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ABSTRACT

Morphology of neurons in the medial preoptic area (mPOA) of the rat: Sexual dimorphisms and prenatal stress effects. Kimberly M. Gerecke, MA Psychology, University of Richmond, 1997; Dr. Craig H. Kinsley, Director.

Androgens masculinize brain development and behavior, and alterations in early hormone exposure modify development. We examined sexual dimorphisms and prenatal stress (PS) effects on the morphology of mPOA neurons. Timed-mated rats were subjected to thrice-daily heat, light and restraint stress from days 15-22 of gestation. In adulthood, the offspring were killed, the brains stained with Golgi-Cox, and the neurons traced for analysis. Sexual dimorphisms in the cumulative length and branching of the dendrites were observed: (Control Females>Control Males). Stress effects were seen in the males in somal area, perimeter, and dendritic length: (PS males>Control males). Stress effects in the females were observed in the measure of apical dendritic branchings, and cumulative dendritic length: (PS Females<Control Females). Sex

steroids, and PS alteration of hormones, may induce fundamental alterations of neuronal populations in specific brain regions.

I certify that I have read this thesis and find that, in scope and quality, it satisfies the requirements for the degree of Master of Arts.

7/31/97

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MORPHOLOGY OF NEURONS IN THE MEDIAL PREOPTIC AREA (mPOA) OF THE RAT: SEXUAL DIMORPHISMS AND PRENATAL STRESS EFFECTS

By

KIMBERLY M. GERECKE

B.S., Muskingum College, 1994

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Sexual differentiation is dependent on the presence or absence of the Y chromosome. Located on the Y chromosome is the testis determining factor gene (TDF) which allows for the differentiation of fetal tissue into the gonads and the subsequent secretion of testosterone. Sexual differentiation is due to the influence of gonadal steroids (Ward & Weisz, 1984), mainly estradiol (Patchev, Hayashi, Orikasa, & Almedia, 1995). Estrogens are the mediators of sexual dimorphism of neural circuits controlling gonadotropin release and sexual behavior (Patchev et. al., 1995). In males the steroid action is mainly prenatal, whereas in females differentiation is due to the absence of steroid hormones in the early postnatal period (Beyer & Feder, 1987; Goy & McEwen, 1980), roughly extending to neonatal day 10 (Jacobson, Davis, & Gorski, 1985; Reier, Cullen, Froelich & Rothchild, 1977). Low levels of estrogen are required for feminized brain development, whereas high levels of estrogen generated by the aromatization of

testosterone are required for masculinization (Kawata, 1995). This differentiating action of androgen is evidenced by manipulation of the hormone environment; perinatal castration of genetic male rats demasculinizes and feminizes sexual behavior and its associated neural substrates, and the injection of androgens into genetic females masculinizes and defeminizes brain and behavior (Beyer & Feder, 1987; Ward, 1972).

The processes involved in the masculinization and defeminization of the male brain are compromised by prenatal stress due to stress-induced disruption of fetal androgen levels (Alonso, Castellano, & Rodriguez, 1991; Barbanzanges, Piazza, LeMoal, & Maccari, 1996; Grisham, Kerchner, & Ward, 1991; Humm, Lambert, & Kinsley, 1995; Keshet & Weinstock, 1995; McEwen, 1994; Ward, 1972; Ward & Weisz, 1984; Ward & Weisz 1980). Maternal stress alters testosterone concentration in the rat fetuses by shifting the testosterone surge that normally occurs at day 18 postconception to

day 17 (Ward & Weisz, 1980). Hormonal influence on the brain is permanently altered due to the desynchronization between the testosterone surge and maturation of the central nervous system. These stress induced changes in testosterone levels exert permanent effects due to the occurrence of this alteration during the critical period for normal CNS masculinization (Ward & Weisz, 1984).

Prenatal stress alters normal sexual differentiation, therefore causing striking physical, behavioral, and physiological modifications. Reductions in body weight, decreased anogenital distance, and decreased adrenal and testicular weights are seen in offspring of mothers exposed to maternal stress (Barlow, Knight, & Sullivan, 1978; Dahlof, Hard, & Larsson, 1978; Rhees & Flemming, 1980). Prenatally stressed males are demasculinized in their sexual behavior, exhibiting significantly fewer ejaculatory responses, and a diminished luteinizing hormone surge in the presence of receptive females (Kinsley, Mann, & Bridges, 1992). Their sexual behavior is

also feminized, showing high rates of lordotic responsiveness to mounting by another male (Ward, 1972; Ward & Weisz, 1984). Prenatally stressed male sexual behavior has been described as awkward and inefficient, and characterized by a general lack of interest on the part of the male (Humm et al., 1995). Male rats typically show a marked right vs. left place preference (when turning to either the left or the right side in a T-maze) the not seen in females, and prenatal stress diminishes these differences (see, e.g., Kolb & Stewart, 1988); the modification of behavioral lateralization by prenatal stress indicates changes in total brain organization. C-fos, a protein coded for by an immediate early gene, is a marker for activity in the brain, and is normally expressed in the male medial preoptic area (mPOA) during sexual encounters; prenatally stressed males show marked reductions in the number and intensity of neurons expressing the C-fos protein in the mPOA after exposure to a receptive female (Humm et al., 1995).

