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Estradiol and Progesterone Differentially Regulate the Dendritic Arbor of Neurons in the Hypothalamic Ventromedial Nucleus of the Female Rat(*Rattus norvegicus*)

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Abstract

The ventromedial nucleus of the hypothalamus (VMH), with its major subdivisions, the dorsomedial and ventrolateral VMH (dmVMH and vlVMH, respectively), has been studied extensively for its role in female sexual behavior. This behavior is controlled by the vlVMH through the cellular actions of estradiol combined with progesterone. Although the effects of treatment with estradiol alone on neuronal morphology in the vlVMH have been examined, much less is known about the combined effects of estradiol and progesterone on neuronal structure. The present study employed Golgi impregnation to investigate the effects of estradiol treatment alone vs. estradiol combined with progesterone treatment on dendritic arbor of VMH neurons. The dendritic arbor of VMH neurons was somewhat different in the vlVMH vs. the dmVMH, with longer and more dendrites in the vlVMH. Estradiol treatment alone caused a marked reduction in the length of long primary dendrites in the vlVMH, but not in the dmVMH. The estradiol-induced retraction of long primary dendrites in the vlVMH was reversed within 4 hours of progesterone treatment. The differences in the dendritic arbors of dmVMH and vlVMH provide further support for the notion that these two regions have different patterns of neural connectivity. In addition, this study is the first to report opposing effects of estradiol alone vs. estradiol plus progesterone on the dendritic arbor of neurons in the vlVMH. These results suggest a structural mechanism for estradiol alone to have a modest effect on mating behavior while setting the stage for its ample expression.

Keywords

ovarian hormone; plasticity; VMH; dendrite length; Golgi impregnation

The ventromedial nucleus of the hypothalamus (VMH) has been implicated in several functions, but most especially female reproductive behavior (for review see Flanagan-Cato et al., 2001) and organismal energy homeostasis (Brobeck et al., 1943; Elias et al., 2000; Majdic et al., 2002; Powley, 1977; Sawchenko, 1998). These functions may be segregated within the major anatomic subdivisions of the VMH. The dorsomedial subdivision of the VMH (dmVMH) and ventrolateral subdivision of the VMH (vlVMH) are two cell-dense compartments of the VMH clearly discerned with various staining methods. In addition to

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topography, these two subdivisions differ in their axonal projection targets and afferents (Canteras et al.,1994; Ter Horst and Luiten, 1987). The dmVMH has been implicated in energy homeostasis, based partially on its selective expression of leptin receptors (Hakansson, 1998;Mercer et al., 1998). In contrast, the vlVMH has been implicated in female sexual behavior, based in part on its population of neurons that express estradiol and progestin receptors (MacLusky and McEwen, 1980; Pfaff and Keiner, 1973). Immediate early gene studies have supported this double dissociation, with the dmVMH, but not the vlVMH, activated by the administration of the adipocyte hormone leptin (Elmquist et al., 1997; Hubschle et al., 2001). However, only the vlVMH is activated by sexual stimuli (Calizo and Flanagan-Cato, 2003; Flanagan et al., 1993; Flanagan-Cato and McEwen, 1995; Flanagan-Cato et al., 2006; Pfaus et al., 1993). Given the relevance of metabolic status to reproduction, and vice versa (Wade and Schneider, 1992), questions remain regarding the possible parallel processing and/or cross-communication between these two subdivisions of the VMH.

Estradiol and progesterone exert their effects on female rat sexual behavior by binding to their cognate receptors in the vlVMH. Both of these ovarian hormones are required for the full expression of female mating behavior, albeit in qualitatively different ways. Estradiol modestly facilitates receptivity, quantified by a small increase in the lordosis quotient (Beach, 1942; Boling and Blandau, 1939). With the combined treatment of estradiol and progesterone, the lordosis quotient becomes near maximal, and females also exhibit solicitatory and proceptive behaviors not seen in animals treated with estradiol alone. Because estradiol receptor activation is required for the synthesis of progestin receptors in the vlVMH (Blaustein and Wade, 1978; MacLusky and McEwen, 1978; Parsons et al., 1982), progesterone cannot influence mating behavior in the absence of estradiol. Thus, both estradiol and progesterone act on vlVMH neurons to promote female sexual behavior, but through different neurobehavioral mechanisms.

The effects of estradiol treatment on sexual behavior may be mediated by neuronal plasticity in the VMH. Ultrastructural analysis and Golgi impregnation studies have shown that estradiol increases the number of axodendritic synapses and the density of dendritic spines in the VMH of adult female rats (Frankfurt et al., 1990; Frankfurt and McEwen, 1991). More specifically, estradiol increases dendritic spine density specifically in the vlVMH, but not the dmVMH (Calizo and Flanagan-Cato, 2000). Although estradiol's influence on neuronal morphology has been well studied, the effect of progesterone on dendritic structure in the VMH is unknown. In intact animals, neurons in the vlVMH have longer dendrite trees on proestrus than diestrus (Madeira et al., 2001). Given that proestrus is a hormonally dynamic phase of the cycle, it is unclear which ovarian hormone is responsible for this elongation. The present study tested the hypothesis that estradiol and progesterone exert unique effects on VMH neuronal morphology. This study is the first to show that estradiol and progesterone distinctly regulate dendrite length in the vlVMH.

MATERIALS AND METHODS

Animals

Thirty-eight adult Sprague-Dawley female rats (Taconic, Hudson, NY) were group housed in plastic tubs (41 mm × 21 mm × 22 mm) with standard bedding. Rat chow and water were available ad libitum. The colony was maintained on a 12/12-hour reverse light/dark cycle, with lights off at 1100 hours. After a 1-week acclimation period, animals were bilaterally ovariectomized. The ovariectomy was performed with rats under general anesthesia (90 mg/kg ketamine and 9 mg/kg xylazine, both i.p.). After the ovariectomy was completed, rats were given yohimbine (2.1 mg/kg, i.p.) to counteract the anesthesia produced by xylazine. The Institutional Animal Care and Use Committee of the University of Pennsylvania approved all animal procedures.

Experimental design

One week after surgery, animals were assigned to a vehicle control group or one of two hormone treatment groups. The hormones, 17β-estradiol benzoate (EB; 10 μg in 100 μl sesame oil, s.c.) and progesterone (P; 500 μg in 100 μl propylene glycol, s.c.), were given in a specific regimen over 4 days. The vehicle control group (n = 8) received injections of sesame oil (100 μl, s.c.) on days 1 and 2, followed by propylene glycol (100 μl, s.c.) on day 4 between 0800 and 0900 hours. Animals in the vehicle group were sacrificed 4 hours (1200-1300 hours) after the propylene glycol administration.

The two other treatment groups were primed with EB on days 1 and 2. On day 4, they received either vehicle (EB group, n = 10) or P (EBP group, n = 5). Animals in the EB group were killed 4 or 24 hours after the vehicle injection (i.e., 48 or 96 hours after the first administration of estradiol). Animals in the EBP group were killed 4 hours after the progesterone injection on day 4.

Golgi impregnation

Animals were deeply anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (9 mg/kg, i.p.). Brains were quickly removed and prepared according to the FD Rapid Golgi Stain Kit User Manual (FD NeuroTechnologies; Ellicot City, MD). Brains were incubated in a potassium dichromate, mercuric chloride, and potassium chromate solution for 2 weeks. After this incubation, VMH sections (200 μm) were obtained using a vibratome (Vibratome Series 1000). Sections then were placed onto gelatin-coated slides and coverslipped with Permount (Fisher Scientific) immediately after dehydration to eliminate cracking in the sections.

