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David Michael Ayoub

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IN THE PREOPTIC AREA
OF THE MONKEY
BRAIN

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David M. Ayoub

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Table of Contents

Introduction ............................................. 1
Sexual Dimorphism and Differentiation of Brain Function .. 2
Neural Substrates of Sexually Dimorphic Brain Function.. 6
Mechanisms of Sexual Differentiation ...................... 12
Methods .................................................. 22
Results .................................................. 26
Cell Characteristics ...................................... 26
Dendritic Field .......................................... 29
Discussion ............................................... 30
Cell Structure within the Preoptic Area .................. 30
Sex Differences in Cell Morphology ....................... 32
Conclusion ............................................. 34
References .............................................. 35
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ABSTRACT

A variety of morphological sex differences in rodents has been reported in brain structures, particularly within the preoptic area, which might underlie sex differences in reproductive physiology and behavior. Although work with non-human primates has been limited and less conclusive, it appears that the preoptic area in the monkey mediates sexual behavior but, unlike rodents, is non-essential in cyclic neuroendocrine function. In order to investigate possible sex differences within the monkey preoptic area, eight animals (Macaca fascicularis) were sacrificed at approximately eight months of age and Golgi-Cox stained neurons were examined with the aid of a camera lucida. Each nerve cell was classified according to soma size (small, medium, large) soma shape (round, intermediate, oblong), dendritic thickness (thin, thick) and dendritic spine density (sparse, intermediate, spiny). Dendritic field distribution was reconstructed by use of a grid analysis and, in addition, mean total dendritic length and mean number of bifurcations were calculated for each cell. Results indicated that, regardless of sex, dendrites with an increased spined density were commonly thicker and more often associated with larger somata as well as somata that were round. Females had a significantly higher incidence of sparsely spined dendrites; this was especially pronounced in cells with either round or large somata. Dendritic field analysis revealed a significant 20 percent increase in the mean number of bifurcations/cell, although no sex differences were apparent in mean total dendritic length or the dendritic distribution patterns. These sex differences in cell morphology are not likely to result from the current hormone condition (which is similar in both sexes of juvenile monkeys), but probably is an expression of the differentiating effects of gonadal steroid hormones during the prenatal critical period of development.
SEXUAL DIMORPHISM IN THE PREOPTIC AREA
OF THE MONKEY BRAIN

Each mammalian species has evolved two divisions within their population separated by distinct physiological, anatomical and behavioral characteristics. Classification of these two divisions has been distinguished, of course, as male and female. By strict definition, the female possesses the larger gametes, the ova (or egg), whereas the male gamete, the sperm, is considerably smaller. Paralleling the evolution of distinct gametes were many adaptations that assured maximal reproductive success. The male and female have evolved separate internal and external reproductive structures which define the respective roles of fertilization. Furthermore, the mammalian brain evolved the capacity to support dimorphic reproductive functions. Evidence of sexually dimorphic brain function is primarily twofold. Pituitary gonadotropin secretion regulating gametogenesis in the female is cyclic in nature whereas the male secretory pattern is essentially acyclic, or tonic. Dimorphic brain function is also implicated by behavioral observations, especially those related to reproduction.

Research in the last several decades has centered mainly upon understanding the process of sexual differentiation in the brain, but only recently have there been demonstrated several possible underlying mechanisms. Although the rodent is most frequently used as the experimental animal model, the monkey is fast becoming a common subject of research, primarily because of remarkable similarities to the human with respect to its menstrual cycle and, to a lesser degree, its increased cortical dependence of behavior. Because an overwhelming amount of knowledge of the process of brain differentiation has been gathered from rodent studies, the following survey will review evidence
from both the monkey and rodent species, with special attention maintained to species differences.

**Sexual Dimorphism and Differentiation of Brain Function**

In all mammalian species, the differentiation of sexually dimorphic patterns of physiology and behavior is highly dependent upon exposure to gonadal steroid hormones during an early period of development. This critical period of development in rodent species is contained within the first several days after birth (Harris, 1964). If neonatal female rats are exposed to gonadal steroid hormones, they will develop male reproductive tissues, whereas castrated neonatal male rats will not possess normal male reproductive tissues, but those of a normal female (Schultz and Wilson, 1974). Common to most long-gestation mammals, the events which lead to normal sexual differentiation in the monkey appear to cover a several-week long period extending from the time when dimorphism appears in the developing gonads at about day 38 of gestation and ending approximately at day 90 of gestation (Goy, 1966). If pregnant rhesus monkeys are treated with testosterone within the critical period, a genetic female that is born will be highly masculinized (Phoenix, Goy and Resko, 1968; Wells and Van Wagenen, 1954). These pseudohermaphrodites will possess external genitalia similar to those of a normal male, including a fully developed scrotum and a small but otherwise normal penis. Therefore, the presence of gonadal steroid hormones (i.e., testicular androgens) can permanently organize peripheral reproductive tissues into those of a normal male; only in the absence of such hormonal influence can normal development of female reproductive tissues proceed.

It is clear that normal differentiation of the reproductive tract can be altered by the presence of gonadal steroid hormones, but what evidence exists of induced changes in the brain? Much of the evidence regarding sexual
differentiation of the brain is behavioral, and thus indirect. Male and female rodents differ in the predominant type of sexual posture they assume during copulation. These heterotypical behaviors can be reversed if neonatal genetic females are exposed to appropriate amounts of testosterone or if genetic males are castrated at birth (Levine, 1966). Goy and Phoenix (1971) found that prepubertal female rhesus monkeys treated prenatally with testosterone, in contrast to untreated females, displayed frequencies of mounting behavior closely resembling those of normal males. These pseudohermaphrodites exhibited mature mounting behaviors indistinguishable from those of normal males, characterized by foot-clasping of the partner's ankles during copulation. Male-like mounting behaviors were frequently displayed throughout adulthood as well (Goy, 1970; Goy and Phoenix, 1971), suggesting the permanency of the organizing effects of prenatal testosterone.

Dimorphic behaviors which are influenced by prenatal androgens are not limited to reproductive behaviors, per se. In rodents, sex differences in several nonreproductive behaviors have been reported, including running wheel activity (Gentry and Wade, 1976) aggression (Powell, Francis and Schneiderman, 1971) and feeding (Wade, 1976). Harlow (1965) was among the first to demonstrate the existence of certain sexually dimorphic behavior patterns in normal infant rhesus monkeys, measured in terms of the frequency of occurrence. Goy and Phoenix (1971) reported that prior to puberty, masculinized female rhesus monkeys were similar to normal male monkeys with respect to frequencies of threat, play initiation, play pursuit, and rough-and-tumble play. In adulthood, masculinized females also displayed more aggressive behaviors than female controls, and these levels of aggression approach those seen in normal adult males (Eaton and Rasko, 1974). It should be noted that sexually dimorphic
behavior in infancy can not be attributed to postnatal hormonal conditions, as very little sex difference in testosterone levels during adolescence is known to exist (Kimble, 1973). Evidently, prenatal androgen exposure can organize dimorphic behavior during infancy; however, this must include contributions of social experience which has been found necessary for normal development of all sexually dimorphic social behaviors (Goy and Goldfoot, 1974; Goy and Resko, 1976).

