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Gonadal Steroids Regulate Neural Plasticity in the Sexually Dimorphic Nucleus of the Preoptic Area of Adult Male and Female Rats

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Key Words
Estrogen · Soma size · Androgens · Neuronal plasticity · Sexual dimorphism

Abstract
Background: The densely staining sexually dimorphic nucleus of the preoptic area (SDNPOA) is profoundly affected by gonadal steroids during perinatal development. ***Methods:*** We tested whether the SDNPOA in rats also remains responsive to gonadal hormones in adulthood. ***Results:*** Castration of 60-day-old male rats led to a reduction of soma size in SDNPOA neurons 28 days later, but not 14 days later. In contrast, the SDNPOA volume in males was unaffected by adult castration, increasing somewhat between 60 and 88 days of age in both castrated males and sham controls. For female rats ovariectomized at 60 days of age, testosterone treatment resulted in a significantly larger SDNPOA soma size after either 14 or 28 days of treatment, compared to blank-treated controls. Testosterone-treated ovariectomized females also had a modestly larger SDNPOA volume after 28 days, but not 14 days, of treatment compared to blank-treated ovariectomized animals. A second experiment revealed that these effects in females were due to ovariectomy: both SDNPOA soma size and regional volume shrank in females that were ovariectomized compared to females subjected to sham surgery. The effects of ovariectomy were blocked by testosterone treatment. The cessation of testosterone treatment in females returned SDNPOA soma size and regional volume to that of control-treated ovariectomized females. ***Conclusion:*** These results indicate considerable plasticity of the adult rat SDNPOA, and that (1) circulating androgens are required to maintain soma size, but not regional volume in males, and (2) ovarian steroids maintain both soma size and regional volume of the SDNPOA in females, an effect that can be mimicked with testosterone treatment and is fully reversible in adulthood.

Introduction

Sexual differentiation of the rat brain depends on steroid hormones during early development and adulthood. These 2 developmental periods of steroid sensitivity are often categorized as either organizational, occurring perinatally and having a permanent effect, or activational, occurring postpubertally and having a transient effect [1]. For example, the sexually dimorphic nucleus of the preoptic area (SDNPOA) has a greater volume in males than in females [2–7], and this sex difference appears to follow the organizational paradigm of being permanent-

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ly affected by perinatal steroids [4]. Similarly, female rats gestating between 2 male fetuses have a larger adult SDNPOA than females gestating between 2 females [8], presumably because they are exposed to prenatal androgens. A high proportion of neurons in the SDNPOA and the rest of the medial preoptic area contain gonadal steroid receptors [9–11], suggesting that steroids may act directly upon this region to exert these effects. The most abundant testicular steroid, testosterone, appears to masculinize the perinatal SDNPOA volume in males by acting on estrogen receptors via its aromatized metabolites, rather than by acting on androgen receptors [4, 12].

Soma size [2, 6], neuronal number and neuropil volume [7] are all sexually dimorphic in the adult SDNPOA and are likely to contribute to the overall sex difference in volume; these parameters are larger in males than in females. The volume of the SDNPOA of genetic male rats with a dysfunctional-androgen-receptor gene [the testicular feminization mutation *(tfm)* allele] is similar to that of wild-type male littermates [2, 12], consistent with the notion that estrogen receptors rather than androgen receptors regulate volume. However, neuronal soma size in the SDNPOA of Tfm males is significantly reduced compared to normal male littermates, and not significantly different from females [2]. These findings suggest both that sex differences in regional brain volume need not correlate with sex differences in neuronal cell size, and that androgen receptors play a role in maintaining neuronal soma size in the rat SDNPOA, although at what point during ontogeny androgen receptors exert this effect has not been explored.

The SDNPOA has received considerable attention as a morphological signature of the organizational hypothesis, and therefore research has focused primarily on manipulating perinatal hormones rather than adult circulating hormones. To evaluate whether adult circulating hormones also influence the morphology of the adult SDNPOA, we examined adult male rats 14 and 28 days after castration, and adult female rats treated with testosterone for 14, 28 or 56 days after ovariectomy, to see if adult circulating hormones play a role in maintaining SDNPOA volume and soma size. While castration of male rats had no effect on SDNPOA volume, it reduced the size of neuronal somata in the nucleus. In females, ovariectomy itself reduced regional volume and soma size in the SDNPOA, an effect that could be averted with testosterone treatment. These results indicate that the rat SDNPOA retains plasticity in response to steroids in adulthood.

