

Representing Sex in the Brain, One Module at a Time

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Sexually dimorphic behaviors, qualitative or quantitative differences in behaviors between the sexes, result from the activity of a sexually differentiated nervous system. Sensory cues and sex hormones control the entire repertoire of sexually dimorphic behaviors, including those commonly thought to be charged with emotion such as courtship and aggression. Such overarching control mechanisms regulate distinct genes and neurons that in turn specify the display of these behaviors in a modular manner. How such modular control is transformed into cohesive internal states that correspond to sexually dimorphic behavior is poorly understood. We summarize current understanding of the neural circuit control of sexually dimorphic behaviors from several perspectives, including how neural circuits in general, and sexually dimorphic neurons in particular, can generate sexually dimorphic behaviors, and how molecular mechanisms and evolutionary constraints shape these behaviors. We propose that emergent themes such as the modular genetic and neural control of dimorphic behavior are broadly applicable to the neural control of other behaviors.

Introduction

Men and women exhibit sex differences in behaviors that immediately enhance reproductive success as well as in tasks that involve higher cognitive function. It is actively debated whether such sex differences are genetically wired or a byproduct of societal influences. While the jury may be out for the underpinnings of these behaviors in humans, research in model organisms leaves little doubt that such manichean distinctions between nature and nurture are simplistic. Indeed research on diverse animals unequivocally demonstrates the importance of both genes and experience on sexually dimorphic behaviors. Nevertheless, these studies underscore the primacy of genetically programmed mechanisms that control the development and activation of the neural circuits underlying these behaviors.

Sex-typical displays of behaviors such as mating and aggression are genetically hardwired in the sense that they can be displayed by animals without training. The activation of the underlying neural circuits is controlled by sensory cues as well as by physiological signals such as sex hormones. Such external and internal control mechanisms ensure that these social behaviors are displayed in the appropriate context. Many animals, including mice, secrete pheromones, chemosensory cues that signal social and reproductive status to other members of the species, to initiate social interactions (Karlson and Lüscher, 1959). Sex steroid hormones secreted by the gonads are the critical internal signals that control these behaviors in vertebrates (McEwen, 1981). The identity of the pheromone and hormone-responsive neural circuits that drive specific sexually dimorphic behaviors remains elusive. By contrast, we have significant insight whereby chemosensory input and sex hormones control the development or activation of specific neurons that influence

these behaviors (Liberles, 2014; Morris et al., 2004; Touhara and Vosshall, 2009; Wu and Shah, 2011).

Our Review discusses the mechanisms that regulate sexually dimorphic behaviors in mammals with a specific focus on mice and the assumption that similar mechanisms are likely to operate in humans. The literature on sexually dimorphic behaviors in other organisms has been reviewed elsewhere (Baum, 2003; Cahill, 2006; Crews and Moore, 2005; Dickson, 2008; Manoli et al., 2006; Moore et al., 2005; Newman et al., 1997; Perkins and Roselli, 2007; Portman, 2007; Wade and Arnold, 2004; Wallen, 2005). We focus largely on sex differences in mating and aggression because the underlying neural pathways have been studied in some detail. We do not list all known cellular or molecular sexual dimorphisms in the nervous system because these have been documented extensively (Cahill, 2006; Cooke et al., 1998; Simerly, 2002; Vries, 1990). Where instructive, we discuss findings in other model organisms, especially flies, that provide insight into the neurobiological basis of sex differences in behavior.

A Framework to Understand How the Brain Can Generate Sexually Dimorphic Behaviors

Males and females transform sensory input into sexually dimorphic behaviors, suggesting that such behaviors are generated by neural circuits that differ between the sexes. This insight has led to a highly successful effort to identify anatomical or molecular sex differences in neuronal populations in order to gain an entry point into the neural circuits underlying gender-typical behaviors (Cachero et al., 2010; Cahill, 2006; Cooke et al., 1998; Jarrell et al., 2012; Liu and Sternberg, 1995; Nottebohm and Arnold, 1976; Raisman and Field, 1971; Simerly, 2002; Vries, 1990; Yu et al., 2010). How these genes or neurons control neural circuit

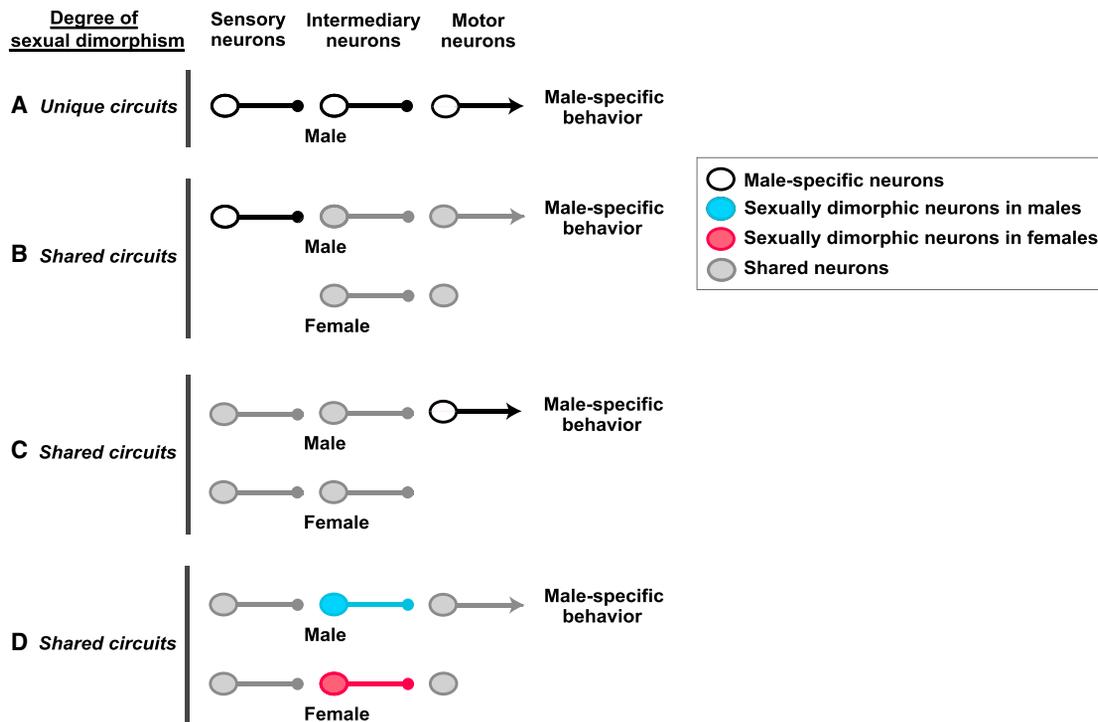


Figure 1. Neural Circuits that Can Generate Sexually Dimorphic Behaviors

Simplified wiring diagram of some neural circuit configurations that can generate sexually dimorphic behaviors. Although only circuits driving male-specific behaviors are shown for clarity, similar circuits will exist for female-specific behaviors. The axon termini of all neurons except those of motor neurons end in small solid circles to show that they may transmit effectively excitatory, inhibitory, or neuromodulatory output. Termini of male motor neurons are shown as arrows to illustrate stimulation of the muscle groups required for the behavioral display. By contrast, female motor neurons are not shown to have termini to depict lack of activation of the male-specific behavioral program.

(A) The entire neural circuit for generating a male-specific behavior is only present in males.

(B) Sensory neurons unique to males feed into a shared neural circuit to activate a male-typical behavior.

(C) Motor neurons unique to males are regulated by a shared neural circuit to activate a male-typical behavioral response.

(D) Sensory and motor neurons are shared between the sexes but there are sex differences in intermediary neuronal populations. Most sex differences in intermediary neurons appear to be quantitative rather than qualitative in mice; in other words, the comparable neuronal population is shared between the sexes, but it displays cellular or molecular sexual dimorphisms that permit activation of the behavior only in males.

function is unclear because a neural circuit that controls a sexually dimorphic display has yet to be delineated from sensory input to motor output.

Absent the complete delineation of such a neural circuit, we envision several mutually nonexclusive neural circuit wiring diagrams that enable sexually dimorphic output (Figure 1). In the most extreme case, such a neural circuit is unique to one sex. One example may be the circuit for penile muscles involved in coitus, which are controlled by motor neurons in the spinal nucleus of the bulbocavernosus (SNB), a population of neurons largely absent in females (Breedlove and Arnold, 1980). Given that wild-type females of many species can display some male-type mounting behavior (see later), if the neural pathway controlling these penile muscles is one component of a singular male sexual behavior circuit then at least some neural centers presynaptic to the SNB are likely to be shared between the sexes. In this scenario, the gender dimorphism in SNB neurons represents an example of a shared neural circuit that differs between the sexes at the level of motor neurons. Sex differences at the level of sensory input can also drive sexually dimorphic behaviors. One example of a neural circuit with well-defined sensory sex

differences is that underlying female pheromone-elicited chemotactic flight in the male moth *Bombyx mori* (Nakagawa et al., 2005; Sakurai et al., 2004; Touhara and Vosshall, 2009). Male but not female moths express chemoreceptors for the pheromone mixture emitted by females, and the antennal sensory neurons expressing these receptors project to unique targets in the male antennal lobe. However, the chemotaxis elicited by the presence of the female pheromone engages output pathways that control flight, a behavior shared between the two sexes.

