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Sex differences in *Drosophila* behavior: Qualitative and Quantitative Dimorphism

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Abstract

The importance of sex as a biological variable is being recognized by more and more researchers, including those using *Drosophila melanogaster* as a model organism. Differences between the two sexes are not confined to well-known reproductive behaviors, but include other behaviors and physiological characteristics that are considered “common” to both sexes. It is possible to categorize sexual dimorphisms into “qualitative” and “quantitative” differences, and this review focuses on recent advances in elucidating genetic and neurophysiological basis of both qualitative and quantitative sex differences in *Drosophila* behavior. While sex-specific behaviors are often mediated by sexually dimorphic neural circuits, quantitative sexual dimorphism is caused by sex-specific modulation of a common neuronal substrate.

1. Introduction

The significance of sex as a biological variable in animal physiology and behavior (including humans) has been underappreciated at times (for recent reviews, see [1–3]). Recently, however, both basic and clinical researchers have widely recognized that the genetic, neuronal, and physiological mechanisms underlying sexual dimorphisms are important topics of research [4–6]. The common fruit fly *Drosophila melanogaster* offers an intriguing platform to study sex differences from genetic, neurobiological, and behavioral perspectives. Unlike in most vertebrates, sex determination in *Drosophila* does not involve hormones. Instead, it is mediated exclusively by a genetic cascade originating from the composition of the sex chromosomes [7]. Stereotypical and robust sex-specific differences in behavior provide both qualitative and quantitative readouts of the effects that genes and neurons exert on sexually dimorphic behavior. This review focuses on recent findings on the genetic and neurophysiological mechanisms underlying sex differences in *Drosophila* behavior. While most researchers use one of the two sexes in experiments, I attempt to focus on comparative studies that involve both sexes wherever possible.

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2. Qualitative and quantitative sexual dimorphism in *Drosophila*

Many behavioral traits show sex differences. While such differences often result in a continuous spectrum of behavior, a dichotomy of “qualitative” and “quantitative” differences can provide a useful working framework [8]. Here, I will first briefly describe qualitative sexual dimorphism in *Drosophila* behavior (including sex-specific behaviors), followed by a description of quantitative differences.

Like many animal species [8], the vast majority of qualitative behavioral differences between the two sexes in *Drosophila* are related to reproduction. Male courtship behavior is among the most intensely studied examples [7]. In response, females either accept or reject male courtship, and upon mating, begin ovipositing [9]. Anatomical constraints restrict some of these behaviors to one sex (e.g., oviposition to females). Other behaviors, such as generation of a courtship song [10], are not associated with such an obvious anatomical constraint, but are exclusively expressed by one sex in natural conditions. Some behaviors are expressed by both sexes in a comparable context, yet the accompanying motor programs have sex-specific patterns. This is the case for some actions during aggressive interactions [11].

The neuronal basis of *Drosophila* sexual behavior has been extensively reviewed in the past few years [7, 9, 12–14]. Two sex-determining transcription factors, *doublesex* (*dsx*) and *fruitless* (*fru*), play critical roles in specifying numerical and morphological sexual dimorphism of the neurons expressing them. Between 1,700 and 3,300 *fru*-expressing neurons [15, 16] and 400 – 700 *dsx*-expressing neurons [17–19] are found in the male central nervous system, whereas ~3,000 *fru*-expressing neurons [16] and 300 – 400 *dsx*-expressing neurons [18, 19] are found in the female central nervous system. These neurons control many, if not all, aspects of sex-specific behaviors. In the first section, I will review recent findings concerning neuronal populations at the intersection of *dsx* and *fru*. While gene structure and molecular function of *dsx* and *fru* are important topics themselves, I will defer discussion of these topics to other excellent reviews [7, 20, 21].

In contrast, much less attention has been paid to quantitative sexual dimorphisms in *Drosophila* behavior. Recently, however, intriguing findings are uncovering genetic and neural bases of behaviors that shows quantitative differences between males and females. In the latter sections of this review, I will specifically focus on two such behaviors: sleep, and chemosensory-guided behavior. While both males and females exhibit these behaviors, sexually dimorphic modulatory mechanisms quantitatively alter them in context-specific manners.

