Alterations of medial preoptic area neurons following pregnancy and pregnancy-like steroidal treatment in the rat

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ABSTRACT: There is a marked increase in the maternal behavior displayed by a female rat following pregnancy—due primarily to exposure to the gonadal hormones progesterone and estradiol (P and E2, respectively). We examined Golgi-Cox silver-stained, Vibratome-sectioned neurons visualized and traced using computerized microscopy and image analysis. In Part One, we examined the hormonal-neural concomitants in the medial preoptic area (mPOA), an area of the brain that regulates maternal behavior, by comparing cell body size (area in μm2; also referred to as soma and perikaryon) in the mPOA cortex of five groups (n = 4–6/group) of ovariectomized (OVX-minus), diestrous, sequential P and E2-treated (P+E2), late-pregnant, and lactating rats; for Part Two, we examined a subset of mPOA neurons, which were traced in their entirety, from these same subjects. In Part One, whereas there was no difference between OVX-minus and diestrous females, both had smaller somal areas compared to OVX-P+E2-treated and late-pregnant females. The area of the soma returned to diestrous/OVX-minus levels in the lactating females. We found no change among the five groups in area of cell body in cortical neurons, which generally lack steroid receptors. In Part Two, which included a more detailed morphometric analysis of mPOA neurons, we examined several additional measures of dendritic structure, including number of proximal dendritic branches (the largest proximal dendrite was defined as the one with the largest diameter leaving the soma); cumulative length of the largest proximal dendrite; area of the cell body; number of basal dendrites; cumulative basal dendritic length; number of basal dendritic branches; and branch-point (distance from cell body to first branch of largest proximal dendrite). Again, we found similar effects on cell body size as in Part One, together with effects on number of basal dendritic branches and cumulative basal dendritic length in pregnant and P+E2-treated groups compared to OVX, diestrous, and lactating. An increase in somal area denotes increased cellular activity, and stimulatory effects on additional neuronal variables represents modifications in information processing capacity. Pregnancy and its attendant hormonal exposure, therefore, may stimulate neurons in the mPOA, which then contribute (in an as yet undetermined manner) to the display of maternal behavior. During the postpartum lactational period, when cues from pups primarily maintain maternal attention, the neuronal soma appears to return to a pre-pregnancy, non-hormonally dependent state, whereas other aspects of the dendrite remain altered. Collectively, these data demonstrate a striking plasticity in the brains of females that may be reflected in modifications in behavior. © 2001 Elsevier Science Inc.

KEY WORDS: Cell body/perikaryon/soma, Hypothalamus, Learning and memory, Maternal behavior, Neuronal plasticity.

INTRODUCTION

Pregnancy substantially modifies female behavior. Among these changes is a long-lasting modification in the female’s behavior toward young. In the hormonal profile characteristic of pregnancy, progesterone (P) and estradiol (E2) play significant roles in the transition from non- to high responsiveness to neonates [2]. The transition occurs because of the temporal patterning of exposure to E2 and P during pregnancy, in particular, the progressive alteration of the ratio of the two hormones. Other substances, e.g., prolactin, placental lactogens, oxytocin, endogenous opioids, etc. are also important to the processes of pregnancy and parturition, as well as maternal behavior (see, e.g., [2,6,7,24]).

The hormones of pregnancy exert significant changes in behavior and physiological regulation in the female, with the immediate onset of maternal behavior—characteristic of the postpartum female—being the most striking. What attributes of steroid hormone exposure are responsible for such marked change? Several studies have reported that P and E2 modify the structure of the neuron in the adult female brain, increasing the concentration of apical dendritic spines in hippocampal neurons [34,60–62]. The latter effects occur with relatively short exposure to the hormones, primarily E2, during the female’s estrous cycle. If the level or pattern of E2 and P associated with the estrous cycle is prolonged or increased, as occurs during pregnancy, would there be even greater effects on the morphology of the neuron? Further, because relative steroid hormone exposure changes or is low during lacta-
tion [2,8,39], alteration in the structure of neurons at that time may also be evident.

