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Sex differences in the neural circuit that mediates female sexual receptivity

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Abstract

Female sexual behavior in rodents, typified by the lordosis posture, is hormone-dependent and sex-specific. Ovarian hormones control this behavior via receptors in the hypothalamic ventromedial nucleus (VMH). This review considers the sex differences in the morphology, neurochemistry and neural circuitry of the VMH to gain insights into the mechanisms that control lordosis. The VMH is larger in males compared with females, due to more synaptic connections. Another sex difference is the responsiveness to estradiol, with males exhibiting muted, and in some cases reverse, effects compared with females. The lack of lordosis in males may be explained by differences in synaptic organization or estrogen responsiveness, or both, in the VMH. However, given that damage to other brain regions unmasks lordosis behavior in males, a male-typical VMH is unlikely the main factor that prevents lordosis. In females, key questions remain regarding the mechanisms whereby ovarian hormones modulate VMH function to promote lordosis.

Keywords

estradiol; estrogen receptor; hypothalamus; lordosis; reproductive behavior; sexual differentiation; ventromedial nucleus

I. INTRODUCTION

The basic pattern of sex differences in mating behavior has been conserved by evolution. As a case in point, in the well-studied insect model system, *drosophila melanogaster*, females display a receptive posture while approached from behind for insemination by males [1]. This invertebrate model system has proven useful for the dissection of the genetic controls of these behaviors. Such sex-specific mating behaviors also are seen in mammals. The laboratory rat is a widely used, tractable model system for the study of the neural controls that underlie mammalian mating behavior [2]. Rather than direct effects of sex chromosomes on the patterning of brain sex differences, as occurs in insects, abundant evidence suggests that in rodents the presence or absence of gonadal steroids during development exerts epigenetic effects in the brain to specify the adult mating behavior [3].

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In particular, the absence of hormone action during development is associated with lordosis behavior in adult female rats.

During the hours just before ovulation, a female rat becomes sexually receptive. At this time, courting males approach the female and attempt to mount her to elicit the receptive stance in females, termed the lordosis reflex. This reflex is gated by the sequential actions of the ovarian hormones estradiol and progesterone. Ovariectomy prevents the display of the lordosis reflex, and this effect is reversed by estradiol and progesterone replacement [4]. The lordosis response is not exhibited by intact males or even by adult castrated males treated with ovarian hormones [5], although there are some illuminating exceptions to this general rule, to be discussed below. Therefore, this behavior has provided a platform to examine the neurological mechanisms underlying a sex-specific mammalian behavior. The goal of this review is to consider what has been learned about sex differences in the neural circuitry that underlies female rat mating behavior.

The hypothalamic ventromedial nucleus (VMH) is a critical site of ovarian hormone action to permit the lordosis response, as detailed below. Axonal projections from the VMH to the periaqueductal gray enable the lordosis reflex by modulating posture-control relays to the reticular formation [6;7]. A few sex differences have been observed in the periaqueductal gray itself [8]. Given that considerably more attention has been focused on sex differences in the VMH than its downstream relays, the present review will attend to the ontogeny of the VMH and the various sex differences that have been observed. I also will consider mechanistic studies that have begun to elucidate the manner in which sex differences are established in the VMH. Lastly, I will discuss how the pattern of sex differences in the VMH may elucidate the neural components that contribute to VMH function.

II. THE BEHAVIORAL SIGNIFICANCE OF THE VMH

Numerous lines of evidence support the important role of the VMH in female rat sexual behavior. First, the VMH can retain estradiol and progesterone [9;10], based on its ability to express estrogen and progestin receptors [11;12;13]. The local application of ovarian hormones to the VMH is sufficient for the behavioral effectiveness of these hormones [14;15;16], which further implicates steroid receptors in the VMH as a major target in the control of lordosis. Moreover, VMH lesions disrupt sexual behavior in female rats [17;18], as do transections of the axonal projections that arise from the VMH and descend to the midbrain [19]. Conversely, electrical stimulation of the VMH enhances lordosis responses in the VMH [20]. Finally, female sexual behavior induces immediate early gene expression in the rat VMH, indicative of neuronal activation [21;22;23;24;25;26]. These results are consistent with electrophysiological studies that showed that VMH neurons are responsive to flank and VCS stimulation [27]. Thus, the fundamental role of the VMH in the control of reproductive behavior in female rats is well established.

Beyond laboratory rats, the importance of the VMH for female sexual behavior has been documented in a range of other mammals. For example, the VMH contributes to the lordosis response in guinea pigs [28], hamsters [29;30;31], cats [32], and sheep [33]. Studies in reptiles, such as whiptail lizards [34;35], have indicated that this neural mechanism is phylogenetically old. Furthermore, electrophysiological recordings have supported an active role of the VMH in female sexual behavior in non-human primates [36]. Although mice have not been the mainstay for the behavioral neuroscience of female reproductive behavior, transgenic mice have been tremendously informative regarding the role of developmental transcription factors and ovarian hormone receptors in VMH structure and function [37;38;39;40;41]. The similarities and differences between mice and rats make the case for both species providing valuable complementary information [42]. Thus, it remains true that

the elucidation of the neural mechanisms within the VMH that mediate female mating behavior in laboratory rats may pertain to diverse vertebrate species.

With regard to the human VMH, neuroanatomical studies suggest a similar organization compared with other species, including its cytoarchitecture, source of afferents, and the peptide content of its afferents [43;44;45;46;47]. In addition, the human VMH expresses estrogen, androgen and oxytocin receptors [48;49], as seen in other species, and a variety of sex differences have been reported in the human VMH [50]. A functional imaging study suggested that a sex-specific activation of the ventromedial hypothalamic area occurs in humans after exposure to putative pheromones [51]. These findings suggest that many aspects of the human VMH have been conserved, and that it is not only sexually dimorphic, but it may process sexually relevant information.

