unequal variances and sample sizes. Calculations were done by the method of F. E. Satterthwaite [*Biom. Bull.* **2**, 110 (1946)] as described in G. W. Snedecor and W. G. Cochran, *Statistical Methods* (Iowa State Univ. Press, Ames, ed. 7, 1980), pp. 96–98.
11. J. L. Goldstein, R. G. Anderson, M. D. Brown, *Nature (London)* **279**, 679 (1979); M. C. Willingham and I. Pastan, *Cell* **21**, 67 (1980).
12. V. A. Lennon, in *Cholinergic Mechanisms and Psychopharmacology*, D. J. Gender, Ed. (Plenum, New York, 1978), pp. 77–92; J. P. Merlie, S. Heinemann, J. M. Lindstrom, *J. Biol. Chem.* **254**, 6320 (1979); C. G. Reiness, C. B. Weinberg, Z. W. Hall, *Nature (London)* **274**, 68 (1978); D. Axelrod, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4823, (1980).
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Sex Differences in Dendritic Structure in the Preoptic Area of the Juvenile Macaque Monkey Brain

Abstract. *Quantitative analysis of Golgi-stained neurons in the preoptic area of the brain of prepuberal* Macaca fascicularis *monkeys indicated structural differences between males and females. Neurons of males had more dendritic bifurcations and a higher frequency of spines. The bifurcation difference appeared in all cell types and was concentrated in the ventrolateral preoptic area. The spine difference was greatest in the central region of the preoptic area. No differences in gross measurements of this brain region were found. These results suggest that sexual dimorphism in the function of the monkey preoptic area may be based on differences in neuronal structure.*

Sex differences in adult mammalian reproductive physiology and behavior depend largely on differences in the organization of the brain, which are activated in the adult by circulating hormones (*1*). To a great extent, these sex differences in brain organization arise in response to gonadal steroid hormones present in the circulation during sensitive periods before or after birth (*2*). Recent research has indicated that sexual dimorphism in brain function may reflect differences in brain structure. For example, the preoptic area of the hypothalamus, which appears critical to male copulatory behavior and which is also involved in phasic female endocrine regulation in rodents (*3, 4*), exhibits sexual dimorphism in synaptic termination patterns (*5*), regional cell size and packing density (*6*), and dendritic density patterns (*7*). Sex differences have also been demonstrated in other parts of the rodent brain (*8*) and in the size of certain brain nuclei and one type of nerve cell in avian species (*9*).

We know relatively little about sex differences in the structure of the primate brain. There are sex differences in the overall size of the brain in humans and other primates (*10*), and there is evidence for human sex differences in morphological brain asymmetry (*11*). At a finer structural level, the only primate sex difference reported to date is in larger nerve cell nuclei in the amygdala of male than of female squirrel monkeys (*12*). These were intact adults whose gonads were releasing different hormones. Thus the nuclear size differences could reflect dynamic responses to circulating hormones rather than a stable sex difference (*13*). Because the preoptic area shows the greatest variety of reported structural dimorphisms in rodents, it seems a likely site for structural dimorphism at the cellular level in primates. This region is essential to normal male sexual behavior in macaques. Although it appears to be unnecessary for cyclic gonadotrophin regulation in primates in contrast to rodents (*3*), it probably plays a modulatory role (*14*). We used quantitative measurements of Golgi-stained neurons in the juvenile macaque monkey preoptic area and report here evidence for structural sexual dimorphism prior to the age of onset of adult sex differences in circulating hormones, which might affect these measures (*15*).

Four male and four female *Macaca fascicularis* monkeys, between 8 months and 2 years of age, were used. The preoptic area (POA) and surrounding tissue was dissected from the brain, block-stained according to a Golgi-Cox procedure (*16*), embedded in celloidin, and sectioned at a thickness of 100 μm in the coronal plane. Each subject was assigned a numerical code that did not reveal its sex. For sampling purposes, the POA was defined as a rectilinear solid lying under the anterior commissure (*17*). All well-impregnated neurons whose processes were not grossly attenuated by sectioning or otherwise obscured were traced by hand at ×500 magnification using a camera lucida microscope attachment. No attempt was made to follow dendritic processes in adjacent sections. Neurons were subclassified on the basis of dendritic spine frequency (*18*). (The location of each soma with regard to the brain's midline and the center of the anterior commissure was also recorded.) A total of 809 neurons from females and 980 neurons from males were drawn. The drawings were analyzed by counting and measuring the projected length of each dendritic branch. Branches were grouped in terms of their order away from the cell body, where first order refers to a branch from the cell body, second order, a branch beyond a bifurcation, and so forth.

The POA neurons were predominantly bipolar cells with a relatively simple dendritic field. Two major types of sex differences were evident. (i) There were approximately 20 percent more dendritic branches per neuron in males than in females (Table 1). The mean number of branches in every male exceeded that in every female. There was no statistical difference in the number of branches

Table 1. Brain measurements. Data are expressed as weighted mean ± standard error of the mean based on four individual subject means (*19*). Abbreviations: M, male; F, female; S, sparse; CC, corpus callosum; AC, anterior commissure; and IC, internal capsule.

