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Progesterone receptor expression in the brain of the socially monogamous and paternal male prairie vole

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Abstract

Differences in the social organization and behavior of male mammals are attributable to species differences in neurochemistry, including differential expression of steroid hormone receptors. However, the distribution of progestin receptors (PR) in a socially monogamous and spontaneously parental male rodent has never been examined. Here we determined if PR exists and is regulated by testicular hormones in forebrain sites traditionally influencing socioreproductive behaviors in male prairie voles (*Microtus ochrogaster*). We hypothesized that PR expression in male prairie voles would differ from that described in other male rodents because PR activity inhibits parental behaviors and social memory in laboratory mice and rats. Adult male prairie voles received a sham surgery, were gonadectomized, or were gonadectomized and implanted with a testosterone-filled capsule. PR immunoreactivity (PRir) was measured four weeks later in areas of the hypothalamus and extended amygdala. A group of gonadally intact female prairie voles was included to reveal possible sex differences. We found considerable PRir in all sites examined. Castration reduced PRir in males' medial preoptic nucleus, anteroventral periventricular nucleus, ventromedial hypothalamus, and posterodorsal medial amygdala, and it was maintained in these sites by testosterone. This is the first study to examine PR expression in brain sites involved in socioreproductive behaviours in a socially monogamous and spontaneously paternal male rodent. Our results mostly reveal cross-species conservation in the distribution and hormone sensitivity of PR expression. Because PR interferes with aspects of sociality in other male rodents, PR may eventually be found to have different neurobiological actions in male prairie voles.

Keywords

hypothalamus; infanticide; monogamy; progesterone; sex difference; social behavior

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1. Introduction

Male mammals differ tremendously in their social organization and the behaviors they display within their species-specific contexts (Clutton-Brock, 2009). Laboratory studies examining the neurobiology underlying differences in social organization and behavior have often compared socially monogamous and biparental male rodents with those that are socially non-monogamous and uniparental (*i.e.*, the mother is the only caregiver) to better understand the proximate and ultimate causes of their markedly different behavioral repertoires. For example, male laboratory rats, most male inbred mice, and males of some species of voles are polygamous and also do not typically display caregiving behaviors. Conversely, other male rodents including prairie voles (*Microtus ochrogaster*) form lifelong pairbonds after mating and are highly parental even without sexual experience (Young et al., 2011). Studies examining neurobiological correlates of these species differences in sociality have implicated numerous peptide systems in the forebrain. Male prairie voles have 3-6-fold greater oxytocin receptor expression in the nucleus accumbens and bed nucleus of the stria terminalis (BST) compared to polygamous and non-parental male voles. Prairie voles also differ from polygamous voles in their vasopressin V1a receptor expression in the BST, ventral pallidum and olfactory bulb (Insel and Shapiro, 1992; Insel et al., 1994), and in their corticotropin-releasing hormone (CRH) receptor expression in many areas of the brain (Lim et al., 2005).

In addition to neuropeptides, forebrain steroid hormone receptor expression differs between paternal and monogamous rodents and uniparental and polygamous ones. Cushing and colleagues have shown that the paternal and socially monogamous pine vole (*M. pinetorum*) has lower estrogen receptor isoform alpha (ER) expression in the medial amygdala and BST compared to the polygamous and non-parental montane vole (*M. montanus*) (Cushing and Wynne-Edwards, 2006). They also found that the highly parental male Djungarian hamster (*Phodopus sungorus*) has lower ER expression in the BST than does the less parental male Siberian hamster (Cushing and Wynne-Edwards, 2006). Even within prairie voles, more social members of the species have lower ER expression in the medial preoptic area, ventromedial hypothalamus, and BST compared to less social individuals (Cushing et al., 2004). A relationship between sociality and androgen receptor expression in these brain sites has not been found, though (Cushing et al., 2004). The functional significance of these differences in ER expression is demonstrated by the reduced sociality in male prairie voles with viral vector-mediated overexpression of ER in the medial amygdala or BST (Cushing et al., 2008; Lei et al., 2010).

