Research report

Pubertal and seasonal plasticity in the amygdala

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Abstract

The present experiments investigated the effects of pubertal maturation and photoperiod on the size of brain regions that mediate mating behavior in the male Syrian hamster. We hypothesized that the low levels of reproductive behavior exhibited by prepubertal and photoinhibited males would be correlated with morphological changes in the neural circuit that mediates mating behavior. We found that the Nissl-stained cross-sectional area of the posterodorsal subdivision of the medial amygdala was significantly smaller in prepubertal and photoinhibited males compared to photostimulated adult males. These differences appear to be caused by a decrease in somal size of individual cells in the ventral aspect of this nucleus. We also found that prepubertal males have a larger anterior subdivision of the medial amygdala (MeA) compared to adults. This difference in the MeA does not appear to be caused by alteration in somal size since somal size did not differ significantly between juveniles and adults. It is concluded that the neural circuit that mediates male mating behavior in this species is capable of significant morphological plasticity during both pubertal development and in adulthood. Furthermore, these alterations may reflect underlying mechanisms of the deficits in sexual behavior exhibited by prepubertal and photoinhibited males.

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Topic: Hormones and development

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1. Introduction

Mating behavior in the male Syrian hamster is dependent upon both circulating steroid hormones and pheromonal cues from the estrous female [42]. These two signals are processed in a steroid-sensitive neural circuit that has been well defined and functionally characterized in the adult male [22,23,28,38,39,41]. How this neural circuitry may change with reproductive status is less understood. Prepubertal males and adults that have been exposed to short photoperiods (i.e., less than 12 h of light per day) engage in little, if any, mating behavior [4,24–27], whereas photostimulated adult males (i.e., housed in day lengths exceeding 12 h) normally engage in sexual behavior whenever they are presented with a receptive female.

These different behavioral phenotypes imply plasticity within the neural circuit mediating mating behavior during pubertal development and with changes in environmental photoperiod.

Both prepubertal and photoinhibited adult hamsters have relatively low levels of circulating androgen [1,24,26,37]. Across several mammalian species, removal of gonadal hormones in adult males results in a decrease in somal size of individual cells and/or the overall volume of nuclei essential for male reproductive behavior, including the medial nucleus of the amygdala [7,18] and the sexually dimorphic nucleus of the medial preoptic area [2,6,35]. Therefore, we predicted that the low testosterone levels characteristic of the prepubertal and photoinhibited hamster would be associated with a decrease in size of hypothalamic and amygdaloid nuclei within the neural circuit that mediates mating behavior. We provide evidence here that cell groups within the amygdala do undergo significant morphological change during normal life events.
such as pubertal development and seasonal (e.g., photoperiodic) transitions.

2. Materials and methods

2.1. Experimental animals

Twenty-four male Syrian hamsters (*Mesocricetus auratus*) bred at Michigan State University (East Lansing, MI, USA) were used in these experiments. All animals were housed in clear polycarbonate cages (37.5×33×17 cm) with ad libitum access to food (Teklad Rodent Diet No. 8640, Harlan, Madison, WI, USA) and water. Photoperiod in the vivaria was adjusted depending on the experiment (see below) and room temperature was maintained at 21±2°C. All animals were treated in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and the protocols were approved by the Michigan State University All-University Committee for Animal Use and Care.

2.2. Experimental design

2.2.1. Experiment 1: puberty

Twelve male Syrian hamsters were weaned from their mothers at 21 days of age and singly housed. The vivarium was on a 14 h light/10 h dark light–dark schedule (lights on at 12:00 h EST). At 28 days of age (pubertal, *n*=6) or 49 days of age (adult, *n*=6), animals were weighed, given an overdose of sodium pentobarbital (130 mg/kg, i.p.) and a blood sample was collected. After expression of the seminal fluid, seminal vesicle and paired testis weights were recorded. Immediately after tissues were collected, animals were intracardially perfused with 100 ml of phosphate-buffered saline (PBS) rinse followed by 150 ml of 4% paraformaldehyde. Brains were then postfixed in 4% paraformaldehyde for 1 h and then transferred to a 20% sucrose solution in PBS. Forty-eight hours later, 40-μm coronal sections were made on a cryostat and stored at −20°C in cryoprotectant.

2.2.2. Experiment 2: photoperiod

Twelve male Syrian hamsters were weaned from their mothers at 21 days of age and housed in triads. The vivarium was on a 14 h light/10 h dark light–dark schedule (long days; lights on at 12:00 h EST). At 56 days of age, six animals were maintained on the long photoperiod and six animals were transferred to a short photoperiod in which animals experienced an 8 h light/16 dark light–dark schedule (lights on at 09:00 h EST). At 16 weeks of age, all animals were weighed and given an overdose of sodium pentobarbital, after which tissues were collected and brains were cut in the same manner as described for Experiment 1.