The question we asked, therefore, was to what extent would the hormones of pregnancy and lactation (primarily P and E2) and/or prolactin influence neurons in the mPOA of the female rat, a region that responds to gonadal steroid stimulation with facilitation of maternal behavior [45]? We examined neurons in the mPOA and cortex of females from different reproductive states, including diestrous, late pregnancy, lactation, ovariectomized or ovarioctomized plus a hormone treatment that stimulates maternal behavior. Cortical neurons were included as a steroid-absent control site, because there are few steroid receptors present in the cortex [40,52,57] and, therefore, few direct morphological changes in neuronal structure would be expected. Together, the data shed light on hormone-neuron interactions that have primarily maternal behavioral implications.

MATERIALS AND METHODS

Animals

Adult nulliparous female Sprague-Dawley rats (Crl:CD[SD]BR) originally purchased from Charles River Laboratories, Inc. (Wilmington, MA, USA) and maintained as breeding stock were used in the present study. The females were isolated in 20 × 45 × 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 (on from 0500–1900 h) 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 those prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council (DHHS publication No. [NIH] 85-23, Revised, 1985).

Experimental Groups and Procedure

Five groups of adult (80–100 days) females were used: Intact diestrous virgins (n = 6; group diestrous), sacrificed and their brains removed on the day of diestrous (one or two) (Diestrus 0); diestrous females were used because they represent a state of naturally low estrogen and low P without the artificiality of the OVX;), virgin, ovariectomized and implanted with blank Silastic capsules (n = 6; group OVX-minus); virgin, ovariectomized and implanted with P and E2-containing Silastic capsules (n = 6; group OVX + P + E2); late pregnant, day 21 (n = 6; group late-pregnant); and day 5–6 postpartum lactating (n = 4; group lactating). All surgery was performed under ether anesthesia.

For the OVX + P + E2 group, females were implanted subcutaneously with sequential steroid-containing Silastic capsules in a regimen that stimulates maternal behavior [1]. After OVX, the females were implanted with three 30-mm Silastic capsules containing P. The capsules remained for 11 days, at which time they were removed and replaced with a 2-mm E2-containing capsule that remained for 10 days. The OVX-minus group was implanted with/removed of blank capsules at times similar to the OVX + P + E2 group. These groups were sacrificed and the brains removed on day 21 of capsule implantation.

The late-pregnant and lactating groups were timed mated in our laboratory. The day that sperm was observed in the vaginal lavage was designated Day 1 of pregnancy, at which time the females were individually housed. The late-pregnant animals were sacrificed on day 21 of pregnancy and the brains removed. On the day of parturition, the lactating females had their litters culled to 10 pups, which remained with the female. The lactating group was sacrificed on days 5–6 postpartum and the brains removed. All surgery and brain removal occurred between 1200 and 1500 h.

Silastic Capsule Preparation

The Silastic implants were prepared using a procedure adapted from [1]. The implants were constructed from tubing (#602-305) purchased from Dow-Corning (Midland, MI, USA). The implants were packed with crystalline steroid (either P or E2; Sigma, St. Louis, MO, USA) and the ends sealed with Silastic cement. After drying, the capsules were washed in ethanol and incubated for 24–48 h in physiological saline at room temperature so stable steroid infusion would commence immediately upon implantation.

Golgi-Cox Staining Procedure

The protocol we used is an adaptation of that published in [53] and modified for short-term staining ([23; Kinsley and Ruscio, unpublished observations]. Animals were sacrificed 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 that follows the plane-of-angle provided in the atlas by Paxinos and Watson [49]; Kopf Instruments, Tujunga, CA, USA). The sections were placed in standard Golgi-Cox stain (potassium dichromate and mercuric chloride, plus potassium chromate and potassium tungstate in dH2O) for 10–14 days, after which they were blocked again (to include the mPOA), super-glued to a metal chuck, and alternate sections were cut at 100 μm (using a Vibratome) into a physiological saline bath. Slices were placed on “subbed” slides and allowed to dry, after which they were exposed for 5-min to the alkalizing solution of lithium hydroxide, which reacts with the Golgi-Cox heavy metal deposits in the neuron to produce the black product characteristic of the stain. They were next run through a dehydration series of alcohols and xylenes. Slides were coverslipped with Permount and allowed to dry.

