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Estrogen Receptor Alpha Distribution and Expression in the Social Neural Network of Monogamous and Polygynous *Peromyscus*

Bruce S. Cushing¤*

Department of Zoology, University of Maryland, College Park, MD, United States of America

¤ Current address: Department of Biological Sciences, The University of Texas at El Paso, El Paso, TX 79968 United States of America
* bcushing@utep.edu

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Abstract

In microtine and dwarf hamsters low levels of estrogen receptor alpha (ERα) in the bed nucleus of the stria terminalis (BST) and medial amygdala (MeA) play a critical role in the expression of social monogamy in males, which is characterized by high levels of affiliation and low levels of aggression. In contrast, monogamous *Peromyscus* males display high levels of aggression and affiliative behavior with high levels of testosterone and aromatase activity. Suggesting the hypothesis that in *Peromyscus* ERα expression will be positively correlated with high levels of male prosocial behavior and aggression. ERα expression was compared within the social neural network, including the posterior medial BST, MeA posterodorsal, medial preoptic area (MPOA), ventromedial hypothalamus (VMH), and arcuate nucleus in two monogamous species, *P*. *californicus* and *P*. *polionotus*, and two polygynous species, *P*. *leucopus* and *P*. *maniculatus*. The results supported the prediction, with male *P*. *polionotus* and *P*. *californicus* expressing higher levels of ERα in the BST than their polygynous counter parts, and ERα expression was sexually dimorphic in the polygynous species, with females expressing significantly more than males in the BST in both polygynous species and in the MeA in *P*. *leucopus*. *Peromyscus* ERα expression also differed from rats, mice and microtines as in neither the MPOA nor the VMH was ERα sexually dimorphic. The results supported the hypothesis that higher levels of ERα are associated with monogamy in *Peromyscus* and that differential expression of ERα occurs in the same regions of the brains regardless of whether high or low expression is associated with social monogamy. Also discussed are possible mechanisms regulating this differential relationship.

Introduction

Estrogen receptor alpha (ERα) plays a critical role in the expression of male social behavior [1,2]. Comparative studies of closely related rodent species have shown that the distribution of

ERα within the limbic system is associated with mating strategies and levels of male prosocial behavior. In microtines (Microtus), prairie (*Microtus ochrogaster*)[3], pine (*M. pinetorum*)[4], and Mandarin voles (*M. mandarinus*)[5], and dwarf hamsters (*Phopodus sp*) high levels of male prosocial (positive affiliative) behavior [3], which includes the formation of long-term pair bonds and parental care, is associated with low levels of ERα in the medial amygdala (MeA) and/or the bed nucleus of the stria terminalis (BST)[3–6]. This is in contrast to closely related polygynous species such as montane voles (*M. montanus*) [4] and unrelated species such as rats [7,8] and mice [9] where both males and females display high levels of ERα in the MeA and BST. Even within species there is a strong inverse correlation between male prosocial behavior and ERα. In prairie voles, the classification of social monogamy (see below) was based upon the behavior of prairie voles in Illinois [10,11]. In contrast males from Kansas display significantly lower levels of prosocial behavior and higher levels of ERα in the MeA and BST than males from Illinois [4]. Neonatal castration of male prairie voles disrupts subsequent expression of male prosocial behavior and the ability of vasopressin to stimulate pair bonds [12], and this is associated with over-expression of ERα in neonatally castrated adult males [13]. Finally, viral vector enhancement of ERα has provided direct evidence for the role of ERα in males, as increasing ERα expression in the MeA [14] and the BST [15] significantly reduced the expression of male prosocial behavior in prairie voles.

Social monogamy is classified as a suite of behaviors, not sexual exclusivity, that include pair bonding, biparental care and low levels of male aggression [11,16,17]. It has been hypothesized that the evolution of high levels of male prosocial behavior is associated with a decrease in male aggression, as high levels of male aggression would conflict with male parental care [18]. In voles male aggression is correlated with ERα expression, with male prairie voles displaying lower levels of aggression than montane [19] and meadow voles (*M. pennsylvanicus*) [20], and Illinois male prairie voles are less aggressive than Kansas males [21]. Male ERα knock-out mice display little or no aggression compared with wild-type males [1]. In dwarf hamsters male aggression is seasonal. During the breeding season or on long day cycles, when males are highly social, they display lower levels of aggression and lower levels of ERα, while males on a short day cycle are typically more aggressive and there is a significant increase in ERα in the MeA and BST [22].

