Received: 3 September 2019 | Revised: 8 October 2019 | Accepted: 28 October 2019

DOI: 10.1111/gbb.12623

Genes, Brain and Behavior

REVIEW

The neurogenetics of sexually dimorphic behaviors from a postdevelopmental perspective

Nicole Leitner | Yehuda Ben-Shahar

Department of Biology, Washington University in St. Louis, St. Louis, Missouri

Correspondence
Yehuda Ben-Shahar, Department of Biology, Washington University in St. Louis, St. Louis, MO.
Email: benshahary@wustl.edu

Funding information
National Institute of Neurological Disorders and Stroke, Grant/Award Number: R21NS089834; National Science Foundation, Grant/Award Numbers: 1322783, 1707221, 1754264

Abstract

Most sexually reproducing animal species are characterized by two morphologically and behaviorally distinct sexes. The genetic, molecular and cellular processes that produce sexual dimorphisms are phylogenetically diverse, though in most cases they are thought to occur early in development. In some species, however, sexual dimorphisms are manifested after development is complete, suggesting the intriguing hypothesis that sex, more generally, might be considered a continuous trait that is influenced by both developmental and postdevelopmental processes. Here, we explore how biological sex is defined at the genetic, neuronal and behavioral levels, its effects on neuronal development and function, and how it might lead to sexually dimorphic behavioral traits in health and disease. We also propose a unifying framework for understanding neuronal and behavioral sexual dimorphisms in the context of both developmental and postdevelopmental, physiological timescales. Together, these two temporally separate processes might drive sex-specific neuronal functions in sexually mature adults, particularly as it pertains to behavior in health and disease.

KEYWORDS

biological sex, *Drosophila melanogaster*, *Mus musculus*, sex determination, sexual dimorphism, sexual reproduction

1 | INTRODUCTION

The term "sex" often intuitively refers to behaviors directly associated with mating. However, from a pure biological perspective, sex represents a highly derived suite of anatomical, physiological and behavioral traits that support sexual reproduction in the majority of animal clades. Most sexually reproducing animal species depend on two morphologically, physiologically and behaviorally distinct male and female sexual forms. Typically, males contribute genetic material (sperm), while females provide both genetic material and the cellular environment to the developing embryo (egg). Although sexual reproduction is almost universal among metazoa, the molecular, genetic and cellular mechanisms that promote either male or female developmental fates are diverse across taxa. In broad terms, sex determination can be initiated by either environmental cues such as population density and temperature, or via genetic factors such as chromosomal architectures and specific allelic combinations.[1-4] Regardless of the initial cue that triggers the sex-determination pathway, the commitment of neurons and other cell lineages to differentiation as either a male or a female is usually thought to be determined early in embryonic development.[2,5-8] However, more recent studies suggest that, even in animal clades with robust male vs female developmental pathways, sex-specific neuronal morphology and function are more plastic than previously assumed and are likely dependent on both sex-specific developmental processes, as well as active, postdevelopmental maintenance of sex-specific states at the cellular and chromosomal levels in both vertebrates and invertebrates.[3,9-12]

Understanding how sex-determination pathways and, potentially, postdevelopmental maintenance, affect sexually dimorphic neural

development, differentiation and behavior is not only a fascinating biological phenomenon but is also important for understanding how biological sex impacts the incidence, expression and penetrance of neural and behavioral pathologies.[13-17] While the effects of sex on pathologies associated with various syndromes is relatively well known[18-20] how biological sex might affect postdevelopmental physiology in general, and behavioral traits in particular, remains mostly a mystery. For example, why addiction, depression and schizophrenia are reportedly more common in women,[21-23] while attention-deficit/hyperactivity disorder and other impulse control disorders are reportedly more common in men[24,25] is poorly understood at the molecular and cellular levels.

Here, we explore how biological sex is defined at the genetic, molecular and cellular levels, its effects on neuronal development and function, and what might be the relative contributions of developmental and postdevelopmental processes to observed behavioral differences between adult males and females. We primarily use studies in genetically tractable animal models to provide a new, synthetic perspective on the possible extended actions of the sex-determination pathway beyond its well-established role as a binary developmental axis. Particularly, we argue that sex-specific behaviors are complex, continuous traits that are driven by both neuronal differentiation decisions during development, as well as postdevelopmental, sex-specific maintenance and homeostasis pathways. Together, these two potentially independent biological processes provide the molecular and neuronal substrates for the maintenance of adaptive, sexually dimorphic behaviors, and might explain at least some of the effects of biological sex on neural and behavioral pathologies.

2 | SEX DETERMINATION AT THE CELLULAR AND MOLECULAR LEVELS

In the majority of sexually reproducing animals, a single genome has the potential for encoding two phenotypically distinct morphs that exhibit dramatic sex-specific morphological and behavioral traits. Yet, the specific genetic and developmental mechanisms that drive sexual dimorphisms are not known in most animal species. Data from a few species across many phylogenetic clades suggest that the specific genes and signaling pathways that drive sex-specific differentiation are diverse. Yet, the overall molecular logic behind the developmental processes that differentiate males and females seems fairly conserved.[1,2,26] Thus, regardless of the initial trigger for sex-specific developmental programs, a cell lineage that becomes committed to a biological sexual fate seems to follow a sex-specific epigenetic differentiation program. This program, in turn, supports sex-specific cellular functions via sex-biased transcriptional and physiological states.[27-34]

Because of their genetic tractability, the neuronal sex-determination pathways of the fruit fly *Drosophila melanogaster* and the lab mouse *Mus musculus* are particularly well described. Both species produce sexually dimorphic male and female forms, which exhibit multiple sex-dependent morphological, neuroanatomical and behavioral traits.[3,35,36] Both species also have evolved a heterogametic XY-dependent chromosomal sex determination system (Figure 1 A,B). However, studies at the molecular and cellular levels have revealed fundamental mechanistic differences between these two species in terms of how biological sex might be translated into sex-dependent brain and behavioral differences. In *Drosophila*, neuronal sex-specific differentiation is determined via cell-autonomous pathways.[37-39] Classical genetic studies have indicated that although *Drosophila* males are XY and females are XX, it is the ratio of X chromosomes to autosome sets (X:A ratio) that specifies male-vs-female cell fate.[40,41] Subsequently, the expression levels of key X-linked genes that are dose-sensitive promote differentiation towards male or female fates (Figure 1 A).[41,42] More recently, it has been suggested that ploidy-dependent, X-encoded signal elements are more likely to be the direct drivers for male vs female differentiation.[43] Subsequently, the dose of the X chromosome promotes a downstream sequence of sex-specific transcriptional and mRNA splicing events that promote sex-specific cellular and tissue differentiation.[42,44,45]

