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Alterations of Sex-Typical Microanatomy: Prenatal Stress Modifies the Structure of Medial Preoptic Area Neurons in Rats

ABSTRACT: *Prenatal stress disrupts normal sexual differentiation and behavior with concomitant alterations in brain development; however, its effects on the cytoarchitecture of neurons in the sexually dimorphic medial preoptic area (mPOA) of the hypothalamus is not known. Morphometric analysis of the mPOA of adult rats showed sex differences as neurons from control females had significantly greater numbers of basal dendritic branches and cumulative basal dendritic length as compared to control male neurons. Prenatal stress significantly altered these sexual dimorphisms, as prenatally stressed (P-S) males had increased measures of cell body area, perimeter, cumulative basal dendritic length, and branch point numbers as compared to control males. Prenatal stress also altered the cytoarchitecture in the female mPOA neurons as P-S female neurons had significantly greater measures for primary dendritic branch number and a trend towards significance for several additional measures as compared to control females. Therefore, there are significant effects of both sex and prenatal stress on neuronal architecture in the mPOA that may help to explain the well-documented alterations in reproductive behaviors observed in P-S animals.* © 2011 Wiley Periodicals, Inc. Dev Psychobiol 54: 16–27, 2012.

Keywords: gestational stress; maternal behavior; neuroanatomy; olfaction; parental/paternal behavior; sexual behavior; sexual differentiation; sexual orientation; steroid hormones; rats

INTRODUCTION

The long-lasting effects of prenatal and early postnatal sexual differentiation of the brain ultimately lead to the many adult sex differences in behavior, physiology, and anatomy. These effects are due largely to the early and permanent influence of gonadal steroids (Ward & Weisz, 1984) predominantly testosterone (T), its metabolite estradiol (Patchev, Hayashi, Orikasa, & Almeida, 1995), and estradiol-induced increases in prostaglandin-E(2) (Amateau & McCarthy, 2004). Interference with this delicate process can result in life-long modifications of sexually dimorphic brain function and behavioral outcomes. For example, a variety of prenatal stressors significantly modify the basic organizational program of sexual differentiation, due primarily to disruption of fetal T levels and their synchronization with neurodevelopmental epochs (Alonso, Castellano, & Rodriguez, 1991; Barbazanges, Piazza, Le, & Maccari, 1996; Grisham, Kerchner, & Ward, 1991; Humm, Lambert, & Kinsley, 1995; Keshet & Weinstock, 1995; McEwen, 1994; Ward, 1972; Ward & Weisz, 1980; Ward & Weisz, 1984), alterations which have subsequent and significant effects on behavior.

Both males and female offspring are affected by prenatal stress. For example, prenatally stressed (P-S) males display reductions in aggressive behaviors and shorter latencies to respond parentally to pups (Harvey & Chevins, 1985; Kelley, 1988; Kinsley & Bridges, 1988; Kinsley & Svare, 1986). Similarly, P-S females display reductions in maternal and aggressive behavior, fertility, and fecundity (Herrenkohl, 1979; Kerchner, Malsbury, Ward, & Ward, 1995; Kinsley, Mann, & Bridges, 1988a; Kinsley, Mann, & Bridges, 1988b; Kinsley & Svare, 1988). In addition, both sexes display alterations in estradiol-induced (Kinsley & Bridges, 1987) and stress-induced prolactin secretion (Kinsley, Mann, & Bridges, 1989) as well as a differential sensitivity to opiates (Kinsley et al., 1988a) as a consequence of prenatal stress.

Marked reductions in male-typical sexual behavior are, however, the most prevalent and the most striking of the many effects reported on behavior and physiology as a result of exposure to stress in utero. For instance, the sexual behavior of P-S males is demasculinized, as they display little copulatory behavior (Ward, 1972), significantly fewer ejaculatory responses and a diminished luteinizing hormone (LH) surge (Kinsley, Mann, & Bridges, 1992) when exposed to sexually receptive females. Their sexual behavior is also feminized, as measured by high rates of lordotic responsiveness to mounting by another male (Ward, 1972; Ward et al., 1984). These effects have been extensively replicated and remain valuable measures of prenatal stress effects (see Ward & Ward (1985) for review).

