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The Volume of a Sexually Dimorphic Nucleus in the Ovine Medial Preoptic Area/Anterior Hypothalamus Varies with Sexual Partner Preference

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Sheep are one of the few animal models in which natural variations in male sexual preferences have been studied experimentally. Approximately 8% of rams exhibit sexual preferences for male partners (male-oriented rams) in contrast to most rams, which prefer female partners (female-oriented rams). We identified a cell group within the medial preoptic area/anterior hypothalamus of age-matched adult sheep that was significantly larger in adult rams than in ewes. This cell group was labeled the ovine sexually dimorphic nucleus (oSDN). In addition to a sex difference, we found that the volume of the oSDN was two times greater in female-oriented rams than in male-oriented rams. The dense cluster of neurons that comprise the oSDN express cytochrome P450 aromatase. Aromatase mRNA levels in the oSDN were significantly greater in female-oriented rams than in ewes, whereas male-oriented rams exhibited intermediate levels of expression. Because the medial preoptic area/anterior hypothalamus is known to control the expression of male sexual behaviors, these results suggest that naturally occurring variations in sexual partner preferences may be related to differences in brain anatomy and capacity for estrogen synthesis. (*Endocrinology* 145: 478–483, 2004)

DOMESTIC RAMS DISPLAY distinct variations in sexual behavior that make them a unique and valuable model to study the biological underpinnings of sexual partner preferences. Most domestic rams are sexually active with females and are classified as female-oriented. However, approximately 8% of rams display sexual partner preferences for other males and therefore are classified as male-oriented (1, 2). The male-oriented sexual preference of rams does not appear to be related to dominance or flock hierarchy (3). No early social factors have been identified that can predict or alter sexual partner preference in rams (1, 4). Male-oriented rams are not female-like in their sexual behavior. Rather, they execute a typical male copulatory motor pattern that is directed at rams instead of ewes. Sexual partner preference does not appear to be regulated by hormonal status in adulthood. Pinckard *et al.* (5) demonstrated that castration reduces mounting in both female- and male-oriented rams, but does not alter their choice of sexual partners. Moreover, variations in basal concentrations of testosterone in adult rams do not correspond with differences in mate preference (6).

In the absence of compelling social or hormonal factors that can explain the observed variations in the sexual partner preferences of rams, it seems likely that neural mechanisms are involved. The medial preoptic area/anterior hypothalamus (MPOA/AH), a region known to be critical for the expression of masculine sexual behavior in most mammalian species (7), is clearly one region to consider. The MPOA/AH comprises a steroid-sensitive brain region that contains high concentrations of androgen and estrogen receptors (8). Conversion of testosterone to estradiol by cytochrome P450 aromatase within the MPOA/AH is an important part of the mechanism by which androgens facilitate male sexual behaviors (9). Higher concentrations of aromatase have been measured in the MPOA/AH of female-oriented rams than that of male-oriented rams (10). Within the MPOA/AH of several species, including humans, sexually dimorphic cell groups have been identified that are significantly larger in males than in females (11). In rats, Gorski *et al.* (12, 13) identified a sexually dimorphic nucleus (SDN) in the preoptic area (SDN-POA). The SDN-POA has been associated with two distinct aspects of male sexual behavior, male copulatory motor patterns (7) and sexual partner preference (14, 15). In male rats treated perinatally with the aromatase inhibitor 1,4,6-androstatriene-3,17-dione, SDN volume correlates positively with male-typical sexual behavior and female-directed partner preference (16). The third interstitial nucleus of the anterior hypothalamus (INAH3) in humans, which exhibits positional and cytoarchitectonic similarities to the SDN-POA of the rat (17), has been found to be significantly larger in heterosexual men than in homosexual men and women (18), although the extent of this difference has recently been disputed (19). In animal studies, masculinization of the SDN depends primarily on testosterone

Abbreviations: BnST, Bed nucleus of the stria terminalis; INAH3, third interstitial nucleus of the anterior hypothalamus; IOD, integrated OD; MPNc, central division of the medial preoptic nucleus; MPOA/AH, medial preoptic area/anterior hypothalamus; oSDN, ovine SDN; SDN, sexually dimorphic nucleus; SDN-POA, SDN in the preoptic area; SSC, single-strength sodium citrate; vPVN, ventral portion of the magnacellular paraventricular nucleus.