In contrast to flies, in which the Y chromosome does not determine male cellular identity, genes on the mammalian Y chromosome contribute both to promoting male-specific cellular fate and suppressing female-specific developmental pathways[3,46,47] (Figure 1B). Specifically, epigenetic regulation of the expression of the Y-linked gene *Sry* in early developmental stages leads to the development of male-specific gonads and suppresses female-specific gonads.[27,47,48] Subsequently, the sexually differentiated gonads release sex-specific hormonal signals that induce and coordinate sexually dimorphic somatic and neuronal cellular differentiation at the organismal level.[49,50] In recent years, the commonly accepted view that mammalian sex determination is a gonad-centric developmental process has been challenged; biological sex also appears to affect the differentiation of various somatic tissues (including neurons) via cell-autonomous processes. Examples include some male-specific traits driven by the nongonadal effects of Y-linked genes, female-specific traits driven by the female-enriched expression of genes that escape X-inactivation, and parental imprinting of X-linked genes that drive sexual dimorphisms via gonad-independent pathways.[51-53]

Advances in the molecular analyses of genomic and chromatin architectures reveal that, like other canonical pathways that determine cell-fate, the sex-determination pathway affects cellular differentiation via whole-genome epigenetic remodeling of both X-linked and autosomal genes,[27] as well as the transcription of sex-specific isoforms of transcription factors. In species with genetic sex determination, sex-dependent epigenetic modulation of gene expression is also essential for solving the ploidy issue associated with the chromosomal sex determination system. In XY, and similar heterochromosomal sex determination systems, sex-chromosome hemizygosity in males generates a gene dose problem, which has been solved differently in different animal lineages.[54,55] For example, in *Drosophila*, the expression levels of X-linked genes are upregulated in males via multiple independent mechanisms such as transcriptional elongation,[56] selection against X-linked genes with male-biased expression,[57] and other mechanisms for the epigenetic transcriptional upregulation by the male-specific lethal ribonucleoprotein complex[58,59] (Figure 1A). As in *Drosophila*, the dosage compensation of

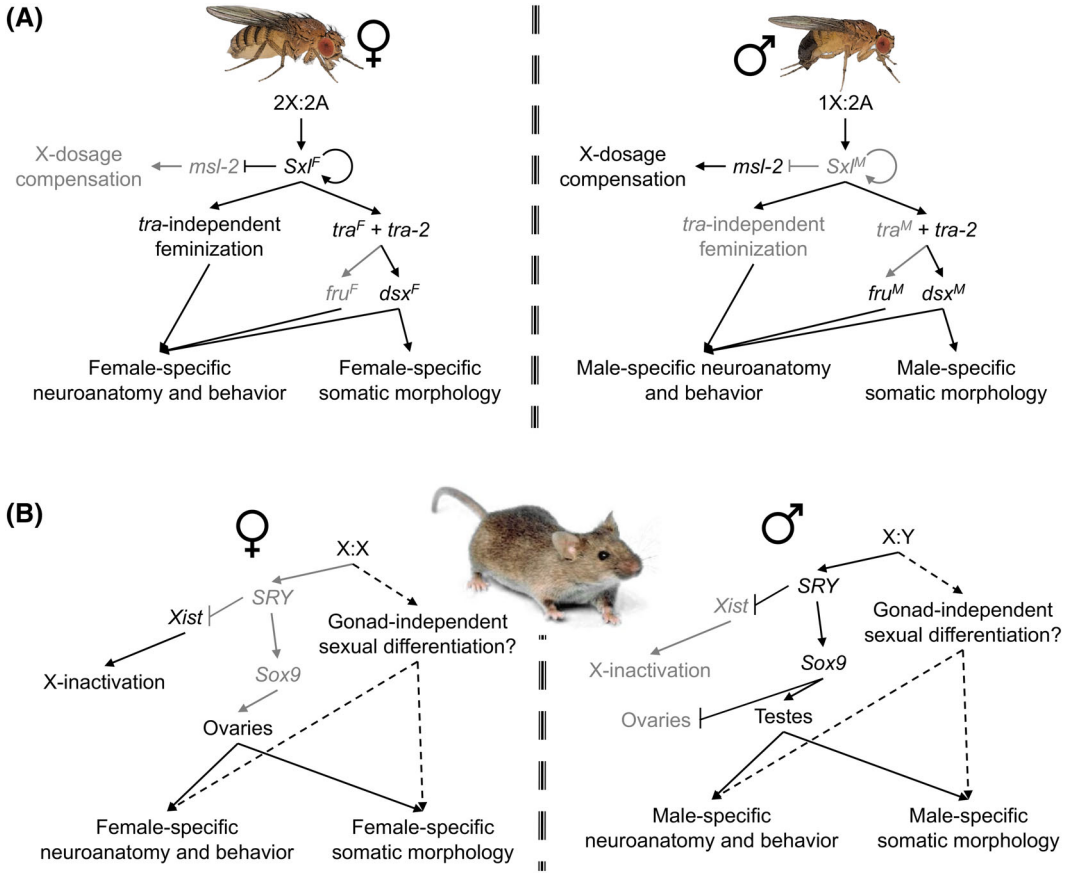

FIGURE 1 Sex determination pathways. A, The fruit fly *D. melanogaster*. Molecular sensing of the X:A chromosome ratio leads to the transcription of the master switch gene *Sex lethal* (*Sxl*), which is translated into protein only in females. Subsequently, *Sxl* acts as an m^6A-dependent female-specific splicing factor. The presence of the SXL protein in females then drives female-specific mRNA splicing of its immediate target, *transformer* (*tra*), which produces a functional protein only in females. The *tra-2*/tra^F protein complex generates female-specific transcripts of the transcription factors *doublesex* (*dsx*) and *fruitless* (*fru*) in females, while its absence in male tissues generates their male-specific counterparts. Together, the male-specific forms of *fru* and *dsx* seem to be sufficient for the differentiation of neural circuits that drive some of the key male-specific behaviors associated with courtship. In particular, it appears that the transcriptional activity of *fru* drives the sexual identity of the neurons that specifically underlie male courtship behaviors. Although not as well understood at the molecular level, recent studies indicate that *Sxl* also drives sex-specific neuronal differentiation via a *tra*-independent pathway, which suggests that the traditional view of *fru* expression as a "sex-circuit" genetic marker is likely too conservative. B, The mouse *M. musculus*. In mice and other mammals, sex determination is based on the presence of the Y chromosome. In chromosomal males (XY), the early expression of the Y-linked master regulator gene *Sry* activates molecular pathways that differentiate the gonad into testes and repress ovary development. The immediate downstream target of *Sry* is *Sox9*, which itself activates genes that promote testes development and suppress ovary development. *Sry* also blocks X-inactive specific transcript (*Xist*) to prevent X inactivation. The absence of *Sry* promotes ovary development and dosage compensation of X-linked genes via allelic silencing by the *Xist* complex. Once differentiated, the gonads produce testosterone in males but remain quiescent in females, which subsequently leads to male- vs female-specific development by regulating downstream, sex-specific gene expression in neuronal and other somatic tissues. In addition to the gonad-dependent sex determination pathway, recent studies indicate that Sry-independent pathways are also likely to drive sexually dimorphic traits in the brain and other somatic tissues. Later in development, sex hormones resurge to produce secondary sexual characteristics and act on the nervous system to drive sex-specific neuroanatomy and behavior (not depicted)

the mammalian X chromosome depends on key nucleoprotein complexes that epigenetically modulate the architecture of the X chromosome in a sex-specific manner. However, in contrast to *Drosophila*, mammals have evolved a female-centric solution to the X gene dosage problem by epigenetically inactivating one copy of the majority of X-linked genes, de facto generating a gene dose that is identical to that in males[60] (Figure 1B). Surprisingly, recent studies have indicated that at least 15% of human X-linked genes escape the inactivation process in females, possibly pointing to selection of inactivation-resistant genes on the X chromosome as a mechanism for driving female-enriched gene expression.[61] Nevertheless, sex determination is not only important for the transcriptional regulation and activity of X-linked genes. In fact, genetic and genomic approaches have demonstrated that sex-dependent differentiation broadly affects transcriptional activity of diverse genes across the whole genome in a cell-lineage specific manner.[62-64] Although predicting which genes might be under the control of

the sex determination pathway is difficult, studies of the two key sexually dimorphic transcription factors *fruitless* (*fru*) and *doublesex* (*dsx*) in *Drosophila* indicate that many genes with sex-specific functions are under direct transcriptional control of the sex determination pathway in a tissue-specific manner.[63-66]

