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Partial Demasculinization of Several Brain Regions in Adult Male (XY) Rats with a Dysfunctional Androgen Receptor Gene

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ABSTRACT

The adult rat posterodorsal medial amygdala (MePD) is sexually dimorphic in regional volume and neuronal soma size, both of which are larger in males than in females. This sexual dimorphism is entirely dependent on adult circulating levels of testicular androgens, and both androgen and estrogen treatment can masculinize MePD structure. We examined male rats that are rendered androgen-insensitive by the testicular feminization mutation (*tfm*) of the androgen receptor (AR) gene to determine how a dysfunctional AR affects this and other brain sexual dimorphisms. In adult wild-type rats, the MePD in males had a greater regional volume, rostrocaudal extent, and soma size than in females. In genetic males, defective ARs affected some but not all of these indices: MePD volume and soma size in *tfm* males were intermediate between those of wild-type males and females, but the rostrocaudal extent of the MePD was unaffected by the mutation, being as great in *tfm* males as in wild-type males. Regional volume and soma size in the suprachiasmatic nucleus was reduced in *tfm* males compared with wild-type males, suggesting that AR normally affects this region in male rats. Interestingly, whereas volume of the sexually dimorphic nucleus of the preoptic area was unaffected by the *tfm* allele, soma size in this region was reduced in *tfm* males compared with wild-type males. Although estrogen receptor activation has been shown to be vital for masculinization of the rodent brain, our results indicate that ARs also contribute to this process in several brain regions. J. Comp. Neurol. 487:217–226, 2005.

Indexing terms: medial amygdala; sexually dimorphic nucleus of the preoptic area; suprachiasmatic nucleus; androgen insensitivity; sexual differentiation; *tfm*

Mammals exhibit sex differences in behaviors and brain morphology that are influenced by gonadal hormones during development and adulthood. For example, the medial amygdala is a sexually dimorphic brain area implicated in the display of male sexual behaviors (Meisel and Sachs, 1994). Both the morphology of this region and the behaviors associated with it are influenced by gonadal hormones. In adult rats, the posterodorsal aspect of the medial amygdala (MePD) is sexually dimorphic and depends on sex differences in circulating *adult* androgens (Cooke et al., 1999). As in other mammalian brain regions such as the sexually dimorphic nucleus of the preoptic area (SDN-POA), the encapsulated region of the bed nucleus of the stria terminalis, and the bed nucleus of the accessory olfactory tract (Segovia and Guillamon, 1993), the volume of the adult MePD is larger in males than in females. However, only in the MePD can the sex difference be eliminated by adult hormone manipulations.

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Current evidence suggests that gonadal hormones affect the adult morphology of the MePD by acting via androgen receptors (ARs) and/or estrogen receptors (ERs). The presence of AR and ER mRNA (McAbee and DonCarlos, 1998; Shughrue et al., 1997) and protein (Shughrue and Merchenthaler, 2001; Roselli, 1991; Li et al., 1997; Greco et al., 1998a) in neurons of the MePD suggests that these neurons may be directly influenced by these hormones. Estrogen levels in the MePD are also likely to be higher than in other areas, because aromatase activity in the medial amygdala as a whole is among the highest in the rat brain (Roselli et al., 1985). These findings are consistent with the idea that testosterone can either act as an androgen and/or be aromatized to an estrogen to influence MePD morphology.

A recent study treating castrated rats with estradiol (E2) or the nonaromatizable androgen dihydrotestosterone (DHT) indicates that *both* androgenic and estrogenic metabolites of testosterone influence the adult morphology of the MePD. Systemic treatment with DHT in adult castrated males maintains MePD soma size but not volume, whereas E2 treatment maintains both measures, suggesting that gonadal hormones may act via ER *and* AR to influence MePD morphology. These results also suggest that other factors besides soma size, such as neuron number or dendritic branching, contribute to volume differences, because increases in MePD soma size after DHT treatment did not lead to increases in volume (Cooke et al., 2003).

To address further the role of endogenous steroids in the sexually dimorphic MePD, we studied male rats with the testicular feminization mutation (*tfm*) of the AR gene, which renders them insensitive to androgens. *tfm* male rats have above normal levels of circulating testosterone (Roselli et al., 1987), but because they have a mutation in the AR gene involving a single nucleotide substitution (Yarbrough et al., 1990), only 10–15% of the AR protein binds androgen (Naess et al., 1976). As *tfm* rodents have decreased AR binding but apparently normal ER binding (Attardi et al., 1976), these animals offer a way to examine the contribution of the AR to adult MePD morphology in gonadally intact genetic males. Distribution of ER subtypes have not been studied in *tfm* rodents, so effects mediated by ER alpha or beta cannot be distinguished by this model.

We compared regional volume and soma size in the MePD of adult *tfm* males with that of wild-type male and female littermates. We also examined the SDN-POA of these animals, which is thought to be masculinized through activation of ER during a perinatal sensitive period (Dohler et al., 1984). If masculinization of the SDN-POA requires ER but not AR stimulation, then *tfm* males would be expected to display a masculine SDN-POA, as suggested by Gorski and Jacobsen (Gorski et al., 1981). Finally, we examined the suprachiasmatic nucleus (SCN) in these animals as a control nucleus in which morphology was not expected to depend on either AR or ER activation. We found evidence that a defective AR gene affects the morphology of all three brain regions.

MATERIALS AND METHODS

Animals

Littermates (n = 15/group) of wild-type male, female (*tfm* carriers and noncarriers), and affected male *tfm* rats from our colony at Michigan State University, aged 85–95 days, were used. These animals approximate a Long-Evans strain, as female carriers of the mutation have been crossed with commercially supplied Long-Evans males for over 10 generations. After weaning, animals were housed three to a cage (males, *tfm*s, and females separately) in standard rat cages with food and water freely available. Lights were turned off at 1900 hours and on at 0700 hours. Animal care followed standards set by the National Institutes of Health and were approved by the institutional animal care and use committee at Michigan State University.