Methods

Animals and Treatment

Experiment 1

Male and female Long-Evans rats from Charles River (Wilmington, Mass., USA) were housed 2–4 to a cage and maintained under standard laboratory conditions (12 h of darkness/12 h of light, 22 ± 1°C) with food (86/40, Harlan Teklad, Ind., USA) and water ad libitum. After 1 week of acclimation, when animals were 60 days of age, surgeries and hormone capsule implantations were performed under isoflurane inhalant anesthesia using aseptic procedures.

Animals were randomly assigned to 1 of several groups: males were either castrated or sham castrated and sacrificed 14 or 28 days later; females were ovariectomized and implanted subcutaneously near the nape of the neck with 2 testosterone-filled or 2 blank Silastic capsules (each 20 mm effective release length, 30 mm total length, i.d. 1.57 mm; o.d. 3.18 mm). Capsules were incubated for 48 h in phosphate-buffered saline (pH 7.4) before being implanted. Females were sacrificed either 14 or 28 days after treatment began.

Experiment 2

To further explore the effects of testosterone on SDNPOA volume and soma size in adult female rats revealed in experiment 1, a second cohort of Long-Evans rats was obtained from Charles Rivers. Sixty-day-old female rats were randomly assigned to 1 of 6 groups: sham ovariectomy plus blank Silastic capsules and sacrificed either 56 days or 84 days later; ovariectomy plus blank capsules and sacrificed either 56 or 84 days later; ovariectomy plus testosterone-filled capsules and 56 days later either sacrificed, or had testosterone capsules removed and sacrificed 28 days after that (i.e., 84 days after the initial surgery). Capsules used were as described above and replaced in kind every 28 days for all animals to ensure that testosterone levels were maintained in testosterone-treated females. Animal care and handling were conducted according to the National Institute of Health guidelines, and all manipulations were approved by the Michigan State University Institutional Animal Care and Use Committee.

Perfusion

At sacrifice, all animals from both experiments were weighed and overdosed with sodium pentobarbital (120 mg/kg, i.p.). Deeply anesthetized animals were transcardially perfused with 0.9% saline solution followed by 10% buffered formalin (approx. 250–300 ml each). Deep anesthesia was noted by a lack of reflexes to tail and foot pinch as well as lack of corneal reflex. Gonadectomies and hormonal implants were confirmed at time of sacrifice. Brains were removed and postfixed for at least 30 days in 10% neutral buffered formalin.

Histology

Experiment 1

Brains were blocked and cryoprotected overnight at 4°C in a 20% phosphate-buffered sucrose (pH 7.4) prior to coronal sectioning on a freezing sliding microtome set at 40 μm using MultiBrain Technology (Neuroscience Associates, Tenn., USA). An embedding matrix ensured there were no lost sections and preserved hemispheric orientation. Brains were sectioned throughout the entire region of interest and alternate sections mounted

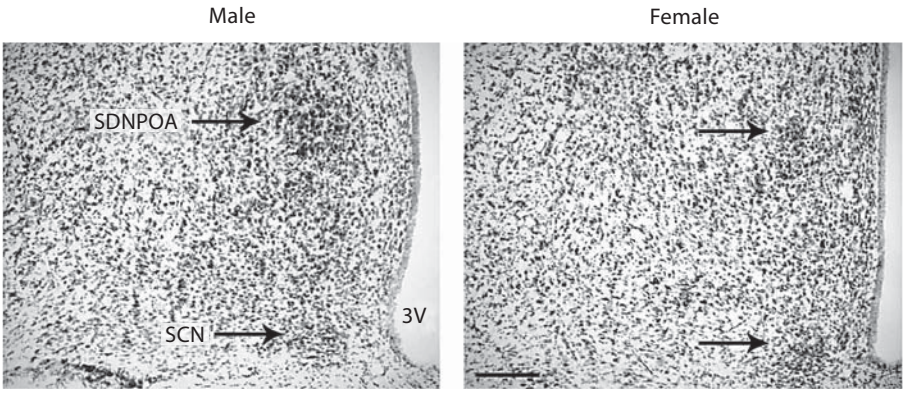

Fig. 1. The SDNPOA of adult rats. In these photomicrographs of Nissl-stained coronal sections, the SDNPOA of an adult male rat is larger than that of a female. The suprachiasmatic nucleus (SCN) lies ventral to the SDNPOA near the third ventricle (3V) at the midline in these sections. Scale bar = 250 μm.

onto gelatin-subbed slides with a random start to ensure that every section had an equal probability of being chosen for sampling. Mounted tissue was allowed to air dry, stained with thionin for the Nissl substance and coverslipped with Permount.

