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Molecular and neural control of sexually dimorphic social behaviors

Taehong Yang[1] and Nirao M Shah[1,2]

Sexually reproducing animals exhibit sex differences in behavior. Sexual dimorphisms in mating, aggression, and parental care directly contribute to reproductive success of the individual and survival of progeny. In this review, we discuss recent advances in our understanding of the molecular and neural network mechanisms underlying these behaviors in mice. Notable advances include novel insights into the sensory control of social interactions and the identification of molecularly-specified neuronal populations in the brain that control mating, aggression, and parental behaviors. In the case of the latter, these advances mark a watershed because scientists can now focus on discrete neural pathways in an effort to understand how the brain encodes these fundamental social behaviors.

Addresses
[1] Department of Anatomy, University of California San Francisco, San Francisco, CA 94158, United States
[2] Center for Reproductive Sciences, University of California San Francisco, San Francisco, CA 94158, United States

Corresponding author: Shah, Nirao M (nms@ucsf.edu)

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Introduction

Sexually dimorphic behaviors such as mating and aggression are instinctual in the sense that they can be displayed without prior training, and they are stereotyped and elicited by ethologically relevant stimuli in a laboratory setting. The fact that these behaviors do not require training is consistent with the notion that they are under selection and developmentally hard-wired, and therefore accessible to unbiased molecular genetic approaches to characterize the underlying neural circuits. Together, these features make them attractive candidates for understanding how the brain encodes complex social interactions.

Despite the instinctual nature of these behaviors, they are only displayed in the presence of specific sensory stimuli emanating from conspecifics. In the case of mice, as in many other animals, these sensory cues are largely pheromonal in nature. Pheromones are chemosensory cues that signal social or reproductive status to other individuals within the species [1]. They are detected by sensory neurons in the nose that reside in the vomeronasal organ (VNO) or the main olfactory epithelium (MOE). In males, genetic studies show that both the VNO and MOE are required for the display of male territorial aggression and mating [2–6]. In females by contrast, both the MOE and VNO are required for female receptivity and maternal aggression, and maternal retrieval of pups requires the MOE, and redundantly, the VNO [7,8]. The identity of the pheromones that elicit these behaviors, the specific chemoreceptors that detect them in the nose, and the neural circuits downstream of MOE or VNO are the subject of ongoing research.

In addition to external, sensory cues such as pheromones, the display of sexually dimorphic behaviors is also regulated by internal, physiological signals. In the case of mice and many other vertebrates, the development and activation of neural circuits underlying these behaviors is controlled by sex hormones such as estrogens, progesterone, and testosterone [9–12]. These hormones bind to cognate nuclear hormone receptors that are also essential for sex-specific social interactions. The sex of the brain is determined during a critical developmental window that varies in different vertebrates, but in mice occurs in the perinatal period. At this time, the ovaries are quiescent and there is a male-specific surge in circulating testosterone that masculinizes the brain. Subsequent to this early event, both ovarian as well as testicular sex hormones play essential roles in the maturation of the brain in the two sexes. In males, testosterone is also converted into estrogens by the enzyme aromatase which is expressed in discrete neuronal populations [13,14]. Both aromatase as well as estrogen receptors are also essential for male-pattern displays of mating and territorial aggression [12]. The relative contributions of testosterone and estrogen signaling in masculine behaviors has been resolved by recent genetic studies [14–16]. Findings from these studies show that the developmental or organizational effects of testosterone on masculinizing the brain during the critical perinatal period are mediated by estrogens; both estrogens and testosterone are subsequently required for the male-typical expression of social behaviors.

In summary, sex hormone signaling is necessary and sufficient to set up sexual differentiation of the brain

and behavior. Although other pathways have also been implicated in the control of sexually dimorphic behaviors [17], sex hormone-regulated pathways are the predominant internal physiological signals that control these social behaviors. Similarly, pheromones provide the predominant sensory cues in eliciting these behaviors in the appropriate social context. Accordingly, our review covers recent progress in understanding how these internal and external cues regulate sex-specific displays of social behaviors. As we will illustrate throughout this review, a dominant theme to emerge from these studies is the exquisite genetic specification of different neural pathways to regulate distinct aspects of social interactions.