Sex	Branches per neuron					Dendritic length (μm)	Percent neurons classified			Gross brain size	
	Order				Total		S	Intermediate	Spiny	CC to AC (mm)	Midline to IC (mm)
	1	2	3	4							
M	2.41 ± 0.04	3.05 ± 0.12	1.26 ± 0.10	0.26 ± 0.05	7.02 ± 0.29	299.4 ± 13.1	32.3	44.2	23.5	6.31 ± 0.06	2.36 ± 0.09
F	2.39 ± 0.02	2.64 ± 0.11*	1.01 ± 0.10	0.14 ± 0.04	6.19 ± 0.27†	277.2 ± 8.2	38.6	40.9	20.5‡	6.16 ± 0.08	2.44 ± 0.10

*$F(1, 6) = 6.58$; $P < .05$. †$F(1, 6) = 6.00$; $P < .05$. ‡χ^2 (2) = 7.4; $P < .025$ (*21*).

arising from the soma. The total projected dendritic length did not differ [but see (*19*)]. Thus, the sexes differed in the tendency for dendrites of POA neurons to bifurcate. (ii) Females had a higher relative frequency of neurons classified as sparsely spined and correspondingly fewer neurons classified as spiny (Table 1) (*20*).

The difference in neuronal branching was largely confined to the ventrolateral part of the sample area. In contrast, the sex difference in neuronal spine frequency was evident throughout the sample region, although the difference tended to be most pronounced in the middle third of the dorsal-ventral extent of the sample area (*21*).

Neither of these sex differences appears to be attributable to sex dimorphism in the gross size of the brain region under study. (i) The sample region was adjusted for individual differences in brain size (*17*). (ii) Our measures of the distances between the corpus callosum and the anterior commissure and between the midline and the medialmost extent of the internal capsule revealed no statistical or apparent sex dimorphism (Table 1). The difference in branching does not seem to arise from an orientation difference, since the percent of branches truncated by the section plane was 18 percent in males and 20 percent in females.

The spine frequency difference is surprising in light of the previously reported tendency for non–stria terminalis preoptic afferents to terminate more frequently on spines in female than in male rats (*5*). Overall, in these macaques, the recipient neurons do not seem to reflect such a characteristic. Instead, the sex differences reported here (more branched dendrites with higher numbers of spines in males) suggest that neurons of males may be differentiated to provide more, and perhaps different types of, synaptic connections.

To our knowledge, these data are the first to suggest a sex difference in the fine structural organization and, perhaps, the functional "wiring diagram" of a primate brain. Since we used juvenile animals in which circulating gonadal steroid hormone differences are minimal (*15*), it seems unlikely that these differences depend on gonadal steroid influences at the time the animals were studied. Thus the differences in bifurcation tendency and spine frequency probably reflect either the masculinizing influence of testosterone in the male during the prenatal sensitive period or some other manifestation of the presence of different sex chromosomes. Although the functional significance of this and other POA sex differences at the cellular level remain uncertain, they may be related to sex differences in the function of the POA in reproduction in the monkey.