3. *dsx*, *fru* and sex-specific behaviors

A common notion is that *dsx* and *fru* each define specific cell types, which can be further subdivided into smaller subclasses [18, 22, 23]. In particular, many classes of neurons that express sex-specific transcripts of *fru* are functional subunits for sexual behaviors (reviewed in [7, 9, 14]). However, one complication is that expression patterns of *fru* and *dsx* do not define mutually exclusive populations of neurons. Instead, *dsx* and *fru* create at least three

distinct “cell types” (Figure 1A): (1) neurons that express both *dsx* and *fru* (called “class A” in this review), (2) neurons that express *dsx* only (“class B”), and (3) neurons that express *fru* only (“class C”). Immunohistochemistry studies have revealed only a small number of clusters of class A neurons, which are often in close proximity to class B or class C neurons [18, 24, 25]. Class A neurons are collectively necessary for male courtship behavior [26], and include three major clusters with specific functions for sexual behaviors: (1) the “P1/pC1” cluster, which is located at the mid-posterior part of the central brain, (2) the “TN1” cluster, which is located in the thoracic ganglia, and (3) the abdominal ganglion (Abg) cluster (Table 1, Figure 1B). Increasing genetic access to specific subpopulation within each cluster (Table 2) has begun to reveal that class A, class B, and class C neurons can assume distinct functions in controlling sex-specific behaviors. While most publications only refer to neurons-of-interest as “*fru*-expressing” or “*dsx*-expressing”, it would be useful to clarify which of these three types their neurons-of-interest belong to.

3.1 “P1/pC1” cluster: a “center for sexual behaviors” with multiple functions

Within the dorsal posterior part of the fly brain is a cluster of *fru*-expressing neurons called “P1” neurons, and a cluster of *dsx*-expressing neurons called “pC1” neurons [15, 17, 27]. In the male brain, the P1 and pC1 populations of neurons overlap (i.e., some P1/pC1 neurons express both *dsx* and *fru*) [25, 28] (Table 1). In flies that lack the male-specific isoform of *dsx*, at least a subset of male P1 neurons is absent [29]. This implies that male P1 neurons are class A, although this has not been determined. In contrast, male pC1 neurons include class B neurons [30]. The class A P1/pC1 cluster is absent in females [29, 31]. However, in females, the neuronal lineage that generates the male P1/pC1 cluster gives rise to *fru*-expressing neurons that appear morphologically distinct from pC1 neurons [23, 27], suggesting that class C P1/pC1 neurons exist in females. As was discussed elsewhere [7, 9, 14, 32], this sexually dimorphic cluster plays major roles in sex-specific behaviors, both in males and females. Recent studies have uncovered a surprising degree of multifunctionality associated with this cluster.

In males, artificial activation of class A and/or class B P1/pC1 neurons consistently induces courtship behavior [33–42]. When another male is present, however, activation of a subset of presumably class A P1/pC1 neurons induces aggressive behavior [43, 44]. Activation of class B P1/pC1 neurons also induces aggression toward males without courtship behavior [30]. Curiously, Koganezawa et al. reported that presumably class A P1/pC1 neurons induce very little aggressive behavior [30]. One possible explanation for this discrepancy is that the GAL4 drivers used by the different groups label functionally different subclasses of class A P1/pC1 neurons. In fact, male class A P1/pC1 neurons can be morphologically classified into at least 10 subclasses [45].

In females, class B P1/pC1 neurons are involved in controlling receptivity to male courtship [31, 39]. Surprisingly, strong activation of these neurons in females induces male courtship-like behavior [39]. Moreover, activation of class B P1/pC1 neurons via a different GAL4 driver induces female aggressive behavior [30]. There are only ~10 class B P1/pC1 neurons in females (Table 1), and it is currently unclear whether these different types of behavioral effects are caused by specific subpopulations of these neurons. Lastly, chromosomally

female flies (e.g. those carrying two copies of the X chromosome) that express a male-specific isoform of *fru* perform at least some form of male courtship behaviors [28, 46], even though they apparently lack class A P1/pC1 neurons [29]. It remains unclear which neurons within the central brain mediate courtship behavior in this genotype.

Although the “P1” and “pC1” nomenclatures have been used to describe neurons labeled by a variety of genetic reagents (Table 2), comparison of cell numbers (Table 1) suggest that each reagent likely labels only a subset of P1/pC1 neurons. As stated above, these subsets may define functionally distinct subpopulations that may play specific roles in sexual behaviors, or may be involved in processing specific sensory modalities. Such findings may clarify whether the P1/pC1 cluster controls multiple types of behaviors through a population coding mechanism, or through cell-type specific division of functions within this population [32]. In either case, comparison of data using different genetic reagents requires caution, even if the same terminology (such as “P1” or “pC1”) is used to refer to the neurons of interest. This point also applies to other neuronal populations below.