Although the above studies and logic make a strong case for the hypothesis that estrogen decreases or inhibits male prosocial behavior the question remains if this is the "universal" pattern associated with social monogamy. This may be particularly relevant in *Peromyscus* where studies indicate that both testosterone and estrogen facilitate male prosocial behavior and different patterns of male aggression. In the socially monogamous California mouse (*P. californicus*) estrogen enhances male parental behavior [23] and the onset of male parental behavior is correlated with a significant increase in aromatase, the enzyme that converts testosterone to estradiol [24]. Additionally, increasing testosterone levels in response to social interactions facilitated the expression of parental behavior. Following courtship males with higher testosterone levels were more likely to approach pups [25]. The expression of male aggression also differs in *Peromyscus* with the polygynous white-footed mouse (*P. leucopus*) displaying lower levels of aggression than the monogamous California mouse [26]. In repeated aggression tests male California males display a winner effect, with males becoming increasingly dominant after winning an aggressive encounter while this does not occur in white-footed mice [26]. However treatment with testosterone stimulated a winner effect in white-footed males, suggesting they are both capable of responding to steroids and that higher steroid levels are associated with increased aggression [27]. *Peromyscus* (both white-footed and *P. polionotus*, the beach mouse) are similar to dwarf hamsters in that males display much higher levels of aggression during short days, which is associated with increased ERα expression [28]. While these studies

support the hypothesis that ERα expression is critical in the regulation of male social behavior in *Peromyscus*, they suggest that the role of ERα in regulating male prosocial behavior may differ from dwarf hamsters and microtines. Therefore, the goal of this study was to determine the pattern of ERα expression in *Peromyscus* based upon reproductive strategy and if they are the same or differ from other socially monogamous species. This was done using immunocytochemistry to examine ERα expression in two polygynous species, the white-footed mouse and deer mouse (*P. maniculatus*), and two socially monogamous species the California mouse [29,30] and the beach mouse (*P. polionotus*)[31,32].

Materials and Methods

Animals

Peromyscus were purchased from the Peromyscus Genetic Stock Center (University of South Carolina, Columbia, SC) and shipped to the University of Maryland. All animals were individually housed, sexually naive adults. Animals were maintained on a 14:10 h light/dark cycle and provided food (Purina Rat Chow) and water *ad libitum*. Brains were collected from males and females of each species (*P. maniculatus*, BW stock, $n = 7$ males, 6 females; *P. leucopus*, LL stock, $n = 5$ males, 5 females; *P. californicus*, IS stock, $n = 5$ males, 4 females; *P. polionotus*, PO stock, $n = 6$ males, 6 females). To avoid complications associated with estrus, vaginal lavages were performed on females (for method details see [3]) to determine stage of estrus and tissue was collected only during diestrus. All procedures reported in this study were approved by the University of Maryland IACUC prior to any research being conducted and were within the guidelines established by the National Institutes of Health Guide for the Care and Use of Animals.

Localization of ERα immunoreactivity

Mice were deeply anesthetized with a mixture of Ketamine (67.7 mg/kg) and Xylazine (13.33 mg/kg) prior to transcardial perfusion with 4% paraformaldehyde and 2.5% acrolein in 0.1 M potassium phosphate buffered saline (KPBS; pH 7.6). Brains were removed and stored in 25% sucrose at 4°C until sectioned at 30 μm using a freezing sliding microtome. Sections were stored in cryoprotectant at −20°C until processed using standard Vector ABC immunocytochemistry (ICC) staining for ERα. Sections were rinsed in 0.05 M KPBS and then incubated at room temperature in 1% sodium borohydride for 20 min to neutralize acrolein and rinsed in KPBS, before being incubated in rabbit ERα polyclonal antibody (anti-ERα C1355, Millipore) at a concentration of 1:100,000 in 0.05 M KPBS +0.4% Triton X for 1 h at room temperature and then for 48 h at 4°C. This antibody binds to both free and bound receptors [33], reducing variation in staining due to potential differences in circulating hormone levels, and has been previously validated in *Peromyscus* [28,34]. Following incubation in the primary antibody sections were rinsed in KPBS and then incubated for 1 h at room temperature in biotinylated goat, anti-rabbit IgG (Vector Laboratories, Burlingame, CA) at 1:600 dilution in KPBS + 0.4% Triton X. Sections were then rinsed in KPBS and incubated for 1 h at room temperature in an avidin—biotin peroxidase complex (Vectastain ABC kit-elite pk-6100 standard, 4.5 μl A and 4.5 μl B per 1 ml solution, Vector Laboratories) in KPBS + 0.4% Triton X. Sections were rinsed in KPBS followed by rinses in 0.175 M sodium acetate. ERα was visualized by incubation in a nickel diaminobenzidine chromogen solution for 15 min. Lastly, sections were rinsed, dehydrated in alcohol, and mounted to slides using Histoclear and Histomount. To assure that staining was associated with the primary antibody the above protocol was followed absent the use of the primary antibody (Fig 1).

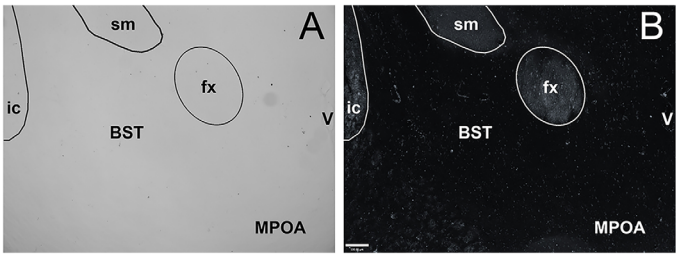

Fig 1. Bright field (A) and dark field (B) images of the same section taken at 10x showing the lack of specific staining in the bed nucleus of the stria terminalis (BST) and medial preoptic area (MPOA) following omission of the primary antibody. fx, fornix, sm, stria medullaris, ic, internal capsul, and V, third ventricle. Scale bar in B represents 100 microns.

