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Photoperiod alters central distribution of estrogen receptor α in brain regions that regulate aggression

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Abstract

Testosterone or its metabolite, estrogen, regulates aggression in males of many mammalian species. Because plasma testosterone levels are typically positively correlated with both aggression and reproduction, aggression is expected to be higher when males are in reproductive condition. However, in some photoperiodic species such as Siberian hamsters (*Phodopus sungorus*), males are significantly more aggressive in short day lengths when the testes are regressed and circulating testosterone concentrations are reduced. These results led to the formation of the hypothesis that aggression is modulated independently of circulating steroids in Siberian hamsters. Thus, recent studies have been designed to characterize the role of other neuroendocrine factors in modulating aggression. However, aggression may be mediated by testosterone or estrogen despite basal concentrations of these steroids by increasing sensitivity to steroids in specific brain regions. Consistent with this hypothesis, we found that males housed under short days have increased expression of estrogen receptor α in the bed nucleus of the stria terminalis, medial amygdala, and central amygdala. Neural activation in response to an aggressive encounter was also examined across photoperiod.
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Keywords: Aggression; Estrogen receptor alpha; Photoperiod; c-Fos; Immediate-early gene; Siberian hamster

Introduction

Although there are exceptions, testosterone (T) or T metabolites such as estradiol (E) are typically associated with aggression. In mice, castration decreases intermale aggression and T implants restore aggression (Beeman, 1947; Brain and Haug, 1992; Rubinow and Schmidt, 1996); neonatal castration also reduces aggression later in life even after administration of exogenous T (Edwards, 1969; Motelica-Heino et al., 1993). Because of this correlation, aggression is generally expected to be higher in seasonal breeders during the breeding season when testes are fully functional, although this is not always the case. Siberian hamsters (*Phodopus sungorus*) reproduce under long photoperiods but are non-aggressive in resident–intruder tests. In Siberian hamsters, aggression is significantly higher under short photoperiods when the testes are regressed and aggression is negatively correlated with both testes weight and plasma T concentrations (Jasnow et al., 2000). This and ambiguous findings in other studies have led to the conclusion that T does not have a primary role in regulating aggression under short days. However, the observation that aggression is expressed when serum T is basal does not rule out the involvement of T. Aggression may be regulated by interactions between multiple factors, including changes in steroid sensitivity through changes in receptor expression. If this hypothesis is correct, then the role of T in photoperiodic regulation of aggression may merit reevaluation.

Short photoperiods may increase the availability of or sensitivity to T or E through increases in aromatase activity or changes in steroid receptor patterns. Testosterone is converted to E intracellularly by aromatase and the resulting E can bind estrogen receptors to impact behavior (Trainor and Marler, 2002). Estrogen receptor alpha (ERα) is a likely candidate for regulation of aggression in hamsters. Comparative studies and studies with ERα knockout mice support a role for ERα in aggression (Cushing and Kramer, 2005a,b; Cushing and Wynne-Edwards, 2006; Ogawa et al., 1998a,b; Scordalakes and

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Rissman, 2004; Trainor et al., 2006). High numbers of ERα in brain regions regulating aggression such as the bed nucleus of the stria terminalis (BST) and medial amygdala (MeA) may be sufficient to increase aggression even when plasma T concentrations are relatively low. Conversely, animals from long photoperiods may be non-aggressive despite high plasma T, if relevant brain regions are rendered insensitive due to low numbers of ERα. Previous studies examining the effect of photoperiod on receptors reported little difference but the resolution was limited by available techniques (Bittman et al., 1990; Callard et al., 1986; Glass et al., 1984).

Our primary objective was to test the hypothesis that changes in aggression in response to photoperiod are correlated with site-specific changes in ERα. If this hypothesis is true, males housed under short days are predicted to have relatively high ERα in brain regions regulating aggression such as the BST and MeA. The importance of ERα in these particular brain regions has been highlighted by studies comparing males from species with markedly different levels of positive and negative social behaviors as well as by studies making comparisons within mouse strain against aggression (Cushing and Kramer, 2005a; Cushing and Wynne-Edwards, 2006; Trainor et al., 2006). To disentangle the individual roles of photoperiod and testes size in regulating steroid sensitivity we also examined ERα in a group of short-day refractory male hamsters that had undergone testicular recrudescence such that they exhibited long-day like testes even though they remained under short day lengths. Our secondary objective was to investigate neural responses to social stimuli by short day- versus long day-housed males. Seasonal behavioral differences may result from photoperiod-induced differences in the pattern of neural activation (i.e., neural processing) in response to a given social cue. Aggression is a complex behavior and involves processing in multiple brain regions; an examination of neural activation in groups differing in aggression would provide some insight as to the neural control of photoperiod-sensitive male–male aggression. Neural activation in specific brain regions can be assessed by examining immediate-early gene expression, such as c-Fos expression. One region that might be expected to play a role in this form of aggression is the central amygdala. Because short photoperiods are associated with anxiety-related behavior (Prendergast and Nelson, 2005; Pyter and Nelson, 2006) and anxiety is related to aggression (Clement and Chapouthier, 1998; Bosch et al., 2005), we expected increased c-Fos expression in the central amygdala and basal lateral amygdala of groups with increased aggression.

