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Photoperiod-Dependent Response to Androgen in the Medial Amygdala of the Siberian Hamster, *Phodopus sungorus*

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Abstract The medial nucleus of the amygdala (MeA) is a steroid-sensitive region that has been implicated in the expression of behaviors such as mating and aggression. The male Siberian hamster (*Phodopus sungorus*) uses light cues to regulate its reproductive neuroendocrine system, reducing androgen synthesis in the autumn and increasing it in the spring. There is also evidence that short photoperiods reduce the sensitivity of the brain to the behavioral effects of androgen. The authors tested the hypothesis that MeA neurons are less responsive to androgen in short photoperiods by comparing the regional volume and average soma size of the four MeA subnuclei (anterodorsal [MeAD], anteroventral [MeAV], posterodorsal [MePD], and posteroventral) in adult male hamsters that had been castrated and then implanted with capsules containing either testosterone (T) or nothing. Animals from each group were housed in either long or short photoperiods for 15 weeks. MeAD and MeAV somata displayed photoperiod-dependent responses to androgen, increasing in size after T treatment only in long days. In contrast, the average soma size and the regional volume of the MePD subnucleus were significantly larger in T-treated males regardless of photoperiod. The authors conclude that photoperiod influences the sensitivity of the MeA to androgen.

Key words amygdala, neural plasticity, seasonal rhythms, gonadal steroids, melatonin

In seasonally breeding mammals, parturition is timed to coincide with increased food availability to maximize reproductive success (Bronson and Heideman, 1994). Day length, the cue seasonal breeders use to regulate their behavior, is transduced by the suprachiasmatic nucleus into nightly secretions of melatonin. Decreasing day lengths during the transition from summer to winter lead to prolonged nightly secretions of melatonin. Longer periods of melatonin secretion suppress the gonadotropin-releasing hormone system and increase its sensitivity to negative feedback, reducing androgen synthesis by the testes (reviewed in Turek and Van Cauter, 1994). In Siberian hamsters (*Phodopus sungorus*), the transition from summer to winter also stimulates a change in pelage color and a reduction in body weight (Gorman and Zucker, 1995).

The medial nucleus of the amygdala (MeA) has been implicated in the chemosensory control of mating behavior in the Syrian hamster (Lehman et al.,

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1980; Wood and Coolen, 1997) and in the feedback regulation of luteinizing hormone release (Kostarczyk, 1986). In the Syrian hamster, the MeA receives inputs from the main and accessory olfactory bulbs and projects to the bed nucleus of the stria terminalis and medial preoptic area (Coolen and Wood, 1998; Kevetter and Newman, 1981; Scalia and Winans, 1975). Studies of gonadal steroid receptor expression have shown that the posterodorsal (MePD) subnucleus expresses androgen and estrogen receptors more densely than the other subnuclei of the MeA (Shugrue et al., 1997; Simerly et al., 1990; Wood and Newman, 1995), and experiments manipulating gonadal hormones in adult animals suggest that the MePD subnucleus displays more steroid-dependent neurochemical plasticity than the other subnuclei (Malsbury and McKay, 1994; Simerly and Swanson, 1987; Swann and Newman, 1992; Wang and De Vries, 1995). Together, these data suggest that fluctuating androgens may influence chemosensory behavior by affecting MePD anatomy. Consistent with this hypothesis, we have found that the regional volume and mean cross-sectional area of MePD neurons in adult male rats are sensitive to circulating androgens (Cooke et al., 1999) and are correlated with changes in the expression of sexual arousal (Cooke, 2001).