3 | SEXUAL DIFFERENTIATION OF THE NERVOUS SYSTEM

Regardless of the specific mechanism that might drive sex determination in different species, the decision to develop as either a male or female is typically associated with the development of a sex-specific nervous system, and subsequently sex-specific behaviors. How sex determination pathways affect neuronal differentiation and circuit development at the molecular and genetic levels is still mostly unknown, though studies in genetically tractable model animals have revealed some of the underlying mechanisms. In mammals, for example, current models suggest that once the gonads differentiate as either testes (male) or ovaries (female), they secrete sex-specific hormones that sexually differentiate the neural scaffolding in the developing embryo (Figure 1 B). In males, the testes secrete testosterone to masculinize the brain, while in females, the ovaries remain quiescent, resulting in feminization.[49,50,67,68] Later in mammalian development, further functionalization of sexually dimorphic neural circuits depends on the identity of the circulating gonadal hormones.[49,50] In particular, testosterone (males), and estrogen and progesterone (females) are required during this time for driving the genetic and neuronal networks that support sex-specific behaviors such as male mounting behavior and aggression, or female sexual receptivity and maternal care.[67] These hormones promote sexually dimorphic neuronal morphology and synaptic connectivity primarily at the transcriptional level by activating sex-specific neuronal gene networks via hormone-binding nuclear receptors and cell surface hormone receptors.[68] However, emerging data suggest that neuronal sex determination in mammals and other vertebrates also utilizes gonad-independent, cell-autonomous pathways.[69,70] For example, the sex chromosome complements (ie, whether an individual has a set of XX or XY chromosomes), appears to contribute directly to sex differences in the number of dopaminergic and vasopressinergic neurons in the brain.[7,71]

In *D. melanogaster*, the sexually dimorphic neural circuits underlying behavior are exceptionally well characterized. In this system, sexually dimorphic neural circuits are not specified by the coordinated actions of hormones and do not depend on any Y-linked genes. Instead, sequential, cell-autonomous transcription and alternative mRNA splicing events define the sexual identity of each neuron independently.[38,39,44,45,72-79] In particular, sex-specific transcripts of the downstream transcription factors *doublesex* (*dsx*) and *fruitless* (*fru*) are important for the expression of sexually dimorphic nervous systems and behaviors.[38,80,81] Together, the male-specific forms of *fru* and *dsx* seem to be sufficient for the differentiation of neural circuits that drive some of the key male-specific behaviors associated with courtship. In particular, it appears that the *fru* locus confers a sexual identity to developing neurons underlying adult courtship behaviors, presumably by acting as a transcriptional switch to turn on or off sex-specific patterns of gene expression that ultimately shape sex-specific neural circuits.[37,45,77-79,82-87]

Although not as well described at the molecular and cellular levels, many animal species have evolved sex determination mechanisms that do not necessarily depend on sexually dimorphic developmental pathways (Figure 2). For example, some animal species can switch between male- and female-specific behaviors and physiology as sexually mature adults. Thus, neuronal and sexual dimorphisms can be achieved at the physiological, postdevelopmental timescale. Prime examples are found in teleost fishes, many of which naturally switch sex at some point in their adult life, often in response to changes in their social environment.[32,88,89] Unlike the gonad-driven sex determination in mammals we describe above, brain development in these sequential vertebrate hermaphrodites precedes gonad development and differentiation. In fact, recent studies suggest that the regulation of both gonad and brain sex are, to some extent, decoupled in these fishes via the action of independent sex-determination epigenetic programs and gene networks in each tissue.[88,90,91] Thus, in these hermaphroditic animals, the environment directly influences the biological sex of the brain independent of the sexual identity of the gonads throughout development and adulthood. In fact, sex change appears to be initiated in the brain, which is not surprising given that this is the first tissue that senses and responds to changes in the social environment. Sex-specific remodeling of the gonadal tissues subsequently follows.[32,88,92,93]

Another example of a plastic sex determination system is exhibited by species with simultaneous hermaphrodites, in which male and female tissues coexist within a single individual. Such systems are common in diverse invertebrate phyla including the platyhelminthes, annelids and molluscs. In these species, biological "sex allocation" might be considered more quantitative than qualitative, defined at any one moment by investment in either male or female gamete production.[94] In some molluscs, for example, sperm transfer is often unilateral, meaning that individuals must assume either a female or male role in a given copulation act.[95] Thus, there is a systemic bias towards one sex or the other, if only for a moment, in which the individual has to rapidly commit to a male or female identity. Thus, simultaneous hermaphrodites face the unique challenge of strictly coordinating their two independent sexual functions on a highly dynamic timescale in response to environmental cues so as to prevent them from being performed simultaneously. In response to mating opportunities, for example, *Ophryotrocha diadema* flatworms dynamically shift from female to male sex allocation when increasing mating opportunities, which suggests a mechanism by which dynamic sex allocation enables optimal fitness.[96]

Understanding simultaneous hermaphroditism at the level of neural circuits and behavior is particularly challenging because these species are able to switch between "male" and "female" behavioral states at the physiological timescale. Therefore, in contrast to species with "fixed," developmentally regulated sex determination, simultaneous