Interestingly, the VMH has been implicated in both male and female mating behavior [24;52;53], although the role of the VMH in male mating behavior is in need of further study. In this regard, it is important to remember that while females experience cyclic variations in gonadal hormones, the levels in males remain steady across days. A better understanding of VMH neural circuitry will provide a better explanation the neural basis of sex-specific, hormone-gated behaviors. Next, I will consider the ontological events that establish the VMH as a first step towards explaining the sex differences in the structure and function of the VMH.

III. GENERAL FEATURES OF THE VMH

A. DEVELOPMENT OF THE VMH

The VMH is defined as an elliptical condensation of neurons surrounded by a cell-poor fiber-rich zone in the caudal, mediobasal hypothalamus. This basic structure is apparent before birth in rats. As with other hypothalamic cell groups, VMH development begins with neurogenesis in the proliferative zone of the third ventricle. In the case of the VMH, neurons are born around embryological day 10 (E10; as reviewed by [54]). Terminally mitotic cells migrate ventrolaterally along the processes of radial glia and then differentiate into neurons. A discernable cytoarchitectonic VMH is visible between E18 and E19 in rats [55]. Axonal projections then are established and synaptic inputs are arranged. The surrounding neuropil that makes the VMH so conspicuous histologically includes dendrites extending from VMH neurons, and these dendrites provide a receptive zone for axons arising from other brain regions [56].

An array of transcription factors contributes to the ontogeny of hypothalamic neurons during brain development. For example, *Islet-1* is a marker for the developing hypothalamus [57]. *Nkx2.1* has a narrower role, establishing the medial basal hypothalamus [58]. More restricted still, the normal development of the VMH depends on the expression of a transcription factor known as SF-1, officially designated NR5A1, encoded by the *FTZ-F1* gene, with a rat homolog identified as Ad4BP [59;60;61]. This protein is a member of the orphan nuclear receptor superfamily, and within the brain it is uniquely expressed in the VMH [61]. When the expression of SF-1 is disrupted, a striking malformation of the VMH occurs, with various cell types inappropriately positioned within or outside the nucleus [62;63;64]. When SF-1 is selectively disrupted in the brain, the resulting malformation of the VMH is associated with impaired lordosis behavior [65].

In sum, the VMH develops as a typical hypothalamic nucleus, although it is unique in its expression and developmental regulation by SF-1, which contributes to the spatial organization of VMH neurons. With the importance of the developmental positioning of VMH neurons in mind, it becomes clear that another key feature of VMH function would be

the chemical phenotypes of its neurons. As discussed below, several important phenotypic markers have emerged for VMH neurons, although our understanding is not yet complete.

B. CYTOARCHITECTURE OF THE VMH

The VMH has been parceled into two hemi-ovals, the dorsomedial (DM-VMH) and the ventrolateral (VL-VMH), with a narrow cell-poor central region between them [56;66;67;68;69]. The DM-VMH and the VL-VMH differ in their patterns of gene expression, as summarized in Table 1. Soma size in the VL-VMH is larger than soma size in the DM-VMH and the central region [70]. Analyses of the subdivision-specific afferents and projection targets also indicate unique patterns of connectivity for these subdivisions [67]. The surrounding shell, also referred to as the fiber plexus, the neuropil, or the lateral rim, contains axonal processes from other brain regions containing neurotransmitters and modulators, including norepinephrine, serotonin, gonadotropin releasing hormone, and oxytocin [71;72;73]. There also are sparse neurons found in the shell [74].

VMH neurons maintain a simple dendritic tree, with the following characteristics: a single very long primary dendrite, a few much shorter primary dendrites, and a few secondary dendrites [75]. The long primary dendrite may be uniquely positioned to contact afferents terminating in the fiber plexus surrounding the VMH. The length of these long primary dendrites are regulated by a variety of physiological conditions [76;77;78;79], thus potentially titrating VMH sensitivity to extranuclear inputs. The short primary dendrites, in contrast, may be situated to receive input from local interneurons. In this way, individual VMH neurons can integrate local computations with afferent inputs arriving from other brain regions.

At the ultrastructural level, both excitatory and inhibitory synaptic contacts exist in the VMH, with approximately half of these remaining after deafferentation of the VMH [80]. About one third of the synapses are axospinous and appear to be exclusively excitatory based on their ultrastructure. A relatively small portion, approximately 10 to 15 percent, of the axodendritic synapses are inhibitory, again based on ultrastructure. Thus, the VMH is rife with intranuclear connectivity, including excitatory inputs to dendritic spines.

Several lines of evidence suggest that the VMH is not homogenous, but instead is comprised of several cell types (summarized in Figures 1 and 2). For example, several markers are co-localized with the alpha subtype of the estrogen receptor (ER- α), whilst others are not, as detailed below. It should be noted that many studies have used nuclear labeling to identify the ER- α -expressing cells. Given that membrane-associated ER- α may be more difficult to visualize with immunohistochemistry, it remains unclear whether these same neurons also mediate the membrane-based effects of estradiol discussed below.

As a second cell type, my laboratory has observed that the ER- α -containing neurons are largely a separate population from those neurons with axonal connections to the periaqueductal gray [81;82;83], with approximately 15 to 25% overlap. Somewhat surprisingly, mating-induced Fos expression occurs mainly in neurons that lack both ER- α and axonal projections to the midbrain [82], thus indicating a third cell type. Likewise, other groups have observed that mating cues activate Fos expression in many VMH neurons that lack immunostaining for nuclear receptors for ovarian hormones [84;85]. In particular, these papers quantified that less than half of the mating cue-induced Fos labeling was found in neurons with estrogen or progesterone receptors. Although the neurochemistry of these mating-activated neurons remains undetermined, previous work from our laboratory suggests that such neurons alter their synaptic connectivity in response to estradiol treatment [75]. Thus, elements of the VMH circuit that participate in lordosis behavior includes ER- α -containing neurons, midbrain-projecting neurons, and neurons that neither express a key estrogen

receptor nor project to the critical downstream relay, but are activated during mating. Further studies are needed to clarify the extent of involvement of these neurons that neither express a key estrogen receptor nor project to the critical downstream relay in mediating lordosis behavior.