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and/or its estrogenic metabolites acting during early development (11). The question thus arises whether sexual partner preferences in rams are associated with morphological differences in the MPOA. We hypothesized that a sexually dimorphic cell group exists in the MPOA/AH of sheep and is larger in animals exhibiting female mate preferences (female-oriented rams) than in those exhibiting male mate preferences (male-oriented rams and ewes).

Materials and Methods

This research was conducted in accordance with the principles and procedures outlined by the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. This work was approved by the Institutional Animal Care and Use Committees of Oregon Health and Science University and Oregon State University.

Animals and behavioral classifications

Twenty-seven adult sheep (~4 yr old) of mixed Western breeds were used in this study. The sheep were obtained from the United States Sheep Experiment Station in Dubois, Idaho, and reared in same sex groups under conditions that were described previously (10, 20). Beginning at approximately 16–18 months of age, rams were given sexual behavior tests so that they could be classified according to their sexual partner preference. All behavioral testing was conducted during the breeding season. The complete testing procedure has been described previously and consisted of tests with estrous females followed by sexual partner preference tests (10, 21, 22). Briefly, each ram was paired for 30 min with three unrestrained estrous ewes on 18 separate occasions during a 2-month period. The number of courtship behaviors, mounts, and ejaculations were recorded. After the 7th to 11th test, an overnight test was conducted with each ram that had not exhibited sexual activity. The overnight test consisted of placing each ram into a pen for 16–20 h with three estrous ewes that were painted with mineral oil/cement pigment mixture above the base of the tail. At the end of the overnight test the brisket area of the rams was checked for pigment. The presence of pigment on the male indicates that the male mounted the ewe. These sexual behavior tests identified rams that mounted and ejaculated with estrous females (*i.e.* potential female-oriented rams) and those that did not (*i.e.* potential male-oriented rams).

Three weeks after the last sexual behavior test, rams that did not mate estrous females were isolated for 7–9 d and then given two separate partner preference tests. A subset of all female-oriented rams (n = 4 of 8 rams) were also given sexual partner preference tests and shown to exclusively mate with ewes. Briefly, the partner preference test consisted of a 30-min test during which rams were given the opportunity to mate with two estrous ewes and two randomly selected males that were restrained simultaneously in stanchions. Test subjects were free to choose among the four stimulus animals or remain neutral. The partner preference testing was repeated two more times during the following breeding season when the rams were 28–30 months old. Rams that exclusively mounted other rams were classified as male-oriented rams, whereas rams that exclusively mated ewes were confirmed as female-oriented rams. After the tests were completed, rams of mixed sexual preference were housed together and were not permitted physical contact with females.

Age-matched experimental ewes born at the United States Sheep Experiment Station were maintained in same sex groups except for 1 wk during their first and second years when they were bred. The ewes used in the current study were reproductively sound and sexually experienced. All ewes lambed at least once during their first 2 yr. The rams and ewes were trucked to Oregon State University in the spring after all behavioral characterizations were completed. They were housed in single sex groups in large fenced pastures with free access to water.

Tissue collection

Tissue was obtained from eight female-oriented rams, nine male-oriented rams, and 10 luteal phase ewes during the breeding season. The ewes were synchronized by giving two im injections of 17β-estradiol (500 μg/d) plus an im injection of the prostaglandin analog Estrumate (250 μg; Bayer Corporation, Agricultural Division, Shawnee Mission, KS) on the second day. A vasectomized ram was used to test behavioral estrus on the third day, and tissues were collected 10 d later during the midluteal phase. The sheep were euthanized with an overdose (15 mg/kg) of sodium pentobarbital (Euthozol; Delmarva Laboratories, Inc., Midlothian, VA). The head was removed and perfused through the carotid arteries with 250 ml physiological saline containing heparin (150,000 U/liter), then with 4 liters of ice-cold 4% paraformaldehyde in borate buffer (pH 9.5). Brains were weighed and then dissected into hypothalamic and temporal lobe tissue blocks, immersion fixed in 4% paraformaldehyde for an additional 2 h, cryoprotected in 10% glycerol followed by 20% glycerol, frozen, and stored at −80 C (23). Diencephalic tissue blocks were coronally sectioned (30-μm thick) into four parallel series and mounted onto microscope slides. One series of brain sections, consisting of every fourth tissue section, was stained with thionin (0.02%). An adjacent series was processed for *in situ* hybridization using a sheep-specific [^{33}P]-labeled aromatase cRNA probe as described below. All processing of brain tissue was performed without knowledge of the sex or behavioral classification of the donor.