Given that most behaviors are common to the two sexes, males and females probably share many components of a neural circuit that drives a behavior of the opposite sex. For example, biting during intermale aggression, feeding, and maternal retrieval of a wandering pup all entail locomotion and coordinated jaw movements. In such instances, sexually dimorphic behavior is likely to emerge from sex differences in neuronal populations inserted (intermediary neurons in Figure 1) within shared neural circuits. Consistent with this notion, most cell or molecular sex differences in neuronal populations are quantitative rather than all-or-nothing qualitative sexual dimorphisms. We anticipate that real world circuits underlying dimorphic behaviors are

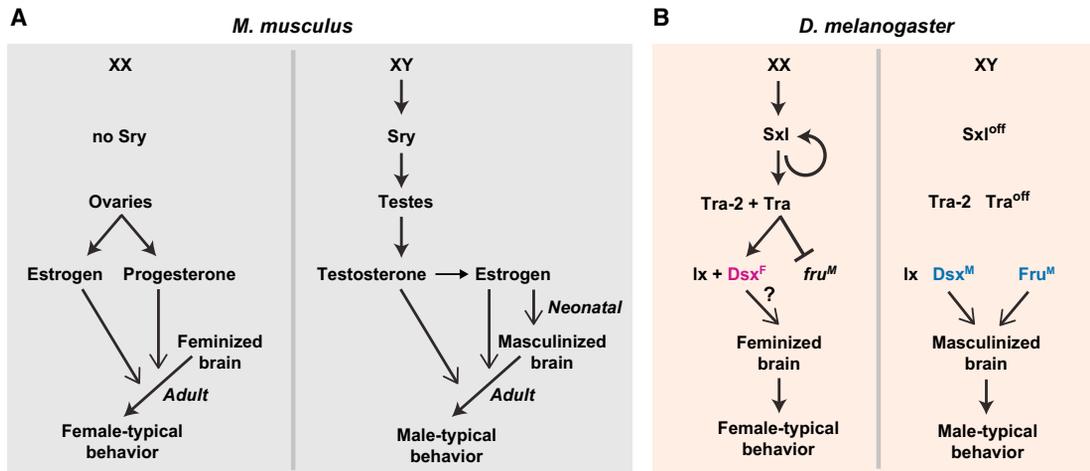


Figure 2. Sex Determination and Sexual Differentiation of Behavior

(A) In mice, the presence or absence of *Sry* drives the differentiation of the bipotential gonad into testes or ovaries, respectively. Sex hormones released into the circulation by the gonads act on their cognate receptors to organize the brain during development and to control the activation of sex-typical behaviors in the adult. In males, estrogen organizes the neural substrates for behavior neonatally, and both estrogen and testosterone activate these pathways for male-typical behavior in adults. In the absence of the neonatal organizational effect of estrogen, the default differentiation program of the brain is female although this hormone may be important in adolescence for maturation of the neural substrates underlying female sexual receptivity (not shown) (Bakker et al., 2002; Brock et al., 2011). Both estrogen and progesterone activate the neural circuit underlying this behavior in adult females.

(B) The sex of a fruit fly is determined in a cell-autonomous manner, with the expression of *sex lethal* (*Sxl*) specifying a female differentiation program. Sex-specific splice forms of *doublesex* (*Dsx*) and *fruitless* direct the cell-autonomous differentiation of neurons that control sex-typical behaviors. *Ix*, *intersex*; *Tra*, *transformer*.

likely to be a composite of the wiring diagrams we have discussed here.

A Primer on Sex Determination and Sexual Differentiation

Whether a sexually dimorphic behavior is innate, in the sense that it can be displayed without prior training, or experiential, animals determine sex early in their development, and sex determination initiates many irreversible sexual differentiation events that influence how the genome and the environment interact to influence gender-specific behaviors. Prior to sex determination, which occurs at midgestation in mice, the brain and gonadal primordia are bipotential and can differentiate in a male- or female-typical pattern.

The Y chromosome is determinative for the male sex (Figure 2). A single Y-linked locus, *Sry*, is necessary and sufficient to masculinize the embryo (Gubbay et al., 1990; Koopman et al., 1991). *Sry* encodes a transcriptional regulator (but see Lalli et al., 2003), and its expression in the bipotential gonads drives their differentiation into testes. Testicular hormones subsequently drive male-pattern sexual differentiation of the body as well as the brain. The embryo is prepatterned to differentiate as a female in the absence of functional *Sry* such that the gonads differentiate into ovaries. Moreover, it is the absence of testicular hormones rather than the presence of ovarian hormones that initiates feminization of the body and the brain (Jost, 1983). Thus, the default mammalian body plan is female.

Although early feminization of the body and the brain is independent of the ovarian hormones estrogen and progesterone, sexual maturation and function of various tissues are controlled by these sex steroids. These hormones regulate sexual differentiation and behavior in females via nuclear hormone receptors

encoded by distinct but homologous genes, *ERα* (or *Esr1*) and *ERβ* (or *Esr2*) for estrogen and *PR* (or *Pgr*) for progesterone (Figure 3) (Burriss et al., 2013). The testes secrete testosterone, which masculinizes the external body phenotype and the brain by signaling through its nuclear hormone receptor, the androgen receptor (AR) (Figure 3) (Burriss et al., 2013). As we discuss later, many actions of testosterone are mediated subsequent to its conversion into estrogen locally in the brain and activation of signaling via *ERα* or *ERβ*.

Sex chromosome-linked genetic loci that remain to be identified may also directly control the development and function of neural circuits underlying innate social behaviors (Bonthuis et al., 2012; Carruth et al., 2002; Dewing et al., 2003; Grgurevic et al., 2012; De Vries et al., 2002; reviewed in McCarthy and Arnold, 2011). These loci subtly regulate male mating and aggression in a manner that mirrors the sex chromosome complement rather than circulating sex hormones. Thus, in contrast to the profound influence of sex hormones on sexually dimorphic displays, such genetic loci appear to exert modulatory effects on these behaviors. In addition to such sex chromosome-based mechanisms, potentially hormone-independent epigenetically regulated gene expression patterns may also regulate sexual differentiation of the brain. Recent studies have identified many potentially imprinted genes that are transcribed, often in a sexually dimorphic manner, in the developing and adult brain (DeVeale et al., 2012; Gregg et al., 2010a, 2010b). The functional relevance of these loci to sexually dimorphic behaviors is presently unclear, but other imprinted genes have indeed been implicated in the control of sexually dimorphic behaviors, including aggression (Garfield et al., 2011; Lefebvre et al., 1998; Li et al., 1999). In summary, gonadal sex hormones appear to act as the master regulators of sexual differentiation in mammals, and hormone-independent

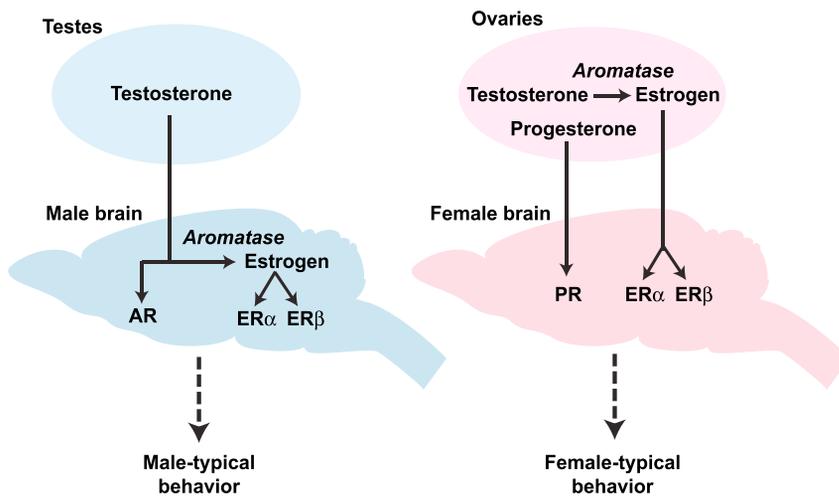


Figure 3. Sex Hormone Control of Sexually Dimorphic Behaviors

Sex hormones produced in the gonads cross the blood-brain barrier and bind to hormone receptors in neurons to regulate sex-typical behaviors. In males, testosterone directs behavior by binding to its receptor AR or it is converted via aromatase into estrogen, which binds to its cognate receptors ER α and ER β . In females, estrogen and progesterone direct behavior via their cognate receptors ER α and ER β and PR, respectively.

genetic loci may exert modulatory control of sexually dimorphic behaviors.