Prenatal stress has also been shown to modify normal neuroanatomical differentiation. For example, sexual dimorphisms have been found in the preoptic nucleus, hypothalamus, stria terminalis, amygdala, and hippocampus (Kawashima & Takayri, 1994). The sexually dimorphic nucleus (SDN) of the preoptic area can be altered by androgen administration or deprivation (Gorski, Gordon, Shyrne, & Southam, 1978; Gorski, Harlan, Jacobson, Shyrne, & Southam, 1980), as well as prenatal stress, which demasculinizes the SDN. Normally, the SDN in juvenile and adult males is two times larger than in females, but males exposed to prenatal stress have a SDN that does not differ significantly from females (Anderson, Rhees, & Fleming, 1985). Cell densities within other regions of the preoptic area are similarly altered (Fleming, Anderson, Rhees, Kinghorn, & Bakaitis, 1986). Daily administration of testosterone propionate during the perinatal period to female rats inhibits the normal apoptosis seen in the female SDN (Dodson & Gorski, 1993). The

dorsolateral nucleus of the lumbar spinal cord, and the spinal nucleus of the bulbocavernosus (SNB) also show a similar pattern of sexual dimorphism, and prenatal stress causes a significant reduction in these motor neuron numbers (Grisham, Kerchner, & Ward, 1991). Taken together, these data suggest that prenatal testosterone has a sparing effect on neurons, inhibiting the apoptosis, or programmed cell death, which occurs in the absence of androgens.

Prenatal stress also has effects in areas of the CNS not directly related to sexual behavior. Normally males have a greater cortical thickness in the right hemisphere; gonadectomized males and prenatally stressed males do not exhibit this difference as the left hemisphere is thickened. The typical hemispheric differences are found in intact males and females, as well as gonadectomized females, suggesting that it is the action of gonadal androgens which suppresses cortical enlargement in the left hemisphere (Kolb & Stewart, 1988; Fleming et. al., 1986).

The effects of prenatal stress have been well documented from the behavioral to the nuclear level, yet little is known about the effects of prenatal stress on the morphology of neurons themselves. Sex differences in the morphology of neurons were not recognized until 1971, when Raisman and Field first reported that females have a greater number of dendritic spines on neurons in the dorsal mPOA than males (Raisman & Field, 1971). However, the majority of research involves the influence of hormones on neuronal networks and synaptic transmission. Concentrations of neurons expressing calcitonin gene-related peptide (CGRP) are modulated by steroid hormones, and are sexually dimorphic with regards to distribution and number in areas sensitive to hormonal effects, particularly the SNB and mPOA (Herbison, 1992). Male and female rats have receptors for estradiol, androgens, and progesterone in similar levels and brain regions irrespective of sex; the responses, however, to the hormones are sex-specific, as the binding patterns of estradiol from

aromatized testosterone as compared to circulating estrogen do differ (McEwen, 1994). Estrogen is known to exert excitatory effects on neurons by enhancing firing (Beyer & Feder, 1987), increasing the processes of nerve fibers (Kawata, Yuri, and Morimoto, 1994), enhancing neuritic outgrowth and elongation both *in vitro* (Diaz, Lorenzo, Carrer, & Caceres, 1992) and *in vivo*, and promoting the formation of synapses in the CA1 region of the hippocampus over the course of the estrous cycle (McEwen, 1994).

As steroid hormones are strongly involved in cell to cell communication (Kawata, 1995), the behavioral, physiological and neuroanatomical modifications of prenatal stress are indicative of changes in total brain organization. The purpose of this research was to assess sex differences and prenatal stress effects on the morphology of mPOA neurons. Sex differences have been seen in hippocampal and spinal cord neurons; we therefore expected to find significant differences in mPOA neurons of control males and

females, and prenatally stressed males and females. It was hypothesized that female neurons would show enhanced neuritic outgrowth due to the absence of androgens; high levels of estradiol promote neuronal growth, and as we were analyzing adult animals, we expected to see the effects of elevated levels of this hormone in the females. Additionally, patterns of outgrowth in prenatally stressed animals were expected to be intermediate to the sexual dimorphisms, as prenatal stress has been shown to have these effects on behavior and the SDN-POA (Fleming et. al., 1986).

Method

Subjects

Adult nulliparous female Sprague-Dawley rats, offspring of stock originally purchased from Charles River Laboratories, Inc. (Wilmington, MA), were timed mated in our lab. The day sperm was observed in the vaginal lavage was designated Day 1 of pregnancy, at

which time the females were isolated in 20 x 45 x 25 cm polypropylene cages, which floors were covered with pine shavings. Food (Purina rat chow) and water were available *ad lib*, and all animals were housed in light (on from 0500-1900 h) and temperature (21-25°C) controlled testing rooms for the duration of the experiment. Animals used in this study were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the University of Richmond, and those prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council.