On the day of sacrifice, animals were injected IP with an overdose of sodium pentobarbital (120 mg/kg). Deep anesthesia was noted by lack of reflexes to tail and foot pinch as well as lack of a corneal reflex. Animals were then perfused transcardially with 0.9% saline, followed by 10% neutral buffered formalin (~300 mL/animal). Phenotype was confirmed at sacrifice by examining external genitalia and the gonads. (Adult *tfm* males have small, undescended testes and a blind vagina.) Brains were removed and placed into the same fixative solution. Body weights and anogenital distance were measured before sacrifice.

Histology

After at least 1 month fixation in formalin, the brains were placed overnight in 20% phosphate-buffered sucrose (pH 7.4) at 4°C prior to slicing. Each brain was scored along the left cortex to mark laterality, blocked at the cerebellum and olfactory tubercle, and coronally sliced on a freezing sliding microtome set to 40 μm. Sections were collected into a phosphate buffer (0.1 M PO_4, 0.1% gelatin, 0.3% Triton; pH 7.4); every third section was mounted onto gel-subbed glass slides, with a random start from the first series of each brain to ensure that every section had an equal probability of being chosen for sampling. Missing sections due to damage were replaced by one of the two remaining sections within that interval, as determined by a coin flip, or a space was left on the slide. Mounted tissue was allowed to air-dry, stained with thionin for Nissl substance, and coverslipped with Permount.

Analysis

An investigator, blind to group status, measured the regional volume of the MePD, SDN-POA, and SCN on both sides of the brain. MePD boundaries were determined following nomenclature from a standard rat atlas (Paxinos and Watson, 1998) using criteria from Hines et al. (1992) and Canteras et al. (1995). The MePD is located in the medialmost aspect of amygdala, and abuts the ventrolateral margin of the optic tract (Fig. 1). The MePD contains larger and more darkly stained cells with higher packing density than the surrounding relatively cell-sparse area. The following landmarks indicated that the caudalmost aspect of the MePD occupied the same rostrocaudal level of the brain in all three groups of animals: size and shape of the lateral ventricle, position and shape of the optic tract with respect to the MePD, and size and shape of the stria terminalis (Fig. 1). Moreover, the caudalmost end of the MePD shows a distinct encapsulation by the stria terminalis and therefore was chosen as the anchor point in all measurements. Boundaries of the intensely staining cells of the SDN-POA were separate from lighter surrounding areas as delineated by Bloch and Gor-

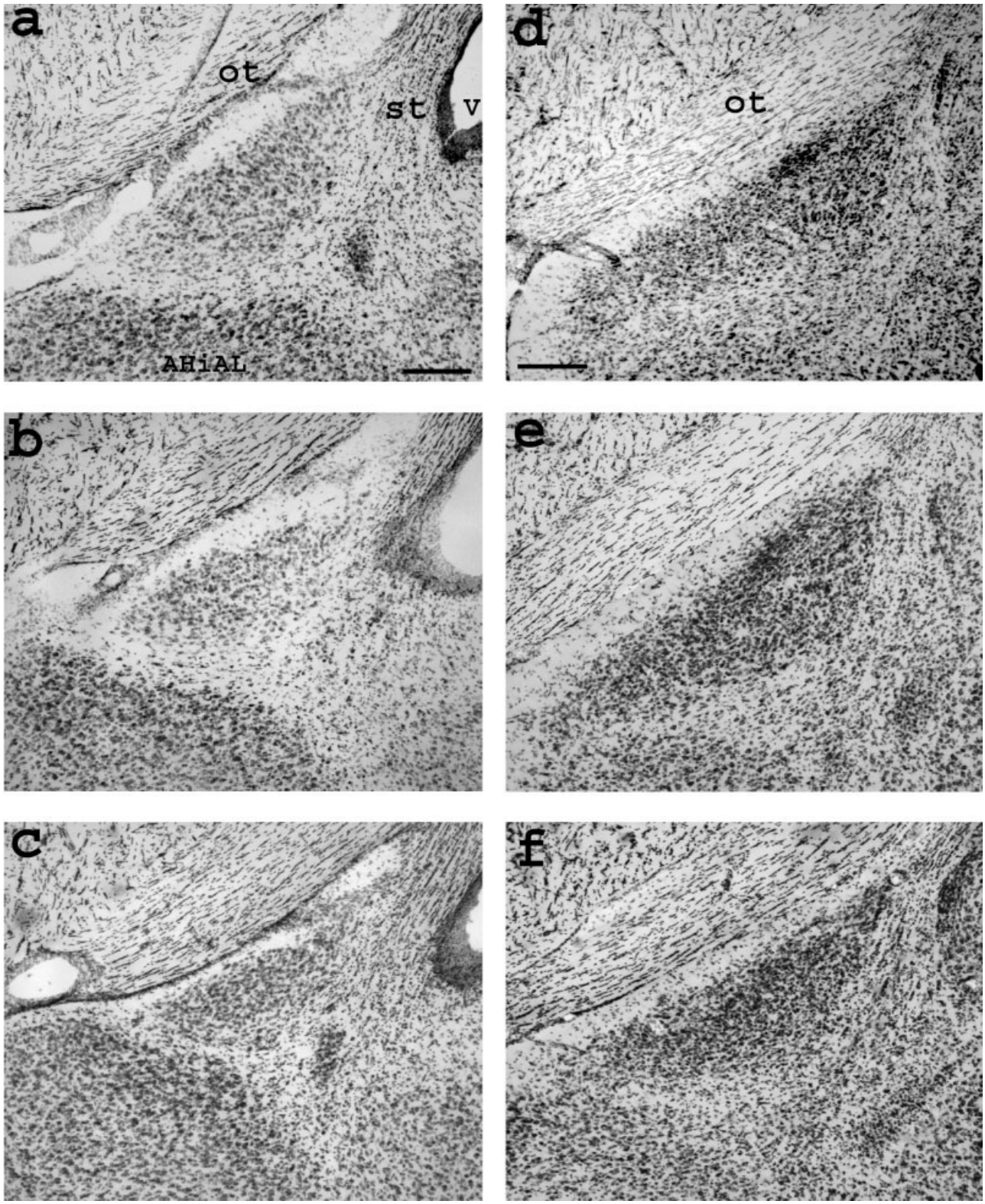
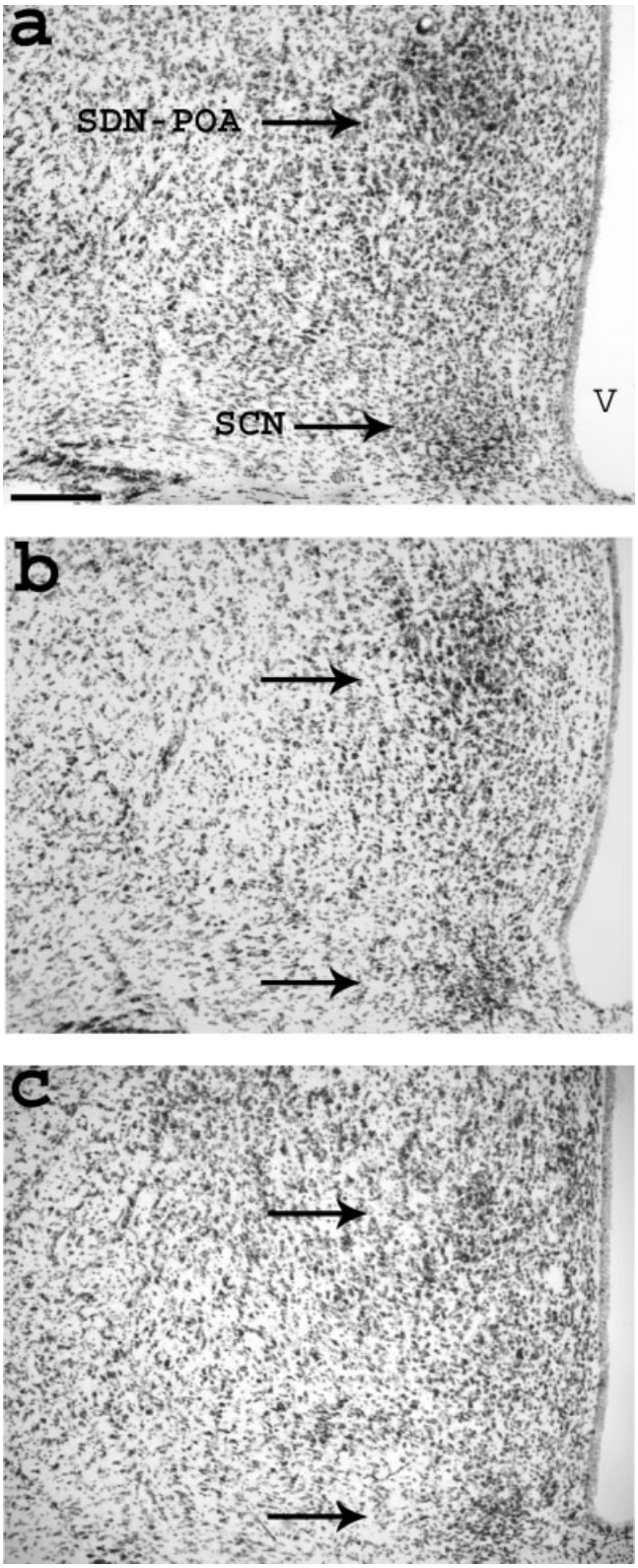


