Research Article

Pregnane Xenobiotic Receptor Knockout Rats Show Decrements in Exploratory, Anti-Anxiety, Social and Sexual Behaviors that Can be in Part Rescued by Allopregnanolone, Medroxyprogesterone, or Mifespristone

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Abstract

Progestogens are secreted by ovaries, adrenals, placenta, central and peripheral nervous systems. The prenane xenobiotic receptor (PXR) is localized to the liver, kidney, and midbrain ventral tegmental area, where progestogen also have their effects to mediate behaviors related to rewards, reinforcement and reproduction. PXR plays an important role to regulate activity of cytochrome P450 gene, CYP3A4, which regulated steroid biosynthesis and clearance of it and many other factors. Here, we utilize PXR knock out rats (PXR KO) and Sprague Dawley wild type controls to test the hypothesis that PXR is involved in progestogen-sensitive variations in behaviors (exploration, anti-anxiety behavior, social and/or sexual behavior). These behaviors were compared between ovariectomized PXR KO or wildtype control rats that has been administered 2-4 hours earlier SC oil vehicle or a clinically relevant progestogen (allopregnanolone 10 mg/kg SC; medroxyprogesterone acetate 10 mg/kg SC; mifepristone 20 mg/kg SC). We hypothesized that if progestogen-sensitive exploration, anxiety, social, and sexual behavior require actions through PXR, then there would be expected changes in these behaviors among WT but not PXR KO rats. When administered vehicle, PXR KO rats exhibited less social, anti-anxiety, and sexual behavior than did WT control rats. Allopregnanolone and medroxyprogesterone acetate, but not mifepristone, reduced anti-anxiety behavior. Medroxyprogesterone Acetate and Mifepristone inhibited lordosis among WT and PXR KO rats respectively. Progestogens also facilitated behaviors of rats. Allopregnanolone and Medroxyprogesterone Acetate enhanced lordosis of PXR KO rats. Medroxyprogesterone Acetate and Mifepristone increased exploration and social behavior. These findings were consistent with our hypothesis that if progestogen-sensitive exploration, anxiety, social, and sexual behavior involve actions through PXR, then there would be changes in these behaviors among PXR KO compared to wildtype, control rats. Follow-up work will examine further the role of PXR in One ('s) Health.

Introduction

Progestogens are hormones that plays a crucial role in reproductive cycles, such as regulating the signs of proestrus and receptivity in rodents, as well as influencing pregnancy and parturition [1]. The C21 progestogens, pregnenolone, progesterone and 17-hydroxyprogesterone are of key importance [2]. Pregnenolone is a precursor to all steroid hormones. Progesterone, the natural product of the ovaries, is necessary for the establishment and maintenance of pregnancy [2]. Synthetic relatives of 17-hydroxyprogesterone have higher progestogenic activity than progesterone [2]. Medroxyprogesterone acetate is used as a contraceptive in combination with estrogen at a dosage that is higher than is used for cancer treatment [3]. Mifepristone, an anti-progestogen, is used with a prostaglandin for early termination of pregnancy [3].

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Progesterone and synthetic progestogens differ in their metabolism and actions at target tissues, particularly within the central nervous system [4]. Progesterone has actions at various membrane targets in the brain [4]. Progesterone is also metabolized through actions of 5α-reductase and 3β-hydroxysteroid dehydrogenase to form allopregnanolone, which has $GABA_A$ agonist like effects in the brain [4]. However, the synthetic progestogens medroxyprogesterone acetate and mifepristone neither metabolize to form allopregnanolone nor have actions at $GABA_A$ receptors [4]. One membrane receptor that progesterone, allopregnanolone, medroxyprogesterone acetate and mifepristone bind is the pregnane xenobiotic receptor (PXR).

