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Photoperiod and social cues influence the medial amygdala but not the bed nucleus of the stria terminalis in the Siberian hamster

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Abstract

We investigated whether the posterodorsal nucleus of the medial amygdala (MePD) and the posteromedial nucleus of the bed nucleus of the stria terminalis (BSTpm) undergo structural changes in response to photoperiod or social environment in the Siberian hamster, a seasonally breeding rodent. Adult male hamsters were either kept in long days (LD; 15:9 h light:dark) from birth or were transferred at 12–16 weeks of age to short days (SD; 8:16) and housed with a male conspecific for 11 weeks. Other males were transferred to SD but were housed with an unrelated female conspecific from LD. Males transferred to SD without a female cagemate displayed testicular regression, but males transferred to SD with a female cagemate did not. The regional volume and average soma size of the BSTpm and the MePD were estimated using Nissl-stained brain sections. Neither photoperiod nor social condition modified either of the BSTpm measures. Among males housed in same-sex groups, the average soma size in the MePD was significantly smaller in SD males than in LD males. Cohabitation with a female resulted in MePD volumes indistinguishable from LD males. These results indicate that the MePD, a nucleus implicated in socio-sexual behavior, can respond to photoperiodic as well as to social cues. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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The Siberian hamster (*Phodopus sungorus*) synchronizes its reproductive behavior with the seasons via photoperiodic control of the gonadotropin-releasing hormone system. Short day-lengths increase the duration of nightly melatonin secretion, which reduces gonadotropin secretion by the pituitary and thus androgen synthesis in the testes (reviewed in Ref. [18]). It has been previously shown that the morphology of the spinal nucleus of the bulbocavernosus (SNB), a neuromuscular system involved in reproduction, is plastic in response to changing photoperiods in Siberian hamsters [11] and that social cues can attenuate the response to photoperiod. Males that were given a female cagemate upon transfer to short days did not respond to the photoperiod cue, maintaining testes weights and the morphology of the SNB [12].

The medial amygdala (MeA) and bed nucleus of the stria terminalis (BST) have been implicated in the appetitive aspects of male sexual behavior in rats and Syrian hamsters [14,15,19]. Both the MeA and BST receive inputs from the accessory olfactory bulb [16] and project to the medial preoptic area and mediobasal hypothalamus [5,13]. In rats and hamsters, the posterodorsal nucleus of the MeA (MePD), and the posteromedial nucleus of the BST (BSTpm) express gonadal steroid receptors in adulthood [17,20] and display structural and neurochemical plasticity in response to changing androgens [7,9,10]. These observations suggest that naturally fluctuating androgens in a seasonally breeding rodent such as *P. sungorus* might influence MePD and/or BSTpm morphology. The number of arginine vasopressin immunoreactive cells is seasonally variable in the MePD of *P. sungorus[3,4]*, but to date there has been no study of seasonal plasticity in the regional volume of the MePD or BSTpm. Therefore we have undertaken a morphological analysis of the MePD and the BSTpm in Siberian hamsters housed in different social and photoperiodic conditions.

Twenty-one male and six female Siberian hamsters were gestated and raised in long days (LD; 15 h light and 9 h dark (15L:9D), lights on at 02:00 h) until 3 to 4 months of age. At this time, the males were randomly assigned to three groups, with littermates distributed among groups. Six males were moved to short photoperiods (8L:16D, lights on at 10:00 h)

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and were housed with a non-sibling male (SD + male). Seven males were transferred to SD and paired with an unrelated virgin female from LD (SD + female). Seven males were kept in LD and were housed with a non-sibling male (LD + male).

After 10–11 weeks, the pelage color of the animals was recorded, the animals were weighed, sacrificed with pentobarbital sodium and perfused with PBS followed by 10% buffered formalin. The testes and brains were extracted and weighed. Brains were stored in buffered formalin 12–14 months before sectioning. Fixed brains were transferred to 20% sucrose in phosphate buffer overnight, scored on the right hemisphere to mark laterality, and frozen-sectioned coronally at 60 μm, with a sampling ratio of 1:2. Sections were mounted on gelatin-coated slides, dehydrated and stained with thionin.