Evolution of the Mechanisms Underlying Sex Determination and Sexual Differentiation

Despite the near universal nature of gender differences in reproductive behavior in animals—even birds, bees, and fleas do it—one theme emerging from work in different species is that the molecular control of sexual determination and differentiation has evolved rapidly even among closely related species (Cline and Meyer, 1996; Marín and Baker, 1998; Matson and Zarkower, 2012). In contrast to the close conservation of most genetic pathways regulating development, there is little similarity between the pathways controlling sex determination and early sexual differentiation of the brain in flies and mice (Figure 2). *Sex lethal*, *transformer*, *doublesex*, and *fruitless* in the fly may be analogous to *Sry* and the genes encoding sex hormone receptors in mice, but they are not encoded by orthologous genes. *Doublesex* may be an exception in the sense that orthologs have been found in flies, worms, and vertebrates, but whether it performs identical functions across diverse animals is unclear. Sex determination and differentiation do appear operationally similar between flies and mice in that they are controlled by a regulatory cascade, initiated by *sex lethal* in flies and *Sry* in mice (Figure 2). However, sex determination and sexual differentiation in the fly are cell autonomous such that each cell undergoes a cell fate decision to become a male or female cell. By contrast, sex determination of the gonads in mice leads to the secretion of sex hormones that act non-cell autonomously elsewhere in the body and the brain to guide sexual differentiation. In contrast to the mouse, the default fly body plan, in the absence of *sex lethal* but with the appropriate chromosomal complement, appears to be male (Figure 2).

Surprisingly, although the genes involved in sex determination and differentiation have diverged rapidly, some of the same neurotransmitter mechanisms appear to be utilized for reproductive behaviors across multiple species (Asahina et al., 2014; Caldwell et al., 2008; DeVries et al., 1997; Garrison et al., 2012; Winslow et al., 1993; Young et al., 1997). Whether such shared signaling pathways always represent evolutionary conservation

or rather reflect, in some cases, convergent evolution of a limited set of interneuronal communication signals remains to be determined. In either instance, it will be important to understand the selective advantage of using a particular neurotransmitter to control similar reproductive

behavior across species. Similar to the genes regulating sex determination and differentiation, the specific displays of sexually dimorphic behaviors also evolve quickly. This has been well documented in the divergence of the stereotyped male courtship ritual across various drosophilid species (Spieth, 1952). For example, male flies of different drosophilid species, similar to male songbirds of different species, sing a species-specific song to which a female fly of the corresponding species is most attracted (Konishi, 1985; Wheeler et al., 1991). By contrast, male fruit flies utilize chemosensory pathways to recognize conspecific females and to reject potential mates from other species (Fan et al., 2013). Thus, there can be rapid divergence of molecular and behavioral reproductive mechanisms between the sexes and across species. Such divergence is to be expected perhaps because these mechanisms are subject to sexual selection and are critical to maintain reproductive isolation and continued propagation of individuals within a species.

Pheromonal Control of Sexually Dimorphic Behaviors Pheromonal Control of Mating and Aggression in Mice

In mice and many other animals, pheromones detected by sensory neurons in the nose are the predominant sensory cues that trigger mating and aggression. Pheromones are recognized by chemosensory neurons located in two sensory epithelia in the nose, the main olfactory epithelium (MOE), and the vomeronasal organ (VNO) (Figure 4) (Zufall and Leinders-Zufall, 2007). Neurons in these two sensory epithelia each express G protein-coupled receptor (GPCR)-type chemoreceptors from unrelated gene families, suggesting that they detect distinct chemosensory cues (reviewed in Liberles, 2014; Touhara and Vosshall, 2009).

Traditional lesioning studies have long implicated pheromonal signaling via the VNO in the control of male mating and aggression (Wysocki and Lepri, 1991). However, genetic studies reveal surprising complexity in the chemosensory control of these behaviors. Male mice with intact MOE but genetically disabled for VNO signaling mate with females essentially normally but exhibit a profound reduction in intermale aggression (Kim et al., 2012; Leypold et al., 2002; Stowers et al., 2002). By contrast, males with an intact VNO but genetically disabled for

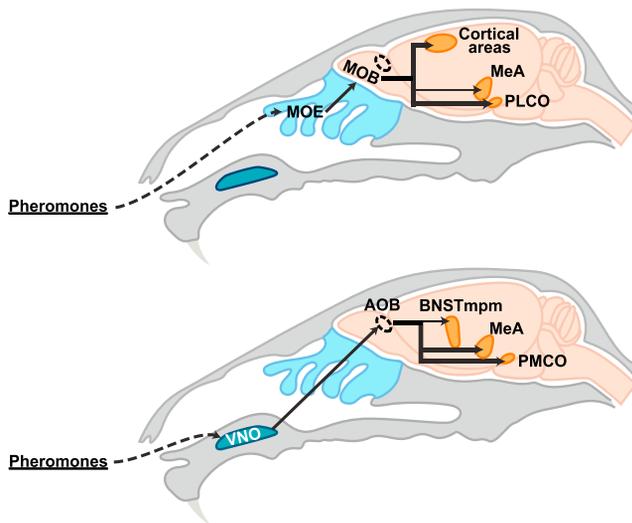


Figure 4. Pheromone-Sensing Pathways

Schematic representing that pheromone-sensing neurons in the main olfactory epithelium and vomeronasal organ activate distinct neural pathways. Dashed oval represents the AOB. Thin arrows to the MeA and BNSTmpm depict relatively minor projections to these areas from the MOB and AOB, respectively.

MOE signaling exhibit a profound reduction in mating and intermale aggression (Mandiyan et al., 2005; Wang et al., 2006; Yoon et al., 2005). Thus, intermale aggression relies on both the MOE and VNO, whereas male-typical sexual displays require an intact MOE but not the VNO (Figure 5A).

Males and females genetically disabled for VNO signaling exhibit male-pattern sexual behavior with mice of either sex (Kimchi et al., 2007; Leybold et al., 2002; Stowers et al., 2002). These findings suggest that male pheromones detected by the VNO normally inhibit sexual behavior and trigger aggression in males (Figure 5A). However, females normally do not attack male mice, and there appears to be no qualitative sex difference in the expression of the chemoreceptor repertoire in the VNO. It is likely therefore that females do not attack males because of a sexual dimorphism downstream of vomeronasal sensory input in the neural circuit that mediates aggression. Females of many species, including mice, exhibit low-frequency male-pattern mating toward conspecific females (Baum et al., 1974; Beach, 1947; Jyotika et al., 2007; Kimchi et al., 2007; Spors and Sobel, 2007). This suggests that the neural circuit for masculine sexual behavior is present in both sexes, but it is normally inhibited by sensory input from the VNO (Figure 5A). One signal that overrides this sensory inhibition is the male sex hormone testosterone, as adult wild-type females supplemented with testosterone display male-pattern mating toward females at male-typical frequencies (Figure 5A) (Edwards and Burge, 1971a). These studies suggest a model in which sex differences in the neural circuit underlying male sexual behavior regulate the probability of displaying this behavior such that VNO sensory input decreases and testosterone increases this probability (Figure 5A).

These genetic studies also show that the VNO and MOE are required for female behaviors, including sexual receptivity and maternal aggression, a behavior that nursing females display to-

ward unfamiliar intruder mice (Fraser and Shah, 2014; Gandelman, 1972; Leybold et al., 2002; Wang and Storm, 2011). Numerous pheromones that regulate sex-typical behaviors or physiology have been identified. The identification of the chemoreceptors that recognize these pheromones will permit delineation of the neural circuits that respond to these cues. This should already be feasible in the case of female sexual behavior. ESP1 is a peptidergic pheromone found exclusively in postpubertal male lacrimal gland secretions (Kimoto et al., 2005), and ESP1 and its chemoreceptor V2Rp-5 are required for the normal, high levels of female sexual receptivity (Haga et al., 2010). These studies now provide a molecular genetic means to trace V2Rp-5-expressing neurons into the central circuits that control female sexual behavior.

Neural Circuits that Transduce Pheromonal Cues into Sexually Dimorphic Behaviors

The anatomic segregation of MOE and VNO neurons is maintained in their central projections, which innervate the main olfactory bulb (MOB) and the accessory olfactory bulb (AOB), respectively (Figure 4) (Dulac and Wagner, 2006; Zufall and Leinders-Zufall, 2007). Mitral and tufted cells, projection neurons of the MOB, send axons to olfactory cortical regions associated with learning of general odorants as well as to the posterolateral cortical amygdala (PLCO), a region that may regulate instinctual behaviors (Figure 4) (Kang et al., 2011a; Scalia and Winans, 1975; Shipley and Adamek, 1984; Sosulski et al., 2011). Thus, the MOE pathway may control innate and learned odor-guided behaviors via projections to distinct brain regions.