The pregnane X receptor (PXR), identified in 1998 is a member of the nuclear receptor (NR) superfamily and is expressed in liver and intestine in all mammalian species examined to date [5]. The PXR is also referred to as the steroid and xenobiotic sensing nuclear receptor (SXR) or nuclear receptor subfamily 1, group I, member 2 (NR1I2). [5] PXR is a nuclear receptor transcription factor, with a ligand-binding domain and a DNA-bind domain; it can detect foreign substances and upregulate proteins to detoxify and clear them [5]. PXR activation is ligand dependent. After a ligand binds to PXR, PXR dimerizers with 9-cis retinoic acid receptor (RXR) and subsequently binds to the PXR response element. [5] This leads to up and down regulation of many genes involves involved in the metabolism of drugs, hormones, nutrients and toxins [6]. One of the most salient is the cytochrome P450 gene, CYP3A4 which participates in the metabolism of more than %50 of marketed drugs [6].

To test the hypothesis that PXR is involved in central actions of progestogens', allopregnanolone, medroxyprogesterone acetate, mifepristone or vehicle were administered to PXR knockout (PXR KO) or wildtype control (WT) rats. Progestogen-sensitive behaviors were examined. We hypothesized that if progestogen-sensitive exploration, anxiety, social, and sexual behavior invole actions through PXR, then there would be expected changes in these behaviors among PXR KO rats.

Methods

Methods and research protocol were pre-approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Alaska, Fairbanks (UAF). 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.

Rat Strain

PXR knockout rats (PXR KO) have a functional biallelic 20bp deletion within Nr1/2 gene and lack induction of cytochrome p4503A. Breeder pairs for PXR KO (-/-) rats were purchased from Sage Laboratories (St. Louis, MO), USA). Control, Sprague Dawley wildtype (+/+) breeder pairs were also obtained from Sage Laboratories.

Ovariectomy and Progestogen Administration

Adult rats 60-90 days of age were ovariectomized. Seven to ten days later rats were administered ligands for PXR, two to four hours prior to testing. Rats were randomly assigned to receive Allopregnanolone (10 mg/kg, SC or oil vehicle), medroxyprogesterone acetate (10 mg/kg, SC or oil vehicle) or mifepristone (20 mg/kg, SC or oil vehicle) prior to behavioral testing. There were N=24 WT and N=26 PXR KO rats in the experiment.

Controls WT (n=6) and PXR KO (n=8) were administered oil vehicle. Allopregnanolone was administered to WT (n=7) and PXR KO (n=8). Medroxyprogesterone acetate was injected to WT (n=7) and PXR KO (n=6). Mifepristone was administered to WT (n=4) and PXR KO (n=4).

Behavioral Testing

The Any-Maze tracking system from Stoelting (Wood Dale, IL) was used for behavioral testing. On the day of testing, rats were transported in their home- cages on a cart to the testing area. Rats were weighed, injected, and singly housed in a clean cage before testing in a battery of tests described below. Rats were housed on a reversed light cycle, so that testing occurred in the middle of dark cycle when they would normally be actively.

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 and observed for 300 seconds. The number of entries to the outer, central, and inner 8 squares is recorded. Central and inner 8 square entries are used as an index of anti-anxiety behavior. The total number of entries is considered a measure of exploratory behavior.

Elevated plus maze

The elevated plus maze is elevated 50 cm off of the ground. It has 2 closed arms (49 cm long, 10 cm wide), enclosed by walls (30 cm high) and 2 open exposed arms of the same size. Rats are placed at the junction of the open and closed arms. The number of entries, and amount of time spent on the open and closed arms are recorded for 300 seconds. The number of entries made, and time spent in the open arms are used as an index of anti-anxiety

behavior. The number of total arm entries are considered a measure of exploratory behavior in the plus maze.

Social Interaction

The experimental rat and a conspecific (ovariectomized Sprague-Dawley rat) are placed in opposite corners of the open field (76 × 57 × 35 cm). Time spent by the experimental rat engaging in social interaction (sniffing of conspecific, following with contact, anogenital investigation, crawling over and under conspecific, tumbling, and grooming) is recorded for 300 seconds.

Sexual Behavior

The experimental rat remained in the open field and the conspecific was removed. A male rat that had tested positive for sexual vigor, with a stimulus female rat, prior to its use in the experiment, was placed in the opposite corner of the open field from the experimental rat. The male was left with the Experimental rat until a mating sequence had ended, or 300 seconds had passed with no contacts. The percentage of sexual contacts that elicited a lordosis response (% lordosis), the intensity of the lordosis response on a scale of 0-3 [7], the percentage of sexual contacts that elicited a proceptivity (hops, darts, ear wiggles; % proceptivity) or aggression (bites, rears, tail rattles, submissions; % aggression) were recorded.

