Published in final edited form as:

Curr Opin Physiol. 2018 December ; 6: 89–95. doi:10.1016/j.cophys.2018.08.003.

Neural control of sexually dimorphic social behaviors

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Abstract

Sexually reproducing animals display sex differences in behavior. Although many of these sex differences in behavior are acquired with experience, sexually dimorphic behaviors such as mating and aggression are innate in the sense that they can be displayed without prior training or experience. In this review, we present recent advances in our understanding of the neural control of such innate sexually dimorphic social behaviors, with a focus on sexual behavior and aggression in flies and mice. We provide a brief overview of fundamental processes that regulate sexual differentiation in these animals to provide a framework within which more recent advances can be understood. We discuss advances in sensory, neuromodulatory, neural circuit, and experiential regulation of sexually dimorphic social behaviors.

Keywords

neural circuits; sexually dimorphic behaviors; sexual; differentiation

Introduction

Sex differences in social behaviors such as mating, aggression, and parental care are developmentally hard-wired such that they can be displayed without prior training or experience. Such genetic programming presumably ensures that adult animals can display appropriate behaviors at the right time and circumstance to maximize reproductive success. Accordingly, these social interactions can be characterized readily in the laboratory using ethologically meaningful stimuli in otherwise naïve animals. Studying sexually dimorphic social behaviors therefore affords the opportunity to understand naturally occurring behavior using modern genetic tools to access underlying neural circuits.

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AUTHOR DECLARATION TEMPLATE

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

In fruit flies (*Drosophila melanogaster*), sex determination proceeds cell autonomously in all tissues via a series of alternative splice events in key transcription factors [1]. This leads to expression of sex-specific splice forms of the transcription factors encoded by *fruitless* (*fru*) and *doublesex* (*dsx*). As described in the accompanying review by Asahina, their expression leads to sexual differentiation of different classes of neurons in the fly nervous system.

In mice, as in humans and many other mammals, sex of the gonads is determined by *Sry*, a gene located on the Y chromosome, such that its expression is necessary and sufficient for masculinization of the midgestation bipotential gonad [2]. In turn, the testes produce testosterone, which acts via its cognate receptor, the androgen receptor (AR), to masculinize the brain and the rest of the body. In the absence of *Sry*, the gonads differentiate into ovaries, which produce estrogens (17 β -estradiol being the most potent estrogen *in vivo*) and progesterone that enable maturation of the female phenotype. Curiously, in mice and many other vertebrates, testosterone can act as a prohormone whose products dihydrotestosterone (DHT) and 17 β -estradiol also function to masculinize the body and the brain. Indeed, DHT is critical for masculinization of the human external genitalia. The conversion of testosterone into 17 β -estradiol catalyzed by the enzyme aromatase seems especially critical in masculinizing the brain, with a large body of pharmacological and genetic studies documenting this role of estrogens in rodents [2,3]. Sex hormones act in a two-step manner to regulate sexual differentiation: during a critical developmental window (organizational phase), they irreversibly differentiate the neural substrate for behavior in the two sexes, and in adults (activational phase), they acutely and reversibly enable physiological functioning of this developmentally patterned neural substrate to drive sexually dimorphic social behaviors. The ovaries are quiescent perinatally in mice whereas neonatal testes produce testosterone which is aromatized in the brain; in this manner, the perinatal male brain is differentially exposed to 17 β -estradiol that masculinizes the brain [2–4]. Both estrogen and testosterone serve activational functions in males whereas estrogens and progesterone serve this function in females. Estrogens and progesterone act via cognate nuclear receptors (Esr 1, 2 or ER α , β ; Pgr or PR) to regulate sexual differentiation and function. The use of conditional alleles and specific Cre drivers has provided new insights into the distinct functions of testosterone and estrogen signaling in male social behaviors. Estrogen signaling is essential to masculinize the developing bipotential brain whereas testosterone signaling serves to amplify the intensity or frequency of adult male sexual and territorial displays. Furthermore, estrogen appears to signal at least in part by means of ER α in GABAergic neurons to regulate male mating and urine marking, a component of male territorial aggression [5]. Presumably, estrogen signals redundantly via its two cognate nuclear receptors to masculinize the neural substrate for other aspects of mating and territorial aggression. The role of other receptors for these sex hormones in sexually dimorphic social behaviors remain poorly understood [2]. Nuclear receptors for sex hormones are thought to not only regulate gene expression but they may also participate in rapid non-transcriptional responses to hormones, although the role of such rapid signaling in mammalian social behaviors remains to be explored thoroughly [2].