AOB projection neurons send their axons to the medial amygdala (MeA) and the posteromedial cortical amygdala (PMCO) and, to a lesser degree, to the medial division of the posteromedial bed nucleus of the stria terminalis (BNSTmpm) (Figure 4) (von Campenhausen and Mori, 2000; Scalia and Winans, 1975; Winans and Scalia, 1970). As we discuss later, these regions influence the display of most sexually dimorphic behaviors. Both the VNO and MOE regulate male-pattern sexual displays and intermale aggression, suggesting crosstalk between or convergence of these two chemosensory circuits. Indeed, mapping of the efferent connections of the projection targets of the MOB and AOB reveals such convergence in the BNST and several hypothalamic nuclei (Figure 5B) (Kevetter and Winans, 1981a, 1981b; Licht and Meredith, 1987; Meredith, 1998; Shipley et al., 1996). In addition some, but not all, studies find that the MeA may also receive afferents from the MOB, thereby providing a site of convergence of MOE and VNO pathways just one synapse removed from the nose (Kang et al., 2009, 2011b; Licht and Meredith, 1987; Sosulski et al., 2011).

The finding that male and female chemosensory neurons express the same repertoire of pheromone receptors immediately poses the question as to how shared sensory input is used to generate sexually dimorphic output. At least in flies, some pheromone-sensing neurons that elicit distinct behaviors in both sexes in response to the same pheromone project to central circuits that are sexually dimorphic (Datta et al., 2008; Kohl et al., 2013; Ruta et al., 2010). How such sex differences direct sexually dimorphic responses in flies is still an open question. In mice, many sex differences have also been described in central olfactory pathways (Guillamón and Segovia, 1997). How such dimorphic neurons in the mouse brain are connected with

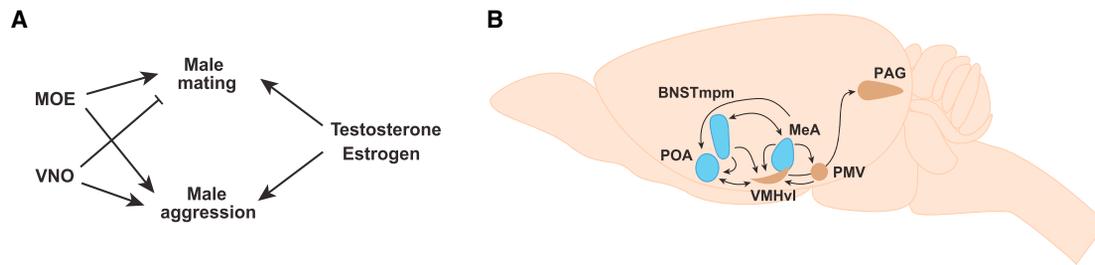


Figure 5. Sensory and Hormonal Control of Sexually Dimorphic Behaviors

(A) Control of male-pattern mating and aggression by chemosensory input and sex hormones.

(B) Schematic representing extensive interconnections between hypothalamic and amygdalar nuclei that regulate sexually dimorphic behaviors. These areas process pheromonal information, and subsets of adult neurons within each of these regions express sex hormone receptors; neurons within some of these regions (blue) also express aromatase. PAG, periaqueductal gray; PMV, ventral premammillary nucleus; POA, preoptic hypothalamus; VMHvl, ventrolateral component of the ventromedial hypothalamus.

pheromone-sensing neurons in the nose is unclear. These sex differences in mice develop under the control of sex hormones, and in adult animals, the central neurons that relay olfactory information are rich in sex hormone receptor expression, thereby affording the potential for sexually dimorphic regulation of the physiology and function of olfactory pathways (Figure 5B). Such convergence of sensory input and hormonal signals in shared neural circuits probably allows the animal to assimilate information about the external world and internal physiological states and to generate sexually dimorphic behaviors.

Hormonal Control of Sexually Dimorphic Behaviors Historical Framework for Understanding Hormonal Control of Sexually Dimorphic Behaviors

The influence of gonadal secretions on behavior and physiology has long been appreciated. Studies in birds in the 19th century correlated the presence of male song or sexual displays with the presence of testicular secretions (reviewed in Fusani, 2008). Subsequent work in male rodents showed that testosterone restored sexual and aggressive displays in adult castrates (Beeman, 1947; Shapiro, 1937; Stone, 1939). Similarly, ovarian extracts or estrogen and progesterone could elicit estrus and sexual receptivity in castrate female rodents (Allen et al., 1924; Dempsey et al., 1936; Ring, 1944; Wiesner and Mirskaia, 1930). Such studies therefore indicated that adult sex hormones were necessary and sufficient for the display of mating, aggression, and the estrous cycle.

Some of the earliest work elucidating a developmental role for gonadal hormones focused on the neural control of the estrous cycle (Harris, 1937, 1964; Harris and Jacobsohn, 1952; Pfeiffer, 1936; Sawyer et al., 1949). These studies showed that the neonatal hypothalamus was bipotential and that testosterone irreversibly inhibited the neonatal hypothalamus from supporting normal ovarian function and estrous cyclicity. By contrast, neonatal castration permitted the male hypothalamus to support ovarian function and the estrous cycle in adult life.

These developmental and adult effects of sex hormones on endocrine physiology were subsequently shown to be equally applicable to reproductive behaviors (Arnold, 2009; Phoenix et al., 1959). In mice, neonatal testosterone irreversibly defeminized sexual receptivity and elicited male-typical territorial aggression upon adult provision of testosterone (Bronson and

Desjardins, 1969; Edwards, 1968, 1969, 1971). Such work revealed that the testes acted on a bipotential brain during a critical perinatal developmental window to regulate adult behaviors. During this period, the ovaries appeared unnecessary for the subsequent display of female sexual behavior. In accord with these experimental observations, the testes secrete testosterone during this critical period, whereas the ovaries are quiescent. The onset and duration of this critical period of sexual differentiation of the brain is species specific, and in mice it extends from late gestation into the first few days after birth (Corbier et al., 1992; Motelica-Heino et al., 1993; Pang and Tang, 1984). In contrast to the perinatal requirement of gonadal hormones in males, the adult display of gender-typical behaviors requires gonadal hormones in both sexes. The enduring, developmental influence of sex hormones is referred to as their organizational function, whereas the reversible, adult role of sex hormones in the acute regulation of physiology and behavior is referred to as their activational function (Figure 6A) (Phoenix et al., 1959). The notion of distinct developmental, including peripubertal, and adult roles of sex hormones is a cornerstone of contemporary understanding of how these steroids control sexually dimorphic behaviors (reviewed in Arnold, 2009; Schulz et al., 2009).

An important advance in understanding the hormonal control of sex-typical behaviors came about from observations that many developmental and adult effects of testosterone were mimicked by estrogen (Antliff and Young, 1956; Ball, 1937; Bronson and Desjardins, 1968; Edwards and Burge, 1971b; Finney and Erpino, 1976; Gorski and Wagner, 1965; Södersten, 1973; Wallis and Luttge, 1975; Whalen and Nadler, 1963). Given that testosterone or a related androgen is a precursor for estrogen in vivo (Figure 3), Naftolin proposed that circulating testosterone in males is converted into estrogen in specific brain regions via the action of the enzyme aromatase, a proposal referred to as the aromatization hypothesis (MacLusky and Naftolin, 1981; Naftolin et al., 1971a, 1971b). Studies in diverse vertebrates now support such local synthesis of estrogen in the male brain, although the extent of the masculinizing effects of estrogen appears to be species specific (Amateau et al., 2004; Balthazart and Ball, 1998; Baum, 2003; Finkelstein et al., 2013; Forlano et al., 2006; Holloway and Clayton, 2001; Lephart, 1996; Lieberburg et al., 1979). How is the brain of the female mouse protected