Seasonal changes in circulating androgens suggest that, as in birds (Brown and Bottjer, 1993; DeVoogd and Nottebohm, 1981), morphological plasticity may occur in the brain of seasonally breeding mammals. In fact, we have found that MePD somata are significantly smaller in gonadally intact male *P. sungorus* with photoperiod-induced winter phenotypes compared to those with summer phenotypes (Cooke et al., 2002). Although the reduced androgens in the short-day animals (evidenced by their lighter testes and seminal vesicles) may account for our observations, experiments with Syrian hamsters suggest that photoperiod also directly affects the substrates of mating behavior, since androgen treatment in short days fails to restore copulation as readily as it does in long days, despite the photoperiod-invariant responses to androgen in the flank glands (Campbell et al., 1978; Morin and Zucker, 1978). Thus, it is possible that photoperiod interacts with MePD steroid responsiveness, concomitant with changes in circulating androgen. Evidence in favor of this hypothesis comes from Tubbiola and Bittman (1994), who showed that tritiated naloxone binding is reduced by testosterone (T) treatment more effectively in long days than in short days. Similarly, Bittman et al. (1996) found that the number of arginine vasopressin (AVP) immunoreactive cells in the *P. sungorus* MePD subnucleus was significantly reduced in the brains of short-day + T hamsters compared to long-day + T hamsters, indicating that photoperiod had influenced the capacity of the MePD subnucleus to synthesize and express AVP in response to androgen. Furthermore, we found that photoperiod can have androgen-independent effects on the size of motoneuron endplates from the spinal nucleus of the bulbocavernosus, a neuromuscular system involved in copulation in Siberian hamsters (Hegstrom et al., in press).

We sought to evaluate whether photoperiod affects MeA steroid responsiveness in *P. sungorus* using Nissl-stained brain sections. Thus, we compared the average soma size and regional volume of the MeA subnuclei in castrated male hamsters treated with T or empty capsules and housed in long or short photoperiods.

MATERIALS AND METHODS

Hamsters were gestated and reared under 15 hours of illumination followed by 9 hours of dark (15:9 LD). At 3.5 months of age, animals were castrated under ketamine cocktail (ketamine 90 mg/kg; xylazine 10 mg/kg) and implanted subcutaneously with Silastic capsules (3.2 mm o.d., 1.6 mm i.d., 4 mm effective length) containing either T or nothing. Immediately after surgery, the animals were randomly assigned to either long-day or short-day (8:16 LD) photoperiods. All males were housed either singly or with other males at 22 °C with food and water provided ad libitum. After 15 weeks, the animals were weighed, euthanized with pentobarbital sodium, and exsanguinated with phosphate buffered saline (pH 7.2), followed by 1% paraformaldehyde with 2.5% gluteraldehyde fixative. Brains and seminal vesicles (including seminal fluid) were harvested, weighed, and postfixed in 10% buffered formalin for 6 months. All procedures were conducted with prior approval from the University of California Animal Care and Use Committee, Berkeley.

Prior to sectioning, fixed brains were transferred to 20% sucrose solution overnight for cryoprotection and scored on the right hemisphere to mark laterality. Brains were frozen-sectioned coronally at 60 µm into phosphate buffer. Alternate sections were mounted on coded, gelatin-coated slides, which were then dried overnight and stained with thionin.