A second line of evidence regarding differentiation of the brain resides in the dimorphic pituitary function of gonadotropin secretion regulating gametogenesis. Normal adult females exhibit a cyclic surge of the gonadotropin, luteinizing hormone (LH) inducing ovulation, whereas the male releases pituitary gonadotropins in a relatively constant, or tonic pattern. Rodent studies revealed that genetic females that had received androgen treatments at birth lost all cyclic pituitary ability in adulthood (Gorski, 1968; Whalen and Edwards, 1967). A closer look at the masculinized female monkey demonstrated that, despite the presence of male external genitalia, normal internal female reproductive organs were present (Goy and Resko, 1972; Phoenix, 1974). Furthermore, at puberty, normal menstrual cycles began, although several months late, with the menstrual flow passing through the penile urethra. Thus, unlike the rodent, cyclic pituitary functions, as well as related internal reproductive tissues, are not irreversibly affected by early androgenic exposure. The inability of prenatal androgens to induce acyclicity in female monkeys was also reported by Goy (1970).

Further distinctions between prenatal response to androgens in rodents and primates with respect to neuroendocrine function were studied by Karsch, Dierschke and Knobil (1973). When castrated adult male rhesus monkeys were given exogenous administration of estrogen, mimicking the normal preovulatory
estrogen rise in females, a subsequent surge of pituitary gonadotropin was produced which was indistinguishable from that observed in identically treated females. In contrast, exogenous estrogen treatment in normal male and masculinized female rats failed to induce gonadotropin surges (Niell, 1972). These important distinctions seen between monkeys and rodents suggest that the effects of androgen on the differentiation of neural substrates mediating gonadotropin release are different. Although both adult male rodents and monkeys normally develop acyclic pituitary function, only in the monkey is this condition reversible. Acyclicity in the normal adult male monkey is continually maintained, perhaps by absence of sufficient circulating estrogen levels and/or by inhibitory influence of testicular androgens.

Other fundamental differences in the mechanisms which govern the surge mode of gonadotropin secretion in monkeys and rodents have also been noted. For example, in order to elicit LH surges in adult female monkeys, plasma estrogen levels not only must exceed a critical threshold value, but this level must be maintained for a period of no less than 12 hours (Knobil, 1974). Response was even more complete if estrogen plasma levels are gradually incremented over a 36 to 42 hour period. The rat brain, however, can respond to increasing estrogen levels within hours of the onset of the stimulus (Caligaris, Astrada and Taleisnik, 1971). Furthermore, effective levels of circulating estrogen produced only a single gonadotropin surge in the monkey, even if elevated levels were maintained for weeks. The rat is capable of responding to high estrogen levels with repetitive gonadotropin surges on a daily basis (Legan and Karsch, 1975). Lastly, the gonadotropin surge mechanism of the rat is coupled to a diurnal light-dark cycle (Schwartz, 1970; Schwartz and McCormack, 1972), whereas the monkey is known to ovulate independently of such cues (Knobil, 1974).
In summary, it has been clearly demonstrated that gonadal steroid hormone exposure during the critical period of development in the monkey or rodent results in peripheral masculinization of reproductive tissues as well as reproductive and certain nonreproductive behaviors that are normally dimorphic. In contrast, cyclic gonadotropin mechanisms are not functionally masculinized in the monkey brain, whereas androgen exposure in neonatal rodents permanently renders subjects acyclic. Other differences between species with respect to neural control of ovulation were cited. Although these studies do not provide evidence of neural substrates which might be involved in regulation of sexually dimorphic behavior and physiology, they do provide the necessity of a more thorough understanding of the levels in the brain at which such dimorphisms may exist.

**Neural Substrates of Sexually Dimorphic Brain Function**

There is little question that the differentiating effects of gonadal steroid hormones during critical periods of development are mediated by the brain. This implies that certain brain regions are at least functionally altered by such hormonal influences.

The hypothalamus, a small area at the base of the brain, is the most intimately related central neural tissue to the pituitary gland. It is well recognized that neurons of the hypothalamus are capable of synthesis and secretion of small peptides which are released into the capillaries of the hypothalamic-portal system and subsequently modify pituitary synthetic and secretory activities (Harris, 1955; Szentágothai, Flerko, Hess and Halász, 1968). One such peptide, known as luteinizing hormone-releasing factor (LH-RH), regulates the activity of luteinizing hormone in the anterior lobe of the pituitary. As previously mentioned, it is the pattern in which LH is released that is sexually dimorphic.
Numerous studies of ovulatory regulation in the adult rat have provided a great deal of evidence implicating hypothalamic and extrahypothalamic involvement. Neural transections which separated the medial basal hypothalamus (MBH) from the preoptic-anterior hypothalamic area (POA-AHA) prevented LH surges, and subsequently, ovulation (Halász and Gorski, 1967; Köves and Halász, 1970). Although cyclicity was blocked in 100 percent of the experimental rats, absence of gonadal atrophy suggested that a tonic LH secretion similarly seen in the normal male rat was operating, and the mechanism responsible for its activity probably resided in the MBH. This was supported from observations of a similar absence of gonadal atrophy in male rats with identical brain transections (Halász and Pupp, 1965).

From these findings it would seem that gonadotropin function is regulated at two distinct levels in the rat brain. The first level of control, represented by the MBH, is probably responsible for a tonic gonadotropin mechanism which maintains the integrity of testicular activity in males and mediates follicular ripening in the female ovary. In normal female rats, the tonic mechanism of gonadotropin control is, in turn, controlled by a second level which appears responsible for preovulatory LH surges. These studies have suggested that at least part of this cyclic mechanism resides in the POA-AHA.

Further implicating the preoptic area in ovulatory control in rats was the demonstration that POA lesions completely eliminated LH surges (Barracough, Yrarrazaval and Hatlan, 1964; Gray, Södersten, Tallentire and Davidson, 1978). Electrical and electrochemical stimulation of the POA has been found to induce preovulatory surges of LH (Clemens, Shaar, Kleber and Tandy, 1971; Cramer and Barracoulgh, 1971, Terasawa and Sawyer, 1969). Crystalline implants of
testosterone propionate placed in the POA of neonatal rats was extremely successful in the masculinizing process (Nalder, 1968; Wagner, Erwin and Critchlow, 1966). This finding is consistent with the discovery of a large number of steroid receptors in the neonatal POA (Pfaff, 1968; Stumpf, 1968; Stumpf, Sar and Keefer, 1974). It is also worth noting that certain limbic structures, especially the amygdala and hippocampus, have been implicated in neural ovulatory control (Ellendorf, 1978; Gorski, 1974; Kawakami and Terasawa, 1972; Velasco and Taleisnik, 1969a,b). Nonetheless, it is currently accepted that the POA is the single most important site of the organizing action of androgens with respect to gonadotropin secretion in rodents.

Similar studies attempting to localized brain regions which regulate male reproductive behavior have also implicated involvement of the preoptic region. Lesions of medial preoptic-anterior hypothalamic regions resulted in loss of copulatory behavior in male rats (Lisk, 1968; Von de Poll and van Dis, 1979). The importance of this region is further supported by the finding that crystalline testosterone propionate implants in the medial preoptic area effectively restored male sexual behavior in castrated male rats, while implantation in surrounding regions was less effective. Electrical stimulation of the POA in male rats also enhanced male sexual behavior (Bermant, Glickman and Davidson, 1968; Malsburg, 1971; Vaughn and Fisher, 1962).

