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PARENTAL REGULATION OF CENTRAL PATTERNS OF ESTROGEN RECEPTOR α

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Abstract—Reduced levels of estrogen receptor alpha (ERα) in the medial amygdala (MeA) and bed nucleus of stria terminalis (BST) have been hypothesized to play a significant role in the expression of male behaviors associated with monogamy. Therefore, the regulation of ERα could be a critical factor in determining male behavior and the evolution of monogamy. Central expression of ERα immunoreactivity was compared in hybrid offspring from crosses between two phenotypically distinct populations of prairie voles (*Microtus ochrogaster*). Illinois voles (IL) are socially monogamous and display low levels of ERα, while Kansas voles (KN) display some characteristics associated with polygyny and have higher levels of ERα. In offspring from hybrid crosses, the pattern of ERα expression was dependent upon parentage; the two types of hybrid crosses did not produce the same ERα pattern in the offspring. In the BST and MeA, hybrid males expressed ERα patterns consistent with those of males from their mother's population, while hybrid females had ERα patterns typical of females belonging to their father's population. The parental-specific patterns of ERα expression are suggestive of genomic imprinting, therefore, the vole ERα (*Esr1*) gene was cloned and sequenced, and examined for allele-specific expression. Results from this study indicate that while maternal factors may play a major role the expression of ERα in their male offspring, genomic imprinting is unlikely to be involved, suggesting another mechanism is responsible. **© 2006 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: ERα, genomic imprinting, intraspecific variation, *Microtus*, monogamy.

In mammals the evolution of social monogamy requires changes in two factors characteristic of polygyny. There must be a decrease in male characteristics typically associated with polygyny such as aggression and mate searching behavior, accompanied by an increase in the expression of affiliative/prosocial behaviors including the formation of long-term pair bonds and paternal care. It has been hypothesized that one mechanism involved in facilitating the change from polygyny to monogamy is a change in the expression of estrogen receptor alpha (ERα) (Cushing et al., 2004). Studies using adult ERα knockout mice indicate that ERα has a masculinizing affect (Scordalakes et al., 2002; Scordalakes and Rissman, 2004), and during development estrogen facilitates the initial masculinization of male neural structures and behaviors (Kendrick and Drewett, 1980; Hutchinson, 1997; Han and De Vries, 2003). Therefore, a reduction in the expression of ERα could reduce the polygynous male phenotype and permit the expression of social monogamy (Cushing et al., 2004; Cushing and Wynne-Edwards, 2006).

A comparison of ERα between two phenotypically distinct populations of prairie voles (*Microtus ochrogaster*) supports this hypothesis. Prairie voles from Illinois (IL) are socially monogamous (Getz et al., 1981; Getz and McGuire, 1997) and display high levels of prosocial behavior, while prairie voles from Kansas (KN) are less social (Cushing et al., 2001) and display some characteristics associated with polygyny, such as high levels of male–male aggression (Rose and Spevak, 1978; Gaines et al., 1985) (for complete details on morphological, physiological, and behavioral differences see Cushing and Kramer, 2005a). In contrast to KN males, adult IL males display little or no ERα in the bed nucleus of the stria terminalis (BST) or in the medial amygdala (MeA) (Hnatczuk et al., 1994; Cushing et al., 2004), two regions of the brain that regulate affiliation and aggression (Cushing et al., 2004).

Assuming that interpopulational differences are the product of natural selection, then the prairie vole represents a unique system to study the mechanisms involved in regulating mating strategies. Because there are significant and consistent populational differences in behavior within prairie voles, a comparative approach to understanding the evolution of social monogamy is not confounded by species differences. The evolution of monogamy represents a potential conflict for males, involving a tradeoff between increasing fitness by fathering offspring with multiple females or investing heavily in the product of one female. It has been argued that in polygynous species there is a selection conflict between males and females (Trivers, 1972). It is assumed that the objective of a polyg-

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Abbreviations: ANOVA, analysis of variance; ARC, arcuate nucleus; BST, bed nucleus of the stria terminalis; ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; IK, offspring resulting from an Illinois dam×Kansas sire; IL, voles from an Illinois population, offspring from IL dam×IL sire; IR, immunoreactivity; KI, offspring resulting from Kansas dam×Illinois sire; KN, voles from a Kansas population, offspring from KN dam×KN sire; KPBS, potassium phosphate–buffered saline; LS, lateral septum; MeA, medial amygdala; MPOA, medial preoptic area; VMH, ventromedial nucleus of the hypothalamus.

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ynous male is to maximize the resources the female commits to his offspring. In contrast, the female must balance resource allocations between the current and future offspring. This theoretical conflict may have led to the evolution of genomic imprinting (Haig and Westoby, 1989; Moore and Haig, 1991; Haig, 1992). Genes regulated by genomic imprinting are differentially expressed from the maternally and paternally inherited alleles, with one allele expressed while the other is epigenetically silenced. This silencing is believed to depend, at least in part, on methylation of DNA and modification of histone proteins, and is fully reversible in the germline (Verona et al., 2003). In monogamous species, however, there is biparental care and, theoretically, the dam and sire have an equal commitment to the current offspring, eliminating conflict between the male and female (Moore and Haig, 1991; Mochizuki et al., 1996). It has been suggested that imprinting should be lost in monogamous species, although this prediction has yet to be supported by empirical data (Vrana et al., 1998).