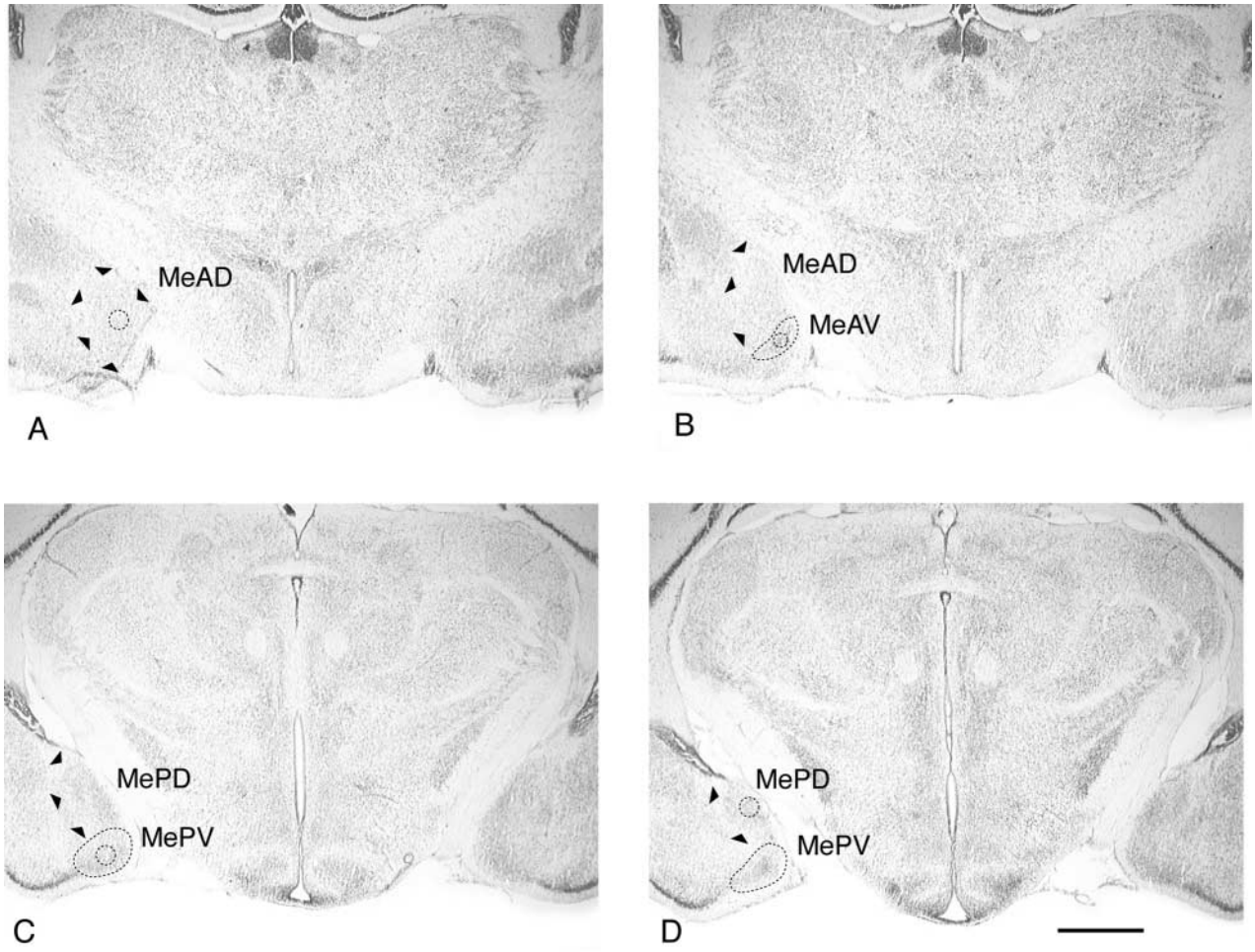

Figure 1. Photomicrograph of medial nucleus of the amygdala subnuclei in *Phodopus sungorus*, outlined with arrowheads and dashed lines. MeAD = anterodorsal subnucleus; MeAV = anteroventral subnucleus; MePD = posterodorsal subnucleus; MePV = posteroventral subnucleus. Dashed circles indicate locations of somal sampling within each subnucleus. Bar = 0.5 mm.

The cytoarchitecture of the hamster MeA was compared to that of the mouse with an atlas (Franklin and Paxinos, 1997); subnucleus boundaries and cytology were found to be similar between the two species. A single investigator unaware of the group membership of the sections used a microprojector to trace the right and left MeA subnuclei (anterodorsal [MeAD], anteroventral [MeAV], MePD, and posteroventral [MePV]) (Fig. 1) in every section where the subnucleus was detected. If tissue damage prevented the complete drawing of a subnucleus, the subnucleus from that animal was not included in the analysis. Tracings were digitized with a flatbed scanner, and the total area of each subnucleus in each hemisphere was measured with Image software from the National Institutes of Health. The volume of each subnucleus in each hemisphere was estimated by summing the total areas of the drawn subnuclei and multiplying the sum by the section thickness (60 μm) and the sampling ratio (2).

From each animal, 20 to 25 neurons from each of the subnuclei in each hemisphere were traced at 630X magnification using a camera lucida attached to a Zeiss compound microscope. The region of somal sampling was within the approximate center of each subnucleus (Fig. 1) or was centered on the regions reported to display a dense expression of gonadal steroid receptors in the Syrian hamster MePD subnucleus (Wood and Newman, 1995). Only those cells with a visible nucleolus and cytoplasm were selected for tracing. The average soma size for each subnucleus was calculated by measuring the areas of the drawn somata and computing the mean cross-sectional area for each subject.