In both flies and mice, these distinct sexual differentiation mechanisms converge on common cellular processes such as apoptosis, neuronal morphological differentiation, and axon pathfinding to regulate the formation of sexually dimorphic neural circuits. Many such

neural pathways have been identified in both flies and mice, and these have provided a useful entry point to understand the neural control of sexually dimorphic social behaviors. Recent reviews, including by Asahina in this volume and others [6], provide excellent overviews on the neural control of courtship behavior in flies and parental behavior in mice; accordingly, we have restricted our review to mating and aggression in mice and, as needed, to courtship in flies to illustrate points relevant to our discussion.

Sensory control of sexually dimorphic social behaviors

In both flies and mice, auditory, visual, and chemosensory cues govern the initiation and interpretation of sexually dimorphic displays. During courtship, *D. melanogaster* males vibrate a single wing to produce a species-specific song comprising at least three unique “syllables”: a low frequency hum called “sine” song, and two types of high frequency “pulses” [7]. Recent studies have revealed how dynamic acoustic and visual information relayed between mating pairs shapes courtship decisions in both sexes. Male flies change the amplitude of their pulse song depending on visual estimates of their distance from the female [8]. On the other hand, female flies appear to be specially tuned to male song signals. Clemens and colleagues showed that female flies modulate their walking velocity in response to long-timescale features of male song [9]. Recording from auditory neurons in females has enabled the generation of computational models that predict female behavioral responses to natural courtship song [9]. Courtship behaviors in flies are also gated by chemosensory cues that are sensed by ionotropic gustatory and olfactory receptors expressed in sensory neurons in various appendages and mouth parts. Recent advances in chemosensory modulation of fly courtship have been discussed in this issue by Asahina, and we restrict our review of this literature to highlight a recent finding in flies [10] that echoes the sex hormone-regulated sexual maturation process in mice discussed in our Introduction. Older male flies are more successful at mating than newly eclosed males and win over younger males in competition assays. Remarkably, this maturation is controlled by juvenile hormone that acts on olfactory sensory neurons expressing the chemoreceptor Or47b. Juvenile hormone increases the sensitivity of Or47b to palmitoleic acid, a cuticular pheromone that promotes courtship, thereby enabling more successful courtship in older males. This process appears analogous to the activational action of sex hormones in mice and raises the intriguing possibility that other aspects of courtship behavior, including development of the underlying circuit (analogous to the organizational action of sex hormones), may also be regulated by hormonal signaling in flies.

Although the overall displays of sexually dimorphic social behaviors are often conserved at a coarse level, many features of these behaviors can differ even between closely related species. Ding and colleagues have recently shown that qualitative differences in the courtship songs of two species, *D. melanogaster* and *D. yakuba*, are not due to changes in central song-command neurons (P1 neurons), but instead may be caused by changes in circuitry downstream of descending neurons that drive song [11]. In a separate study, Ding and colleagues found that strain- and species-specific differences in sine song characteristics have arisen from mutations at the locus encoding the slowpoke ion channel, although the neural site of action of this widely expressed gene is presently unknown [12]. Differences in behavior can also be caused by changes in peripheral, cognitive mechanisms. Ahmed and

colleagues [13] show that the chemoreceptor genes Gr32a and Gr33a which are known to inhibit mating of atypical targets in *D. melanogaster* males [14–16] do not do so in *D. simulans* males. By contrast, they find that a member of the pickpocket ion channel family, Ppk25, that is known to promote courtship in *D. melanogaster* males [17] also performs a similar function in *D. simulans* males. Together, these studies highlight conserved and divergent genetic and neural mechanisms underlying the evolution of sexually dimorphic courtship behaviors.