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Image analysis

Slides were coded and images captured on a PC and analyzed using IPLab (Scanalytics, Fairfax, VA) by an experimentally-blind scorer. IPLab generates a segment layer based upon intensity of the staining and a minimum contiguous pixel area representing a single "cell". Only cell that were darker than background are segmented and regions larger than 10 pixels considered represented stained cells. Number of cells are counted by the program within the region of interest (ROI). Specific regions/nuclei were determined using established landmarks and the Paxinos and Watson rat atlas [35]. Image analysis was used to determine the number of cells expressing ERα-IR in the medial preoptic area (MPOA), BST posterior medial division, arcuate nucleus (ARC), ventromedial nucleus of the hypothalamus (VMH), and MeA posterodorsal. These areas were chosen because they are involved in a variety of aspects of reproduction and social behavior. One third of the total brain sections for each animal were stained. This was done to assure that matched regions were available from each individual [3,4,34]. The matched representative section containing the nucleus/area of interest was counted bilaterally and then cell counts for each hemisphere were averaged. It should be noted that volume of the region of interest was not measured, so that differences reported could be associate with relatively more cells in a region. These methods are published and have been previously established in assessing ERα immunoreactivity in *Peromyscus* [34] as well as *Microtus* [3] and dwarf hamsters [4].

Statistics

Because ERα was predicted *a-priori* to be sexually dimorphic between species differences were analyzed separately for each sex by brain region using a one-way ANOVA [3–5,13]. If the ANOVA was significant, a Fisher's PLSD was used to make post-hoc pair-wise comparisons. Within species between-sex differences were analyzed for each brain region using a *t*-test. Differences were considered significant if $P < 0.05$.

Results

Between Species

There was a significant difference between males in the expression of ERα in the posterior medial division of the BST ($F_{3,17} = 4.5$, $P < 0.05$). The difference was due to significantly lower ERα-IR in white-footed mice and deer mice compared with both beach mice and California mice ($P < 0.05$ Figs 2 and 3). There was no significant difference in other regions or between females (Fig 2).