3.2 “TN1” neurons: generating the male-specific courtship song

Male flies generate courtship song by vibrating a wing. The courtship song consists of two sound components: pulse song and sine song [7, 10]. Stimulation of *dsx*- and *fru*-expressing neurons drives the generation of courtship song even in a headless fly [47, 48]. This suggests that the neural components necessary to generate courtship song, namely motoneurons that control wing motions [49, 50] and a so-called “pattern generator” [47], reside in the ventral nerve chord.

A male-specific cluster of *dsx*-expressing neurons located between the pro- and mesothoracic ganglia is named TN1 [17, 18, 25, 28]. Many *fru*-expressing neurons are also found in this region [15, 16, 22, 23, 51], and *fru* expression partially overlaps with *dsx* [25, 28]. This suggests that class A, class B, and class C neurons all exist in this area. Interestingly, class C neurons include motoneurons that innervate muscles involved in controlling the wings [19, 22, 28, 50]. Although the name “TN1” was originally given to *dsx*-expressing (class A or B) neurons, I will extend discussion to the class C neurons near the TN1 cluster in the following section.

A subset of class A TN1 neurons, named vPR6 [22], is primarily involved in producing pulse song [34, 52], whereas a morphologically distinct subset, called TN1A, primarily controls production of sine song [52]. Although the expression of *fru* in TN1A has not been directly determined, wiring of these neurons to the downstream motoneuron, hg1 (which controls sine song [50]), requires *dsx* [25, 52]. Thus, as with the P1/pC1 cluster, different subpopulations of the TN1 cluster are involved in specific aspects of a sexual behavior. Since the wing-control muscle ps1 primarily controls pulse song [50], vPR6 may synapse onto another *fru*-expressing motoneuron that innervates ps1 [50]. Functions of other types of TN1 neurons [28, 52] are currently unknown, although *fru*-expressing (class A or C) interneurons, called vMS11 (whose cell bodies reside near the TN1 cluster), influence pulse song production [34].

How females [39, 47] and chromosomally female individuals that express the male-specific isoform of *fru* [28, 46, 47] are capable of producing courtship song remains an open question. Because these individuals lack TN1 (Table 1), their production of male courtship song must depend on as yet uncharacterized neurons.

3.3 Abdominal ganglion (Abg) neurons: controlling copulation in both sexes

The Abg contains anatomically and functionally heterogeneous neuronal populations that are involved in coordinating copulation-related behaviors in both males and females. Both *dsx* and *fru* are expressed in numerous Abg neurons in both sexes [15–18, 22–24, 28, 51]. Although relatively poorly characterized, all class A, class B, and class C neurons seem to be present in Abg, at least in males [18, 24, 28].

In males, ~80 glutamatergic, *dsx*-expressing motoneurons serve to maintain copulation, whereas ~150 GABAergic, *dsx*-expressing neurons locally inhibit glutamatergic neurons, thereby promoting the termination of copulation [19]. Among them, ~12 GABAergic class B neurons specifically control the duration of copulation [53]. Four *fru*-expressing (class A or C), corazonin (*crz*)-expressing neurons promote ejaculation [54]. These *crz*⁺ neurons (also cholinergic) are likely upstream of a subset of ~20 male-specific, *fru*-expressing serotonergic neurons (called SAbg) that innervate male internal reproductive organs [24, 54]. Approximately 8 *dsx*-expressing, serotonergic neurons (class A or B) in this region are also necessary for successful copulation [55]. Whether these neurons overlap with the *fru*-expressing SAbg neurons downstream of the above-mentioned *crz*-expressing neurons is unclear.

Functionally diverse Abg neurons are also present in females. First, female-specific class C ascending neurons, called SAG neurons, receive input from sex peptide sensory neurons (SPSNs) located in the uterus [56]. SPSNs receive sex peptide (SP) from a male during copulation [57–59], and induce post-mating behavioral changes, including rejection of male courtship. It has been proposed that binding of SP by SPSNs reduces neuronal transmission from these neurons to the upstream SAG neurons, and that this reduced SAG neuronal activity causes post-mating behavioral changes [56]. Signaling from SPSNs to SAGs is gated by other female-specific, *dsx*-expressing (class A or B), local interneurons in Abg that co-express myoinhibitory peptide (MIP) [60]. *dsx*-expressing ascending neurons in Abg may include other types of neurons involved in female receptivity [61], but the functions of these neurons are currently uncharacterized. Lastly, *fru*-expressing (class A or C) Abg local interneurons labeled by *Abd-B* are necessary for female copulation [62], whereas *dsx*-expressing, octopaminergic neurons promote post-mating behavior [63]. Relationships between these neurons in a copulation-controlling circuit remains to be resolved. Further characterization of Abg neurons in both males and females will be necessary to integrate the above findings, and to assign precise functions to Abg neurons.