Image Analysis and Quantification

Part One. The mPOA region and corresponding cortex (as a control condition; see Introduction) were visualized (by investigators naive to the experimental condition) under low magnification (×100) using a Zeiss Axioskop microscope fitted with an Hitachi CCD camera. Only neurons with completely stained cell bodies that were separable from the surrounding neurons and processes were used in the present analysis. We used Golgi-Cox-stained neurons for examining cell bodies (as opposed to a Nissl stain) because of the way in which the neurons could be separated and examined, and rapidly digitized in close detail at high magnification. The following procedure was used to identify and measure the cell body: Each candidate neuron was viewed, brought into clear focus, and the entire cell body identified within its largest plane of focus. The focus was changed to ×400, the entire digitized image was thresholded (between the values 0–255) and, using a PC with a frame-grabber card interfaced to a software package (“Bioquant,” R&M Biometrics, Nashville, TN, USA), each cell body was pseudocolored until completely filled. The pseudocolored cell body was then automatically traced at ×400 by the computer and its area (μm2) calculated. Only neurons whose somas were fully in focus were captured and counted. A total of 50 neurons (25 mPOA and 25 cortical) were traced and measured per animal. Means for each animal (based on the 25 mPOA and cortical neurons) were calculated and combined to form group means. Coordinates for both mPOA and corresponding general cortex were between bregma 0.20 and −0.92 [49]. Cortical neurons, included at random from its different layers corresponding to the mPOA sections, were imaged and traced in a similar fashion to mPOA neurons as a non-hormone control.
Part Two. A separate random sample of Golgi-stained mPOA neurons from the same brains from Part One was used in this morphometric analysis, with care taken to ensure that no duplication of neurons (with Part One) occurred. In the analysis for Part Two, then, we traced entire well-impregnated neurons (save dendritic spines, which were sparse in the mPOA neurons) at \( \times 1000 \), and therefore more closely examined specific neuronal characteristics that were not available in Part One. For example, using a Zeiss Axioplan microscope and Optronics color camera, and a software package expressly designed to trace and record neuronal morphometry ("Neurolucida"; Microbrightfield, Inc., Burlington, VT, USA), we were able to measure the following neuronal features: the number of proximal dendritic branches (the proximal dendrite was defined as the dendrite with the largest diameter leaving the soma, after Uylings et al. [58]); cumulative length of the proximal dendrite; area of the cell body; number of basal dendrites; cumulative basal dendritic length; number of basal dendritic branches; and branch-point (distance from cell body to first branch of principal dendrite). For each of the groups in Part Two, the numbers of neurons that were imaged, traced, and subsequently measured were as follows: OVX-minus: \( n = 17 \); diestrous: \( n = 11 \); OVX+P and E\(_2\): \( n = 14 \); late-pregnant: \( n = 19 \); lactating: \( n = 17 \).

Statistical Analysis

In Part One, two one-way analyses of variance (ANOVA) were performed on the dependent variable of area of the soma of mPOA and cortical neurons using means per animal. (A two-way mixed design using mPOA and cortical neurons as within-group variables was not used because the comparison between the two [viz., mPOA and cortex] was not a meaningful one for our purposes.) Post-hoc examinations using the Least Significant Differences (LSD) test [21] were performed on the significant omnibus \( F \)-test.

In Part Two, a multivariate analysis of variance (MANOVA) was used, with LSD tests as post-hocs. In all cases, differences were considered significant if \( p \leq 0.05 \).

RESULTS

Part One

The ANOVA indicated that there was a highly significant effect of hormonal condition on the somal area of mPOA neurons, \( F(3,23) = 8.284, p < 0.0003 \). For cortex, as expected, no effect of hormonal condition was evident, \( F(3,23) = 2.20, p > 0.10 \). Thus, hormonal condition affects the structure (soma) of neurons in mPOA, a hormone-responsive region (see Fig. 1).

For the mPOA somal areas, post-hoc analyses using the LSD test (critical value = 79, \( p < 0.04 \)) revealed the following pattern: Groups diestrous, OVX-minus and lactating, which did not significantly differ among themselves, were significantly smaller in somal area compared to groups OVX+ P+E\(_2\) and late-pregnant. The latter two groups were not significantly different from each other. A larger number of subjects or a decrease in the variability (or both) might have produced a significant difference between the
OVX+P+E₂ and late-pregnant females (with the latter females having the largest somal areas), a trend that may merit discussion (see below). Thus, being hormonally replete affects the area, and presumably the activity, of mPOA neurons.