These alterations in behavior may be influenced by interference with the marked effects of hormones on normal organization and activity of regional groups of neurons/nuclei. Sexual dimorphisms have been identified in the size of the preoptic nucleus, stria terminalis, amygdala, hippocampus (Kawashima & Takagi, 1994), anterior commissure (Ac; Jones et al., 1997), and certain nuclei of the ventromedial nucleus of the hypothalamus (VMH; Flanagan-Cato, 2000). In the medial preoptic area (mPOA), a region important for sexual behavior and physiology, there are sex differences in the morphology of astrocytes in this region (Amateau & McCarthy, 2002). In addition, the activity of neurons is altered by prenatal stress as P-S males show marked reductions in the number and intensity of neurons expressing the immediate early gene protein c-fos, as well as NMDA receptor phosphorylation (Dominguez et al., 2007) after exposure to sexually receptive females (Humm et al., 1995). Estrogen exerts excitatory effects in both male and female neurons by enhancing firing rates (Beyer & Feder, 1987), increasing the number of processes of nerve fibers (Kawata, Yuri, &

Morimoto, 1994), enhancing neuritic outgrowth and elongation both in vitro (Diaz, Lorenzo, Carrer, & Caceres, 1992), and in vivo (Cooke & Woolley, 2005; Gerrits et al., 2008), and promoting the formation of synapses in the CA1 region of the hippocampus over the course of the estrous cycle (McEwen, 1994; Yankova, Hart, & Woolley, 2001). Thus, because neuronal networks and activity can also be influenced by steroid hormones, such activity is likely sensitive to modification by exposure to prenatal stress.

The effects of steroid hormone exposure on the cytoarchitecture of neurons in several hypothalamic brain nuclei have been reported; however, these effects may be specific for discrete subregions. For instance, estradiol alone stunts the elongation of primary dendrites in ventrolateral VMH whereas the dorsomedial subdivision is largely unaffected (Griffin & Flanagan-Cato, 2008). Interestingly, a subpopulation of neurons of the VMH that are involved in the female rat lordosis response (Daniels, Miselis, & Flanagan-Cato, 1999) did not express receptors for estrogen (E_2), suggesting that the effects of the hormone on circuit modulation were via unknown indirect mechanisms (Daniels & Flanagan-Cato, 2000). As steroid hormones normally induce significant behavioral, physiological, and neuroanatomical sexual dimorphisms, it follows that there are also sex differences in the morphology of mPOA neurons. Further, given the mPOA's role in regulating parental responses, which are significantly affected in both P-S males and females (Harvey & Chevins, 1985; Kinsley & Svare, 1986; Kelley, 1988; Kinsley et al., 1988a), potential neuronal structural effects of P-S in this region are worth examining.

Sexual dimorphisms in the cytoarchitecture of neurons, including size of neuronal cell soma and dendritic length, have been reported in the mPOA (Madeira, Leal & Paula-Barbosa, 1999). Additionally, it has been shown that prenatal stress can alter the dendritic morphology of neurons in several meocorticolimbic structures including the nucleus accumbens and hippocampus (Martinez-Tellez, Hernandez-Torres, Gamboa, & Flores, 2009); however, the effects of prenatal stress on neuronal cytoarchitecture of the mPOA in adult offspring have not been described. We show here that neurons in the adult mPOA show significant sexual dimorphisms in morphology, and that these organizational effects are modified by exposure to prenatal stress.

MATERIALS AND METHODS

Animals

Adult (90–120 days) nulliparous female Sprague–Dawley rats (Crl:CD[SD]BR), offspring of stock originally purchased

from Charles River Laboratories, Inc. (Wilmington, MA) were timed mated in our laboratory. The day that a vaginal plug, or sperm in the vaginal lavage, was observed was designated day 1 of pregnancy. The females were then removed from the mating cage and housed individually in standard rat cages (20 cm × 45 cm × 25 cm polypropylene cages), the floors of which were covered with pine shavings. Food (Purina rat chow) and water were available ad libitum and all animals were housed in light (0500–1900 hr) and temperature (21–24°C) controlled testing rooms for the duration of the present work. 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 in accordance with the *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (National Research Council, 2003; University of Richmond's assurance number: A3615-01).

Prenatal Stress Procedure

On the morning of gestation Day 15, females were randomly assigned to one of two groups. One group of pregnant animals ($N = 11$) was exposed to a regimen of heat and restraint stress comprised of placing the female into a 11.3 cm × 7.0 cm × 10.0 cm Plexiglas restraint tube over which were poised two 100 W flood lights. This stress procedure, which produces nearly 350 foot candles of illumination and an ambient temperature within the restraint tube of approximately 36°C, was conducted three times daily for 30 min (separated by 4-hr intervals beginning at 0830), beginning on day 15 of gestation and continuing through Day 22. This heat, light, and restraint stress procedure has been used successfully with both rats and mice by our laboratory (Miller, Mueller, Gifford, & Kinsley, 1999) and others (Ward et al., 1984), allowing for the control of the duration and intensity of the stress without adaptation by the animals. This procedure has also been shown to induce similar effects on rat physiology and behavior as those observed following peripheral administration of corticotropin-releasing factor to the dam (Williams, Hennessy, & Davis, 1998), as well as to significantly increase plasma corticosterone levels in the fetuses (Lesage et al., 2004). The second group of females (non-P-S Controls) received routine maintenance plus daily handling at the times that the P-S dams were handled. Following the stress procedure pregnant females were allowed to remain in standard housing undisturbed until parturition.