In contrast to male reproductive behaviors, the display of lordosis behavior in female rats does not seem to require the preoptic area. Lesion of this region either facilitated or had no effects on lordosis (Gray, et. al., 1978; Power and Valenstein, 1972; Rodriguez-Sierra and Terasawa, 1979).

Work on the determination of neural substrates involved in dimorphic reproductive function in primates has been limited and less conclusive than
similar rodent studies. Krey, Butler and Knobil (1975), in an attempt to localize the neural components involved in estrogen regulation of gonadotropin secretion in the rhesus monkey, used a modified "Halasz" knife in order to separate the medial basal hypothalamus from all other neural pathways. In animals with complete or anterior deafferentation of the MBH, spontaneous and induced gonadotropin surges were observed, suggesting that the sites of central components controlling tonic and cyclic gonadotropin secretion in the rhesus monkey may reside within the MBH–hypophysial unit.

In contrast, Norman, Rasko and Spies (1976) reported that bilateral lesions of the ventral preoptic-anterior hypothalamus blocked ovulation and rendered the hypothalamo-hypophysial axis incapable of producing spontaneous or induced gonadotropin surges. They suggested that failure of the isolation of the MBH from the POA–AHA to disrupt cyclic pituitary function shown by Krey, et. al. (1975) could be accounted for by rapid neural regeneration of afferent MBH connections or by the transport of a humoral factor, such as a neurotransmitter or releasing-hormone, by some non-neural pathway.

Plant and his colleagues repeated this study by placing bilateral lesions in the rostral hypothalamus of the adult female rhesus monkey resulting in extensive destruction of the ventromedial POA–AHA (Plant, Moossy, Hess, Nakai, McCormack and Knobil, 1979). However, these animals were able to demonstrate gonadotropin surges in response to exogenously induced increments of serum estrogen concentration. The apparent discrepancy from the findings of Norman et. al. (1976) was explained by differences in stereotoxic procedure and subsequent experimental protocol. Norman et. al. (1976) injected a radioopaque dye into the lateral ventricle in order to visualize the third ventricle; however, this dye has been demonstrated to have severe inflammatory effects on the central nervous system, possibly rendering the animals acyclic. A more plausible
explanation of this discrepancy is a difference in quantitative interpretation of qualitatively similar results, since it has been demonstrated that a significant proportion (20%) of female monkeys with regular menstrual cycles fail to respond to positive feedback action of estrogen (Dierschka, Yamaji, Karsch, Wieck, Weiss and Knobil, 1973). Norman et. al. (1976) found that two animals with "effective" lesions did indeed ovulate and Plant et. al. (1979) did acknowledge that not all animals with extensive bilateral destruction of the POA-AHA were capable of gonadotropin surges.

In view of these findings, it would seem that the monkey, in contrast to the rat (Halász and Gofski, 1967; Köves and Hálassz, 1970) does not require a signal from the POA-AHA necessary for the initiation of preovulatory gonadotropin surges. That the basic surge mechanism resides in the MBH-hypophysial unit was further substantiated by the demonstration of estradiol-induced gonadotropin surges in female rhesus monkeys after complete aspiration of all neural tissue dorsal and anterior to the optic chiasm (Hess, Wilkins, Moore, Chang, Plant, McCormack, Nakai and Knobil, 1977). Furthermore, other studies have demonstrated the presence of gonadotropin surges in female rhesus monkeys with bilateral disconnection of the MBH-hypophysial unit, further supporting the work of Krey et. al. (1975) (Ferin, Antunes, Zimmerman, Dyrenfurth, Franz, Robinson and Carmel, 1977; Knobil, 1974).

Although these findings support the idea that the medial basal hypothalamus initiates the cyclic response in female monkeys, it is unlikely that male and female brains do not differentiate with some respect to neuroendocrine control mechanisms and that at least some of the influence of the MBH upon the pituitary gland is extrahypothalamic in nature. Pfaff and his co-workers found that tritiated estradiol binds to remarkably similar regions in the monkey and rat.
brain, including extrahypothalamic structures such as the bed nucleus of the
striata terminalis, portions of the amygdala and the preoptic area (Pfaff, Gerlach,

A second line of evidence of extrahypothalamic input to the monkey MBH
arises from the fact that most non-human primates are seasonal breeders. Although
the rat brain is sensitive to the changes in light on a daily basis, the monkey
may be sensitive to changes in day length over periods of the entire year.
Environmental cues such as these are likely to express themselves to brain
functions through the suprachiasmatic nucleus and preoptic area (Silver and
Brand, 1979). Indeed, electrical stimulation of the preoptic-suprachiasmatic
region has been shown to evoke low-level gonadotropin release in monkeys (Spies,
Norman, Quandri and Clifton, 1977). This strongly suggests that this region
influences the MBH-hypophysial axis.

In contrast to regulation of gonadotropin secretion, regulation of sexual
behavior in the monkey seems to be highly influenced by experimental manipulation
of the POA-AHA and limbic structures. Slipp, Hart and Goy (1978) demonstrated
that bilateral lesions placed in the medial POA-AHA of male rhesus monkeys
greatly reduced or eliminated the display of manual contacts of the partner, as
well as mounts, intromissions and ejaculations. It is interesting to note a
lack of interference with masturbation, indicating that copulatory behavior and
masturbation seem to be different behavioral expressions of male sexuality.
Perachio, Harr and Alexander (1979) demonstrated that electrical stimulation of
the POA elicited mounting behavior, with or without intromission, and thrusting;
however, ejaculation could not be elicited. Furthermore, testosterone propionate
implants restored sexual activity in adrenalectomized-ovariectomized estrogen-
treated female rhesus monkeys when localized in similar regions (Everitt and
Herbert, 1975).
The amygdala also seems to play an important role in sexually dimorphic behavior. Amygdaloid lesions in male stump-tailed macaques produced a decrease in aggression and an increase in sexual behavior; lesions in females increased aggression (Kling, 1974, 1975). Interestingly, Bubenik and Brown (1973) have reported a sex difference in neuronal nuclear sizes within the medial amygdaloid nucleus, with males possessing significantly larger nuclear diameters.

In summary, the preoptic area plays a central role in sexually dimorphic reproductive functions in both rodents and monkeys. The monkey POA is highly implicated in regulation of male sexual behavior and probably influences sexual behavior and hypothalamic gonadotropin regulation in females, although the basic surge mechanism, in contrast to the rat, resides within the MBH-hypophysial unit. The existence of steroid hormone receptors in the monkey POA suggests at least a site at which hormones may direct brain function, and perhaps even present a substrate for the organizational effects of prenatal androgens.

