SEXUALLY DIMORPHIC REGIONS IN THE MEDIAL PREOPTIC AREA AND THE BED NUCLEUS OF THE STRIA TERMINALIS OF THE GUINEA PIG BRAIN: A DESCRIPTION AND AN INVESTIGATION OF THEIR RELATIONSHIP TO GONADAL STEROIDS IN ADULTHOOD¹

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Abstract

Sexually dimorphic regions are described in two areas of the guinea pig brain: the medial preoptic area (MPOA) and the bed nucleus of the stria terminalis (BNST). The volume of a darkly staining portion of the MPOA is approximately 4-fold larger in male than in female guinea pigs, and the volume of a darkly staining portion of the BNST is approximately 36% larger in male than in female animals. The sex differences in both of these areas are present in animals that have been gonadectomized as adults as well as in intact animals, suggesting that they result from differences between the sexes in the hormonal environment during early development. Both the MPOA and the BNST bind high levels of gonadal steroids early in life, during the period when functional differentiation occurs. It is possible that dramatic morphological sex differences characterize such steroid-binding areas. Furthermore, these sexually dimorphic areas may form an anatomically and functionally interrelated system. Attention to these possibilities may help elucidate more precisely the neural basis for sexually dimorphic functions, as well as the basic mechanisms underlying sexual differentiation of behavior and the brain.

Gonadal hormones have powerful influences on sexual differentiation of the brain in mammals. For example, a genetic female rat that is treated with testosterone during the perinatal period of development will be masculinized with respect to a variety of neural functions, including gonadotropin regulation, reproductive behavior, food intake, aggression, and performance on certain types of learning tasks. Although these influences have been studied most extensively in the rat, similar effects have been documented in mice, hamsters, gerbils, guinea pigs, dogs, cattle, ferrets, sheep, marmoset and rhesus monkeys, and even, to some extent, in human beings (for reviews see Gorski, 1979; Goy and McEwen, 1980; Hines, 1982).

A recent development in this field has been the identification of sex differences in the structure of the brain that may underlie these functional changes. There are sex differences in dendritic

branching patterns in the preoptic area of the rat, hamster, and rhesus monkey (Greenough et al., 1977, 1981; Meyers and Gordon, 1982) and in the synaptic organization of the preoptic area, the hypothalamic arcuate nucleus, and the medial amygdala of the rat (Raisman and Field, 1973; Matsumoto and Arai, 1981; Nishizuka and Arai, 1981). In addition to these relatively subtle differences in the ultrastructure of the male and female brain, there is a dramatic sex difference in the morphology of the rat medial preoptic area (MPOA) (Gorski et al., 1978, 1980; Hsu et al., 1980; Bleier et al., 1982; Young, 1982). A nucleus in this area, which is called the sexually dimorphic nucleus of the preoptic area (SDN-POA), is severalfold larger in male rats than in female rats (Gorski et al., 1978). The sex difference is so large that it can be seen in stained sections without magnification, and it reflects, at least in part, a difference between male and female animals in the number of neurons in the region (Gorski et al., 1980). A similar dimorphism has been described in the MPOA of the gerbil (Yahr and Commins, 1982) and the ferret (Tobet et al., 1983).

Most of these neural sex differences appear to result from the action of steroid hormones during the period of sexual differentiation. Neurons in the areas that show morphological sex differences bind high levels of steroids during early development (Sheridan et al., 1975; Vito et al., 1979), at the time when hormonal manipulations influence sexual differentiation of function. Also, hormonal manipulations at this time influence the sex differences in these neural regions (Raisman and Field, 1973; Matsumoto and Arai, 1981; Nishizuka and Arai,

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Figure 1. Photomicrographs illustrating a sex difference in the MPOA of the guinea pig brain. A darkly staining portion of this region (arrows) is severalfold larger in the male (right) than in the female (left) brain. The illustration is of 60- μ m frozen sections cut in a coronal plane corresponding to that of de Groot in the rat and stained with thionin. The sections are from the anterior-posterior midpoint of the sexually dimorphic region in the intact male and female animals with volumes closest to the means for their respective groups. AC, anterior commissure; QC, optic chiasm.