Prenatal Stress Procedure

On the morning of gestation day 15, females were randomly assigned to one of two groups. One half of the pregnant females was exposed to a regimen of heat and restraint stress comprised of placing the female into a 11.3 cm x 7.0 cm x 10.0 cm Plexiglas

restraint tube over which was a 100 watt flood light beginning on day 15 of gestation and continuing through day 22. This stress procedure utilizing heat, light, and restraint, has been used successfully by our laboratory and others, allows for the control of variables such as the intensity and duration of the stress, and the dams do not adapt to this stress (Ward & Weisz, 1984). Subjects were exposed to three 30-minute stress sessions each day at 0830, 1230, and 1630 h. The second group of females (non-PS Control) received routine maintenance only. At parturition the litters were culled to 7-10 pups of both sexes, and the pups were cross fostered. In order to control for litter effects, only one male and one female from each litter were subjects in the experiment. The remaining animals were utilized in other laboratory investigations. The final ns for each group were as follows: control males, n=7; PS males, n=5; control females, n=6; PS females, n=6.

Histology and Brain Sectioning

In adulthood (50-60 days of age) animals were killed, the brains removed, and blocked in the coronal plane in three sections. The sections were placed in Golgi-Cox stain for 14 days-2 months, after which they were blocked again to include the mPOA, and cut at 100 microns on a Vibratome. Eight to ten sections were taken from each brain, and the slices were placed on subbed slides and allowed to dry. The slides were then exposed for five minutes to the alkalizing solution of lithium hydroxide; this reacts with the Golgi-Cox heavy metal deposits in the neuron to produce the black product characteristic of the stain. The slides were then run through a dehydrating series of alcohols and xylene, coverslipped with Permount, and allowed to dry.

Image Analysis and Quantification

A 300 micron x 300 micron region of the dorsal mPOA was used in this analysis. This region of the mPOA was visualized under low magnification using a Zeiss Axioplan microscope fitted with a color Optronics CCD camera. Only neurons that were completely stained, and fully discernable were used in the analysis. Neurons were traced at 40x using the Neurolucida tracing software (Microbrightfield, Burlington, VT). The entire neuron was traced, save for dendritic spines, and the following variables were recorded: number of branches on the apical dendrite (the thickest primary dendrite, extending from the apex of the soma), cumulative apical dendrite length, length of the apical dendrite shaft (distance from the soma to the first node), area of the perikarya, perimeter of the perikarya, number of basal dendrites, number of basal dendritic branches, and cumulative length of the basal dendrites. Spine densities were not recorded, as the staining of the spines was not

consistent or clear enough to differentiate actual spines from artifacts of the stain. An average of 13 neurons per animal were traced for analysis. All slides were coded, and therefore all data was collected without knowledge of the sex or treatment condition of the animal.

Statistics

Means for each animals across each dependent variable were used for the analysis. Tests for homogeneity of variance (Cochran's D) revealed nonhomogenity among variables; therefore, nonparametric tests were employed. Individual Mann-Whitney U tests were performed for each variable, and comparisons between groups for each variable were analyzed using the one-tailed p value. The SPSS statistical package for PC was used for all statistics.

Results

Number of Apical Dendrite Branches

No significant sex differences were observed between controls ($U=19.0$, $p>0.84$). Stress effects were not observed between male controls and PS males ns($U=9.0$, $p>0.20$), but were seen in the females, with PS females having significantly fewer branches than control females ($U=4.5$, $p<0.03$). There was also a significant difference ($U= 4.0$,$p<0.05$) between PS males and control females, but not between control males and PS females ns($U=9.0$, $p>0.10$).

Cumulative Apical Dendritic Length

No differences were observed between male and female controls ns($U=12.0$, $p>0.23$). No stress effect was seen in the males ns($U=10.0$, $p>0.27$), but a trend was seen in the females ns($U=6.0$, $p>0.06$), with females controls having longer cumulative length than PS females. Male controls did not differ significantly from PS

females ns(U=18.0, p>0.73), but female controls did differ significantly (U=3.0, p<0.03) from PS males.

Length of Apical Dendrite from Soma to First Node

There was no significant sex difference ns(U=10.0, p>0.14). A stress effect was observed in the males, with male controls having a significantly shorter apical shaft than PS males (U=4.0, p<0.03). Female controls did not differ from PS females ns(U=16.0, p>0.82), or from PS males ns(U=9.0, p>0.33). Male controls did not differ from PS females ns(U=14.0, p>0.37). PS males did not differ from PS females ns(U=10.0, p>0.43).

Somal Area

No sex differences were observed between control groups ns(U=14.0, p>0.37). There was an effect of stress in the males, with PS males having significantly larger somal area than control males

(U=4.0. p<0.03). No stress effect was seen in the females ns(U=17.0, p>0.94). Female controls did not differ from PS males ns(U=10.0, p>0.43), and male controls did not differ significantly from PS females ns(U=13.0, p>0.29). PS male and PS female somal areas were not significantly different ns(U=10.0, p>0.43).

Perimeter

There was no significant sex difference for perimeter of the soma (U=11.0, p>0.18). An effect of stress was seen in the males, as PS males had a greater cell perimeter than control males (U=5.0, p<0.05), and there was a trend for the effect in the females, with PS females having a smaller perimeter than control females ns(U=6.0, p>0.06). There were no differences between control males and PS females, or control females and PS males. PS males and PS females did differ, as PS males had a significantly larger perimeter than PS females(U=1.0, p<0.01).