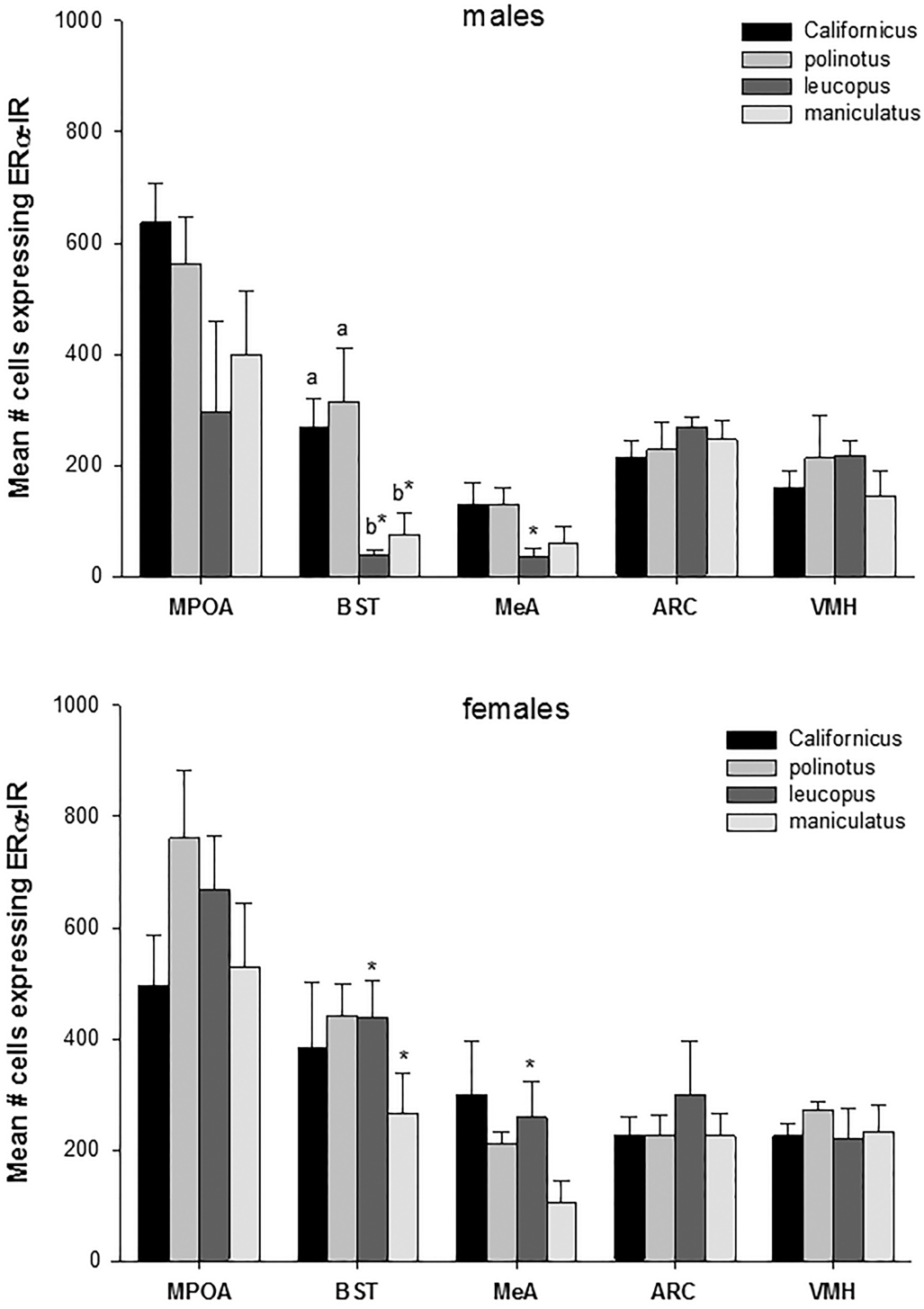

Fig 2. Shows the mean expression of ERα-IR by region in male and female *Peromyscus*. Polygynous males, *P*. *leucopus* and *P*. *maniculatus*, displayed significant higher levels of ERα-IR in the BST than socially monogamous males. Within species ERα-IR was sexually only in polygynous species with females displaying significantly higher levels in the BST than males in female *P*. *leucopus* more than *P*. *leucopus* males in the MeA. * = significant within species difference $P < 0.05$; different letters indicate significant between species within sex differences by region $P < 0.05$.

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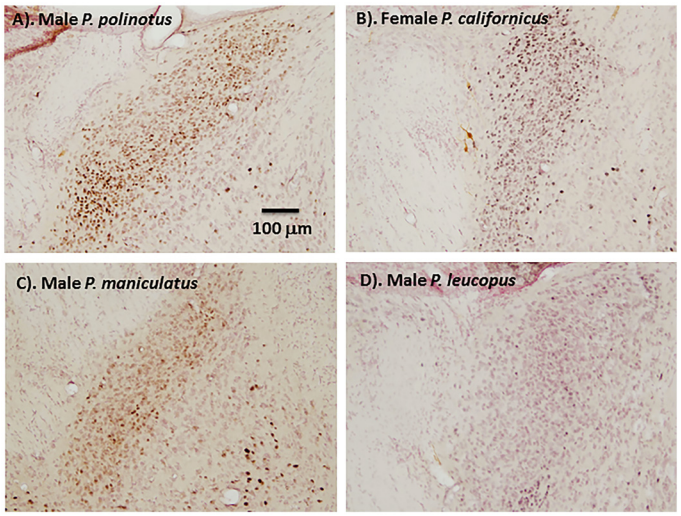

Fig 3. Representative photomicrographs (100x) of BST in male (A, C, D) and female (B) *Peromyscus*.— = 100µm.

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Within Species—sexual dimorphism

ERα-IR was sexually dimorphic in the polygynous species. Male white-footed mice expressing significantly fewer ERα-IR cells than females in BST posterior medial and posterior MeA posterodorsal ($t_7 = 5.2$, $P < 0.01$, $t_7 = 3.8$, $P < 0.01$, respectively) and deer mice males significantly less in the BST ($t_9 = 2.4$, $P < 0.05$).

Discussion

As predicted, male ERα-IR expression was correlated with mating strategy and male social behavior. Socially-monogamous male California and beach mice displayed significantly higher levels of ERα-IR in the BST than their polygynous counter parts. ERα-IR was sexually dimorphic in the BST and/or MeA of the polygynous deer and white-footed mice, with females expressing significantly higher levels than males. Finally, ERα expression in the VMH and MPOA in *Peromyscus* differed from that reported in most other rodents. In rats, mice, voles, and dwarf hamsters ERα expression is sexually dimorphic with females expressing higher levels than males [4–9], while in all four *Peromyscus* ERα expression in the VMH and MPOA was not sexually dimorphic.

There are three potentially important points associated with these findings. First, the differential expression of ERα between polygynous and monogamous species supports the behavioral studies in *Peromyscus* indicating that greater sensitivity to estrogen, through greater ERα expression, is at least in part responsible for high levels of male prosocial behavior and aggression [23,28]. Second, that the brain regions in which ERα differs between males of closely related polygynous and monogamous species is consistent across rodent genera. Finally, and perhaps most informative, is that the results suggest that natural selection has acted differentially on the same underlying mechanism(s) to produce convergent behavioral evolution.

Male Prosocial behavior

Although high levels of male prosocial/parental behavior have typically been inversely correlated with gonadal steroid levels in most rodent [36] and humans [37] empirical studies using *Peromyscus* suggest a positive correlation. In the socially monogamous California mouse high

levels of prosocial behavior, especially parental behavior, are associated with high levels of estrogen/aromatase activity [23,24]. Seasonal changes in male *Peromyscus* behavior are correlated with changes in ERα expression [28], and estrogen and aromatase activity enhance male prosocial behavior [23,24]. A positive correlation between gonadal steroids and male prosocial behavior may not be limited to *Peromyscus*, as male Volcano mice (*Neotomodon alstoni*) displayed increase parental care when T levels were relatively high [38].