**Part Two**

Again, reproductive/hormonal condition had a significant effect on general neuronal structure. The omnibus $F$-test revealed that there were significant effects for somal area, $F(4,73) = 5.70, p < 0.0001$; number of basal dendritic branches, $F(4,73) = 3.93, p < 0.006$; and cumulative basal dendritic length, $F(4,73) = 3.99, p < 0.006$. There were trends for number of proximal dendritic branches ($p < 0.085$) and cumulative length of the proximal dendrite ($p < 0.061$). No differences were observed for number of basal dendrites and branch-point.

The post-hoc tests revealed that, as in Part One, diestrous, OVX-, and lactating females, which did not significantly differ among themselves, had significantly smaller ($p < 0.05$) somal areas compared to groups OVX + P + E₂ and late-pregnant, themselves not differing (see Table 1 for soma and additional measures).

For number of basal dendritic branches, a similar overall pattern was observed, with diestrous, OVX-minus, and lactating, which did not differ among themselves, having significantly fewer basal dendritic branches compared to late-pregnant ($p < 0.02$; $p < 0.05$ for late-pregnant vs. lactating; see Fig. 2). Late-pregnant and OVX+P and E₂ were not significantly different from each other, but OVX+P+E₂ was significantly different from diestrous, OVX-minus, and lactating.

Finally, for cumulative basal dendritic length of mPOA neurons, OVX+ P+E₂, late-pregnant females and lactating (which were not significantly different from one another) had significantly longer basal dendritic length compared to diestrous and OVX-minus ($p < 0.01$). No significant differences were observed between diestrous and OVX-minus females (see Fig. 3).

Figure 4 displays photomicrographs focusing on Golgi-Cox-stained cell bodies from the mPOA of a very different pair of hormone groups, diestrous and late-pregnant female rats. Note the increase in the overall perikaryon area of the late-pregnant females compared to the diestrous females apparent at both low ($\times200$) and high ($\times1000$) magnification.

**DISCUSSION**

The present data suggest the presence of an interesting plasticity in the brains of adult females—a plasticity that correlates potential neuronal activity with maternal behavioral potentials. First, in Part One, females with low P and E₂ levels, (diestrous and OVX-minus) have the smallest mPOA somal area and are not different from each other. Second, late-pregnant and OVX+P+E₂ females, both of which are significantly different from the former two groups, have the largest somas, and those of late-pregnant females, though not significantly larger than those of hormone-treated females, have a trend toward the largest of all. Lastly, postpartum, lactating females, with relatively low P and E₂ levels [2,8,39,54] display a somal area that is similar to the OVX-minus and diestrous groups and significantly smaller than OVX+P+E₂ and late-pregnant female. The latter data suggest a return to the non-hormonal neuronal structure—at least with regard to cell body area—at a time when maternal behavior is fully evident (≈ postpartum days 5–6), possibly owing to direct pup-stimulation.

There are also effects on other aspects of the mPOA neuron in females from different reproductive states. In Part Two we examined additional morphometric characteristics of neurons from diestrous, OVX-minus, late-pregnant, OVX+ P+E₂, and lactating females. Again (using a different image analysis package designed for neuron tracing), differences were observed for somal area, with diestrous, OVX-minus, and lactating, which did not significantly differ among themselves, having significantly smaller somal area (see also Table 1) relative to OVX+P+E₂ and late-pregnant, themselves not differing.

The number of basal dendritic branches was also affected by hormonal condition, in a direction similar to the cell body data, with diestrous, OVX-minus, and lactating, not different among themselves, having significantly fewer basal dendritic branches compared to late-pregnant ($p < 0.02$; $p < 0.05$ for late-pregnant vs. lactating; see Fig. 2). Late-pregnant and OVX+P and E₂ were not significantly different from each other, but OVX+P+E₂ was significantly different from diestrous, OVX-minus, and lactating.