**Mechanisms of Sexual Differentiation of the Brain**

The search for mechanisms which could underlie sex differences in brain function outlined above has primarily focused upon those hypothalamic and limbic structures involved in reproductive physiology and behavior. At the molecular level, Gorski (1971b) suggests several events which could result from prenatal exposure to gonadal steroid hormones after binding to a receptor molecule at the cell membrane of target tissue. These events include (a) changes in membrane configuration, (b) production of the secondary messenger, cyclic AMP, (c) activation of a carrier mechanism, (d) synthesis of a new products (e.g., enzymes, proteins, neurotransmitters, releasing factors) and finally, (e) conversion of androgen to another active form. Functional correlates
might include changes in probability of neuronal firing, alterations in neural transmission physiology, or changes in synthetic, metabolic or secretory activity. Indeed, several chemical synthesis inhibitors have been reported to greatly interfere with neonatal action of androgen in the rat (Gorski, 1971a; Salaman, 1974). That prenatal androgen may influence synthetic processes in the brain does not demonstrate an exact mechanism for masculinization but provides a basis of understanding the very nature of hormonal influences on the developing brain. From this initial understanding of hormone action, several mechanisms of sexual differentiation have been proposed.

Sex Differences in Steroid Receptors

It has been suggested that neonatal exposure to androgen may alter the integrity of the steroid hormone receptor site in neural tissues, perhaps by influencing synthesis and renewal processes or disrupting the biochemical pathways which lead to the transcription and translation of genomic information. Several reporters have shown a decreased estradiol binding capacity in the hypothalamus in androgenized rats (Lisk, 1971; Maurer and Woolley, 1975; Tuohimaa and Johansson, 1971) and hamsters (DeBold, 1978), while other brain regions less involved in reproductive function were unaffected. This suggests that neonatal androgen may interfere with the normal development of estrogen receptor proteins in the hypothalamus, resulting in possible alterations of hypothalamic functions.

Another plausible cause of differential brain development resides in a sex difference in receptor location; however, no conclusive evidence exists which would support this notion. In rodents, estradiol receptors are highly concentrated in the hypothalamus, preoptic area and amygdala (Pfaff, 1968; Keiner, 1973).
Remarkably similar binding regions exist in the monkey brain (Pfaff et al., 1976).

**Sex Differences in Neurotransmitter Function**

Since the biogenic amines appear to be involved in neuroendocrine functions (Fuxe, Hokfelt, Agnati, Lofström, Everitt, Johansson, Johansson, 1976; Kalra, 1976), steroid hormone action during development may produce significant and permanent functional consequences. Fuxe, Hokfelt and Nilsson (1972) reported that the characteristic cyclic change in dopamine turnover in the female rat was eliminated by neonatal administration of androgens. Sex differences in adult amine content have been observed and appear to be modified by perinatal hormone exposure (Giualan, McEwen and Pohorecky, 1974; Hardin, 1973a, Lodasky and Gazira, 1970). Hyyppä (1971) revealed increased neurotransmitter levels of serotonin (5-HT) and norepinephrine in the adult female rat compared to the adult male. With respect to the enzymes involved in the metabolism of catecholamine and 5-HT, monoamine oxidase activity was similar in both sexes during the neonatal period (Hardin, 1973b) but became higher in the brains of female rats during adulthood (Kamberi and Kobayashi, 1970). These studies suggest that hormonal influences differentially affect the enzyme and neurotransmitter synthesis, storage and release processes, as well as the corresponding receptor sites where these functions occur.

A recent study by Dyer, McCleod and Elendorf (1976) provides evidence that can be interpreted in terms of possible disturbances in neurotransmitter function. Using the technique of orthodromic and antidromic indentification of POA neurons, they reported that preoptic cells which project to the medial basal hypothalamus received significantly more synaptic connections from the amygdala in males than females or neonatally castrated males, with androgenized
females falling into an intermediate position. This provides evidence that the connections between the amygdala and POA may be functionally altered by neonatal androgen exposure. In addition, POA neurons which were not influenced by stimulation of the medial basal hypothalamus fired twice as fast in normal females and neonatally castrated males than either normal males or masculinized females.

These differences in responsivity of the POA cells may reflect a difference in "affective connectivity", meaning that disturbances in neurotransmitter processes, rather than actual dimorphisms in morphological connectivity, may exist and underlie differences in electrophysiological neural transmission.

Morphological Sex Differences

This final possibility of steroid hormone action has drawn the most attention, not only because of its suggestion that early hormone environment can induce permanent structural changes in the brain, but also because of the considerable experimental support it has gained in the last decade.

Intracellular level. Early studies of sex differences in brain structures have focused primarily upon cellular sizes, including nuclear, nucleolar and somal measurements. Because nuclear and nucleolar size has been reported to be highly sensitive to a wide variety of metabolic and hormonal conditions (Ift, 1964; Szenthágothai et. al., 1968) data must be reviewed with great caution.

Dörner and Staudt (1968, 1969) demonstrated that female and castrated male rats possessed greater nuclear volume in cells of the POA and ventromedial hypothalamus than males of neonatally castrated males given testosterone replacement therapy. In their work adult females were ovariectomized, and all animals received identical testosterone treatment for 29 days prior to sacrifice,
thus eliminating possible differential effects of adult hormonal environment. Staudt and Dörner (1976) have also found a sex difference in cell nuclear sizes in the central and medial portions of the amygdala using similar experimental control conditions. Female and neonatally castrated male rats possessed greater cell nuclear volume than females given neonatal testosterone or males castrated beyond the critical period of sexual differentiation.

Sex differences in cellular measures have also been reported in non-human primates. Bubenik and Brown (1973) demonstrated that neuronal nuclear diameters of medial amygdaloid nerve cells were approximately 10 percent larger in male squirrel monkeys (see Figure 1). Nuclear sizes did not differ in the cerebral cortex or in the suprachiasmatic nucleus; however, no conclusions were drawn from data collected from the medial preoptic area or arcuate nucleus because of a broad variance in the cell diameter frequency distribution. This suggests that the preoptic area and arcuate nucleus may possess more than one cell population which could mask any existing sex difference.

Insert Figure 1 here

Intercellular level. The first "classical" morphological study was presented by Raisman and Field (1971, 1973) based upon their electron microscopic examination of the rat preoptic area. In order to investigate the neural connections between the amygdala-stria terminalis system and the preoptic area, synapses residing within the strial portion of the POA were classified as axons whose terminals contacted either dendritic spines or the dendritic shaft. Lesion of the stria terminalis produced an orthograde terminal degeneration process, enabling further classification of synapses: degenerating (amygdaloid) and
nor degenerating (other afferents) axons. Raisman and Field discovered a significantly higher proportion of non-amygdaloid spine synapses in female rats than males (see Figure 2). No sex differences were found in frequencies of non-amygdaloid spine or shaft synapses or either type of amygdaloid termination patterns. Furthermore, this dimorphic wiring pattern was highly dependent on neonatal hormone exposure. Animals classified as "cyclic" (i.e., normal females, neonatally castrated males and females treated with exogenous testosterone beyond the critical period of development) had nearly twice as many non-amygdaloid spine synapses than "acyclic" counterparts (i.e., normal males, females given exogenous testosterone at birth and males castrated beyond the critical period of development). Interestingly, this difference is restricted to the strial portion of the preoptic area. Comparable counts of synaptic types were taken in parts of the ventromedial hypothalamic nucleus receiving strial projections and in the septal nucleus, but no sex differences were found.