4. Regulation of sleep: sex-invariant behavior regulated via sex-specific mechanisms

Recent studies have begun to illuminate sex-specific modulatory mechanisms that alter the amount of sleep in both males and females (Figure 2). Well-characterized genetic and neuronal sleep mechanisms make *Drosophila* sleep a powerful model system for understanding the origins of this quantitative sexual dimorphism in behavior.

While virgin and mated males sleep similar amounts, mating reduces daytime sleep in females [64–66], likely to meet demands of feeding and oviposition [66]. This may account for the longstanding observation that females are more active than males during the day [67]. Like receptivity changes described above [56], this post-mating reduction in sleep is largely mediated by the SPSN-SAG axis [64–66]. How signal from SAGs modulate the core sleep-controlling circuit is an intriguing question. One potential link is the class B pC1 neurons in females, which promote receptivity to male courtship [31]. Activation of this cluster modestly increases sleep, partially mimicking the high levels of sleep exhibited by virgin females [68].

While males do not change the amount of sleep after mating [64], males housed with females sleep significantly less than those housed with other males [69–72], and exhibit increased courtship behavior [71, 72]. Sexually satiated males sleep more than sexually inexperienced males, even in the presence of females [72]. These data suggest that male sexual arousal and sleep drive are inversely correlated. In fact, activation of the class A P1/pC1 neurons greatly reduces male sleep [68, 71, 72], even at low levels of activation that do not induce overt courtship behavior [68]. P1/pC1 neurons make mutually excitatory connections with *fru*-expressing circadian clock neurons, the DN1s [68, 70, 73]. DN1 neurons are sexually dimorphic in terms of numbers (males have more DN1 neurons than females) [70] and daily activity patterns [74]. Also, a group of octopaminergic neurons, called MS1 neurons, promote wakefulness in males, presumably by influencing the activity of *fru*-expressing neurons [72]. Thus, DN1 and MS1 neurons are the neural substrates that modulate sleep drive by affecting male sexual arousal. In particular, interactions between P1/pC1 and DN1 neurons may account for a sexually dimorphic sleep-regulating mechanism.

5. Regulation of chemosensory behavior: ethologically relevant modulations in both sexes

Many *Drosophila* pheromone receptors have been identified [14, 75, 76]. While some pheromone compounds may be differentially represented in each sex via sexually dimorphic neuronal wiring [77–81], sex-specific modulation of chemosensory inputs (pheromones or other chemicals) has generally not been investigated. In this section, I will specifically focus on recent findings that illuminate sex-specific modulation of chemosensory systems, and the resulting quantitative sexual dimorphisms in neurophysiology and behavior (Figure 3).

Mating increases feeding quantity specifically in females [64, 82]. Mated females show elevated gustatory preference, relative to both virgin females and males, to amino acid-rich

substrates [83–86], salt [87], and polyamines [88]. Olfactory preference to polyamines is also elevated in females after mating, to a level comparable to that shown by males [88]. All these substrates are sensed by chemosensory neurons that express the ionotropic receptor, Ir76b [89], but amino acid-sensing taste neurons, salt-sensing taste neurons, and polyamine-sensing olfactory neurons are distinct from each other [85, 89, 90]. A mutation of the sex peptide receptor (SPR) abolishes these mating-dependent changes in gustatory and olfactory preference [83, 88]. Once again, post-mating increases in salt and amino acid appetites depend on the SPSN-SAG axis [82, 87]. In contrast, post-mating changes in olfactory and gustatory preference to polyamines are mediated by SPR in the sensory neurons themselves [88]. There, MIP (the ancestral SPR ligand) serves as an autocrine signal to alter the response of Ir76b-expressing neurons to polyamines in a female-specific manner [88]. Thus, the SP system modulates multiple chemosensory-guided behaviors using distinct molecular and neuronal mechanisms.

In addition, mated females oviposit preferentially on acid- and polyamine-containing sweet substrates (more so than is seen with virgin females) [88, 91–93]. Two separate mechanisms can account for the attraction to acid-containing substrates: (1) acid- and salt-sensing Ir76b-expressing tarsal neurons [93], and (2) mechanosensory stimuli from the oviduct (caused by the physical presence of eggs) [94]. Attraction to amine-rich substrates is mediated by Ir76b-expressing olfactory sensory neurons [90]. In summary, Ir76b-expressing sensory neurons detect multiple known chemical compounds that are relevant for chemosensory-guided behaviors, and their activity is modulated in a female-specific, mating-dependent manner.