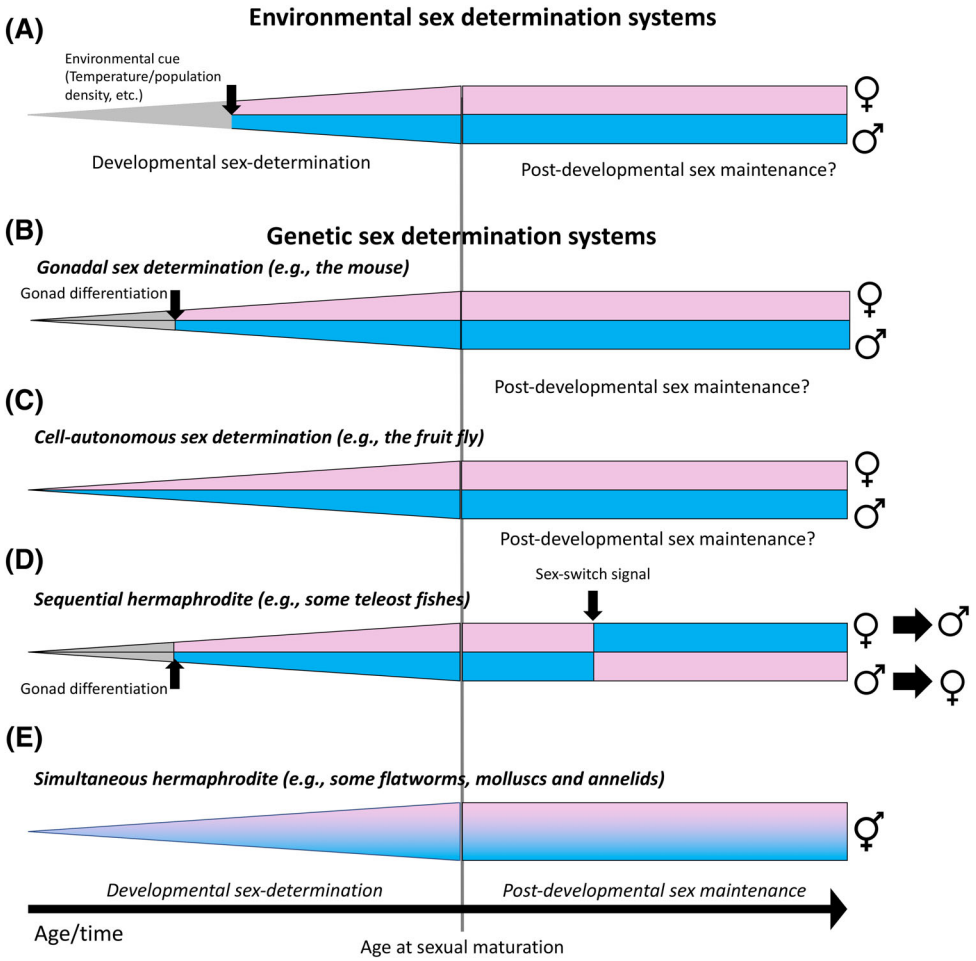

FIGURE 2 The role of developmental processes and postdevelopmental maintenance to sex-specific behaviors in different sex determination systems. A, In an environmental sex determination system, a sexually undifferentiated embryo (represented by the gray region) adopts one sex based on an environmental cue such as temperature or population density. In contrast, in genetic sex determination systems, sex is often specified by chromosomal architectures (eg, XY heterogameity) or other genetic factors. B, Most mammals, such as the mouse, exhibit gonadal sex determination, in which the genetic factors determine the sex-specific fate of bipotential gonads (represented by gray with a midline to separate biological sexes). Once differentiated, the gonads coordinate the sexual differentiation of all subsequent tissues. C, In fruit flies, on the other hand, sex determination is cell-autonomous rather than coordinated, such that sex is conferred independently to each cell of the developing embryo based on the ratio of X to autosomal chromosomes. In (A-C), early developmental mechanisms are the predominant driver of sexual dimorphisms, while postdevelopmental mechanisms might play only a minor role in maintaining these dimorphisms. D, In contrast to the fixed sex systems in (B) and (C), sequential hermaphrodites exhibit labile sex determination, with sexes that are temporally distinct. These organisms often begin life as one sex and naturally switch to the opposite sex later in life, often due to external environmental signals. In some cases, individuals can reverse sex multiple times throughout their lifespan. E, Many invertebrates exhibit simultaneous hermaphrodism, whereby individuals possess the anatomy and behaviors associated with male and female sexes. In these organisms, sexual identity is highly fluid, though individuals often assume only one sex role at a given time. In (D) and (E), postdevelopmental mechanisms might play a more prominent role in regulating sexual dimorphisms, whereas developmental sex determination plays a comparatively minor role in establishing anatomy and sex-specific behavior. The sex-determination systems depicted here are not necessarily mutually exclusive nor do they represent the full diversity of sex-determination systems among the metazoa. Pink denotes the female pathway and blue denotes the male pathway

hermaphrodites must use the same neural circuits to drive sex-specific behaviors. While the exact mechanisms that enable this remarkable plasticity remain unknown for most species in this group, studies on the great pond snail, *Lymnaea stagnalis*, suggest that in these species male and female behaviors are controlled by anatomically independent brain regions. At any given time, when one of the sex-specific neural circuits is active, activity in the other sex-specific circuit is silenced.[97,98] Although the factors mediating rapid, sex-specific behavioral switches are not entirely known, an interneuron in the brain that receives input from both male and female circuits, known as the "ring neuron," has been implicated in coordinating the neuronal and behavioral switch between "male" vs "female" states in this species.[99-101]

The remarkable sexual plasticity exhibited by sequential and simultaneous hermaphroditic species thus suggests the intriguing hypothesis that the "fixed" contribution of developmental pathways to sex determination in mammals might be a derived trait; furthermore, this might suggest that the binary "male" vs "female"

differentiation in "fixed"-sex species (such as the mouse and *Drosophila*), often assumed to be genetically determined, is in fact a continuous trait that is also influenced by postdevelopmental plastic processes. Sexually dimorphic behavioral and neuronal traits might thus depend on genetic mechanisms that actively maintain the sexual identity of neurons and circuits at the postdevelopmental, physiological timescale.

4 | POSTDEVELOPMENTAL MAINTENANCE OF SEXUALLY DIMORPHIC BEHAVIORS

To date, the majority of studies in genetically tractable model animals have primarily focused on the role of "hardwired" developmental processes in determining sexually dimorphic neuronal and behavioral traits. However, emerging data indicate that postdevelopmental, active maintenance of sexual dimorphisms at the level of neurons, circuits and behavior is important as well. Here, we present a unifying model for understanding behavioral sexual dimorphisms; in particular, we propose that behavioral sexual dimorphisms are generated and maintained by integrating inputs from both relatively "hardwired" developmental programs, as well as postdevelopmental, active homeostatic maintenance of sex-specific neuronal and behavioral traits. Under this mechanistic framework, the dramatic differences in how biological sex is determined in different species might be explained, at least in part, via different selective forces on the "hardwired" elements at the developmental timescale vs more plastic postdevelopmental processes at the physiological timescale (Figure 2). Sexually dimorphic behaviors, for example, might be regulated by the species-specific balance between these two processes. If true, then we would expect that the anatomy and transcriptional states of some neurons are established by hardwired, sex-specific developmental processes to establish some components of sex-specific morphology and physiology; in contrast, other neurons might remain sexually undifferentiated during development, but later turn on sex-specific postmitotic transcriptional programs that are required for sexual behavior in mature individuals. Since these alternative models are not mutually exclusive, we also expect that to exert their sexually dimorphic functions, some neurons require both sexually dimorphic differentiation and development, as well as continuous homeostatic maintenance of their sex-specific physiological state postmitotically. For example, the sexually dimorphic axonal wiring patterns of some neurons might be specified early in development, but are not able to execute their sex-specific functions without the continuous expression of sex-specific transcriptional networks.