Sensory control of sexually dimorphic social behaviors

A comprehensive survey of progress in uncovering specific classes and sources of pheromones and chemoreceptors that regulate sexually dimorphic social behaviors is beyond the scope of this article, and it has also been the subject of numerous excellent reviews [1,18–20], including one in this issue by Stowers and Liberles. Suffice it to say that not only are we beginning to understand the diversity of pheromones that mice can detect due to large, unbiased screens [21,22•,23•], but various groups are beginning to link specific pheromones to particular chemoreceptors and behavioral output. It seems clear that males and females use a diversity of pheromones to engage in social investigation, sexual behavior, and territorial behaviors, including intermale aggression [22•,23•,24,25,26,27,28••,29,30,31•]. An emerging theme from recent studies of pheromone-elicited responses is that these responses can be modulated by physiological status or prior experience [28••,31•,32].

In addition to acting as positive signals that trigger instinctual behavioral responses, pheromones may also inhibit such behaviors. Indeed, a recent study by Liberles and colleagues identified a peptide pheromone ESP22 secreted by lacrimal glands in juvenile mice that inhibits adult males from mating with them [33••]. ESP22 is sufficient to inhibit sexual displays from adult males, and it acts by activating sensory neurons in the VNO. Indeed, adult male mice mutant for Trpc2, a cation channel required for sensory signal transduction in the VNO, display sexual behavior toward juvenile mice. This inhibitory signaling system has evolved presumably to inhibit male sexual behavior toward reproductively futile targets such as juvenile conspecifics. This study also informs our understanding of the behavior of male mice null for Trpc2. Previous work shows that Trpc2 null males attempt to mate with both adult males and females [2,3]. These findings are compatible with a model wherein the VNO simply provides a tonic inhibitory signal that inhibits all male sexual behavior in wildtype males. However, the findings by Liberles' group provide direct evidence that in fact pheromones such as ESP22 function to inhibit male sexual displays. This notion is in accord with other studies that show that the VNO is also required for the normal display of male sexual behavior toward wildtype females [4]. Thus, detection of distinct pheromone ligands by the VNO, presumably via distinct chemoreceptors expressed in non-overlapping sensory neurons, may either activate or inhibit male sexual behavior. It is therefore possible that adult males also produce pheromones that inhibit mating attempts by other males, and impaired VNO function in Trpc2 null males enables sexual displays toward other males. In summary, there has been enormous progress in understanding the sensory mechanisms that guide pheromone-mediated control of sexually dimorphic social behaviors. The specific neural pathways that transmit recognition of identified pheromones to the central circuits discussed below to regulate these behaviors remain to be characterized.

Central control of sexually dimorphic social behaviors

Two distinct but related approaches underlie recent advances in the identification and functional characterization of central molecular and neural pathways that control sexually dimorphic social behaviors. The first approach utilizes current understanding of hormonal or molecular control of these behaviors to identify genes and neurons expressing these genes as an entry-point into the underlying neural circuits. The second approach utilizes markers of neural activity such as Fos as an entry-point into the neural circuits underlying a given behavior. Both approaches have naturally also been guided by classical lesion studies that have identified broad domains within heterogeneous brain regions such as the amygdala or hypothalamus as being relevant for mating, aggression, or parental care. In the sections that follow, we have provided a synthetic overview of recent advances in the field, combining where possible studies that utilized one or the other approach.