Experiment 2

Brains were treated the same way as for experiment 1 except they were sectioned in-house, no embedding matrix was used and the cortex was scored on the right to keep track of side.

Stereological Analysis

SDNPOA Volume

A Zeiss Axioplan 2 Microscope along with Stereo Investigator Software (Microbrightfield, Colchester, Vt., USA) was used to analyze the tissue from both experiments. The SDNPOA cross-sectional area was traced using a ×5 objective and then volume was reconstructed using NeuroExplorer Software (Microbrightfield), multiplying total cross-sectional area calculations by the section thickness (40 μm) and section-sampling interval [2]. The SDNPOA was traced rostral to caudal by an investigator without knowledge of the treatment, who drew the boundaries of the SDNPOA (fig. 1) according to descriptions given by Canteras et al. [13] and by Bloch and Gorski [3].

SDNPOA Neuronal Soma Size

Neurons were identified by the presence of a distinct Nissl-stained cytoplasm and nucleolus. Four to 5 neuronal somata were sampled on each side of the brain from each section throughout the rostrocaudal extent of the nucleus using a ×63 objective, totaling 6–10 sections per animal. The fractionator probe (Stereo Investigator) randomly chose spots within the traced perimeter of the nucleus, and the nearest neuronal somata was traced to provide an unbiased sample of 24–40 neurons per side per animal. The 2-dimensional profile of the selected neuronal somata was then traced and the area calculated by the software. A single mean soma size was calculated for each animal, and these were averaged across animals in each group, with n representing the number of animals.

Statistical Analysis

Group differences in body weights were assessed using independent t tests. Laterality was assessed by paired t tests comparing regional volume or soma size in the left versus right hemisphere in each group. As this analysis revealed no lateral asymmetry for any measure in any group, subsequent analyses were conducted on bilateral volume and somal area measures. For experiment 1, SDNPOA measures were analyzed by ANOVA within each sex, with androgen status and survival time as independent factors. For experiment 2, the factors were hormone treatment and survival time. When ANOVA revealed significant interactions, Fisher's LSD post hoc tests were conducted pairwise to determine which differences were statistically significant. All tests were two-tailed with n representing the number of animals in each group (fig. 2–4).

Results

Body Weights

In experiment 1, castrated males had a significantly lower body weight than sham males at both 14 and 28 days after surgery ($p < 0.05$; table 1). The average body weights of females also conformed to expectation: at both 14 and 28 days after ovariectomy, blank-treated females weighed less than testosterone-treated females, but these differences were not statistically significant. In experiment 2, where females were examined after longer treatment regimens, the effects of testosterone were statistically significant after both 56 and 84 days of treatment, and sham females weighed significantly less than blank-treated ovariectomized females ($p < 0.05$, two-tailed).

SDNPOA Morphology

Males

There was no significant main effect of castration on male SDNPOA regional volume, nor any interaction of surgery and survival time ($p > 0.20$). However, there was a significant main effect of survival time ($p < 0.001$), indicating that SDNPOA volume was still growing between 74 and 88 days of age (fig. 2a). In contrast, there was no main effect of survival time on neuronal soma size in the