A single investigator, unaware of the group membership of each specimen, outlined the boundaries of the BSTpm using a microprojector at 25×. Fifteen to 25 somata from the BSTpm in each of the animals were also traced using a camera lucida attached to a compound microscope using approximately 630× (Fig. 1A). Likewise, the boundary of the MePD was drawn bilaterally using a microprojector, and 20–25 somata in the MePD were traced using the camera lucida (Fig. 1B). Drawings were digitized with a flatbed scanner, captured by Adobe Photoshop and analyzed with NIH Image software. The volume of each nucleus was estimated by summing the areas of the drawn subnuclei and multiplying the sum by the section thickness (60 μm) and the sampling interval (2). Because circulating androgens are low in SD [2] and because we have observed the regional volume of the rat MePD to shrink after castration [7], we hypothesized that BSTpm and MePD measures would be smaller in SD males compared to males kept in LD. Likewise, because we previously found that having a female cagemate prevented males from reducing androgen production in response to SD, we predicted that SD + female animals would have larger brain measures than SD + male animals. Thus, we used one-tailed *t*-tests to compare data from the various groups.

SD + male hamsters had reduced testes weights (51.4 ± 14.3 mg) compared to LD + male hamsters (693.7 ± 93.8, $t(13) = 6.31$, $P < 0.0001$). Five of the six SD + male hamsters displayed a winter-like pelage at the time of sacrifice, further indicating that they had responded to photoperiod. SD + female males failed to show an SD-induced involution of the testes (testes weight: 842.0 ± 123.7 mg) or change in pelage color, indicating that social cues inhibited the response to photoperiod, as reported earlier [12]. Mixed sex groups in SD were sexually active, with 71% of the pairs producing one or more litters, as reported elsewhere [12]. As suitable brain sections were not obtained from all of the animals from the previous study [12], the means listed above vary slightly from the earlier report.

The BSTpm displayed no effects of hemispheric laterality (data not shown). Thus, the bilateral volumes were averaged and used in the analysis. Mean bilateral volume and soma size showed no effect of photoperiod or social condition (Fig. 2A,B).

No effect of hemispheric laterality was detected in MePD volume. The mean bilateral MePD was significantly larger in SD + female males compared to SD + male males ($t(11) = 2.8$, $P = 0.008$; Fig. 3A) but no significant effect of photoperiod on MePD volume was detected (LD + male vs. SD + male; $t(13) = 1.59$, $P = 0.06$; Fig. 3A).

Analysis of MePD neurons revealed that LD + male hamsters had significantly larger somata than SD + male hamsters ($t(13) = 1.9$, $P = 0.03$). Social condition did not exert a detectable effect on MePD soma size as SD + male hamsters had slightly, but not significantly, smaller MePD somata than SD + female males ($t(11) = 1.55$, $P = 0.07$; Fig. 3B).

MePD neurons in SD + male hamsters were significantly smaller than those from LD + male hamsters. We