Molecular control of sexually dimorphic social behaviors

As discussed in the Introduction, sex hormones govern the entire repertoire of sexually dimorphic behaviors. What are the molecular mechanisms whereby sex hormones regulate these diverse social behaviors? The titers of sex hormones in the circulation are sexually dimorphic and these hormones can regulate gene expression by binding to their cognate nuclear hormone receptors. Accordingly, a recent study used unbiased gene expression profiling and identified numerous sex hormone-regulated sex differences in gene expression in various hypothalamic and amygdalar nuclei in the adult mouse brain [34•]. Moreover, analysis of mice mutant for individual genes expressed in a sexually dimorphic manner revealed restricted deficits in one or a few components of sex-specific social behaviors such that other interactions as well as general sensorimotor coordination and motivated behaviors were unaffected. We discuss one of these

dimorphically-expressed genes, Cckar, that is required for female sexual behavior in detail below. Together these findings suggest a model wherein sex hormones control the repertoire of sexually dimorphic social behaviors in a modular manner such that sex hormone-dependent, genetically separable signaling networks regulate the display of distinct aspects of sex-specific social interactions [12,34•]. Such modularity in the control of complex social behaviors also seems to operate at the level of molecularly-specified neuronal populations as described below. More recently, studies of unrelated behaviors such as tunneling in *Peromyscus* have also suggested a modular genetic architecture underlying different components of the behavior under consideration [35,36]. Thus, modular control of behavior may be a general principle whereby the brain controls diverse behaviors.

How sex hormones control gene expression during the critical perinatal period and subsequently to drive sexual differentiation of the brain and behavior is largely unknown. However, a recent study in rats and mice shows that the early masculinizing surge of sex hormones during the critical period may inhibit DNA methylation by modulating the activity of DNA methyltransferases (DNMT) in the preoptic hypothalamus (POA) [37], a sexually dimorphic brain region previously implicated in parental care (see below) and male mating. Indeed, pharmacological inhibition of DNMT activity or neonatal deletion of the *de novo* DNA methyltransferase DNMT3a in the POA masculinized sexual behavior subsequently in females. It will be important in future studies to link changes in DNA methylation of specific DNA sequences directly with sexually dimorphic expression of particular genes in identified neurons in the POA, and ultimately tie in such changes with behavioral output. Previous studies have demonstrated that in fact adult female mice treated with testosterone display male sexual behavior [38]. This suggests that the early DNA methylation events can be reversed by adult exposure to testosterone; alternatively, testosterone can also activate male sexual displays in females via other mechanisms.

Both oxytocin and serotonin have been implicated in the control of social interactions and reward [39,40], and several groups have recently studied the role of these neuromodulatory signaling systems in mating, aggression, or parental behaviors. For example, oxytocin signaling in prefrontal and auditory cortical inhibitory neurons is critical in female mice for social investigation of males and pup retrieval, respectively [41••,42]. By contrast, serotonin signaling regulates mating partner preference in both males and females [43,44]. Intriguingly, targeted deletion of the oxytocin receptor in serotonergic neurons essentially abrogates male aggression [45], thereby demonstrating control of distinct sexually dimorphic social behaviors by serotonergic neurons. How such oxytocin, serotonin, and other neuromodulatory signaling pathways intersect with the hypothalamic or amygdalar neuronal populations (see below) that regulate these behaviors is poorly understood.

Molecularly identified neurons that control female sexual receptivity

As mentioned above, Cckar was identified in a screen for genes expressed in a sexually dimorphic manner in adult mice [34•]. Cckar is a G-protein coupled receptor for the neuropeptide cholecystokinin (CCK), and it is expressed in a sexually dimorphic manner in several brain regions, including the ventrolateral compartment of the ventromedial hypothalamus (VMHvl). Male mice constitutively mutant for Cckar do not have overt deficits in social interactions whereas mutant females show a specific diminution in sexual receptivity. Administration of Cckar antagonists to wildtype females in estrus also reduces sexual receptivity, suggesting that this receptor normally functions in the adult animal to promote this behavior. Cckar expression in the VMHvl is largely restricted to females, where its expression peaks at estrus. Previous studies have provided strong evidence for a critical role of the VMH in regulating female sexual behavior [12], and in fact >95% of Cckar neurons in the VMHvl express progesterone receptor (PR) [46••], which is also required for this behavior [12]. Genetically targeted ablation of PR-expressing neurons in the VMHvl of adult females led to a profound diminution of female sexual receptivity [46••]. Importantly, neither Cckar mutants nor females lacking PR (and Cckar) expressing neurons in the VMHvl show deficits in the estrous cycle or in other behavioral tests, suggesting a restricted deficit in female mating behavior. Given that the majority of PR-expressing neurons also express Cckar, these findings are consistent with the interpretation that Cckar functions in VMHvl neurons to regulate female sexual behavior. Previous work has also implicated CCK in regulating sexual receptivity in female rodents [47], and it will be important in future studies to identify the source of CCK to Cckar-expressing VMHvl neurons.