Taken together, a key structural feature of the VMH is its functionally distinct domains, with the VL-VMH likely to be especially important for mating behavior. VMH neurons have a signature dendritic tree that allows for the integration of intrinsic and extrinsic activity and exhibits plasticity. Finally, at least three cell types within the VMH contribute to the control of female sexual behavior, although the connectivity between these cell types and key aspects of their neurochemistry has not been determined.

C. NEUROCHEMISTRY OF THE VMH

Several receptors, neuropeptides, and transcription factors intrinsic to the VMH are common across VMH subdivisions, whereas others are found in one region and not the other, as listed in Table 1. Many of the markers specifically localized within the VL-VMH have been implicated in the control of female sexual behavior, most notably estrogen receptor subtype α (ER- α) [86]. The aggregation of these lordosis-modulating factors within the VL-VMH has contributed to the notion that the control of female sexual behavior is housed within the VL-VMH rather than the VMH more broadly.

An important neurotransmitter in the VMH is gamma-aminobutyric acid (GABA). GABA is present in the VMH, based on immunoelectron microscopy [87], as well as light-level immunohistochemistry [88], which revealed terminals labeled for the GABA biosynthetic enzyme glutamic acid decarboxylase-65 (GAD-65). Electrophysiological recordings of dissociated VMH neurons found spontaneous GABAergic inhibitory postsynaptic currents [89], which suggested that the GABA neurons may be local interneurons. Numerous studies have indicated that GABA activity in the VMH is regulated by estradiol levels and modulates the lordosis response (as reviewed by [90]); [91]. Activity in these GABA neurons may facilitate reproductive behavior, as infusion of a GABA_A receptor agonist into the VMH promotes sexual behavior [92], whereas a local infusion of a GABA_A receptor antagonist has an inhibitory effect [91]. Thus, the presence of GABA in the VMH is behaviorally relevant. However, it is important note that GAD65 and 67 are not synthesized by VMH neurons [93]. Therefore, the GABAergic influences on VMH neurons arise from cell bodies that reside elsewhere.

Excitatory neurotransmission in the VMH also has been implicated in lordosis. The critical ER- α -containing neurons express the vesicular glutamate transporter, indicating that their main neurotransmitter is glutamate [94]. Another receptor abundant in the VL-VMH is the oxytocin receptor (OTR), which also promotes female sexual behavior [72;95;96;97]. Likewise, neuronal nitric oxide synthase (nNOS), enkephalin and substance P have been shown to promote lordosis [98;99;100;101]. Each of these VL-VMH-specific factors is co-localized with estrogen receptors in the VMH [102;103;104], and estradiol treatment up-regulates the levels of these factors in the adult female VMH [105;106;107;108]. Thus, estradiol acts within glutamatergic neurons, in which the enhanced production and release of enkephalin, substance P and nitric oxide promotes sexual behavior. Oxytocin also acts on these neurons to promote lordosis. Thus, several neurochemical properties of ER- α -containing neurons have been revealed, although the specific role of each of these signals in the context of the local circuit is not yet clear.

In contrast, cholecystokinin is found in VMH neurons that do not co-express estrogen receptors [109]. When administered into the VMH, cholecystokinin has an inhibitory effect

on female sexual behavior [110]. Thus, cholecystokinin-expressing neurons must play a distinct role in the VMH local circuitry compared with the ER-expressing neurons.

In sum, investigations of phenotypic markers in the VMH are in accordance with the aforementioned tracing studies and Fos analysis studies in that both sets of literature suggest that the VMH consists of several cell types. Although several neurochemical features of the ER- α -containing neurons have emerged (summarized in Figure 2), it remains unclear how the GABA-ergic input and cholecystokinin-labeled neurons correspond to the Fos-activated neurons and to the projection neurons. It also seems likely that there are subpopulations amongst the ER- α -containing neurons.

IV. SEX DIFFERENCES IN THE VMH

A. NEUROCHEMISTRY

A fundamental difference between the male and female VMH is its responsiveness to steroid hormones in adulthood. In particular, males exhibit higher levels of both the androgen receptor and aromatase, the enzyme that converts testosterone to estradiol, than females in the VL-VMH [111;112]. Conversely, the female manifests more receptors for estradiol, including both ER- α and ER- β subtypes [113;114;115], and for progesterone [116;117;118] than males. Thus, it may not be surprising that a number of estradiol-induced changes in the VMH are more robust in females than males, as detailed below. Nevertheless, there are several cases in which estradiol-induced changes persist in males or occur in the reverse direction.

In addition to the levels of steroid receptors, many of the gene products uniquely expressed in the VL-VMH, with known contributions to lordosis behavior, also exhibit sex differences in their expression. As summarized in Figure 2, these VMH neurochemical signaling molecules can be organized into three categories. The first category includes receptors for estradiol and progesterone, but also includes GABA and enkephalin. Each of these is found in greater amounts in females, is increased when estradiol levels are elevated, and promotes lordosis behavior in females [119;120;121;122]. Thus, these signals may activate a lordosis-specific network within the VMH.