In mice, pheromones are detected by sensory neurons in the vomeronasal organ (VNO) and the main olfactory epithelium (MOE), two major chemosensing epithelia found in distinct locations in the nose [18]. A major research direction in the field has been to match chemically identified pheromones with specific receptors expressed in the VNO or MOE and to subsequently delineate the central neural circuits that regulate the behavioral or other effects of such pheromones. Elegant work by the Touhara group [19] has shown that the male lacrimal gland secretes the pheromone Esp1 (Exocrine Gland-Secreting Peptide 1) in tears that is recognized by V2Rp5, a G-protein coupled receptor expressed in VNO sensory neurons. Esp1 promotes sexual behavior in females in a V2Rp5-dependent manner, although in many instances this enhancement of sexual behavior follows pre-exposure of females to Esp1. In follow up work [20], mapping neural activity induced by Esp1 and circuit manipulation studies have delineated a neural pathway emanating from V2Rp5 neurons that includes the medial amygdala posteroventral region (MeApv), the ventromedial hypothalamus dorsal region (VMHd), and the dorsal periaqueductal gray (PAGd) that enhances female sexual behavior. The PAG has previously been implicated in female sexual receptivity [21], but the identification of MeApv and VMHd, regions traditionally thought to regulate defensive behaviors [22–24], is surprising. Establishing the specific molecular identity of neurons in MeApv and VMHd that promote female sexual behavior, and understanding how this pathway intersects with the pathway that includes the ventrolateral region of the VMH (VMHvl) and is essential for female sexual behavior [2,25], will be an important future direction in this field.

In addition to promoting female sexual behavior, Esp1 also appears to signal via V2Rp5 to induce females to abort pregnancy when they encounter males that secrete Esp1 at levels different than their mates [26]. This pregnancy block, also referred to as the Bruce effect, elicited by Esp1 relies on the female memorizing her mate's signature level of Esp1 in tears via poorly understood mechanisms. These authors provide evidence to suggest that the Bruce effect is also induced by other male pheromones, consistent with previous work [27]. Finally, Esp1 also appears to promote inter-male aggression in a V2Rp5-dependent manner [28]. How Esp1 regulates these distinct behaviors between sexes and even in females is poorly understood; similarly, how Esp1 functions in more naturalistic settings in which females encounter a complex mix of pheromones represents an interesting avenue for future studies. How different circuits are engaged by Esp1 to promote one or the other behavior at the expense of others is likely contingent on context-dependent modulation of neural responsivity in females as well as sex differences in neural circuits, as shown for the male pheromone CVA in flies. This pheromone promotes sexual receptivity in females Whereas it promotes aggression and inhibits mating in males (reviewed in [29]). These divergent responses are governed, at least in part, by sex differences in central wiring of the

chemosensory pathway that detects CVA [30,31]. Contextual modulation of sexually dimorphic social behaviors by pheromones is consistent with experiments that show gating of behavioral responses to pheromones by the estrous cycle [32]. Similarly, although past work has indicated that male pheromones elicit aggression by other males, more recent work shows that male-derived pheromones also inhibit aggression from males that intrude on a resident male's territory (see below; [33]).

Control of sexually dimorphic social behaviors by central circuits

Recent studies have delineated the circuitry underlying male-specific courtship behaviors in the fly [34,35]. These studies have revealed that olfactory and gustatory neurons are connected to the P1 cluster of neurons, master controllers of male courtship drive, via distinct neural components that actuate male-typical courtship behaviors. These sensory neurons respond to male- and female-specific pheromonal cues and either inhibit or excite P1 neurons, respectively. Information from courtship-promoting pheromonal cues is carried by PPN1 neurons in the Ventral Nerve Cord and activates P1 to promote courtship, while inhibitory cues are sensed by a parallel pathway to inhibit P1 neurons through GABAergic mAL neurons. These studies highlight how distinct sensory pathways converge onto sexually dimorphic central neurons that globally control male-specific courtship drive.

Traditional studies in rodents over several decades have identified many brain regions that regulate sexually dimorphic social behaviors, including the medial amygdala (MeA), preoptic hypothalamus (POA), bed nucleus of the stria terminalis (BNST), and ventromedial hypothalamus (VMH) [2]. More targeted manipulations of specific neuronal subsets in these brain regions have revealed a great deal of specificity in control of complex social behaviors. Some of the first molecular genetic studies identified PR/ER α -expressing neurons in the ventrolateral region of the ventromedial hypothalamus (VMHvl) that regulate mating in both sexes and territorial aggression in males [25,36]. ER α signaling in PR/ER α VMHvl neurons also appears essential for these behaviors [37,38]. These studies foreshadowed the explosion of reports characterizing the role of other molecularly defined neuronal pools in regulating different sexually dimorphic social behaviors. Subsequent studies identified galanin-expressing neurons in the preoptic hypothalamus (POA) [39] and dopaminergic neurons in the anteroventral periventricular nucleus (AVPV) [40] that regulate parental behavior and, more subtly, other social and non-social displays. These studies have been reviewed recently [6,41] and are not discussed in detail here.