Morphological analysis

Sections containing the VMH were viewed with a BX50 research microscope (Olympus; Central Valley, PA). Neurons were considered to be in the dmVMH vs. the vlVMH based on the borders of the subdivisions according to Paxinos and Watson (1986; Fig. 1). For atlas plates in which the VMH was not subdivided, neurons were considered to be in the dmVMH or vlVMH if they were located in the dorsal or ventral portion of the VMH, respectively. VMH neurons were then visualized at ×1,000 and traced via camera lucida. Next, the tracings were scanned with a Hewlett Packard Scanjet 3970, and dendrite length was measured in NIH Image v.1.62. Only neurons in which the dendritic arbor was clearly discernible in one 200-μm section were analyzed.

Dendrites were categorized as either primary or secondary based on whether they extended directly from the soma or branched from a primary, respectively. Primary dendrites were further categorized as the longest or short, based on length as in previous studies (Calizo and Flanagan-Cato, 2000, 2002). By definition, each neuron possessed only one long primary dendrite (LPD). All other primary dendrites were considered “short primary dendrites” (Fig. 2). After classifying dendrites into these categories, dendrites were determined to be ramifying in one of four directions: dorsomedial, ventromedial, dorsolateral, or ventrolateral (Calizo and Flanagan-Cato, 2000). All morphological analyses were performed with the experimental group concealed.

Data and statistical analysis

After Golgi impregnation, some sections were labeled too intensely to identify accurately the dendritic arbor of VMH neurons. This study included only animals with a minimum of four neurons in which all the dendrites could be easily distinguished from other dendrites of neighboring neurons. Twenty-three animals met these criteria, and nine were excluded. Among these 23 animals, a subset of the vehicle and estradiol alone groups was further examined for

neurons within the dmVMH. All comparisons between the dmVMH and vlVMH were made using only neurons from vehicle-treated animals. Dendrite length was based on an average of triplicate measurements. This measure was specific to each dendrite type of each neuron, not a summation of total dendrite length for a neuron. The dendrite length for the short primary dendrite represented an average when there was more than one of this dendrite type per neuron. Values for each animal were calculated as an average of the values per neuron. One of two investigators measured dendrite length. The interobserver reliability was 0.99. The *n* presented in all figures and in the Results reflects the number of animals per group.

To determine whether dendrite directions were different from those predicted by chance, a χ^2 test was employed. To assess multiplegroup comparisons, a one-way ANOVA was conducted and, if warranted, followed by the Bonferroni-corrected *t*-test. When comparing only two groups, a *t*-test was employed assuming equal variances. Averages are expressed as the mean ± SE. Significance was set at $P < 0.05$.

Image acquisition and processing

All photomicrographs were acquired with a charge-coupled device digital color camera attached to a BX50 research microscope (Olympus). Images were then stored in the TIFF format using the Spot RT software v.4.0 (Diagnostic Instruments; Sterling Heights, MI). Next, the contrast and brightness of images were modified in Microsoft Powerpoint v.11.3.5 (Microsoft Corporation; Redmond, WA). Finally, the images were resized for publication in Adobe Photoshop v.7.0 (Adobe; San Jose, CA).

RESULTS

Neuronal location and general dendritic arbor

This study included 209 neurons along the rostralcaudal axis of the VMH. Among these, 128 neurons (22 animals, four to seven neurons per animal) were localized within the vlVMH and 81 neurons (15 animals, four to six neurons per animal) were located in the dmVMH (Fig. 1). In the vlVMH, 53 and 75 neurons were found in the rostral (2.30-2.56 mm posterior of bregma; plates 27 and 28) vs. caudal (2.80-3.30 mm posterior of bregma; plates 29-31) aspects, respectively (Paxinos and Watson, 1986). Among the neurons analyzed in the dmVMH, 31 neurons were located in the rostral plane (Fig. 1A) and 50 neurons were found in the caudal plane (Fig. 1B). Chi-squared analysis did not reveal significant differences in the rostrocaudal distribution of the analyzed dmVMH and vlVMH neurons based on treatment group.

Neurons pooled from both VMH subdivisions in all treatment groups displayed attributes consistent with previous findings. Each neuron in the VMH included an average of 1.6 ± 0.1 short primary dendrites (Calizo and Flanagan-Cato, 2000, 2002). The LPDs (143.6 ± 7.0 μm) were substantially longer than both secondary and short primary dendrites ($F_{2,69} = 76.9$, $P < 0.05$, *t*-test with Bonferroni correction for multiple comparisons). Overall, the average length of short primary dendrites (64.1 ± 2.9 μm) was significantly greater than those of secondary dendrites (42.8 ± 3.2 μm, $P < 0.05$, *t*-test with Bonferroni correction for multiple comparisons). Although not analyzed, dendritic spines were discernible on all dendrites examined. Figure 2 illustrates a typical Golgiimpregnated VMH neuron with labeled dendrite types. Figure 3 shows an example of an LPD ramifying from the cell body of a neuron in the vlVMH.

Differences between VMH subdivisions

The first goal of this experiment was to compare the dendrite morphology observed in the two major VMH subdivisions. As mentioned above, all comparisons between the dmVMH and the vlVMH were restricted to animals in the vehicle group. Short primary dendrites and LPDs within the dmVMH and vlVMH exhibited different directional preferences (Fig. 4). Short

primary dendrites in the dmVMH did not extend in a preferred direction, but their counterparts in the vlVMH were more likely to radiate in the dorsomedial direction (Fig. 4A; $P < 0.05$, χ^2 test). Likewise, LPDs on neurons in the dmVMH did not selectively extend toward any particular quadrant. In contrast, LPDs on neurons in the vlVMH preferentially ramified in the ventrolateral direction (Fig. 4B; $P < 0.001$, χ^2 test). There was also a pattern for LPDs to extend toward the opposite VMH subdivision. For example, 22% of LPDs in the vlVMH ramified dorsomedially toward the dmVMH, whereas only 13% of neurons in the dmVMH extended in the dorsomedial direction. Similarly, 31% of LPDs in the dmVMH extended toward the vlVMH compared with only 13% of LPDs in the dmVMH that reached in the dorsomedial direction.

In addition to dendrite directional preference, the dmVMH and vlVMH also exhibited a difference in the number and length of dendrites. Neurons in the vlVMH had significantly more dendrites per neuron. This increase in dendrite number was specific to secondary dendrites (Fig. 5A,B; $P < 0.05$, *t*-test). Not only did neurons in the vlVMH have more secondary dendrites, but they also had longer secondary dendrites than neurons in the dmVMH. Figure 5C shows the increased lengths of secondary dendrites in the vlVMH compared with the dmVMH. This increase was specific to secondary dendrites branching from LPDs in the vlVMH (Fig. 5D). In the vlVMH, neurons with LPDs extending in the ventrolateral direction were markedly longer than their counterparts in the dmVMH (215.5 ± 28.1 μm vs. 138.2 ± 10.3 μm; $P < 0.05$, *t*-test).

Dendrite-specific effects of estradiol

The second question addressed by this experiment was whether estradiol and progesterone differentially regulate neuronal morphology in the VMH. The EB group included animals that were sacrificed 48 or 96 hours after the first injection of estradiol. Measurements from these animals were nearly identical to each other in every aspect and, therefore, were combined into one group for analysis. Several parameters did not vary across the three treatment groups. Ovarian hormone treatment did not alter the number or the length of short primary dendrites in the dmVMH or vlVMH. Moreover, hormone treatment did not alter the directional preferences of long and short primary dendrites in either subdivision or secondary dendrite length (data not shown). Finally, estradiol administration alone did not influence dendrite number or length in the dmVMH.