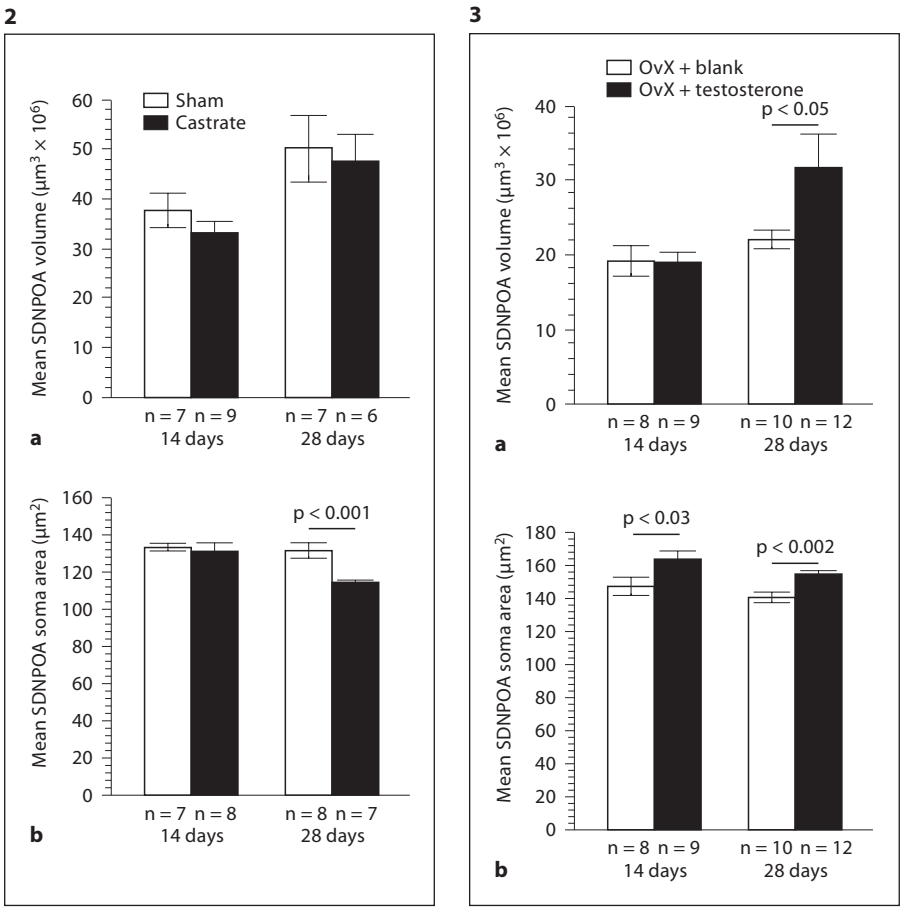

Fig. 2. Effects of castration on the SDNPOA of adult male rats. **a** Mean SDNPOA volume (±SEM) for male rats subjected to either castration or sham surgery at 60 days of age and examined 14 or 28 days later. There was significant growth of the SDNPOA volume during this period, which was unaffected by castration. **b** SDNPOA soma area is stable in gonadally intact males, but shrinks after castration, an effect apparent 28 days, but not 14 days, after surgery.

Fig. 3. Effects of testosterone treatment on the SDNPOA of ovariectomized adult female rats (experiment 1). **a** Mean SDNPOA volumes (± SEM) in ovariectomized (OvX) female rats given either testosterone-filled or blank Silastic capsules were equivalent in the 2 groups 14 days after surgery, but was larger in testosterone-treated females 28 days after surgery. **b** Average SDNPOA soma size was greater in ovariectomized females given testosterone than in blank-treated females, both 14 and 28 days after surgery.

Table 1. Body weights after surgery

Group	Body weight, g	
	14 days	28 days
Experiment 1		
Male shams	377.0 ± 5.3	461.7 ± 13.9
Male castrates	330.8 ± 7.2	382.3 ± 9.1
Female OvX + blank	258.5 ± 6.6	297.1 ± 13.3
Female OvX + testosterone	264.7 ± 6.3	319.2 ± 8.8
	56 days	84 days
Experiment 2		
Female shams	303.9 ± 8.3	308.0 ± 11.4
Female OvX + blank	344.9 ± 18.3	366.4 ± 10.0
Female OvX + testosterone	385.4 ± 7.7	413.9 ± 12.3[1]

Except for females in experiment 1, the body weight of each group was significantly different ($p < 0.05$, two-tailed) from the other same-sex groups at each age.

[1] The female OvX + testosterone group sacrificed at 84 days had the testosterone capsules removed at day 56.

SDNPOA. There was also no main effect of androgen treatment, but there was a significant interaction ($p < 0.05$). Post hoc tests revealed that SDNPOA somata were smaller in castrated males than sham-operated males 28 days after surgery ($p < 0.001$), an effect that was not seen 14 days after surgery (fig. 2b).