Insert Figure 2 here

These findings seem incompatible with those which have suggested a sexually differentiated input from the amygdala into the preoptic area (Dyer et. al., 1976; Velasco and Talesnik, 1969a); however, the effect of stimulation of a group of afferents to neurons depends not only on the impulses generated in those afferents, but also in the balance of all other inputs to that cell. Thus, it is possible that a fixed number of amygdaloid afferents could be more effective in altering the firing pattern if that cell receives more spine synapses from another, non-amygdaloid pathway.
Multicellular level. In a second major study involving structural brain
dimorphisms, Gorski and his co-workers demonstrated the existence of a
marked sex difference in the size of the densely staining portion of the medial
preoptic nucleus (MPON) (Gorski, Gordon, Shryne and Southam, 1978). The volume
of this densely staining area was estimated to be several times greater in males than
in females, while no differences were found in such brain measurements as gross
size and weight (see Figure 3). This dimorphism of nuclear staining volume was
independent of adult hormonal exposure; however, normal male rats had significantly
larger volume than castrated males. The medial preoptic nuclear volume in
both neonatally castrated males and masculinized females was significantly
greater than normal females.

Insert Figure 3 here

It is important to note that nuclear volume was not similar in neo-
natally castrated males and normal females or between androgenized females and
normal males. In other words, complete sex reversal of this dimorphism could
not be achieved by reversing neonatal hormonal environments. This suggests
that the perinatal hormonal influences may act in conjunction with a genetic
factor to produce a sexual dimorphism. It is equally possible that the
development of dimorphic nuclear volume began prior to the period within which
experimental manipulations were performed. Thus, the period of maximum
sensitivity to hormonal induction of the enlargement of the MPON may have
occurred prior to castration or exogenous testosterone treatment, permitting
only partial sex reversal of this dimorphism. In either case, nuclear volume,
per se, did not correlate with gonadotropin regulation, i.e., females and
neonatally castrated males exhibited cyclic patterns of LH secretion but both groups had significantly different medial preoptic nuclear volume. A similar lack of correlation existed in the dimorphism of sexual behavior.

More recent studies have attempted to define the nature of this dimorphism of nuclear volume (Gorski, Harlan, Jacobson, Shryne and Southam, 1980). It was found that the larger nuclear volume in males was characterized by an increased neuronal density per unit area of the medial preoptic nucleus resulting from increases in cell body sizes, rather than increases in the number of cells or decreases of similar parameters in the female brain. Furthermore, developmental patterns of this differentiation have shown that the sexual dimorphism residing in the medial preoptic nucleus in the adult rat developed within the first 10 days of postnatal life (Jacobson, Shryne, Shapiro and Gorski, 1980).

The third major finding which revealed a morphological basis of sexual dimorphism was produced by Greenough and his co-workers in 1977 (Greenough, Carter, Steerman and DeVoogd). In this study, Golgi-stained neurons of the dorsomedial preoptic area in the hamster were quantified in such a way to determine patterns of branching dendrites. The results (see Figure 4) clearly demonstrated a sexual dimorphism. The female dendritic density was concentrated lowest in the region corresponding to the highest dendritic density in the male. No difference was found in cell body location. It was apparent that the male possessed neurons whose dendrites were favoring the central portion of this region, whereas the female neuronal, dendritic density was greatest in the immediate regions surrounding this central portion. Unpublished data obtained concurrently with this work by the Greenough lab indicated that
neonatally castrated males and masculinized females possessed dendritic patterns intermediate to those of normal adult male and female hamsters. Furthermore, similar dimorphic distributions were not found in the ventromedial anterior hypothalamus.

Insert Figure 4 here

In an attempt to explain how steroid hormones might induce dimorphic connectivity, Greenough et al. (1977) suggest several possible developmental processes which might occur: (a) hormones may affect neuronal neurite growth rates by increasing or decreasing the rate of proliferation, (b) hormones might influence synaptic contact formation between neuronal populations whose neurites were proximate during development, either by inhibiting or mediating such a process, (c) hormones might influence chemoaffinity patterns which would affect directed growth patterns, and finally (d) selective preservation may be occurring, with hormonal influences determining the survival or degeneration of neural connections.

Greenough (in preparation) also reported that from day 10 to day 30 of postnatal life, an absolute loss of first order dendritic branches (dendrites emanating from soma) occurred while higher order branches increased in length and frequency. This simultaneous loss and gain of dendrites seems to be the mechanism by which neuronal polar dendritic fields develop, producing region 1 assymetry in dendritic distribution.

Work by Toran-Allerand (1976) suggests a possible model for investigation of the morphogenetic effects of gonadal steroids on the developing preoptic area. By observing the influence of exogenous steroids on neurite (axons and/or dendrites)
outgrowth of POA-AHA tissue cultures, it was found that addition of estradiol or testosterone to a culture medium supporting mouse tissue resulted in the dramatic stimulation of neuritic outgrowth. Blocking estrogen receptors eliminated these effects (Toran-Allerand, 1980). These studies suggest that steroid-induced differences in neuritic growth patterns play an important role in the neurogenesis of sexual differentiation.

In summary, the preoptic area has been shown to possess several marked structural dimorphisms which are dependent upon early hormonal environment; however, in all cases precise functional significance of these structural differences remains unknown. It is certainly not unreasonable to suspect that these morphological differences are in some way responsible for sex differences in reproductive brain function. Importantly, clear morphological sex differences in mammals have been demonstrated solely in the rodent brain. Although Bubenik and Brown (1973) have reported sex differences in the amygdaloid nucleus of the squirrel monkey, lack of adequate control groups prevent distinction of this dimorphism as a result of the early organizing influence of gonadal steroid hormones or the activational effects of hormones in adulthood.

Summary and Proposal

That the rodent brain is sexually dimorphic with respect to reproductive physiology and behavior is well documented. Sex differences are implicated in both the regulation of pituitary gonadotropin secretion and in various behaviors, especially those associated with reproduction. The preoptic area has been shown to be essential in regulating preovulatory gonadotropin surges in the female rodent, and is also necessary for normal male sexual behaviors. Furthermore, anatomical dimorphisms exist at the level of the preoptic area
in rodents which could underlie sex differences in brain function. The preoptic area also appears essential for reproductive behaviors in the male monkey; however, gonadotropin surge mechanisms in the female monkey brain can operate independent of this region, although a subtle regulatory role undoubtedly exists.

Thus, the purpose of this study was to investigate possible anatomical sex differences in the monkey preoptic area. Although species differences in the neural substrates regulating gonadotropin secretion existing between monkeys and rodents reduce the likelihood of morphological dimorphisms, behavioral sex differences clearly exist at this level. The monkey brain is of considerable interest because it is more similar to the human brain: monkeys display an increased cortical determination of behavior and possess menstrual cycles similar to those of humans. Because of these similarities, inferences to the human can be made with greater ease.

METHODS

Subjects

Experimental subjects in this study were Macaca fascicularis, or long-tailed macaques. Animals were born and reared at the University of Washington Regional Primate Research Center in Seattle, Washington, where they were sacrificed at approximately eight months of age, well before puberty. Data for this study was collected from a total of eight monkeys divided equally among sex.

Histological Procedures

Tissue blocks which encompassed the preoptic area were removed from left and right sides of the brain and placed into a Golgi-Cox solution.
(5% K₂Cr₂O₇, 5% HgCl₂, 5% K₂Cr₂O₇) for approximately 8-16 days, as test sections for each set of blocks were used to optimize stain quality. The brain tissue was then embedded in 16 percent celloidin, hardened with chloroform fumes and mounted on wooden blocks. With the use of a microtome, coronal sections 100 µm thick were obtained and developed with 16 percent NH₃ preceding dehydration with successive alcohol baths of increasing concentration. Sections were then mounted on slides in Permount with a cover-slip.