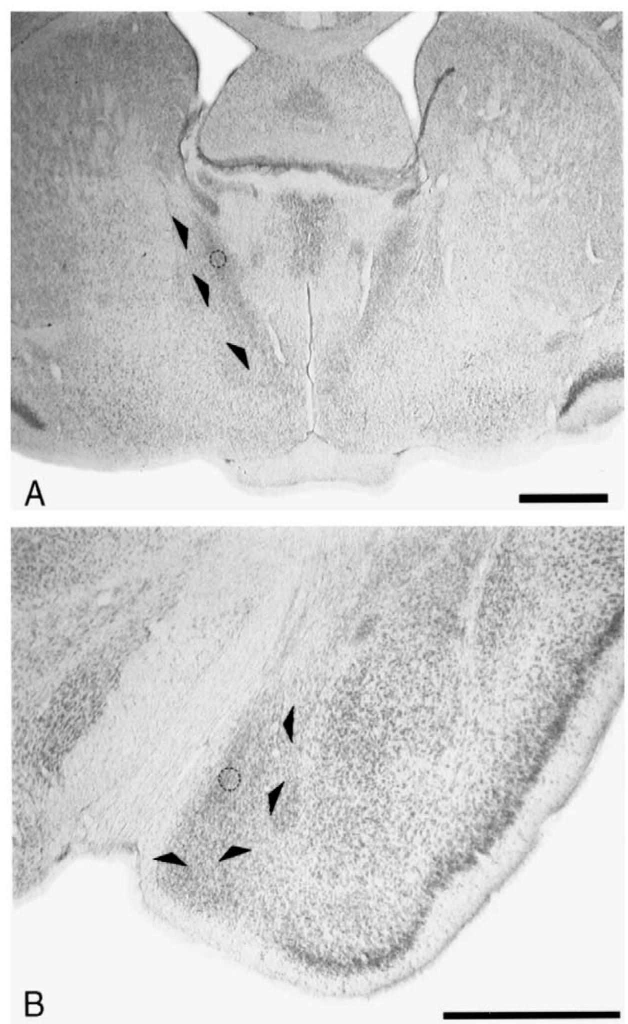

Fig. 1. Photomicrograph of Nissl-stained sections containing the BSTpm (A) and MePD (B); circles indicate locations of somal sampling within each subnucleus. Calibration bar = 1.0 mm.

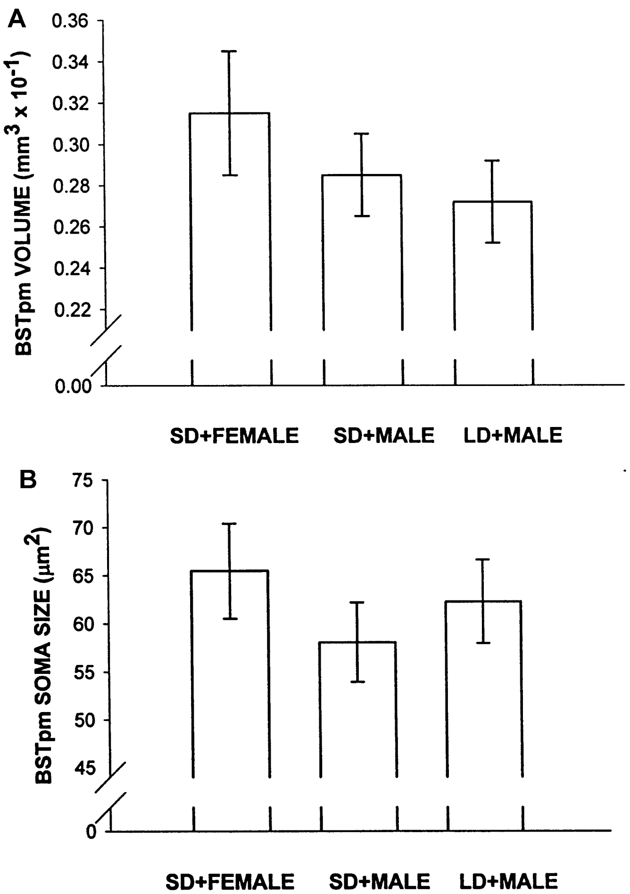

Fig. 2. Mean ± SEM volume (A) and soma size (B) of the BSTpm in male hamsters from different social and photoperiodic conditions. No effects of photoperiod or social environment were detected.

previously observed MePD soma size to be modulated by circulating androgens in the rat [7]. The greatly diminished testes weights in the SD + male hamsters, compared to LD + male hamsters, suggest that they experienced winter-like levels of circulating androgen, as would be expected from previous reports. Reduced androgen in these animals may account for the observed photoperiodic effect on MePD somata.