Number of Dendrites

No sex differences were observed between control groups ns($U=10.0$, $p>0.14$). There was no stress effect in the males ns($U=15.5$, $p>0.78$), or in the females ns($U=15.0$, $p>0.70$). There were no differences between any of the remaining groups.

Number of Dendritic Branches

There was a significant sex difference in the number of basal dendrite branches, with female controls having more branches than male controls ($U=7.5$, $p<0.05$). Male controls did not differ significantly from PS males ns($U=16.0$, $p>0.88$), but there was a trend for PS females to have less branches than female controls ($U=6.5$, $p>0.06$). PS males were significantly different than female controls ($U=4.0$, $p<0.05$), having less dendritic branching. No differences were observed between control males and PS females ns($U=17.0$. $p>0.63$), or between the stressed animals ns($U=10.5$, $p>0.43$).

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Cumulative Dendritic Length

There was a significant sex difference, as female controls had a greater cumulative length than control males ($U=0.0$, $p<0.00$). Male controls had significantly shorter cumulative length compared to PS males ($U=1.0$, $p<0.01$), and female controls had significantly longer total length than PS females ($U=3.0$, $p<.02$). Male controls did not differ from PS females ns($U=10.0$, $p>0.14$), whereas female controls did differ from PS males ($U=4.0$, $p<0.05$). PS groups did not differ ns($U=10.0$, $p>0.43$).

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Discussion

The sex differences in neuronal morphology which we observed illustrate expected sexual dimorphisms. There was a dramatic difference in the cumulative length of the basal dendrites, with the control females having the longest processes of the four groups, and the control males having a mean cumulative length 53% of that of the female controls. Additionally, female neurons had a greater mean number of dendritic branches than the male neurons. The nonsignificance of comparisons of the number of basal dendrites suggests that there are some aspects of morphology that are set during the early migrational phase, and are not as vulnerable to hormonal modifications as aspects that arise after neurogenesis, during the differentiation phase of the dendrites (Geröchs, Réthelyi,

& Halász, 1986; Hammer & Jacobson, 1984; Jacobson et. al., 1985; Reier et. al., 1977).

Some authors have reported that androgens can promote neuritic outgrowth *in vitro*. When cultures of dissociated hypothalamic fetal rat neurons were raised in medium containing estradiol, the male neurons showed a greater neuritic outgrowth than neurons from female fetuses; however, the majority of this growth was axonal outgrowth (Diaz et. al., 1992). In another *in vitro* study, Kawashima and Takagi in 1994 reported that testosterone administration to the medium did not affect the mean value of the total process length, although it did increase the distribution and number of process branchings. Neither estradiol nor 5alpha-DHT administration had an effect on dendritic length, but both increased branching when added to the cell culture medium. The authors of this experiment speculated that the cell densities in the culture, as well as the dosage of the hormones may have been

factors for their results (Kawashima & Takagi, 1994). Testosterone exposure *in vitro* produces neuritic outgrowth that is dose dependent (Kandel, Schwartz, & Jessel, 1995), and may not accurately reflect the levels of steroid hormones seen in *in vivo* models. It is important to note that hypothalamic neurons have no distinctive polarity, so the question of hormone action on axons versus dendrites is still considered controversial (Kawata, 1994). Additionally, the neurons which are responding with outgrowth have been characterized as neurons which contain estrogen receptors (Kandel et. al., 1995; Kawata, 1994). The mPOA of the female rat contains more neurons with estrogen receptor immunoreactivity than the male (Kawata et. al., 1994), and the greater mean cumulative length that we observed in the females may be a reflection of an enhanced estrogen response of the female mPOA.

Only a few studies have looked specifically at the morphology of neurons in the mPOA in *in vivo* models. Cherry and colleagues in

1992 concluded that dendritic arborization is a function of gonadal steroidal exposure, as they found that males had longer dendrites with greater arborizations than females in a sexually dimorphic region of the ferret mPOA. These data are not readily generalizable to the rat, as in the ferret the nucleus analyzed is completely absent in the female; the corresponding structure in rats is present in both sexes, and is sexually dimorphic with regard to size. It has been found that exogenous estrogen, or estrogen and progesterone, administration to female rats did not alter cell body area, number of dendritic branch points, or the number of primary dendrites of neurons in the hypothalamus when evaluated as a function of age (Frankfurt, 1994). The dendritic spine density of these neurons was significantly affected; this effect, however, was specific to the ventromedial hypothalamic nucleus of the hypothalamus (VMN), as compared to the DMN. It is interesting to note that only the VMN shows a priming of progesterone receptors by estrogen

administration, indicating that it is both estrogen and progesterone that are required in some regions for plasticity. The dendritic responses we observed may be due to the fact that we allowed for the females to cycle normally, permitting the full expression of plasticity seen as a function of reproductive state (Wooley & McEwen, 1992).