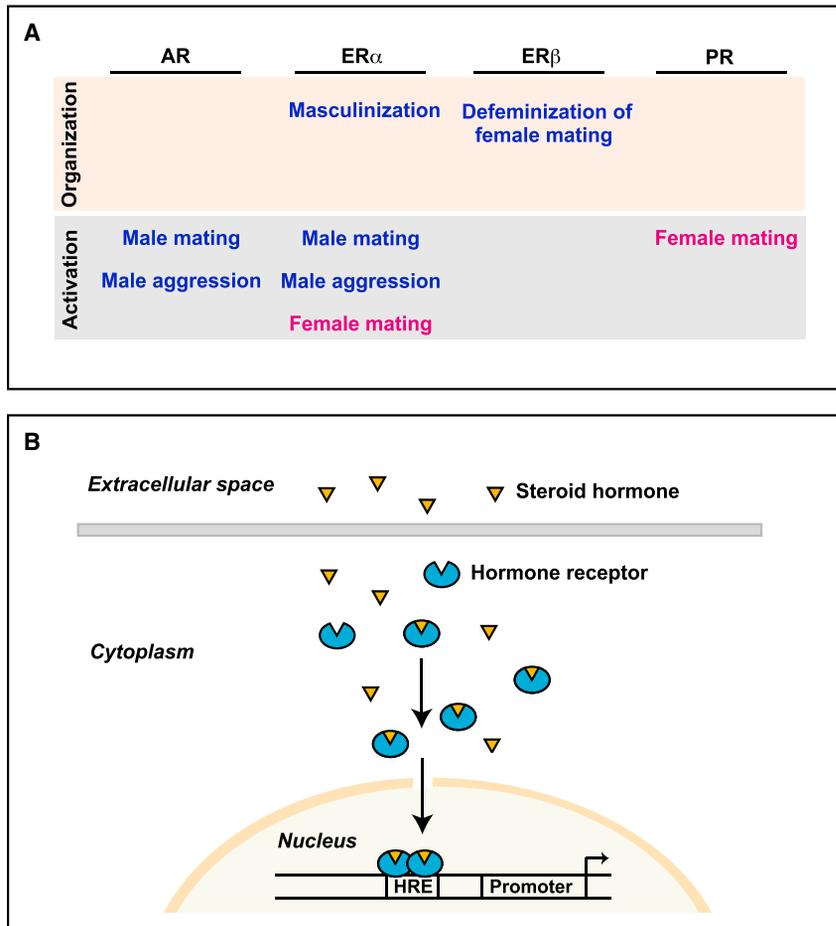


Figure 6. Mechanism and Function of Sex Hormone Action

(A) Function of sex hormone receptors in the nervous system during development (organization) and adult life (activation). Not shown is the requirement of estrogen to feminize sexual behavior, which probably occurs via ER α subsequent to the neonatal organizational phase but prior to adulthood (Bakker et al., 2002; Brock et al., 2011).

(B) Schematic illustrating sex hormone action via nuclear hormone receptors. Sex hormones are steroids that can cross the blood-brain barrier and cell membranes to bind their cognate receptors. Hormone-bound receptor translocates to the nucleus, where it can regulate transcription of target genes by directly binding to specific DNA sequences. HRE, hormone response element.

The evidence that estrogen signaling is required for male behaviors is convincing. Male mice null for aromatase exhibit profound deficits in mating and aggression (Honda et al., 1998; Matsumoto et al., 2003; Toda et al., 2001a, 2001b). Targeted deletions of ER α and ER β show that ER α and ER β are required for male sexual behavior, whereas only ER α is essential for adult male-typical aggression (Krege et al., 1998; Ogawa et al., 1997, 1999, 2000; Scordalakes and Rissman, 2003; Wersinger et al., 1997). As discussed earlier, estrogen is also sufficient to masculinize the brain during development. Supplementing neonatal females with estrogen suffices for the later display of

from the developmental, masculinizing effects of estrogen? As we noted above, the ovaries are quiescent during this critical period and there is consequently little, if any, estrogen synthesis in female embryos. Moreover in mice and many other species, the embryonic liver-derived α -fetoprotein sequesters circulating estrogen produced by the ovaries (Bakker et al., 2006; MacLusky and Naftolin, 1981). Together, these studies make a compelling case for estrogen in regulating differentiation of the male brain and behavior.

Recent Insights into the Hormonal Control of Sexually Dimorphic Behaviors

Recent genetic studies have provided previously unanticipated insights into the mechanisms whereby sex hormones control dimorphic behaviors (Figure 6A). Females constitutively null for PR or ER α are not sexually receptive, thereby confirming the importance of the cognate hormones in this behavior (Blaustein, 2008; Kudwa and Rissman, 2003; Lydon et al., 1995; Ogawa et al., 1996, 1998; Rissman et al., 1997). By contrast, ER β is not required for female sexual receptivity, but it appears essential to defeminize this behavior in male mice (Krege et al., 1998; Kudwa and Rissman, 2003; Kudwa et al., 2005; Ogawa et al., 1999). Thus, it appears that the neonatal testosterone surge in male mice defeminizes sexual behavior at least in part subsequent to conversion into estrogen and activation of ER β .

male pattern territorial behaviors, albeit at lower levels than normally observed in wild-type males (Wu et al., 2009). Thus, neonatal estrogen masculinizes the female as observed by her male-typical aggression toward males. Strikingly, this neonatal estrogen also drives subsequent territorial marking (Wu et al., 2009), which can be displayed without social interactions and may therefore be an objective surrogate for an internal representation of one aspect of masculinity. These masculine behaviors in females neonatally provided with estrogen are abolished upon removal of the ovaries, indicating that they are dependent on gonadal estrogen release (Wu et al., 2009). Thus, estrogen exposure is sufficient to masculinize the brain during development, and adult estrogen permits low-intensity male-typical behavioral displays in the adult. More broadly, these studies show that the adult gonads of either sex can support male-type behaviors provided that the neonatal mouse has been exposed to estrogen.

In contrast to the masculinization effected by estrogen, testosterone signaling via AR appears essential to scale the intensity of masculine behaviors. Male mice constitutively mutant for AR do not display male-typical mating or aggression (Ono et al., 1974; Sato et al., 2004). Such males have a normal neonatal testosterone surge, but the testes atrophy subsequently, leading to a loss of circulating testosterone in adults (Sato et al., 2004). Consequently, these males are developmentally masculinized,

and the behavioral deficits can be rescued to a large degree with testosterone (or estrogen) in adult life (Olsen, 1992; Rosenfeld et al., 1977; Scordalakes and Rissman, 2004; Wu et al., 2009). Very few cells express AR in the wild-type mouse brain at the time of the neonatal surge of testosterone, whereas many cells in regions such as the BNSTmpm, POA, and MeA express one or both nuclear ERs and aromatase (Juntti et al., 2010). The adult pattern of sexually dimorphic AR and aromatase expression is established several days after the neonatal testosterone surge, and this masculinization is dependent on estrogen (Juntti et al., 2010; Wu et al., 2009). Taken together, these studies suggest that testosterone signaling via AR is not required to masculinize the neural substrates for behavior, but rather it plays an activational role in male behavioral displays. Strikingly, this hypothesis has been validated in male mice bearing a nervous system-restricted deletion of AR (Juntti et al., 2010; Raskin et al., 2009). Deletion of AR in the brain prior to the perinatal testosterone surge generated male mutants with masculinized genitalia, male-typical testosterone titers, and a loss of AR expression in the adult brain. These mutant males exhibited a male-typical repertoire of mating and territorial aggression, albeit with a reduced frequency or intensity of specific components of these behaviors (Juntti et al., 2010). Thus, testosterone signaling via AR is not required for developmental masculinization of the brain, but it is essential to amplify the adult display of male-typical mating and territoriality.

These genetic studies make a compelling case for the primacy of estrogen in developmental masculinization of the circuits for mating and territorial aggression and for a dual control by testosterone and estrogen signaling in the adult display of these behaviors. A surprising finding from such studies is the circumscribed distribution of aromatase-expressing neurons (Figure 5B), which localize to subsets of cells within a few regions, including the BNSTmpm, MeA, and the preoptic hypothalamus (POA) in mice (Wu et al., 2009). This limited distribution of aromatase-positive neurons suggests that estrogen may signal to a few critical centers to regulate all male-typical social behaviors. Alternatively, as has been suggested in birds (Balthazart and Ball, 1998; Peterson et al., 2005), estrogen may be distributed widely from such circumscribed sites of synthesis. It is unclear whether such aromatase-expressing neurons function solely as neuroendocrine cells that synthesize estrogen or rather participate directly within the neural circuits that regulate sexually dimorphic behaviors.