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References and Notes

1. R. W. Goy and B. S. McEwen, *Sexual Differentiation of the Brain* (MIT Press, Cambridge, Mass., 1980).
2. N. J. MacLusky and F. Naftolin, *Science* **211**, 1294 (1981); C. H. Phoenix, R. W. Goy, A. A. Gerall, W. C. Young, *Endocrinology* **65**, 369 (1959); C. H. Phoenix, R. W. Goy, J. A. Resko, in *Perspectives in Reproduction and Sexual Behavior*, M. Diamond, Ed. (Indiana Univ. Press, Bloomington, 1969), pp. 33–49.
3. C. A. Barraclough, S. Yrarrazoval, R. Hatlan, *Endocrinology* **75**, 838 (1964); D. M. Cramer and C. A. Barraclough, *ibid.* **88**, 1175 (1971); G. Bermant, S. E. Glickman, J. M. Davidson, *J. Comp. Physiol. Psychol.* **65**, 118 (1968); R. D. Nadler, *ibid.* **66**, 157 (1958); R. D. Lisk, *Brain Res.* **5**, 306 (1968); A. A. Perachio, L. D. Marr, M. Alexander, *ibid.* **177**, 127 (1979); J. C. Slimp, B. L. Hart, R. W. Goy, *ibid.* **142**, 105 (1978).
4. B. Powers and E. S. Valenstein, *Science* **175**, 1003 (1972). A ventral region (medial preoptic nucleus) seems to be most critical to rodent gonadotrophin surges [J. A. Clemens, E. B. Smalstig, B. D. Sawyer, *Endocrinology* **99**, 728 (1976); S. J. Weigand, E. Terasawa, W. E. Birdson, R. W. Goy, *Neuroendocrinology* **31**, 147 (1980)].
5. G. Raisman and P. M. Field, *Science* **173**, 731 (1971); *Brain Res.* **54**, 1 (1973).
6. R. A. Gorski, J. H. Gordon, J. E. Shryne, A. M. Southam, *Brain Res.* **148**, 333 (1978); R. A. Gorski, R. E. Harlan, C. D. Jacobson, J. E. Shryne, A. M. Southam, *J. Comp. Neurol.* **193**, 529 (1980); C. D. Jackson, V. D. Csernus, J. E. Shryne, R. A. Gorski, *J. Neurosci.* **1**, 1142 (1981).
7. W. T. Greenough, C. S. Carter, C. Steerman, T. J. DeVoogd, *Brain Res.* **126**, 63 (1977).
8. A. Matsumoto and Y. Arai, *Neuroendocrinology* **33**, 166 (1981); D. W. Pfaff, *J. Endocrinol.* **36**, 415 (1966); J. Yanai, *Acta Anat.* **103**, 150 (1979).
9. T. J. DeVoogd and F. Nottebohm, *J. Comp. Neurol.* **196**, 309 (1981); M. E. Gurney and M. Konishi, *Science* **208**, 1380 (1980); F. Nottebohm and A. P. Arnold, *ibid.* **194**, 211 (1976).
10. R. L. Holloway, *Am. J. Phys. Anthropol.* **53**, 109 (1980); C. de Lacoste-Utamsing and R. L. Holloway, *Science* **216**, 1431 (1982); F. Tilney and H. A. Riley, *The Brain from Ape to Man* (Hoeber, New York, 1928).
11. A. Rubens, M. Mahowald, J. Hutton, *Neurology* **26**, 620 (1976); J. Wada, R. Clarke, A. Hamm, *Arch. Neurol.* **32**, 239 (1975).
12. G. A. Bubenik and G. M. Brown, *Experientia* **29**, 619 (1973).
13. ———, *ibid.* **30**, 378 (1974); J. Szentágothai, B. Flerkó, B. Mess, B. Halász, *Hypothalamic Control of the Anterior Pituitary, An Experimental-Morphological Study* (Akademiai Kiado, Budapest, 1962).
14. Cyclic gonadotrophin surges occur in animals with severe damage anterior to the medial basal hypothalamus [T. M. Plant, J. Moossy, D. L. Hess, Y. Nakai, J. T. McCormack, E. Knobil, *Endocrinology* **105**, 465 (1979); D. L. Hess, R. M. Wilkins, J. Moossy, J. L. Chang, T. M. Plant, J. J. McCormack, Y. Nakai, E. Knobil, *ibid.* **101**, 1264 (1977)]. However, electrical stimulation of the preoptic-suprachiasmatic region can evoke gonadotrophin release, suggesting a modulatory role for this region [H. G. Spies, R. L. Norman, S. K. Quandri, D. K. Clifton, *ibid.* **100**, 314 (1977)].
15. J. A. Resko, *ibid.* **87**, 680 (1970).
16. H. Van Der Loos, *Monatsschr. Psychiatr. Neurol.* **132**, 331 (1956).
17. The POA was defined as a rectilinear solid extending 0.2 mm anterior to the furthest anterior extent of the midline anterior commissure, 0.2 mm anterior to furthest posterior extent of the midline anterior commissure, ventrally from the anterior commissure's lowest point to a distance equal to 40 percent of the distance from midline corpus callosum to midline anterior commissure, and laterally, beginning 0.3 mm from the third ventricle, for half the distance to medial extent of the internal capsule. These measurements were designed to equate for individual differences in brain size. Numbers of neurons from individual subject were females: 273, 161, 195, 180; males: 354, 269, 221, 136. The smaller number of neurons in two subjects reflects loss of tissue from one hemisphere as a result of sampling for another study.
18. Neurons were categorized as sparsely spined (fewer than one per ten linear micrometers of dendrite), intermediate (one to five spines per ten micrometers), or spiny (more than five per ten micrometers). Dendrites of a given neuron had generally equivalent spine frequency. For consistency, however, the dendrite parallel to the section plane with the visibly greatest spine frequency was selected, and the region of this dendrite with highest spine frequency, usually approximately one soma diameter from the soma, was counted.
19. Analysis of variance differences reported as statistically significant used one score per subject, the overall mean of its neurons, or a total of eight scores per analysis. If each neuron is used as an independent measure from the respective sex, males exceed females in total projected dendritic length per neuron [$F(1, 1787) = 11.66$, $P < .001$]. The number of third-order branches per neuron also differed by this less conservative statistic [$F(1, 1787) = 12.56$, $P < .001$)]. The number of primary, or first-order, dendrites did not differ ($F = 0.19$).
20. Each neuron was treated as an independent measure from the appropriate sex. Analysis was performed on a 2 (sex) by 3 (spine frequency categories) matrix.
21. Based on chi-square tests in which dorsal, middle, and ventral and medial-lateral equal-sized volumes through the sample area were examined. There was a nonsignificant sex difference in the number of cells sampled across these six subregions, but the differences in branch number and spine density were not uniquely associated with the most disparate regions. Dendritic density distribution differences of the type described in the hamster (*7*) appear to occur in these analyses, but resolution of these differences for groups is complicated by individual size differences in sample areas.
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