At parturition the litters were culled to 7–10 pups, and the rats were housed 2–3 of each sex per cage until testing. In order to control for litter effects, only one male and one female from each litter were used as subjects in the experiment; remaining animals were utilized in other laboratory investigations. As has been previously reported (Kerchner et al., 1995; Keyser-Marcus et al., 2001; Kinsley & Bridges, 1988; Kinsley et al., 1989), we did not see significant differences in litter sizes between stressed and control females. The following n's were used in the present work: Control males ($n = 7$); control females ($n = 6$); P-S males ($n = 5$); P-S females ($n = 6$).

Golgi-Cox Staining Procedure

We used Golgi-Cox-stained neurons for examining cell body anatomy (as opposed to, e.g., a Nissl stain) because the entire neuronal anatomy could be separated and examined in close detail at high magnification in our preparation as in previously published work (see below). The protocol we used is an adaptation of that published by Swaab and Fliers (1985), and modified for short-term staining (Kinsley and Ruscio, unpublished data; Keyser-Marcus et al., 2001; Kinsley et al., 2006; Lambert et al., 2000). Adult offspring (90–120 days) were rapidly euthanized and the brains were removed and blocked in the coronal plane in three sections beginning 1.0 mm anterior to the optic chiasm using a brain matrix, a mold which follows the plane-of-angle provided in the atlas by Paxinos and Watson (1986; Kopf Instruments, Tujunga, CA). The sections were placed in freshly prepared standard Golgi-Cox stain [a solution of 1.2% $K_2Cr_2O_7$ and 1.2% $HgCl_2$ (w/v in dH_2O)]; combined with a solution of 4.0% K_2CrO_4 and 2.5% K_2WO_4 (w/v in dH_2O) and incubated for 10–14 days. Impregnated sections were then rinsed in saline, blocked again into smaller sections that included the mPOA, and then soaked in a 20% sucrose solution for 2 days for cryoprotection. The tissue sections were then super-glued to a metal chuck, and 100 μm sections were sliced into a physiological saline bath using a Vibratome. The slices were placed on subbed slides coated with albumin and K_2CrO_4 and allowed to dry. To develop, the slices were exposed for 5 min to the alkalizing solution of lithium hydroxide (.5% LiOH and 15.0% KNO_3 w/v in dH_2O), which reacts with the Golgi-Cox heavy metal mercuric deposits in the neuron to produce the black product characteristic of the stain. The slides were then dehydrated in graded alcohols followed by xylenes, coverslipped with Permount, and allowed to dry.

Image Analysis and Quantification

Investigators naive to the experimental condition visualized the mPOA region under low magnification (×10) using a Zeiss Axioplan microscope fitted with a new model Optronics cooled CCD camera. The coordinates for the mPOA regions we examined were between bregma .20 and −.92 (Paxinos & Watson, 1986), similar to Keyser et al. (2001). Using the third ventricle and Ac as lateral and dorsal boundaries, a 300 μm × 300 μm digitized box incorporating the dorsal mPOA, was superimposed over the magnified tissue section and neurons whose cell bodies fell within this box were designated as candidate neurons for analyses. Each candidate neuron was viewed through the microscope and its image brought into crisp focus through both the objectives as well as on a high-definition wide-panel LCD monitor. To be selected for further analysis, neurons had to have completely stained cell bodies and processes that were contrasted and obviously separable from the surrounding neuropil. Beginning with the cell body and its proximal dendrites identified within the largest plane of focus, candidate neurons were imaged in the X-, Y-, and Z-planes through the entire extent of the dendritic arbor to ensure that they could be traced in their entirety. Neurons which fit the above criteria were selected as

final candidates for tracing, and those used in the reported analyses were selected randomly from among the final candidate neurons throughout the entire range of the bilateral mPOA, similar to that previously reported (Madeira, Leal, & Paula-Barbosa, 1999).

For analyses, a software package expressly designed to trace and record neuronal morphometry (Neurolucida; Microbrightfield BioSciences, Inc., Burlington, VT) was used to outline the morphology of neurons at total magnification of $\times 1,000$ [$\times 10$ ocular, $\times 100$ oil-immersion objective (NA = .75)]. The following neuronal morphology variables were recorded and analyzed: Number of branches and cumulative dendritic length of the primary dendrite (defined as the thickest primary dendrite, extending from the apex of the soma); length of the primary shaft (distance from the soma to the first node); area of the cell body; perimeter of the cell body; number of basal dendrites; number of basal dendritic branches; and cumulative length of basal dendrites. An average of 13 neurons per animal for each group (males and females, P-S and Control) was traced for analyses. Because of the specific criteria for analysis, a range from a minimum of 10 neurons to the maximum of 15 was used for analysis. All slides were coded and all data were collected blind, without knowledge of the sex or treatment condition of the slide/subject. Codes for the groups were not revealed until after analysis of the data.