Mechanisms Whereby Sex Hormones Control Sexual Differentiation of the Nervous System and Behavior

The cellular mechanisms activated by sex hormone receptor signaling to drive sexual differentiation are similar, if not identical, to those that control neuronal number, morphology, projection patterns, and gene expression during neural development. Such mechanisms as they relate to sexual differentiation have been thoroughly reviewed elsewhere (Forger and de Vries, 2010; McCarthy and Arnold, 2011; Morris et al., 2004; Simerly, 2002; Toran-Allerand, 1984), and we do not discuss these here. By comparison, we know surprisingly little about the molecular pathways activated by sex steroids to regulate these cellular processes. In addition to binding the nuclear hormone receptors discussed above, sex hormones are also thought to

bind transmembrane receptors. However, with the exception of GPR30, a GPCR for estrogen, such transmembrane receptors for other steroid hormones remain to be identified definitively (Burris et al., 2013; Revankar et al., 2005). GPR30 appears not to be required for sexually dimorphic behaviors in mice (Otto et al., 2009; Wang et al., 2008). Many studies also indicate that nuclear receptors for sex hormones can effect rapid changes in cellular function by participating in cytoplasmic or membrane-associated signaling pathways that do not lead to changes in gene expression (Foradori et al., 2008; Henderson, 2007; Lishko et al., 2011; Micevych and Dominguez, 2009; Vasudevan and Pfaff, 2008). This is surprising because the standard view of nuclear hormone receptors has been that they are transcription factors that can bind to consensus DNA sequence elements in the genome to regulate gene expression (Figure 6B) (Burris et al., 2013). These nontranscriptional modes of sex hormone signaling remain poorly understood in the sense that we do not know the specific protein domains of sex hormone receptors involved in these pathways. At least in the case of ER α , genetic studies show that the DNA-binding domain is essential for the normal display of sexually dimorphic behaviors (Jakacka et al., 2002; McDevitt et al., 2007, 2008). These findings suggest that ER α , and perhaps the other nuclear receptors for sex hormones, largely regulates sexually dimorphic behaviors by controlling gene expression. However, no direct transcriptional targets of sex hormone signaling have been identified in the nervous system. In other words, we have yet to learn of a genetic locus whose expression in the nervous system is sexually dimorphic and controlled via occupancy of *cis*-regulatory DNA sequences by a sex hormone receptor.

We imagine that sex hormones regulate gene expression programs that correspond to their enduring organizational and transient activational roles in the developing and adult animal. The long-term developmental effects of sex hormone signaling are probably reflected in sex differences in morphological features and epigenetic control of gene expression (reviewed in McCarthy and Nugent, 2013). Epigenetic programming would permit expression of distinct genes and behaviors upon exposure to the same sex hormone in adult life; for example, such mechanisms may explain why estrogen elicits intermale aggression and sexual receptivity in adult castrate males and females, respectively (Edwards and Burge, 1971b). The extent and specificity of epigenetic marks programmed by sex hormones in the brain remain to be determined. In any event, sex hormone receptors have been shown to associate, at least in cell lines, with histone-modifying enzymes that can activate or repress gene expression, and it will be important in future studies to identify the genes regulated by this mechanism in the brain (Leader et al., 2006; Rosenfeld et al., 2006).

In contrast to the lack of insight on the molecular mechanisms of sex hormone action, many sex differences in gene expression have been identified, and at least some of these are dependent on sex hormones. The nuclear sex hormone receptors and aromatase display some of the most obvious and well-characterized differences in gene expression in adult mice (Grgurevic et al., 2012; Shah et al., 2004; Wersinger et al., 1997; Wu et al., 2009; Xu et al., 2012; Yang et al., 2013; Zuloaga et al., 2014). There are many other genes whose expression in the mouse

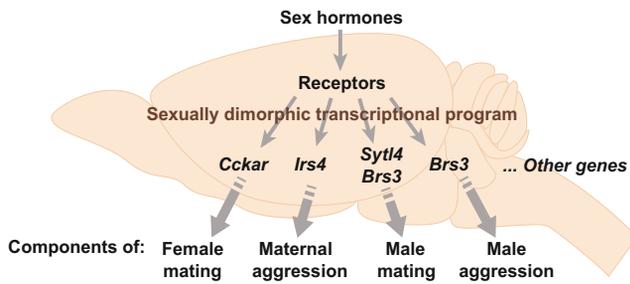


Figure 7. Modular Genetic Control of Sexually Dimorphic Behaviors by Sex Hormones

This model proposes that sex hormones control a sexually dimorphic transcriptional program in the nervous system such that individual dimorphically expressed genes control one or a few components of a sex-typical behavior. This model is supported by work showing that genes downstream of sex hormone signaling (*Brs3*, *Cckar*, *Irs4*, *Sytl4*) are required for the normal display of sexual or aggressive displays (Xu et al., 2012). Many genes downstream of sex hormone signaling still remain to be identified.

brain is also sexually dimorphic (Dewing et al., 2003, 2006; Edelman et al., 2007; Gagnidze et al., 2010; Rinn et al., 2004; Vries, 1990; Wolfe et al., 2005; Xu et al., 2012; Yang et al., 2006). Despite the identification of such dimorphically expressed genes in the brain, there has been no concerted effort to understand whether they influence sexually dimorphic behaviors. A recent study utilized expression profiling to identify many novel sex differences in gene expression in specific centers in the adult mouse hypothalamus, BNST, and MeA (Xu et al., 2012). These gene expression dimorphisms do not correspond to absolute sex differences in cell number, and they often only label a subset of neurons within a brain region. These dimorphic expression patterns are regulated by circulating sex hormones in the adult, although it is unclear whether these genes are direct transcriptional targets of sex hormone receptors. Strikingly, mice singly mutant for these genes exhibited specific deficits in one or a few components of male or female sexual behavior (*Brs3*, *Sytl4*, *Cckar*), intermale aggression (*Brs3*), or maternal care (*Irs4*) while maintaining a sex-typical repertoire of other behaviors (Figure 7). Each of these genes is expressed in particular neurons within the hypothalamus and the MeA or elsewhere, implicating one or more neuronal pools in distinct components of sex-typical behaviors. This notion has been validated in the case of *Cckar*, a GPCR for the neuropeptide cholecystinin. *Cckar* is required for the normal high levels of female sexual receptivity (Xu et al., 2012), and it labels a subset of neurons within the VMH that is also required for the display of this behavior (Yang et al., 2013). More generally, the specificity of behavioral deficits observed in mice mutant for such genes is in complete contrast to the global behavioral deficits observed in castrated mice or mice mutant for sex hormone receptors. These findings suggest a model in which individual dimorphic behaviors or components thereof are controlled in a modular manner by genetically separable pathways that are downstream of sex hormone signaling (Figure 7). This genetic modularity in the control of complex sexually dimorphic social behaviors may allow for evolutionary selection of the components of these behaviors that are critical for reproductive success. As we discuss later, such genetic modularity is probably a

general principle that underlies other innate behaviors in diverse animals.

What Is the Function of Sexually Dimorphic Neuronal Populations?

Numerous cell and molecular sex differences have been identified in neurons and other cell types in virtually every sexually reproducing species. In some cases, perhaps especially so in invertebrates, the sex differences are qualitative such that particular neurons are unique to one sex (Figure 8). In such instances, it can be straightforward to determine the function of sexually dimorphic neurons. For example, the male-specific P1 neuronal cluster expresses *Fru^M* and appears necessary and sufficient for initiation of singing during courtship (Kimura et al., 2008; Kohatsu et al., 2011; von Philipsborn et al., 2011).

Functional analysis of sexual dimorphisms is complicated in cases in which sex differences represent quantitative rather than qualitative cell or molecular differences in neurons. A quantitative sex difference may represent a dimorphism in the same cell or molecular feature in shared neurons. It is conceivable that smaller subsets of neurons embedded within such shared but quantitatively dimorphic neuronal populations are, in fact, unique to one sex. Such smaller subsets may only become apparent upon careful examination of cellular features such as axon projections or gene expression. It is unclear how quantitative sex differences relate to sexually dimorphic behaviors in the two sexes (De Vries and Boyle, 1998). It is possible that a dimorphic neuronal population is functional in both sexes, and it regulates the probability of displaying the same behavior, thereby leading to a sex difference in this behavior (Figure 8). In the extreme version of this model, the dimorphic neurons in one sex are nonfunctional, and the probability of displaying the behavior is zero. A quantitative sex difference could also permit the neurons to be functionally bivalent such that they regulate distinct sexually dimorphic behaviors in both sexes.

We recently examined the function of the quantitative sex difference in the number of PR-expressing neurons in the mouse VMHvl (Figure 5B) (Yang et al., 2013). There are more PR-positive neurons in the female VMHvl, and these neurons also express *Cckar* and *ER α* (Yang et al., 2013). Similar to PR and *ER α* , *Cckar* is also essential for normal female sexual receptivity (Xu et al., 2012). Importantly, PR-expressing VMHvl neurons arise from the same developmental lineage in both sexes (Grgurevic et al., 2012), thereby affording functional characterization of developmentally related dimorphic neurons. We found that genetically targeted ablation of these PR-expressing neurons in the adult female VMHvl led to a profound reduction in female sexual receptivity (Yang et al., 2013). Ablation of the corresponding male neurons resulted in significant reduction in male sexual behavior and aggression. Thus, PR-expressing VMHvl cells constitute a functionally bivalent group of sexually dimorphic neurons (Yang et al., 2013). It will be interesting to test whether all quantitative sex differences in the mouse brain are also functionally bivalent.