Neurons of animals in both the estradiol alone and the estradiol combined with progesterone groups contained fewer dendrites than those in the vehicle group (Fig. 6; $F_{2,20} = 4.3$, $P < 0.05$, *t*-test with Bonferonni correction for multiple comparisons). This estradiol-induced reduction in dendrite number was specific to a loss of secondary dendrites (Fig. 6B; $F_{2,20} = 4.0$, $P < 0.05$, *t*-test with Bonferonni correction for multiple comparisons) and was not recovered within 4 hours of progesterone administration. More specifically, estradiol treatment decreased the number of secondary dendrites branching from LPDs, not those branching from short primary dendrites in the vlVMH (Fig. 6C; $F_{2,20} = 8.0$, $P < 0.05$, *t*-test with Bonferonni correction for multiple comparisons).

Estradiol and progesterone exert distinct effects on LPD length

The hormone treatments exhibited marked effects on LPD length in the vlVMH. Treatment with estradiol alone reduced the lengths of LPDs by 50 μm (31%) compared with vehicle (Fig. 7A; $F_{2,20} = 3.6$, $P < 0.05$, *t*-test with Bonferonni correction for multiple comparisons). Progesterone treatment reversed this estradiol-induced reduction in LPD length, restoring lengths to those equivalent of the vehicle group ($P < 0.005$, *t*-test with Bonferonni correction for multiple comparisons). These opposing actions of ovarian hormones were specific to LPDs extending in the ventrolateral direction. Estradiol decreased LPD length on these dendrites

extending ventrolaterally (Fig. 7B; $F_{2,18} = 3.7$, $P < 0.05$, *t*-test with Bonferonni correction for multiple comparisons), and there was a trend for progesterone to reverse the estradiol-induced reduction on these dendrites ($P < 0.07$, *t*-test with Bonferonni correction for multiple comparisons).

DISCUSSION

The goal of this study was two-pronged. The first set of analyses compared the patterns of dendritic morphology in the dmVMH and vlVMH, predicted to be different based on prior evidence that these two regions subserve different functions. In this regard, several differences were found between the dendritic arbors of dmVMH and vlVMH neurons, suggesting a simpler dendritic tree in the dmVMH. The effect of estradiol treatment on LPD length was restricted to the vlVMH. The second analysis determined whether estradiol alone vs. estradiol combined with progesterone treatment have distinct effects on VMH neuronal morphology, as predicted by the hypothesis that changes in VMH dendrites correspond to changes in reproductive behavior. In support of this hypothesis, the hormone treatments had differential effects on LPD length in the vlVMH, with estradiol treatment reducing LPD length. Subsequent treatment with progesterone rapidly reversed the effect of estradiol on LPD length within 4 hours.

It is noteworthy that the VMH neurons analyzed in the present study had characteristics similar to those noted in previous Golgi impregnation, Lucifer yellow, and DiI cell-filling studies (Calizo and Flanagan-Cato, 2000; Flanagan-Cato et al., 2006; Frankfurt et al., 1990; Madeira et al., 2001; Millhouse, 1979-1981). For example, in all studies, neurons in both VMH subdivisions had relatively sparsely branched dendritic trees, exhibiting three to six dendrites. In addition, the dendrite categories, along with associated average lengths, continue to appear valid. Thus, VMH neurons in both subdivisions may be organized to integrate distally arriving cues on LPDs and local inputs on short primary dendrites. Overall, the dendritic arbor of VMH neurons has remained consistent regardless of the labeling method.

Neuronal morphology in the dmVMH and vlVMH

Neurons in the vlVMH contained more secondary dendrites than their counterparts in the dmVMH, and those that branched from LPDs extended farther than secondary dendrites in the dmVMH. Previous Golgi impregnation work also had observed that neurons in the vlVMH have longer dendrites than those in the dmVMH, although all dendrite types were combined in that study (Madeira et al., 2001). The present work identifies the number and length of secondary dendrites as the main difference in dendritic arborization between the two VMH subdivisions. These differences would produce a greater dendritic surface for synaptic contact in the vlVMH compared with the dmVMH. The increased dendrite surface may enable vlVMH neurons to integrate a larger range of interoceptive and environmental cues to optimize mating behavior.

In addition to the quantitative difference in potential inputs, these subdivisions also may differ qualitatively with regard to source of inputs as seen in differences for directional preference. Almost half of the LPDs in the vlVMH extended ventrolaterally toward the nearby fiber plexus. Many extranuclear afferents to the VMH do not penetrate the VMH but rather terminate in a neuropil laterally adjacent to the VMH (Millhouse, 1973a). For example, oxytocinergic and noradrenergic afferents are found in this fiber plexus and have been implicated in the regulation of female rat sexual behavior (Etgen et al., 1992; Schumacher et al., 1990). These differences in input number and source may contribute to the distinct regulatory roles of the vlVMH and dmVMH.

Despite the biochemical evidence that suggests different functions of the vlVMH (e.g., the estrogen receptor-α) and dmVMH (e.g., the leptin receptor), these two regions also may exhibit

cross-communication (DonCarlos et al., 1991; Hakansson, 1998; Mercer et al., 1998; Pfaff and Keiner, 1973; Simerly et al., 1990; Shrugue et al., 1992). Axonal tract tracing studies have indicated that these two subdivisions send axonal projections to each other (Ter Horst and Luiten, 1987). Our data expand on this finding by demonstrating that neurons in each region extend dendrites toward the other subdivision. These axonal projections and dendrite extensions may contribute to the critical interrelations between metabolic and reproductive status (Wade and Schneider, 1992).

Dendritic branching

The effects of estradiol on VMH neuronal morphology were confined to the vlVMH, consistent with previous reports that the dmVMH does not respond to estradiol treatment with changes in synapse number or dendritic spine density (Calizo and Flanagan-Cato, 2000; Nishizuka and Pfaff, 1989). This is consistent with other evidence, discussed above, that the vlVMH, and not the dmVMH, controls reproductive functions. Because no effect of estradiol treatment was observed in the dmVMH, this region was not examined for possible subsequent effects of progesterone. In the vlVMH, estradiol treatment alone reduced dendrite number, specifically the amount of secondary dendrites emanating from LPDs. This reduction in dendrite number was not affected by a 4-hour exposure to progesterone. Maravall and colleagues (2004) have shown that sensory experience can remove and rearrange secondary dendrites while the number of primary dendrites remains stable in the barrel cortex. Recently, workers in our laboratory have demonstrated that sexual experience decreases dendritic spine density only on secondary dendrites in the vlVMH (Flanagan-Cato et al., 2006). Taken together, these studies demonstrate that secondary dendrites are important sites for structural plasticity. The present finding that estradiol acts on secondary dendrites suggests that sexual behavior may be regulated by changes in this highly plastic dendrite type.

Dendrite length

A novel finding reported here is that estradiol and progesterone exert opposing actions on VMH neuronal morphology. In the vlVMH, the effect of the ovarian hormones on dendrite length was restricted to LPDs and their secondary dendrites. As discussed above, the LPDs may be uniquely positioned to respond to extranuclear inputs. Previous work in our laboratory using cell filling to visualize neurons indicated that estradiol reduces dendritic spine density on LPDs (Calizo and Flanagan-Cato, 2002). That study did not observe a significant estradiol-induced reduction in dendrite length. However, reanalysis of those data indicates a similar trend for estradiol to retract LPDs by 24% on neurons in the vlVMH but not the dmVMH. Those data did not reach statistical significance mainly because the number of neurons per animal within the vlVMH was smaller than the present study. Collectively, the reduction in length and spine density on LPDs after estradiol treatment suggests that these dendrites may be associated with the regulation of sexual behavior. Revealing the inputs to LPDs in the vlVMH would be an important step toward further dissecting this behaviorally relevant circuit.