Aggression

Although studies on the role of estrogen and ERα in male aggression have produced mixed results, when a direct relationship has been shown ERα is typically associated with increased aggression [1,39]. This includes socially monogamous microtines, dwarf hamsters [22], and *Peromyscus* [28]. "Seasonal" changes in ERα are associated with higher levels of aggression. In monogamous dwarf hamsters aggression occurs primarily during the non-breeding season, triggered by short day length and region-specific increases in ERα expression [22]. The polygynous white-footed mouse also displays increased aggression during short days [28]. In contrast male prairie voles only show high levels of aggression following the initial bond formation and mating with a female [17]. This very selective mate guarding may have evolved because following the initial mating, female prairie voles are sexually receptive and mate during delivery, "partum" receptivity, which occurs in the nest eliminating the need for mate "guarding" during subsequent receptive periods. If high levels of ERα expression are necessary for the expression of aggression then this may explain, at least in part, why selection has acted differentially in the California mouse. In contrast to prairie voles, female California mice undergo a more typical "post-partum" estrus that occurs 48 hr after birth and frequently seek extra-pair copulations [40]. In the field, female *Peromyscus* are more active and significantly more likely to be trapped when in estrus, while female voles are significantly less likely to be captured in estrus [41]. Therefore, male California mice may need to display high levels of male/male aggression to defend their territory and mate, while also displaying high levels of bi-parental care. This means that the selective forces acting on monogamous *Peromyscus* and voles are significantly different and means that California mice contradict the hypothesis that the evolution of social monogamy involves both an increase in male prosocial behavior and a decrease in male aggression [19].

The role of the BST and MeA

Although prosocial behavior and aggression are regulated by complex neural circuits the differential expression of ERα associated with mating strategy appears to be limited to the BST and the MeA. The BST and MeA are derived from the same embryonic brain regions with the BST considered to be part of the extended amygdala and there are efferent and afferent neural connections between the two nuclei [42,43]. The BST and MeA are the initial regulators of many social interactions and are among the first regions to show neuronal activation during social contact, as they receive direct neural stimulation from both the olfactory and accessory olfactory bulbs [44–47]. They have been directly implicated in the regulation of a variety of social behaviors, including parental behavior [48,49], the formation of male-female pair bonds [14,50,51], and aggression [52,53]. In California mice, lesions of the amygdala disrupted parental behavior in males but not females [54]. In addition, these regions are sexually dimorphic in polygynous *Peromyscus* and monogamous microtines and dwarf hamster [3–6] suggesting that they are playing an essentially different role in males and females. Taken together the relationship of the MeA and the BST to critical sensory input, response to social cues, and connections with other critical regions of the brain means that differential expression of essential receptors,

such as ERα, within either or both regions would have a major impact on the expression of social behavior [14,15].

Mechanisms regulating Prosocial Behavior

The fact that both high and low levels of ERα are associated with high levels of male prosocial behavior raises the obvious questions of how and why differential expression produces convergent behaviors and mating strategies. This question and the possible answer represent the potential for significant advancement of our understanding of the regulation of male social behavior and should stimulate empirical studies. Many of the studies using voles (*Microtus sp*.) have focused on the role of vasopressin in males, V1a receptor expression, and/or variation in microsatellite length of the V1aR gene (for review see [55]). However this relationship does not exist in many other rodent taxa, and in fact it has been observed that outside the genus Microtus there is no correlation between microsatellite length and mating strategy [56]. In *Peromyscus*, the lack of a relationship between male social behavior and V1aR microsatellite length has led to the conclusion that additional mechanisms must be involved [57].

It has been hypothesized that the expression of social monogamy is, at the very least, an interaction between steroids and neuropeptides [13], as gonadal steroids play a major regulatory role in the effects of vasopressin and oxytocin. Social recognition, an essential aspect of long-term pair bond formation, has been hypothesized to be the product of an estrogen-dependent four-gene interaction, consisting of vasopressin, oxytocin and estrogen receptors [58]. In male mice it has recently been suggested that social disorders, such as autism and schizophrenia, which are characterized by an inability to bond and display affiliative behaviors, are also the product of an interaction between estrogen receptors and gene expression of vasopressin and oxytocin [59]. This may explain why, when studied independently, gonadal steroids often regulate many of the same behaviors as vasopressin and oxytocin (for review see [13,58,59]). Therefore, a logical extension of these two hypotheses is that social monogamy is also the result of the interplay of steroids and neuropeptides. If social monogamy is the result of a dynamic interaction of these factors then, while changing one may disrupt the expression of social behavior, changing more than one in a coordinated fashion could produce the same suite of behaviors.