In males, age-dependent sensitization of pheromone-sensing olfactory sensory neurons elevates mating success. Seven-day old males have higher courtship success than 2-day old males, and this difference depends on the odorant receptor, Or47b [95]. Concomitantly, Or47b-expressing olfactory neurons become more sensitive to the courtship-promoting pheromone, palmitoleic acid, as male flies age [95]. This male-specific modulation is mediated by juvenile hormone, which acts directly on Or47b-expressing neurons through its receptor, methoprene-tolerant (Met) [95]. Or47b-expressing olfactory neurons project to one of a few sexually dimorphic glomeruli [16, 96]. It will be interesting to determine whether other chemosensory systems that exhibit morphological sexual dimorphism (which are often involved in detecting sex-specific compounds) [77–80] are modulated by a male-specific mechanism, and how juvenile hormone can exert such a sex-specific effect.

6. Conclusion

It appears that qualitative dimorphism in behavior is primarily controlled by *fru*- or *dsx*-expressing neurons, whereas quantitative dimorphism involves sexually dimorphic modulation of neural components that are common to both sexes. Males and females experience different types of physiological changes during life, and they must have evolved to adjust their behaviors using sexually dimorphic mechanisms.

Recent advances in our understanding of the neural circuits controlling sex-specific behaviors have been driven by a growing variety of genetic reagents (Table 2). Commonly used nomenclatures (such as “P1” or “pC1”) may not provide sufficient resolution to classify

functionally relevant neuronal groups. Therefore, a comprehensive and well-defined cell type classification system that incorporates neuronal lineages [23, 27, 97, 98], morphologies [22, 23, 45, 99], connectivity maps [100–102], and gene expression profiles [103–105] across sexes will be necessary. Implementing the class A, B, and C system proposed here could be a small step toward facilitating this process. It is also important to recognize that behavioral phenotypes may become apparent only in a specific condition, and therefore, the reported behavioral phenotype may not exclude involvement of the neurons-of-interest in other types of behavior.

Quantitative sexual dimorphisms in behavior covered in this review are often modulated by sex-specific neuronal mechanisms, such as courtship-promoting P1/pC1 neurons in males, or the SPSN-SAG axis in females. It is therefore tempting to speculate that roles of currently uncharacterized sexually dimorphic neurons may include modulation of behaviors common to both sexes in a sexually dimorphic manner. At the same time, it is important to remember that sexually dimorphic, non-neuronal physiological or genetic factors (such as maternally inherited mitochondrial genes) can contribute to the expression of behaviors [106–109]. Like in studies using vertebrates, most *Drosophila* studies use only one sex. Comparative approaches that use both sexes, common genetic reagents, and standardized behavioral paradigms will greatly facilitate our understanding of the genetic, physiological, and neural mechanisms that create sexual dimorphism in *Drosophila* behavior.

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Abbreviation

<i>fru</i>	<i>fruitless</i>
dsx	<i>doublesex</i>
SP	sex peptide
SPR	sex peptide receptor
SPSN	sex peptide sensory neuron
crz	corazonin
MIP	myoinhibitory peptide

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Highlights

- *Drosophila* exhibits qualitative and quantitative sexual dimorphisms in behavior.
- A class of sexually dimorphic neurons may contain multiple subclasses.
- Sex differences can be generated by sex-specific neuronal modulations.
- Comparative studies on physiology and behavior across sexes are informative.