The neural network necessary for parental and other social behaviors in rodents not only contains high densities of estrogen and androgen receptors (Koch and Ehret, 1989; Li et al., 1997; Pfaff and Keiner, 1973; Simerly et al., 1990), but is also exquisitely responsive to progesterone. Progesterone mediates many of its neurobehavioral functions by acting on two isoforms of a classic nuclear receptor, PRA and PRB (Wagner, 2006; Mani et al., 2006), that differ in their ratio depending on the brain site and hormone milieu (Carrillo-Martínez et al., 2011). PR relevance for social behavior in male rodents is readily demonstrated by progesterone receptor knockout mice (PRKOs), which exhibit aberrations in most social behaviors examined including responses to pups and copulation (Lydon et al., 1995; Phelps et al., 1998; Schneider et al., 2003).

PR expression within the rodent brain, particularly within the hypothalamus/preoptic area, is influenced by gonadal hormones. Indeed, numerous investigations have demonstrated that estradiol or testosterone treatment increases brain PR binding, PR mRNA levels, and PR protein expression (Blaustein and Turcotte, 1989; Blaustein et al., 1980, 1988; Brown et al., 1987, 1996; Etgen, 1985; Lauber et al., 1991; Moguilewsky and Raynaud, 1979; Scott et al.,

2002; Shughrue et al., 1997; Simerly et al., 1996; Warembourg et al., 1989). What is most striking about this hormone-mediated regulation of PR expression in the brain is that it is evolutionarily conserved and present in many non-mammalian species including whiptail lizards (Godwin and Crews, 1999), chickens (Gasc and Baulieu, 1988), and frogs (Roy et al., 1986).

In the present study we investigated whether PR expression in neural sites traditionally involved in mammalian social behaviors differs between male prairie voles and what has typically been reported in non-parental, polygamous, and less gregarious male rodents (including laboratory rats and mice). This was of interest because the inhibition of parenting in non-parental male mice requires the presence of PR (Schneider et al., 2003), so in prairie voles one may expect a paucity of PR in brain sites such as the preoptic area (POA) and bed nucleus of the stria terminalis (BST) that promote parental behaviors (Numan and Insel, 2003). Additionally, we were interested in whether the gonadal hormone upregulation of PR seen in polygamous rodents would also exist in prairie voles. As noted above, the upregulation of preoptic and hypothalamic PR by steroid hormones is thought to be an evolutionarily conserved phenomenon, but given the spontaneous paternal behavior of male prairie voles, it could be counterproductive to have PR in their POA upregulated by testicular hormones. To accomplish these goals, we examined PR immunoreactivity in select regions of the male prairie vole forebrain in animals that were gonadally intact, castrated, or castrated and subcutaneously implanted with a testosterone-filled capsule. A group of gonadally intact virgin female prairie voles, which are induced ovulators with very low circulating ovarian hormones in the absence of male cues (Carter et al., 1989), were also examined.

2. Results

PRir was present in all six brain sites analyzed in all groups of voles (Fig. 1). In the medial preoptic nucleus (MPN), Sham males had very high levels of PRir that were reduced by castration and maintained by testosterone ($F(3,23) = 9.00, p < 0.001$; Fig. 2, Fig. 3). Females had relatively low PRir in the MPN, similar to castrated males. In the anteroventral periventricular nucleus (AVPV), high levels of PRir were found in Sham males, GDX+T males, and females. PRir in the AVPV of GDX males was significantly lower than all of these groups ($F(3,20) = 5.358, p < 0.009$; Fig. 3).

In the principal nucleus of the bed nucleus of the stria terminalis (pBST), GDX+T males had significantly more PRir than did GDX males ($H = 11.75, p < 0.008$; Fig. 4); no other group comparisons in this site were statistically significant. In the posterodorsal medial amygdala (MeApd) the pattern of differences among groups was similar to that found in the MPN - very high PRir in Sham and GDX+T males and significantly lower PRir in GDX males and females ($F(3,20) = 6.22, p < 0.005$; Fig. 4).