Because of the strong role of hardwired developmental processes in the sex determination of the genetically tractable fruit fly and mouse models, the developmental perspective of sex-differences in neuronal and behavioral traits is dominant. In contrast, fewer studies have considered a theoretical framework that integrates the roles of both developmental processes and postdevelopmental maintenance in driving and maintaining sexually dimorphic behaviors. Although direct empirical data in support of active maintenance of sexually dimorphic gene networks in postmitotic neurons is lacking, emerging data suggest that it is likely an important, yet somewhat neglected, aspect of sexually dimorphic behavioral traits.

Current empirical support for the importance of both developmental and postdevelopmental physiological processes in driving sexually dimorphic behaviors is evident from systems-level analyses of brain transcriptomes in sexually mature *Drosophila* fruit flies,[63,102-105] mice,[106] humans,[107] and other animals.[108,109] For example, several recent studies of pheromone-sensing neurons in *Drosophila* have shown that the axons of a specific sexually dimorphic subset of these neurons, present only in the male forelegs, cross the thoracic midline.[12,110-112] Surprisingly, although the male-specific axonal midline crossing phenotype is determined early in pupal development, the postmitotic genetic feminization of these neurons late in development still leads to abnormally high male–male attraction and changes in gene expression without any observed impact on axonal patterns.[12] Thus, in addition to their developmentally determined sexually dimorphic wiring pattern, these neurons appear to require active maintenance of a male-specific transcriptional network for proper function. Furthermore, numerous studies have documented the postmitotic expression of the sex-specific mRNAs of the sex determination transcription factors *fru* and *dsx* (Figure 1A). This indicates that the sexually dimorphic functions of these factors do not end when the sexually dimorphic elements of the nervous system complete their development.[83,87,102,113-115] That the sex determination pathway continues functioning into adulthood to drive behavior is also supported by studies in *Drosophila*, which show that the genetic feminization of specific elements in the olfactory system of adult males is associated with same-sex male courtship behavior.[116,117] Furthermore, postdevelopmental genetic manipulations of the sex-specific splicing complex *tra* and *tra-2* (Figure 1A), for example, can induce male-like courtship behaviors in biological females.[118] Similarly, genetic manipulations that affect male-specific splicing of *fru* can lead to partial expression of male-like courtship behavior in females,[82] and ectopic expression of the female-specific tra^F allele in postmitotic neurons of adult males feminizes male behavior by suppressing courtship.[119] Independent of its action in the postdevelopmental nervous system, the fly's sex determination pathway plays an important role in the fat body and oenocytes, regulating the sex-specific synthesis and/or release of various cuticular hydrocarbons used by male and female flies as mating pheromones.[120-122] Together, these data indicate that the sex determination pathway plays an essential postdevelopmental role in regulating mating behaviors by simultaneously regulating the production, perception and neural integration of mating-related signals.

Similarly to *Drosophila*, the active maintenance of biological sex of postmitotic neurons is also likely to be important for sexually dimorphic behaviors in mammals.[123,124] For example, neural circuits underlying male-specific courtship and aggression behavior in mice, which are silent in females, can be activated postnatally by surgically removing the chemosensory vomeronasal organ.[125] Moreover, loss of testosterone via the castration of males feminizes sexually dimorphic anatomical regions of the amygdala involved in male sexual behavior, alters adult neurogenesis in the vomeronasal system, and elicits male-male sexual attraction.[10,11]

5 | CONCLUSIONS

Together, these studies strongly suggest that the active maintenance of biological sex is not unique to the sexually labile hermaphrodites. Rather, the processes underlying sexual dimorphisms in behavior across diverse taxa might share more similarities than previously appreciated. Possibly, all sexually reproducing species depend on both fixed developmental and active, postdevelopmental maintenance components for sexualizing the nervous system. Thus, sex determination systems that appear highly disparate may simply differ in the ratio of developmentally regulated "hardwired," vs the more plastic, actively maintained components of the sex-determination pathway in the nervous system. Consequently, the balance between the contributions of genetic networks that regulate behavior at the developmental vs the physiological timescales is, by itself, a trait that can be shaped by natural selection. The nature of the balance between these two temporally distinct processes can therefore lead to dramatic differences in the level of sex-specific neuronal and behavioral traits exhibited by specific animal clades, as well as between individuals in a single population.

The emergence of biological sex as a system that enables the development of at least two phenotypically distinct organisms from a single genome remains one of the most fascinating, yet somewhat mysterious, aspects of animal evolution. However, studying sex determination at the cellular, genetic and molecular levels has implications that are far broader than understanding sexual behaviors. Fully understanding how single genomes can encode multiple different forms at both developmental and physiological timescales, for example, might shed light on the consequences and effects of biological sex on human behavior in health and disease.

ACKNOWLEDGMENTS

The authors thank members of the Ben-Shahar lab for helpful comments on drafts of this manuscript. Work in the Ben-Shahar lab has been supported by grants R21NS089834 from the NIH, and 1754264 and 1707221 from the NSF to Y.B.-S.

ORCID

Nicole Leitner https://orcid.org/0000-0001-9507-0551
Yehuda Ben-Shahar https://orcid.org/0000-0002-2956-2926