Figure 2. The sex difference in the MPOA of the guinea pig brain in intact and gonadectomized animals. The volume of the region is greater in male than in female animals and is unaffected by gonadectomy in adulthood. Data are means $\pm$ SEMs. ***, $p < 0.001$ for comparison to comparable female group.

1981). In fact, in the case of the SDN-POA, the early hormonal environment may determine the size of the nucleus. Administration of testosterone to genetic female rats during late prenatal and early postnatal life produces an SDN-POA that is as large as that of a normal male rat (Döhler et al., 1982). In contrast, hormonal manipulations in adult rats do not affect the size of the nucleus (Gorski et al., 1978).

Most of our knowledge of neural sex differences has come from studies of altricial rodents, particularly the laboratory rat. A precocial rodent, the guinea pig, may provide a preferable model for human reproductive development. Guinea pigs resemble human beings, and differ from rats, in undergoing sexual differentiation prenatally (Phoenix et al., 1959; Goy and McEwen, 1980) and in having an estrous cycle with a true luteal phase (Brown-Grant and Sherwood, 1971). In this paper we report sex differences in the guinea pig brain. Specifically, we describe and quantify a sex difference in the guinea pig MPOA that appears to correspond to the rat SDN-POA. In addition, we report a similar sex difference in the guinea pig bed nucleus of the stria terminalis (BNST), another neural region that binds high levels of steroids during the period of sexual differentiation (Sheridan et al., 1975). Finally, we report that neither of these structural sex dimorphisms appears to result from sex differences in the adult hormonal environment. Some of these data have been presented previously in an abstract (Hines et al., 1983).

Materials and Methods

Subjects. The brains of 32 adult guinea pigs (aged 5 to 9 months) were studied. The animals were of a genetically heterogeneous stock ("Topeka") and had been bred in the colony at the Wisconsin Regional Primate Research Center. Eighteen of the guinea pigs (9 males and 9 females) had been gonadectomized as part of a behavioral study (Hines et al., 1982a). Fourteen of the guinea pigs (7 males and 7 females) were gonadally intact.

Procedures. Gonadectomies were conducted under Metofane anesthesia at least 2 weeks before sacrifice. For sacrifice, animals were given an overdose of Nembutal and were perfused intracardially with 0.9% saline followed by 10% neutral formalin. Brains were removed and postfixed in this formalin solution for approximately 14 days, then

Figure 3. Photomicrographs illustrating a sex difference in the BNST in the guinea pig. A darkly staining portion of this region (arrows) is larger in the male (top row) than in the female (bottom row) brain. The illustration is of 60- μ m frozen sections taken in a coronal plane corresponding to that of de Groot in the rat and stained with thionin. The sex difference is shown at four levels (A through D). A is approximately 150 μ m caudal to the beginning of the region. B, C, and D are each approximately 300 to 350 μ m further caudal, and D is approximately 150 μ m rostral to the end of the region. The sections are from the intact male and female animals in which the volumes of the sexually dimorphic region were closest to the means for their respective groups. FX, fornix; LV, lateral ventricle; OT, optic tract.

frozen-sectioned at 60 μ m in a coronal plane corresponding to that of de Groot (1959) in the rat, and stained with thionin.

For quantitative analysis, serial sections from these brains were projected onto paper at $\times 43$ magnification, and the boundaries of portions of the MPOA and BNST were traced. The brains of all 32 animals were included in analysis of the MPOA. The brains of 27 animals (7 intact males, 6 intact females, 9 gonadectomized males, and 5 gonadectomized females) were included in analysis of the BNST.

