these behaviors were compared between PXR knockout (PXR KO) rats and their wildtype counterparts in behavioral estrous. We hypothesized that if the natural rise in progestogen-sensitive exploration, anxiety, social, and sexual behavior requires actions through PXR, then there would be expected decrements in these behaviors among PXR KO compared to wildtype, control rats in behavioral estrous (proestrus). Further, we expected that these behavioral decrements would be amplified when a primary source of progestogens, ovaries, was removed. We hypothesized that if central PXR is a mechanism for

central production of progestogens to mediate behavioral responses, then decrements in exploration, anxiety, social, and/or sexual behavior would be even more salient among ovariectomized PXR KO, compared to wildtype, control rats. The primary goal was to address issues of behavioral specificity of PXR.[5,6]

Methods

All methods were pre-approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Alaska, Fairbanks. All methods were per the National Institute Guide for Animal Care and Use. All research was conducted in the Biological Research and Diagnostic Building (BiRD) animal facility at The University of Alaska-Fairbanks (UAF).

Rat Strain

Rats were n=18 Sprague Dawley controls and n=24 PXR knockout rats, on a Sprague- Dawley background. PXR KO rats have a functional biallelic 20bp deletion within Nr1/2 gene and lack induction of cytochrome p4503A. PXR KO rats were derived from homozygous (-/-) breeder pairs purchased from Sage Laboratories. The Sprague Dawley wildtype (+/+) controls were derived from breeder pairs also obtained from Sage Laboratories. Adult rats were group housed until testing.

Estrous Cycle

Rats in Experiment One, Rats were gonadally-intact, adult, female rats, were approximately 60-90 days of age. Their vaginal epithelium was collected (between 0900-1100 with reversed light cycle: lights off:0600; on:2000). Epithelium was examined under a light microscope for presence of different cell types that predominant in each stage of the estrous cycle. Rats were cycled through two normal estrous cycles (4-5 day cycle) prior to testing. Vaginal epithelium of proestrous rats was characterized by nucleated cells, 4-5 days after the previous preponderance of this cell type. Rats were tested on the evening of proestrus, which corresponds to behavioral estrus, when female rats are receptive to social and sexual contacts from female and male rats, respectively. There were N=28 rats in this experiment; n=12 wild type rats and n=16 PXR KO rats.

Ovariectomy

Rats in Experiment Two, Rats were ovariectomized. Seven to ten days later, rats were administered oil vehicle one hour prior to behavioral testing. There were N=14 rats in this experiment; n=6 wild type rats and n=8 PXR KO rats.

Behavioral Testing

Behavioral data was collected simultaneously by an experimenter and the Any-Maze tracking system (Stoelting, Wood Dale, IL). On the day of testing, rats were transported in their home- cages on a cart to the testing area. Rats were singly housed in a clean cage immediately before testing in a battery of test.

Open Field

The open field (76 × 57 × 35 cm) has a 48-square grid floor (6×8 squares, 9.5 cm/side), with 24 peripheral, 16 central, and 8 inner squares. Rats are placed in the open field, observed for 5 mins, while the number of entries made to the outer, central, and inner 8 squares is recorded. The number of central and inner 8 square entries made by experimental groups (PXR KO) compared to controls is utilized as an index of anti-anxiety behavior. The total number of entries made by experimental groups compared to controls is an index of exploratory behavior.

Elevated plus maze

The elevated plus maze consists of 2 arms (49 cm long, 10 cm wide), enclosed by walls 30 cm high and 2 exposed arms. The arms of the apparatus are elevated 50 cm off of the ground. Rats are placed at the junction of the open and closed arms. The number of entries, and amount of time spent on the open (compared to closed) arms, are recorded for 5 min. The number of entries made, and time spent, in the open arms in experimental groups compared to controls is reported here as indices of anti-anxiety-like behavior. The number of total arm entries are reported as exploratory measures in the plus maze.