Females

In experiment 1, there were no significant main effects on SDNPOA volume in females, but there was a significant interaction ($p < 0.05$). There was no effect of testosterone treatment on SDNPOA volume at 14 days, and post hoc tests found no volume change in ovariectomized blank-treated females between the 2 adult ages ($p = 0.2$; fig. 3a). However, SDNPOA volume was larger in testosterone-treated females than in blank-treated females 28 days after surgery and capsule implantation ($p < 0.05$; fig. 3a). For SDNPOA somata size, there was no significant effect of survival time ($p > 0.20$), but there was a significant main effect of androgen treatment as somata size was larger in ovariectomized females given testosterone

than those given blank capsules ($p < 0.01$; fig. 3b). Although there was no significant interaction of the factors, post hoc tests suggest that the effect of testosterone in ovariectomized females may be greater after 28 days of treatment ($p < 0.002$) than after 14 days ($p < 0.03$).

These results suggested that steroid hormones affect both SDNPOA regional volume and soma size in adult females, which was probed further in experiment 2. For SDNPOA volume, there were no significant main effects of either treatment or survival time ($p > 0.20$), but there was a significant interaction ($p < 0.05$). Post hoc probing revealed that the more prolonged testosterone treatment (56 days) of ovariectomized females again resulted in greater SDNPOA volume than in ovariectomized females given blank capsules ($p < 0.01$; fig. 4a), extending the results of experiment 1. However, the additional control females, which had been subjected to sham ovariectomy, also had a greater SDNPOA volume than blank-treated ovariectomized females ($p < 0.05$), indicating that ovariectomy itself shrinks SDNPOA volume by approximately 25%. This finding was confirmed in females examined 84 days after surgery: sham-ovariectomized females again had a larger SDNPOA regional volume than ovariectomized females given blank capsules ($p < 0.01$). SDNPOA volume in gonadally intact females was also greater than in females that had been treated with testosterone for the first 56 days and then had the androgen withdrawn 28 days before sacrifice.

Neuronal soma size in females followed a similar pattern. Again, only the interaction of treatment and survival time reached significance in ANOVA. Post hoc tests indicated that soma size was smaller in blank-treated ovariectomized females than in either testosterone-treated ovariectomized females or sham-operated females after 56 days of treatment ($p < 0.02$; fig. 4b). The difference between ovariectomized females given blanks and sham females persisted 84 days after surgery at 60 days of age ($p < 0.001$). In ovariectomized females that had been treated with testosterone for 56 days, then had testosterone withdrawn for 28 days before sacrifice, SDNPOA soma size was smaller than in sham females ($p < 0.001$) and no larger than in ovariectomized females that had never received testosterone (fig. 4b).