The activational effects of cyclical gonadal hormones in the female brain are not likely illustrated by studies done in pre-puberal rats, but very little work to date has been done using adult animals in which the morphology of mPOA neurons was examined. Comparison of the cortical thickness and dendritic trees of rats at weaning age compared to adulthood found that the sex differences seen in adulthood in these regions are not identical to those seen in the younger animals (Seymoure & Juraska, 1993). Greenough and colleagues in 1977 found that adult male golden hamsters had a nonsignificant tendency for longer dendrites in the mPOA as

compared to adult females. It may be important to note that behaviorally, female golden hamsters dominate males in direct encounters. and are equal, if not greater, than the male in aggressive behavior towards a variety of stimuli; however, the female rat is less likely to try to establish dominance (Floody, 1983). Considering the enormity of behavioral differences which have been attributed to subtle variations in brain function and connectivity, the behavioral dimorphisms between these species may be significant in terms of differences in brain function and therefore neuronal structure.

It is more likely that the results we obtained as compared to other work can be attributed to regional differences. Kolb has demonstrated that male rats have more dendritic material in one subfield of the prefrontal cortex, but when the other subfield was measured the opposite was found (Kolb & Stewart, 1991). Human females have been shown to have a greater neural density in layers II and IV of the posterior temporal cortex, but not in any other layer

(Witelson, Glezer, & Kigar, 1995). Seymoure and Juraska in 1992 also noted differences in sexual dimorphism of the morphology of related subfields of the rat visual cortex significant enough to treat the different layers separately. As we sampled throughout the mPOA without restriction to a particular nucleus, it is possible that our results reflect the average of morphological characteristics of neurons in the mPOA, possibly accounting for a great deal of the variation that we encountered in our analyses.

It is known that prenatal stress shifts the testosterone surge from the fetal gonads from embryonic day 18 to 17, and that fetal testosterone levels are markedly decreased in the male fetuses for the duration of the period of the stress (Ward & Weisz, 1984). In essence, the PS males show a decrease in testosterone levels at the same time control males have an increase in fetal testosterone titers. This absence of the appropriate level of testosterone during the critical period of development has significant effects on the

morphology of male mPOA neurons. PS males exhibited demasculinization and feminization in measures of the apical dendritic shaft, dendritic length, and area and perimeter of the soma. Although neurogenesis and migration may not be sexually dimorphic (Lawerence & Raisman, 1980; Reier et. al., 1977) the development and differentiation of neurons in the mPOA is sensitive to steroid hormones (Jacobson et. al., 1985; Lawerence & Raisman, 1980; Reier et. al., 1977), and, as indicated by these data, is modified by prenatal stress. The changes in cell area and perimeter reflect alterations in cell growth and shape, and this may be due to direct stimulatory action upon the neuron, or through the removal of the protective effects of testosterone in apoptosis. The increased cell death associated with a decrease in testosterone levels in the sexually dimorphic nucleus (Grisham, Kirchner, & Ward, 1991) may require the neurons to increase neuritic outgrowth in order to make appropriate connections. The morphological differences between PS

and control males that we found mirror the modifications in brain connectivity, neuroanatomy, and function that have been demonstrated in previous anatomical and behavioral studies (Gorski et. al., 1978; Gorski et. al., 1980; Humm et. al., 1995; Ward, 1970; Ward & Weisz, 1984).

Prenatal stress exposure also acted to defeminize and masculinize females on several measures. PS female apical dendrites were significantly less arborized, and there was a tendency for the apical dendrites to be shorter than control females. The PS female group had a mean cumulative dendritic length that was 68% of that of the control females, and tended to have less arborization of their basal dendrites as well. It is interesting to note that these differences were masculinized to the extent that they did not differ from control males on these two measures. Taken together, these data suggest that prenatal stress acts to modify normal neural differentiation in females.

The organizational effects of hormones in PS females may be disrupted by alterations in corticosterone levels, which could then act to disrupt differentiation of the gonadotropin regulatory mechanisms. Sex differences have been found in the expression of corticotropin releasing hormone, hippocampal and hypothalamic glucocorticoid receptor densities, diurnal corticosterone secretion, and sensitivity to exogenous estradiol (Patchev et. al., 1995), indicating that gonadal steroids play a modulating role in the function of the hypothalamic-pituitary-adrenal (HPA) axis. The alterations in female sexual behavior by prenatal stress indicate modification of the responsiveness of neurons to the activational effects of adult hormone levels.

The prenatal stress modifications in morphology that we oberserved in females may also be due to a direct action of corticosterone on neurons. High levels of glucocorticoids are neurodegenerative (Sapolsky, 1992; Uno, Tarara, Else, Suleman, &

Sapolsky, 1989; Uno et. al., 1994; Wooley, Gould, & McEwen, 1990). Prenatal stress, modulated by high corticosterone levels in the dam (Ward & Weisz, 1984), has been shown to exert the same neurodegenerative effects on hippocampal neurons as that seen due to high glucocorticoid levels. Macaque monkeys whose mothers were treated with dexamethasone, a potent glucocorticoid, show a dose-dependent degeneration of CA2 and CA3 hippocampal neurons, and smaller soma and poorly developed dendritic branching of the remaining neurons, effects which persist and are evident in adulthood (Uno et. al., 1994). When corticosterone levels are maintained at basal levels in the dam during stress, this reduction in hippocampal neurons is not seen (Barbanzanges, Piazza, LeMoal, &Maccari, 1996).