REFERENCES

1. Beukeboom LW, Perrin N. *The Evolution of Sex Determination*. 1st ed. Oxford: Oxford University Press; 2014.
2. Bachtrog D, Mank JE, Peichel CL, et al. Sex determination: why so many ways of doing it? *PLoS Biol*. 2014;12(7):e1001899.
3. Capel B. Vertebrate sex determination: evolutionary plasticity of a fundamental switch. *Nat Rev Genet*. 2017;18(11):675-689.
4. Knoedler JR, Shah NM. Molecular mechanisms underlying sexual differentiation of the nervous system. *Curr Opin Neurobiol*. 2018;53:192-197.
5. Bull JJ. *Evolution of Sex Determining Mechanisms*. Menlo Park, CA: Benjamin/Cummings Pub. Co., Advanced Book Program; 1983.
6. Hodgkin J. Problems and paradigms: genetic sex determination mechanism and evolution. *Bioessays*. 1992;14(4):253-261.
7. Carruth LL, Reisert I, Arnold AP. Sex chromosome genes directly affect brain sexual differentiation. *Nat Neurosci*. 2002;5(10):933-934.
8. Smith CA, McClive PJ, Western PS, Reed KJ, Sinclair AH. Conservation of a sex-determining gene. *Nature*. 1999;402(6762):601-602.
9. McCarthy MM, Arnold AP. Reframing sexual differentiation of the brain. *Nat Neurosci*. 2011;14(6):677-683.
10. Cooke BM, Tabibnia G, Breedlove SM. A brain sexual dimorphism controlled by adult circulating androgens. *Proc Natl Acad Sci*. 1999;96(13):7538-7540.
11. Schellino R, Trova S, Cimino I, et al. Opposite-sex attraction in male mice requires testosterone-dependent regulation of adult olfactory bulb neurogenesis. *Sci Rep*. 2016;6(1):1-15.
12. Lu B, Zelle KM, Seltzer R, Hefetz A, Ben-Shahar Y. Feminization of pheromone-sensing neurons affects mating decisions in drosophila males. *Biol Open*. 2014;3(2):152-160.
13. Abel KM, Drake R, Goldstein JM. Sex differences in schizophrenia. *Int Rev Psychiatry*. 2010;22(5):417-428.
14. McCarthy MM, Arnold AP, Ball GF, Blaustein JD, De Vries GJ. Sex differences in the brain: the not so inconvenient truth. *J Neurosci*. 2012;32(7):2241-2247.
15. Uddin M, Koenen KC, de Los Santos R, Bakshis E, Aiello AE, Galea S. Gender differences in the genetic and environmental determinants of adolescent depression. *Depress Anxiety*. 2010;27(7):658-666.
16. Altemus M, Sarvaiya N, Neill Epperson C. Sex differences in anxiety and depression clinical perspectives. *Front Neuroendocrinol*. 2014;35(3):320-330.
17. Scharfman HE, MacLusky NJ. Sex differences in the neurobiology of epilepsy: a preclinical perspective. *Neurobiol Dis*. 2014;72:180-192.
18. Bashamboo A, McElreavey K. Human sex-determination and disorders of sex-development (DSD). *Semin Cell Dev Biol*. 2015;45:77-83.
19. Dotto GP. Gender and sex-time to bridge the gap. *EMBO Mol Med*. 2019;11(5):e10668.
20. Bashamboo A, McElreavey K. Mechanism of sex determination in humans: insights from disorders of sex development. *Sex Dev*. 2016;10(5–6):313-325.
21. Kuehner C. Why is depression more common among women than among men? *Lancet Psychiatry*. 2017;4(2):146-158.
22. Li R, Ma X, Wang G, Yang J, Wang C. Why sex differences in schizophrenia? *J Transl Neurosci (Beijing)*. 2016;1(1):37-42.
23. Becker JB, McClellan ML, Reed BG. Sex differences, gender and addiction. *J Neurosci Res*. 2017;95(1–2):136-147.
24. Fattore L, Melis M. Editorial: exploring gender and sex differences in behavioral dyscontrol: from drug addiction to impulse control disorders. *Front Psych*. 2016;7:19.
25. Martin J, Walters RK, Demontis D, et al. A genetic investigation of sex bias in the prevalence of attention-deficit/hyperactivity disorder. *Biol Psychiatry*. 2018;83(12):1044-1053.
26. Gempe T, Beye M. Function and evolution of sex determination mechanisms, genes and pathways in insects. *Bioessays*. 2011;33(1):52-60.
27. Kuroki S, Matoba S, Akiyoshi M, et al. Epigenetic regulation of mouse sex determination by the histone demethylase Jmjd1a. *Science*. 2013;341(6150):1106-1109.
28. Piferrer F. Epigenetics of sex determination and gonadogenesis. *Dev Dyn*. 2013;242(4):360-370.
29. Crews D. Epigenetic modifications of brain and behavior: theory and practice. *Horm Behav*. 2011;59(3):393-398.
30. Nugent BM, McCarthy MM. Epigenetic underpinnings of developmental sex differences in the brain. *Neuroendocrinology*. 2011;93(3):150-158.