Statistical Analyses

Two-way analysis of variance for multiple dependent measures, MANOVAs, with Pillai's trace as the test statistic index (0-1) was used to determine how much the explanatory variables genotype (PXR KO; WT) and progestogen (vehicle, allopregnanolone, medroxyprogesterone acetate, mifepristone) accounted for the response variable, behaviors (open field, elevated plus maze, social, sexual behaviors). This index reveals the overall net change in behaviors but not how any behavior may be different due to the explanatory variables. To address this, one-way analyses of variance, ANOVAs, with Bonferroni corrections, were used as post-hoc tests. The alpha level for statistical significance was $p=0.05$.

Results

Pillai's trace revealed a significant interaction between the explanatory variables, genotype X progestogen ($V = 0.54$, $F(12,123) = 2.26$, $p = 0.01$). When administered vehicle PXR KO rats exhibited less anti-anxiety, social, and sexual behavior than did their wildtype (WT) counterparts. PXR KO, compared to WT, rats made fewer inner entries in the open arms of the elevated plus maze and spent less time in social interaction when administered vehicle (Figure 1, Table 1).

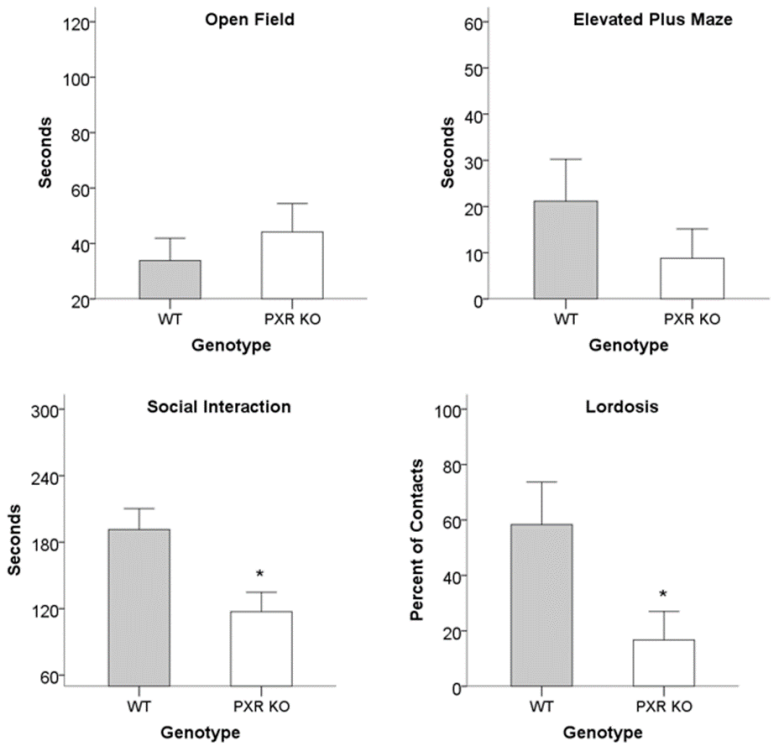

Figure 1: Ovariectomized PXR KO, compared to wildtype (WT) control, rats demonstrated less time in social interaction with a conspecific and had lower percentage of lordosis (sexual) responses to mounting by male rats. Mean ± SEM.

Table 1: Mean +/- standard error of the mean for behavioral parameters of ovariectomized rats administered vehicle not shown in Figure 1.

	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)

Allopregnanolone, compared to vehicle, increased exploration and decreased social interaction in WT rats. Allopregnanolone reversed anti-anxiety behaviors of WT and PXR KO rats and increased lordosis among PXR KO rats (Figures 1 & 2, Tables 1 & 2).