Finally, for cumulative basal dendritic length of mPOA neurons, OVX+ P+E₂, late-pregnant females and lactating (which were not significantly different from one another) had significantly longer basal dendritic length compared to diestrous and OVX-minus ($p < 0.01$). No significant differences were observed between diestrous and OVX-minus females (see Fig. 3).

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>Diestrous</th>
<th>OVX-Minus</th>
<th>OVX + P + E₂</th>
<th>Late Pregnant</th>
<th>Lactating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of proximal dendritic branches</td>
<td>1.09 (0.33)</td>
<td>1.29 (0.26)</td>
<td>1.72 (0.47)</td>
<td>1.78 (0.17)</td>
<td>1.65 (0.35)</td>
</tr>
<tr>
<td>Cumulative proximal dendritic length</td>
<td>297 (43)</td>
<td>195 (19)</td>
<td>372 (62)</td>
<td>365 (110)</td>
<td>216 (34)</td>
</tr>
<tr>
<td>Number of basal dendrites</td>
<td>2.09 (0.26)</td>
<td>2.70 (0.30)</td>
<td>2.39 (0.29)</td>
<td>2.11 (0.19)</td>
<td>2.42 (0.24)</td>
</tr>
<tr>
<td>Branch-point</td>
<td>21.0 (2.37)</td>
<td>25.0 (3.35)</td>
<td>23.34 (5.72)</td>
<td>18.24 (3.15)</td>
<td>25.0 (6.64)</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>17</td>
<td>14</td>
<td>19</td>
<td>17</td>
</tr>
</tbody>
</table>

Numbers are the mean (± SEM). Lengths are in microns and area in square microns. n, number of neurons traced from each group, randomly chosen from the subjects in Part One.

* Significantly different from ovariectomized (OVX) + progesterone (P) + estradiol (E₂) and late-pregnant groups at least at $p < 0.05$ (similar to Part One data. See Fig. 1, as well, for data from Part One).
mPOA, an area that regulates maternal behavior. Hence, hormone-neuron interactions involving the shape or size of the neuronal cell body may influence the pattern of different behaviors characteristic of the female over the various reproductive stages represented.

Gubernick et al. [16] reported that the mPOA of parental male and female mice (the bi-parental *Peromyscus californicus*) undergo significant alterations following the advent of parenthood. The volume of the nucleus increased in females, eliminating the sex difference that existed pre-parenthood. Further, in females with reproductive experience, somal area was significantly increased compared to virgin females. We found a decrease (relative to OVX+P+E2 and late pregnant groups) in somal area during lactation, whereas Gubernick et al. ([16]; who did not control for hormonal status) found an increase. The mPOA appears to be pliable, and dependent, at least in part, on gonadal steroid stimulation.

In the rat, pregnancy lasts approximately 22–23 days. Progesterone levels, by day 3 of pregnancy, have risen substantially and undergo an additional peak by day 15, well into the last third of pregnancy [1,2]. As parturition approaches, levels of P decline precipitously, an abrupt reduction associated with, and apparently required for, both parturition and the onset of parental behavior [8]. The pattern of E2 roughly parallels that of P, though with significantly lower levels [1]. What is mainly different between the two steroid hormones is the pattern that occurs as parturition is imminent. As P declines, E2 displays a slight rise [1,2] that also is associated with the initiation of parental behavior in the female, perhaps through the stimulation of prolactin [5]. It is changes in E2 that appear to be mainly involved in the marked changes in hippocampal dendritic spines reported [34,60–62], though E2 can stimulate prolactin release by itself [25,43]. Numan et al. [47] have demonstrated that the number of mPOA neurons that contained receptors for P was higher toward the end of pregnancy when compared to either early pregnancy or lactation. Hence, the effect on cell bodies being reported here may be regulated, at least in part, by P. That we have observed the greatest increase in somal area in the late-pregnant group, therefore, may be related to the peri-parturitional changes occurring at the time, including increases in placental lactogens to which the P and E2 group would not be exposed (see [6,7]). Females begin to display greater interest in young at this time (days 20–22 of pregnancy) as well [32].