In situ hybridization

A sheep-specific [^{33}P]-labeled P450$_{AROM}$ cRNA probe was used for *in situ* hybridization as previously described (23). Briefly, the labeled cRNA probe was purified through a G-50 Nick column (Pharmacia Biotech, Inc., Alemeda, CA). The purified probe was heated at 65 C for 5 min with 500 μg/ml yeast tRNA and 50 μM dithiothreitol in water before being diluted to an activity of 1.0×10^7 dpm/ml with hybridization buffer containing 50% formamide, 10 mM Tris-HCl, 0.2 M NaCl, 1 mM EDTA, 1× Denhardt's solution (1× = 0.02% ficoll, 0.02% polyvinylpyrrolidone, and 0.02% acetylated BSA), and 10% dextran sulfate. To begin hybridization, tissue sections were thawed and treated with Proteinase K (10 μg/ml) for 30 min. They were acetylated in 0.1 M triethanolamine containing 0.25% acetic anhydride, dehydrated through a series of ethanol solutions, and desiccated under vacuum for at least 2 h. The hybridization solution was pipetted onto the sections (80 μl/slide), covered with a glass coverslip, and sealed with DPX mounting media (Gallard-Schlesinger Industries, Inc., Carle Place, NY) before incubation for 18 h at 58 C. After hybridization, the slides were washed four times (5 min each) in 4× single-strength sodium citrate (SSC; 0.15 M NaCl and 0.015 M Na citrate) before RNase digestion (20 μg/ml for 30 min at 37 C), rinsed at room temperature in decreasing concentrations of SSC that contained 1 mM dithiothreitol (2, 1, and 0.5×, for 10 min each) to a final stringency of 0.1× SSC at 65 C for 30 min. Sections were dried and apposed to BioMax MR film (Eastman Kodak, Rochester, NY) for 1 wk and then dipped in NTB-3 nuclear tract liquid emulsion (Eastman Kodak) and exposed for 3 wk before being developed according to the manufacturer's directions. The slides were then counterstained with Gill's 3× hematoxylin diluted 1:12 with water and coverslipped. Control sections incubated with [^{33}P]-labeled sense strand riboprobe lacked hybridization signal.

Image analysis

Images of autoradiographic films were collected at 3200 dpi with an Epson (Long Beach, CA) 1640SU flatbed scanner. Images of thionin-stained sections were captured on a Zeiss Axiophot 2 microscope (Zeiss, Oberkochen, Germany) with a 1.25× Plan Neofluar lens (numerical aperture 0.035) and an Optronics International (Chelmsford, MA) video camera. Analysis of images was performed on a Macintosh (Cupertino, CA) iMac G4 using NIH Image, version 1.62 (NIH Image is available from http://rsb.info.nih.gov/nih-image/Default. html). Images of each section from each animal were made into a stack and scaled. Two or three investigators unaware of animal classifications independently made measurements. The means of these measurements were used for statistical comparisons.

We previously reported (23) that the sheep brain contains a densely packed cluster of cells in the MPOA/AH that exhibits a high level of P450$_{AROM}$ mRNA expression. This region constitutes the central division of the medial preoptic nucleus (MPNc) and resembles the SDN-POA of rats because of its anatomical location and cytoarchitectonic character-

istics. We, therefore, considered the sheep MPNc a likely candidate for a SDN.