Fig. 1. The posterodorsal medial amygdala (MePD) as revealed by Nissl stain of coronal sections from a wild-type male (top), a *tfm* male with a dysfunctional androgen receptor (middle), and a female rat (bottom). The panels on the left are from the caudalmost appearance of the MePD, which served as an anchor point to assess changes in the nucleus across the rostrocaudal dimension. The appearance of the MePD, as well as the optic tract (ot), the stria terminalis (st), the anterolateral part of the amygdalohippocampal transition area (AHiAL), and the lateral ventricle (v) are equivalent in wild-type males (**a**), *tfm* males (**b**), and females (**c**), indicating that the caudal termination of the MePD occurs in the homologous region of the brain across groups. The panels on the right are from the approximate middle of the rostrocaudal extent of the MePD where the nucleus is larger in wild-type males (**d**) than in females (**f**) and is intermediate in size in genetic males with a dysfunctional androgen receptor (**e**). The MePD extends farther rostrally in wild-type males and *tfm* males than in females (see text). Scale bar = 250 μm in a (applies to a–c), d (applies to d–f).

ski (1988a), and similarly the SCN highly contrasted with the surrounding areas (Fig. 2), as delineated in Paxinos and Watson (1998).



StereoInvestigator (Microbrightfield, Colchester, VT) software was used to estimate the volume of the MePD, SDN-POA, and SCN and the average neuronal soma size within each brain region of both sides of the brain. A digital camera captured at 50× an image containing the area of interest from a Zeiss (Axioplan) compound microscope, and a computer mouse was used to trace the perimeter of the area of interest on a computer monitor in successive sections throughout the rostrocaudal axis. Total volume for each nucleus in each hemisphere was calculated taking into account sampling ratio (one of every three sections) and section thickness (40μm). Data from animals with damaged or missing tissue in the region of interest were excluded from the analysis. To estimate rostrocaudal extent, the number of sections used for volume estimates were counted. For soma size estimates, neurons were randomly selected by the StereoInvestigator software, which positioned points within the traced sections without bias for location or appearance so that an investigator could trace the soma of the nearest neuron. An average of 5 neuronal somata from each section was measured throughout the MePD (sampling 25–55 neurons/side), SDN-POA (15–30 neurons/side), and SCN (20 neurons/side) from each hemisphere, traced at 630×. Neuronal somata measures were averaged within each hemisphere yielding a mean soma size per hemisphere for each animal. Neurons were identified by the presence of a distinct Nissl-stained cytoplasm and nucleolus.