The female cagemate caused male hamsters to ignore winter-like photoperiods and remain reproductively active, as indicated by dark pelage color, summer-like testes weights and the presence of litters [12]. MePD volume was also significantly greater in SD + female males than in SD + male males and was statistically indistinguishable from that of LD + male hamsters. Because MePD volume in rats is sensitive to circulating androgens [7], the present results are likely due to social cues causing the SD + female males to retain high androgen levels, which then influenced MePD volume. But it is also possible that socio-sexual behavior may have influenced MePD volume independently of androgen.

The apparent dissociation of MePD volume and soma size (volume affected by social stimuli but not photoperiod, while soma size was affected by photoperiod but not social stimuli) may simply reflect Type II statistical error, as the non-significant differences were in the expected direction and approached significance. On the other hand, we have seen dissociation of those two measures in other studies [6].

Contrary to expectation, the BSTpm did not display any of the effects observed in the MePD, although the pattern of average soma size resembled that seen in the MePD. We hypothesized that both subnuclei would respond similarly to our manipulations because they both contain gonadal steroid receptors in the adult rat [17], are reciprocally connected in the Syrian hamster [8] and because Alheid and others have argued that the BST and MeA belong to the 'medial extended amygdala', a unitary structure separated during ontogeny by the internal capsule [1]. Our results suggest that, despite their interconnectedness and ontogenetic commonality, the BSTpm and MePD in the Siberian

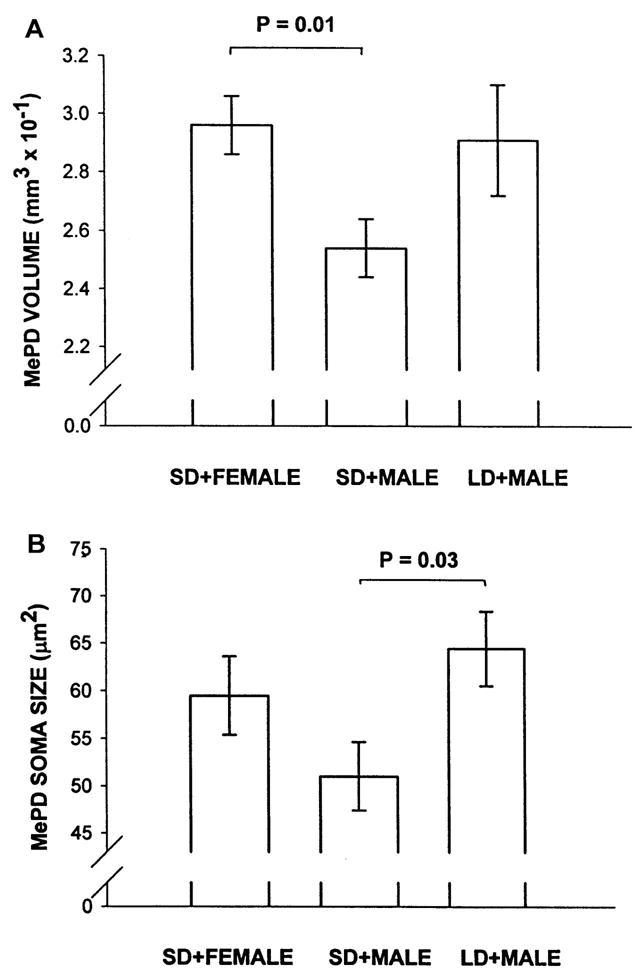

Fig. 3. Mean ± SEM volumes (A) and soma sizes (B) of the MePD in male Siberian hamsters. MePD volume displayed an effect of social environment (SD + female vs. SD + male, $**P = 0.01$), but not of photoperiod (LD + male vs. SD + male, $P = 0.06$). MePD somata showed an effect of photoperiod (SD + male vs. LD + male, $*P = 0.03$), but not of social condition (SD + female vs. SD + male, $P = 0.07$).

hamster respond differently to manipulations of photoperiod and the social environment.

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