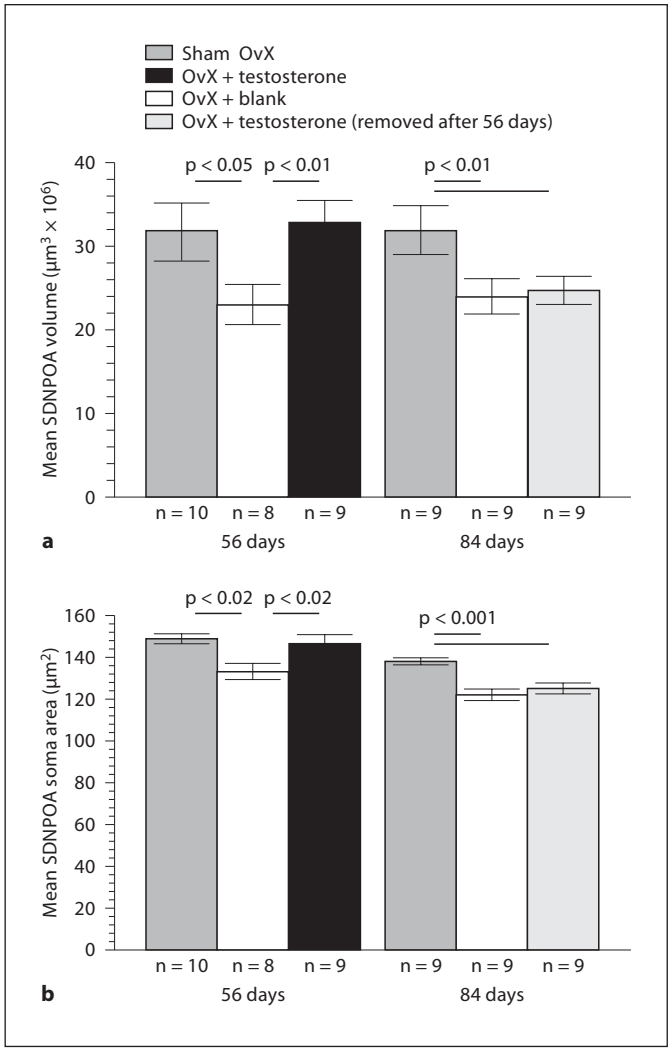

Fig. 4. SDNPOA volume and soma size is maintained by ovarian hormones in adult female rats (experiment 2). **a** Mean SDNPOA volume for adult females subjected to either ovariectomy (OvX) or sham OvX then given either testosterone-filled or blank capsules and sacrificed 56 or 84 days later. Testosterone capsules were removed from some females after 56 days of treatment and those females were sacrificed 28 days later (84 days after initial surgery) to assess whether the effects of testosterone are reversible. Volume of the SDNPOA was smaller in control-treated OvX females than either sham females or OvX females given testosterone. When testosterone was removed, SDNPOA volume was reduced 28 days later, comparable to the size of the SDNPOA in OvX females that never received testosterone. **b** Average SDNPOA soma size was also smaller in ovariectomized females than sham females at both time points. Testosterone treatment averted this effect of ovariectomy, but when testosterone was withdrawn, SDNPOA somata shrank to the size of OvX females that had never received it.

Discussion

As had been reported by Gorski et al. [4], we found that castration of adult male rats did not reduce the volume of the SDNPOA. Indeed, we documented a significant ex-

pansion of SDNPOA volume between 74 and 88 days of age, growth that was equivalent in males that had been castrated at 60 days and in gonadally intact males (fig. 2a). This result is consistent with previous findings that dramatic changes in the SDNPOA continue throughout at least the first 10 days of postnatal life [5]. We conclude that a more modest growth rate continues up to at least 88 days of age. Thus, the perinatal steroid actions that masculinize SDNPOA volume appear to be permanent in males, or at least not dependent on circulating testicular steroids after 60 days of age. However, soma size of neurons in the male SDNPOA shrinks after castration in adulthood. This effect appears to develop slowly, as it was not present 2 weeks after castration but was apparent after 4 weeks (fig. 2b). Thus, there appears to be an activational effect of androgen on neuronal soma size, but not regional volume, in the male rat SDNPOA. This dissociation of soma size and regional volume probably reflects the small fraction of overall regional volume accounted for by somata volume, and suggests that steroids act upon partially independent mechanisms to affect these 2 morphological features.

In female rats, steroid manipulations in adulthood had several effects we did not anticipate. Our first cohort suggested that testosterone treatment in adulthood could expand the volume of the SDNPOA in females, since ovariectomized females given testosterone had larger SDNPOA volumes 4 weeks later than ovariectomized females given blank capsules (fig. 3a). When following up on these results in experiment 2, the addition of another control group, sham-ovariectomized females, revealed that in fact it was the ovariectomy that caused the SDNPOA to shrink compared to gonadally intact females, an effect that was apparent both 56 and 84 days after surgery (fig. 4a). Interestingly, testosterone treatment of ovariectomized females blocked this reduction, suggesting that testosterone was compensating for the loss of ovarian steroids that normally maintain SDNPOA volume in females. It is not entirely clear whether ovariectomy causes SDNPOA volume to shrink or merely prevents it from growing, but comparisons of blank-treated ovariectomized females at 74, 88 and 116 days of age (fig. 3a, 4a) suggest that the female SDNPOA may be growing slightly during this period of ontogeny. In that case, the testosterone may be more accurately regarded as promoting a gradual growth that would have occurred if the ovaries had been left intact. We did not conduct experiments to determine which ovarian secretions normally promote SDNPOA volume in female rats. In any case, the maintenance of SDNPOA volume by testosterone following ovariectomy was shown to be only a transient effect, since cessation of testosterone treatment after 56 days resulted, 28 days later, in an SDNPOA volume that was no different from ovariectomized females that had never received testosterone (fig. 4a).