This hypothesis is supported by studies in the socially monogamous prairie vole (*M. ochrogaster*) where neonatal castration eliminated the expression of adult male alloparental behavior [60], the formation of pair bonds [12] and disrupted the ability of centrally administered vasopressin to stimulate the formation of a partner preference [12]. Neonatal castration did not alter V1aR expression [12], but did produce a significant over-expression of ERα in the BST, MeA, MPOA, and VMH in adult males [61]. Direct manipulation of increasing ERα in male prairie voles inhibited prosocial behavior [14,15], suggesting the possibility that increasing ERα may have inhibited the response to vasopressin. However, if V1aR expression had also been manipulated then the effect could have been different. This concept is supported by the previous findings on the expression of ERα and AVP in the PVN of *Peromyscus* sp. In the species used in this study there was no correlation between ERα or AVP in PVN and mating strategy [34]. However there was an inverse relationship between number of cells in the PVN expressing ERα and AVP [34], suggesting that differential patterns of expression could be associated with the production of similar prosocial behavioral patterns.

In conclusion, ERα expression in *Peromyscus* varies based upon mating strategy, supporting the hypothesis that ERα plays a critical role in the expression of male mating strategies and prosocial behavior. Further, the results indicate that the BST and/or MeA are critical regions in the regulation of mating strategy and male prosocial behavior. However in *Peromyscus*, in

contrast to previously studied rodents, it is the monogamous males that express higher levels of ERα while ERα is sexually dimorphic in the polygynous species. Finally, the results indicate that to fully understand the regulation of mating strategies it is necessary to study the mechanisms that regulate prosocial behavior and aggression in concert rather than independently.

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Author Contributions

Conceived and designed the experiments: BSC. Performed the experiments: BSC. Analyzed the data: BSC. Contributed reagents/materials/analysis tools: BSC. Wrote the paper: BSC.

References

1. Ogawa S, Washburn TF, Taylor J, Lubahn DB, Korach KS, Pfaff DW. Modifications of testosterone-dependent behaviors by estrogen receptor-alpha gene disruption in male mice. Endocrinology 1998; 139:5058–69. PMID: 9832445
2. Perry AN, Paramadilok A, Cushing BS. Neonatal oxytocin alters subsequent estrogen receptor alpha protein expression and estrogen sensitivity in the female rat. Behav Brain Res 2009; 205:154–61. doi: 10.1016/j.bbr.2009.08.021 PMID: 19703497
3. Cushing BS, Razzoli M, Murphy AZ, Epperson PM, Le W-W, Hoffman GE. Intraspecific variation in estrogen receptor alpha and the expression of male sociosexual behavior in two populations of prairie voles. Brain Res 2004; 1016:247–54. PMID: 15246861
4. Cushing BS, Wynne-Edwards KE. Estrogen receptor-alpha distribution in male rodents is associated with social organization. J Comp Neurol 2006; 494:595–605. PMID: 16374794
5. Hnatczuk O, Lisciotto C, DonCarlos L. Estrogen and progesterone receptor immunoreactivity (ER-IR & PR-IR) in specific brain areas of the prairie vole (*Microtus ochrogaster*) is altered by sexual receptivity and genetic sex. J Neuroendocrinol 1994.
6. Pei-Yuan Z, Fa-Dao T, Hui X, Rui J. Inter-sexual variation in social interactions and distribution of estrogen receptor alpha in the brain of mandarin voles *Microtus mandarinus*. Acta Zool Sinica 2008.
7. Simerly RB, Chang C, Muramatsu M, Swanson LW. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. J Comp Neurol 1990; 294:76–95. PMID: 2324335
8. Yokosuka M, Okamura H, Hayashi S. Postnatal development and sex difference in neurons containing estrogen receptor-alpha immunoreactivity in the preoptic brain, the diencephalon, and the amygdala in the rat. J Comp Neurol 1997; 389:81–93. PMID: 9390761
9. Brown AE, Mani S, Tobet SA. The preoptic area/anterior hypothalamus of different strains of mice: sex differences and development. Dev Brain Res 1999; 115:171–82.
10. Thomas JA, Birney EC. Parental care and mating system of the prairie vole, *Microtus-ochrogaster*. Behav Ecol Sociobiol 1979; 5:171–86.
11. Getz LL, Carter CS,Gavish L. The mating system of the prairie vole, *Microtus ochrogaster*: field and laboratory evidence for pair-bonding. Behav Ecol Sociobiol 1981; 8:189–194.
12. Cushing BS, Okorie U, Young LJ. The effects of neonatal castration on the subsequent behavioural response to centrally administered arginine vasopressin and the expression of V1a receptors in adult male prairie voles. J Neuroendocrinol 2003; 15:1021–6. PMID: 14622431
13. Cushing BS, Kramer KM. Mechanisms underlying epigenetic effects of early social experience: the role of neuropeptides and steroids. Neurosci Biobehav Rev 2005; 29:1089–105. PMID: 16099507
14. Cushing BS, Perry A, Musatov S, Ogawa S, Papademetriou E. Estrogen receptors in the medial amygdala inhibit the expression of male prosocial behavior. J Neurosci 2008; 28:10399–403. doi: 10.1523/JNEUROSCI.1928-08.2008 PMID: 18842899