While genomic imprinting has been associated with genes that regulate fetal growth and resource allocation across the placenta (Costancia et al., 2002; Sibley et al., 2004), there also may be selective advantages associated with regulating the behavior of progeny (Lefebvre et al., 1998; Li et al., 1999). In a socially monogamous species there may be benefits to producing highly social offspring, i.e. offspring that stay at the nest longer and provide care for siblings. However, this would reduce the probability that these individuals would themselves reproduce. In contrast, offspring that dispersed would have higher individual fitness. While these different strategies have been argued at the species level, these differences should also exist between populations of the same species that practice different reproductive strategies. If this is the case, then KN prairie voles may benefit from producing less social offspring, while IL prairie voles may benefit by producing socially monogamous offspring.

If ERα plays a significant role in the differential expression of social behavior within populations of prairie voles, then the expression of ERα could be under the control of genetic or non-genetic factors. To test this we generated interpopulational crosses and examined the resulting patterns of ERα expression in the F_1 hybrid offspring (experiment 1). After finding that ERα expression differed between the two types of hybrids, we tested for genomic imprinting of the *Esr1* gene (experiment 2).

EXPERIMENTAL PROCEDURES

Experiment 1: ERα expression in hybrids

Animals. A laboratory colony of prairie voles was established using animals trapped near Urbana, Illinois (IL) and near Lawrence, Kansas (KN). Subjects of this study were of the F_3 or F_4 generation. Four groups of breeding pairs were established: IL dam×IL sire (IL), KN dam×KN sire (KN), KN dam×IL sire (KI), IL dam×KN sire (IK). Breeding pairs were housed in polycarbonate cages (24×45×20.5 cm) under a 14/10-h light/dark cycle and provided with high fiber rabbit chow (PMI Nutrition International, Brentwood, MO, USA) and water *ad libitum*, and cotton batting for nest material. Housing the dam and sire together is standard procedure for breeding prairie voles as this species is highly social and often lives in communal groups in the wild (Getz and McGuire, 1997). Breeding pairs were checked daily for new litters. On the day of birth (D1), pups were sexed and marked for identification. At 21 days of age (D21), pups were weaned and housed in same-sex sibling pairs.

All animal husbandry and experimental procedures were approved by the appropriate Institutional Animal Care and Use Committee and were within the guidelines established by the National Institutes of Health Guide for the Care and Use of Animals. Care was taken to minimize any distress or pain experienced by the animals. Using power analyses, the experiments were designed to minimize animal usage.

Tissue collection and immunocytochemistry. At 60–90 days of age, brains were collected to perform immunocytochemical analyses for the distribution of ERα. Female prairie voles do not have a spontaneous estrous cycle and plasma estradiol remains low until the female is exposed to a novel male (Carter et al., 1980, 1989). However, vaginal lavages were conducted after tissue collection to verify that females were not in estrus at the time of tissue collection. Because animals from the same litter are not statistically independent, brains were collected from no more than one male and one female per litter (n=5–7 per sex/per group). Animals were deeply anesthetized with a mixture of ketamine (67.7 mg/kg) and xylazine (13.3 mg/kg) and transcardially perfused with 4% paraformaldehyde and 2.5% acrolein in 0.1 M potassium phosphate–buffered saline (KPBS; pH 7.6). Fixed brains were stored in 25% sucrose at 4 °C until sectioned at 30 μm using a freezing sliding microtome. Sliced tissue was stored in cryoprotectant (Watson et al., 1986) until processed for ERα immunoreactivity (IR) using ABC immunocytochemistry (ICC).

Prior to incubation in the primary antibody, tissue was rinsed in 0.05 M KPBS and then incubated for 20 min in 1% sodium borohydride. After rinsing tissue with KPBS, sections were incubated with rabbit ERα polyclonal antibody (Upstate Biotechnology, Waltham, MA, USA; anti-ERα C1355) at a dilution of 1:100,000 in 0.05 M KPBS–0.4% Triton X-100 at room temperature for 1 h and at 4 °C for 48 h. Following incubation in C1355, tissue was rinsed in KPBS and then incubated for 1 h at room temperature in biotinylated goat anti-rabbit IgG (1:600 dilution in 0.4% Triton X-100). Sections were then incubated 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, Burlingame, CA, USA) for 1 h. Sections were then rinsed in KPBS followed by rinses with 0.175 M sodium acetate. Finally, ERα was visualized using a nickel sulfate–diaminobenzidine chromogen solution (250 mg nickel II sulfate, 2 mg DAB, 8.3 μl 3% H_2O_2 per 10 ml sodium acetate). Tissue was mounted onto subbed glass slides and air-dried overnight. To aid in identifying specific brain regions, slides were counterstained with Neutral Red prior to dehydration in ascending ethanol solutions, cleared in Histoclear (National Diagnostics, Atlanta, GA, USA), and coverslipped using Histomount (National Diagnostics).

The primary antibody used for ERα binds to both free and bound receptors, eliminating variation in staining due to potential differences in circulating hormone levels (Murphy et al., 1999). The antibody was generated against the last 15 C-terminal amino acids of the rat ERα protein; this region shares no homology with estrogen receptor beta (ERβ). Controls were run to verify the specificity of the primary antibody. Tissue was incubated in the secondary antibody without prior incubation in the primary antibody. A second control was run by incubating the tissue in the primary antibody plus the peptide against which the antibody was generated; the concentration of the peptide was 10× the concentration of the antibody. In neither case was staining observed.

ERα-IR was quantified in the following areas: the ventral portion of the lateral septum (LS), medial preoptic area (MPOA),

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