Statistical analysis

Body weight and anogenital distance were each analyzed by one-way ANOVA with Fisher's PLSD post hoc tests to determine which groups of animals differed significantly from one another. For each brain measure, groups were compared using a mixed-design 2 × 2 ANOVA with genotype (male, female, or *tfm*) as an independent variable, and hemisphere (left, right) as a repeated measure. When there was no significant effect of hemisphere nor a significant interaction between genotype and hemisphere, the means for each hemisphere were collapsed, and one-way ANOVAs were conducted to determine which genotypes differed from one another. If there was a significant main effect of laterality, or an interaction of laterality and genotype, we conducted matched-pairs t-tests within each genotype to ask which groups displayed a significant hemispheric asymmetry. If there were significant main effects of both genotype and hemisphere, but no interaction between the two, we conducted separate one-way ANOVAs on each hemisphere to determine which genotypes were significantly different from one another in each hemisphere. For each analysis, Fisher's PLSD post hoc tests were used to determine

Fig. 2. The volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) as revealed by Nissl-stained coronal sections is larger in a wild-type male (**a**) than in a wild-type female rat (**c**). SDN-POA volume in a *tfm* rat, a genetic male with a dysfunctional androgen receptor (**b**), is masculine: larger than in females and equivalent to that of males. The sexual dimorphism in the volume of the suprachiasmatic nucleus (SCN), although more subtle than in the SDN-POA, again reflects a larger nucleus in wild-type males (top) than in females (bottom), but the SCN volume in *tfm* males (middle) is feminine: less than that of wild-type males and equivalent to that of females. Scale bar = 250 μm in a (applies to a–c).

TABLE 1. Body Weight and Anogenital Distance of Subjects[1]

Subject	Body weight (g)	AGD (mm)
Males (n = 18)	489.4 (±11.7)	42.6 (±0.6)
TFMs (n = 16)	368.8 (±12.9)	17.8 (±0.7)
Females (n = 19)	287.6 (±5.9)	13.1 (±0.4)

[1]Average body weights and anogenital distance (AGD) for each group, with standard errors of the means in parentheses. For each measure, all groups are significantly different from each other (one-way ANOVA for each measure; post hoc tests indicate all $P < 0.0001$).

which groups of animals differed significantly from one another. A two-tailed probability value of 0.05 was used as the significance criterion, with N representing the number of animals in all analyses.

Adobe Photoshop (version 7.0) was used to store and manipulate photographs. No processing of images occurred except for resizing.

RESULTS

The body weights of *tfm* males were significantly heavier than those of females and significantly lighter than those of wild-type males (one-way ANOVA, $P < 0.0001$ for all; Table 1).Similarly, anogenital distance was also intermediate in *tfm* males ($P < 0.0001$ for all) but much closer to that of females than that of wild-type males (Table 1). Several landmarks confirmed that the caudalmost aspect of the MePD arises in the same region of the brain in all three groups. These landmarks include the caudalmost occurrence of a prominent stria terminalis, distinctive shape and size of the lateral ventricle, and the abutment of the optic tract on the dorsomedial corner of the MePD (Fig. 1). Furthermore, the size of the MePD was equivalent in this caudalmost section across the three groups. There was more variability across the groups in the rostral extent of the MePD, with the nucleus ending sooner in females than in the other two groups.

MePD

Regional volume. MePD volume in *tfm* males was significantly greater than in females, yet significantly less than in wild-type males, whether considering the left hemisphere, right hemisphere, or the two hemispheres combined (one-way ANOVAs, $P < 0.001$ for all). This meant that there was also a significant sex difference in MePD volume among the wild-type animals. The mean total volume of the MePD in females was 59% of that in wild-type males, comparable to previous reports (65%; Cooke et al, 1999). In wild-type males and *tfm* males, MePD volume was asymmetrical, with the right hemisphere volume greater than the left ($P < 0.03$ for both, matched-pairs t-tests), but there was no asymmetry in MePD volume in females ($P = 0.55$; Fig. 3).

Soma size. There was no significant laterality of MePD soma size in any of the groups of animals ($P > 0.10$ for all, two-way ANOVA), so we used soma size averaged across the two hemispheres to compare the different groups of animals. As with MePD regional volume, soma size of MePD neurons in *tfm* males was significantly larger than in females ($P < 0.005$) yet significantly smaller than in wild-type males ($P < 0.03$; Fig. 3). Among the wild-type males and females there was also a large sex difference in MePD soma size ($P < 0.0001$). Thus, there is a robust sex difference such that wild-type males have larger MePD volumes and soma size than females, with *tfm* males displaying intermediate values that are significantly different from both females and wild-type males.