A second category includes signals that are elevated in males compared with females, and are known to inhibit lordosis. This category includes cholecystokinin, glutamate, and serotonin [107;123]. For cholecystokinin, the inhibitory effect on behavior in females depends on the normal lack of exposure to gonadal steroids during development in females [124]. Thus, developmental effect of hormones on sex differences in the VMH (discussed in more detail below) includes determining the responsiveness of the adult VMH to some of these modulators of lordosis. There is a sex difference in glutamate receptors, specifically the *dl*- α -amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptor subunit 2/3 with males exhibiting a diminished level of estradiol-induced up-regulation compared with females [125]. This is reminiscent of the aforementioned blunted effect of estradiol on enkephalin in males, and may be secondary to lower levels of ER- α . Further investigation is needed on glutamate's behavioral effects and the sex differences and hormonal regulation of its receptors. With regard to serotonin, projections arrive from the dorsal raphe nucleus [126], and serotonin suppresses lordosis when released in the VMH [127]. A recent study elegantly demonstrated that there are substantially more serotonin-labeled fibers in the male VMH compared with females [88]. Although the total number of neurons in the VMH is the same in males versus females, more neurons receive serotonin inputs in males. This increase in serotonin innervation is specific for non-ER- α -containing neurons, as the percentage of ER- α -containing neurons that are innervated by serotonin is approximately 70 percent in

both males and females. Thus, the pronounced sex difference in the serotonin innervation of the VMH consists of a doubling of the input to the non-ER- α -containing cells in males.

In a third category, some neuromodulators, are more abundant in males, yet are known to increase lordosis behavior. For the oxytocin receptor, which promotes lordosis behavior in females, males have a substantially higher level of receptor binding activity and mRNA expression compared with females [128;129]. Likewise, males express higher levels of nNOS than females [130]. One explanation for this pattern of results is that oxytocin receptors and nNOS may generically promote reproductive behavior, whether mounting or lordosis, in both males and females, respectively. In fact, central injections of oxytocin promote sexual behavior in males [131]. Thus, these neuromodulators may not influence a sex-specific posture, but may activate a circuit within the VMH that promotes mating in a non-sex specific way.

In closing this section, a complex picture has emerged regarding sex differences in the neurochemistry of the VMH. Overall, males and females seem to express the same signaling molecules in the VMH, with differing mechanisms in the regulation of these signals. The fact that some signals that promote lordosis in female are present in higher amounts in males suggests that some elements of VMH activity are not sex-specific with regard to facilitating reproductive behavior. On the other hand, some sex differences in classical neurotransmitters do correlate with the ability to express lordosis behavior. A critical difference is the lower level of ER- α in males, which may explain the blunted estradiol-mediated changes in the expression of several genes in males compared with females. In this way, developmental exposure to steroids, to be discussed shortly, may epigenetically blunt the effect of estradiol on a number of genes that are estrogen-responsive and promote lordosis in females. This may include downstream effects on neural plasticity, as discussed next.

B. SYNAPTIC ORGANIZATION

The volume of the adult VMH is approximately 25% larger in males compared with females, based on Nissl stain analysis [70;132;133;134], and this sex difference is most pronounced in the VL-VMH [134]. This volume difference is secondary to males having a larger neuronal soma size than females, rather than males having a greater total number of neurons [70;134]. In addition, the space occupied by neuropil in the VMH is greater in males than females [70]. At the ultrastructural level, several laboratories have reported a higher density of spine and shaft synapses in males compared with females [135;136;137], although this is not always reported [70;138]. As mentioned above, serotonin innervation contributes to the greater levels of synaptic input in the male VMH [88]. Given that dendritic spines are induced and maintained by neuronal activity, the higher level of spine density in the male VMH implies intrinsic differences in basal synaptic activity. Overall, the sex difference in the volume of the VMH is based on males exhibiting the same number of neurons, albeit of greater size and with more synaptic inputs. Thus, to understand the sex-specific function of the VMH, it is necessary to delve into its synaptic organization.

Connectivity within the VMH is regulated quite differently in males versus females. Estradiol treatment decreases dendritic spine density in adult male rats [139;140], whereas estradiol generally increases spine density and axospinous synapses in females [75;141;142;143] (but see [81]). Estradiol is a physiological regulator of spine density in male rats based on the evidence that estradiol treatment reverses the castration-induced increase in dendritic spine density in the male VMH [139;140]. The spine density on short primary dendrites, but not other dendrite types, is markedly increased by estradiol treatment in females, possibly via afferent input from nearby ER- α -containing interneurons [75]. It remains unclear whether the effect of estradiol on dendritic spines in males is confined to the

same dendrite types that are affected in the opposite direction in females. A mechanistic accounting for this opposite effect of estradiol in males versus females has not been provided, and the known effects of estradiol on local neuromodulators, as discussed above, do not explain it. It seems likely that the sex difference in the density of synapses in the VMH is not merely quantitative, but that it represents a fundamentally different kind of synaptic organization that allows estradiol to have such radically different re-wiring effects.

We recently conducted a comparison of the VMH dendritic arbor in males versus females to test the hypothesis that a sex difference might be localized to a specific dendrite type [77]. VMH neurons were visualized with Golgi impregnation. Male rats displayed significantly longer dendrites than females in both the DM-VMH and the VL-VMH. Longer dendrites were observed in males for all dendrite types, regardless of which direction the dendrites extended. The elongated male VMH dendrites may provide additional sites to process input from both local interneurons and extranuclear afferents. In addition, the longer dendrites may be needed to traverse a greater VMH area to reach the surrounding fiber plexus. This sex difference occurred regardless of the hormonal status of the females, and is thus a true sex difference.

To date, relatively few studies have investigated possible sex differences in the connectivity between the VMH and other brain regions. One such study observed a sex difference in the innervation of the VL-VMH from the fornix, with the male VMH receiving approximately 25 percent more terminals than the female VMH [136]. It seems likely that similar differences occur with other afferents to the VMH, contributing to the sex difference in the volume of neuropil and the density of synapses. Likewise, sex differences in the outflow from the VMH to the periaqueductal gray, either in terms of the density of the projection, or the subdivisions being targeted, may be critical for the behavioral specificity of the VMH.