The full anterior-posterior extent of the MPNc was determined from the aromatase mRNA expression pattern, and the same range of sections was used for measurements in the adjacent, Nissl-stained series. The MPNc begins anteriorly near the point where the anterior commissure crosses the midline and extends posteriorly to where it lies ventral to the anterior extent of the bed nucleus of the stria terminalis (BnST). The BnST was measured from the anteriormost position, dorsal of the MPNc, to the posteriormost position, ventral of the stria terminalis. The ventral portion of the magnacellular paraventricular nucleus (vPVN) was measured anteriorly where it becomes distinct from the suprachiasmatic nucleus and posteriorly to a position where it reached 1.5 mm dorsal to the floor of the third ventricle.

For quantitative analysis of aromatase mRNA expression, the outlines of the MPNc and BnST were traced by hand from autoradiographic images and analyzed for cross-sectional area and OD by computer. Volume was calculated by multiplying the distance between sections that were positive for autoradiographic signal by the average cross-sectional area of the signal. A measurement of background OD was made for each animal from a region of size similar to the MPNc, but from an area on the film where there was no specific signal. To compare aromatase mRNA expression levels, the corrected integrated OD (IOD) was calculated from the autoradiograms by subtracting the background density from the signal OD in the MPNc or BnST and then multiplying this value by the cross-sectional area. The IOD was summated from all sections sampled to calculate the total IOD.

To determine the boundaries of cell groups, outlines of the MPNc and the vPVN were traced from thionin-stained sections, and the cross-sectional areas were measured by computer. The length of the MPNc and the vPVN was calculated by multiplying the number of sections in which the cell group appears by the distance between sections (accounting for the sections present in other series). Cell group volume was calculated by multiplying the length by the average of the cross-sectional areas. The number and size of neurons were measured from each Nissl-stained section. To do this, a density slice was performed to distinguish cells from background, and particle analysis was performed to exclude stained objects smaller than 50 μm^2 and larger than 1200 μm^2. These limits were determined by measuring hand-traced neurons and were set to include neurons that touch. Given these parameters, all neurons within the outlined boundaries were automatically counted by the NIH Image software, including those touching the inside of the border, and their sizes (in microns squared) were calculated. The neuron count represents the sum of cells for the total number of sections sampled from each animal. Neuron density was calculated by dividing the neuron count for a section by its cross-sectional area. The neuron densities were then averaged for each nucleus.

In 11 brains (four ewes, four female-oriented rams, and three male-oriented rams), nuclei from both left and right hemispheres were traced. Because no significant side differences were found for any measure in which the three nuclei were analyzed by paired Student's *t* tests, only the left side was used for group comparisons. The size of neuronal soma was analyzed in these same brains, and no difference in neuron size was observed among the three groups ($P > 0.05$; data not shown).

Blood sampling and testosterone measurements

Three daily blood samples were drawn between 0900 and 1100 h from the jugular veins of conscious rams 2 wk before they were killed. Testosterone was measured in serum aliquots by RIA after extraction with ethyl ether and chromatography on Sephadex LH-20 columns as previously described (24). The assay extraction efficiency for testosterone was 68.9 ± 1.7%. Interassay and intraassay coefficients of variation were 7.8 and 6.4%, respectively.

Statistical analysis

Behavioral data were analyzed by Mann-Whitney *U* tests. Morphometric and expression data were analyzed by one-way ANOVA followed by *post hoc* Newman-Keuls multiple comparisons tests. *P* values less than 0.05 were considered significant for both the parametric and nonparametric tests. All statistical analysis was performed using GB-STAT software version 7.0 (Dynamics Microsystems, Inc., Silver Spring, MD).

Results

The rams designated as female-oriented in this study mated vigorously and repeatedly with estrous ewes in the sexual performance tests (mean = 3.3 ± 0.2 ejaculations/30 min). The rams designated as male-oriented never copulated in the performance tests. Table 1 contains data on the average behaviors exhibited during sexual partner preference tests by the male-oriented rams and a subset of the female-oriented rams that were used. Obvious differences in mate preferences were evident between groups. Rams categorized as female-oriented displayed significantly more mounts and ejaculations with ewes than with stimulus rams, whereas rams categorized as male-oriented displayed more behaviors directed at stimulus rams than at ewes in all categories except mount attempts. Serum concentrations of testosterone were not significantly different between female-oriented (5.6 ± 2.0 ng/ml) and male-oriented rams (3.6 ± 1.6 ng/ml).