Follow up studies with ER α -expressing neurons in the VMHvl indicate that these neurons also regulate maternal aggression, demonstrating an important role for physiological context in modulating this behavior [42]. In addition, this study by Lin and colleagues also showed a profound role of effect of genetic background in aggression in virgin females, with SW but not C57Bl6/J females attacking juvenile mice ("bully" test for aggression; [43]); correspondingly, optogenetic stimulation could elicit aggression in SW but not C57Bl6/J virgin females. These data point to important genetic modifiers of aggression in sexually naïve females. This study also identified additional heterogeneity within the VMHvl consistent with previous gene expression profiling studies [44]. In keeping with such molecular heterogeneity, work by Wang and colleagues showed that the immediate-early

gene Fos is induced in a subset of ER α + VMHvl neurons in males that are attacked [45]; whether this indicates ongoing neural activity during the attack episodes or rather reflects later events remains to be determined. A recent study has suggested that VMH neurons may also regulate aggression-seeking behavior by male mice [46], but whether these neurons also express PR/ER α and are identical to those that are necessary and sufficient for male aggression is unknown. Together, studies of this important brain region have revealed new insights into the regulation of distinct sexually dimorphic social behaviors. They have also highlighted the need to explore physiological and social context-related variables to more precisely define the behavioral contribution of this small neuronal population. Echoing an emerging theme in other brain regions, these studies raise important questions about what constitutes a neuronal type even within this circumscribed population of ~2,000 neurons (per hemisphere). Studies of PR/ER α -expressing VMHvl neurons are poised to test the relation between molecularly defined subtypes of neurons and the behaviors encoded by the entire population. There may be a relatively simple correspondence between molecular subtypes and different behavioral functions or there may be more complex interactions between these neuronal subsets as discussed elsewhere [2]. Studies of GABAergic neurons in the MeA are instructive here [47,48]. These neurons were shown to regulate both male mating and aggression whereas aromatase+ neurons, which constitute a subset of the GABAergic pool in the posterodorsal MeA, regulate aggression. These findings suggest a correspondence between molecular subtypes and behavior, although additional studies that exclude other mechanisms of behavioral control are needed.

Studies of hypothalamic control of parental behaviors raise similar issues on a larger scale. There is strong evidence showing that ER α as well as galanin+ POA neurons and dopaminergic AVPV neurons all contribute to parental displays [39,40,49,50]. These neurons constitute at best partially overlapping populations, raising the question as to their relative roles in parental care; functional studies to understand the nature of interactions between these neuronal types will be critical to understand how this complex social behavior is regulated. In addition to parental care, these three neuronal populations also regulate social behaviors such as male mating, territorial aggression, and infanticide, as well as locomotion. These distinct behaviors could be regulated by distinct subtypes within these three populations, by their projections to distinct regions as suggested by studies of galanin+ POA neurons in parental care [49], or by more complex circuit interactions. Together, these studies offer a glimpse into the near future, with many more molecularly-specified neuronal populations that regulate one or more social behaviors in a modular manner likely to be identified at a rapid pace. How these different neuronal populations assemble to generate sexually dimorphic social behaviors with species-specific motor patterns and ongoing sensory input remains an outstanding problem in this field. Here too, studies of PR/ER α VMHvl neurons in male aggression may shed light on the neural circuits underlying complex social interactions. Elegant work by Lin and colleagues revisited the role of the lateral septum in inhibiting aggression to show that GABAergic neurons in this region send projections to the VMH and can inhibit ongoing male aggression without modulating male sexual behavior [51]. Further work is needed to test whether these septal neurons are presynaptic to PR/ER α VMHvl neurons and to exclude the role of other hypothalamic projections of septal neurons in regulating aggression. We expect that similar studies will

ultimately reveal postsynaptic targets of PR/ER α VMHvl neurons that regulate different social behaviors and may also shed light on the role of sexually dimorphic projections emanating from these neurons [25].

Influence of neuromodulation and past experience in sexually dimorphic social behaviors

Signaling by neuropeptides and other small molecules such as biogenic amines at traditional fast chemical synapses can have profound modulatory effects that reconfigure neuronal populations within a network [52,53]. In addition, such neuromodulators can diffuse over larger distances or act extra-synaptically such that anatomical tracing studies may not provide an adequate description of the circuit underlying a particular behavior. Oxytocin acts as a hormone as well as a modulatory neuropeptide [54], and previous work had implicated oxytocin signaling in the MeA in regulating recognition of females by male mice [55]. A recent study [56], employing a slightly different behavioral paradigm, has gone further, providing evidence that oxytocin acts on aromatase+ neurons to regulate the preference of male mice for female pheromones. Findings from circuit manipulation studies further indicate that the source of this oxytocin is the paraventricular nucleus of the hypothalamus. In a twist on such neuropeptide modulation of sexual preference, a previous study showed that progesterone acting via a non-canonical membrane receptor gates female sexual behavior by modulating responsivity of VNO sensory neurons [32].