In summary, the synaptic organization of the VMH differs by virtue of males having more dendritic surface, including more synaptic contacts. At least some of this additional connectivity in males arises from a higher density of input coming from other brain regions. Another fundamental sex difference in the synaptic organization of the VMH is the direction of changes in wiring wrought by estradiol. Future studies should address how that additional information processing in the male VMH yields distinct neurobehavioral functions.

C. MECHANISMS OF HORMONE-INDUCED SEX DIFFERENCES

Neurological sex differences can arise from two very different mechanisms: direct effects of genes expressed by sex chromosomes and epigenetic effects mediated by the developmental activation sex steroid receptors [144]. In the case of the VMH, neonatal exposure to gonadal hormones can explain many of the sex differences that have been documented; in fact neonatal manipulation of exposure to testosterone and estradiol can reverse the sex differences in the VMH studied to date. Thus, neonatally castrated males have a female-like VMH, and, conversely, females treated neonatally with testosterone or estradiol have a male-like VMH, according to studies assessing a variety of parameters. For example, the perinatal testicular secretion in males contributes to the masculinization of the general volume of the VMH [133], the number of synapses [136;137], including the serotonergic innervation [88], the pattern of gene expression [115], and the adult pattern of electrophysiological activity [145]. Therefore, although neuronal effects of sex chromosomes may mediate some sex differences in the brain, dichotomies in the VMH seem to be largely explained by the developmental, organizing surge of testosterone.

The developmental surge in testicular secretions occurs between the ages of E18 and E19 in male rats [146]. Several biochemical sex differences in the VMH emerge in close succession to this event. For example, on E22 the male VMH contains half the level of the serine/

threonine kinase Raf-1 compared with females [147]. On the day of birth, the male VMH contains twice the number of cells labeled with phosphorylated CREB compared with females [148]. Another sex difference, in this case GABA-A receptor activity, emerges shortly after birth, with a slower decay of the GABA-A-mediated current in males compared with females [149]. Finally, between PN5 and PN14, males express less ER- α and ER- β than females [150;151]. This may reflect auto-down-regulation based on the aromatization of testosterone and subsequent estradiol action in males. Thus, sexual dichotomies in VMH biochemistry and electrophysiology are temporally linked with an organizational surge in testosterone.

A detailed study of the sex difference in dendritic spines in the VMH has provided insights into the mechanisms of estradiol-induced sexual differentiation in the brain [152]. As mentioned above, males exhibit higher levels of dendritic spines in the VMH compared with females. Likewise, the level of the spine-specific protein spinophilin is approximately twice as high in males compared with females in the hypothalamus. The effect of perinatal estradiol to increase spinophilin levels is mediated by glutamate. Specifically, blockade of glutamate receptors prevented an estradiol-dependent increase in spinophilin levels. More specifically, estradiol treatment increased the depolarization-induced release of glutamate. The timing of this presynaptic effect of estradiol on glutamate release occurs within three hours after estradiol treatment. This time frame challenges the traditional view that estradiol masculinizes the brain mainly through a transcriptional mechanism. In fact, the translation inhibitor cyclohexamide did not interfere with estradiol-induced glutamate release. Instead, an inhibitor of phosphatidylinositol 3 (PI3) kinase blocked the effects of estradiol on both glutamate release and spinophilin levels. Indeed, estradiol treatment increased levels of phosphorylated Akt, a substrate of PI3 kinase. Subsequent to estradiol-induced glutamate release, there is a postsynaptic increase in the levels of phosphorylated mitogen-activated protein kinase (MAPK). The inhibition of MAPK phosphorylation blocked the effect of glutamate on spinophilin levels, and presumably spine formation. Taken together, masculinization of the VMH involves a cascade of presynaptic PI3 kinase activity, which phosphorylates Akt, which then promotes glutamate release. Consequently, glutamate binds to its receptors post-synaptically, which in turn leads to MAPK activation, which then promotes spinophilin expression and spine formation. Thus, control of glutamate activity in this early time window is a critical feature of the sexual differentiation of synaptic organization in the VMH. The receptor mechanisms mediating this action of estradiol is unclear.

While the aforementioned study implicates a novel mechanism of estradiol action in mediating sex differences in the VMH, at least for the masculinization of dendritic spines, there are parallel roles for the classic sex steroid receptors. Recent work has established the involvement of both ER- α and ER- β in VMH sexual differentiation [150;151;153]. In addition to these critical organizational mechanisms in the VMH involving estradiol, androgen receptors also contribute to the sex differences in the size of the VMH, as shown in rats with mutant androgen receptors [134]. It is possible that androgen receptor activation is important for the control of the expression of the aromatase enzyme [111], which would suggest that estrogen receptors are involved afterall. Androgen-based mechanisms also extend to sex differences in neurochemistry. For example, males express higher levels of nNOS than females. This male pattern of nNOS expression is blocked by a loss of effective androgen receptors [130]. It should be noted that androgen receptors and aromatase are expressed in the VMH in modest amounts compared to other brain regions, but limbic regions with high expression of androgen receptors and aromatase provide afferents to the VMH [11]. As for the outer limits during which gonadal steroids can masculinize the VMH, a postnatal time course analysis indicated that the adult pattern of sex-specific connectivity is established by PN45, but not at PN20 [137]. Thus, the secretion of gonad hormones

during puberty may normally contribute to the final pattern of male and female VMH circuitry in adults.