Molecularly identified neurons that control aggression and male sexual behavior

Decades of lesion as well as stimulation studies had identified a center in the caudal and ventral hypothalamus in cats as well as rodents that was critical for attack behavior [12,48]. These studies did not distinguish whether such manipulations affected fibers of passage or resident neurons in this area. A recent study localized aggression-eliciting neurons in male mice within or close to the VMHvl and further showed that neurons in this region were also activated during male aggression [49]. Strikingly, genetically targeted ablation of PR-expressing neurons in the male VMHvl led to significant reduction in male aggression as well as specific deficits in male sexual behavior [46••]. However, males lacking these neurons could distinguish between the sexes and also marked their territory, indicating that behavioral deficits in mating and aggression did not

represent pervasive deficits in related social behaviors. A related study used optogenetics to show that activity of an overlapping population of VMHvl neurons that expresses estrogen receptor alpha (Esr1 or ERα) is necessary and sufficient for male aggression [50••]. Remarkably, stimulation of these neurons at lower intensity elicited male sexual displays toward both male and female mice whereas stronger stimulation elicited attacks toward both sexes. Together with other findings, these studies have identified long-sought neurons in the hypothalamus that control aggression, and unexpectedly they have also revealed a previously unknown function of this region in male sexual behaviors [46••,50••]. The control of both mating and aggression by PR and Esr1 expressing neurons may reflect further molecular and functional heterogeneity within this population. Alternatively, it may reflect differential neural network dynamics or recruitment that drive mating or aggression depending on context or other variables.

These studies in the VMHvl have a surprising parallel with studies that reveal dual control of male mating and aggression by GABAergic medial amygdalar (MeA) neurons in a stimulation intensity-dependent manner [51••]. The MeA processes pheromonal cues in urine in a sexually dimorphic manner, is active during social interactions, and has been implicated in sexual and aggressive behaviors by previous studies [52–54]. Male aggression elicits Fos induction preferentially in GABAergic but not glutamatergic neurons within the MeA [51••]. Low intensity optogenetic stimulation of GABAergic neurons in males elicited social grooming or sexual displays toward males or females whereas high intensity stimulation elicited aggression toward both sexes. There is a surprising antagonistic relation between glutamatergic and GABAergic MeA neurons such that stimulation of the former elicits self-grooming and inhibition of social behaviors whereas stimulation of the latter drives social behaviors and inhibits self-grooming. At least in the MeA, the dual control of male mating and aggression is regulated by molecularly distinct groups of neurons. Aromatase expressing neurons are restricted to a subset of GABAergic neurons exclusively within the posterodorsal MeA, and targeted ablation or silencing of these aromatase-expressing neurons reduces male aggression without altering mating, sex discrimination, or territorial marking behaviors [55••]. MeA neurons that specifically regulate male sexual but not aggressive behaviors remain to be molecularly identified.

In related studies, aromatase-expressing GABAergic neurons in the posterodorsal MeA are also required for maternal aggression [55••], a behavior exhibited by nursing dams toward unfamiliar intruders in their cage. Importantly, mice exhibit distinct patterns of fighting during male territorial and maternal aggression, and these two forms of aggression are regulated by distinct sensory, hormonal, and molecular pathways. Together, these findings therefore demonstrate that aromatase-expressing neurons within the posterodorsal MeA regulate different behaviors in the two sexes [55••].