In the ventrolateral division of the ventromedial nucleus (VMNvl), Sham and GDX+T males did not differ in their PRir and both groups had greater levels than did GDX males. PRir in females was also greater than that found in GDX males ($F(3,24) = 3.13, p < 0.05$; Fig. 5, Fig. 6). There were no significant differences among groups in their PRir in the arcuate nucleus (ARN) ($F(3,22) = 0.459, p > 0.71$; Fig. 6).

In addition to the brain sites analyzed in detail above, we observed considerable PRir in the gonadally intact male prairie vole anterior hypothalamus, paraventricular nucleus, lateral hypothalamus, and posteromedial division of the cortical amygdala. Low levels of PRir were found in the claustrum, retrochiasmatic nucleus, reticular thalamic nucleus, deep

mesencephalic nucleus, substantia nigra (pars compacta), and the VTA. Very little or no PRir was observed in any other forebrain or midbrain sites.

3. Discussion

The present study examined PRir in male prairie vole forebrain sites that have well-established roles in mammalian reproductive physiology and behavior. PRir was found in all six brain sites we analyzed, similar to what has been found in numerous studies reporting abundant PR mRNA, binding, or immunoreactivity in these sites in gonadally intact or gonadal hormone-treated castrated male laboratory rats, mice, and guinea pigs (Blaustein et al., 1980, 1988; Brown et al., 1987, 1996; Lauber et al., 1991; Scott et al., 2002). We did not necessarily expect this conservation across species, though. We hypothesized that PRir would not be found in abundance particularly in the POA and BST of the spontaneously paternal prairie vole because progesterone inhibits parental behaviors in other rodents. For example, progesterone suppresses immediate-early gene activity in the POA and BST, as well as the onset of maternal behavior, in female rats (Bridges et al., 1978; Sheehan and Numan, 2002). Furthermore, mutant male mice that cannot express PR are less infanticidal and more parental compared to wildtype males (Schneider et al., 2003). Consistent with this result, wildtype male mice chronically treated with the PR antagonist RU-486 are highly paternal (Schneider et al., 2003). It is clear from the present results, however, that an absence of PR in the POA and BST does not contribute to the spontaneous paternal behavior of male prairie voles. It also might have been possible that PRir would be low in the male prairie vole MeApd, a major source of the extra-hypothalamic arginine-vasopressin (AVP) that promotes prairie vole paternal behavior (Wang et al., 1994). Many extra-hypothalamic AVP-synthesizing cells contain PRir, and their AVP expression is negatively influenced by progesterone (Auger and Vanzo, 2006; Rood et al., 2008) in a manner that impairs male social recognition and behavior (Bychowski and Auger, 2012). Even so, similar to non-parental male laboratory rats and mice, our gonadally intact male prairie voles had high PRir in the MeApd. It would be valuable to know whether the distribution of PRir in these and other sites differs between male prairie voles and non-monogamous male voles. If non-monogamous male rats and mice are any indication, a conservative prediction is that there may be little or no difference, although relative levels of expression may differ among species.

Instead of inhibiting parenting behaviors in male prairie voles, perhaps PRs are permissive for it. As noted above, paternal voles have lower ER expression in the POA and BST compared to closely related, non-parental voles (Cushing and Wynne-Edwards, 2006). ER expression in some brain areas is blunted by progesterone (Blaustein and Brown, 1984; Don Carlos et al., 1995), a phenomenon thought to be involved in terminating estrus behavior, so a similar PR-mediated downregulation of ER may be necessary for paternal behavior in male prairie voles. This may partly explain why paternal behavior in male prairie voles and dwarf hamsters is unaffected by adult castration or aromatase inhibition. That is, PR-mediated suppression of estrogenic activity may be required for their paternal behavior (Hume and Wynne-Edwards, 2006; Lonstein and De Vries, 1999). This would be in line with De Vries (2004), who suggested that high circulating testosterone in males requires compensatory mechanisms that prevent the hormone from interfering with male-atypical behaviors, such as parenting. Experimentally blocking PR's influence in the presence of high estradiol (naturally found in males after the aromatization of testicular testosterone) might reveal such a phenomenon in male prairie voles. The source of progesterone binding to PRs and potentially influencing male prairie vole behavior is unknown, but prairie voles have extremely high adrenal glucocorticoid release (Taymans et al., 1997) and this might extend to their adrenal release of other steroids including progesterone (Chrousos et al., 1984; Hnatzuk and Morrell, 1995).