Our results are the first to identify a putative SDN in the sheep brain. Based on its anatomical location, cytoarchitecture, and sexual dimorphic volume, we have designated this nucleus the ovine SDN (oSDN) of the MPOA/AH. The oSDN, which appears to be homologous with the MPNc in the sheep brain, is situated bilaterally in the caudal portion of the MPOA approximately 1.5 mm lateral to the third ventricle and about 2.5 mm dorsal to the vPVN (Fig. 1). As defined by Nissl staining, the oSDN is irregular in shape and contains densely packed, heavily stained neurons. One-way ANOVA ($F_{2,24} = 17.2$; $P < 0.0001$) followed by *post hoc* Newman-Keuls tests revealed that the volume of the oSDN was

TABLE 1. Averages of behaviors exhibited by female-oriented and male-oriented rams during exposure to two estrous ewes and two rams in four separate 30-min sexual partner preference tests

Behaviors	Female-oriented rams (n = 4)		Male-oriented rams (n = 9)	
	Estrous ewe stimulus	Ram stimulus	Estrous ewe stimulus	Ram stimulus
Precopulatory behaviors[a]	31.1 ± 7.7	19.8 ± 8.9	3.5 ± 0.5	34.6 ± 9.8[b]
Mount attempts	0.2 ± 0.2	0	0	0.4 ± 0.2
Mounts	9.7 ± 2.6	0.1 ± 0.1[c,d]	0	16.2 ± 3.3[b]
Ejaculations	2.6 ± 0.5	0[c]	0	0.6 ± 0.2[c]

Before sexual partner preference tests, rams were given performance tests with estrous ewes. Those that mounted and ejaculated were designated as potential female-oriented rams; those that did not were potential male-oriented rams. Exclusive female-oriented rams and male-oriented rams were identified by results in the sexual partner preference tests.

[a] Precopulatory behaviors include: numbers of genital sniffs, foreleg kicks, vocalizations, and Flehmens (curling of the upper lip, presumably to facilitate pheromone detection).

[b] $P < 0.01$ compared with response with an estrous ewe.

[c] $P < 0.05$ compared with response with an estrous ewe.

[d] Value represents a single mount by one female-oriented ram on the final test. Behavior data were analyzed by Mann-Whitney *U* tests.

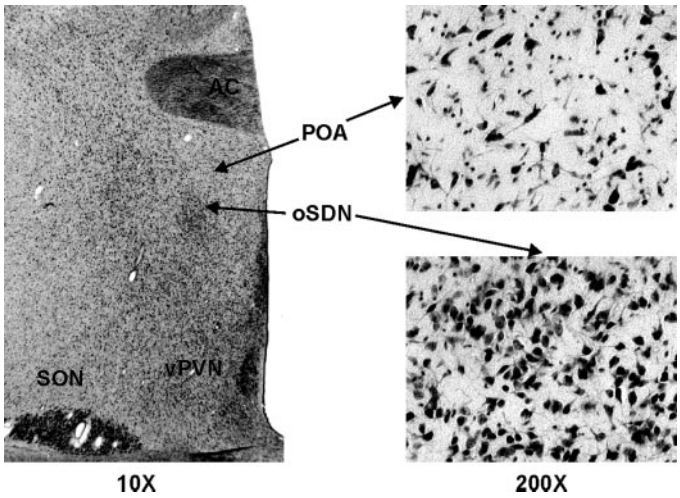

FIG. 1. Location of the oSDN. Coronal view through the MPOA/AH showing the oSDN in a female-oriented ram. *Insets*, Cellular density of the oSDN and adjacent preoptic area at magnification ×200. AC, Anterior commissure; POA, preoptic area; SON, supraoptic nucleus.