The exact neurophysiological and behavioral consequences of estradiol-induced retraction of LPDs in the vlVMH are not known. One possible scenario is that estradiol curbs the influence of extranuclear projections, such as oxytocinergic and noradrenergic inputs, to preclude female mating behavior. Although it is often noted that estradiol promotes mating behavior, it also may invoke mechanisms to limit this behavior until progesterone levels rise. One such mechanism is the activation and internalization of the mu-opioid receptor. Mills and colleagues (2004) have shown that estradiol treatment alone activates and internalizes the mu-opioid receptor in the female rat medial preoptic nucleus. Subsequent administration of progesterone reverses the activation and internalization of the mu-opioid receptor, thereby enhancing female sexual receptivity. Here we show that, consistent with this notion of progesterone reversing

the effects of estradiol, subsequent acute progesterone treatment prompted LPD elongation within 4 hours.

A weakness with this correlation between dendrite length and behavior is that the LPDs are fully extended in the vehicle treatment group, the group expected to display the lowest levels of sexual responsiveness. A possible explanation for this paradox may be that estrogen receptor activation has parallel effects on other cellular processes (Pfaff and Schwartz-Giblin, 1988), such as increased spine density on short primary dendrites (Calizo and Flanagan-Cato, 2000). Thus, the vehicle control group may have properly positioned LPDs but hyporesponsive neurons. The combination of estradiol and progesterone may provide the necessary scenario of excitability and connectivity.

A recent study in our laboratory revealed that food deprivation also reduced the length of LPDs in the vlVMH, but not the dmVMH (Flanagan-Cato et al., 2008). This result was surprising given that the dmVMH has been more associated with the regulation of energy balance, as discussed above. Taken together with the present results, shorter LPDs in the vlVMH may be associated with various conditions that inhibit mating behavior, ranging from food restriction to elevated levels of estradiol in the absence of progesterone. This interpretation is based on the notion that morphological changes in the vlVMH mediate the regulation of reproductive behavior. Alternatively, brain regions may differ in their ability to exhibit dendrite plasticity. The vlVMH may have a bias to use rewiring as a mechanism to modify neurobehavioral functions, including reproduction and energy balance, to a greater extent than the dmVMH.

The unique actions of progesterone

The current results have a direct bearing on a study of vlVMH neuronal morphology in intact females at two different stages of the estrous cycle. In particular, dendrites were longer on the afternoon of proestrus, the phase during which plasma levels of both estradiol and progesterone peak, compared with diestrus, when both estradiol and progesterone levels are quite low (Madeira et al., 2001). From this experimental design, it cannot be determined which ovarian hormone promotes the increase in dendritic length observed on proestrus. By examining ovariectomized, hormone-treated female rats, the present results clarify the dendrite elongation seen on proestrus by implicating progesterone, rather than estradiol. Given the rate at which progesterone treatment reextends LPDs (within 4 hours), it is possible that the hormone may be exerting a nongenomic effect on dendrite length. Our design did not test for nongenomic effects of progesterone by analyzing a progesterone-alone group but rather utilized the physiological context of the estrous cycle, where plasma levels of progesterone surge after levels of estradiol peak. The reduced dendrite length observed on diestrus may seem at odds with the LPD length displayed in our vehicle-treated group. However, it should be noted that, during diestrus, LPDs are subject to the effects of low levels of estradiol, the delayed effects of progesterone, or both. Therefore, the vehicle-treated group does not accurately represent diestrus. Overall, our results confirm and extend the previous work on ovarian hormones and vlVMH neuronal morphology.

Potential mechanisms

The present study contributes to our understanding of hormone-induced plasticity in the vlVMH, but questions remain regarding the cellular mechanisms. Additional studies are needed to determine whether factors released from axons in the VMH neuropil promote the retraction and/or elongation of LPDs. Estradiol treatment has been shown to increase levels of the GAP-43 in the vlVMH, a protein implicated in axonal elongation (Lustig et al., 1991; Shughrue and Dorsa, 1993). Previous studies suggest that the effect of estradiol treatment on dendrite length may be region and/or cell specific, having both elongated dendrites in the hippocampus and cerebellum (Audesirk et al., 2003; Sasahara et al., 2007) and retracted dendrites in

serotonergic neurons (Lu et al., 2004). In the cerebellum, brain-derived neurotrophic factor (BDNF) has been associated with estradiol-induced dendritic growth (Sasahara et al., 2007). Tran and colleagues (2006) have detected BDNF in the vlVMH. Ovarian hormones may regulate the expression of BDNF to alter dendritic length in this subdivision. However, the exact hormonal milieu that may regulate BDNF transcription in this region is unclear (Singh et al., 1995; Sugiyama et al., 2003; Toran-Allerand, 1996; Tran et al., 2006).

The findings of this investigation may pertain to the regulation of the localization of oxytocin receptors in the vlVMH by estradiol and progesterone. Estradiol treatment increases oxytocin receptor mRNA and binding in the vlVMH (Bale and Dorsa, 1995; De Kloet et al., 1986). In estradiol-primed rats, progesterone treatment is associated with a migration of oxytocin receptor binding toward the laterally adjacent fiber plexus, a region with oxytocin-containing axons (Schumacher et al., 1990). The present results suggest that the apparent migration of receptors induced by progesterone may be accomplished in part by progesterone's ability to reextend LPDs.

A previous study in Syrian hamsters found that estradiol treatment increased dendrite length in the vlVMH, but not the dmVMH (Meisel and Luttrell, 1990). The apparent discrepancy with the present finding may be due to a species difference. The control of reproductive behaviors is highly species specific. Based on the existing literature, it seems likely that novel mechanisms underlie the estradiol-mediated retraction of dendrites in the female rat vlVMH.

The current study has several limitations, mainly related to the Golgi impregnation methodology. For example, the chemistry of this technique may label a particular subset of neurons, rendering the results less generalizable to the entire VMH. Another potential caveat is that some dendrites, particularly LPDs in the dmVMH, may be oriented in a manner that causes the dendrite not to be fully contained in one 200-μm-thick coronal section. As stated previously, only neurons in which the dendritic arbor was clearly discernible in one section were analyzed. Nevertheless, if distal dendrites have exited the section, the length may be underestimated. Using sagittal sections instead of coronal sections would provide an alternative approach to assess the lengths of dendrites that extend orthogonally to the coronal plane. However, all neurons examined contained a single LPD that was much longer than the other dendrite types. The reduction in LPD length was striking after estradiol treatment even if the original lengths of LPDs were somewhat underestimated.

This study did not directly quantify the extent to which LPDs protrude out of the VMH proper. Previous studies have shown that dendrites of neurons in the vlVMH do extend into the surrounding fiber plexus (Daniels and Flanagan-Cato, 2000; Millhouse, 1973b). Recent work from our laboratory has shown that the distance between the cluster of neurons containing estrogen receptor-α in the vlVMH and the oxytocinergic fibers running lateral to the VMH proper is 50 μm (unpublished observations). Therefore, it seems likely that LPDs in the vlVMH, particularly those extending in the ventrolateral direction (average length = 215.5 μm), reach into the surrounding fiber plexus. Combining immunofluorescence and cell filling would allow for the exact mapping of dendrites in relation to the VMH border. This approach would provide an opportunity to document close appositions between LPDs from the vlVMH and fibers just lateral to the VMH and compare the extent to which LPDs protruded outside the VMH border.

Another concern in interpreting these results is that the reduction of LPD length may actually reflect a thinning of distal dendrites, occluding the Golgi impregnation, rather than shortening of the dendrites. If estradiol does induce thinning, rather than a retraction, of the dendrite, then that also would have a major impact on the synaptic integration of neurons in the vlVMH. Future followup studies employing other cell-filling methods may clarify whether estradiol shortens vs. thins distal dendrites.