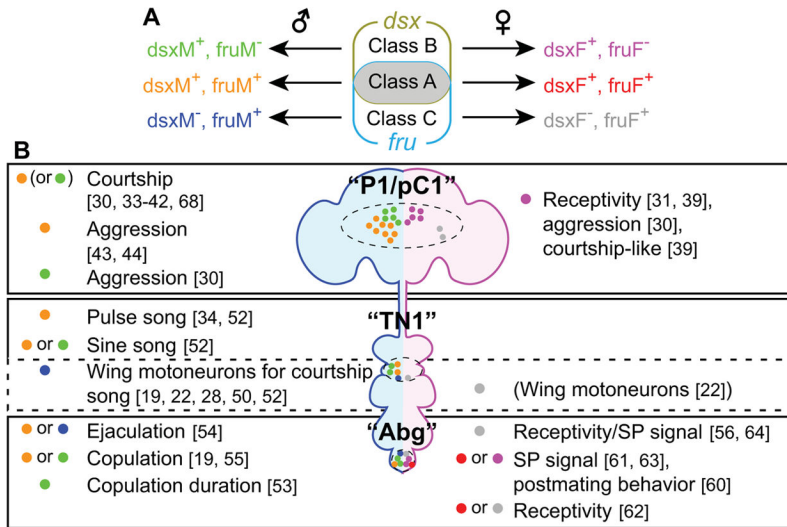


Figure 1. Cell types specified by *dsx* and *fru*

A. *dsx* and *fru* expression are partially overlapping, creating three “cell types”. Since both *fru* and *dsx* produce sex-specific transcripts (*fruM* and *fruF* for *fru*, *dsxM* and *dsxF* for *dsx*), class A, class B, and class C neurons in both sexes express a unique combination of sex-specific transcripts. **B.** Summary of behaviors influenced by the manipulation of class A, class B, or class C neurons in “P1/pC1” (*fru*-expressing (P1) neurons are also referred to as pMP-e [23] or pMP4 [22]), “TN1”, or “Abg” clusters (see text for details). Colors of dots correspond to cell types in **A**. Motoneurons near the TN1 cluster do not express *dsx* (see text for details), but are shown here in a broken line box as relevant neurons. Neurotransmitter and/or neuromodulator expression is omitted for clarity, but these details often provide further information concerning the identity of neurons.