These results support the hypothesis that sex-specific behaviors are a function of sexual dimorphisms in neural substrate and total brain organization. Further work needs to be done in order

to better characterize the morphology of neurons in specific brain regions; due to the inherit variability within the brain in regards to sexual dimorphisms, as well as the high variability seen in the analyses of our study, it may be necessary to restrict sampling to a particular nucleus within the POA. Due to the lack of information regarding neural morphology in adult animals, particularly in adult females, investigations of neuronal morphology as a function of estrous cycle are warranted (Wooley & McEwen, 1992).

There is considerable evidence which demonstrates the direct relationship between brain connectivity and behavior, a relationship due to hormonal influences on early neuronal development. The present data suggest that sex steroids, and prenatal stress alteration of these hormones, may induce fundamental alterations of neuronal populations in specific brain regions. The net result may be altered information processing, and hence, behavioral anomalies. The prenatally-stressed animal has been characterized as being very

different than its non-stressed counterparts from behavior to neuroanatomy. Thus, effects on behavior may reflect striking alterations of neural morphology.

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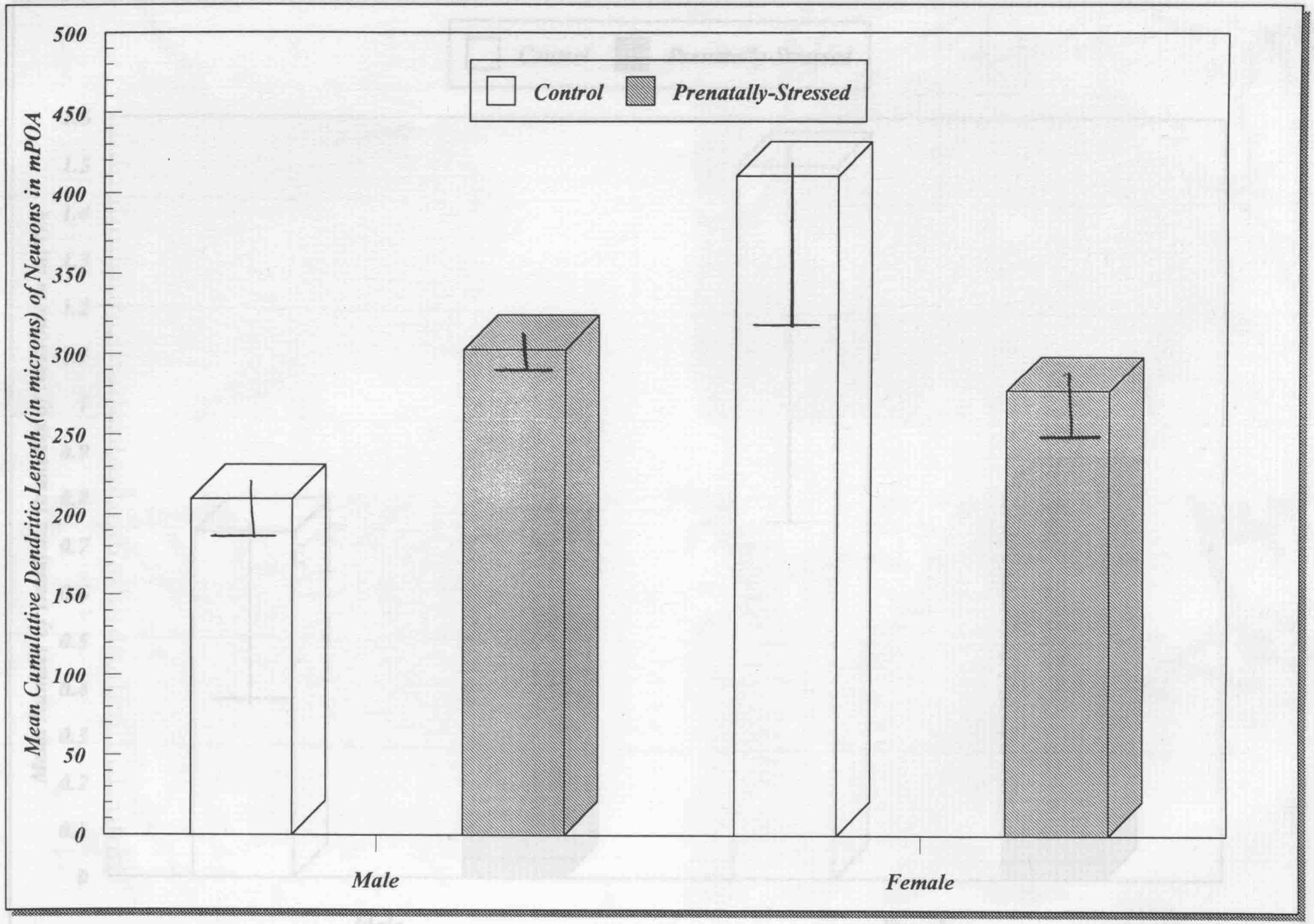
Figure Captions