In conclusion, these results address several issues regarding dendrite morphology in the VMH. First, they document differences between the dendritic arbors of neurons in the two main subdivisions of the VMH, which may contribute to their distinct functions. Second, they demonstrate that ovarian hormones regulate dendrite length, in addition to the previously observed changes in dendritic spine density. The changes in dendrite length may affect the number and source of vlVMH synaptic input. Finally, the present findings indicate distinct effects of estradiol and progesterone on dendrite structure that is correlated with their roles in female rat sexual behavior. Taken together, these results portray unique and dynamic effects of ovarian hormones on vlVMH connectivity. Future studies will be aimed at elucidating the biochemical mechanisms underlying these structural changes as well as their behavioral consequences.

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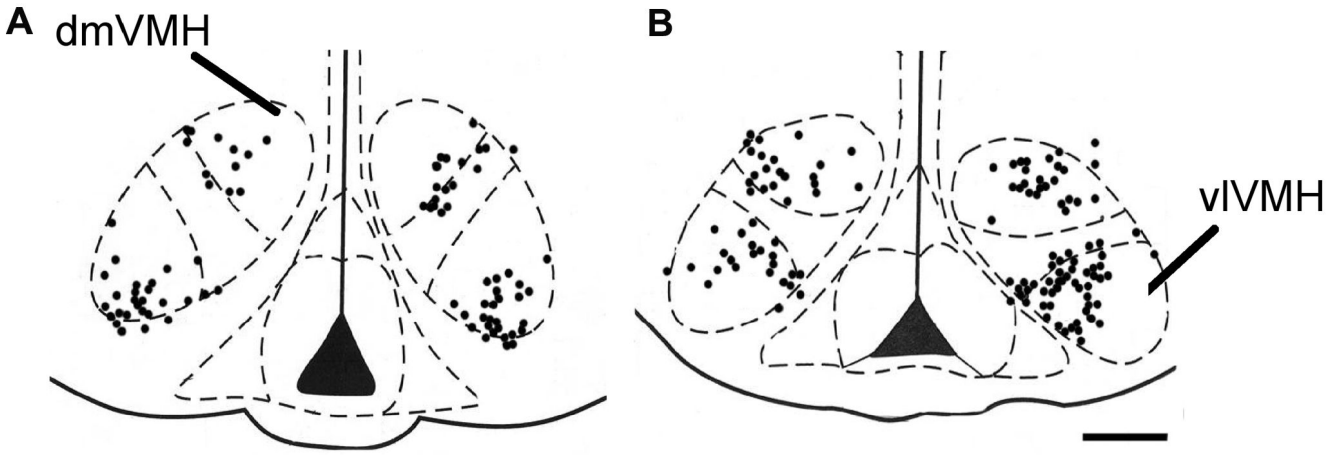

Fig. 1.

Locations of the Golgi-impregnated neurons surveyed in both subdivisions of the VMH. In total 209 neurons were examined in both the dorsomedial subdivision of the VMH (dmVMH) and the ventrolateral subdivision (vlVMH). Sections located 2.30-2.56 mm posterior to bregma (plates 27 and 28 from Paxinos and Watson, 1986) were classified as being rostral (**A**). **B:** Caudal sections of the VMH were those that were 2.80-3.30 mm posterior to bregma (plates 29-31 from Paxinos and Watson, 1986). This figure is a modified version of Figures 30A and 32B from Paxinos and Watson (1998). Scale bar = 400 μm.

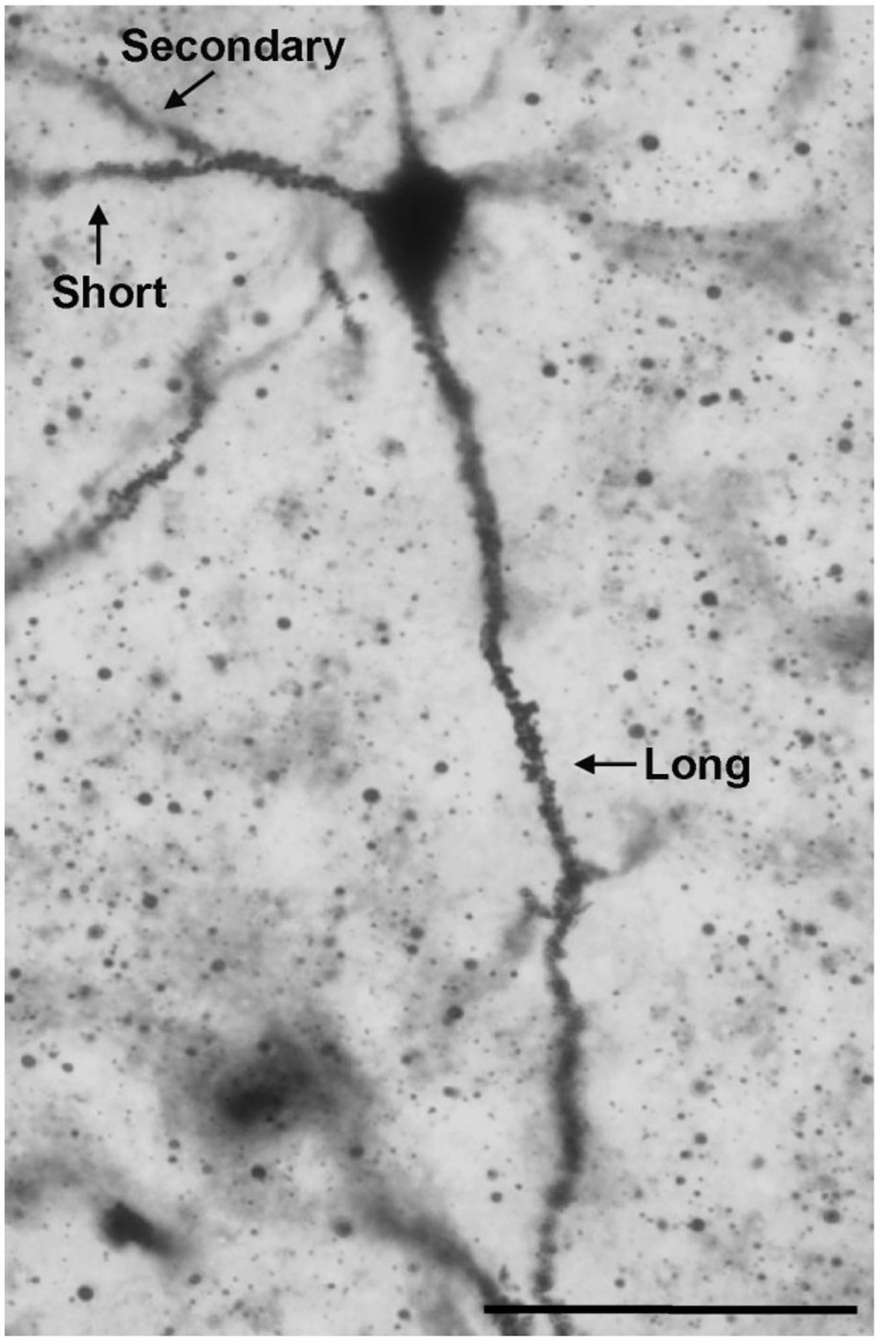

Fig. 2.

Photomicrograph of a representative Golgi-impregnated VMH neuron. Each VMH neuron had a single long primary dendrite (LPD; 137.2 ± 3.4 μm long), and all other primary dendrites were classified as short (64.1 ± 2.7 μm long). Any dendrite branching from a primary dendrite was labeled as a secondary dendrite (39.7 ± 2.3 μm long). All dendrites were statistically significantly different from each other with regard to length. LPDs were the longest, and short primary dendrites were longer than secondary dendrites ($F_{2,69} = 76.9$; $P < 0.05$, *t*-test with Bonferroni correction for multiple comparisons). Scale bar = 100 μm.