Statistical Analysis

A two-way analysis of variance [ANOVA; sex (male/female) by condition (prenatal stress/control)] was originally performed, followed by a test for homogeneity of variance (Cochran's test). The Cochran's test demonstrated significant heterogeneity of variance in the data, not surprising given the variability inherent to neuronal morphology. We therefore used nonparametric statistical tests thereafter. Individual Mann–Whitney U-tests (one-tailed) were used for the subsequent analyses. In all cases, differences were considered significant at $p \leq .05$.

We also conducted comparisons between P-S males and Control females, and between P-S females and Control males. Where a significant sex difference exists, these comparisons help to shed light on the degree to which prenatal stress may demasculinize and feminize males, and where it may masculinize/defeminize females. That is, in those cases where a sex difference was observed, any between-sex/group comparisons that are not significantly different (e.g., between P-S males and Control females, and between P-S females and Control males) may suggest the gender-altering effects of prenatal stress.

RESULTS

Sexual Dimorphisms in Cytoarchitecture

Several sexual dimorphisms in the mPOA morphological measures of neurons were noted in between Control males and females. No sex differences were observed in the comparisons of median values for number of branches or cumulative length of the primary dendrites, somal area and perimeter, or the total number of basal dendrites (Tab. 1). However, the basal dendrites of Control female neurons were increased in length and had more elaborate branching than those of Control males. Compared to Control males, the basal dendrites of Control females had a greater cumulative length ($U = .0$, $p < .00$; Figs. 1 and 2C, Tab. 1). By counting the number of branch points, we found that the neurons of Control females had a significantly greater number of basal dendritic branches compared to male Controls ($U = 7.5$, $p < .05$; Fig. 2B; Tab. 1). Thus, neurons in the mPOA show striking sex differences in their architecture.

Effects of Prenatal Stress in Males

The mPOA neurons of P-S males displayed marked morphological changes compared to control males. P-S males had significantly longer primary dendritic shafts, as measured by the length to the first node ($U = 4.0$, $p < .03$); larger somal area ($U = 4.0$, $p < .03$); and larger somal perimeter ($U = 5.0$, $p < .05$), compared to male Controls (Tab. 1). In addition, the cumulative length of the basal dendrites in P-S males was larger than in Control males ($U = 1.0$, $p < .01$; Figs. 1 and 2C, Tab. 1). There was no prenatal stress effect on the number of basal dendrites ($U = 15.5$, $p = .78$), or their branches ($U = 16.0$, $p = .88$; Fig. 2C, Tab. 1).

In contrast, for the majority of major comparisons, neurons in the mPOA of P-S males did not significantly differ from those of Control females. For instance, the length of the primary dendritic shaft ($U = 9.0$, $p = .33$), somal area ($U = 1.0$, $p = .33$), and somal perimeter ($U = 12.0$, $p = .31$) of P-S males did not differ significantly from female Controls (Tab. 1), suggesting feminization. Whereas the cumulative length of basal dendrites in P-S males was significantly greater than that of Control males, they were significantly shorter than those of Control females ($U = 4.0$, $p = .05$; Fig. 2C, Tab. 1) and thus represented a more intermediate morphology. Therefore, for many measures the effect of prenatal stress was sufficient to demasculinize/feminize the morphology of neurons in the mPOA of males.

Effects of Prenatal Stress in Females

Prenatal stress also significantly affected neuronal morphology in females. P-S females had significantly fewer branch points on their primary dendrites ($U = 4.5$, $p = .03$; Fig. 2A, Tab. 1) and shorter cumulative length of the basal dendrites ($U = 3.0$, $p < .02$; Fig. 2C, Tab. 1) compared to Control females. In addition, there

Table 1. Median Values of Measures for Medial Preoptic Area Neurons in Prenatally Stressed and Control Male and Female Rats

	Male		Female	
	Control	Stress	Control	Stress
PrimDB#	1.13	.62	1.11^{b,*}	.87
PrimDeL	240	169	308^{b,***}	207
Area	194^{b,*}	236	216	218
Den#	1.92	1.93	2.26	2.20
DeBr#	.73^{a,*}	.71	1.5^{b,***}	.83
CumBasDenL	212^{a,b,**}	302	411^{b,*}	279
Perim	56^{b,*}	61	60^{b,***}	48
Dist 1stNode	23^{b,*}	33	29	25

PrimDB#, primary dendrite branch number; PrimDeL, primary dendrite length (μm); Area, area of the perikaryon (μm^2); Den#, dendrite number; DeBr#, dendrite branch number; CumBasDenL, cumulative basal dendrite length (μm); Perim, perimeter of perikaryon (μm); Dist1stNode, distance between perikaryon and primary node of apical dendrite (μm).

^{a}Significant sex difference (for Controls only).

^{b}Significant difference between same sex groups.

*Significant at $p < .05$.