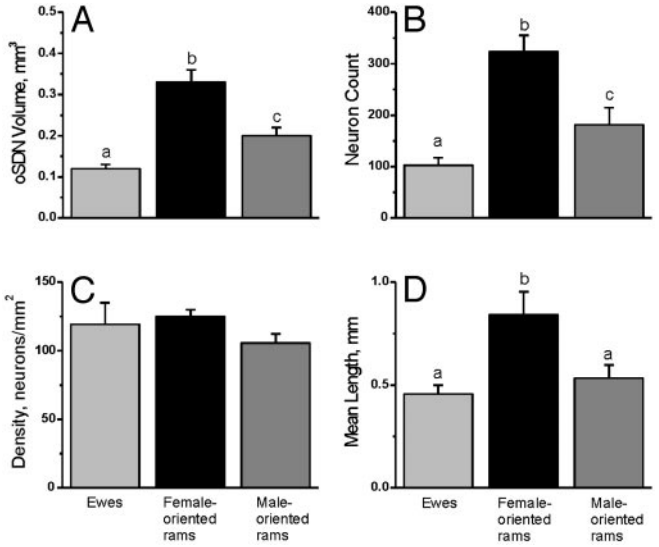

FIG. 2. Differences of the oSDN among female-oriented rams (n = 8), male-oriented rams (n = 9), and luteal phase ewes (n = 10). A, Differences in oSDN volume. B, Differences in neuron counts of the oSDN. C, Differences in neuron density of the oSDN. D, Differences in mean length of the oSDN. Data are presented as means ± SEM. *Bars* marked with *different letters* differ significantly ($P < 0.05$).

significantly greater in female-oriented rams than in male-oriented rams and significantly larger in male-oriented rams than in ewes (Fig. 2A). Group differences in cell counts were also evident ($F_{2,24} = 16.5$; $P < 0.0001$; Fig. 2B). The oSDN in female-oriented rams contained significantly more neurons than in male-oriented rams, which contained more than in ewes. Cell densities in the oSDN were not different ($F_{2,24} = 0.56$; $P = 0.58$; Fig. 2C). Group differences in oSDN volumes and cell numbers appeared to be attributable to variations in the length of the oSDN ($F_{2,24} = 7.36$; $P = 0.003$; Fig. 2D).

One-way ANOVA revealed significant group differences in the volume of the oSDN as defined by the autoradiographic pattern of aromatase mRNA expression ($F_{2,24} = 18.9$; $P < 0.0001$; Fig. 3A). The volume of expression was significantly greater in female-oriented rams than in ewes and male-oriented rams, but the volume of aromatase mRNA expression was not different between male-oriented rams and ewes. Significant group differences were also apparent in the level of aromatase mRNA when expressed as the total IOD in the oSDN ($F_{2,24} = 3.74$; $P = 0.03$; Fig. 3B). Aromatase mRNA levels in oSDN were significantly greater in female-oriented rams than in ewes, whereas male-oriented rams exhibited intermediate levels of expression. Mean brain weights ± SEM were recorded from six ewes (98.3 ± 3.5 g), four female-oriented rams (104.0 ± 6.1 g), and six male-oriented rams (102.8 ± 2.8 g). No significant differences were found among groups ($F_{2,13} = 0.24$; $P = 0.80$), suggesting that there were no group differences in brain sizes that could account for differences in the morphometric features of the oSDN. By comparison to the oSDN, the morphometric features of the vPVN (Table 2) and the expression of aromatase mRNA in the BnST (Table 3) did not exhibit differences in relation to sex or mate preference. Thus, differences in brain morphology and aromatase expression that were related to sex and sexual partner preference appeared to be specific to the oSDN and not the result of systematic variations in brain size, tissue preparation, or shrinkage.

Discussion

The present results indicate that the volume of the oSDN and the level of aromatase expression within this nucleus may be related to the expression of sexual partner preferences. Sheep exhibiting a female mate preference (female-oriented rams) had a significantly larger oSDN containing more neurons and greater aromatase mRNA expression than sheep showing a male mate preference (male-oriented rams and ewes). Our study is the first to demonstrate that there is an association between natural variations in sexual partner preferences and brain structure and function in nonhuman male animals.