The dynamic hormonal modifications in the female appear to stimulate neuronal changes that may result in long-lasting behavioral events. For example, as discussed in [62] vis-à-vis female sexual behavior, P has a biphasic effect on neuronal structure (dendritic spines on hippocampal CA-1 neurons). Progesterone administered shortly after E2-exposure increases spine density, whereas longer-term exposure (upwards of 18 h) results in a decline in spine density similar to that observed in OVX females. Such an effect may relate to P involvement in the onset of maternal behavior, where its withdrawal is necessary for the exhibition of the behavior [8]. It is not clear, however, to what extent somal areas might be affected, though two studies [13,62] reported no change in cross-sectional cell body area in hippocampal CA-1/3 or granule cell neurons.

Nevertheless, because of the pattern of hormone exposure characteristic of pregnancy, it is interesting to speculate about global changes occurring in the brain of the female following the hormonal changes accompanying sexual receptivity and subsequent pregnancy. That no significant differences were found in the observed cortical neurons from the different hormonal groups suggests that the relative lack of steroid receptors therein [40,52,
57] may account for the absence of direct stimulation of somal size. There is some evidence, however, that steroidal environment does affect the cortex, particularly synaptic connections [19, 35, 41, 42]. Further, the cortical neurons chosen came from the same sections that contained the mPOA neurons, so effects in other portions of cortex cannot be ruled out. Lastly, we have preliminary data (2–3 neurons/animal) showing that the bed nucleus of the stria terminalis (BNST), a hormone-receptor rich area [57], does not appear to show the same degree of plasticity as the mPOA (Kinsley, unpublished observations). Thus, some specificity may be inherent to the mPOA, a hormonal responsiveness that governs its role in maternal behavior.

That P and E2 are capable of stimulating maternal behavior [2], are able to alter the density of dendritic spines in the adult female rat brain [34, 60–62], and may modify the area of mPOA neuronal cell bodies ([16], present data) suggest corresponding intracellular events. McEwen and his colleagues have shown that brief (hours) and long-term (up to 15 days) exposure to gonadal steroids can alter dendritic spine density and nuclear structure [18, 62]. What is happening at the cellular level to cause such changes? The somal hypertrophy suggests augmented cellular metabolism and genomic transcription. For instance, Miller and Erskine [36], using electron microscopy, found that short-term exposure to E2 resulted in somal and nuclear hypertrophy in neurons from the ventromedial nucleus of the hypothalamus of female rats. Coincident with the many changes they observed in morphology, they reported a significant increase in cytoplasmic lysosomes, and in stacking of rough endoplasmic reticulum. The pattern of neuronal changes at this ultrastructural level suggests increases in both cellular metabolism and protein synthesis. Further, synaptic alterations also occur.

Garcia-Segura et al. [12], who discuss evidence for gonadal steroid re-organization of neuron structure in general, report changes in number of axo-somatic synapses in arcuate nucleus during the female estrous cycle (though, we should point out, the changes may not be attributable to changes in the soma, per se).

The development of maternal responsiveness through hormonal modifications may result in permanent neuronal changes [24, 25]. For instance, the mPOA, which has a high concentration of E2 and P receptors [11, 44, 48], may be particularly sensitive (as opposed to BNST discussed above). Once established, however, maternal behavior is preserved independent of direct hormonal regulation. That is, once neuronal networks that elicit maternal behavior are hormonally activated, the behavior is retained or easily activated for long periods of time [2, 24, 38]. Such “adult organizational” changes in the brains of primiparous and multiparous females are believed to be structural. Modney and Hatton’s [37] observations suggest that the neuronal modifications are influenced by stimulation provided by pups (viz., suckling stimulation), but the effects may have been initiated by steroidal exposure during pregnancy.

Hormonal activation of mPOA neurons may increase the processing of information relevant for the exhibition of maternal behavior. For example, Numan and Numan [46] reported that maternal behavior is accompanied by neural c-fos activation in mPOA, an effect that is reduced by morphine [56]. (Morphine also disrupts maternal behavior [4, 14]). Perhaps mPOA neurons ex-