Both PR-expressing neurons in the VMHvl and aromatase-expressing neurons in the posterodorsal MeA control distinct behaviors in the two sexes. Such sexual dimorphism in neuronal function reflects underlying sex differences in the neurons themselves, and indeed previous work has identified differences in gene expression or neuronal projections in these neurons [14,34•,46••]. Although the MeA and VMH are inter-connected, it is unlikely that MeA neurons that are required for aggression synapse directly on to PR (and Esr1) expressing VMHvl neurons. This is because the latter population is glutamatergic and is also required for aggression whereas MeA neurons are GABAergic. This suggests that if these two neuronal populations are part of the same circuit then there is likely to be an intermediary group of neurons that is also GABAergic. In fact, a recent study has described a GABAergic set of neurons in the lateral septum that projects to the VMH and inhibits male aggression [56•]. However, these neurons project widely within the hypothalamus, and it is presently unclear whether they synapse directly on to PR (or Esr1) expressing VMHvl neurons. Similarly, whether these neurons are synaptically linked with MeA neurons that regulate aggression is also unknown. Nevertheless, this study provides a candidate set of GABAergic neurons that may link MeA and VMHvl neurons that regulate male aggression.

Molecularly identified neurons that control parental behaviors

Female mice exhibit maternal behavior toward pups even as virgins whereas male mice are infanticidal toward pups. This aggressive behavior is transiently inhibited in males that have ejaculated and sired pups such that they exhibit parental behaviors during a time window that is programmed to begin at the time of parturition [57]. Dulac and colleagues recently showed that pup-directed aggression by males is dependent on VNO signaling, and Trpc2 null males show parental behaviors even as virgins [58••]. This group found Fos induction upon parenting behaviors in the POA, a region previously implicated in maternal behaviors. There is significant overlap between Fos and galanin-expressing neurons in the POA, suggesting a functional role for these neurons during parenting in males and females. Indeed targeted ablation of galanin-expressing POA neurons reduced parenting in females and mated males, and it also elicited pup-directed aggression in virgin females but not in mothers or mated males. By contrast, optogenetic stimulation of these neurons reduced pup-directed aggression in virgin males and induced parenting behaviors. It is likely that these galanin-expressing POA neurons are heterogeneous because Dulac and colleagues observed subtle changes in other social and locomotor behaviors [58••]. Nevertheless, this important study has identified long-sought neurons that regulate maternal care as well as pup-directed aggression in virgin males.

Using a complementary approach, Kimchi and colleagues focused on neurons that express tyrosine hydroxylase (TH) in a sexually dimorphic manner in the anteroventral periventricular nucleus (AVPV) of the hypothalamus [59••]. Targeted ablation of TH-expressing neurons, which are more numerous in females, reduced maternal behaviors whereas their activation increased the display of these behaviors. By contrast, ablation of these neurons in males increased aggression whereas their activation decreased this behavior. The functional or anatomical connectivity between these TH-expressing AVPV neurons and the adjacent galanin-expression POA neurons is unknown. However these TH-expressing neurons do project directly to oxytocinergic neurons in the paraventricular hypothalamus, and optogenetic stimulation of TH-expressing neurons in the AVPV increases oxytocin in the circulation. Thus, these studies outline a neural pathway that regulates levels of oxytocin, a neuropeptide hormone long associated with maternal behaviors.

Conclusion

There have been remarkable advances in our understanding of the control of complex, sexually dimorphic social behaviors by genetically-identified neuronal populations. It seems reasonable to assume that these behaviors are regulated by separable genetic and neuronal populations. How these neuronal populations communicate with each other as well as the outline of the overall neural circuits within which these neurons function is poorly understood. Similarly, we do not yet have a reasonably complete description of sex hormone-regulated genetic networks that control distinct social behaviors. How sex hormone-regulated genetic networks interact with other molecular pathways that regulate dimorphic behaviors is also poorly understood [17]. We anticipate that future comparative studies of these sex hormone-regulated genetic networks will yield novel insights into evolutionary processes that have led to changes in the repertoire of social behaviors in different species.