**Significant at $p < .01$.

***Trend towards significance at $p < .06$.

was a trend for P-S females to have shorter overall length of the primary dendrite ($U = 6.0$, $p = .06$; Tab. 1) and smaller somal perimeter ($U = 6.0$, $p = .06$; Tab. 1). Prenatal stress modified the neurons to the extent that they were not significantly different as compared to males for primary dendrite branch points ($U = 9.0$, $p = .10$; Tab. 1) and basal dendritic length ($U = 10.0$, $p = .14$; Tab. 1). Thus, prenatal stress also
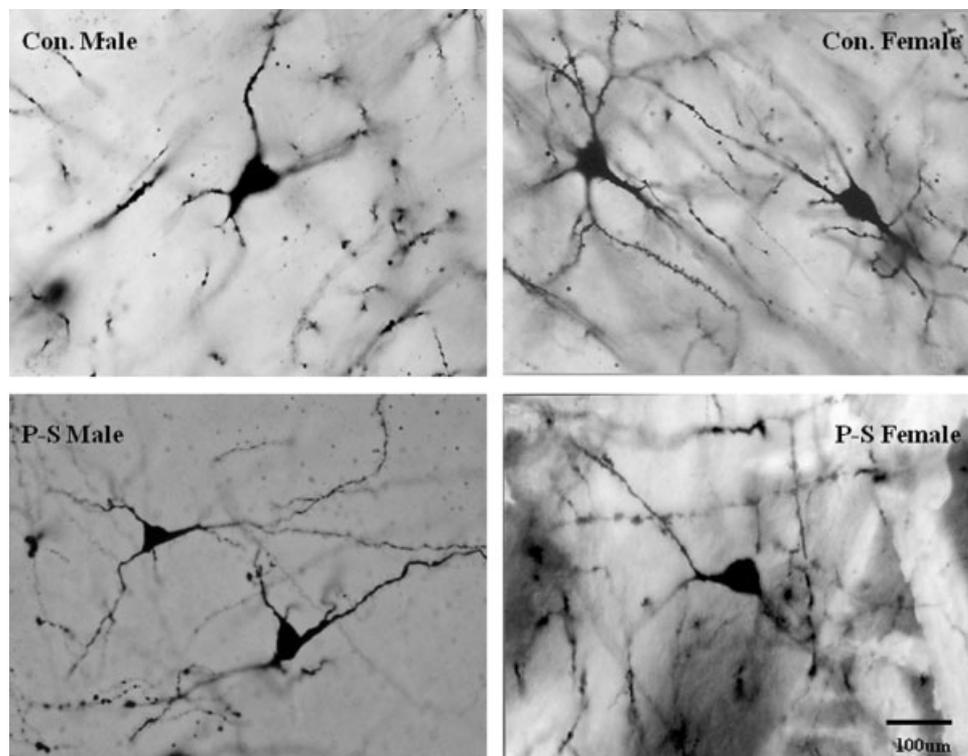

FIGURE 1 Representative images of Golgi-Cox stained neurons from prenatally stressed and Control male and female rats. In general, there were sex differences, as well as differences between Controls and prenatally stressed animals. Scale bar = 100 μm.
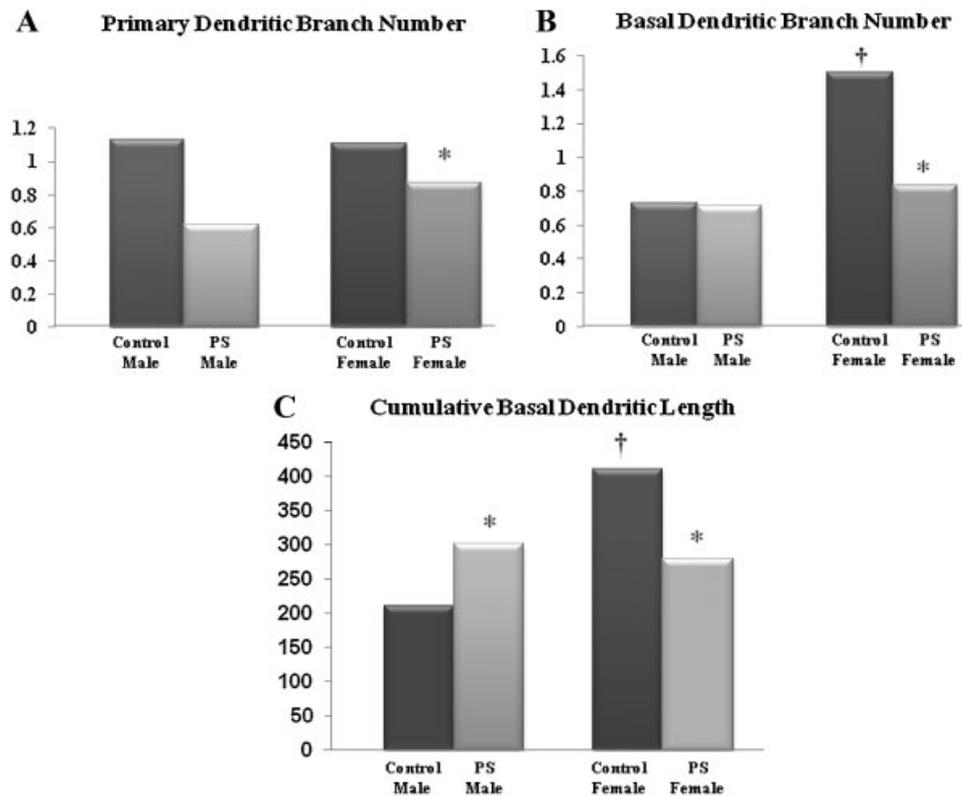
A   **Primary Dendritic Branch Number**