In addition to permanent effects on the VMH morphology and circuitry, developmental exposure to steroids affects later responsiveness to estradiol, as mentioned above. In particular, a number of estradiol-mediated effects are weaker in adult males compared with females. For example, estradiol does not induce progesterin receptor expression in males, despite this being a classic effect of estradiol in the female VMH [117]. As mentioned above, estradiol treatment enhances enkephalin expression in females but not males [119]. One possibility is that this reduced estrogen responsiveness in males merely represents a quantitative difference in estrogen receptors between the sexes. Given that males express lower levels of estradiol receptors than females, the blunted capacity to respond to estradiol may be a consequence of fewer receptors. The lack of estradiol-induced progesterin receptors in the male VMH would certainly explain the lack of effect of progesterone on oxytocin receptor binding in males than has been observed in females [154]. Alternatively, the sex difference in estrogen responsivity may reflect epigenetic modifications on the accessibility of estrogen-regulated genes. This would be manifested as qualitative differences in hormone-induced genotropic effects later in life, compared with females. Another possible source of quantitative sex differences in the responses to estradiol treatment may occur if the effects depend on transynaptic actions. Given that the organization of sex differences in the VMH includes robust differences in connectivity, and given that some effects of estradiol are secondary to neurotransmission, the effect of estradiol to indirectly influence the pattern of gene expression may be sex-specific. Given that these mechanisms are not mutually exclusive, sex differences in estradiol responsiveness could be *both* quantitative and qualitative.

In addition to distinct responses in adult males versus females to the genotropic actions of estradiol, it remains unclear whether the sex differences in the synaptic organization of the VMH are quantitative or qualitative. As mentioned in the previous section, male rats may require additional dendritic surface to process more inputs, with the source of those inputs being identical to females. Conversely, sex differences in synaptic connectivity may represent categorical differences in the afferents to the VMH and/or the targets of its axonal projection [24]. In this way, the greater number of synapses in male VMH may reflect a categorical difference in computational processing, rather than simply stronger excitatory or inhibitory influences. To date, there has not been a quantitative comparison of the density and source of VMH afferents in males versus females.

In summary, the extant studies have established that during development, gonadal hormones produce long-term changes in the wiring of the VMH as well as its responsiveness to estradiol in adulthood. These mechanisms are summarized in Figure 3. Although in some cases cellular mechanisms have been further elaborated, many questions remain regarding the functional consequences of the dichotomies in VMH connectivity and transcriptional potential.

V. STRUCTURE-FUNCTION RELATION OF THE VMH AND LORDOSIS

As noted thus far, certain attributes of the VMH are not sex-dependent. For example, both males and female exhibit an ovoid structure with neurochemically distinct dorsomedial and ventrolateral regions, each comprised of equivalent numbers of neurons with simple dendritic arbors. However, within this overall structure, specific dimensions are dichotomous for the male versus female VMH, including the levels of certain signaling molecules, the pattern of synaptic organization, and the responses to adult hormone

exposure. Each of these specific differences may contribute to the sex-specific behavioral functions of the VMH.

An obstacle to explaining how the sex differences observed in the VMH provide a biological substrate for sex-specific mating behaviors lies in the fact that the neurochemistry and neural plasticity that allow the female VMH to promote lordosis remain only partly understood. As mentioned above, the normal fluctuations in estradiol and progesterone across the estrous cycle are critical for controlling the timing of lordosis behavior in intact females based on their actions in the VMH. A number of neurochemical changes take place in the VMH when estradiol levels become elevated, as already described. Ovarian hormones also reorganize neuronal connections within the VMH. Golgi impregnation studies showed that the number of dendritic spines in the VMH correlates with reproductive behavior across the estrous cycle. In particular, the density of dendritic spines in VMH neurons is highest at the time when ovarian hormones are elevated [142]. In ovariectomized rats, estradiol treatment increases the number of spines on the dendrites of VMH neurons two-fold [142]. Similar effects of estradiol to increase synaptic connectivity in the VMH have been reported with a variety of methods [70;141;142;143]. This finding also was replicated and extended using the cell filling technique [75]. Keeping in mind that the dendritic tree of VMH neurons includes a single long primary dendrite that extends toward the lateral afferent fiber field, as well as several short primary dendrites [56;75], my laboratory tested the hypothesis that these dendrite categories represent different roles in neuronal computation. In support of this notion, we found that the estradiol treatment induced a robust increase in dendritic spine number specifically on the short primary dendrites. Thus, the estradiol-induced spines in the VMH may signify additional local connectivity within the VMH induced by estrogen to mediate reproductive behavior. We also demonstrated that these changes occur on VMH neurons that do not express nuclear ER- α nor send axonal projections to the periaqueductal gray [75;81].

At the same time that ovarian hormones promote the formation of dendritic spines on short primary dendrites, they dynamically regulate the length of the long primary dendrites. In particular, estradiol treatment causes a marked shortening of these dendrites, a processes that is reversed with subsequent progesterone treatment. This regulation of dendrite extension was first observed with Golgi analysis, which allowed for straightforward measurement of dendrite length [76]. A subsequent electron microscopy analysis of the lateral fiber plexus verified that estradiol treatment caused an attrition of dendritic profiles in that region [155]. This pattern is consistent with findings reported from intact cycling female rats [70]. Thus, estradiol acts to shorten the long primary dendrites, removing them from the lateral fiber plexus at the same time that the short primary dendrites have heightened excitatory input. Intriguingly, a neurochemical marker for the dendrites that remain after estradiol treatment is oxytocin labeling within these dendrites [155]. Thus, ovarian hormones re-wire VMH dendrites with notable spatial and neurochemical specificity.

Putting these findings together with our knowledge of VMH neurons, the following working model emerges. As the estrous cycle proceeds and estradiol levels rise, ER- α neurons respond with changes in the transcription of a host of signaling and effector proteins. Given that these are glutamate neurons, the release of glutamate is likely to increase. As suggested in the developing brain, these neurons may also have a rapid, non-genotopic response to increase the release of glutamate [152]. We propose that a second cell type then responds to the glutamate released by the ER- α -containing neurons by developing dendritic spines at these sites of contact on their short primary dendrites (Figure 1). The emergence of these dendritic spines may enhance the neurotransmission between the ER-containing neurons and this second cell type. The identity of this second cell type may be the mating-activated neurons and/or the cholecystokinin-expressing neurons. It is also possible that estradiol

initially acts on extranuclear afferents to transynaptically increase spines [80]. Based on this change in excitability after the marked increase in dendritic spines, these neurons may impinge on the projection neurons, which send their axons to the periaqueductal gray, to exert effects on behavior.