Conflict of interest statement

Nothing declared.

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The authors identify long-sought hypothalamic neurons that regulate male aggression and female sexual receptivity. The authors develop a novel caspase-based genetic tool to conditionally ablate targeted neurons to show that PR-expressing VMHvl neurons regulate these distinct behaviors in the two sexes. In addition, the authors show that these neurons are also required for the wildtype display of male sexual behavior. The authors show that these neurons are sexually dimorphic for gene expression and long-distance projections, thereby providing a potential mechanism underlying their sexually dimorphic behavioral functions.

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The authors use optogenetic manipulation of Esr1-expressing neurons in the VMHvl (that overlap with PR-expressing neurons in this region) to show that activity of these neurons is necessary and sufficient for male aggression. Lower intensity stimulation elicits male mounting toward mice of either sex whereas high intensity stimulation elicits aggression toward males and females.

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The authors find that male aggression induces Fos preferentially in GABAergic MeA neurons and that activity of these neurons is necessary and sufficient for male aggression. As in the VMHvl, low intensity stimulation promotes male mounting whereas high intensity stimulation drives aggression. Activation of these neurons inhibits the self-grooming behavior promoted by glutamatergic MeA neurons, and in turn activation of the latter neuronal subset promotes self-grooming and inhibits social behaviors.

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55. •• Unger EK, Burke KJ, Yang CF, Bender KJ, Fuller PM, Shah NM: **Medial amygdalar aromatase neurons regulate aggression in both sexes**. *Cell Rep* 2015, **10**:453-462.
Aromatase is required for diverse male-typical social behaviors, but it is unclear whether different aromatase-expressing neuronal subsets control one or all of these behavioral programs. Moreover, the function of these neurons in the female brain is unknown since there is essentially no detectable level of circulating testosterone in females that can be aromatized into estrogens in the brain. The authors find that aromatase-expressing MeA neurons are required for wildtype displays of male territorial aggression as well as maternal aggression. These neurons represent a subset of GABAergic MeA neurons and they are not required for sexual behavior, thereby suggesting that the dual control of male mating and aggression by GABAergic MeA neurons results from molecular and functional heterogeneity within this population.

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Lesions of the lateral septum elicit rage response (septal rage). The authors find that inhibition of lateral septal neurons promotes aggression whereas stimulation of GABAergic lateral septal neurons, in particular their projections to and near the VMHvl, inhibits male aggression. This study provides an important potential afferent input source to PR and Esr1 expressing VMHvl neurons that are necessary and sufficient for male aggression.

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Classic studies have implicated POA neurons in maternal behavior and shown that a male mouse switches from pup-directed aggression to parental care during a time window after mating when his sexual partner is likely to be nursing their litter. The authors identify Fos induction in galanin-expressing POA neurons during parental behavior and show that these neurons are required for nursing in females and parental males and further that virgin females lacking these neurons switch from nursing to pup-directed aggression. Activation of these neurons in suppresses pup-directed aggression and elicits components of nursing behavior.

59. •• Scott N, Prigge M, Yizhar O, Kimchi T: **A sexually dimorphic hypothalamic circuit controls maternal care and oxytocin secretion**. *Nature* 2015, **525**:519-522.
The authors show that TH-expressing neurons in the AVPV are critical for maternal behaviors in females and suppress aggression in males. The authors perform elegant optogenetic-mediated circuit mapping to show a functional connection from these AVPV neurons to oxytocin-expressing neurons in the paraventricular hypothalamus. Furthermore, excitation of AVPV neurons elicits an increase in circulating oxytocin. Thus these studies link AVPV neurons required for maternal behaviors with oxytocin, a neuropeptide hormone long known to be important for this behavior.