Thus, it appears that SDNPOA volume in adulthood is regulated somewhat differently in male versus female rats. Our hormone manipulations had no effect on this measure in males. In contrast, in females the loss of ovarian secretions prevents the maintenance or growth of SDNPOA volume, an effect that can be averted with testosterone treatment. We conclude that SDNPOA volume in adult rats shows evidence of modest steroid responsiveness, but only in females. This hormonal responsiveness of adult female SDNPOA volume may have been demonstrated in Gorski et al. [4]. In that report, adult females, ovariectomized and treated with an oil vehicle for 2 weeks before sacrifice, had a smaller mean SDNPOA volume than ovariectomized females given testosterone propionate for 2 weeks. Analysis of these data was by a one-way ANOVA across several groups, including animals treated with estrogen plus progesterone, propylthiouracil or water deprivation (fig. 3 in Gorski et al. [4]). There was no overall main effect of these treatments, but the mean SDNPOA volume for the females treated with androgen is greater than for control ovariectomized females and the standard error bars do not overlap, so comparing only those 2 groups might have revealed a statistically significant difference. Table 1 in that same publication [4] provides the means and standard errors for SDNPOA volume relative to brain weight. An estimated t test from these data indicates that ovariectomized females given either androgen or estrogen plus progesterone had significantly larger relative SDNPOA volumes than oil-treated ovariectomized females ($p < 0.05$, two-tailed, our analysis). The paper does not examine sham-ovariectomized females for comparison, so no effect of ovariectomy per se could have been detected. The effect of estrogen plus progesterone on the ovariectomized rats in Gorski et al. [4] also suggests that these ovarian steroids help maintain SDNPOA volume. It further suggests that ovarian steroids exert this effect via estrogen and/or progestin receptors rather than androgen receptors. That idea is strengthened by the entirely male volume of the SDNPOA in Tfm rats [2] – if androgen receptors are responsible for SDNPOA volume, one would expect it to be smaller in Tfm animals than in wild-type males. In that case, the effect of testosterone treatment on SDNPOA volume in females in the present study might be working via aromatization and activation of estrogen receptors.

In contrast, neuronal soma size in the rat SDNPOA appears to remain steroid responsive in adulthood in both sexes. Loss of androgen following castration of adult males causes SDNPOA somata to shrink, presumably in response to the loss of testicular steroids. This waxing and waning of SDNPOA neuronal size may reflect a neural plasticity that was important for the ancestors of laboratory rats, since they were probably seasonally breeding rats in which the reproductive system was suppressed for part of the year [14, 15]. Gonadectomy also leads to shrinkage of SDNPOA neuronal somata in females, and testosterone is one candidate for mediating this effect of the ovary. We did not conduct any experiments to determine whether testosterone acts upon estrogen receptors or androgen receptors to affect SDNPOA soma size in females. However, it may be that androgen receptors mediate some of the effects of steroids on this parameter because SDNPOA soma size is smaller in Tfm male rats than in wild-type males [2]. Indeed, in that study, average SDNPOA soma size in females was between those of Tfm males and wild-type males, without being significantly different from either, which further suggests that androgen receptors are activated in females to maintain SDNPOA soma size. The present results would suggest that the ovaries normally provide ligands for that effect. Thus, androgen receptors may act to maintain neuronal soma size in the SDNPOA of both sexes, while estrogen receptors may affect regional volume of the same nucleus. The sensitive period for steroid effects on SDNPOA volume may be entirely perinatal in male rats, but continues to a modest degree into adulthood in females. We did not count the number of neurons in the SDNPOA of females in this study [7], but presumably it was unchanged since the periods of neurogenesis and apoptosis in the nucleus have been well characterized and fall within the perinatal period [16, 17].

While the SDNPOA has not been considered to play a role in feminine behaviors, because females have an SDNPOA and it appears to be steroid responsive in adulthood, it would be interesting to know what functions are mediated by this nucleus in female rats and whether hormones modulate those functions. The medial POA as a whole has been implicated in copulatory pacing in female rats [18], which offers one candidate for such a function. Further experiments delineating the function of the SDNPOA, in either sex, might clarify the significance of this neural plasticity.