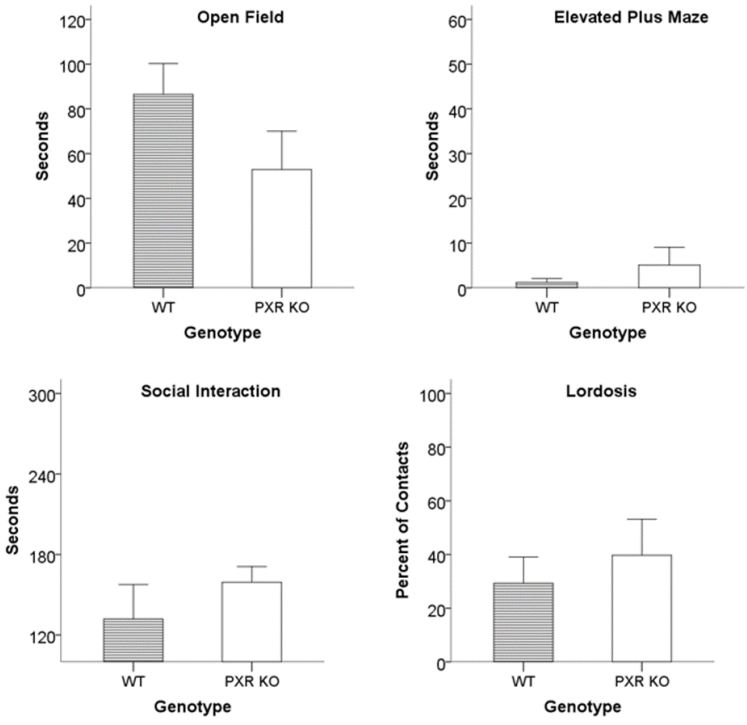

Figure 2: Treating rats with Allopregnanolone (10mg/kg, SC1 h prior to behavioral testing) increased exploration and decreased social interaction in wildtype (WT) rats when compared to vehicle-treated rats. Treatment with Allopregnanolone reversed anti-anxiety behaviors of WT and PXR KO rats and increased lordosis response to sexual contacts among PXR KO rats. Mean ± SEM. See Figure 1 for Vehicle comparison.

Table 2: Mean +/- standard error of the mean for behavioral parameters of ovariectomized rats Administered Allopregnanolone not shown in figure 1.

	WT (n=7)	**PXR KO (n = 8)**
Open field		
Total entries	279.7 (±27.7) (n = 7)	199.3 (±31.9)*
Inner 8 entries	24.6 (±4.9) (n = 7)	16.9 (±4.2)
Elevated plus maze		
Open Arm Entries	0.2 (±0.2) (n = 5)	0.6 (±0.3)
Closed arm entries	3.8 (±1.2) (n = 5)	2.5 (±0.7)
Closed arm time	298.8 (±0.9) (n = 7)	294.9 (±4.0)
Reproductive behaviors		
Lordosis rating	0.5 (±0.1) (n = 7)	0.8 (±0.2)
Proceptivity quotient	0 (n = 7)	20.8 (±12.9)
Aggression Quotient	19.0 (±12.8) (n = 7)	29.3 (±8.7)

Medroxyprogesterone Acetate, compared to vehicle, increased exploration and social interaction with a conspecific, reduced anti-anxiety behavior in the elevated plus maze, while inhibiting lordosis of WT but facilitating it among PXR KO rats (Figures 1 & 3, Tables 1 & 3).