PRir in most brain sites examined was very high in our gonadally intact males, was considerably reduced in the castrated voles, and maintained at high levels in castrated voles implanted with a testosterone-filled capsule. It should be noted that standard immunohistochemical procedures do not readily detect membrane or other extra-nuclear steroid receptors, which have important neurobehavioral functions (Mani and Blaustein, 2012), so the hormone influences on PR expression observed in our voles probably reflect changes mostly occurring in nuclear PR. Direct comparison of our results with previous studies examining the effects of gonadal hormones on neural PR expression or binding in other castrated male animals is difficult because these studies invariably involved acute, rather than chronic, hormone treatments. Even so, our results are very consistent with the reduced PR expression observed in the preoptic area and hypothalamus post-castration in male rats and guinea pigs, and increase after a single injection of estradiol benzoate or testosterone propionate (Blaustein et al., 1980; Brown et al., 1987; Etgen, 1985; Lauber et al., 1991; Moguilewsky and Raynaud, 1979). It remains to be determined what steroid receptor(s) mediates testosterone's ability to maintain high PRir in male prairie voles. As far as we are aware, there is no evidence that PR is upregulated by androgen receptor activity within the brain, so it is more likely that PRir was increased after testosterone's aromatization to estradiol and the subsequent stimulation of estrogen receptors. This is known to be the mechanism for testosterone's upregulation of PRir in the MPN of male rat fetuses (Quadros et al., 2002) and previous studies of other neural systems or parental behavior in prairie voles do reveal almost identical effects of exogenous testosterone and estradiol (Cavanaugh et al., 2010; Lonstein and De Vries, 1999; Lonstein et al., 2002, 2005). Future studies giving castrated male prairie voles estradiol, or intact prairie voles an aromatase inhibitor, would help confirm that an ER-mediated mechanism is involved in how testosterone influences their PRir. A notable exception to the hormone effects we found on PR expression was the male prairie vole ARN, which was unaffected by castration or testosterone. This result is consistent with the similar percentage of cells expressing PR mRNA found in the ARN of castrated male rats treated with either oil, EB or TP (Lauber et al., 1991), although Brown et al. (1987) reported that castrated male rats show a tremendous increase in PR binding in the ARN 48 hr after a single injection of EB.

The group of female prairie voles included in our study provides some insight into progesterone's role in their socioreproductive behavior. Unlike the cyclic ovarian function of rats and mice, virgin female prairie voles have tonically low ovarian activity until urinary cues from males initiate a very prolonged behavioral estrus (over 24 hr; Witt et al., 1988) and ovulation is triggered by the vaginocervical stimulation received during copulation (Carter et al., 1995). Despite these species differences in ovarian function, some PRir was found in the same brain sites in female prairie voles as in female rats and mice. Studies of PR expression in other induced-ovulating female mammals have revealed some species differences, but no consistent differences between spontaneous and induced-ovulators have emerged (Baum et al., 1986; Bayliss et al., 1991; Caba et al., 2003; Camacho-Arroyo et al., 2007). We do not know if PRir in the sites we quantified increases in female prairie voles exposed to male cues or injected with EB, as is the case for estradiol-treated female rats, mice, and guinea pigs (with the exception of the MeA; Blaustein and Turcotte, 1989). The only other study of PR expression anywhere in the female prairie vole brain examined PR binding in females naturally induced into estrus by male cues, and found that PR binding in the mediobasal hypothalamus was increased 48 hr after male exposure when compared to unstimulated females (Cohen-Parsons and Carter, 1988). It is unclear if this increased PR binding is estradiol-dependent because ovarian estradiol rises quickly after male exposure but seems to return to baseline well within 48 hr (Carter et al., 1989). In fact, this increased mediobasal hypothalamic PR binding temporally corresponds better to the rise in progesterone seen in *ad-lib* mated female prairie voles and may be involved in terminating

their behavioral estrus (Carter et al., 1989; Cohen-Parsons and Carter, 1988), as it does in some other species.