In summary, we confirmed earlier reports that SDNPOA volume is independent of androgen in adult male rats [4], as it is unchanged after castration versus sham surgery. However, in females it appears that ovarian steroids normally help maintain the growth and maintenance of SDNPOA volume. Unlike the apparently permanent 'organizational' effect of perinatal testicular steroids on male SDNPOA volume, the ovarian maintenance of female SDNPOA volume is a more transient, 'activational' effect. Neuronal somata within the SDNPOA also respond to both gonadectomy and androgen treatment in adult rats of both sexes, and these effects are transient in nature. These results demonstrate that the SDNPOA retains some plasticity in terms of steroid responsiveness into adulthood and suggest that SDNPOA morphology depends on ovarian steroids in female rats.

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References

1 Arnold AP, Breedlove SM: Organizational and activational effects of steroid hormones on vertebrate behavior. Horm Behav 1985; 19:469–498.

2 Morris JA, Jordan CL, Dugger BN, Breedlove SM: Partial demasculinization of several brain regions in adult male (XY) rats with a dysfunctional androgen receptor gene. J Comp Neurol 2005;487:217–226.

3 Bloch GJ, Gorski RA: Cytoarchitectonic analysis of the SDN-POA of the intact and gonadectomized rat. J Comp Neurol 1988; 275:604–612.

4 Gorski RA, Shryne JE, Southham AM: Evidence for a morphological sex difference within the medial preoptic area of the rat brain. Brain Res 1978;148:333–346.

5 Jacobson CD, Shryne JE, Shapiro F, Gorski RA: Ontogeny of the sexually dimorphic nucleus of the preoptic area. J Comp Neurol 1980;193:541–548.

6 Gorski RA, Harlan RE, Jacobson CD, Shryne JE, Southam AM: Evidence for the existence of a sexually dimorphic nucleus in the preoptic area of the rat. J Comp Neurol 1980; 193:529–539.

7 Madeira MD, Leal S, Paula-Barbosa MM: Stereological evaluation and Golgi study of the sexual dimorphisms in the volume, cell numbers, and cell size in the medial preoptic nucleus of the rat. J Neurocytol 1999;28:131–148.

8 Pei M, Matsuda K, Sakamoto H, Kawata M: Intrauterine proximity to male fetuses affects the morphology of the sexually dimorphic nucleus of the preoptic area in the adult rat brain. Eur J Neurosci 2006;23:1234–1240.
9 Jacobson CD, Arnold AP, Gorski RA: Steroid autoradiography of the sexually dimorphic nucleus of the preoptic area. Brain Res 1987;414:349–356.
10 Simerly RB, Chang C, Muramatsu M, Swanson LW: Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. J Comp Neurol 1990;294:76–95.
11 Bloch GJ, Kurth SM, Akesson TR, Micevych PE: Estrogen-concentrating cells within cell groups of the medial preoptic area: sex differences and co-localization with galanin-immunoreactive cells. Brain Res 1992;595:301–308.
12 Jacobson CD: The Characterization, Ontogeny and Influence of Androgen on the Sexually Dimorphic Nucleus of the Preoptic Area; PhD dissertation, University of California, Los Angeles, 1980.
13 Canteras NS, Simerly RB, Swanson LW: Organization of projections from the medial nucleus of the amygdala: a PHAL study in the rat. J Comp Neurol 1995;360:213–245.
14 Forger NG, Breedlove SM: Seasonal variation in mammalian striated muscle mass and motoneuron morphology. J Neurobiol 1987;18:155–165.
15 Cooke BM, Hegstrom CD, Breedlove SM: Photoperiod-dependent response to androgen in the medial amygdala of the Siberian hamster, *Phodopus sungorus*. J Biol Rhythms 2002;17:147–154.
16 Jacobson CD, Gorski RA: Neurogenesis of the sexually dimorphic nucleus of the preoptic area in the rat. J Comp Neurol 1981;196:519–529.
17 Davis EC, Popper P, Gorski RA: The role of apoptosis in sexual differentiation of the rat sexually dimorphic nucleus of the preoptic area. Brain Res 1996;734:10–18.
18 Yang LY, Clemens LG: MPOA lesions affect female pacing of copulation in rats. Behav Neurosci 2000;114:1191–1202.