In this model, estradiol treatment stimulates the expression of gene products that promote lordosis in the ER- α -containing neurons, and enhances the excitability of a second set of neurons. As mentioned above, the long primary dendrites of some neurons disengage from extranuclear afferents in the lateral fiber plexus until progesterone comes along. Thus, the behavioral effects of progesterone may derive from the re-establishment of sensory or arousal cues arriving from other brain regions and impinging on the long primary dendrites.

With these inferences about the effects of lordosis-promoting hormones on the dendritic tree of VMH neurons, one can consider the dendrite morphology in males. As mentioned above, VMH neurons in males have the same dendrite components, although the dendrites are longer and they receive more synaptic input. Thus, male VMH neurons do not simply resemble those of a female without ovarian hormones, which would entail a low number of dendritic spines. Just as the neurochemistry of the male VMH does not mirror that of a female without ovarian hormone exposure, as described below, the synaptic organization of the male VMH is not simply that of a non-estrous female. Thus, the masculinization of the VMH is more than imposing the synaptic arrangement seen in a non-estrus female.

The VMH expresses the oxytocin receptor, nNOS, enkephalin and substance P, all of which promote lordosis in females [72;95;96;97;98;99;100;101]. These factors are also present in males, but in some cases to a lesser degree. Thus, the lack of lordosis behavior in males may result, in part, from a relative dearth of lordosis-promoting factors in the VMH. Likewise, males exhibit higher levels of cholecystokinin and serotonin in the VMH than females [88;107]. Given that cholecystokinin and serotonin have inhibitory effects on female sexual behavior in the VMH [110;156], the sex difference in cholecystokinin and serotonin in the VMH may contribute to the lack of lordosis behavior in males. Thus, a combination of low amounts of lordosis-promoting signals and high levels of lordosis-inhibiting signals may be responsible for the lack of female sexual behavior in males (summarized in Table 2). An exception to this scheme is glutamate levels, as discussed above.

The notion that sex-specific behavior is controlled by the ratio of lordosis promoting and inhibiting substances in the VMH is challenged by reports that under certain circumstances, lordosis behavior can be observed in males when primed with estradiol and progesterone. One example is the transection of dorsal afferents to the anterior hypothalamus, implicating the lateral septum as a lordosis-inhibiting system [157]. The unmasking of lordosis in males with this denervation persisted when a concomitant VMH lesion was performed. Thus, circuits outside the VMH are sufficient to support lordosis behavior in males. Another situation that reveals a latent lordosis circuitry in adult males is the loss of serotonergic innervation to the hypothalamus [156;158]. Furthermore, given that the extent of the lesions were not verified, the change in behavior may have reflected a loss of serotonin input elsewhere, such as to the lateral septum. The lateral septum was already implicated in inhibiting lordosis [159;160]. It is unknown whether or not these interventions cause a sex reversal in the levels of lordosis-promoting and lordosis-inhibiting factors in the VMH. Given that the unmasking of lordosis persists after the VMH has been lesioned, any feminization that may occur in VMH neurochemistry or neural circuitry does not seem to mediate the sex reversal of reproductive behavior in these conditions. This VMH-independent expression of lordosis in males questions the importance of sex differences in the VMH as being responsible for the lack of lordosis in males. Furthermore, a developmental treatment that masculinizes the serotonin innervation to the VMH does not

concomitantly abolish lordosis behavior [88]. Thus, despite the host of sex differences in the VMH and the abundant evidence that the VMH controls lordosis behavior in females, there has not been a clear demonstration that masculinization of the VMH is the key factor that prevents males from exhibiting lordosis behavior. A transgenic experiment that allowed a VMH-specific, developmentally controlled manipulation of estradiol and androgen action could test the hypothesis that masculinization of the VMH is necessary and sufficient for the sex-specificity of lordosis. Although such an experiment would not be trivial to conduct, it is need to demonstrate that masculinization of the VMH and only the VMH is critical for the lack of lordosis in males.

It may be useful to note that there are several other examples of sexually dimorphic brain structures that are associated with known dichotomous effects on behavior or physiology that exhibit much greater sex differences in size than the VMH. For example, in songbirds, areas that control the male-typical behavior of singing, such as the high vocal center, are three times larger in males than females [161]. An example in the mammalian brain is the pathway that controls the female-typical positive feedback effect of estradiol to trigger gonadotropin secretion and ovulation. This circuit, which is twenty times larger in males versus females, includes a projection from the principal nucleus of the bed nuclei of the stria terminalis to the anteroventral periventricular nucleus [162]. The modest sexual dimorphism of VMH size, which is 20% larger in males in its most dimorphic subregion, the VL-VMH [134], may suggest that masculinization of VMH, or the lack thereof, does not dictate the sex-specificity of lordosis.

If it is true that the masculinization of the VMH is not the key to preventing lordosis in males, the functional significance of sex differences in the VMH must be addressed. A clue to this question may be the many documented sex differences in the VMH pertain to the response to estradiol. In addition to the control of female mating behavior, estradiol also contributes to the regulation of body weight [163;164;165;166]. This particular role of estradiol may also be mediated in the VMH [167]. It has been noted that the effect of estradiol on body weight is opposite for males and females [168]. Thus, the relatively weak sex difference in VMH size may be a reflection of the relevant function not being completely dichotomous between males and females, such as body weight regulation.