Table 1. Effect of photoperiod, castration, and testosterone treatment on body, brain, and seminal vesicle weights in Siberian hamsters.

Group		*Body (g)*	*Seminal Vesicle (g)*	*Brain (g)*
Long day + T	(n = 8)	53.6 ± 1.79*	0.90 ± 0.16**	0.632 ± 0.009
Long day + B	(n = 5)	42.8 ± 2.23	0.03 ± 0.00	0.656 ± 0.012
Short day + T	(n = 7)	49.6 ± 1.91*	1.13 ± 0.06**	0.643 ± 0.012
Short day + B	(n = 7)	42.4 ± 3.51	0.04 ± 0.00	0.620 ± 0.019

NOTE: Data are expressed as mean ± SEM. Castrated adult males housed in long days (15:9 LD) and short days (8:16 LD) were treated with capsules containing either testosterone (T) or nothing (B). Data were collected after 15 weeks of treatment.
*Significantly different compared to blank-treated controls exposed to the same photoperiod, $p = 0.01$.
**$p < 0.0001$.

Morphometric data were initially analyzed using mixed-design three-way analysis of variance (ANOVA) (Statview 5.0, Abacus Concepts, Berkeley, CA), with hemisphere as a repeated measure and day length and androgen as independent variables. When no significant effects of laterality were found, the values from the two hemispheres were averaged and each subnucleus was analyzed with two-way ANOVA (with androgen treatment and photoperiod as independent variables). If an interaction between day length and androgen was detected, the groups were compared using independent t tests, with $p < 0.05$ (two-tailed) as the criterion for significance. Body, brain, and seminal vesicle weights were also subjected to two-way ANOVA.

RESULTS

Body, Brain, and Seminal Vesicle Weights

As expected, seminal vesicle weights were significantly greater in T-treated males ($p < 0.0001$), irrespective of day length. Body weight was increased in those hamsters given T ($p = 0.01$), also irrespective of photoperiod. However, brain weight was not affected by androgen or photoperiod (Table 1). Therefore, the absolute volumes of the MeA subnuclei were used in the subsequent analyses.

MeA Somata Size

No effects of laterality were found in any subnucleus (ps > 0.20), so the somata from both hemispheres were averaged for further analysis.

Within the anterior nuclei, photoperiod-dependent responses to androgen were found. No main effects of androgen or photoperiod were detected in MeAD somata, but there was a significant interaction between the main factors, $F(1, 19) = 8.25$, $p = .009$, which was due to androgen increasing soma size only in long days. This was verified by a t test between T-treated and blank-treated (B-treated) animals in the two photoperiods (long day: T, B $t[11] = 3.25$, $p = .007$; short day: T, B $t[12] = -0.47$, $p = 0.6$). The MeAV subnucleus displayed a main effect of androgen, $F(1, 19) = 9.2$, $p = 0.006$, and an interaction, $F(1, 19) = 7.48$, $p = 0.01$, which was again due to T exerting a significant effect in long days but not in short days (long day: T, B $t[9] = 3.7$, $p = 0.004$; short day: T, B $t[11] = 0.27$, $p = 0.7$) (Fig. 2).

The posterior nuclei displayed photoperiod-independent responses to androgen. MePD somata displayed a main effect of androgen, $F(1, 19) = 22.06$, $p = 0.0002$, with soma size increasing in the presence of T, and no effect of photoperiod. There was a weak but insignificant interaction between photoperiod and androgen ($p = 0.06$). Similarly, the MePV subnucleus displayed a main effect of androgen, $F(1, 19) = 4.38$, $p = 0.04$, no effect of photoperiod, and no interaction (ps ≥ 0.1) (Fig. 2).

MeA Regional Volumes

Only two of the four MeA subnuclei had a significant main effect of laterality. The left MePV subnucleus was significantly larger than the right, $t(19) = 2.32$, $p = 0.03$, and the right MePD subnucleus was significantly larger than the left, $t(22) = -2.41$, $p = 0.02$. However, for neither subnucleus was there any interaction of hemisphere with either photoperiod or androgen. Thus, the lateral asymmetry appeared to be unrelated to either manipulation, so bilateral volumes of the subnuclei were averaged for further analysis by two-way ANOVA.