Social Interaction

The experimental subject and a conspecific (Sprague-Dawley, ovariectomized female) are placed in opposite corners of the open field. Time spent by the experimental rat engaging in social interaction (crawling over and under partner, sniffing of partner, following with contact, anogenital investigation, tumbling, and grooming) with the conspecific is recorded for 5 mins.

Sexual Behavior

The experimental subject remained in the open field and the conspecific was replaced by a male rat. The male rat had tested positive for sexual vigor, with a stimulus female rat, prior to his use in the experiment. The male was left with the Experimental rat until a mating sequence had concluded. The percentage of sexual contact that elicited a lordosis response (% lordosis), the intensity of the lordosis response on a scale of 0-3,[7] the percentage of sexual contact that elicited a proceptivity (hops, darts, ear wiggles; % proceptivity) or aggression (bites, rears, tail rattles, submissions; % aggression).

Metabolism

Plasma cholesterol was measured using the FDA approved PRIMA Cholesterol and Triglycerides 2-in-1 Home Test/Meter Kit Monitoring System. Plasma glucose was measured using the True Result Glucose Monitoring System. The PXR KO produces no overt phenotype under normal laboratory conditions,[4,5] but may influence cholesterol metabolism or be activated by behaviors, such as those expressed during behavioral testing. Before assessing effects on behavior, we first investigated if metabolism was affected in PXR KO or WT rats. We found that neither plasma cholesterol nor glucose levels differed between SD/WT and SD/PXR KO regardless of behavioral testing (data not shown).

Statistical Analyses

Multiple analyses of variance, MANOVAs, with Pillai's trace was used as the test statistic index (0-1) of how much the explanatory variable (genotype: PXR KO or control, WT, Sprague-Dawley) effected the response variable (open field, elevated plus maze, social, sexual behaviors). However, this index does not tell you which dependent measures are different from one another. To address this, *post-hoc* one-way analyses of variance, ANOVAs, were used to compare between- and within-group differences. The alpha level for statistical significance was p=0.05.

Results

Among proestrous rats, genotype, as an explanatory variable, had statistically significant overall effects (V = 0.32, F(4,23) = 2.72, p = 0.05) on behaviors, the response variable, i.e. open field, elevated plus maze, social interaction, and reproductive behaviors, shown in Figure 1. Proestrous PXR KO, compared to control, WT, Sprague-Dawley, rats exhibited significantly less time exploring the squares in the center of the open field ($p<0.05$). PXR KO, compared to control, rats made significantly fewer entries to the 8-inner squares of the open field and to the open arms in the elevated plus maze. PXR KO, compared to control, rats had significantly higher aggression quotients (Table 1).

Among ovariectomized rats, Pillai's trace revealed significant overall effect of the explanatory variable, genotype (V = 0.24, F (4,10) = 2.26, p = 0.05) on behaviors, the response variables, open field, elevated plus maze, social interaction, and reproductive behaviors, shown in Figure 2. Ovariectomized PXR KO, compared to control, rats spent less time in social interaction with a conspecific and made fewer lordosis responses to sexual contact. PXR-KO, compared to control, rats made fewer entries in the open arms of the elevated plus maze and demonstrated lower lordosis ratings (Table 2).