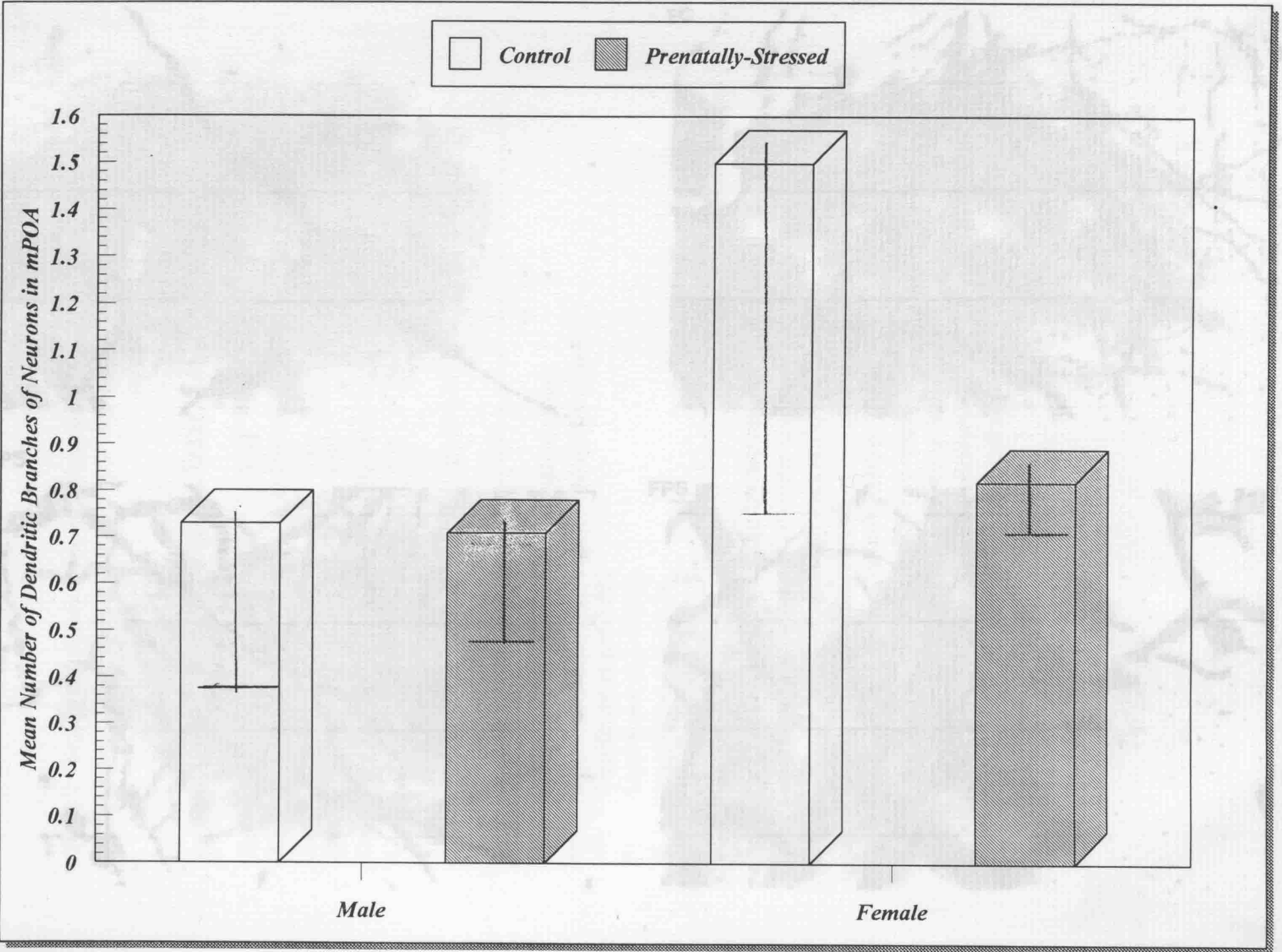
Figure 1. Mean cumulative dendritic length (in microns) of neurons in the mPOA. There is a significant sex difference, and a stress effect in the males and the females. No differences were seen between control males and PS females.

Figure 2. Mean number of dendritic branches of neurons in the mPOA. There is a significant sex difference, and a trend for a stress effect in the females.

Figure 3. Photomicrographs of representative neurons from control males (MC), control females (FC), prenatal-stressed males (MPS), and prenatal-stressed females (FPS) on the measure of cumulative dendritic length.

Table 1. Values (medians) of medial preoptic area neurons for prenatally stressed and control male and female rats.