2.3. Histology

Brain sections were rinsed in PBS to remove the cryoprotectant, mounted on gelatin coated slides, and stained with thionin. The sections were cleared in xylenes and coverslipped with Permount. Slides were coded so that the experimenter was blind to age and photoperiod conditions during slide selection and microscopic analysis.

2.4. Microscopy

The medial preoptic nucleus (MPN, Fig. 1A), the postero medial subdivision of the bed nucleus of the stria terminalis (BNSTpm, Fig. 1B), the magnocellular region of the medial preoptic nucleus (MPNmag, Fig. 1B), the anterior subdivision of the medial amygdala (MeA, Fig. 1C), and the posterodorsal subdivision of the medial amygdala (MePD, Fig. 1D) were selected for analysis because they are components of the circuit that mediates male reproductive behavior in the Syrian hamster [38,41]. All areas are readily identifiable in Nissl-stained sections. For each brain region, the cross-sectional area was mea-
sured bilaterally in two tissue sections, which were anatomically matched across animals. For the MPN, MeA, and MePD, analyzed sections were separated by 160 μm. Because of the relatively short rostral to caudal extent of the BNSTpm and MPNmag, the two sections used for measurement of these nuclei were separated by 80 μm.

Cross-sectional area of a nucleus was obtained by capturing the area of interest under a 4× objective and 10× eyepiece with a Sony XC-77 video camera and displaying the image on a monitor using NIH Image 1.56 software. The nucleus was outlined on the screen, and the area (μm²) was computed by NIH Image.

Brain regions that were significantly affected by puberty or photoperiod were further investigated by measuring areal cell density (cells per unit area) and the somal area of individual cells contained within those regions. In the MePD, we obtained separate areal density and somal area measurements in the dorsal and ventral aspects of this nucleus since the MePD is relatively large, while the MeA was not further subdivided since its absolute size and cell number are much smaller than those of the MePD.

Cell density and somal area measurements of individual cells were made in the same brain sections that were analyzed for cross-sectional area of the brain region. Areal cell density was determined by centering the brain areas under a 10× objective, and then increasing the magnification to 40×. Cells that appeared within the area (15 625 μm²) of a 125 × 125 μm ocular grid centered in the eyepiece were tallied. All data are expressed as the mean number of Nissl-stained cells/15 625 μm². Somal area of individual cells was measured by centering the brain region of interest under a 4× objective and then increasing the magnification by changing to the 40× objective. On each side, 20 cells within the brain region were randomly chosen for analysis. To be used for somal area measurements, cells had to have a clearly defined nucleus and could not be overlapping any other cell. Cells were then outlined and the area (μm²) was computed by NIH Image.

2.5. Radioimmunoassay

Plasma testosterone concentrations were measured using the Coat-A-Count Total Testosterone Kit (Diagnostic Products, Los Angeles, CA, USA). This assay has been previously validated in our laboratory for the measurement of plasma testosterone concentrations in Syrian hamsters [30]. In Experiment 1, the lower limit of detectability of the assay was 0.2 ng/ml and the intra-assay coefficient of variation (CV) was 11.9%. In Experiment 2, the lower limit of detectability of the assay was 0.1 ng/ml and the intra-assay CV was 7%.

2.6. Statistical analyses

Within an experiment, tissue weights, hormone levels, cell counts, and area measurements were analyzed by t-tests. Differences were considered significant if \( P<0.05 \). All data are presented as mean±standard error of the mean (S.E.M.).

3. Results

3.1. Experiment 1: puberty

Adult males had heavier body, paired testis, and seminal vesicle weights and higher circulating levels of testosterone compared to the prepubertal males \( (P<0.05, \text{Table 1}) \).

The mean cross-sectional area of the MePD was significantly larger in adult males compared to prepubertal males \( (P<0.05, \text{Figs. 2 and 3}) \). Further investigation of the MePD revealed that cell density was equivalent in prepubertal and adult males in both the dorsal and ventral aspects of this nucleus \( (\text{Table 2}) \). However, mean somal area was greater in the ventral, but not dorsal, aspect of the MePD in adults compared to prepubertal animals \( (P<0.05, \text{Table 3}) \).