A creative study addresses the mechanisms whereby sexual behavior might recruit reward pathways in the brain [57]. Kimchi and colleagues observed a large increase in dopamine in the nucleus accumbens in male mice interacting with females. Strikingly, this transient increase in dopamine was absent in males engineered to be mutant for Trpc2, an ion channel critical for signal transduction in response to pheromonal cues in the VNO [18]. Trpc2 mutant males display no sexual preference, mating equivalently with males and females, but preference appears to be restored by optogenetic stimulation of dopaminergic termini in the nucleus accumbens concomitant with exposure to female cues. These data suggest the intriguing possibility that even sexual preference may be influenced by reward pathways in wildtype mice. Alternatively, the pairing of dopaminergic release with female pheromones may reflect an artifact of pairing a rewarding stimulation with what is an otherwise neutral cue to these mutants. Nevertheless, this is an exciting finding that provides an entry-point into studying how reward pathways may intersect with circuits that mediate innate social behaviors. Findings in flies [58] complement these results: activation of male-specific neurons expressing the neuropeptide corazonin, which promotes ejaculation, was shown to be rewarding to males and mimics naturally evoked post-copulatory responses.

Work in mice provides dramatic examples of the role of social context or experience in modulating the display of mating and aggression. The groups of Anderson and Dulac used miniscopes to image calcium signals in PR/ER α + VMHvl neurons and largely GABAergic MeA neurons from male mice, respectively [59,60]. These studies yielded a rich dataset of neuronal activity in relation to different components of male social behaviors. The most striking conclusions however relate to findings showing that sex-specific neuronal activity in

sexually naïve males only emerges subsequent to social interactions with female mice. It is difficult presently to exclude the possibility that there are molecularly defined subtypes of neurons in these regions whose activity would provide sex recognitive signals even in naïve males. Such specificity would suggest developmental hard-wiring of sex recognition, and it would be in line with the notion that sexually naïve males can display typical routines of mating and aggression toward females and males, respectively. Regardless, how social experience shapes sex recognition in these important nodes in the circuits underlying social interactions remains to be determined.

A recent study highlighted the importance of considering social context and past experience when testing the function of a neuronal population in social behavior [33]. Studies over several decades have indicated that the caudal hypothalamus contains an attack center later localized to PR/ER α VMHvl neurons ([25,36]; reviewed in [2]), that upon stimulation elicits unfettered aggression. Yang et al [33] provided further evidence in support of the notion that activation of these neurons elicits aggression: abrogation of pheromone sensing or castration still elicited aggression in males. In fact, these males even threatened a mirror image. Nevertheless, stimulation of these neurons in group housed males failed to elicit aggression when such males were inserted into the cage of a resident male. This inhibition of aggression is mediated by sensing pheromones from the resident male such that group housed males genetically disabled from sensing pheromones attacked a resident male following stimulation of these VMHvl neurons. These studies provide some of the first evidence for pheromones that inhibit male aggression.

Conclusion

As discussed above, we expect that the discovery of additional centers that regulate sexually dimorphic social behaviors in a modular manner will continue unabated. In addition, advances in our understanding of how contextual cues provided by experience, dominance hierarchies [61,62], and even circadian rhythm [63] modulate sexually dimorphic social behaviors will provide richer insights into how animals behave under more ethologically relevant settings. We are excited by recent advances in whole brain imaging and manipulation technologies [45,64–67] that will facilitate a complete delineation of neuronal populations that may participate in different social behaviors. Together, these complementary approaches will provide new insights into the neural control of these primal behaviors.

Acknowledgments

We thank members of the Shah lab for comments on the manuscript. This work was supported by grants to G.L.M. (postdoctoral fellowship from American Association of Anatomists) and N.M.S. (NIH R01NS049488, R01NS083872, R01MH108319).

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Highlights

- Molecularly defined neuronal populations underlying different sexually dimorphic social behaviors have been identified
- These populations control social behaviors in a modular manner
- Neural circuits within which these neurons function are beginning to be delineated
- Newer technologies will facilitate brain-wide mapping of these cells and circuits