31. Forger NG. Past, present and future of epigenetics in brain sexual differentiation. *J Neuroendocrinol*. 2018;30(2):e12492.
32. Todd EV, Ortega-Recalde O, Liu H, et al. Stress, novel sex genes, and epigenetic reprogramming orchestrate socially controlled sex change. *Sci Adv*. 2019;5(7):eaaw7006.
33. Anreiter I, Biergans SD, Sokolowski MB. Epigenetic regulation of behavior in *Drosophila melanogaster*. *Curr Opin Behav Sci*. 2019;25: 44-50.
34. Radhakrishnan S, Literman R, Neuwald JL, Valenzuela N. Thermal response of epigenetic genes informs turtle sex determination with and without sex chromosomes. *Sex Dev*. 2018;12(6):308-319.
35. Billeter JC, Rideout EJ, Dornan AJ, Goodwin SF. Control of male sexual behavior in drosophila by the sex determination pathway. *Curr Biol*. 2006;16(17):R766-R776.
36. Schwarz JM. Sex and the developing brain. In: Shansky RM, ed. *Sex Differences in the Central Nervous System*. San Diego, CA: Academic Press; 2016:221-245.
37. Kimura K, Ote M, Tazawa T, Yamamoto D. Fruitless specifies sexually dimorphic neural circuitry in the drosophila brain. *Nature*. 2005; 438(7065):229-233.
38. Rideout EJ, Billeter JC, Goodwin SF. The sex-determination genes fruitless and doublesex specify a neural substrate required for courtship song. *Curr Biol*. 2007;17(17):1473-1478.
39. Robinett CC, Vaughan AG, Knapp JM, Baker BS. Sex and the single cell. II. There is a time and place for sex. *PLoS Biol*. 2010;8(5): e1000365.
40. Bridges CB. TRIPLOID INTERSEXES IN *Drosophila melanogaster*. *Science*. 1921;54(1394):252-254.
41. Cline TW. The Drosophila sex determination signal: how do flies count to two? *Trends Genet*. 1993;9(11):385-390.
42. Keyes LN, Cline TW, Schedl P. The primary sex determination signal of drosophila acts at the level of transcription. *Cell*. 1992;68(5): 933-943.
43. Erickson JW, Quintero JJ. Indirect effects of ploidy suggest X chromosome dose, not the X:A ratio, signals sex in drosophila. *PLoS Biol*. 2007;5(12):e332.
44. Erickson JW, Cline TW. Molecular nature of the Drosophila sex determination signal and its link to neurogenesis. *Science*. 1991;251 (4997):1071-1074.
45. Rideout EJ, Narsaiya MS, Grewal SS. The sex determination gene transformer regulates male-female differences in drosophila body size. *PLoS Genet*. 2015;11(12):e1005683.
46. Arnold AP. Sex chromosomes and brain gender. *Nat Rev Neurosci*. 2004;5(9):701-708.
47. Stevant I, Nef S. Genetic control of gonadal sex determination and development. *Trends Genet*. 2019;35(5):346-358.
48. Kuroki S, Tachibana M. Epigenetic regulation of mammalian sex determination. *Mol Cell Endocrinol*. 2018;468:31-38.
49. Cindy N. Representing sex in the brain, one module at a time. *Neuron*. 2014;82(2):261-278.
50. Yang T, Shah NM. Molecular and neural control of sexually dimorphic social behaviors. *Curr Opin Neurobiol*. 2016;38:89-95.
51. Arnold AP. The end of gonad-centric sex determination in mammals. *Trends Genet*. 2012;28(2):55-61.
52. Arnold AP, Reue K, Eghbali M, et al. The importance of having two X chromosomes. *Philos Trans R Soc Lond B Biol Sci*. 2016;371(1688): 20150113.
53. Arnold AP. A general theory of sexual differentiation. *J Neurosci Res*. 2017;95(1–2):291-300.
54. Zimmer F, Harrison PW, Dessimoz C, Mank JE. Compensation of dosage-sensitive genes on the chicken Z chromosome. *Genome Biol Evol*. 2016;8(4):1233-1242.
55. Sangrithi MN, Turner JMA. Mammalian X chromosome dosage compensation: perspectives from the germ line. *Bioessays*. 2018;40(6): 1800024.
56. Larschan E, Bishop EP, Kharchenko PV, et al. X chromosome dosage compensation via enhanced transcriptional elongation in drosophila. *Nature*. 2011;471(7336):115-118.
57. Parisi M, Nuttall R, Naiman D, et al. Paucity of genes on the drosophila X chromosome showing male-biased expression. *Science*. 2003;299(5607):697-700.
58. Gelbart ME, Kuroda MI. Drosophila dosage compensation: a complex voyage to the X chromosome. *Development*. 2009;136(9):1399-1410.
59. Samata M, Akhtar A. Dosage compensation of the X chromosome: a complex epigenetic assignment involving chromatin regulators and long noncoding RNAs. *Annu Rev Biochem*. 2018;87:323-350.
60. Payer B, Lee JT. X chromosome dosage compensation: how mammals keep the balance. *Annu Rev Genet*. 2008;42(1):733-772.
61. Balaton BP, Brown CJ. Escape artists of the X chromosome. *Trends Genet*. 2016;32(6):348-359.
62. Stévant I, Neirijnck Y, Borel C, et al. Deciphering cell lineage specification during male sex determination with single-cell RNA sequencing. *Cell Rep*. 2018;22(6):1589-1599.
63. Clough E, Jimenez E, Kim Y-A, et al. Sex- and tissue-specific functions of drosophila doublesex transcription factor target genes. *Dev Cell*. 2014;31(6):761-773.
64. Clough E, Oliver B. Genomics of sex determination in drosophila. *Brief Funct Genomics*. 2012;11(5):387-394.
65. Chang PL, Dunham JP, Nuzhdin SV, Arbeitman MN. Somatic sex-specific transcriptome differences in drosophila revealed by whole transcriptome sequencing. *BMC Genomics*. 2011;12:364.
66. Arbeitman MN, New FN, Fear JM, Howard TS, Dalton JE, Graze RM. Sex differences in drosophila somatic gene expression: variation and regulation by doublesex. *G3 (Bethesda)*. 2016;6(7):1799-1808.
67. Xu X, Coats Jennifer K, Yang Cindy F, et al. Modular genetic control of sexually dimorphic behaviors. *Cell*. 2012;148(3):596-607.
68. Schwarz JM, McCarthy MM. Steroid-induced sexual differentiation of the developing brain: multiple pathways, one goal. *J Neurochem*. 2008;105(5):1561-1572.
69. Arnold AP, Chen X, Link JC, Itoh Y, Reue K. Cell-autonomous sex determination outside of the gonad. *Dev Dyn*. 2013;242(4):371-379.
70. Clinton M, Zhao D, Nandi S, McBride D. Evidence for avian cell autonomous sex identity (CASI) and implications for the sex-determination process? *Chromosome Res*. 2012;20(1):177-190.
71. De Vries GJ, Rissman EF, Simerly RB, et al. A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits. *J Neurosci*. 2002;22(20):9005-9014.
72. Steinmann-Zwicky M, Schmid H, Nöthiger R. Cell-autonomous and inductive signals can determine the sex of the germ line of drosophila by regulating the gene Sxl. *Cell*. 1989;57(1):157-166.
73. Possidente DR, Murphey RK. Genetic control of sexually dimorphic axon morphology in drosophila sensory neurons. *Dev Biol*. 1989;132 (2):448-457.
74. Rideout EJ, Dornan AJ, Neville MC, Eadie S, Goodwin SF. Control of sexual differentiation and behavior by the doublesex gene in *Drosophila melanogaster*. *Nat Neurosci*. 2010;13(4):458-466.
75. Ito H, Sato K, Koganezawa M, et al. Fruitless recruits two antagonistic chromatin factors to establish single-neuron sexual dimorphism. *Cell*. 2012;149(6):1327-1338.
76. Rezaval C, Pavlou HJ, Dornan AJ, Chan YB, Kravitz EA, Goodwin SF. Neural circuitry underlying drosophila female postmating behavioral responses. *Curr Biol*. 2012;22(13):1155-1165.
77. Yamamoto D, Koganezawa M. Genes and circuits of courtship behaviour in drosophila males. *Nat Rev Neurosci*. 2013;14(10):681-692.
78. Neville MC, Nojima T, Ashley E, et al. Male-specific fruitless isoforms target neurodevelopmental genes to specify a sexually dimorphic nervous system. *Curr Biol*. 2014;24(3):229-241.
79. von Philipsborn AC, Jorchel S, Tirian L, et al. Cellular and behavioral functions of fruitless isoforms in drosophila courtship. *Curr Biol*. 2014;24(3):242-251.