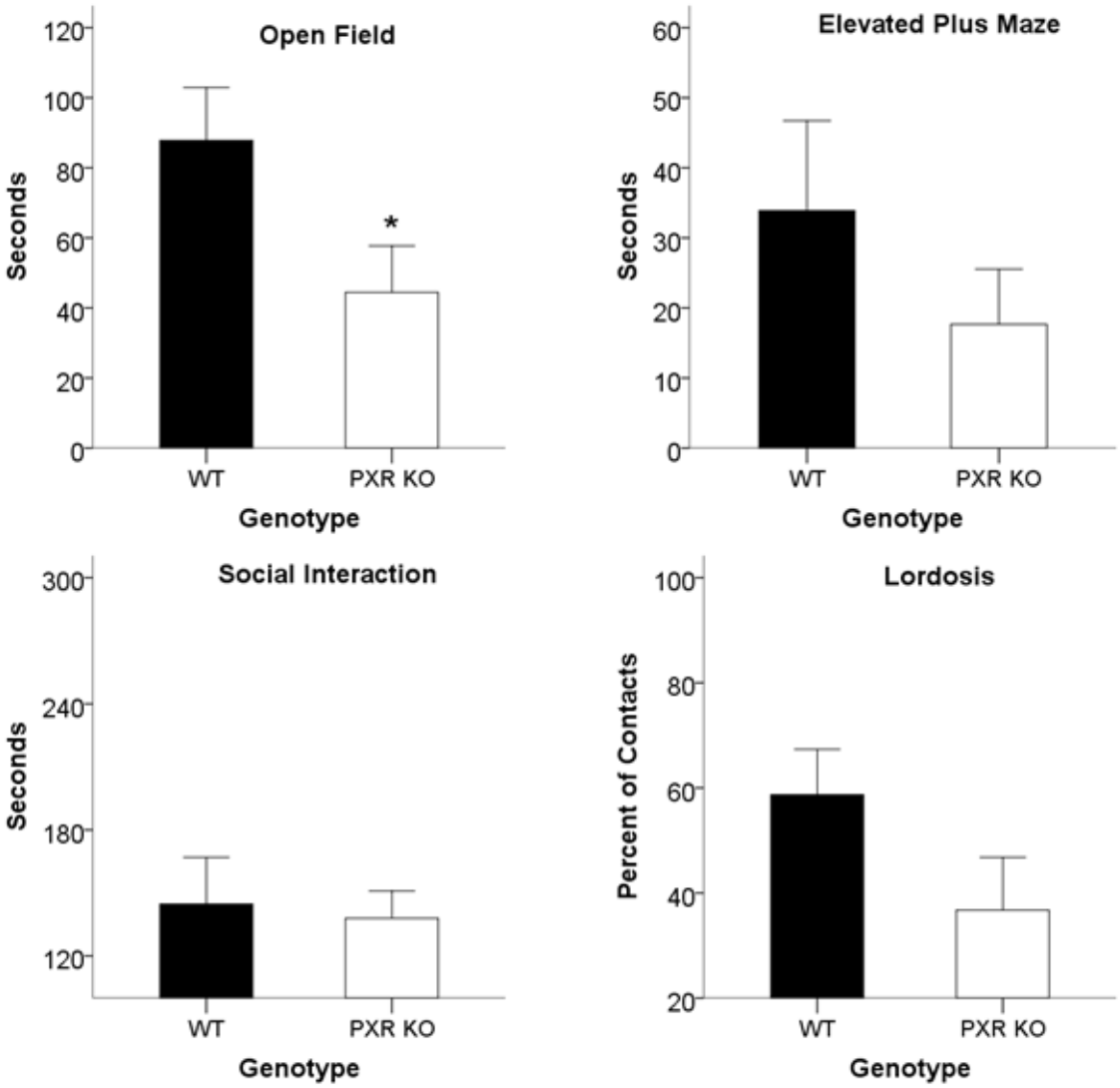

Figure 1: KO of PXR (open bars) and their wild type, Sprague Dawley controls (black bars) decreases anti-anxiety behavior. Mean ± SEM.

Discussion

The objective of this study was to determine the behavioral effects of proestrous and ovariectomy among Sprague-Dawley rats in which expression of the functional PXR was knocked out (PXR KO) or intact (WT). A similar pattern of results was revealed among proestrous and ovariectomized PXR rats to have significantly less exploration in the open field, significantly fewer entries to the open arms in the plus maze, and to have significantly fewer social and/or sexual interaction compared to their wild type counterparts. These findings and interpretation are largely consistent to that of results obtained after acute knock down of PXR with AS-ODNs.[3] A limitation of the acute knock down of PXR with AS-ODNs is that such factors may promote further upregulation of PXR.