Our definition of the MPOA was based on the description of this area provided originally by Gurdjian (1927). The area of interest within the MPOA was a darkly staining group of cells corresponding to the central portion of the medial division of the MPOA, or the SDN-POA, as described in previous studies of the rat (e.g., Gorski et al., 1978; Simerly et al., 1984). Our definition of the BNST was based on the descriptions provided originally by Johnston (1923) and Gurdjian (1927). The specific area of interest within this region was a darkly staining group of cells located in the caudal portion of the medial division of the nucleus. This cell-dense region is surrounded by a cell-sparse zone and has been referred to in the rat and the rabbit as the "encapsulated region" or "special nucleus" of the BNST (Johnston, 1923; Young, 1936; McDonald, 1983). In the guinea pig the region appears rostrally near the base of the fornix, approximately 300 μ m caudal to the sexually dimorphic regions of the MPOA. In subsequent sections its location is dorsal and then lateral, accompanying the stria terminalis beneath the ventricles, and terminating near their lateral extremes.

Each brain was analyzed by three investigators acting independently and without knowledge as to the sex of individual animals. A final outline, made up of those areas included within the boundaries of the sexually dimorphic region by at least two of the three investigators, was then drawn for each brain section, and the area of the outline was

determined using a bit pad and microcomputer. The volumes of the sexually dimorphic portions of the MPOA and BNST were calculated for each animal by summing these area values, multiplying by the thickness of the sections, and dividing by the magnification at which the original tracings were made.

To evaluate whether the sex differences were specific to these two steroid-binding areas, we also assessed the volume of an area that does not bind steroids, the suprachiasmatic nucleus (SCN). In addition, we measured four general indices of brain size: preoptic area height, septal width, and overall brain height and width. These four measurements were taken at the level of the closing of the anterior commissure.

Statistical analysis. Two-way (sex $\times$ gonadal status) analyses of variance (ANOVAs) were used to investigate: (1) possible sex differences in the volumes of the darkly staining portions of the MPOA and the BNST; (2) the influence of gonadectomy on the volumes of these regions; and (3) the relationship of any sex differences in these regions to adult gonadal status. In these analyses both sex (male *versus* female) and gonadal status (intact *versus* gonadectomized) were between-groups factors. Similar two-way ANOVAs were used to analyze control variables: SCN volume, preoptic area height, septal width, and overall brain height and width.

Results

The two-way (sex $\times$ gonadal status) ANOVA of data for the MPOA revealed a main effect of sex ($p < 0.001$). The mean volume of the darkly staining portion of this region was severalfold larger in male than in female guinea pigs (mean $\pm$ SEM = 9.6 ± 0.8 and 2.3 ± 0.3 $\text{cm}^3 \times 10^{-3}$ for male and female animals, respectively). The sexually dimorphic region is illustrated in Figure 1 (see also Fig. 5). There was neither a main

Figure 4. Photomicrographs illustrating the relationship of the sexually dimorphic portion of the BNST to other neural structures. Arrows indicate the sexually dimorphic region in the male (top) and female (bottom) brain. The sections are the same as those illustrated in Figure 3C. CPU, caudate putamen; LV, lateral ventricle; OT, optic tract.

effect of gonadal status, nor an interaction between sex and gonadal status. Subsequent group comparisons indicated that the volume of the darkly staining region of the MPOA was greater in male than in female guinea pigs, regardless of whether they were intact ($p < 0.001$) or gonadectomized ($p < 0.001$). Also, consistent with the lack of a main effect of gonadal status, there were no differences between intact and gonadectomized male animals or between intact and gonadectomized female animals in the volume of this region (Fig. 2).

The volume of the darkly staining portion of the second steroid-binding area, the BNST, was also larger in male than in female guinea pigs ($p < 0.001$ for the main effect of sex in the two-way (sex $\times$ gonadal status) ANOVA). The magnitude of the sex difference was 36% (mean $\pm$ SEM = 34.6 ± 1.2 and $25.5 \pm 1.1 \text{ cm}^3 \times 10^{-3}$ for male and female animals, respectively). This sexually dimorphic region is illustrated in Figures 3 to 5. There were no other significant effects, and group comparisons