80. Siwicki KK, Kravitz EA. *Fruitless*, *doublesex* and the genetics of social behavior in *Drosophila melanogaster*. *Curr Opin Neurobiol*. 2009;19(2):200-206.
81. Evans DS, Cline TW. Drosophila switch gene sex-lethal can bypass its switch-gene target transformer to regulate aspects of female behavior. *Proc Natl Acad Sci*. 2013;110(47):E4474-E4481.
82. Demir E, Dickson BJ. *fruitless* splicing specifies male courtship behavior in *Drosophila*. *Cell*. 2005;121(5):785-794.
83. Manoli DS, Foss M, Villella A, Taylor BJ, Hall JC, Baker BS. Male-specific *fruitless* specifies the neural substrates of *Drosophila* courtship behaviour. *Nature*. 2005;436(7049):395-400.
84. Pavlou HJ, Goodwin SF. Courtship behavior in *Drosophila melanogaster*: towards a 'courtship connectome'. *Curr Opin Neurobiol*. 2013;23(1):76-83.
85. Krupp JJ, Levine JD. Neural circuits: anatomy of a sexual behavior. *Curr Biol*. 2014;24(8):R327-R329.
86. Yamamoto D, Sato K, Koganezawa M. Neuroethology of male courtship in drosophila: from the gene to behavior. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*. 2014;200(4):251-264.
87. Newell NR, New FN, Dalton JE, McIntyre LM, Arbeitman MN. Neurons that underlie *Drosophila melanogaster* reproductive behaviors: detection of a large male-bias in gene expression in fruitless-expressing neurons. *G3 (Bethesda)*. 2016;6(8):2455-2465.
88. Godwin J. Neuroendocrinology of sexual plasticity in teleost fishes. *Front Neuroendocrinol*. 2010;31(2):203-216.
89. Todd EV, Liu H, Muncaster S, Gemmell NJ. Bending genders: the biology of natural sex change in fish. *Sex Dev*. 2016;10(5–6):223-241.
90. Pellegrini E, Mouriec K, Anglade I, et al. Identification of aromatase-positive radial glial cells as progenitor cells in the ventricular layer of the forebrain in zebrafish. *J Comp Neurol*. 2007;501(1):150-167.
91. Le Page Y, Diotel N, Vaillant C, et al. Aromatase, brain sexualization and plasticity: the fish paradigm. *Eur J Neurosci*. 2010;32(12):2105-2115.
92. Liu H, Todd EV, Lokman PM, Lamm MS, Godwin JR, Gemmell NJ. Sexual plasticity: a fishy tale. *Mol Reprod Dev*. 2017;84(2):171-194.
93. Dodd LD, Nowak E, Lange D, et al. Active feminization of the preoptic area occurs independently of the gonads in *Amphiprion ocellaris*. *Horm Behav*. 2019;112:65-76.
94. Scharer L. Tests of sex allocation theory in simultaneously hermaphroditic animals. *Evolution*. 2009;63(6):1377-1405.
95. Koene JM. Sex determination and gender expression: reproductive investment in snails. *Mol Reprod Dev*. 2017;84(2):132-143.
96. Santi M, Picchi L, Lorenzi MC. Dynamic modulation of reproductive strategies in a simultaneous hermaphrodite and preference for the male role. *Anim Behav*. 2018;146:87-96.
97. Leonard JL, Córdoba-Aguilar A. *The Evolution of Primary Sexual Characters in Animals*. Oxford, NY: Oxford University Press; 2010.
98. Koene JM. Neuro-endocrine control of reproduction in hermaphroditic freshwater snails: mechanisms and evolution. *Front Behav Neurosci*. 2010;4:167.
99. Jansen RF, Bos NP. An identified neuron modulating the activity of the ovulation hormone producing caudo-dorsal cells of the pond snail *Lymnaea stagnalis*. *J Neurobiol*. 1984;15(2):161-167.
100. Jansen RF, ter Maat A, Bos NP. Membrane mechanism of neuroendocrine caudo-dorsal cell inhibition by the ring neuron in the pond snail *Lymnaea stagnalis*. *J Neurobiol*. 1985;16(1):15-26.
101. Jansen RF, ter Maat A. Ring neuron control of columellar motor neurons during egg-laying behavior in the pond snail. *J Neurobiol*. 1985;16(1):1-14.
102. Goldman TD, Arbeitman MN. Genomic and functional studies of Drosophila sex hierarchy regulated gene expression in adult head and nervous system tissues. *PLoS Genet*. 2007;3(11):e216.
103. Arbeitman MN, Fleming AA, Siegal ML, Null BH, Baker BS. A genomic analysis of drosophila somatic sexual differentiation and its regulation. *Development*. 2004;131(9):2007-2021.
104. Graveley BR, Brooks AN, Carlson JW, et al. The developmental transcriptome of *Drosophila melanogaster*. *Nature*. 2011;471(7339):473-479.
105. Vernes SC. Genome wide identification of fruitless targets suggests a role in upregulating genes important for neural circuit formation. *Sci Rep*. 2014;4:4412.
106. Dewing P, Shi T, Horvath S, Vilain E. Sexually dimorphic gene expression in mouse brain precedes gonadal differentiation. *Brain Res Mol Brain Res*. 2003;118(1–2):82-90.
107. Leader DP, Krause SA, Pandit A, Davies SA, Dow JAT. FlyAtlas 2: a new version of the *Drosophila melanogaster* expression atlas with RNA-Seq, miRNA-Seq and sex-specific data. *Nucleic Acids Res*. 2018;46(D1):D809-D815.
108. Mank JE, Hultin-Rosenberg L, Webster MT, Ellegren H. The unique genomic properties of sex-biased genes: insights from avian microarray data. *BMC Genomics*. 2008;9:148.
109. Gegenhuber B, Tollkuhn J. Signatures of sex: sex differences in gene expression in the vertebrate brain. *Wiley Interdiscip Rev Dev Biol*. 2019;e348.
110. Lu B, LaMora A, Sun Y, Welsh MJ, Ben-Shahar Y. ppk23-Dependent chemosensory functions contribute to courtship behavior in *Drosophila melanogaster*. *PLoS Genet*. 2012;8(3):e1002587.
111. Thistle R, Cameron P, Ghorayshi A, Dennison L, Scott K. Contact chemoreceptors mediate male-male repulsion and male-female attraction during drosophila courtship. *Cell*. 2012;149(5):1140-1151.
112. Toda H, Zhao X, Dickson BJ. The drosophila female aphrodisiac pheromone activates ppk23+ sensory neurons to elicit male courtship behavior. *Cell Rep*. 2012;1:599-607. https://doi.org/10.1016/j.celrep.2012.05.007(6):599–607.
113. Lee G, Hall JC, Park JH. Doublesex gene expression in the central nervous system of *Drosophila melanogaster*. *J Neurogenet*. 2002;16(4):229-248.
114. Stockinger P, Kvitsiani D, Rotkopf S, Tirián L, Dickson BJ. Neural circuitry that governs drosophila male courtship behavior. *Cell*. 2005;121(5):795-807.
115. Sanders LE, Arbeitman MN. Doublesex establishes sexual dimorphism in the drosophila central nervous system in an isoform-dependent manner by directing cell number. *Dev Biol*. 2008;320(2):378-390.
116. Ferveur JF, Stortkuhl KF, Stocker RF, Greenspan RJ. Genetic feminization of brain structures and changed sexual orientation in male drosophila. *Science*. 1995;267(5199):902-905.
117. O'Dell KMC, Armstrong JD, Yao Yang M, Kaiser K. Functional dissection of the drosophila mushroom bodies by selective feminization ofagenetically defined subcompartments. *Neuron*. 1995;15(1):55-61.
118. Belote JM, Baker BS. Sexual behavior: its genetic control during development and adulthood in *Drosophila melanogaster*. *Proc Natl Acad Sci U S A*. 1987;84(22):8026-8030.
119. Kido A, Ito K. Mushroom bodies are not required for courtship behavior by normal and sexually mosaic drosophila. *J Neurobiol*. 2002;52(4):302-311.
120. Ferveur JF, Savarit F, O'Kane CJ, Sureau G, Greenspan RJ, Jallon JM. Genetic feminization of pheromones and its behavioral consequences in drosophila males. *Science*. 1997;276(5318):1555-1558.
121. Fujii S, Amrein H. Genes expressed in the drosophila head reveal a role for fat cells in sex-specific physiology. *EMBO J*. 2002;21(20):5353-5363.
122. Lazareva AA, Roman G, Mattox W, Hardin PE, Dauwalder B. A role for the adult fat body in drosophila male courtship behavior. *PLoS Genet*. 2007;3(1):e16.
123. Beny Y, Kimchi T. Conditioned odor aversion induces social anxiety towards females in wild-type and TrpC2 knockout male mice. *Genes Brain Behav*. 2016;15(8):722-732.

124. Beny-Shefer Y, Zilkha N, Lavi-Avnon Y, et al. Nucleus Accumbens dopamine signaling regulates sexual preference for females in male mice. *Cell Rep*. 2017;21(11):3079-3088.
125. Kimchi T, Xu J, Dulac C. A functional circuit underlying male sexual behaviour in the female mouse brain. *Nature*. 2007;448(7157): 1009-1014.

How to cite this article: Leitner N, Ben-Shahar Y. The neurogenetics of sexually dimorphic behaviors from a postdevelopmental perspective. *Genes, Brain and Behavior*. 2020;19:e12623. https://doi.org/10.1111/gbb.12623