B   **Basal Dendritic Branch Number**

C   **Cumulative Basal Dendritic Length**

FIGURE 2 Prenatal stress (P-S) alters the morphology of neurons in the mPOA of adult offspring. (A) P-S females have significantly fewer branch points on their primary dendrites as compared to control females. (B) Control females have significantly more elaborate basal dendritic arbors than control males as shown by their significantly greater number of dendritic branch points. Prenatal stress significantly altered this measure, as P-S females have significantly fewer basal dendritic branch points as compared to control females. (C) Control females have significantly longer cumulative lengths in their basal dendrites as compared to control males. However, prenatal stress alters this sexual dimorphism, in that P-S males have significantly longer cumulative length, and P-S females have significantly shorter cumulative length when compared to control males and females, respectively. Bars represent the median values, † indicates $p < .05$ comparing control males and females; * indicates $p < .05$ when comparing P-S versus Controls within each sex.

appears to exert significant organizational effects on the morphology of neurons in the female mPOA.

DISCUSSION

Sex Differences in Morphology of mPOA Neurons

As expected, there was a sex 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 cumulative length 53% of that of the female Controls. Additionally, Control female neurons had a greater number of basal dendritic branches than Control male neurons. In

agreement with other work, we show significant sexual dimorphisms in some cytoarchitectural measures, but not all. For instance, in contrast to our findings, Madeira et al. (1999) reported that neurons in the mPOA of adult male rats had longer dendrites, and no significant differences in dendritic branching when compared to females (Madeira et al., 1999). One possible reason for this discrepancy is that we separately analyzed primary and basal dendrites, whereas Madeira et al. (1999) analyzed these elements collectively. As the sex differences we observed were selective for basal dendrites, it is possible that we identified discrete effects on the neuronal compartments not identified by the previous work.

The reasons behind the specificity of alterations to the basal dendrites is unclear. However, previous work has shown that activity-induced changes in dendritic

morphology can be substantial over a very short period of time, and that these diurnal differences in neuronal morphology were specific to the basilar dendrites (Perez-Cruz, Simon, Flugge, Fuchs, & Czeh, 2009). As well, handling stress selectively alters the basal dendrites in hippocampal CA1 neurons (Horner, O'Regan, & Arbuthnott, 1993). Moreover, brain-derived neurotrophic factor increases the number and branching of primary neuritis, but not total neuritic length or number of second to fifth order branch points as compared to another growth factor, neuregulin-1 beta (Gerecke, Wyss, & Carroll, 2004), suggesting that there are subtle differential effects on neurite growth and elaboration between the two growth factors. Taken together, these data suggest that neurons are extremely dynamic in terms of their morphology, and that different factors may induce unique effects on subcellular compartments. As such, it is not unreasonable to consider that exposure to different sex-steroid hormones may also have similar differential effects on specific neuronal components.

Effects of Prenatal Stress in Males

As hypothesized, prenatal stress-induced significant alterations in numerous measures of male mPOA neurons, inducing a more femalelike pattern of neuronal morphology in the male and a more masculinized one for P-S females. Exposure to prenatal stress in males resulted in a greater length to the first node, increased somal area and perimeter, and greater cumulative length of the basal dendrites. Remarkably, with the exception of cumulative basal dendritic length, the architecture of P-S male mPOA neurons was not significantly different from that of female Controls. These data suggest that the feminizing and demasculinizing effects of prenatal stress also dramatically affect neuronal patterning. These effects may, through as-yet unexamined mechanisms and concomitant alterations of information processing, regulate the physiological and behavioral changes characteristic of the P-S or otherwise differently sexually differentiated organism.

The observed effects in the mPOA are interesting in light of the well-reported effects on such variables as sexual motivation and behavior in males, and parental behavior in both males and females. Interestingly, P-S males respond significantly faster with parental behavior toward young than do control males (Kinsley et al., 1988a). Erskine and Miller (1995) report that the size of the cell body is associated with an increase in the activity of the neuron, with the attendant engorgement indicative of increased gene translation, transcription, and protein synthesis (Erskine & Miller, 1995). In the present work we observed a feminization of the cell bodies of mPOA neurons in the P-S males, an increase in its size that may be associated with the enhancement of parental behavior reported for the P-S male, an effect that is considered to be a form of behavioral demasculinization/feminization.