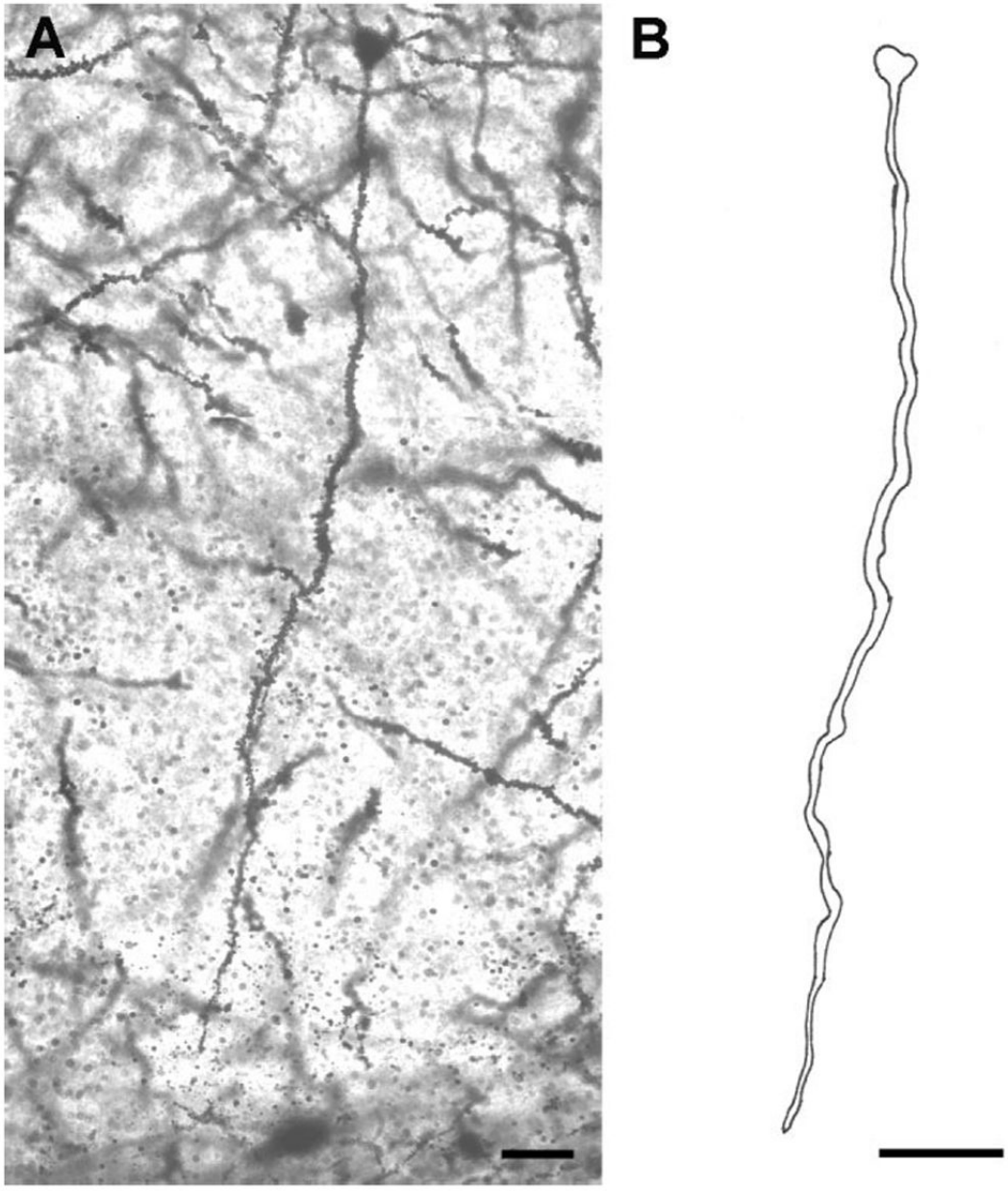

Fig. 3.

A long primary dendrite (LPD) in the vlVMH within the coronal plane of a thick (200 μm) section. **A** depicts a montage of three photomicrographs of an LPD of a neuron in the vlVMH from an ovariectomized female rat in the vehicle group. The associated camera lucida tracing for the LPD is illustrated in **B**. This LPD measured 436.1 μm long. Scale bars = 50 μm.

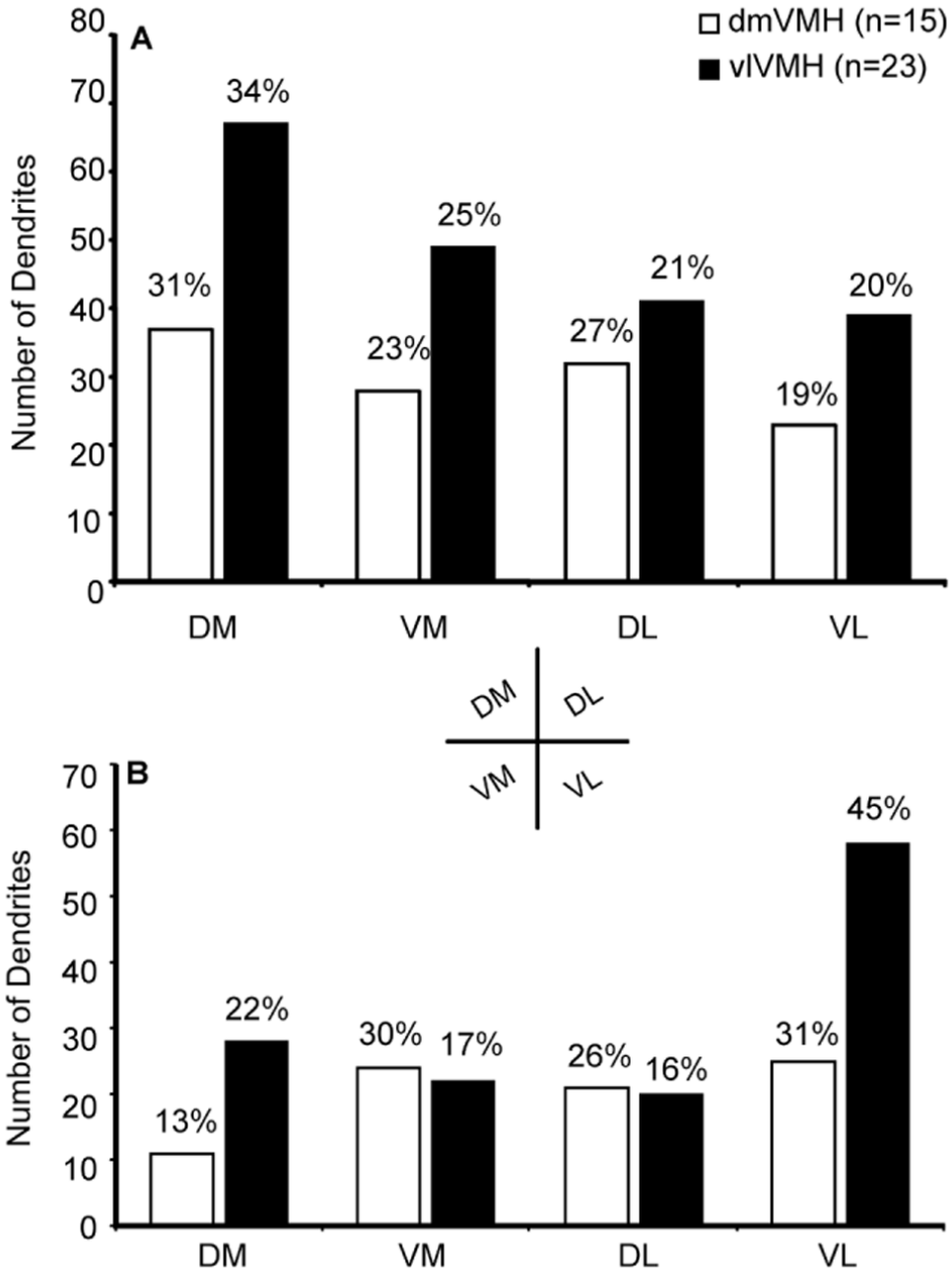

Fig. 4.