Whether a sex difference in neurons is quantitative or qualitative, individual groups of such neurons are dimorphic in multiple dimensions. For example, the PR-expressing VMHvl neurons exhibit sex differences in gene expression, cell density, and

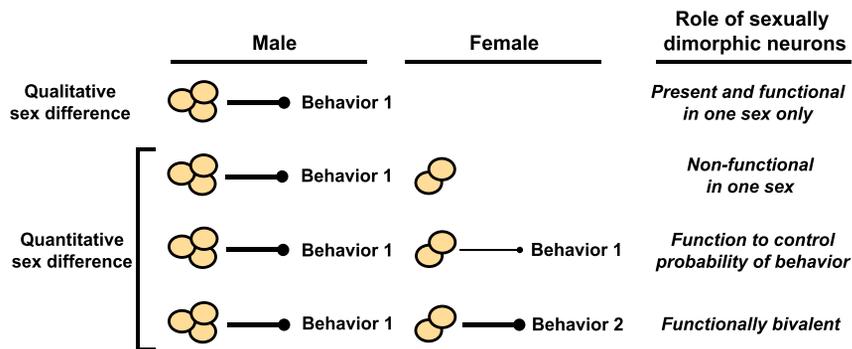


Figure 8. Function of Sexually Dimorphic Neuronal Populations

Neurons present only in one sex (qualitative sex difference) may either activate or inhibit a sexually dimorphic behavior in that sex. More commonly in mice and other vertebrates, a neuronal population is present in both sexes but presents sex differences (quantitative sex difference) in gene expression, cell number, or other cytological feature. In such cases, the neurons may be nonfunctional in one sex, regulate the probability of displaying a sexually dimorphic behavior, or control the display of different sexually dimorphic behaviors in the two sexes (functionally bivalent).

number within this region and in their long-distance projections (Yang et al., 2013). How each of these molecular and cellular sexually dimorphic features relates to sexually dimorphic behavioral output is unknown, and we anticipate that it will be difficult to address this issue with current approaches.

Which Neural Circuits Underlie Mating and Aggression?

Decades of rodent lesion or stimulation studies have revealed a limited and overlapping set of sexually dimorphic brain regions that control sexual behavior and aggression in the two sexes (Figure 5B) (Colpaert and Wiepkema, 1976; Commins and Yahr, 1984; Emery and Sachs, 1976; Goy and Phoenix, 1963; Hennessey et al., 1986; Kondo et al., 1990, 1998; Kruk et al., 1979; Lin et al., 2011; Liu et al., 1997; Olivier and Wiepkema, 1974; Pfaff and Sakuma, 1979a, 1979b; Yamanouchi and Arai, 1985). These centers, including the BNSTmpm, POA, MeA, and VMH, are interconnected and encompassed largely within the pheromone processing neural pathways that regulate these behaviors (Figure 5B) (reviewed in Swanson, 2000). With few exceptions, subsets of adult neurons within these regions also express aromatase or one or more sex hormone receptors in mice (Grgurevic et al., 2012; Shah et al., 2004; Wersinger et al., 1997; Wu et al., 2009; Xu et al., 2012; Yang et al., 2013; Zuloaga et al., 2014). It is presently unclear whether these interconnected pathways are composed of distinct circuits that control each of these behaviors. Alternately, these regions may comprise a single neural circuit controlling both mating and aggression.

In fact, the neurons within these regions (Figure 5B) are molecularly heterogeneous and control diverse behaviors (Choi et al., 2005; Shah et al., 2004; Swanson, 2000; Xu et al., 2012; Yang et al., 2013). How such molecular heterogeneity translates into behavioral pleiotropy is unclear. We have recently examined this issue in the VMHvl and linked a molecularly discrete neuronal population to some, but not all, behaviors controlled by this area (Yang et al., 2013). The VMH is molecularly heterogeneous, and neurons within or adjacent to the VMH regulate female sexual behavior, aggression, defensive reactions to predators, and energy balance in diverse animals, including humans (Goy and Phoenix, 1963; Hess and Akert, 1955; Hetherington and Ranson, 1940; King, 2006; Kow et al., 1985; Kruk et al., 1979; Kurrasch et al., 2007; Lin et al., 2011; Mathews and Edwards, 1977; Musatov et al., 2007, 2006; Olivier and Wiepkema, 1974; Pfaff and Sakuma, 1979a, 1979b; Reeves and Plum, 1969; Robarts and Baum, 2007; Silva et al., 2013; Swaab, 2003; La Vaque and

Rodgers, 1975). Experimental lesions or other manipulations that do not target molecularly distinct neurons within the VMH can yield conflicting behavioral phenotypes (see, for example, Kow et al., 1985; Pfaff and Sakuma, 1979a, 1979b; La Vaque and Rodgers, 1975), presumably reflecting nontargeted manipulation of heterogeneous neurons or fibers of passage within this region. Importantly, none of these studies have determined the role of molecularly distinct VMH neurons underlying female sexual behavior or male aggression. To resolve these issues, we used genetic tools to ablate adult PR-expressing VMHvl neurons, which represent ~10%–20% of all cells within the VMH. We found that these neurons are required for the normal display not only of female sexual behavior and male aggression, but also male sexual behavior (Yang et al., 2013). A role of VMH neurons in male sexual behavior was not revealed even with optogenetic manipulation of molecularly unidentified neurons within this region (Lin et al., 2011), illustrating the power of genetic targeting of molecularly identified neuronal subsets within heterogeneous regions such as the VMH (Yang et al., 2013). Importantly, these PR-expressing neurons are not required for other VMH functions such as maintenance of normal body weight. In summary, molecularly distinct VMH neurons appear to underlie the functional diversity of this region.

How PR-expressing VMHvl neurons influence both male mating and aggression is unclear. In one scenario, this population consists of molecularly distinct neuronal subsets that subserve one or the other behavior in a labeled-line manner (Figure 9A). Thus, activation of the appropriate VMHvl subset would elicit mating or fighting. Our observation that PR-positive VMHvl neurons influence both male mating and aggression, but not feeding, is also in accord with Tinbergen's proposal that these two behaviors are more closely allied to each other than to feeding (Tinbergen, 1951). In his scheme, the reproductive instinct includes mating, aggression, nesting, and parental care but is distinct from the instincts for sleep, feeding, or defense from predators. Tinbergen reasoned that behavioral decisions for instinctual displays are hierarchically organized. In his model, high-level decisions would enable an animal to enter a state promoting reproductive rather than, for example, feeding behavior; a lower-level decision would subsequently enable an animal to mate, fight, or nurse. He further proposed that such a behavioral hierarchy would be reflected in a hierarchical organization of the underlying neural substrates such that behavioral choices would be made and enforced via crossmodal inhibition of neuronal populations

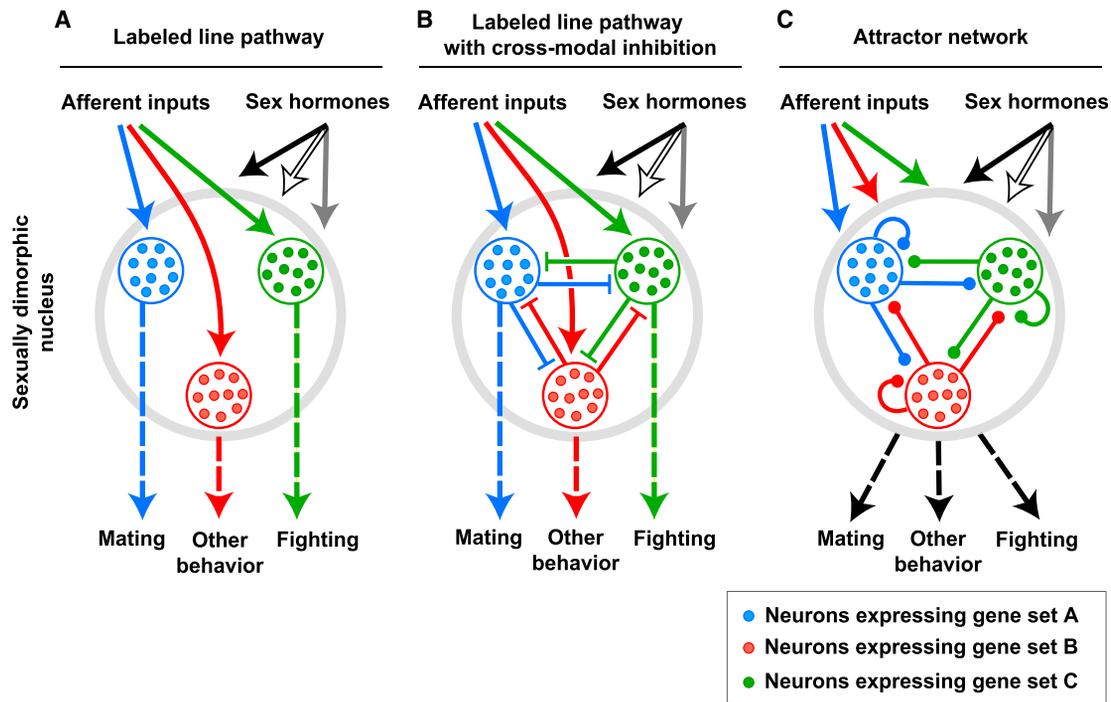


Figure 9. The Relation between Molecular Heterogeneity in and Functional Pleiotropy of Sexually Dimorphic Brain Regions

Some models that can be used to relate molecular heterogeneity to functional diversity are shown.