Figure 5. A reconstruction in the sagittal plane of the sexually dimorphic regions of the preoptic area (SD-POA) and of the bed nucleus of the stria terminalis (SD-BNST). The reconstruction is based on serial analyses of coronal sections from three male and three female guinea pigs. These animals were selected for use in the reconstruction because the volumes of the sexually dimorphic regions in their brains resembled the means for male and female animals. Vertical dashed lines indicate the approximate planes of section illustrated by photomicrographs in Figures 1, 3, and 4. Line 1 corresponds to Figure 1, line 2 to Figure 3A, line 3 to Figure 3B, line 4 to Figure 3C (and to Fig. 4), and line 5 to Figure 3D. AC, anterior commissure; FX, fornix; OC, optic chiasm.

indicated that the volumetric sex difference was present in both intact ($p < 0.01$) and gonadectomized ($p < 0.001$) animals. Furthermore, gonadectomy had no effect on the volume of this region in either male or female guinea pigs (Fig. 6).

The sex differences in the MPOA and the BNST were not due to general differences in the brains of male and female guinea pigs. No sex differences were seen in the volume of the SCN or in preoptic area height, overall brain height, or overall brain width (Table I). Although there was a sex difference in septal width in this study ($p < 0.02$), the difference was much smaller than that seen in either the MPOA or BNST. Also, a previous study did not find a sex difference in this measure (Hines et al., 1982b), and the volumetric sex differences in the MPOA and BNST remained in the present study, even when each animal's score was adjusted for septal width (Table II).

It should be noted that there were differences between intact and gonadectomized animals in two of the general indices of brain size: brain height ($p < 0.004$) and preoptic area height ($p < 0.002$). Regardless of sex, these measures were greater in the gonadectomized than in the intact guinea pigs (Table I). However, these differences probably are attributable to age, rather than to gonadal status. Because of their use in a behavioral study (Hines et al., 1982a), the gonadectomized animals were 1 to 4 months older at sacrifice than the intact animals. Nevertheless, to ensure that these generalized differences in the brain were not distorting results, we reanalyzed the data after adjusting each animal's score for brain height and preoptic area height. Both of these analyses produced the same results as

Figure 6. The sex difference in the BNST of the guinea pig brain in intact and gonadectomized animals. The volume of the region is greater in male than in female animals and is unaffected by gonadectomy in adulthood. Data are means $\pm$ SEMs. **, $p < 0.01$; ***, $p < 0.001$ for comparison to comparable female group.

those reported for unadjusted scores and for scores adjusted for septal width (Table II).

Finally, the brains of intact animals in this study had been analyzed also in preliminary studies of sex differences in the guinea pig MPOA (Hines et al., 1982b) and BNST (unpublished data). Thus, we had an opportunity to assess the reliability of our volume estimation procedure. The Pearson product-moment correlation coefficient for estimates of the volume of the sexually dimorphic region of the MPOA in the 14 animals included in both studies was 0.88 ($p < 0.001$). The correlation for the 13 animals in which the sexually dimorphic portion of the BNST was assessed twice was 0.74 ($p < 0.001$).

Discussion

The data presented in this paper support three major points: (1) there is a sex difference in the MPOA of the guinea pig that resembles the SDN-POA of the rat; (2) there is a similar sex difference in another steroid-binding region of the guinea pig brain, the BNST; and (3) neither of these neural sex differences results from differences between male and female animals in circulating levels of gonadal steroids in adulthood. Each of these points will be discussed in turn.

TABLE I
SCN volume and general indices of brain size in intact and gonadectomized male and female guinea pigs

	Intact		Gonadectomized	
	Male (N = 7)	Female (N = 7)	Male (N = 9)	Female (N = 9)
SCN Volume (cm ³ × 10 ⁻³) ^a	13.77 ± 0.59 ^b	13.27 ± 0.56	13.19 ± 0.58	11.87 ± 0.53
Preoptic Area Height (cm × 10 ⁻¹)	2.66 ± 0.07	2.70 ± 0.07	2.93 ± 0.06	2.88 ± 0.06
Brain Height (cm)	1.16 ± 0.03	1.16 ± 0.02	1.24 ± 0.02	1.22 ± 0.02
Brain Width (cm)	2.06 ± 0.02	2.09 ± 0.03	2.10 ± 0.02	2.11 ± 0.03
Septal Width (cm × 10 ⁻¹)	4.20 ± 0.06	4.10 ± 0.07	4.34 ± 0.06	4.17 ± 0.04

^a For SCN volumes, N = 6 intact males, 7 intact females, 5 gonadectomized males, and 8 gonadectomized females.