In contrast to the MePD, the mean cross-sectional area of the MeA was significantly larger in prepubertal males compared to adult males \( (P<0.05, \text{Fig. 2 and Fig. 4A and B}) \). The cell density and mean somal area of the MeA were not different between prepubertal and adult males \( (\text{Tables 2 and 3}) \). There were no significant differences in the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mean±S.E.M. values for body, paired testis, and seminal vesicle weights and plasma testosterone concentrations ([T])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>Body weight (g)</td>
</tr>
<tr>
<td>Prepubertal</td>
<td>57.6±1.3</td>
</tr>
<tr>
<td>Adult</td>
<td>115.3±4.6*</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
</tr>
<tr>
<td>Short day</td>
<td>152.0±6.4</td>
</tr>
<tr>
<td>Long day</td>
<td>129.1±8.0</td>
</tr>
</tbody>
</table>

\* \( P<0.05 \).
cross-sectional areas of the MPN, BNSTpm, or MPNmag between juveniles and adults.

3.2. Experiment 2: photoperiod

Animals housed under a long photoperiod had significantly heavier paired testis and seminal vesicle weights and significantly higher circulating levels of plasma testosterone compared to the males housed in a short photoperiod ($P<0.05$, Table 1).

Mean cross-sectional area of the MePD was significantly greater in males housed in long days compared with males housed in short days ($P<0.05$, Fig. 5 and Fig. 3). Further investigation of this region revealed that cell density was equivalent in both the dorsal and ventral portions of the MePD of long and short day males (Table 2). Somal area of individual cells was significantly larger in the ventral, but not dorsal, aspect of the MePD of long-day compared with short-day males ($P<0.05$, Table 3). There were no significant differences in the cross-sectional area of the MeA.

### Table 2

<table>
<thead>
<tr>
<th>Region</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prepubertal</td>
<td>Adult</td>
</tr>
<tr>
<td>MePD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorsal</td>
<td>85.1±2.2</td>
<td>82.1±1.6</td>
</tr>
<tr>
<td>Ventral</td>
<td>113.6±3.5</td>
<td>106.9±1.4</td>
</tr>
<tr>
<td>MeA</td>
<td>89.8±2.4</td>
<td>85.3±2.0</td>
</tr>
</tbody>
</table>

* Areal cell densities were not made for the MeA in Experiment 2 because cross-sectional area of this brain region was not significantly affected by photoperiod.
Table 3
Mean±S.E.M. somal area (µm²) for cells in the dorsal and ventral aspects of the MePD and MeA

<table>
<thead>
<tr>
<th>Region</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prepubertal</td>
<td>Adult</td>
</tr>
<tr>
<td>MePD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorsal</td>
<td>52.4±3.0</td>
<td>54.4±1.9</td>
</tr>
<tr>
<td>Ventral</td>
<td>50.1±1.3</td>
<td>55.0±1.8*</td>
</tr>
<tr>
<td>MeA</td>
<td>57.0±2.1</td>
<td>57.9±1.5</td>
</tr>
</tbody>
</table>

*Somal area measurements were not made for the MeA in Experiment 2 because cross-sectional area of this brain region was not significantly affected by photoperiod.

MPN, BNSTpm, MPNmag, or MeA between adult males housed in long or short photoperiods.

4. Discussion

These experiments indicate that specific cell groups within the neural circuit that mediates male mating behavior show significant morphological plasticity during both pubertal development and with change in day length in adulthood. Specifically, the cross-sectional area of the MePD is larger in photostimulated adults compared to either prepubertal or photoinhibited males, while prepubertal males have a larger MeA than adults. Thus, ontogenetic morphological changes are brain region specific and not always in the same direction as changes in testosterone secretion. The MePD and MeA are not only steroid-sensitive [40,41,43], but also process the femalepheromonal cues integral for the initiation of sexual behavior in this species [10,39,42]. Morphological change in these nuclei likely reflects the neural mechanisms underlying the lack of mating behavior observed in prepubertal and photoinhibited males [3,24,27].

The smaller cross-sectional area of the MePD in prepubertal and photoinhibited males is due to reduced somal area of cells specifically in the ventral aspect of this nucleus. Change in cell size affects membrane excitability [19,36] and may also reflect a difference in protein synthesis. Thus, the 10% decrease in somal area of cells in the ventral portion of MePD in reproductively quiescent males implies functional differences within the MePD that are related to reproductive status. The MePD of testosterone-treated, gonadectomized adult hamsters and rats is larger than in respective untreated controls [7,18]. Therefore, it seems likely that the decreased levels of testosterone in prepubertal and photoinhibited males results in a decrease in somal area of cells in the ventral MePD and an overall decrease in the cross-sectional area of the MePD.

The larger cross-sectional area of the MeA in prepubertal compared to adult males was a surprising finding, and does not conform to predictions derived from the assumption that testosterone is trophic for neurons related to reproductive behavior. Furthermore, cross-sectional area of the MeA changed significantly with puberty, but not with photoperiod manipulation in adulthood. Thus, even if the pubertal decrease in MeA size were associated with the rising levels of testosterone, this change is apparently not reversible by a photoperiod-induced decline in testosterone.