15. Lei K, Cushing BS, Musatov S, Ogawa S, Kramer KM. Estrogen receptor-alpha in the bed nucleus of the stria terminalis regulates social affiliation in male prairie voles (*Microtus ochrogaster*). PloS One 2010; 5:e8931. doi: 10.1371/journal.pone.0008931 PMID: 20111713
16. Carter CS, Getz LL. Monogamy and the prairie vole. Sci Am 1993; 268:100–106. PMID: 8516669
17. Winslow JT, Hastings N, Carter CS, Harbaugh CR, Insel TR. A role for central vasopressin in pair bonding in monogamous prairie voles. Nature 1993; 365:545–8. PMID: 8413608
18. Trivers RL. Parental investment and sexual selection. In: Campbell B, editor. Sexual selection and the descent of man, London: Heinemann; 1972, pp. 136–79.
19. Pierce JD, Pellis VC, Dewsbury DA, Pellis SM. Targets and tactics of agonistic and precopulatory behavior in montane and prairie voles: Their relationship to juvenile play-fighting. Aggressive Behav 1991; 17:337–49.
20. Turner BN, Iverson SL. The annual cycle of aggression in male *Microtus pennsylvanicus* and its relation to population parameters. Ecology 1973; 54:967–981.
21. Gaines MS, Fugate CL, Johnson ML, Johnson DC, Hisey JR, Quadagno DM. Manipulation of aggressive behavior in male prairie voles (*Microtus ochrogaster*) implanted with testosterone in Silastic tubing. Can J Zool 1985; 63:2525–8.
22. Kramer KM, Simmons JL, Freeman DA. Photoperiod alters central distribution of estrogen receptor alpha in brain regions that regulate aggression. Horm Behav 2008; 53:358–65. PMID: 18078937
23. Trainor BC, Marler CA. Testosterone promotes paternal behaviour in a monogamous mammal via conversion to oestrogen. Proc R Soc B-Biol Sci 2002; 269:823–9.
24. Trainor BC, Bird IM, Alday NA, Schlinger BA, Marler CA. Variation in aromatase activity in the medial preoptic area and plasma progesterone is associated with the onset of paternal behavior. Neuroendocrinology 2003; 78:36–44. PMID: 12869798
25. Gleason ED, Marler CA. Testosterone response to courtship predicts future paternal behavior in the California mouse, *Peromyscus californicus*. Horm Behav 2010; 57:147–54. doi: 10.1016/j.yhbeh.2009.10.006 PMID: 19833131
26. Oyegbile TO, Marler CA. Weak winner effect in a less aggressive mammal: correlations with corticosterone but not testosterone. Physiol Behav 2006; 89:171–9. PMID: 16859719
27. Fuxjager MJ, Montgomery JL, Marler CA. Species differences in the winner effect disappear in response to post-victory testosterone manipulations. Proc R Soc B-Biol Sci 2011; 278:3497–503.
28. Trainor BC, Rowland MR, Nelson RJ. Photoperiod affects estrogen receptor alpha, estrogen receptor beta and aggressive behavior. Euro J Neurosci 2007; 26:207–18.
29. Gubernick DJ, Alberts JR. The biparental care system of the California mouse, *Peromyscus californicus*. J Comp Psychol 1987; 101:169–77. PMID: 3608423
30. Ribble DO. The monogamous mating system of Peromyscus californicus as revealed by DNA fingerprinting. Behav Ecol Sociobiol 1991; 29:161–6.
31. Blair WF. Population structure, social behavior, and environmental relations in a natural population of the beach mouse (Peromyscus polionotus leucocephalus). The University of Michigan Press; 1951.
32. Foltz DW. Genetic evidence for long-term monogamy in a small rodent, *Peromyscus polionotus*. Am Nat 1981; 117:665–675.
33. Murphy AZ, Shupnik MA, Hoffman GE. Androgen and estrogen (alpha) receptor distribution in the periaqueductal gray of the male Rat. Horm Behav 1999; 36:98–108. PMID: 10506534
34. Kramer KM, Yamamoto Y, Hoffman GE, Cushing BS. Estrogen receptor alpha and vasopressin in the paraventricular nucleus of the hypothalamus in *Peromyscus*. Brain Res 2005; 1032:154–61. PMID: 15680954
35. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. Academic Press; 2007.
36. Newman SW. The medial extended amygdala in male reproductive behavior. A node in the mammalian social behavior network. Ann NYAcad Sci 1999; 877:242–
37. Gettler LT, McDade TW, Feranil AB, Kuzawa CW. Longitudinal evidence that fatherhood decreases testosterone in human males. Proc Nat Acad Sci USA 2011; 108:16194–9. doi: 10.1073/pnas.1105403108 PMID: 21911391
38. Luis J, Ramírez L, Carmona A, Ortiz G, Delgado J, Cárdenas R. Paternal behavior and testosterone plasma levels in the Volcano Mouse Neotomodon alstoni (Rodentia: Muridae). Rev De Biol Trop n.d.; 57:433–9.
39. Trainor BC, Greiwe KM, Nelson RJ. Individual differences in estrogen receptor alpha in select brain nuclei are associated with individual differences in aggression. Horm Behav 2006; 50:338–45. PMID: 16753164

40. Gilbert AN. Postpartum and lactational estrus: a comparative analysis in rodentia. J Comp Psychol 1984; 98:232–45. PMID: 6478785

41. Cushing BS, Cawthorn JM. Species differences in activity patterns during oestrus. Can J Zool 1996; 74:473–9.

42. Northcutt KV, Lonstein JS. Neuroanatomical projections of the species-specific tyrosine hydroxylase-immunoreactive cells of the male prairie vole bed nucleus of the stria terminalis and medial amygdala. Brain Behav Evo 2011; 77:176–92.