IV. SUMMARY AND FUTURE DIRECTIONS

Previous investigations on the mechanisms in the VMH that control female mating behavior have provided a valuable heuristic for understanding the organizational and behavioral effects of steroid hormones at the cellular and molecular levels [152;169]. Nevertheless, this neurobehavioral system still has secrets to reveal about how its circuitry and neurochemistry are configured and regulated to control reproductive behavior. To provide a full account of the neurological mechanisms that operate in the VMH to mediate female sexual behavior, the cell types in the VMH and their connectivity must be further elucidated. Given that ovarian hormones cause specific changes in connectivity, an important goal for my laboratory is to reveal the behavioral significance of ovarian hormone-induced re-wiring of the synaptic organization of the VMH. Although studies of the female VMH can help explain the neural underpinnings of lordosis behavior, comparisons with male sexual behavior are urgently needed to provide unique insights regarding structure-function relationships.

With regard to sex differences in the VMH, there are well-documented differences in a number of neurochemical markers. The role that this shift in the chemical environment of the VMH plays in the sex-specificity of mating behavior is not clear. Likewise, there are sex differences in the synaptic organization of the VMH, but whether or not this represents a

dichotomy in the functional output of the VMH remains unclear. Another fascinating aspect of the sex differences in the VMH is the sex-specific estrogen responsiveness. A number of mechanisms are possible, but the mechanisms of chromatin modifications have not been systematically explored. Finally, it remains to be proven that sex differences in the VMH actually explain the lack of lordosis behavior in normal males. This may be addressed with transgenic experiments that anatomically and developmentally restrict the disruption of estrogen receptors. If the absence of lordosis in males is not controlled by the VMH, the functional significance of the VMH sex differences will require an explanation. Answering questions about the male VMH would represent a major advancement in our understanding of the numerous sex differences in neurochemical and circuitry.

Aside from a general interest in normal biological systems, there are practical reasons to elucidate the mechanisms of sexual differentiation in the brain. In recent years, evidence has accumulated to show that various human-made compounds in our environment, such as bisphenol A (BPA), can have endocrine disrupting effects, including during development. In particular, neonatal exposure to BPA caused a down-regulation of ER- α in the female VMH. These females also exhibited lower levels of mating behavior [170]. BPA is a widely used compound [171], including in our food containers, and recent epidemiological studies have found that a high percentage of the human population with readily detectable levels in their bodies [172;173]. Animal models, including further studies of the VMH, may provide a useful screen for endocrine disruptors, as well as possible antidotes. Basic research on normal VMH responsiveness to hormones will afford a tremendous foundation for future studies to protect neuroendocrine health.

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FIGURE 1.

Working model of the cell types in the VL-VMH, including their dendritic arbors, axonal targets and possible connections between them. A) The ER-containing neurons (gold) are known to release glutamate as their neurotransmitter and may serve as local interneurons. Other neurons (purple) exhibit estradiol-induced increases in dendritic spines. A third cell type (blue) sends descending projections to the periaqueductal gray (PAG) to affect behavior. All three cell types extend long dendrites into the lateral fiber field, which includes axonal fibers containing a variety of peptides and transmitters.

FIGURE 2.

Summary of our current understanding of the neuron types in the VMH. Several lines of evidence indicate that the ER α -containing neurons are largely separate from the PAG-projecting neurons. Various markers have been co-localized with the ER α -containing neurons. The available evidence suggests that there is a third cell type, that is, non-ER α -containing neurons and non-PAG-projecting neurons. Although several features may be attributed to these neurons, it is not yet known if this represents a single cell type.

Abbreviations: Enkephalin, Enk; Estrogen receptor- α ; ER α ; Neuronal nitrous oxide synthase, nNOS; Oxytocin receptor, OTR; Vesicular glutamate transporter 2, vGlut2.

References: [75;81;82;83;84;85;94;102;103;104;109].

FIGURE 3.

Working model of the mechanisms of sexual differentiation in the VMH. Testosterone acts on androgen receptors, which in turn upregulate the enzyme aromatase. Aromatase allows the conversion of testosterone to estradiol, and many masculinizing effects then are mediated by estrogen receptors. ER- α is certainly a critical receptor, but other estrogen receptors are likely to participate as well. Membrane-based actions may mediate the sex differences in dendrite morphology, which lead to permanent sex differences in synaptic organization. In parallel, developmental effects of estrogen receptors may modify the chromatin to permanently alter the responsiveness to sex hormones in adult hood.

Table 1

Phenotypic markers that are specifically localized to the ventrolateral (VL-VMH) versus the dorsomedial (DM-VMH).

	VL, not DM	DM, not VL
Receptors	ER- α , OTR	GHRH-R
Peptides, Growth Factors, signaling	Enk, SST, SubP, CCK, nNOS	NPY, BDNF
Transcription Factors	Islet-1, Nkx2.1	SF-1

Abbreviations: Brain-derived neural growth factor, BDNF; Cholecystokinin, CCK; Enkephalin, Enk; Estrogen receptor- α ; ER α ; Growth hormone releasing hormone receptor, GHRH-R; Neuronal nitrous oxide synthase, nNOS; Neuropeptide Y, NPY; Oxytocin receptor, OTR; Somatostatin, SST; Substance P, SubP. References: [9;57;63;105;106;174;175;176;177;178;179;180;181;182].

Table 2

Proposed differential functions of neurochemical markers in the VL-VMH based on their pattern of sex differences and their effects on lordosis behavior.

Proposed function	Signaling molecule	Sex with higher levels	Effect on lordosis in females
Promotes lordosis (female sex behavior)	ER- α	Females	increase
	ER- β	Females	increase
	Progesterone receptor	Females	increase
	Enkephalin	Females	increase
	GABA	Females	increase
Inhibits lordosis in both sexes	Cholecystokinin	Males	decrease
	Glutamate (+/-)	Males	decrease
	GluR2/3	Males	decrease
Promotes mating in both sexes	Oxytocin receptors	Males	increase
	nNOS	Males	increase

Abbreviations: Estrogen receptor, ER; Neuronal nitrous oxide synthase, nNOS Glutamate receptor subtype 2 and 3, GluR2/3. References: [14;15;16;92;98;99;100;101;107;110;128;129;130]