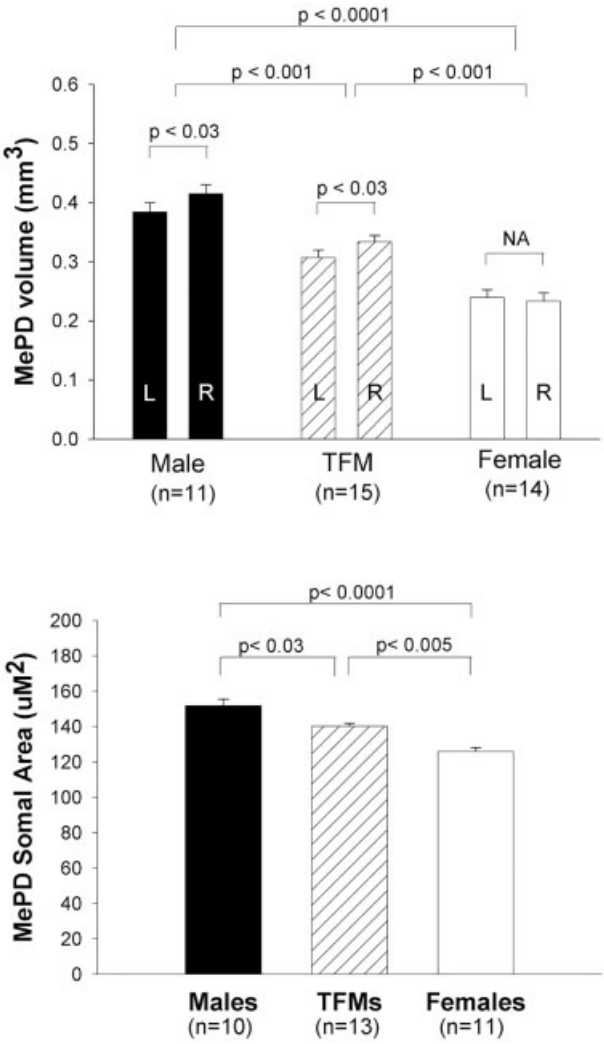


Fig. 3. MePD regional volume (top) and soma size (bottom) in adult *tfm* males and wild-type male and female littermate controls. MePD volume is significantly greater in the right than in the left hemisphere in wild-type males ($P < 0.03$) and androgen-insensitive *tfm* males ($P < 0.03$), but not in females ($P = 0.55$). On each side of the brain, all three groups were significantly different from one another, with values for *tfm*s intermediate between those of males and females ($P < 0.001$ for all). There is also a robust sex difference in MePD soma size ($P < 0.0001$) with wild-type males having larger somata than do females. *tfm* males have significantly smaller somata than wild-type male littermates ($P < 0.03$) but significantly larger somata than female littermates ($P < 0.005$). There was no laterality of MePD soma size in any of the groups. All error bars represent the standard errors of the means (SEM).

Rostrocaudal extent. In both hemispheres, wild-type males had a significantly longer MePD rostrocaudal extent than did females (L: $P < 0.02$; R: $P < 0.0003$; one-way ANOVAs followed by Fisher's PLSD, $P < 0.02$ for all). The *tfm* males were similar to wild-type males on this measure in both hemispheres (L: $P = 0.84$; R: $P = 0.57$) with greater rostrocaudal extents than in females (L: $P < 0.02$;

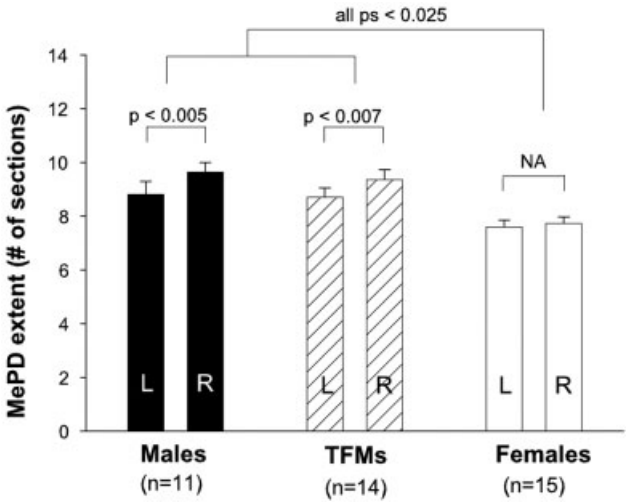


Fig. 4. Mean (± SEM) rostrocaudal extent of the MePD was estimated by counting the number of sections sampled for each animal that contain this region. MePD extent is significantly greater on the right than on the left hemisphere in wild-type males ($P < 0.005$) and androgen-insensitive *tfm* males ($P < 0.007$) but not in females ($P = 0.55$). Wild-type males have a significantly longer extent than did females on each side (L, $P < 0.02$; R, $P < 0.0003$). The *tfm* males are as masculine as wild-type males in this measure.

R: $P < 0.0008$; Fig. 4). As with MePD volume, there was an asymmetry of MePD extent, with the nucleus being longer in the right hemisphere than the left, in wild-type males ($P < 0.005$; matched-pairs t-test) and *tfm* males ($P < 0.007$), but not in females ($P = 0.55$; Fig. 4).

By examining the volume of the MePD at various rostrocaudal levels, we found that MePD volume seemed equivalent across the three groups of animals at the caudalmost region of the nucleus but that differences emerged more rostrally (Fig. 5). Interestingly, wild-type and *tfm* males did not appear to differ in MePD volume at the rostralmost extent of the nucleus, where females displayed no MePD at all.

SDN-POA

Regional volume. There was no significant laterality of SDN-POA volume ($P > 0.60$ for all, two-way ANOVA), so we examined group differences in total regional volume (Fig. 6). Wild-type males had a significantly larger SDN-POA volume than did females ($P < 0.0001$, one-way ANOVA and Fisher's PLSD). The *tfm* males were not significantly different from wild-type males ($P < 0.20$) and, like wild-type males, had a significantly greater volume than did females ($P < 0.0001$; Fig. 2).

Rostrocaudal extent. There was also no laterality of SDN-POA rostrocaudal extent ($P > 0.20$ for all, two-way ANOVA), so we compared mean rostrocaudal extent across the groups of animals. Wild-type males had a significantly longer extent than did females ($P < 0.0001$, one-way ANOVA and Fisher's PLSD). The mean rostrocaudal extent of the SDN-POA of *tfm* males (3.6 ± 0.20) was less than that of wild-type males (4.2 ± 0.28), but this difference was not statistically significant ($P = 0.07$). Rostrocaudal extent of the SDN-POA was greater in *tfm* males than in females (2.3 ± 0.16; $P < 0.003$). In short, as