Sampling Region

The preoptic area was defined as beginning 0.2 mm rostral to the anteriormost extent of the midline anterior commissure and ending 0.2 mm caudal to the posteriormost extent of the midline anterior commissure (see Figure 5). All cell bodies fell within a sampling region beginning 0.1 mm below the anterior commissure and 0.3 mm lateral to midline. The ventral and lateral extensions of the preoptic area were derived from two separate brain size measurements in order to compensate for variances in brain size and/or slight deviations from the coronal plane of section which might have occurred when tissue samples were excised from the brain. The lateral extension of the POA was defined as 50 percent of the distance measured between midline and the medialmost extent of the internal capsule. The ventral extension of the POA was defined as 40 percent of the measured distance between the ventral border of the corpus callosum and the horizontal midline of the anterior commissure (see Figure 6).

Insert Figures 5, 6 here

Data Collection

With the aid of a camera lucida, neurons from well-stained sections were
traced at 500 X magnification. Cell body location of each neuron with respect to the medial and dorsal borders of the preoptic area was recorded to the nearest 0.02 mm. Approximately 25 neurons were traced from each coronal POA section, with an accumulation of 809 cells in the female and 980 cells in the male. Sampling was random and bias was unlikely because all neurons that were well-stained were recorded. Neurons were traced by a single microscopist who had no knowledge of the sex of the individuals.

Data Analysis

Cell characterization. In an attempt to classify different cell characteristics, several criteria were employed that might reveal the existence of distinct cell populations. Each neuron was carefully classified with respect to four cell qualities: (a) soma shape, (b) soma size, (c) dendritic thickness and (d) dendritic spine density.

Cell soma shape was classified as "round" if the ratios of the minimum and maximum edges of the smallest rectangle that could contain the cell body was 1:1 (+ 5%). Cells whose ratios exceeded 2:1 were classified as "oblong", whereas ratios falling between 1:1 and 2:1 were defined as "intermediate".

Because cell soma shapes were quite variable, a maximum diameter measure alone would be a poor indication of cell size. For example, an oblong cell with the same maximum diameter as a round cell would undoubtedly possess less soma volume. A crude measure of cell size was obtained by combining information of soma shape and maximum diameter. All cells with diameters less than 13.0 μm were classified as "small" regardless of shape. Cells with diameters approaching 15.0 μm were also "small" if their shapes were oblong or intermediate. Cells were "large" if the maximum diameters were 23.0 μm or greater regardless of shape. Large cells could have diameters as low as 19.0 μm if they were round and 21.0 μm if they were
intermediate. All remaining cells were classified as "medium" in size. Table 1 summarizes the method of cell size classification.

Insert Table 1 here

Dendritic thickness comprised another type of cell classification. If a neuron's thickest dendritic branch, beginning one soma length from the cell body, exceeded 2.0 μm in width, it was considered "thick", whereas dendritic branches less than 2.0 μm wide were designated "thin".

Dendritic spine density was measured along a neuron's most heavily spined 20.0 μm segment. A neuron was specified as "sparse" if its spine density never exceeded two (two spines/20.0 μm dendrite). Spine densities of ten or greater indicated "spiny" cells, and values ranging from three to nine yielded cells designated "intermediate". A general summary of all cell-type classifications appears in Table 2.

Insert Table 2 here

Statistical analysis was performed upon total cell frequency data using individual means with analysis of variance or chi-squared tests when appropriate.

Dendritic field analysis. To reconstruct dendritic density patterns within the preoptic area, using a method similar to that devised by Greenough et al. (1977), each neuron tracing was centered beneath a grid overlay and intersections between dendritic branches and the 20 μm equivalent grid squares were recorded (see Figure 7). Cell body coordinates were added to the coordinates of each grid
square intersection with respect to the cell body, thus enabling the number of intersections for any 20 μm coronal region to be calculated. For analysis neurons from the right side of the brain were rotated to a mirror image matching the left side, and data from individual coronal sections were collapsed along the anterior-posterior axis. A composite dendrite distribution of the POA was obtained in two ways. First, each grid square was ranked according to hierarchy with respect to the relative amount of dendritic intersections it possessed, and a grid density diagram was created in which darkest areas represented the highest quartile of dendritic density. Secondly, all grid squares were mathematically summed along the dorsal-ventral POA axis from which a graphic representation of regional dendritic distribution was created.

Insert Table 7 here

A second measure of dendritic fields was obtained by measuring total branch lengths and the number of bifurcation (branch) points from each cell. Mean measures were calculated for each neuron and individual means were compared and statistically evaluated by analysis of variance.

RESULTS

CELL CHARACTERISTICS

General Observations

Neurons within the preoptic area were predominantly simple bipolar cells whose dendrites were oriented in a symmetrical manner about the cell body. There appeared to be a wide distribution of the various cell characteristics described above, with the occurrence of all possible combinations. Furthermore, by examining
the relationships between these cell characteristics several patterns were revealed which were consistent across sexes.

Analysing the interactions between cell shape and dendritic spine density, it was evident that as the soma shape distribution passed from round to oblong, the associated dendritic spine density decreased significantly. This pattern, exemplified by both an increased occurrence of sparse dendrites and a decreased incidence of spiny branches (see Figure 8), was prevalent in both sexes, although of greater magnitude in females.

Insert Figure 8 here

Another distinct pattern was revealed from examination of the relationship between soma shape and cell size. Cells with an oblong soma shape rarely (≤ 2.6%) existed as small in size, whereas round somata rarely (≤ 2.0%) were large (see Figure 9). Again, this interaction was clearly present in both sexes.

Insert Figure 9 here

Analysis of the relationship between cell soma size and dendritic spine density revealed another significant pattern. As soma size increased, the associated spine density increased, that is, the relative frequency of spiny dendrites increased while the incidence of sparse branches decreased (see Figure 10). This pattern, too, was clearly prevalent in both sexes.

Insert Figure 10 here
Dendritic spine density also appeared to interact specifically with branch thickness. Figure 11 illustrates the tendency towards an increased spine density as dendrites become thick. This pattern is characterized in both sexes by an increase in the relative frequency of spiny branches and a decline in the occurrence of sparse dendrites.

Insert Figure 11 here

Dendritic thickness, in turn, is highly interactive with cell soma size. Dendritic processes from small cells are predominantly (80-90%) thin, but as soma size increases, the proportions of thin and thick dendrites become approximately equivalent (see Figure 12). No significant relationship was observed between soma shape and dendritic thickness, although thick branches were distributed with a slightly greater frequency in oblong shaped cells.

Insert Figure 12 here

Sex Differences.

Table 3 demonstrates the distributions of all defined cell characteristics. No sex differences were evident within the various classifications of soma size, soma shape or dendritic thickness; however, upon examination of the distribution of various spine densities, it was apparent that the female possessed significantly more sparse dendrites than males.

Insert Table 3 here
In an attempt to reveal the specific source of the sex difference in relative frequency of sparse dendrites, several combinations of cell characteristics were examined. The results (see Table 4) indicate that large somata were more than twice as likely to possess sparse dendrites in females than males. Somata that were oblong maintained a significantly higher proportion of sparse branches in females also; however, cells which were round or small were not dimorphic in this respect. Thus, the overall higher incidence of sparse branches in the female was a result of a higher relative frequency of sparse dendrites from both large somata and those which were oblong.