Fig. 5. Mean cross-sectional area of the MPN, BNSTpm, MPNmag, MeA, and MePD in male hamsters housed in short or long day photoperiods. Asterisk indicates a significant difference. All values are mean±S.E.M.

Fig. 4. Photomicrograph of the MeA in a prepubertal (A) and adult (B) male hamster. Arrowheads outline the outer edge of the MeA. Bar, 200 µm. Abbreviations: ot, optic tract.
secretion. Perhaps the MeA is organized by either steroid-dependent or -independent processes during puberty, after which time structural features that affect the size of the MeA are not modified by photoperiod or gonadal steroids. In fact, castration of adult hamsters does not significantly affect somal size, dendritic length, or dendritic branching of MeA neurons [18]. It would be interesting to determine whether these parameters of MeA neurons could be influenced by steroids prior to or during pubertal development. The pubertal decrease in MeA cross-sectional area is not due to significant changes in either somal size or cell density. We cannot rule out the possibility that changes in dendritic length or branching or glial morphology, all of which can be modified by steroid hormones [5,9,13,18], underlie the pubertal decrease in size of the MeA.

The brain-region specificity of the structural plasticity observed in these experiments provides interesting clues about the nature of ontogenetic change particular to aspects of reproductive behavior. We observed changes in cross-sectional area and/or somal size only in the amygdala, and within the amygdala, the MeA changed only with puberty, whereas the pubertal increase in the size of MePD neurons was reversible in adulthood by exposure to short days. The MeA and MePD subserve different functions in male reproductive behavior. First, MeA receives a substantial direct projection from the accessory olfactory bulb, while the chemosensory projection to the MePD is relatively sparse [8,17]. Conversely, androgen- and estrogen-receptive cells are more numerous within the MePD than MeA [41]. Selective lesions of the MeA completely abolish chemoinvestigatory behavior, but this component of mating behavior is only modestly affected by lesions of the MePD [29]. We have shown that female pheromones elicit an increase in testosterone secretion only after puberty [32]. Thus, pubertal development may involve changes within the MeA that permit appropriate processing and integration of female chemosensory cues, which then remains fixed throughout adulthood. In contrast, cells in the MePD, which is reciprocally connected with the MeA [8], may retain the capacity for plasticity throughout life so that expression of reproductive behavior is possible only under certain steroidal and/or environmental conditions.

We found no significant effects of puberty or photoperiod on the cross-sectional area of the MPN, BNSTpm, or MPNmag. However, it should be noted that the absence of a change in cross-sectional area cannot rule out the possibility that these brain regions may vary in three-dimensional volume with puberty or photoperiodic conditions. With this caveat in mind, these results do underscore two important points. First, morphological plasticity within the behavioral neural circuit is cell group specific, indicating that components of the circuit are differentially affected by endogenous and exogenous agents. Second, somatic growth does not account for increases in the size of MePD with pubertal development since a global increase in the size of all cell groups in adults did not occur.

Puberty and photoperiod affect other cell groups related to reproductive behavior. For instance, the motoneurons of the spinal nucleus of the bulbocavernosus, which innervate the muscles that are attached to the base of the penis and are integral for successful reproduction [33], are affected by puberty in rats [16] and photoperiod in white-footed mice [11] and Siberian hamsters [20,21], such that prepubertal and photoinhibited males have smaller SNB somata than photostimulated adults. The lower amount of androgenic stimulation experienced by prepubertal and photoinhibited males most likely underlies these morphological changes since castration of adults decreases SNB somal size [11,12,16]. Taken together with the present set of experiments, these data indicate that the lack of reproductive behavior exhibited by prepubertal and photoinhibited males is the result of structural changes in the behavioral circuit at the level of both the spinal cord and amygdala.

In sum, this set of experiments shows that the neural circuit that mediates male mating behavior is capable of significant morphological plasticity during both pubertal development and in adulthood. These changes may reflect underlying cellular processes related to the absence of mating behavior in prepubertal and photoinhibited males. It is likely that these morphological changes are brought about by the different hormonal milieus of animals in different stages of reproductive development or status. The smaller MePD observed in photoinhibited adults indicates that the adult mammalian brain is capable of undergoing significant morphological changes from season to season. The plasticity observed in the MePD and MeA during pubertal development suggests that puberty is a period of neural development in which structural alterations in the central nervous system, presumably reflecting alterations in cellular physiology and function, result in maturation of adult behaviors. Indeed, recent studies provide evidence that the human brain undergoes major morphological plasticity during puberty that is correlated with behavioral and psychological changes exhibited during adolescence [14,15,31,34].

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References