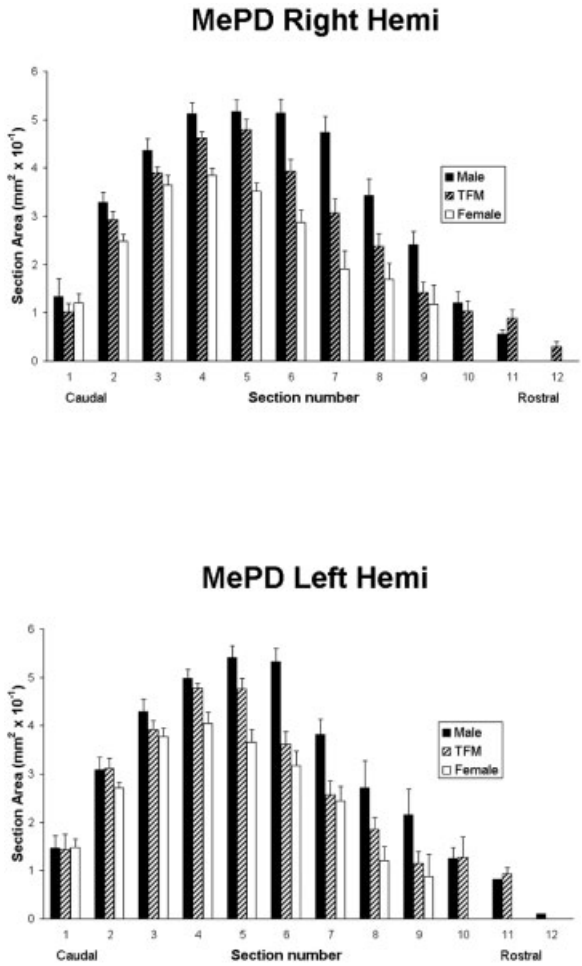


Fig. 5. Rostrocaudal extent of MePD in the right (top) and left hemispheres (bottom) of androgen-insensitive *tfm* males and wild-type male and female littermates binned by average area per section (±SEM). The sex differences in MePD are found primarily in the middle and rostral end of the nucleus. *tfm* males resemble females in some regions, are indistinguishable from males in caudalmost and rostralmost regions, and have volumes intermediate between males and females in others.

with the MePD, it appears that the longer rostrocaudal extent of the SDN-POA that is typical of males does not depend on a functional AR.

Soma size. There was no laterality of SDN-POA soma size ($P > 0.20$ for all), and therefore we used soma size averaged across the two sides to examine group differences. Mean soma size in *tfm* males was smaller than in wild-type males ($P < 0.02$) but not significantly different from females ($P = 0.31$). Comparing wild-type males with females, there was no significant sex difference in this measure ($P = 0.16$, one-way ANOVA and Fisher's PLSD; Fig. 6), although the means of males was greater than that of females. Therefore it is possible that a larger sample size might have detected a sex difference in SDN-POA soma size.

SCN

Volume. There was no significant laterality of SCN volume ($P > 0.05$), so we combined the data across sides.

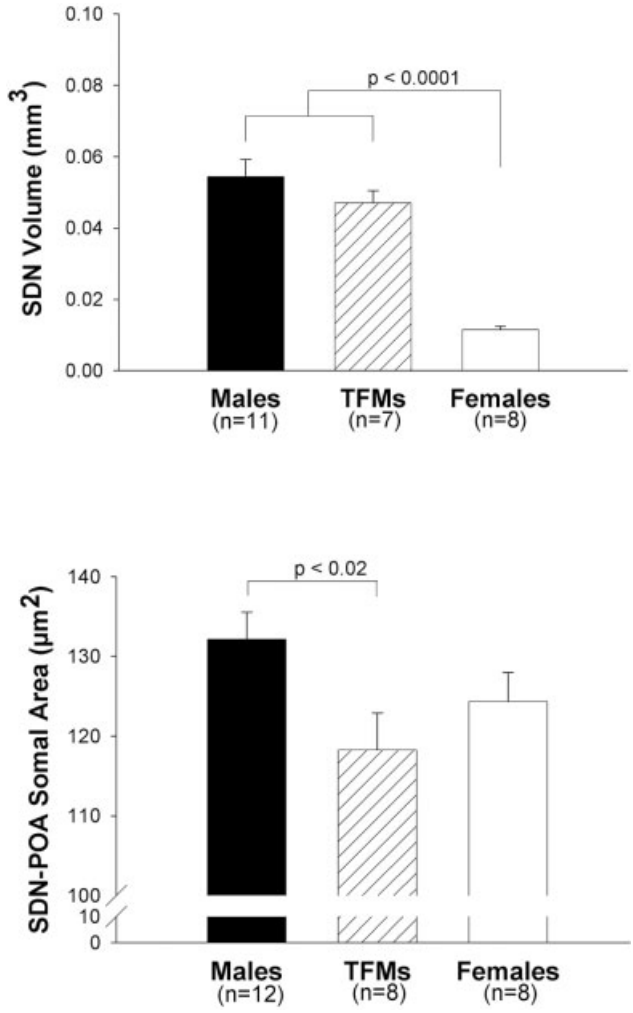


Fig. 6. Total SDN-POA volume (top) and mean cross-sectional area of neuronal somata (bottom) in adult *tfm* males and wild-type male and female littermates. SDN-POA volume is not significantly different between wild-type and *tfm* males, but both groups displayed a greater volume than did females ($P < 0.0001$). In contrast, *tfm* males have smaller somata than wild-type males, indicating a role for AR in maintaining SDN-POA soma size in male rats. However, there is no significant sex difference in the size of SDN-POA somata ($P = 0.16$), nor is soma size in *tfm* males different from that in females ($P = 0.31$).