Insert Table 4 here

DENDRITIC FIELD

In the first analysis of the dendritic field, a density diagram was produced (see Figure 13) which revealed no apparent sex difference. These distribution patterns differed markedly from those described in the hamster by Greenough et. al. (1977) (c.f. Figure 4). Quartiles of the highest density did not seem to cluster in one common area, but rather tended to accumulate in smaller clusters throughout the POA. The graphic presentation of the density distribution (see Figure 14) illustrates the similarity of dendritic field patterns between males and females.

In a second measure of dendritic fields, the mean total dendritic length and number of bifurcations/cell were calculated. The results (see Table 5) indicate that although males and females did not differ in mean total dendritic length, male subjects possessed a significantly greater mean number of bifurcations. Males on the average had 20 percent more bifurcations/cell than females.

Insert Table 5 here
DISCUSSION

The present study demonstrated the existence of several possible cell populations within the preoptic area of the monkey, defined in terms of specific cell characteristics. Bubenik and Brown (1973) first suggested that more than one cell population was present when they discovered a broad heterogeneity in the distribution of neuronal nuclear diameters within the preoptic area of the squirrel monkey. Furthermore, many associations between cell characteristics described in the current study were observed in both males and females, suggesting that certain cell parameters within the monkey preoptic develop independent of sex.

Cell Structure within the Preoptic Area

Thicker dendrites were more likely to possess a higher spine density and extend from larger somata. Although the relationship between soma size, dendritic thickness and spine density is clear, the degree or direction of dependence existing among them is not yet known. For example, a large soma may predispose the growth of thicker branches which become heavily spined; equally possible, a heavily innervated dendrite may grow thicker, requiring greater soma capacity. Interestingly, Feldman and Dowd (1975) have reported an increased spine density along thicker dendrites in pyramidal cells of the rat cortex but did not investigate soma size interactions.

Spiny dendrites were also found to be associated more often with round soma than oblong-shaped cells. This initially was somewhat surprising, considering that oblong cells usually were larger and had thicker dendrites than cells with round somata; therefore, a greater dendritic spine density seen in round cells was not attributed to thicker branches or larger somata. This suggests that round, spiny cells may comprise a distinct cell population whose spine density is independent of those characteristics which appear to dictate the relative
degree of spine density in other populations. Figure 15 illustrates the relationships between the various cell characteristics.

[Insert Figure 15 here]

The finding that oblong cells tended to be large in size and round cells were considerably smaller can be interpreted in two ways. First, a neuron may increase its soma volume in an asymmetrical pattern, either through unidirectional growth or bidirectional growth in opposite directions, transforming an originally small, spherical cell into one that is large and subsequently, oblong. Another plausible explanation for the high incidence of large, oblong cells might result from an insensitivity in the method that was devised to determine relative cell size. Because the calculated cell size was based upon maximum diameter measures, bias may have been created from variances in soma shape. This, however, seems unlikely, because cell body diameters were, in fact, considerably weighted according to their shape prior to size classification in order to prevent such bias.

In light of these distinct relationships which exist between various cell characteristics, the functional significance of such morphological features must be explored before implications of sex differences in cell populations can be determined. Dendritic spines extend from a wide variety of neuron types, and spine density has been shown to be highly sensitive to several metabolic pathological and environmental conditions (Globus, 1975; Schapiro and Vukovich, 1970). In general, spine synapses are thought to be excitatory (Diamond, Gray and Vasagril, 1970); thus a neuron with a spiny dendritic tree would possess a greater likelihood of excitatory function than a neuron with fewer spines. Furthermore, branches with very few spines have been implicated in inhibitory function (Peters and Fairen, 1978; Ribak, 1977).
In more general terms, an increased spine density, as well as increases in dendritic thickness and soma size represent greater neuronal area upon which synapses may form more frequently. Increases in soma size have also been correlated with greater dendritic tree development (Hinds and McNelly, 1976).

Although no specific functional significance has been attributed to various soma shapes, it is believed that thicker dendrites emanating from cell bodies tend to have a gradually tapering base, giving the soma an oblong appearance. Other branches, especially those that are thinner, emerge abruptly from the cell body and achieve uniform width within a very short distance, thus producing a spherical-shaped soma (Peters and Fairen, 1978).

Sex Differences in Cell Morphology

Proper evaluation of existing morphological sex differences in the monkey brain must include consideration of the age at which animals were examined. Through the action of the fetal testis, male monkeys possess significantly higher levels of testosterone prior to day 100 at gestation (Rasko, 1975), after which levels in males and females are relatively comparable until puberty (Kimble, 1973). Subjects in this study were sacrificed postnatally, beyond the critical period but well before puberty. Thus, morphological sex differences do not appear to result from effects of different hormonal conditions, but likely are products of permanent organizational influences of prenatal testosterone or a more direct result of the Y chromosome and Barr body.

Investigation of sex differences in the monkey preoptic area revealed that females possessed a significantly lower spine density, attributed to increased frequencies of sparse branches in cells that were either larger or oblong. It is unlikely that these two dimorphic cell types are mutually exclusive, as about 25 percent of oblong cells are large and vice versa. One interpretation is that
sexually dimorphic cell populations within the preoptic area of the monkey represent an overall functional dimorphism in synaptic transmission. Thus, females possess a relatively greater number of inhibitory synaptic interactions than males. This appears to contradict what might be predicted from Raisman and Field's (1971, 1973) finding that non-striatal synapses in the rat POA were more frequently formed on dendritic spines in the female, relative to the male, suggesting an overall increase in excitatory function. However, this dimorphism in connectivity patterns found in rats was restricted to the striatal portion of the preoptic area; the spatial distribution of sparse cells in monkeys was not examined. Therefore, although female monkeys in this study possessed a significantly higher proportion of cells with relatively low spine density, their distribution pattern may not necessarily eliminate a sexual dimorphism in the striatal preoptic area, if one exists. Furthermore, although overall frequencies of other cell characteristics failed to indicate a sex difference, the distribution of relatively equivalent proportions of these cells may be sexually dimorphic.

The sex difference discovered in the mean number of dendritic bifurcations/cell indicates a potentially dimorphic connectivity pattern is present within the preoptic area despite failure of the grid analysis to reveal a broader level of this dimorphism. Overall mean dendritic lengths were not sexually dimorphic, suggesting that a similar amount of dendritic material is distributed about the cell body and throughout the POA in different patterns between sexes.

How might sex differences in dendritic branching develop? Testosterone has been shown to dramatically enhance neuritic outgrowth patterns in mouse hypothalamic tissue cultures (Toran-Allerand, 1976) and mediate peripheral nerve regeneration in rats (Yu and Srinivasan, 1981). Logically, testosterone present
in significant levels during the prenatal critical period in monkeys might act upon a non-differentiated POA neuron and induce dendritic outgrowth by increasing the number of branches rather than increasing the length of a fixed number of dendrites. Directional growth could be achieved with greater ease by producing multiple branches that produce a regional polarity rather than outgrowth and subsequent "bending" of a pre-existing dendrite. Alternatively, testosterone could act to modify branching patterns by selectively preserving specific branches.