Effects of photoperiod in MeA regional volumes were not observed. MeAD regional volume displayed a main effect of androgen, $F(1, 21) = 4.43$, $p = 0.04$, and no effects of photoperiod or of interaction. MePD vol-

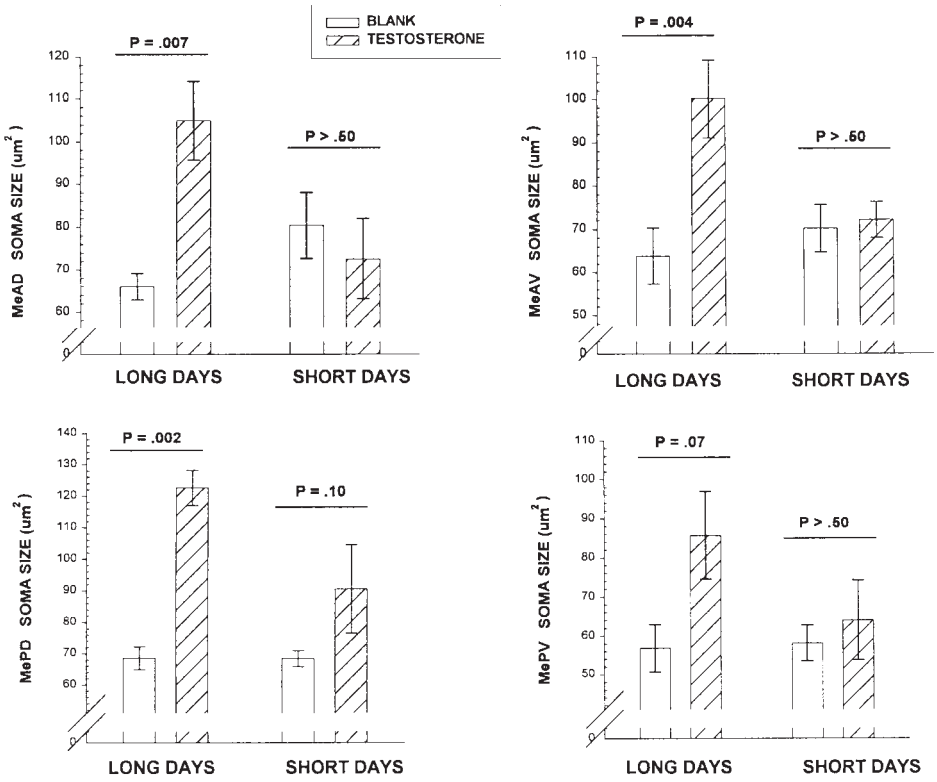

Figure 2. Mean ± SEM of medial nucleus of the amygdala soma size from subregions of the medial amygdala from male *Phodopus sungorus* that were castrated and given blank capsules or castrated and treated with capsules filled with testosterone and maintained for 15 weeks in either long days or short days. Analysis of variance revealed a significant interaction of photoperiod and androgen on the anterodorsal (MeAD) subnucleus and anteroventral (MeAV) subnucleus: Androgen significantly increased soma size in these two subnuclei only in long days. A similar interaction for posterodorsal (MePD) soma size did not reach statistical significance ($p = 0.06$, two-tailed). Posteroventral (MePV) somata revealed only a significant main effect of androgen treatment ($p < 0.05$).

ume also showed an effect of androgen, $F(1, 20) = 6.8$, $p = 0.01$, and no effects of photoperiod or of interaction. In both the MeAD subnucleus and the MePD subnucleus, the effect of androgen was to increase regional volume in T-treated animals compared to castrates given B capsules. No significant main effects or interactions were detected for MeAV or MePV regional volume (Fig. 3).