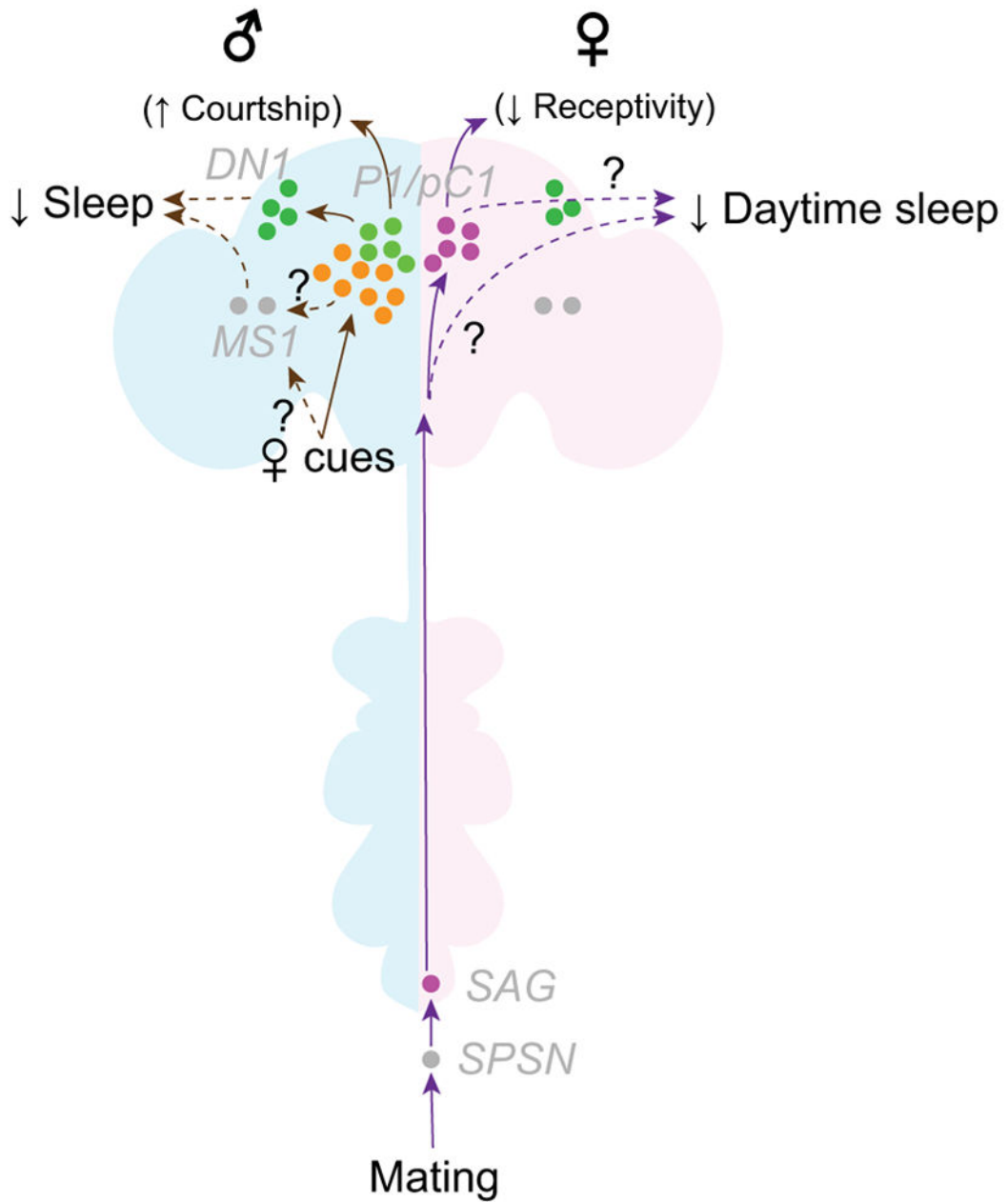


Figure 2. Sex-specific modulations of sleep

Purple arrows indicate female-specific, mating-dependent modulations of sleep, and brown arrows indicate male-specific sleep modulations in response to the presence of female cues. Gray italic letters indicate neuronal names. Solid arrows represent established neuronal connections (but do not necessarily represent monosynaptic connections); dotted arrows represent uncharacterized but potential neuronal connections. Data sources: [31, 56, 64, 65, 68, 71, 72].

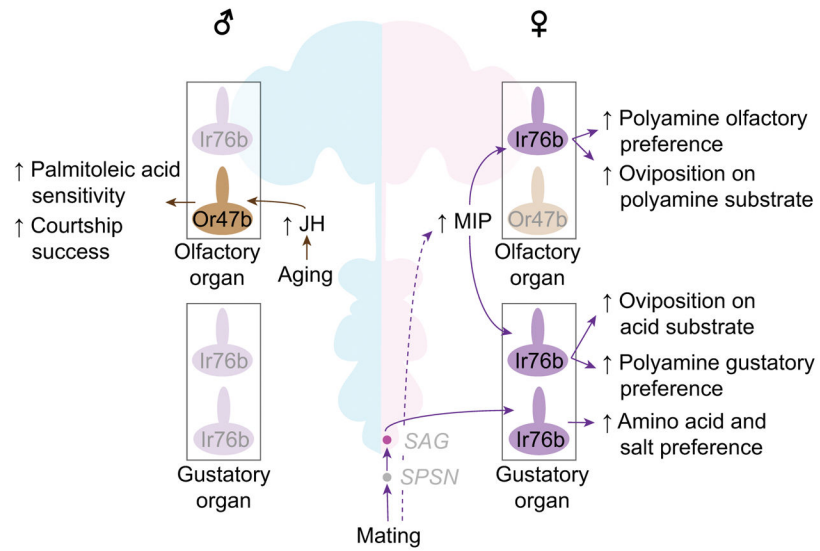


Figure 3. Sex-specific modulations of chemosensory-guided behaviors

Purple arrows indicate female-specific, mating-dependent modulations of chemosensory-guided behaviors, and brown arrows indicate male-specific, age-dependent modulations. Gray italic letters indicate neuronal names. Ir76b subclasses can be further divided by the co-expression of receptors, which are omitted in this panel for clarity. Data sources: [85, 87, 88, 95]. JH: juvenile hormone.

Table 1

Summary of cell numbers in clusters that contain class A neurons.

Sex	“p1/pC1”		“TNI”		“Abg”	
	♂	♀	♂	♀	♂	♀
Overlap of <i>dsx</i> and <i>fru</i> (class A)	18 ^{*1} [25], 34 [28]	0 ^{*2} [31]	9 [25], 17 [28]	N.D.	112 ^{*3} , ♂ [24]	N.D.
<i>dsx</i> -expressing (class A+B)	30-50 [17], 53 [19], 57 [18], 65 [27], 83 ^{*1} [25]	5 [27], 8 [19], 9 [18], 16 ^{*1} [25]	15 [25], 18-24 [17], 22 [18], 23 [19]	0 [25], 0 [18], 0 [19]	200-300 ^{*6} [17], 231 ^{*3} , ♂ [24], 254 ^{*6} [18], 275 ^{*6} [19]	300 ^{*6} [18], 315 ^{*6} [19]
<i>fru</i> -expressing (class A+C)	21 [29], 38 [23], 48 [27], 73 [15], 97 , 177 ^{*4} [16]	7 [27], 9 [23], 163 ^{*4} [16]	83 [15], 105 , 136 [28], 235 , 441 ^{*5} [16]	112 [28], 395 ^{*5} [16]	91 [15], 112 ^{*3} , ♂ [24], 244 , 504 ^{*6} [16]	516 ^{*6} [16]

Cell numbers (bold letters) are based on either immunohistochemistry or genetic reagents targeting *fru*- or *dsx*-expressing neurons (such as knock-in alleles for either gene). Numbers are rounded to the closest integer. Unless otherwise noted, cell numbers are per hemibrain and counted at the adult stage. N.D., no data.