Alterations of the structure of neurons are likewise accompanied by modifications in the activity of those neurons as evidenced by alterations in behaviors. P-S males display a modest LH surge when exposed to sexually receptive females (Kinsley et al., 1992), suggesting a neuroendocrinological insufficiency that leads to under-arousal. This LH response is mediated, in part, through activity of neurons in the mPOA. The lower levels of c-fos expression in the mPOA of P-S males (Humm et al., 1995) and concomitant diminished activity of mPOA neurons may underlie their reduced copulatory behavior (Ward, 1972). The sexual behavior of P-S males is also feminized, as measured by high rates of lordotic responsiveness to mounting by another male (Ward, 1972; Ward & Weisz, 1984). These studies demonstrate the demasculinizing and feminizing effects of P-S on sex-specific behaviors. Thus, P-S-induced effects on the structure of individual neurons and the concomitant alterations in the function of those neurons, may underlie observed variations in sexual behavior.

Effects of Prenatal Stress in Females

Surprisingly, exposure to prenatal stress also defeminized and masculinized the cytoarchitecture of female mPOA neurons on several measures. Whereas it has been reported that P-S masculinizes the size of the rostral Ac in female rats (Jones et al., 1997), the effects of prenatal stress on neuronal morphology in the mPOA are not known. We show here that the primary dendrites of P-S females were significantly less arborized, and trended toward being shorter when compared to control females—that is, they were more malelike. The P-S female group had a mean cumulative basal dendritic length that was 68% of that of the control females, and tended to be less arborized as well. It is interesting to note that these differences are by definition masculinized in that they were not different from Control males on these two measures.

The dendritic alterations we observed in P-S females may be due to the fact that we allowed them to cycle normally, permitting the full expression of plasticity (with associated dendritic spine and other effects) seen as a function of reproductive state (Flanagan-Cato, 2000; Madeira, Ferreira-Silva, & Paula-Barbosa, 2001; Woolley & McEwen, 1992). Gonadal steroids play a modulating role in the function of the hypothalamic-pituitary-adrenal (HPA) axis, as sex differences have

been reported 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). Therefore, the organizational effects of hormones in P-S females may be altered by increased corticosterone levels, which may subsequently act to disrupt normal differentiation of the gonadotropin regulatory mechanisms.

It is also possible that the observed effects of prenatal stress in females may be due to a direct action of corticosterone on developing neurons, as high levels of glucocorticoids are neurodegenerative (Sapolsky, Uno, Rebert, & Finch, 1990; Uno et al., 1994; Uno, Tarara, Else, Suleman, & Sapolsky, 1989; Woolley, Gould, & McEwen, 1990). Prenatal stress induces the same neurodegenerative effects on hippocampal neurons as those induced by exogenous glucocorticoids administration (Ward et al., 1984) and this neurodegeneration persists into adulthood (Uno et al., 1994). Importantly, the P-S-induced neurodegeneration seen in the hippocampus of adult offspring is abolished if corticosterone levels are maintained at basal levels in the dam during stress (Barbazanges et al., 1996). We have previously shown that activity-stress induces atrophy of the apical dendrites in the CA1 and CA3 hippocampal neurons in male rats (Lambert et al., 1998), and others have shown that corticosterone exposure decreases the complexity of the apical tree in developing CA1 neurons (Alfarez et al., 2009). Thus, while the effects on P-S females we observed cannot be attributed to T influences, it is possible that these alterations in the cytoarchitecture of mPOA neurons were induced via exposure to high stress hormones prenatally.

In females, the effects of prenatal stress on physiology and behavior generally are masculinizing. P-S females show poor fertility and fecundity (Herrenkohl, 1979) and are more aggressive than control females (Kinsley et al., 1988a). Further, prolactin (Kinsley & Bridges, 1987; Kinsley et al., 1989) and morphine sensitivity (Kinsley et al., 1988a) are also more malelike than control females. Interestingly, P-S females resemble males in that latencies to display parental behavior are more malelike, namely, longer (Kinsley et al., 1988a). The observed alterations in the cytoarchitecture of P-S female mPOA neurons may subsequently affect the display of parental behavior, or the release of prolactin. Together, the data suggest that the masculinizing effects of prenatal stress on neuronal morphology may underlie behavioral and physiological sexual dimorphisms in mPOA function. Although we do not have data on neurochemical alterations induced by prenatal stress, likely effects at that level, together with structural effects, may unite to bring about behavioral modifications of the sort

well described in the literature. It is of some interest that the effects of P-S on neuronal structure are in accord with other manipulations that have examined neuronal structure associated with specific behaviors. Lambert et al. (2000) reported that activity stress, a significant chronic stressor, reduces neuronal dendritic length in the CA3 region of the hippocampus in a fashion similar to that observed in the P-S females in the present work (Lambert et al., 2000). This similarity of effects for two different types of stressors (activity stress and prenatal stress) suggests a common mechanism in operation that degrades the neuronal structure.