DISCUSSION

We previously reported that photoperiod affects MePD structure in gonadally intact Siberian hamsters (Cooke et al., 2002). MePD neurons were larger in animals kept in long-day photoperiods compared to males transferred to short-day photoperiods. Because androgen secretion is reduced in short days, the effects on MePD morphology could have been due solely to androgen. The present results indicate that fluctuating androgens are indeed affecting MePD morphology in gonadally intact males placed in various photoperiods. However, it also appears that long days, in addition to producing higher levels of circulating androgen, prime the anterior MeA to respond to androgen. Anterior MeA neurons responded to T in long days but failed to do so in short days, lending support to the notion that the neural substrates of mating become less sensitive to gonadal steroids in winter-like photoperiods (Campbell et al., 1978; Morin and Zucker, 1978).

Effects of Androgen and Photoperiod on MeA Somata

Among animals in long-day photoperiods, the somata in the MePD subnucleus displayed the greatest response to androgen, a 79% difference between B- and T-treated animals. In the other three subnuclei, somata were 56% to 59% larger in T-treated castrates than in castrates given blanks in long days.

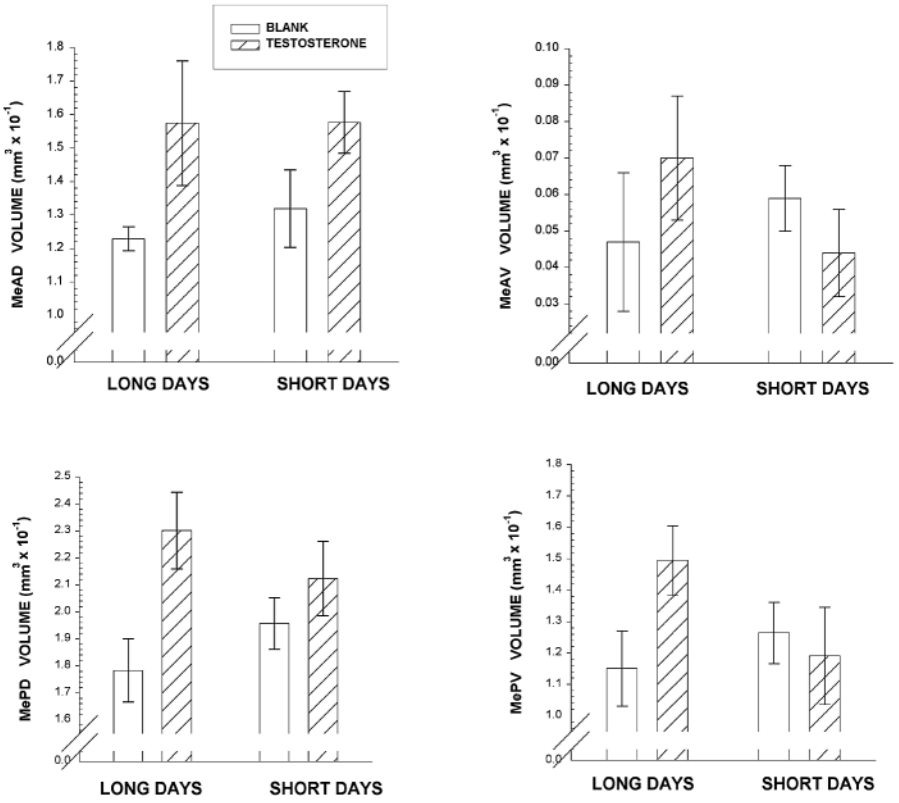

Figure 3. **Mean ± SEM medial nucleus of the amygdala regional volumes from *Phodopus sungorus*. Main effects of androgen treatment were detected in the anterodorsal (MeAD) subnucleus ($p = 0.04$) and posterodorsal (MePD) subnucleus ($p = 0.01$), where testosterone increased regional volume relative to blank-treated castrates. Photoperiod did not significantly influence T responsiveness in any of the subnuclei, that is, there was neither a main effect of photoperiod nor any significant interaction of photoperiod and androgen for these measures. MeAV = anteroventral subnucleus; MePV = posteroventral subnucleus.**

Interestingly, we detected the influence of photoperiod in the anterior, but not the posterior, MeA. In the MeAD subnucleus, the interaction term indicated that photoperiod exerted a permissive effect on the steroid responsiveness of neurons there, allowing T to significantly increase soma sizes only in long days. Likewise, in the MeAV subnucleus, T increased soma sizes in long-day animals but was ineffective in short-day males. In contrast, MePD and MePV somata responded to androgen in either photoperiod, yet even here there was a hint of an interaction ($p = 0.06$, two-tailed) in MePD somata, which suggests that a larger sample might have revealed a greater responsiveness in long days than in short days. Indeed, AVP immunoreactivity was found to be dependent on photoperiod in the posterior, but not the anterior, MeA (Bittman et al., 1996).