Including the group of female prairie voles also allowed us to determine if there are sex differences in PRir in gonadally intact prairie voles. We found that males had more PRir than females in the MPN and MeApd. There is no sex difference in the volume or cell density of the prairie vole MPN that could easily explain this sex difference in PRir (Shapiro et al., 1991). Because the sex differences in PRir in both the MPN and MeApd no longer existed when females were compared to castrated males, the sex differences seem to be due to an activational effect of testosterone on PRir in males as opposed to sexual differentiation of the capacity for prairie voles to express PRir. Most studies of PR mRNA, binding, or immunoreactivity in other animals do report similarly low PR expression in the brains of gonadectomized males and females (Blaustein et al., 1980; Lauber et al., 1991; Migulewsky and Raynaud, 1979). Significant sex differences in hypothalamic PR are often observed in other animals (usually $F > M$) when circulating gonadal hormones are equated at high levels with exogenous steroids (Lauber et al., 1991; Migulewsky and Raynaud, 1979; Brown et al., 1987, 1996), but we have data demonstrating that when female prairie voles are treated chronically with testosterone, PRir in their MPN and MeApd is almost as high as that found in gonadally intact or testosterone-treated castrated males (Williams et al., unpublished data), suggesting little or no sexual differentiation of PR responsiveness to exogenous gonadal hormones in prairie voles.

In sum, differences among rodents in their social repertoire are associated with species differences in the distribution and expression of receptors for many neurochemical systems, including some steroid hormones. It was surprising that the distribution and testosterone regulation of forebrain PR expression in the monogamous and biparental male prairie vole was more similar than different to what has been demonstrated in male laboratory rats, mice, and guinea pigs. Differences among animals in the neurochemical phenotype of forebrain cells expressing PR, or in the intracellular effects of PR activity, may be better predictors of species differences in socioreproductive strategy.

4. Experimental Procedure

4.1 Subjects

Virgin prairie voles (*Microtus ochrogaster*) were born and raised in the Michigan State University breeding colony, which was established in 2002 from breeding stock originating from Illinois voles as previously described (Northcutt and Lonstein, 2007). Animals were maintained on a 14:10-hour light:dark cycle, with an ambient temperature maintained at $\sim 21^{\circ}\text{C}$, and were housed in clear plastic cages ($48 \times 28 \times 16$ cm) containing wood chips, wood shavings, and a substantial hay covering. Water and a food mixture were freely available. Food mixture contained cracked corn, whole oats, sunflower seeds, and rabbit chow (Teklad rodent diet No. 2031) in a ratio of 1:1:2:2. Voles were placed in same-sex sibling groups of 2-3 animals/cage at weaning and maintained in these groups until they were sacrificed.

4.2 Castration and Testosterone Administration

Animals were anaesthetized with a cocktail containing ketamine, xylazine and acepromazine (Northcutt and Lonstein, 2007). Males were either gonadectomized (GDX) or received a sham surgery (Sham) in which an incision was made in the scrotum but the testes were not removed. A group of female prairie voles were also anesthetized and received a sham surgery in which the ovaries were visualized but not removed. Approximately half of the gonadectomized males were immediately subcutaneously implanted with a 2.5-cm-long

Silastic capsule containing testosterone (Sigma, St. Louis, MO) (GDX+T; $n = 7$). The other gonadectomized males ($n = 6$) and all the Sham males ($n = 7$) and the females ($n = 5$) each received an empty Silastic capsule. Four weeks following surgery, subjects were killed and their brains collected as described below. We sacrificed animals four weeks after surgery to more easily compare the results with other castration-induced neural and behavioral effects we have recently reported in male prairie voles that used a similar time of sacrifice (Cavanaugh and Lonstein, 2010; Holmes et al., 2009; Lonstein et al. 2002, 2005; Northcutt et al., 2007). All procedures were in accordance with the Institutional Animal Care and Use Committee at Michigan State University.