Primary dendrites exhibit directional preferences in the vlVMH. **A:** Short primary dendrites displayed a directional preference in the vlVMH (solid bar) but not the dmVMH (open bar). Across all treatment groups, short primary dendrites in the vlVMH extended in the dorsomedial direction more than any other direction ($P < 0.05$, χ^2-test). **B:** Long primary dendrites in the vlVMH subdivision exhibited a directional preference, with the greatest number of dendrites extending in the ventrolateral direction ($P < 0.0001$, χ^2-test). No directional preference was seen in the dmVMH. The numbers above each bar represent the corresponding percentage of dendrites branching in the designated direction (DM, dorsomedial; VM, ventromedial; DL, dorsolateral; VL, ventrolateral). dmVMH, dorsomedial subdivision of the ventromedial

nucleus of the hypothalamus; vlVMH, ventrolateral subdivision of the ventromedial nucleus of the hypothalamus.

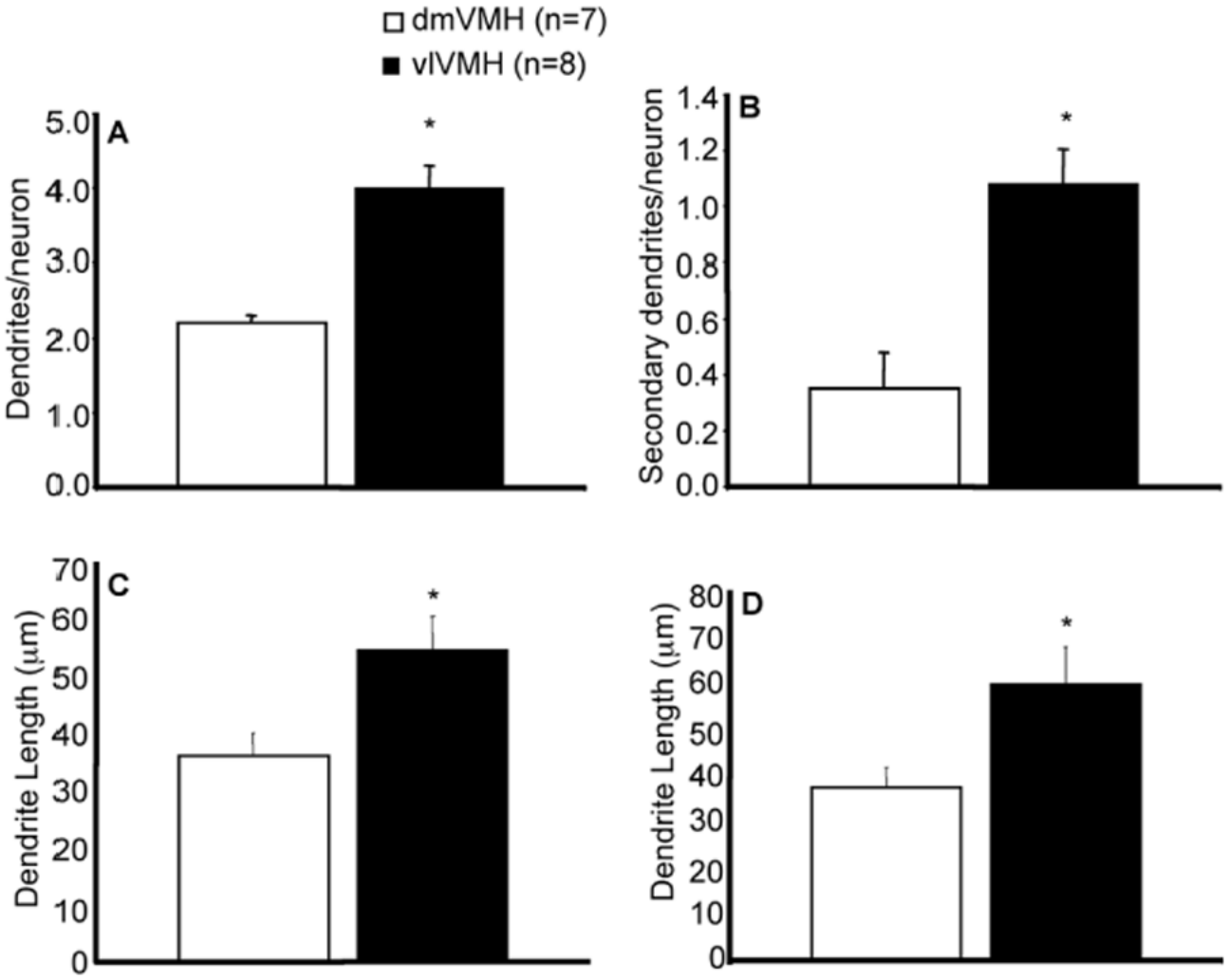

Fig. 5.

Comparison of the dendritic arbors of the dmVMH and vlVMH. Neurons in the vlVMH of vehicle-treated animals contained more dendrites per neuron than their counterparts in the dmVMH (**A**). The increase seen in dendrite number was specific to secondary dendrites (**B**). Neurons in the vlVMH of vehicle-treated animals not only contained more secondary dendrites but these dendrites were also longer compared with the dmVMH (**C**). More specifically, secondary dendrites branching from long primary dendrites (LPDs) extended longer in the vlVMH than in the dmVMH (**D**). *$P < 0.05$, *t*-test. dmVMH, dorsomedial subdivision of the ventromedial nucleus of the hypothalamus; vlVMH, ventrolateral subdivision of the ventromedial nucleus of the hypothalamus.