In the rat, estrogen receptors α and β are expressed mainly, although not exclusively, in the MePD portion of the MeA (Shugrue et al., 1997). MeA androgen receptor expression in rats and Syrian hamsters follows a similar pattern (Simerly et al., 1990; Wood and Newman, 1995). Although there are fewer gonadal steroid receptor-expressing cells in the anterior and ventral MeA subnuclei, such cells may account for the androgen-dependent changes we observed there. Alternatively, the MePD subnucleus or other steroid-sensitive structures may influence the steroid sensitivity of the anterior subnuclei via paracrine effects or axonal connections.

The question remains how neurons are less responsive to androgen in different photoperiods. Because melatonin receptors are not known to be expressed in the MeA, a direct effect of the hormone on this nucleus seems unlikely. However, there are reports of retinal projections to the anterior MeA (Cooper et al., 1994; Johnson et al., 1988), raising the possibility that light cues influence androgen responsiveness directly.

A second way in which photoperiod could affect androgen responsiveness is to reduce the capacity of the brain to synthesize estrogen. It is possible that the trophic effect of androgen we observed was due mainly to estrogen, given that estrogen is sufficient to increase the immunoreactivity for certain peptides

expressed by the rat MePD subnucleus (Micevych et al., 1994; Simerly et al., 1989; Wang and De Vries, 1995) and to increase somal size and dendrite length in the Syrian hamster MePD subnucleus (Gomez and Newman, 1991). Hutchison et al. (1991) described reduced aromatase activity in the anterior hypothalamus of short-day hamsters given T capsules compared to long-day + T hamsters, and Callard et al. (1986) reported 80% lower nuclear estrogen receptor expression in Syrian hamsters acutely injected with T in short-day photoperiods compared to hamsters injected in long-day photoperiods, suggesting that less estrogen was available in short-day + T animals. We found MeAD and MeAV neurons to be less responsive to androgen in short days compared to long days, despite equivalent T treatments (however, see Raouf et al., 2000). A plausible explanation for this finding is that reduced aromatase in our short-day + T animals decreased the amount of the active metabolite (i.e., estrogen) in the MeA and, thus, attenuated the trophic effect of androgen.

Effects of Androgen on Regional Volumes

Unlike our observations of MeA somata, photoperiod did not exert a detectable effect on MeA regional volume. MePD volume was greater in T-treated castrates than in B-treated castrates, an effect that seemed equivalent in either photoperiod. MeAD volume also responded equivalently to castration and T-treatment in long days and short days, unlike MeAD somata. Because we are unaware of any significant neurogenesis in the adult MeA, we presume changes in regional volume reflect somal and dendritic changes. It is unknown why somata within every subnucleus displayed androgen and/or photoperiod-dependent responses, yet only two subnuclei revealed significant changes in regional volume following our treatments.

CONCLUSION

In seasonally breeding Siberian hamsters, short-day photoperiod suppresses reproduction in two ways. First, prolonged melatonin secretion increases the sensitivity of the GnRH system to negative feedback by T, which reduces the secretion of gonadotropin, and therefore androgen synthesis by the testis. Second, short-day photoperiod induces refractoriness to some of the activational effects of T on male sex behavior (Morin and Zucker, 1978). This refractoriness to androgen may be reflected in the MeA, where it has been found that androgen-dependent AVP expression and tritiated naloxone binding are both diminished in short-day + T compared to long-day + T conditions (Bittman et al., 1996; Tubbiola and Bittman, 1994). Here, we find that MeA cell size is also regulated by both androgens and photoperiod. Neuronal somata in the anterior subnuclei responded to androgen in a photoperiod-dependent fashion, increasing in size after T treatment in long days only. The posterior subnuclei, on the other hand, responded to T in either photoperiod.

ACKNOWLEDGMENT

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