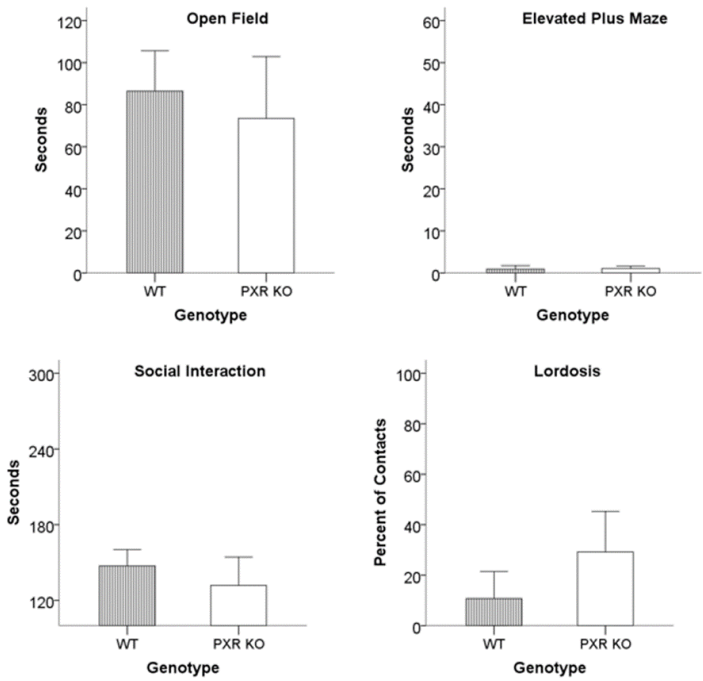

Figure 3: The synthetic progestogen Medroxyprogesterone Acetate (10 mg/kg, SC, 1h before testing) to PXR KO or WT increased exploration in the open field, decreased social interaction with a conspecific, reduced anti-anxiety behavior in the elevated plus maze, while inhibiting lordosis behaviors of WT but facilitating it among PXR KO rats. Mean ± SEM. See Figure 1 for Vehicle comparison.

Table 3: Mean +/- standard error of the mean for behavioral parameters of ovariectomized rats administered Medroxyprogesterone Acetate not shown in figure 3.

	WT (n = 7)	**PXR KO (n = 6)**
Open field		
Total entries	258.6 (±45.6)	264.0 (33.6)
Inner 8 entries	29.6 (±6.9)	15.3 (±4.6)
Elevated plus maze		
Open Arm Entries	0.3 (±0.2)	0.5 (±0.2)
Closed arm entries	1.3 (±0.2)	2.7 (±1.0)
Closed arm time	299.1 (±0.8)	299.0 (±0.6)
Reproductive behaviors		
Lordosis rating	0.1 (±0.1)	0.7 (±0.3)
Proceptivity quotient	0	35.4 (±17.2)
Aggression Quotient	23.2 (±14.5)	28.5 (±13.7)

Mifepristone, versus vehicle, increased exploratory behavior in the open field and inhibited lordosis in WT and PXR KO rats. Social interaction was also greater among PXR KO rats administered Mifepristone compared to vehicle (Figures 1 & 4, Tables 1 & 4).

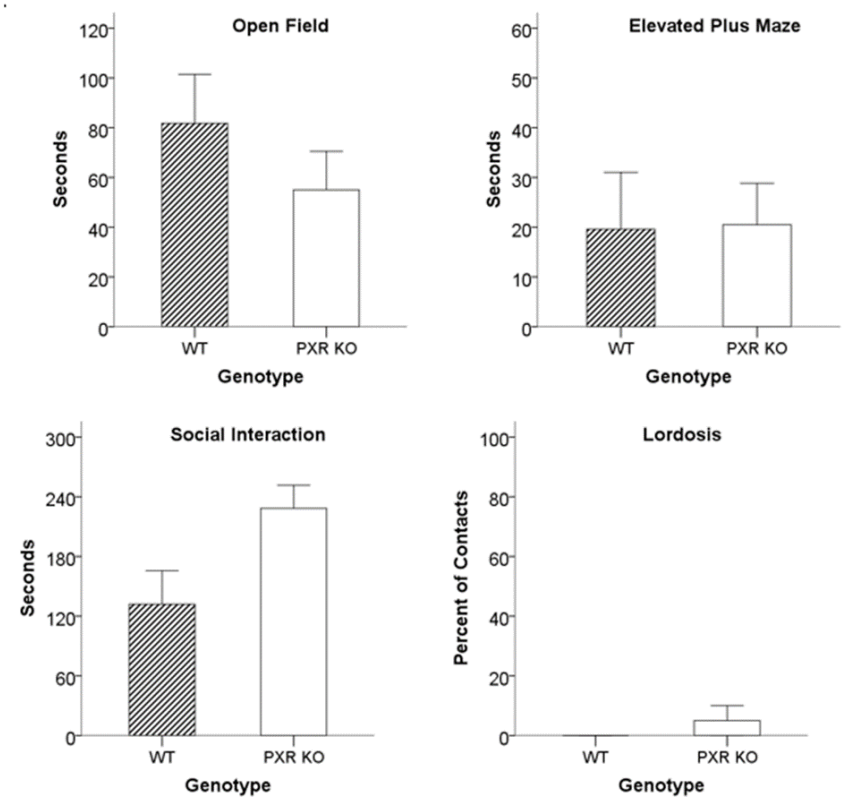

Figure 4: Treating with Mifepristone (20mg/kg, SC, 1h before testing), compared to vehicle, increased exploratory behavior in the open field and inhibited lordosis in WT and PXR KO rats. Social interaction was also greater among PXR KO rats administered Mifepristone compared to vehicle. Mean ± SEM. See Figure 1 for Vehicle comparison.