Other Possible Mechanisms for the Effects of Prenatal Stress on Behavior

Prenatally stressed animals display alterations in opiate sensitivity which control olfactory responsiveness as shown by a reversal of analgesic responses between the sexes (Kinsley et al., 1988a), alterations likely mediated by reductions in opiate receptor content in the caudate putamen, endopiriform cortex, lateral amygdala, and nucleus accumbens (Insel, Kinsley, Mann, & Bridges, 1990). These areas share an olfactory association with the anterior division of the Ac (De Olmos, Alheid, & Beltramino, 1985; Heimer, Alheid, & Zaborsky, 1985; MacLean, 1985), an area that we have shown undergoes significant changes in P-S animals (Jones et al., 1997). The amygdala may also be an Ac/mPOA-related site receiving olfactory information, because of its roles in emotionality, aggression, sexual behavior, and parental behavior, all of which are affected in P-S animals. Thus, the effects on behavior in the P-S animals may be related to P-S-induced modification of endogenous opioid regulation of mPOA activity.

The present data suggest that typical sex-specific developmental factors, and prenatal stress-induced alteration of these organizational effects, may induce early and fundamental alterations of neuronal populations in specific brain regions. The exact translation of these cellular level changes to behavioral differences, however, is difficult to speculate. However, it has been shown that exposure to an enriched environment leads to potent changes in the brains of rats (Bennett, Diamond, Krech, & Rosenzweig, 1964; Kempermann, Kuhn, & Gage, 1997), including significant alterations in the cytoarchitecture of neurons. Specifically, neurons in the CA1 and dentate gyrus of the hippocampus in mice exposed to an enriched environment are significantly larger in soma size and dendritic length (Faherty, Kerley, & Smeyne, 2003). Presumably, these EE-induced neuronal alterations underlie increased performance on spatial learning and memory tasks (Frick & Fernandez, 2003; Frick, Stearns, Pan, & Berger-Sweeney, 2003;

Williams et al., 2001) observed in these animals. Thus, the net result of significant changes in neuronal architecture within the mPOA may also similarly underlie changes in information processing, and subsequently, significant alterations in behavior.

CONCLUSIONS

Due to the inherit variability within the brain with regard 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 subnucleus within the POA. In addition, the cytoarchitecture of neurons in the VMH has been shown to vary across the estrous cycle (Madeira, Ferreira-Silva, & Paula-Barbosa, 2001). Because only a proportion of the animals in this work (~20%) would be expected to be in the same stage of the cycle at the time of sacrifice, these results are from females sampled at various stages of the cycle. However, because of the lack of information regarding neural morphology in mPOA of adult animals, particularly in adult females, investigations of neuronal morphology as a function of estrous cycle are warranted.

Changes in dendritic spines have been correlated with sexual behavior (Flanagan-Cato, Calizo, Griffin, Lee, & Whisner, 2006; Leuner, Glasper, & Gould, 2010; Wright, Burks, & McCarthy, 2008), and with alterations in sex-typic behavior. For example, it has been shown that there is a significantly greater induction of dendritic spines formation on CA1 pyramidal neurons in the hippocampus of masculinized female rats following an acute tail-shock stressor than in normally cycling females (Dalla, Whetstone, Hodes, & Shors, 2009). As spines are sensitive to the effects of stress, we did intend to include measures of spine density in our analysis; however, our examination of spines and sex differences therein did not yield any appreciable numbers of countable spines. We have also reported this relative lack of spines in previous work on this region where we described the numbers of spines on mPOA dendritic branches as "sparse" (Keyser et al., 2001). Moreover, others have reported that dendritic spine number on mPOA neurons diminishes dramatically as animals get older, and in adults the numbers therein are relatively small (Gerocs, Rethelyi, & Halasz, 1986). As such, we restricted our analyses to the described cytoarchitectural measures for the current work. Because the Golgi-Cox stain is taken up by a subset of neurons, it is possible that this technique is not sensitive enough to label sufficient numbers of neurons to statistically compensate for the sparse number of spines for analysis. However, it would be an interesting avenue for future research to use a panel of immunohistochemical markers for the neuronal cytoskeleton and see if possible prenatal stress-induced differences in the small number of spines in this region could be identified.

We show here that prenatal stress alters the structure of neurons in the mPOA of both male and female rats, and that these organizational effects persist into adulthood. These data exemplify other observed effects on the demasculinization and feminization of males and the masculinization of females (Humm et al., 1995; Kinsley et al., 1992; Ward & Renz, 1972; Ward & Weisz, 1984). As this region is an area important for the regulation of sex-typical physiology and behavior the alterations in behavior due to prenatal stress may be a function of concomitant P-S induced alterations in sexual dimorphisms in neural substrate and total brain organization.

NOTES

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