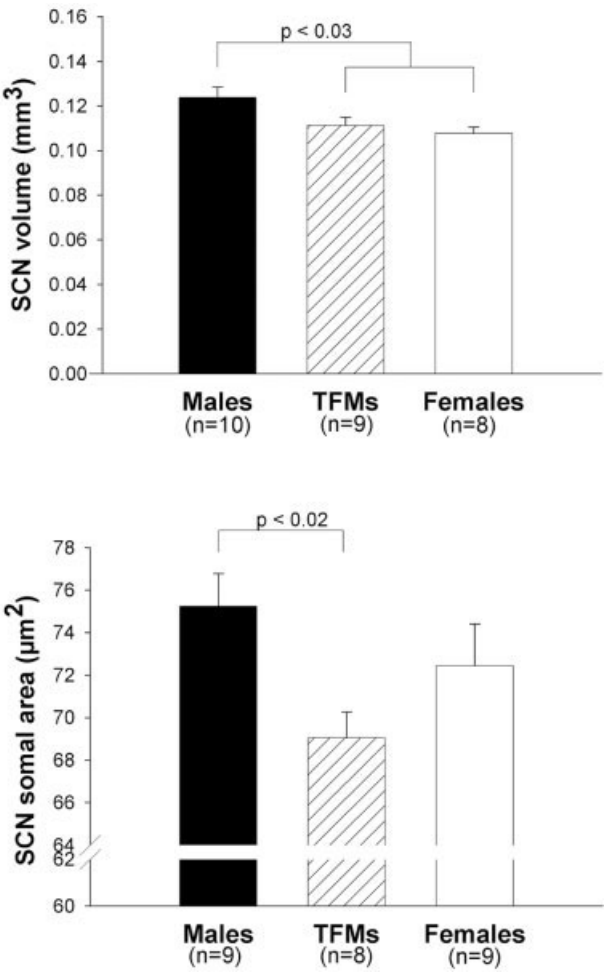


Fig. 7. Total volume (top) and mean cross-sectional area of neuronal somata (bottom) in SCN of androgen-insensitive *tfm* affected males, wild-type males, and female littermates. SCN volume was significantly larger in wild-type males than in control females ($P < 0.01$) with *tfm* males having the same size SCN as female controls ($P = 0.55$). SCN soma area is greater in wild-type males compared with *tfm* males, but there is no significant sex difference in this measure ($P = 0.23$). SCN soma size did not differ between *tfm* males and females ($P = 0.16$).

Wild-type males had a significantly larger SCN volume than did females ($P < 0.01$; Fig. 7). The *tfm* males were similar to female littermates on this measure ($P = 0.55$) and, like female littermates, had a smaller SCN volume than did males ($P < 0.03$).

Rostrocaudal extent and soma size. SCN rostrocaudal extent did not differ across the three groups of animals, nor was there any significant laterality in this measure (data not shown). Whereas there was no significant sex difference in SCN soma size among wild-type males and females ($P > 0.20$), this measure was smaller in *tfm* males than in wild-type males ($P < 0.02$). SCN soma size in *tfm* males did not differ from that found in females ($P = 0.16$). There was no significant laterality of SCN soma size in any group ($P > 0.05$ for all).

DISCUSSION

Although masculinization of the rodent brain is widely assumed to be due to estrogenic metabolites of testosterone interacting with ERs, we found that genetic male rats with a dysfunctional AR were demasculinized to some extent in every nucleus we examined. These results suggest that full masculinization of brain morphology in rats requires activation of ARs. On the other hand, the brains of genetic males with dysfunctional ARs were not entirely feminine in any region examined, which is further evidence that ER activation also plays a role in masculinization of the brain. Taken together, these results indicate that testosterone and its metabolites must activate both ERs and ARs to masculinize the rat brain fully.

The *tfm* male rats have the same or higher circulating testosterone as wild-type males (Roselli et al., 1987), as

might be expected due to lack of negative feedback. Aromatase activity levels remain high in the medial amygdala of *tfm* males, equivalent to that seen in wild-type males. Having higher available testosterone would probably increase the amount of estrogen available for activating ERs in the MePD. If the volume and soma size in the MePD were solely ER dependent, one would expect fully masculine measures, yet the MePD of *tfm* rats was only partially masculinized. The body weights of *tfm* rats were less than that of males, yet the volume of the SDN was not demasculinized, suggesting that these effects were not due to a generalized decrease in brain volume.

We found MePD volume and somata to be greater in males than in females, similar to the results of others (Cooke et al., 1999, 2003; Kerchner et al., 1995; Malsbury and McKay, 1994; Hines et al., 1992). An effect of laterality (R > L hemisphere) in volume was shown by Cooke et al. (2003) in male Long-Evans rats, which we also see in both mutant and wild-type males of the Long-Evans strain, but not in females. In contrast to Cooke et al. (2003), we did not find an effect of laterality on MePD somata in any group. However, we measured somata from throughout the entire MePD, whereas Cooke et al. measured somata from a representative section in the more caudal-medial MePD corresponding to a dense concentration of AR. It may be that somata size in that subregion of the MePD is lateralized in its responsiveness to androgens.

The rostrocaudal extent of the MePD was similar in wild-type and *tfm* males, and both groups of males displayed a greater extent that did females, which indicates that the sex difference in MePD volume reflects differences in both the length and width of the nucleus. The greatest differences between wild-type and *tfm* males in cross-sectional area occurred around the middle third of the nucleus (Fig. 5). However, the region displaying the greatest sex difference in MePD volume was at the rostralmost extent, where *tfm* and wild-type males are equivalent, which suggests that ARs may not contribute to masculinization of this portion of the MePD.

The MePD was the only brain region examined that displayed an asymmetry; specifically, total volume and rostrocaudal extent were greater in the right hemisphere than the left in *tfm* and wild-type males, but not in females. These results indicate that laterality of MePD morphology is a masculine trait that does not depend on the presence of functional AR. Presumably the masculine lateralization of MePD structure is mediated solely by aromatized metabolites of androgen acting on ER.

Circulating gonadal steroids can influence many aspects of cellular morphology, including neuron size and number, dendritic branching, and synaptic connectivity. Because Cooke et al. (2003) showed that changes in MePD volume were not fully explained by changes in soma size following castration, other elements of the neuropil and neuronal number might also contribute to the volume differences. Gomez and Newman (1991) reported diminished dendritic arborization in the medial amygdala of hamsters following castration. Greater number and/or size of glia could also contribute to volume differences. However, recent evidence indicates that female rats have a denser staining of glial fibrillary acid protein in the posterior medial amygdala than do males, suggesting that glia may not account for the increased volume seen in male MePD and implicating neuronal elements instead (Rasia-Filho et al., 2002). Adult neurogenesis cannot be excluded as a contributor to the increased volume of MePD in male rats. For example, female prairie voles given bromodeoxyuridine in adulthood show both labeled neurons and glia in the MePD following exposure to males (Fowler et al., 2002), suggesting that both neurogenesis and gliogenesis can occur in the adult MePD in response to sexually relevant stimuli.