(A) Afferent and sex hormone inputs drive labeled line pathways to control different behaviors. Such a labeled line pathway is a simplified version of a multilayered feedforward network.

(B) Afferent and sex hormone inputs drive labeled line pathways with crossmodal inhibition to control different behaviors.

(C) The heterogeneous neurons constitute an attractor type network with local and recurrent connections. Afferent and sex hormone inputs in conjunction with local circuits lead to a stable state of the network that elicits behavior.

Molecular heterogeneity may afford a single neural circuit to utilize labeled line, labeled line with crossmodal inhibition, and attractor network pathways at distinct synaptic stations. Alternatively, a single neural circuit may consist entirely of one or the other of these pathways.

governing comparable decisions at a similar level of the hierarchy (Figure 9B). In the case of the VMHvl, if the PR-positive cells can be further subdivided molecularly into subsets that are dedicated to male mating or fighting, Tinbergen’s model predicts inhibitory interactions between these two populations. However, any such crossmodal inhibition must be quickly reversible because male mice can mate and fight with the appropriate target when presented simultaneously with a male and female conspecific (Leypold et al., 2002). Although not part of Tinbergen’s original proposal, it is tempting to speculate that there are similar crossmodal interactions even for different instincts, a notion supported by the close proximity of PR-positive VMHvl neurons with VMH neurons that regulate feeding and responses to predators.

Both the labeled-line as well as the crossmodal inhibition models rely on PR-expressing VMHvl neuronal subsets that control either mating or aggression (Figures 9A and 9B). However, it is also possible that the same neurons mediate both male mating and fighting via distinct patterns of neural activity or the release of specific neuromodulators (Figure 9C) (Marder, 2012). In this scenario, the activity of PR-expressing VMHvl output neurons would not only depend on afferent input and hormonal signals but would also be contingent on feedback loops or local networks between these cells. Such recurrent connectivity can lead to secondary network dynamics with stable, or attractor,

states (Hopfield, 1982, 1984). In this model, the output of the PR-positive VMHvl neurons would depend on the dynamics of external input and local connectivity, and it would promote (or inhibit) the display of a particular behavior when a stable state is achieved. Importantly, such a model does not necessitate invoking molecular heterogeneity within the PR-expressing VMHvl neurons, although if the local network consists of both output neurons and interneurons, then these two cell types will be molecularly distinct.

Presumably other models can also be invoked to explain the dual control of mating and aggression by PR-expressing VMHvl neurons. The MeA, BNSTmpm, POA, and other regions implicated in mating and aggression (Figure 5B) are also molecularly heterogeneous and behaviorally pleiotropic. It is possible that one or more of the scenarios we have discussed for the VMHvl (Figure 9) also underlie the functional pleiotropy of these regions. Moreover, whether the entire neural circuit(s) for mating or aggression utilizes one or the other models exclusively is an open question.

A common theme emerging from the studies of mating and aggression in mice and flies is the modular or specialized nature of the function of individual neuronal populations. In other words, distinct neuronal pools appear essential for the performance of different components of the same behavior. Distinct neuronal clusters are also required for the performance of various components of male fly courtship song and copulation

(Kim et al., 2013; Kimura et al., 2008; Kohatsu et al., 2011; von Philipsborn et al., 2011). Our studies with PR-expressing VMHvl neurons show that these neurons control male mating and aggression, but sex discrimination, conspecific grooming, ejaculation, and territorial marking are unaffected (Yang et al., 2013). Recent work from Rao and colleagues indicates that sex discrimination and sexual preference are regulated by serotonergic neurons located in the hindbrain (Liu et al., 2011b). Taken together, these studies suggest that different elements of mating and aggression are encoded, to a large degree, by distinct neuronal populations. It is presently unclear how such functionally modular neurons interact to generate a cohesive display of mating or aggression.

Conclusions and Future Directions

There has been striking progress in understanding how the brain generates sexually dimorphic behaviors in mice. Gonadal sex hormones control the overall repertoire of male and female typical behaviors, and genes downstream of sex hormone signaling appear to control specific components or routines of these behaviors. Most sexual dimorphisms in the brain manifest as hormone-regulated quantitative differences in cell or molecular properties of neurons, and in at least one instance such sexual dimorphisms have been shown to control distinct behaviors in the two sexes (Yang et al., 2013). Coupled with modern neural circuit mapping tools, the recent dramatic advances in systematic gene expression profiling, genetic manipulations, and potential deorphanizing of many pheromone receptors will lead to rapid progress in understanding how sex is represented in the brain and transformed into gender-typical behavior in mammals (Isogai et al., 2011; Luo et al., 2008; Mardis, 2008; Wang et al., 2013; Xu et al., 2012). We anticipate that such insights into how sex differences in the brain control sexually dimorphic behaviors in health will eventually translate to understanding the startling sex differences in many common neuropsychiatric illnesses. Indeed, at least some sexually dimorphically expressed genes in the brain appear to be linked with such human disorders that occur in sex-skewed ratios (Xu et al., 2012).

Many of the same sexually dimorphic brain regions have been implicated in the control of both mating and aggression. Most of these regions contain pools of molecularly heterogeneous neurons, and with few exceptions (Yang et al., 2013), it is unclear whether molecular heterogeneity within a region always translates into functional specialization such that different neuronal pools, marked by unique sets of genes, regulate different behaviors. Alternatively, such molecular heterogeneity could be used to construct unique network dynamics that generate different behaviors based on afferent input, hormonal signals, and past experience. A resolution of this issue of heterogeneity in conjunction with anatomical neural circuit mapping will provide insight as to how the same brain regions encode mating and fighting.

Although mating and aggression are innate behaviors such that they can be displayed without prior training, they are nevertheless modified by past experience. For example, repeated defeat in aggressive encounters renders a male mouse more liable to defeat in subsequent encounters (Russo et al., 2012);

such a submissive male also marks his territory only sparsely in comparison to naive or dominant males (Desjardins et al., 1973). Significant progress has been made in understanding the molecular and cellular basis whereby such social defeat modifies reward, stress, and defense pathways in the brain (Russo et al., 2012), and it will be interesting in future studies to extend these analyses to the core circuits (Figure 5B) that mediate mating and fighting. Mating and aggressive displays are often dramatically plastic in some species; for example, submissive male cichlid fish can, within minutes of removal of a dominant male from their vicinity, start behaving like a dominant male (Fernald, 2012). Mated prairie voles exhibit aggression toward unfamiliar intruders, including those of the opposite sex, whereas sexually naive voles do not typically attack members of the opposite sex (Carter and Getz, 1993; Insel, 1997). We anticipate that work in these model systems will provide insight into the neural substrates for plasticity in otherwise hard-wired behaviors.

Studies in mice reveal a surprising modularity at molecular and cellular levels in the control of mating and aggression. Indeed, gene deletion or genetically targeted functional manipulation of restricted neurons generates very specific deficits in one or more components of sexual or aggressive displays while leaving other components intact. It is tempting to speculate that other components of courtship and territoriality that are learned such as birdsong are also controlled in a modular manner by specific neuronal pools and genetic loci. In fact, we anticipate that modular control of diverse behaviors, learned or otherwise, may turn out to be a general organizing principle for the underlying neural circuits. It will be interesting to test whether other behaviors (such as predator-defense) that are also usually thought to be associated with emotional states are encoded by a modular genetic and neural architecture. Recent studies in *Peromyscus* show that individual parameters of species-specific tunnels built by these mice are controlled in a modular manner by a few genetic loci (Weber et al., 2013). Similarly, different aspects of schooling behavior in stickleback fish also appear to be controlled by distinct genetic loci (Greenwood et al., 2013). Such genetic and neural compartmentalization of behaviors presumably affords rapid evolvability of circuits and behavior, in a manner perhaps analogous to exon shuffling that leads to the generation of genes that encode proteins with novel combinations of functional domains.

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