Conclusion

The present study clearly demonstrated the existence of various cell populations within the preoptic area of the monkey. Sex differences have been found with respect to the overall frequency of sparse dendritic branches; females had a greater incidence of sparsely branched neurons as a result of significant increases in sparse dendrites from cells that were either large or oblong. Furthermore, males possessed nerve cells with a significantly larger number of bifurcation points, suggesting the existence of a sexually dimorphic wiring pattern within the preoptic area. This study appears to be the first in which a morphological dimorphism in primates has been found in reproductively-related brain structures that can not be attributed to differences in current hormonal environment. Although specific functional implications of these sex differences remain uncertain, it is reasonable to believe that any morphological sex difference in brain structures within the preoptic area might underlie differences in reproductive brain function. That these differences have been found in the monkey brain, the possibility of sex differences in the human primate can no longer be discounted.
REFERENCES


Dörner, G. and Staudt, J. Structural changes in the preoptic-anterior hypothalamic area of the male rat, following neonatal castration and androgen substitution. *Neuroendocrinology*, 1968, **3**, 136-140.


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<th>&lt;11.0</th>
<th>11.0-12.9</th>
<th>13.0-14.9</th>
<th>15.0-16.9</th>
<th>17.0-18.9</th>
<th>19.0-20.9</th>
<th>21.0-22.9</th>
<th>23.0-24.9</th>
<th>&gt;25.0</th>
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<td>S</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>Intermediate</td>
<td>S</td>
<td>S</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>Oblong</td>
<td>S</td>
<td>S</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>L</td>
<td>L</td>
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<td>L</td>
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*S = Small, M = Medium, L = Large*
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<td>Oblong</td>
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<td>Thick</td>
<td>Spiny</td>
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TABLE 3
Relative Distribution of Cell Characteristics in Males and Females

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<th>Cell Characteristic</th>
<th>Relative Distribution (%)</th>
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<td>Thin</td>
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* $\chi^2 = 7.40$, df = 2, $p < .025$
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<td>Oblong*</td>
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** F= 9.84, df= 1/6, p < .025  
* F= 7.89, df= 1/6, p < .05
TABLE 5
Dendritic Branch Analysis in Males and Females

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<th>Males</th>
<th>Females</th>
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<td>* Mean # bifurcations/cell</td>
<td>2.30 ± .12</td>
<td>1.88 ± .12</td>
</tr>
<tr>
<td>Mean total dendritic length/cell</td>
<td>306.3 ± 13.1</td>
<td>284.0 ± 8.2</td>
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* F = 6.00, df = 1/6, p < .05
Figure Captions

Figure 1. Frequency distribution of cell diameters in various brain regions of the squirrel monkey. Note the broad heterogeneity in the medial preoptic area (B). From Bubenik and Brown (1973).

Figure 2. Mean incidences of non-striatal spine synapses in the preoptic area of rats under various experimental conditions (MO = males castrated at birth; F16 = females given testosterone on day 16; F = normal females; M = normal males; M7 = males castrated on day 7; F4 = females given testosterone on day 4). From Raism. and Field (1973).

Figure 3. (A) Influence of neonatal hormonal treatments on nuclear volume of the medial preoptic nucleus (MPON) and the suprachiasmatic nucleus (SCN). The number of animals is indicated at the bottom of the bars; ** (p<.01)); *(p<.05).
(B) Location of sexually dimorphic MPON in a coronal section of the brain. From Gorski et. al., (1978).

Figure 4. Relative dendritic distribution throughout the medial preoptic area in hamster. From Greenough et. al., (1977).

Figure 5. Mid-sagittal section of the monkey brain showing the position of the POA sampling region (mm = mammillary body; VM = ventromedial nucleus; DM = dorsomedial nucleus).

Figure 6. Coronal section of the monkey brain illustrating the position of the POA sampling region and the method used to determine its dimensions (cc = corpus callosum; ic = internal capsule; oc = optic chiasm; ac = anterior commissure; x = measure used to determine medial-lateral dimension of POA: Y = measure used to determine dorsal-ventral dimension of the preoptic area).
Figure 7. Method used to determine dendritic distribution in the POA. Hatched lines represent transparent overlay used to quantify dendritic branching (D = dorsal, V = ventral, M = medial, L = lateral). From Greenough et. al. (1977).

Figure 8. Relationship between dendritic spine density and soma shape in males and females. (female, $x^2 = 23.7$, df = 2, $p<.005$; male, $x^2 = 6.23$, df = 2, $p<.05$).

Figure 9. Relationship between soma shape and soma size in males and females. (female, $x^2 = 2.6 \times 10^4$, df = 1, $p<.005$; male, $x^2 = 5.5 \times 10^3$, df = 1, $p<.005$).

Figure 10. Relationship between dendritic spine density and soma size in males and females. (female, $x^2 = 815$, df = 2, $p<.025$; male, $x^2 = 11.1$, df = 2, $p<.005$).

Figure 11. Relationship between dendritic spine density and width in males and females. (female, $x^2 = 11.1$, df = 1, $p<.005$; male, $x^2 = 6.8$, df = 1, $p<.01$).

Figure 12. Relationship between soma size and dendritic width in males and females. (female, $x^2 = 56.9$, df = 2, $p<.005$; male, $x^2 = 23.5$, df = 2, $p<.005$).

Figure 13. Dendritic distributions throughout the POA of the male and female monkey. Darkest regions represent the highest quartile of dendritic density.

Figure 14. Graph of dendritic density collapsed along the dorsal-ventral POA axis. No significant differences exist between the male and female plot.

Figure 15. Relationship between various cell characteristics of POA neurons. Arrows with (+) indicate relationships supported by data, whereas those with (-) are non-supported relationships.
FIGURE 1
FIGURE 2
FIGURE 3
**Figure 8**

Relationship between soma shape and dendritic spine density.

- **Female**
  - Round: 20
  - Intermediate: 30
  - Oblong: 10

- **Male**
  - Round: 30
  - Intermediate: 20
  - Oblong: 10

Legend:
- Sparse
- Spiny
FIGURE 9
RELATIONSHIP BETWEEN SOMA SHAPE AND SOMA SIZE

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<th>RELATIVE FREQUENCY</th>
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<td>Round</td>
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</table>

| Male               |       |       |
| Round              | 30    |       |
| Oblong             | 20    |       |
Figure 10
Relationship between soma size and dendritic spine density

Female

Male

Relative frequency (%)

Cell size

Small  Medium  Large  Small  Medium  Large

SPINY
SPARSE
FIGURE 11
RELATIONSHIP BETWEEN DENDRITIC THICKNESS AND SPINE DENSITY

Relative Frequency [%]

Female

Male

SPARSE SPINY

DENDRITIC THICKNESS

THIN

THICK

THIN

THICK
FEMALE

Anterior Commissure

Third Ventricle

Optic Chiasm

MALE

Anterior Commissure

Third Ventricle

Optic Chiasm

FIGURE 13
FIGURE 14

MEAN DENDRITIC SUM SQUARE

DISTANCE FROM MEDIAL POA BORDER (mm)

- FEMALE
- MALE
FIGURE 15

Diagram showing the relationships between rounded soma shape, increased dendritic spine density, increased soma size, and increased dendritic thickness.