^b Data are means $\pm$ SEM. Values for Preoptic Area Height and Brain Height are greater in gonadectomized than in intact animals ($p < 0.005$), and values for Septal Width are greater in male than in female animals ($p < 0.02$).

The observation of a sex difference in the guinea pig brain similar to the rat SDN-POA disagrees to some extent with a previous report. Bleier et al. (1982) identified a group of cells corresponding to the SDN-POA of the rat in the male, but not the female, guinea pig. In the female animal they could not distinguish this region, because it appeared immediately adjacent to another densely cellular region. In our material a group of cells corresponding to the rat SDN-POA could be distinguished in both the male and female guinea pig, and there was no adjacent densely cellular region in either sex (e.g., see Fig. 1). Although we cannot be certain of the source of the discrepancy between our observations and those reported previously, one possibility involves differences in histological procedures. For example, the previous authors used celloidin-embedded sections, whereas we used frozen sections. The celloidin procedure may have made it more difficult to distinguish subgroups of cells.

As mentioned in the introduction, gross morphological sex differences have been described also in the MPOA of the ferret (Tobet et al., 1983) and the gerbil (Yahr and Commins, 1982). In contrast, there are two reports suggesting that no such sex difference exists in the brain of the mouse (Bleier et al., 1982; Young, 1982). These differences among species may provide a clue as to the function of the SDN-POA. Young (1982), for example, has suggested that masculine sexual behavior, like the SDN-POA, is more strikingly dimorphic in rats than in mice. Based on this suggestion and on a variety of studies indicating involvement of the MPOA in male sexual behavior (e.g., Larsen and Heimer, 1964; Malsbury, 1971; Christensen and Gorski, 1978), Young (1982) proposed that the SDN-POA may be related functionally to male sexual behavior. Our observations regarding the magnitude of the sex difference in the guinea pig MPOA appear to be consistent with this suggestion. The guinea pig volumetric difference (4.3-fold) is greater than that seen in recent studies using the same procedures in the rat (e.g., Döhler et al., 1982).⁴ In keeping with this greater neural dimorphism,

⁴ The original report on the rat SDN-POA (Gorski et al., 1978) found an 8-fold sex difference, but the procedures used in that report differed from those used in the present study and other more recent studies.

TABLE II
Ratio of the volume of the two sexually dimorphic brain regions to general indices of brain size

Ratio	MPOA				BNST			
	Intact		Gonadectomized		Intact		Gonadectomized	
	Male (N = 7)	Female (N = 7)	Male (N = 9)	Female (N = 9)	Male (N = 7)	Female (N = 6)	Male (N = 9)	Female (N = 5)
Volume: Septal Width	21.8	6.2	23.8	5.2	81.3	63.3	83.0	63.1
	±	±	±	±	±	±	±	±
	1.2 ^a	1.2	3.2	0.8	4.8	5.1	2.7	3.0
Volume: Preoptic Height	33.7	9.2	34.3	7.5	126.7	92.6	121.2	90.8
	±	±	±	±	±	±	±	±
	2.0	1.7	4.2	1.2	10.6	7.9	2.2	5.2
Volume: Brain Height	7.7	2.1	8.1	1.7	29.1	21.6	28.6	20.9
	±	±	±	±	±	±	±	±
	0.4	0.4	1.0	0.2	2.4	1.7	0.7	0.9

^aData are means ± SEM. All values for male guinea pigs are significantly greater than those for comparable female guinea pigs ($p < 0.001$) except for BNST values in intact animals ($p < 0.01$).

male sexual behavior, at least as reflected in mounting, appears to be more dimorphic in the guinea pig than in the rat (for example, cf. Phoenix et al., 1959, and Whalen and Edwards, 1967). It should be pointed out, however, that selective lesions of the SDN-POA appear to have no influence on male sexual behavior in the rat, whereas lesions of comparable size in a more dorsal portion of the MPOA impair mounting, intromission, and ejaculation (Arendash and Gorski, 1983). Although alternative explanations may be possible, these data do not support the suggestion that the SDN-POA is involved in male sexual behavior.