Table 4: Mean +/- standard error of the mean for behavioral parameters of ovariectomized rats administered mifepristone not shown in figure 4.

	WT (n = 4)	**PXR KO (n = 4)**
Open field		
Total entries	295.5 (±76.9)	259.5 (±45.7)
Inner 8 entries	22.5 (±12.4)	16.5 (±5.7)
Elevated plus maze		
Open Arm Entries	(n = 0)	2.0 (±0.8)
Closed arm entries	(n = 0)	3.8 (±1.0)
Closed arm time	280.4 (±11.4) (n = 4)	279.5 (±8.3)

Reproductive behaviors		
Lordosis rating	0	0.1 (±0.1)
Proceptivity quotient	0	0
Aggression Quotient	37.5 (±12.5)	50.0 (±0)

Discussion

Genotype and progestogen interacted significantly as explanatory variables to account for 54% of the variance in the response variable, behaviors (exploration, anti-anxiety, social and sexual behavior). When administered vehicle, PXR KO rats exhibited less social, anti-anxiety, and sexual behavior than did WT rats. Allopregnanolone decreased anti-anxiety behaviors of WT and PXR KO rats and increased lordosis among PXR KO rats. Medroxyprogesterone Acetate enhanced exploration and social behavior, reduced anti-anxiety behavior, while inhibiting lordosis behaviors of WT but facilitating it among PXR KO rats. Mifepristone increased exploration and social behaviors, but inhibited lordosis behaviors of PXR KO rats.

These findings are consistent with our hypothesis that if progestogen-sensitive exploration, anxiety, social, and sexual behavior involve actions through PXR, then there would be expected changes in these behaviors among PXR KO compared to wildtype, control rats. When administered vehicle, PXR KO rats exhibited less social, anti-anxiety, and sexual behavior than did WT control rats. Allopregnanolone and medroxyprogesterone acetate, but not mifepristone, reduced anti-anxiety behavior. Medroxyprogesterone Acetate and Mifepristone inhibited lordosis among WT and PXR KO rats respectively. Progestogens also facilitated behaviors of rats. Allopregnanolone and Medroxyprogesterone Acetate enhanced lordosis of PXR KO rats. Medroxyprogesterone Acetate and Mifepristone increased exploration and social behavior.

It is not surprising to see bidirectional behavioral changes with progestogen administration to WT or PXR KO rats. First, with a functional PXR, ligand binding can lead to greater downregulation than upregulation of genes involved with clearance [5]. Second, there are well known differences in sensitivities to progestogens related to their structure and activity, dosage, prior exposure, and an individual's prior responses. For example, women who did not have a history of premenstrual syndrome reported negative mood and physical symptoms, when taking progesterone 400 mg/day, but neither a higher dosage of progesterone nor placebo. Women with prior premenstrual syndrome reported no progesterone-induced symptom cyclicity. The authors concluded that in women without prior premenstrual syndrome, natural progesterone caused negative mood effects similar to those induced by synthetic progestogens but the dose-effect relation was complex [8].

Like all studies, this experiment has its limitations. One criticism regarding 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 progestogens, 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 reproductively-relevant 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 is also a question of sufficient power with the size of group varying between 4 and 8 observations per group. Ovariectomized rats, n=4-8/group in the MANOVA with Pillai's trace accounted for 54% of the covariance (p=0.01). These effects reveal a significant explanatory effect of PXR genotype and progestogens on behaviors (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).

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 [9]. Upon further

inquiry, we learned that PXR is found in liver and kidney. It is highly 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, including progestogens, 50% of marketed medications, household products, pesticides and environmental contaminants. Follow-up work will examine further the role of PXR in One('s) Health [3,5,6,9-11].

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