Sex differences in amygdala morphology and function have also been found in primates. Neurons in the medial amygdala of squirrel monkeys are smaller in females than in males (Bubenik and Brown, 1973). The human amygdala, measured by MRI, is sexually dimorphic beginning at puberty (Brierley et al., 2002). Although a study of the Yakovlev collection of human brains did not find a sex difference in the volume of the medial amygdala (Murphy, 1986), the sample size was small (n = 17) and included data from across the lifespan, which might easily mask a sex difference if it arises during puberty. Allen and Gorski (1990) found a sex difference in the human bed nucleus of the stria terminalis (BNST), part of the so-called extended amygdala that shares connectivity with the medial amygdala.

As a node in the circuit responsible for male sexual behavior, the medial amygdala and the effects of gonadal hormones on it have been well documented. Malsbury and McKay (1994) showed in castrated adult males that systemic testosterone maintains both sexual behavior and the density of substance P staining in the MePD for at least 8 weeks. Lesions of the medial amygdala in inexperienced males leave erections intact during copulation but abolish noncontact erections (NCEs), a measure of sexual arousal (Kondo et al., 1997). Intracranial implants of either DHT or E2 into the medial amygdala delay the loss of NCEs following castration of males (Bialy and Sachs, 2002). Discrete neuronal populations in the MePD show immunostaining for both Fos and AR following ejaculation (Greco et al., 1998b). Taken together, these results suggest that AR activation in the MePD is important for male sexual behavior.

Behavioral studies of *tfm* rats implicate both ER and AR in the activation of male copulatory behaviors. Castrated *tfm* males show mounting and intromissive patterns following treatment with testosterone or estrogen but not DHT, whereas male littermates responded to treatments with DHT as well (Olsen, 1979). These same sexual behaviors were also deficient in gonadally intact *tfm* males in an earlier study (Beach and Buehler, 1977). The present findings suggest two explanations for this deficit. Perhaps *tfm* males experience only a decreased activational effect caused by the loss of functional AR in adulthood, leading to an undermasculinized MePD and SDN-POA. Alternatively, *tfm* males may not receive a fully masculinizing organizational effect if perinatal AR stimulation augments hypothalamic estrogen levels by increasing aromatase activity and therefore increasing local ER activation.

As expected, the volume of the SDN-POA was fully masculine in *tfm* rats, confirming the report of Jacobson and Gorski (Gorski et al., 1981). However, we found that the somata of neurons in the SDN-POA were smaller in *tfm* males than in wild-type males. Whereas Gorski et al. (1981) report a sex difference in neuronal soma size in the SDN-POA, they do not provide mean size or details about the strain or age of the rats examined, so we cannot

readily compare the present study with their results. In our material the mean soma size of SDN-POA neurons in females was less than that of males, albeit not statistically significant ($P = 0.16$, two-tailed). It is possible that with a larger sample size we might have replicated the sex difference in SDN-POA soma size reported by Gorski et al. (1981) and Madeira et al. (1995). In any case, finding that SDN-POA somata are smaller in *tfm* than in wild-type males suggests that a functional AR is required for full masculinization of this characteristic. Dohler et al. (1986) found that SDN-POA volume can be demasculinized by blocking perinatal exposure to estrogen, whereas blocking perinatal androgen binding had no effect. However, Roselli et al. (1987) showed that aromatase was diminished in the medial preoptic area of *tfm* rats compared with normal males, supporting the idea that AR may normally have a role in regulating SDN-POA morphology by augmenting aromatase activity in this region, and in turn providing more ligand to activate ER to increase SDN-POA volume.

If AR activation acts in this manner, it would explain why Lund et al. (2000) found that postnatal androgen receptor blockade with flutamide reduced the SDN-POA volume in adult males to a level comparable to that of control females: AR-mediated conversion of testosterone to estrogen in the hypothalamus may be compromised by lack of AR activation. In *tfm* rats, the decreased hypothalamic aromatase activity caused by lack of AR may be partially compensated for by the elevated circulating testosterone compared with wild-type males. Roselli et al. (1987) found aromatase activity to be normal in the medial-cortical amygdala of *tfm* male rats, so we have no reason to think that AR functions by modulating aromatase activity in the MePD.

We found a sex difference in total SCN volume (males > females). Our absolute estimate of SCN volume in males (0.060 mm^3 per side) is similar to that of Guldner (1983), who reported a unilateral SCN volume of 0.064 mm^3. Robinson et al. (1986) and Gorski et al. (1978) also reported sex differences in volume of SCN, consistent with the present results. However, there are reports of a lack of a sex difference in SCN volume (Madeira et al., 1995), including a subsequent study from Gorski's laboratory (Bloch and Gorski, 1988b), so the sexual dimorphism may be subtle and/or strain dependent. The SCN in mice has a smaller volume in males that are missing the gene for steroid-receptor coactivator-1 (Monks et al., 2003), so perhaps AR activation requires this cofactor to masculinize the SCN. Castrating male gerbils at birth reduces the size of the SCN in adulthood (Holman and Hutchison, 1991), and the human SCN contains ARs (Fernandez-Guasti et al., 2000), all of which is consistent with AR having an influence on this nucleus. Swaab et al. (1992) also found that the human SCN was larger in homosexual men than in heterosexual men. If androgens play a role in human sexual orientation, the morphology of the SCN may serve as a marker for this influence.

The present analysis cannot determine whether the morphological effects of a defective AR are due to the protein dysfunction during the perinatal, pubertal, or adult period because *tfm* rats have defective AR throughout development. For the MePD, stress of the dam on embryonic days 17–21, which drastically reduces available testosterone during the prenatal critical period, does not change MePD volume in adulthood (Kerchner et al., 1995), suggesting that prenatal androgens may not influence adult volume of the MePD. Because adult MePD dimorphism is fully dependent on circulating androgens (Cooke et al., 1999), it may be that AR contributes to MePD morphology primarily in adulthood. Whether AR stimulation acts on the developing and/or the adult SDN-POA or SCN must await further study.

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