In addition to documenting the existence of a sex difference in the MPOA of the guinea pig, the present study described and quantified a sex difference in another steroid-binding region of the brain: the BNST. Specifically, this sex difference involves a darkly staining group of cells located in the caudal portion of the medial BNST. This cell group appears to correspond to the "special" nucleus of the stria terminalis described by Johnston (1923) in the rat and the "encapsulated region" described by Young (1936) in the rabbit. A sex difference in the BNST also has been reported in the hamster, but the dimorphic region in the hamster appears to be rostral to the sexually dimorphic region in the guinea pig BNST and may be continuous with the region corresponding to the SDN-POA of the rat (Bleier et al., 1982). However, our observations in the rat suggest that there is a sex difference in the BNST of this species that corresponds to that seen in the guinea pig (M. Hines, S. Paul, and R. A. Gorski, unpublished data).

Interestingly, the location of the sex difference in the guinea pig BNST coincides roughly with the location of steroid-binding neurons as indicated by autoradiographic studies in this species. Although steroid-binding neurons are distributed throughout the region, the label is heaviest in the caudal BNST (Sar and Stumpf, 1975). Morphological sex differences may characterize areas of the brain that bind gonadal steroids during periods of development when hormones influence functional sexual differentiation. In keeping with this possibility, sex differences have been reported recently in the rat in the volume of two other steroid-binding regions: the medial amygdala (Mizukami et al., 1983) and the ventromedial hypothalamus (Matsumoto and Arai, 1983).

The present study also suggests that neither the sex difference in the MPOA nor the BNST of the guinea pig is influenced by the adult hormonal environment. The sex differences were of comparable size in both intact and gonadectomized animals, and gonadectomy appeared to be without influence on the volume of the sexually dimorphic regions in either sex. Gonadectomized animals were somewhat older than intact ani-

mals, however, and perhaps because they were older, they showed increased brain height and preoptic area height. It is possible, therefore, that decreases in the volume of the sexually dimorphic portions of the MPOA and BNST due to gonadectomy were offset by increases due to age. Several lines of evidence argue against this possibility, however. First, it seems unlikely that age-related increases would be balanced perfectly by gonadectomy-related decreases, particularly in both sexes. Second, adjustment of volume scores by the parameters that showed age-related increases (brain height and preoptic height) failed to reveal effects of gonadectomy. Finally, because the sex differences in the volumes of the darkly staining portions of the MPOA and BNST were present in gonadectomized as well as intact animals, factors other than circulating gonadal hormones must be responsible, at least to some extent, for the differences.

What might such factors be? The possibilities include: (1) the genome, (2) the steroid environment during early development, (3) the steroid environment at a later time (e.g., puberty), and (4) experience. Although none of these possibilities can be eliminated on the basis of the available data, the second seems the most likely. The steroid environment during early development has permanent influences on genital morphology, sexual behavior, and gonadotropin regulation in the guinea pig (Phoenix et al., 1959; Goy et al., 1964; Brown-Grant and Sherwood, 1971; Hines et al., 1982a). In contrast, no comparable influences on sexual differentiation have been linked in this species to the genome, the later hormonal environment, or experience. Furthermore, the volume of the SDN-POA of the rat, which, like that of the guinea pig, is independent of the adult hormonal environment (Gorski et al., 1978), appears to be determined by the early hormonal environment (Döhler et al., 1982).