Nissl staining has been used to define sexually dimorphic nuclei in the MPOA/AH of a number of species, including rats (12, 13), gerbils (25), guinea pigs (26), ferrets (27), quail (28), macaques (29), and humans (17, 18). Like the SDN-POA of the rat, the oSDN comprises the central portion of the MPNc (30), is three times larger in volume, and contains significantly more neurons in rams than in ewes. Like the

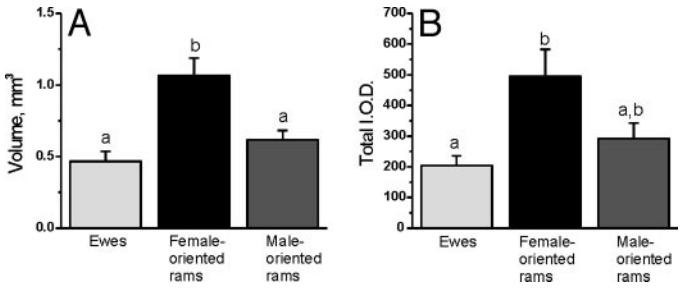

FIG. 3. Differences in the aromatase mRNA expression of the oSDN among female-oriented rams (n = 8), male-oriented rams (n = 9), and luteal phase ewes (n = 10). A, Differences in the volume of aromatase mRNA expression. B, Differences in the total IOD of aromatase mRNA expression of the oSDN. Data are presented as means ± SEM. *Bars* marked with *different letters* differ significantly ($P < 0.05$).

TABLE 2. Morphometric comparison of the vPVN in ewes, female-oriented rams, and male-oriented rams

	Mean volume (mm^3)	Mean length (mm)	Neuron count	Mean neuron density ($cells/mm^2$)
Ewes (n = 9)	0.57 ± 0.03	0.97 ± 0.04	601 ± 48	126 ± 5.66
Female-oriented rams (n = 8)	0.58 ± 0.11	1.07 ± 0.08	592 ± 102	130 ± 5.33
Male-oriented rams (n = 9)	0.64 ± 0.08	1.09 ± 0.09	719 ± 82	126 ± 3.08

The vPVN was outlined in Nissl-stained coronal brain sections and analyzed by NIH Image as described in *Materials and Methods.* One ewe was eliminated from the analysis because the tissue was damaged in processing. Data were compared by one-way ANOVA, and no significant differences were observed among groups for any measure.

TABLE 3. Comparison of aromatase mRNA expression in the BnST of ewes, female-oriented rams, and male-oriented rams

	Mean volume (mm^3)	Mean length (mm)	Total IOD
Ewes (n = 10)	2.59 ± 0.61	0.52 ± 0.05	767 ± 205
Female-oriented rams (n = 8)	2.35 ± 0.64	0.67 ± 0.07	842 ± 160
Male-oriented rams (n = 9)	4.65 ± 0.86	0.67 ± 0.06	1144 ± 175

Film autoradiograms generated by *in situ* hybridization for aromatase mRNA expression were analyzed as described in *Materials and Methods.* Data were compared by one-way ANOVA, and no significant differences were observed among groups for any measure.

SDN of the musk shrew (31) and the quail (28), the oSDN can be specifically identified by the presence of a dense cluster of neurons that express cytochrome P450 aromatase.