![Fig. 3. Mean cumulative basal dendritic length (in µm) of medial preoptic area (mPOA) neurons from females from Part Two. Ovariectomized (OVX) + progesterone (P) + estradiol (E2) and late-pregnant females (which did not differ between them) had significantly longer basal dendritic length compared to diestrous and OVX-minus, but not lactating. The lactating group had significantly longer basal dendritic length compared to diestrous and OVX-minus. No significant differences were observed between diestrous and OVX-minus.](image-url)
posed to considerable amounts of steroid hormones throughout pregnancy alter their ability to respond to the heightened afferent input that will be present upon delivery of the pups. Steroid hormones have the capacity to modify the membrane potentials, electrical activity and interactions inherent to neurons in the hypothalamus [17,33,51]. And, many hormones (prominently E2), can alter the resting and response firing rates of neurons in hypothalamus [50]. Add changes that occur in neurochemistry in the brains of pregnant/parous females (e.g., changes in neuropeptides, opioids, neurotransmitters, etc.; [3,22,26,31]), therefore, and an mPOA neuron from a pregnant or lactating female may be substantially different in form and function from one taken from a virgin female. An accumulation of these modified neurons is reflected in the significant differences in maternal behavior characteristic of the groups observed in the present work.

E2 and P stimulate the proliferation (and subsequent regression) of dendritic spines in hippocampus [62]. If gonadal steroids affect neuronal size/activity in mPOA, it follows that we may expect to see alterations of hippocampal neuron structure in females exposed to the hormones of pregnancy. We have reported that, indeed, females from groups exposed to similar hormonal treatments as those herein display significant increases in dendritic spine density in hippocampal-CA-1 neurons (viz.; late-pregnant = OVX+P+E2 = lactating > diestrous = OVX-minus; [55]; Kinsley et al., in preparation). Therefore, an interesting picture is beginning to emerge regarding gonadal steroid regulation of brain regions involved in the initiation of maternal behavior, as well as those playing a vital, but subordinate role. For instance, hormone-induced modifications in hippocampus may facilitate resource-gathering behaviors in which the female is engaged; and concurrent alterations in both the hippocampus and the mPOA may contribute to the marked behavioral transition characteristic of the maternal female.

Maternal behavior in the rat involves a myriad of new, pup-directed behaviors, in addition to significant modifications of existing ones [15,59]. For instance, the mother must construct and provision a more elaborate nest; be able to engage in the discrete acts of maternal care, such as retrieving, grouping, crouching over and licking her young; be capable of distinguishing her young and friendly/unfriendly conspecifics; be capable of defending her nest and her young from predators or rogue males and females; eat, drink, and sleep in a different pattern than before; forage more efficiently; and remember the location of both food caches and potential danger spots. Recently we reported that the experiences of pregnancy and pup exposure facilitated spatial memory [27], thereby enhancing the female’s overall maternal behavior. In short, there is a marked difference between what she knew (or had to know) before her pups were born and what she must acquire in order for her offspring to survive and thrive.

In conclusion, the present data demonstrate yet another interesting feature of the female brain: The ability of neurons in the mPOA to modify their shapes under hormonal stimulation, likely

FIG. 4. Photomicrographs of representative Golgi-Cox-stained cell bodies from the medial preoptic area (mPOA) of divergent, but natural, pairs of hormone groups, diestrous (A,C) and late-pregnant female (B,D) rats. Note the increase in the overall perikaryon area of the late-pregnant females compared to the diestrous females apparent at both low (×200, A and B; scale bar: 100 µ) and high (×1000, C and D; scale bar: 20µ) magnification.
in preparation for the demands of parental care during the so-called initiation phase, and then to be modified further during the maintenance phase. Two major questions arise from the present work. First, what neurons or neuronal population are changing? Are the effects global ones, or is there a specific type of neuron that is responding to the hormonal stimulation? Neurons expressing γ-aminobutyric acid, an inhibitory neurotransmitter, have been reported to be under E2 influence in arcuate nucleus [12]. Perhaps, in light of recent work demonstrating the involvement of the fosB gene in maternal behavior [9], the swelling neuronal cell bodies, or modified dendritic arbors, are from neurons expressing the aforementioned protein. And second, because reproductive experience (parity) renders highly parental a formerly non-parentally responsive female [24,26], do the neuronal cell bodies of parous females reflect their reproductive status by changing more or less following subsequent pregnancies and lactations? What would the mPOA cell bodies of simultaneously pregnant and lactating females look like? Nevertheless, the present data attest to the subtle plasticity inherent to the brain in general, and to the female brain, in particular.

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