The independence of neural sex differences from the adult hormonal environment in the rat and the guinea pig appears to contrast with the situation in the gerbil. In this species, sex differences in the MPOA seem to be influenced by the adult hormonal environment. For instance, a cell-dense region (called the pars compacta of the sexually dimorphic area), that appears to correspond to the SDN-POA of the rat, is reduced in size by 50% following castration of adult male gerbils (Commins and Yahr, 1984). This difference between gerbils and other rodents in the dependence of neural sex differences on the adult hormonal environment remains to be explained. Like differences between species in the magnitude of the MPOA sex difference, however, it may provide a clue as to the function of the dimorphic region. For example, in canaries, sexually dimorphic neural regions involved in vocal communication are influenced

by hormonal changes in adulthood (Nottebohm, 1980, 1981), and it has been suggested that sex differences in the gerbil brain may be related to another type of communication: scent marking (Yahr and Commins, 1982).

Returning to the hypothesis that neural regions that bind steroids during early development may be characterized by structural sex differences, we would like to suggest also that the sexually dimorphic portions of these areas may form an anatomically and functionally interrelated neural system. There are anatomical connections among the steroid-binding areas, including those in which volumetric sex differences have been identified (the MPOA, BNST, medial amygdala, and ventromedial hypothalamus). The MPOA appears to project to and receive projections from the BNST (Conrad and Pfaff, 1976; Swanson and Cowan, 1979) and the ventromedial hypothalamus (Conrad and Pfaff, 1976; Saper et al., 1976; Swanson, 1976), and may be interconnected with the medial amygdala (Conrad and Pfaff, 1976; Berk and Finkelstein, 1981), although projections between the MPOA and the medial amygdala have not been reported in all investigations (e.g., see Swanson, 1976; Krettek and Price, 1978). Also, in addition to its connections with the MPOA, the BNST has reciprocal connections with the medial amygdala (de Olmos and Ingram, 1972; Conrad and Pfaff, 1976; Krettek and Price, 1978; Swanson and Cowan, 1979) and receives projections from the ventromedial hypothalamus (Saper et al., 1976; Swanson and Cowan 1979). It may also project to the ventromedial hypothalamus (Conrad and Pfaff, 1976), although this projection was not seen in another investigation (Swanson and Cowan, 1979). Similarly, there are reciprocal connections between the medial amygdala and the ventromedial hypothalamus (Saper et al., 1976; Krettek and Price, 1978), in addition to the previously cited connections between these two regions and the MPOA and BNST.

Because neural sex differences have been identified only recently, we do not know whether the projections among these four steroid-binding areas interconnect their sexually dimorphic components. However, the available evidence appears to be consistent with this possibility. For example, Krettek and Price (1978) reported that the medial amygdala "sends fibers to the medial division of the BNST." Although it would be premature to overemphasize this report in the present context, it suggests the possibility of a specific connection between the sexually dimorphic region of the BNST and the medial amygdala. Substantiation of this possibility, and the possibility of other interconnections specific to sexually dimorphic regions, awaits further study.

With regard to functional relationships, lesion, implant, and stimulation studies suggest that the MPOA, BNST, medial amygdala, and ventromedial hypothalamus are involved in control of several sexually dimorphic functions, including gonadotropin regulation, sexual behavior, maternal behavior, marking behavior, avoidance learning, social play behavior, feeding, and regulation of body weight (for reviews, see Kaada, 1972; Pfaff and Keiner, 1973; Saper et al., 1976; Goy and McEwen, 1980; Yahr and Commins, 1982; Beatty, 1984). In the case of at least one of these functions, gonadotropin regulation, the four regions are mutually involved. Again, the available evidence is not sufficient to determine whether these functional relationships are specific to the sexually dimorphic portions of these regions. However, further attention to these sexually dimorphic subregions, and to possible connections among them, may help elucidate more precisely the neural basis of sexually dimorphic functions.

Finally, neural sex differences such as those in the MPOA and the BNST provide an opportunity to investigate the basic mechanisms underlying hormonal influences on sexual differentiation. Identification of a sexually dimorphic neural system might enhance such investigations. For example, one influence

of gonadal steroids on neural development during the period of sexual differentiation is an increase in neurite outgrowth (Toran-Allerand, 1980; Toran-Allerand et al., 1980). The existence of an interconnected, sexually dimorphic neural system might suggest that the role of steroids in sexual differentiation is related, at least in part, to establishing appropriate connections among the components of this system.

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