^{*1} Cells include nearby “pC2” cluster.

^{*2} Inferred by the absence of overlap between *fru* and *dsx* gene products.

^{*3} Cells counted at two-day old pupal stage.

^{*4} Cells include other neurons positioned posteriorly.

^{*5} Cells include other neurons positions in pro- and meso-thoracic ganglia.

^{*6} Cell numbers represent the total number from both hemibrains.

Table 2

List of genetic reagents used to characterize functions of P1/pC1, TN1, and Abg neuronal clusters on *Drosophila* sexual behaviors.

Neuronal cluster	Sex	dsx	fru	Function	Genetic reagent	Ref.	Note
(1) P1/pC1	Male	(+)	+	Courtship	fru ^{NP2} clone	[35]	Called P1
	Male	+	(+/-)	Courtship	dsx ^{GAL4} clone	[37, 38]	Called pC1
	Male	(+)	+	Courtship	NP2361 U fru ^{FLP}	[30, 34, 41]	Called P1
	Male	+	(+/-)	Courtship	GMR71G01-LexA \cap dsx ^{GAL4}	[36, 39, 40, 68]	Called P1/pC1
	Male	(+)	+	Courtship	GMR71G01-LexA U fru ^{GAL4}	[36]	Called P1
	Male	(+)	+/-	Courtship / aggression	P1 ^a -GAL4 (GMR15A01 ^{AD} U GMR71G01 ^{DBD})	[33, 42-44, 68]	Called P1/ P1 ^a
	Male	(+)	+	Aggression	GMR71G01-GAL4 U fru ^{FLP}	[43, 44]	Called P1
	Male	(+)	+	Aggression	GMR15A01-GAL4 U fru ^{FLP}	[43]	Called P1
	Male	+	+/-	Courtship / aggression	NP2361 U dsx ^{FLP}	[30]	Called pC1
	Male	+	-	Aggression	NP2361 U dsx ^{FLP} - fru ^{P1-LexA}	[30]	Called fru-pC1
	Female	+	(-)	Receptivity	GMR71G01-LexA U dsx ^{GAL4}	[31, 39]	Called pC1
	Female	+	(-)	Aggression	NP2361 U dsx ^{FLP}	[30]	Called pC1
	Female	+	(-)	Courtship	GMR71G01-LexA U dsx ^{GAL4}	[39]	Called pC1
	Male	+	+	Courtship pulse song, inter-pulse interval	[VT17258, VT5534, VT19579, VT57239, VT46099] U fru ^{FLP}	[34]	vPR6
(2) TN1	Male	+	N.D.	Courtship pulse song	GMR24B02-LexA U GMR41A01 ^{AD} U dsx ^{DBD}	[52]	TN1C; possibly the same as vPR6
	Male	N.D.	+	Courtship pulse song	VT43702 U fru ^{FLP}	[34]	vMS11
	Male	-	+	Courtship pulse song	GMR14B02-LexA U GMR21A01-GAL4	[50]	Hg1 (motoneuron)
	Male	-	+	Courtship pulse song	GMR52E01-LexA	[52]	Hg1 (motoneuron)
	Male	+	N.D.	Courtship sine song	GMR24B02-LexA U VT017258 ^{AD} U dsx ^{DBD}	[52]	TN1A
	Male	N.D.	+	Ejaculation	crz-GAL4	[54]	Cholinergic
	Male	N.D.	(+)	Ejaculation	Tph-GAL4	[54]	Serotonergic
	Male	+	-	Copulation	NP2719	[53]	GABAergic
	Male	+	N.D.	Copulation	vGlu ^{rAD} U dsx ^{DBD}	[19]	Glutamatergic
	Male	+	N.D.	Copulation	Gad ^{AD} U dsx ^{DBD}	[19]	GABAergic
(3) Abg	Male	+	N.D.	Copulation	Trh-GAL4 U dsx ^{FLP}	[55]	Serotonergic

Neuronal cluster	Sex	<i>dex</i>	<i>fru</i>	Function	Genetic reagent	Ref.	Note
	Female	N.D.	+	Copulation / receptivity	Abd-B-GAL4 U fru ^{FLP}	[62]	
	Female	+	-	Copulation / receptivity	SAG-1-GAL4 (VT50405 ^{AD} U VT7068 ^{DBD}), SAG-2-GAL4 (VT50405 ^{AD} U VT45154 ^{DBD})	[56, 64]	
	Female	+	N.D.	Copulation / receptivity	MIP-GAL4 U dsx ^{FLP}	[60]	