Mean Cumulative Dendritic Length (in microns) of Neurons in mPOA
500
450
400
350
300
250
200
150
100
50
0
Control
Prenatally-Stressed
Male
Female


Control
Prenatally-Stressed
Mean Number of Dendritic Branches of Neurons in mPOA
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Male
Female

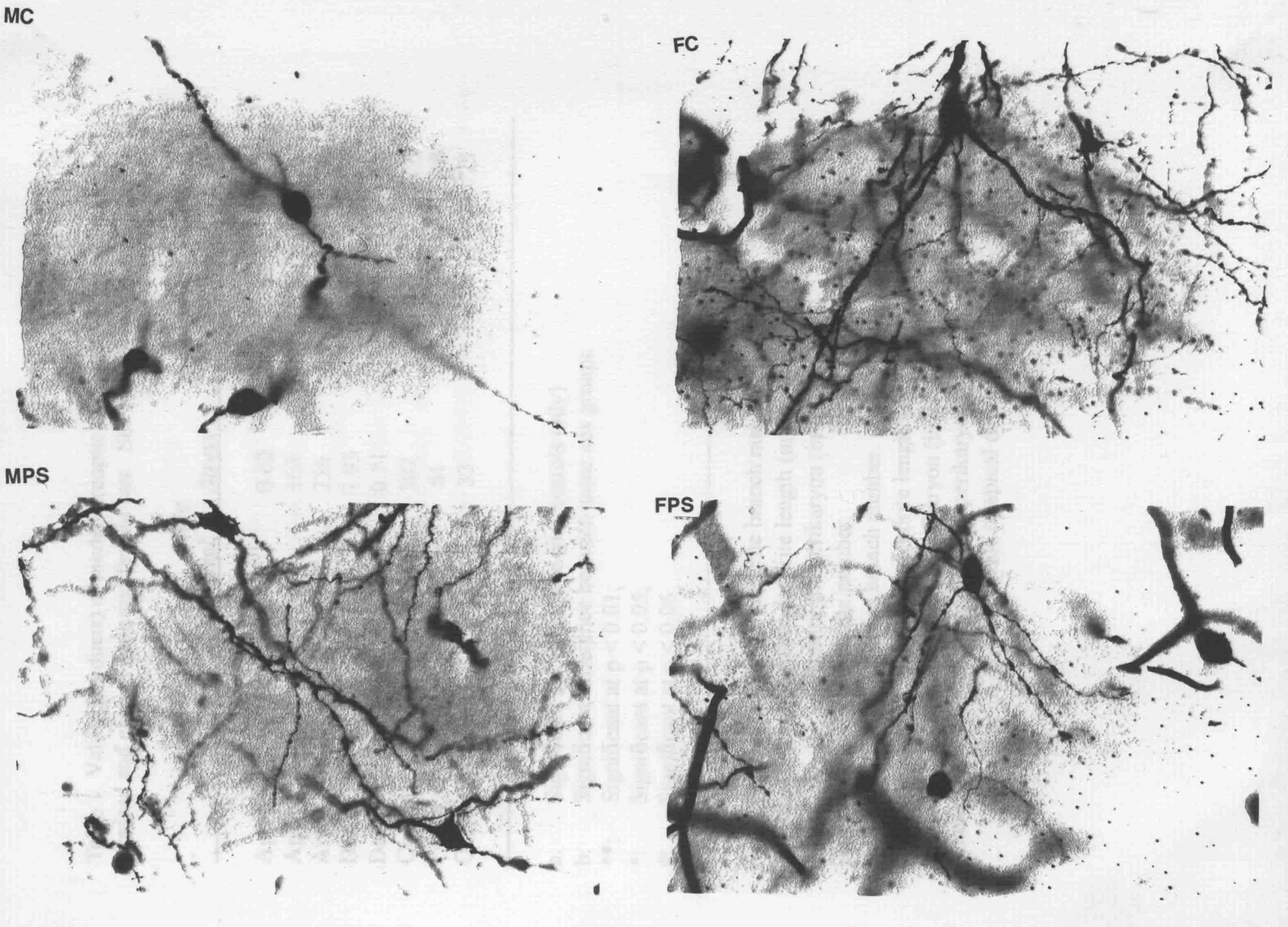

MC
FC
MPS
FPS

Table 1. Values (medians) of medial preoptic area neurons for prenatally-stressed and control male and female rats. See Results for complete details.

	Male		*Female*	
	Control	*Stress*	*Control*	*Stress*
ApDB#	1.13	0.62	1.11 [b*]	0.87
ApDeL	240	169	308 [b#]	207
Area	194 [b*]	236	216	218
Den #	1.92	1.93	2.26	2.20
DeBr#	0.73 [a*]	0.71	1.5 [b#]	0.83
CuDenL	212 [a**b**]	302	411 [b*]	279
Perim	56 [b*]	61	60 [b#]	48
Qmeas	23 [b*]	33	29	25

a: Significant sex difference (in Controls only)
b: Significant difference between same sex groups
**: Significant at $p < 0.01$;
*: Significant at $p < 0.05$;
#: Significant at $p < 0.06$

ApDB#: Apical dendrite branch number.
ApDeL: Apical dendrite length (in μ).
Area: Area of the perikaryon (in μ^2).
Den #: Dendrite number.
DeBr#: Dendrite branch number.
CuDenL: Cumulative dendrite length (in μ).
Perim: Perimeter of perikaryon (in μ).
Qmeas: Distance between perikaryon and primary node of apical dendrite (in μ).

Vita

Kimberly Marie Gerecke graduated from Muskingum College in 1994 with a B.S. in Neuroscience, and received a Master of Arts in Psychology from the University of Richmond in 1997. She is currently pursuing a Doctorate in Behavioral Neuroscience from the University of Alabama at Birmingham, where she is conducting research in recovery of function following stroke and traumatic brain injury.