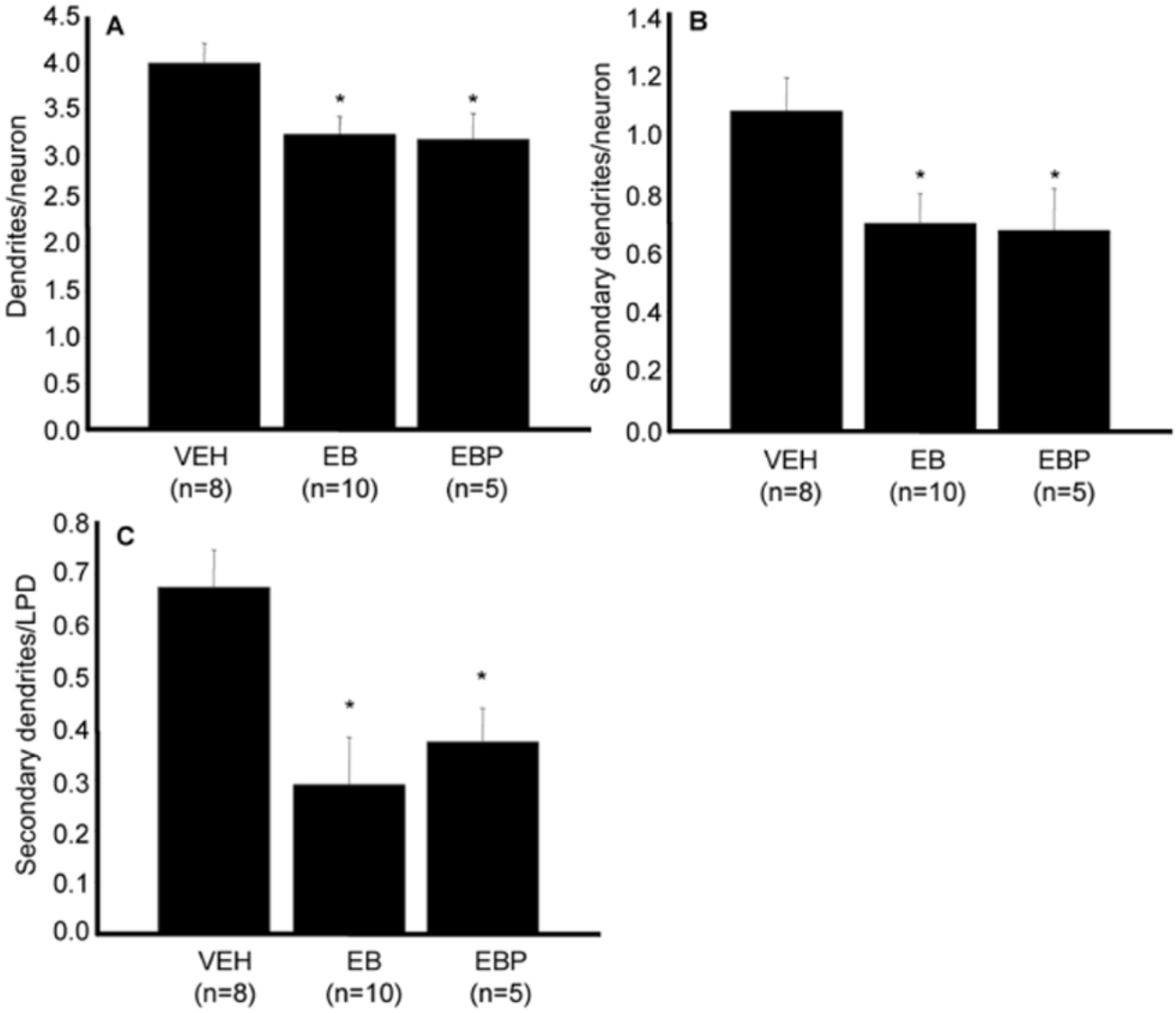

Fig. 6.

Ovarian hormone treatment reduces the number of dendrites per neuron in the vlVMH. Both estradiol alone (EB) and estradiol combined with progesterone (EBP) treatments decreased overall dendrite number in the vlVMH (**A**; $F_{2,20} = 4.3$, ANOVA). The decrease in dendrite number was specific to secondary dendrites in the vlVMH (**B**; $F_{2,20} = 4.0$, ANOVA), particularly those that branched from long primary dendrites (**C**; $F_{2,20} = 8.0$, ANOVA). *$P <$ 0.05, *t*-test with Bonferroni correction for multiple comparisons. dmVMH, dorsomedial subdivision of the ventromedial nucleus of the hypothalamus; vlVMH, ventrolateral subdivision of the ventromedial nucleus of the hypothalamus.

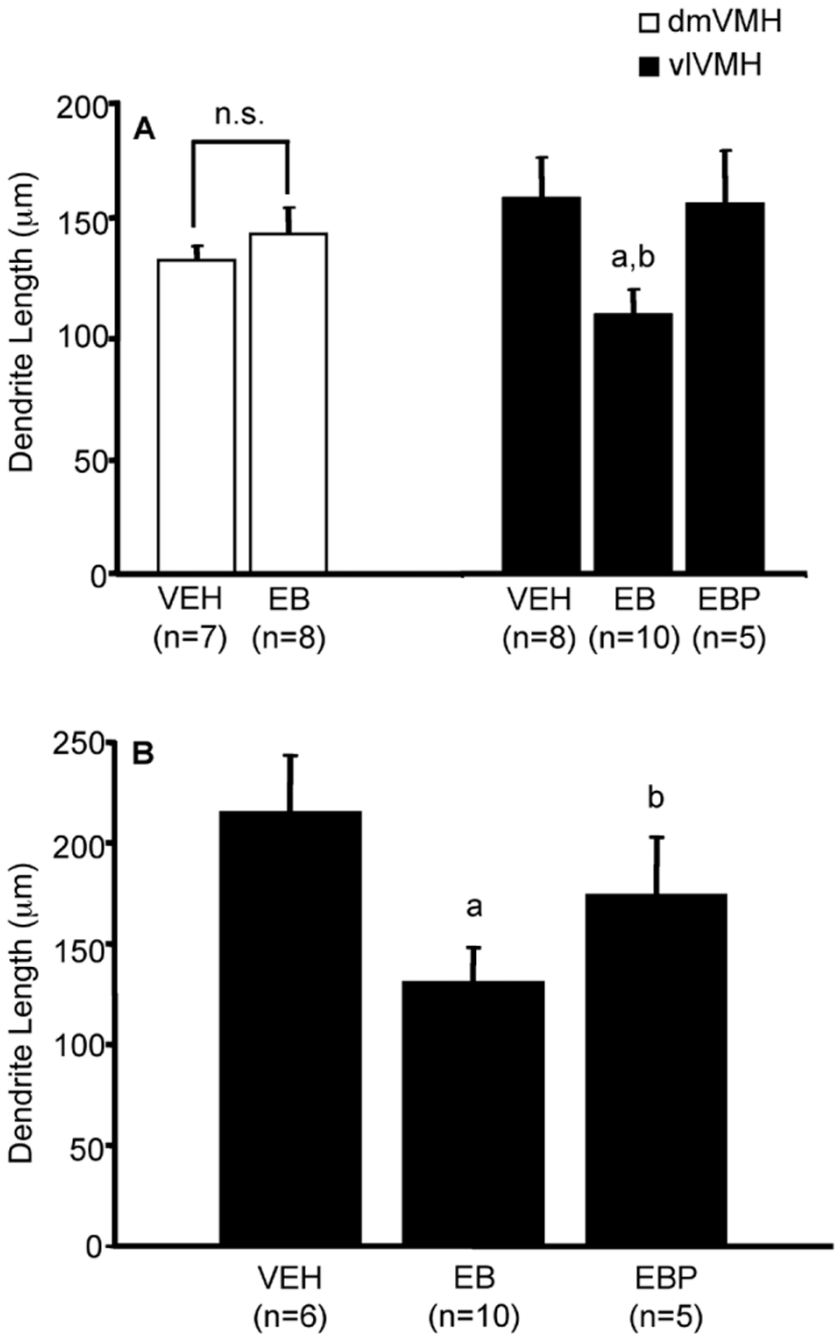

Fig. 7.

Estradiol and progesterone differentially regulate long primary dendrite length in the sexually relevant vlVMH. Estradiol treatment alone (EB) did not alter the lengths (micrometers) of long primary dendrites (LPDs) in the dmVMH (**A**; open bars). Therefore, the neurons of animals treated with both estradiol and progesterone (EBP) were not examined in this region. Estradiol treatment alone caused a 31% decrease in LPD length in the vlVMH (solid bars) compared with vehicle (ANOVA, $F_{2,20} = 3.6$; $P < 0.05$, *t*-test with Bonferroni correction for multiple comparisons). This reduction in dendrite length was reversed within 4 hours of progesterone treatment ($P < 0.05$). These differential effects of estradiol and progesterone were also seen on LPDs in the vlVMH extending in the ventrolateral direction (**B**; $F_{2,18} = 3.7$). In A, the letter

a indicates that the EB group is significantly different from the vehicle group ($P < 0.05$). The letter *b* denotes that the EB group is significantly different from the EBP group ($P < 0.05$). In B, the letter **a** indicates that the EB group is significantly different from the vehicle group ($P < 0.05$); the letter *b* denotes a trend ($P < 0.07$) for the EBP group to be different from the EB group. dmVMH, dorsomedial subdivision of the ventromedial nucleus of the hypothalamus; vlVMH, ventrolateral subdivision of the ventromedial nucleus of the hypothalamus.