To address this, PXR KO rats were used in this experiment. One criticism regarding the experiments using non-induced, genetically modified rodents is that rodents lack the gene at the time they are examined and

throughout their lifespan. Hence, it is not possible to attribute the extent to which the effects of the knockout gene are due to activational, acute effects of steroids, and/or organizing, earlier-on, development effects of steroids, a combination of both or other lifespan effects. Another limitation is that these studies were conducted using Sprague-Dawley rats, which are more in-bred and typically show less of a range of reproductive behaviors than do more outbred Long-Evans rats, typically used in experiments of reproductive behaviors. Examination of these behaviors in rats is preferable to using 129 mice (the typical strain and species used for knockout studies, because they are less likely to reject or have fatality from a mutation) for behavioral assessment, which can be challenging, due to variability in arousal/skittishness. There was sufficient power with 16 PXR KOs and 12 WT proestrous rats using MANOVAs to capture the relationship between PXR as an explanatory variable and behaviors as an outcome variable which accounted for 32% of the covariance (p=0.05). When ovariectomized, 8 PXR KO, and 6 WT, rats in the MANOVA with Pillai's trace accounted for 24% of the covariance (p=0.05). These effects reveal a significant explanatory effect of PXR genotype on behaviors examined (exploration in open field, anti-anxiety-like behavior on the open arms of the elevated plus maze, social behavior with a conspecific and response to sexual approaches).