Methods

Husbandry and treatment groups

Subjects were adult male Siberian hamsters (*P. sungorus*) from breeding pairs in our colony. The colony was derived from animals supplied by Dr. Irving Zucker at the University of California, Berkeley. Animals were provided food (Agway Prolab 3500 HMR) and water *ad libitum*. Offspring were weaned at postnatal day 17–19 and housed in same-sex groups of two or three in polypropylene cages (27.8×7.5×13.0 cm). Subjects were from one of three photoperiod treatments: maintained under short-day (SD) conditions (10L:14D, light offset 18:00 CST) for 6 weeks ($n=10$); maintained under SD >20 weeks (SDR; $n=12$); maintained under long day (LD) conditions (16L:8D, light offset 18:00 CST) for >20 weeks ($n=10$). All husbandry and experimental procedures were approved by the University of Memphis Animal Care and Use Committee and adhere to standards set by the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Measures were taken to minimize animal number required for the study and to minimize any distress or discomfort.

Resident–intruder testing

Subjects were individually housed for at least 1 week prior to testing following Jasnow et al. (2000). Aggression was assessed using a standard resident–intruder test which consists of placing a non-aggressive, dye-marked, stimulus male (intruder) into the home cage of the subject (resident) for 10 min (e.g., Caldwell and Albers, 2004; Demas et al., 2004; Jasnow et al., 2000). Stimulus males were group-housed males from LD conditions of approximately the same age and size as subjects and unrelated to the subjects. Stimulus males were not used more than once per week. All testing was done between 08:30 and 10:00 CST. Tests were recorded to allow behavioral analysis (see below) at a later time.

Tissue collection and immunocytochemistry

At 55–70 min after the resident–intruder test began, animals were deeply anesthetized using a combination of Ketamine (>67.7 mg/kg) and Xylazine (>13.3 mg/kg) and brains collected. This time point was chosen because the protein product of the *c-fos* gene is maximal at 60–90 min following the stimulus for its transcription (Hoffman et al., 1993); in this case, the stimulus is the introduction of an intruder into the resident's cage. Brains were fixed using a spinning immersion technique following Kramer et al. (2006) with 4% paraformaldehyde and 5% acrolein in 0.1 M KPBS (pH 7.6). After 4 h in paraformaldehyde+acrolein fixative brains were placed in 4% buffered paraformaldehyde for 24 h at 4 °C. Brains were then placed in 25% sucrose and stored at 4 °C until sectioning. Paired testes weight (PTW) was obtained for each animal to assess gonadal regression and response to photoperiod.

Brains were sliced at 30 μm on a freezing sliding microtome and free-floating sections were stored in cryoprotectant (Watson et al., 1986) at −20 °C until processed for either ERα or c-Fos. For each animal one half of the brain, alternating sections rostral to caudal, was stained for ERα and the other half was stained for c-Fos. Immunocytochemistry was done using standard ABC immunocytochemistry and following the methods of Kramer et al. (2006). The primary antibody for ERα (Upstate USA, Charlottesville, VA anti-ERα C1355) was used at a dilution of 1:10,000. This antibody binds both free and bound receptors, minimizing variation due to endogenous steroid levels (Murphy et al., 1999). The antibody was generated against the last 15 C-terminal amino acids of the rat ERα protein, a region that shares no homology with ERβ. The specificity of this antibody was tested by omitting the primary antibody from the ICC procedure and by performing ICC after pre-adsorption with the synthetic peptide (10× the concentration of the antibody). Procedures for c-Fos labeling were the same with the exception that the primary antibody was anti-c-Fos (Ab-5, EMD Biosciences, San Diego, CA) used at a dilution of 1:80,000.

Sections were scored by a single experimentally blind scorer. For each brain region, the number of cells expressing ERα- or c-Fos immunoreactivity (IR) was counted bilaterally using ImageJ (Rasband, 1997–2006) image analysis software at 40× magnification and then averaged. Regions analyzed for ERα-IR included the ventral lateral septum (LSV), medial preoptic area (MPOA), bed nucleus of the stria terminalis (BST), arcuate nucleus (ARC), ventromedial nucleus of the hypothalamus (VMH), BLA, CeA and the posterodorsal (MePD) and posteroventral (MePV) divisions of the MeA. Sections scored corresponded to rat Bregma 0.36 (LSV), Bregma −0.84 (MPOA, BST), Bregma −2.28 (ARC, VMH, BLA, CeA) and Bregma −3.00 (MePD, MePV) (Paxinos and Watson, 2005). The regions were chosen for analysis because they are part of the extended social network and play a role in sociosexual behavior including aggression (Halász et al., 2002; Haller et al., 2006; Newman, 1999). Regions analyzed for c-Fos-IR were the basolateral amygdala (BLA, Bregma −2.28), central amygdala (CeA, Bregma −2.28) and paraventricular nucleus of the hypothalamus (PVN, Bregma −1.56). They were analyzed because other studies have shown increased activation in these regions in response to aggressive interactions (e.g., Halász et al., 2002; Haller et al., 2006). Furthermore, they are

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