4.3 Tissue Collection and Immunohistochemistry

Between 1200-1600 hr voles were overdosed with sodium pentobarbital and perfused through the heart with 0.9% saline followed by 4% paraformaldehyde in sodium phosphate buffer (0.1M PB; pH 7.6). Brains were removed, postfixed overnight in 4% paraformaldehyde in PB, and then submerged in a 20% sucrose (0.1M PB) solution for at least 3 days before sectioning into 40 μ m coronal sections on a freezing slide microtome. Sections were stored in a sucrose-based cryoprotectant (30% sucrose, 0.1% polyvinylpyrrolidone-40 in ethylene glycol, 0.1 M PB) at -20° C until immunohistochemical processing.

Immunohistochemistry for progesterone receptors (PRs) was performed on every other section through the brain using procedures similar to what has been previously described (Quadros et al., 2002). Briefly, sections were rinsed three times for 5 min each in 0.05M Tris-buffered saline (TBS; pH 7.6), incubated in 0.1% sodium borohydride for 10 min, rinsed three times in TBS, then incubated in 1% bovine serum albumin, 1% hydrogen peroxide and 20% normal goat serum (NGS) in TBS for 20 min. Sections were then incubated with a rabbit anti-progesterone receptor polyclonal antibody that recognizes both the A and B isoforms (RB-9017-P; Lab Vision, Thermo Scientific, Fremont, CA; 1:400) in 0.3% Triton-X and 2% NGS in TBS for ~16 hr at 4° C. According to the manufacturer, the immunogen for this primary antibody was a recombinant protein encoding human progesterone receptor 412-526aa (see below for further antibody specificity). Sections were rinsed three times in 0.3% Triton-X and 2% NGS in TBS and incubated in a biotinylated goat anti-rabbit secondary antibody (3 μ g/ml; Vector Laboratories, Burlingame, CA) in 0.3% Triton-X and 2% NGS in TBS for one hr at room temperature. Sections were rinsed two times in 0.3% Triton-X and 2% NGS in TBS and two times in TBS alone. Next, they were incubated with avidin-biotin complex (Vectastain ABC kit, Vector Laboratories) for 60 min and following rinses the immunoreactivity was visualized using nickel-enhanced diaminobenzidine (DAB kit, Vector Laboratories) and running the reaction for 10 min, which resulted in a black nuclear stain. Sections were mounted on gelatin-coated microscope slides, rinsed with water, dehydrated, and coverslipped. Immunocytochemical controls were conducted in which the absence of immunoreactivity confirmed specificity of the antisera, and these included assays where vole brain sections were processed in the absence of the primary antisera and where sections were incubated in 1:400 of the primary antisera that had been preabsorbed with a 10-fold concentration excess of human PR B isoform (Fig. 7). The human PR-B protein was obtained from Sf9 insect cells infected with the appropriate PR recombinant transfer plasmids (Tissue Culture CORE Facility, University of Colorado Cancer Center, Denver, CO). In addition, the primary antiserum was incubated with brain sections obtained from PR knockout mice (wildtype mice as the positive control) in which we found an almost complete absence of specific labeling (Mani et al., 1997).