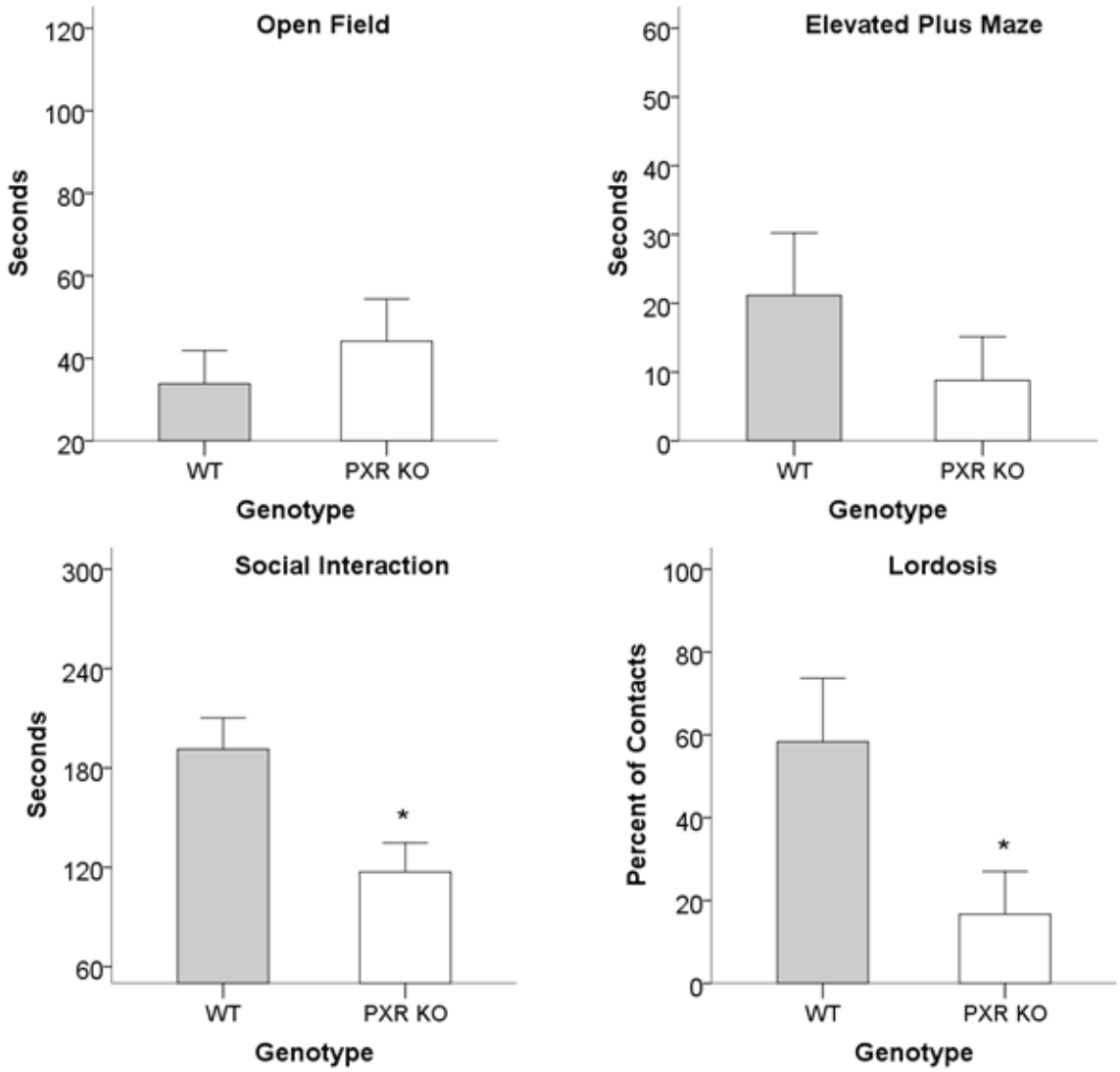

Figure 2: Ovariectomized PXR KO (open bars) and SD (gray bars) rats were affected much the same as PXR KO rats in proestrus (Figure 1). Mean ± SEM.

What are the broader implications of PXR's action and role? We first became interested in PXR when it came up in a microarray analysis of tissues from the midbrain VTA. It was one of ~50 genes and was among a small group that we had neither previously investigated nor were familiar with.[8] Upon further inquiry, we learned that PXR is found in liver and is involved in hepatic clearance of chemicals and toxins, but also many factors from the body and brain. PXR is a transcription regulator of cytochrome P450 gene, CYP3A4. It binds to the response element of the CYP3A4 promoter, as a heterodimer with RXR. It is activated by many factors that induce CYP3A4, such as progesterone, allopregnanolone, medroxyprogesterone acetate, and mifepristone.[4] These are clinically-relevant, positive modulators of PXR. Follow-up work will examine if effects of ovariectomy can be rescued by administering these positive modulators of ligands of PXR.

Table 1 Mean +/- standard error of the mean for behavioral parameters of proestrous rats not shown in figure 1

	wt (n=12)	Pxr ko (n=13-16)
Open field		
Total entries	306.5 (±30.4)	186.9 (±36.6) (n=16)
Inner 8 entries	46.1 (±9.4)	25.4 (±10.7) (n=16)*
Elevated plus maze		
Open Arm Entries	5.1 (±1.7)	2.3 (±1.3) (n=13)*
Closed arm entries	6.6 (±0.9)	4.6 (±1.2) (n=13)
Closed arm time	266.1 (±12.8)	282.3 (±7.9) (n=16)
Reproductive behaviors		
Lordosis rating	1.6 (±0.3)	0.9 (±0.3) (n=16)
Proceptivity Quotient	45.8 (±11.4)	33.0 (±11.3) (n=16)
Aggression Quotient	9.0 (±5.0)	35.0 (±7.3) (n=16)*

*represents signicant $p<0.05$ difference between wt, control Sprague-Dawley rats and Pxr ko rats on this measure.

Table 2 Mean +/- standard error of the mean for behavioral parameters of ovariectomized rats administered vehicle not shown in figure 2

	wt (n=6)	Pxr ko (n=8)
Open field		
Total entries	220.7 (±35.7)	253.6 (±52.5)
Inner 8 entries	16.8 (±5.0)	22.8 (±6.1)
Elevated plus maze		
Open Arm Entries	1.8 (±0.8)	0.4 (±0.3)*
Closed arm entries	3.3 (±0.9)	2.4 (±0.5)
Closed arm time	278.9 (±9.1)	291.2 (±6.3)
Reproductive behaviors		
Lordosis rating	0.7 (±0.2)	0.3 (±0.1)*
Proceptivity quotient	0	0
Aggression Quotient	12.5 (±8.5)	26.0 (±8.2)

*represents signicant difference $p<0.05$ between wt control Sprague-Dawley rats and Pxr ko rats on this measure.

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Conflict of interests

Author declares that there is no conflict of interest.

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