43. Fudge JL, Haber SN. Bed nucleus of the stria terminalis and extended amygdala inputs to dopamine subpopulations in primates. Neuroscience 2001; 104:807–27. PMID: 11440812

44. Kirkpatrick B, Kim JW, Insel TR. Limbic system fos expression associated with paternal behavior. Brain Res 1994; 658:112–8. PMID: 7834331

45. Pfaus JG, Heeb MM. Implications of immediate-early gene induction in the brain following sexual stimulation of female and male rodents. Brain Res Bull 1997; 44:397–407. PMID: 9370204

46. Curtis JT, Wang Z. Forebrain c-fos expression under conditions conducive to pair bonding in female prairie voles (*Microtus ochrogaster*). Physiol Behav 2003; 80:95–101. PMID: 14568313

47. Cushing BS, Mogekwu N, Le W-W, Hoffman GE, Carter CS. Cohabitation induced Fos immunoreactivity in the monogamous prairie vole. Brain Res 2003; 965:203–11. PMID: 12591139

48. Parker KJ, Kinney LF, Phillips KM, Lee TM. Paternal behavior is associated with central neurohormone receptor binding patterns in meadow voles (*Microtus pennsylvanicus*). Behav Neurosci 2001; 115:1341–8. PMID: 11770064

49. De Jong TR, Chauke M, Harris BN, Saltzman W. From here to paternity: neural correlates of the onset of paternal behavior in California mice (*Peromyscus californicus*). Horm Behav 2009; 56:220–31. doi: 10.1016/j.yhbeh.2009.05.001 PMID: 19433091

50. Kramer KM, Choe C, Carter CS, Cushing BS. Developmental effects of oxytocin on neural activation and neuropeptide release in response to social stimuli. Horm Behav 2006; 49:206–14. PMID: 16112115

51. Gabor CS, Phan A, Clipperton-Allen AE, Kavaliers M, Choleris E. Interplay of oxytocin, vasopressin, and sex hormones in the regulation of social recognition. Behav Neurosci 2012; 126:97–109. doi: 10.1037/a0026464 PMID: 22141469

52. Gobrogge KL, Liu Y, Jia X, Wang Z. Anterior hypothalamic neural activation and neurochemical associations with aggression in pair-bonded male prairie voles. J Comp Neurol 2007; 502:1109–22. PMID: 17444499

53. Villalon Landeros R, Morisseau C, Yoo HJ, Fu SH, Hammock BD, Trainor BC. Corncob bedding alters the effects of estrogens on aggressive behavior and reduces estrogen receptor-α expression in the brain. Endocrinology 2012; 153:949–53. doi: 10.1210/en.2011-1745 PMID: 22186416

54. Lee AW, Brown RE. Comparison of medial preoptic, amygdala, and nucleus accumbens lesions on parental behavior in California mice (*Peromyscus californicus*). Physiol Behav 2007; 92:617–28. PMID: 17610916

55. Nair HP, Young LJ. Vasopressin and pair-bond formation: genes to brain to behavior. Physiology 2006; 21:146–52. PMID: 16565480

56. Fink S, Excoffier L, Heckel G. Mammalian monogamy is not controlled by a single gene. Proc Nat Acad Sci USA 2006; 103:10956–60. PMID: 16832060

57. Turner LM, Young AR, Römpler H, Schöneberg T, Phelps SM, Hoekstra HE. Monogamy evolves through multiple mechanisms: evidence from V1aR in deer mice. Mol Biol Evo 2010; 27:1269–78.

58. Choleris E, Gustafsson J-A, Korach KS, Muglia LJ, Pfaff DW, Ogawa S. An estrogen-dependent four-gene micronet regulating social recognition: a study with oxytocin and estrogen receptor-alpha and -beta knockout mice. Proc Nat Acad Sci USA 2003; 100:6192–7. PMID: 12730370

59. Murakami G, Hunter RG, Fontaine C, Ribeiro A, Pfaff D. Relationships among estrogen receptor, oxytocin and vasopressin gene expression and social interaction in male mice. Euro J Neurosci 2011; 34:469–77.

60. Lonstein JS, Rood BD, De Vries GJ. Parental responsiveness is feminized after neonatal castration in virgin male prairie voles, but is not masculinized by perinatal testosterone in virgin females. Horm Behav 2002; 41:80–7. PMID: 11863386

61. Cushing BS, Cawthorn JM. Species differences in activity patterns during oestrus. Can J Zool 1996; 74:473–9.