Anatomic studies (32) in rodents demonstrated that the SDN-POA is predominantly interconnected with the lateral septum, the posterior medial portion of the BnST, the ventrolateral portion of ventromedial hypothalamic nucleus, the posteromedial amygdaloid nucleus, and the ventral premammillary nucleus. The SDN-POA also projects to the ventral tegmental area of the midbrain, as well as the laterodorsal tegmental nucleus of the pons. It is notable that many of these areas are targets for sex steroid hormones and are sexually dimorphic (33). The organization of this circuitry implies that the SDN-POA is involved in the integration of sensory, somatic, and humoral information to coordinate appropriate behavioral and visceral responses to sexual cues. Extensive lesions of the MPOA/AH severely impair copulation in males of all vertebrate species tested (7). In contrast, the effects of small lesions confined to the SDN-POA of rats are much more variable. Small lesions of SDN-POA had no effect on copulation in experienced male rats (34) and produced only a transient increase in the latencies to mount, intromit, and ejaculate in previously inexperienced male rats (35). Similarly, bilateral lesions of the male preoptic nucleus in ferrets produced only small deficits in male copulatory behavior (36). In male gerbils, small bilateral lesions that were localized to either of two regions of the sexually dimorphic area produced copulatory deficits just as severe as a large lesion encompassing more of the MPOA/AH (37). More recent studies implicate the SDN-POA in the control of sexual partner preferences in rats. Bilateral lesions of the MPOA/AH that encompass the SDN-POA change sexual partner preferences from female-oriented to male-oriented in male ferrets and rats (14, 15). Although the exact function and projections of the oSDN in sheep are not yet known, it is possible that a dimorphism in volume and number of cells could bias the processing of sexually relevant sensory cues to shape sexual partner preferences.

We reported previously that aromatase activity in the MPOA is significantly lower in male-oriented rams than in female-oriented rams (10). Using *in situ* hybridization, we found that aromatase mRNA is highly expressed in the MPOA/AH of sheep (23). Quantitative analysis of the volume as well as the total IOD of the autoradiographic aromatase signal in the oSDN revealed both a sex difference in expression (rams significantly greater than ewes) and a difference based on sexual partner preference (female-oriented rams significantly greater than male-oriented rams). The latter result most likely accounts for the difference in aromatase activity that we reported previously and cannot be attributed to differences in circulating testosterone concentrations, which did not differ between male- and female-oriented rams (present results and Ref. 6). Our current results further suggest that the differences in capacity for estrogen synthesis in the MPOA of adult sheep probably arise from differences in the total number of aromatase-containing neurons in the oSDN of ewes, male-oriented rams, and female-oriented rams. Previous studies in rats demonstrated that the capacity for aromatization in the MPOA is a sexually differentiated feature of the brain (38) and that males tend to have more aromatase mRNA-containing neurons than females (39). Whether this difference in aromatase is causally related to the expression of sexual partner preferences is not yet known. However, although sexual partner preferences, like sexual behaviors, are activated by gonadal hormones in adult animals, the sex-specific nature of these preferences is part of the program of brain sexual differentiation established during prenatal and/or postnatal development (40). Thus, the differences in expression of aromatase mRNA and enzyme activity probably reflect differences in neuronal populations and functional circuitry in addition to a deficit in locally active estrogen. In agreement with this conclusion, we previously found that neither castration nor replacement treatment with estradiol altered sexual partner preferences in adult rams (5).

To the degree that cross-species comparisons are valid, our data support the study of LeVay (18), who found that INAH3, the human analog of the rat SDN-POA, is more than twice as large in heterosexual men as in homosexual men and women. However, a more recent replication study by Byne *et al.* (19) found that although there was a trend for INAH3 to occupy a greater volume in heterosexual men than in homosexual men, the number of neurons within this nucleus was not related to sexual orientation. Similar to the results in humans, the variability in oSDN measurements within each group of sheep was large compared with the differences between groups, and it is impossible to predict the sexual partner preference of any individual on the basis of a single brain measurement. Nor do the present data allow us to determine whether the observed differences in the size of the

oSDN are the cause or consequence of an animal's sexual partner preference, or whether the size of the oSDN is influenced by other unidentified variables. However, experiments in several species (11) have shown that the development of sexually dimorphic nuclei within MPOA/AH is the direct result of exposure to testosterone or its metabolites during a critical period in prenatal or early neonatal life. Although it seems likely that the size of the SDN in sheep is also established by testosterone exposure early, this relationship has not yet been established. Experiments are in progress to determine when the neurons composing the oSDN are generated and when they differentiate into a dimorphic nucleus.

In summary, this is the first demonstration in nonhuman animals that there is an association between natural variations in sexual partner preferences and brain structure. Female-oriented rams had the largest oSDN, male-oriented rams were intermediate, and ewes were smallest. Moreover, the presence of aromatase in the oSDN suggests that testosterone metabolism to estradiol occurs in this cell group and may be important for both its development and adult function.

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