4.4 Image and Statistical Analyses

A single representative section from each brain region for each animal was selected for image analysis performed similar to that previously described (Quadros et al., 2002). Representative sections were selected using distinguishing landmarks based on the adult rat brain (Paxinos and Watson, 1998) and were carefully anatomically matched across subjects. Digital images of sections were captured at 100X on an Olympus BX40 microscope attached to a SPOT INSIGHT camera (Diagnostics, Inc.) at a constant exposure time. Captured images were analyzed in ImageJ (<http://rsbweb.nih.gov/ij/>). A shape specific to each site standardized across each section and completely covering the site of interest was superimposed upon the images and the relative amount of PRir in each region determined by measuring the area (μm^2) covered by pixels with an optical density greater than a defined threshold density (2-6 times the standard deviation of the mean background optical density). The mean background density was measured in a region devoid of PRir immediately adjacent to the analyzed region containing PRir. Brain sites analyzed were the rostral anteroventral periventricular nucleus (AVPV), the medial preoptic nucleus (MPN), the principal nucleus of the bed nucleus of the stria terminalis (pBST), the posterodorsal medial amygdala (MeApd), the ventrolateral division of the ventromedial nucleus (VMNvl) and the arcuate nucleus (ARN) (Fig. 1). These regions were selected because, as detailed above, they are components of the neural networks for social behaviors in other adult mammals and PR protein or mRNA in these sites is often found to be hormonally regulated. The PR-immunoreactive area (foreground covered by pixels) on each tissue section was used for statistical analysis.

Each brain region was analyzed separately using a one-way ANOVA followed by post-hoc comparisons using Fisher's LSD for multiple pairwise comparisons. The data from the BST were not normally distributed, so were analyzed instead by a Kruskal-Wallis analysis on ranks followed by Dunn's post-hoc tests. Lost or damaged tissue sections resulted in slightly reduced sample sizes in some groups for some brain sites. Statistical significance was indicated by $p < 0.05$.

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- Progesterone receptor expression was examined in the brain of male prairie voles.
- PR expression in throughout the brain was similar to that in polygamous rodents.
- Castration reduced PR expression in areas of the hypothalamus and amygdala.
- PRs must have a different role in male voles than they do in polygamous rodents.

Figure 1. Schematic representation of prairie vole brain coronal sections where PRir was quantified (areas indicated by hatched marking): A) AVPV, B) MPN, C) pBST, D) MeApd and E) ARN and VMNvl. ac = anterior commissure, fx = fornix, LV - lateral ventricle, mtt - mammillothalamic tract, opt = optic tract.

Figure 2. Photomicrographs of PRir cells in the MPN of female and male prairie voles that were sham gonadectomized (Sham), and males that were gonadectomized (GDX) or gonadectomized and implanted with a testosterone-filled capsule (GDX+T).

Figure 3.

Area (Mean $\pm$ SEM) covered by PRir in the AVPV (top panel) and MPN (bottom panel) of male and female prairie voles that were sham gonadectomized (Sham), and males that were gonadectomized (GDX) or gonadectomized and implanted with a testosterone-filled capsule (GDX+T). Bars with different letters are significant different from each other, $p < 0.05$.

AVPV: N = 4-6 animals per group; MPN: N = 4-7 animals per group.

Figure 4.

Area covered by PRir in the pBST (top panel) and MeApd (bottom panel) of female and male prairie voles that were sham gonadectomized (Sham), and males that were gonadectomized (GDX) or gonadectomized and implanted with a testosterone-filled capsule (GDX+T). Bars with different letters are significant different from each other, $p < 0.05$. Note - pBST data not normally distributed so medians and 25% interquartile ranges are shown. Means $\pm$ SEM are shown for the MeApd. pBST: N = 4-7 animals per group; MeApd: N = 4-9 animals per group.

Figure 5. Photomicrographs of PRir cells in the VMNvl of female and male prairie voles that were sham gonadectomized (Sham), and males that were gonadectomized (GDX) or gonadectomized and implanted with a testosterone-filled capsule (GDX+T).

Figure 6.

Area (Mean $\pm$ SEM) covered by PRir in the VMNvl (top panel) and ARN (bottom panel) of female and male prairie voles that were sham gonadectomized (Sham), and males that were gonadectomized (GDX) or gonadectomized and implanted with a testosterone-filled capsule (GDX+T). Bars with different letters are significant different from each other, $p < 0.05$.

VMNvl: N = 4-7 animals per group; ARN: N = 5-7 animals per group.

Figure 7.
Photomicrographs of PRir in the MPN of a representative male prairie vole after no preabsorption of the primary antiserum (left) or preabsorption of the primary antiserum with an overabundance of PR-B (right).