Published in final edited form as:
Curr Opin Neurobiol. 2010 December ; 20(6): 776–783. doi:10.1016/j.conb.2010.09.006.

Cell Death and Sexual Differentiation of Behavior: Worms, Flies, and Mammals

Nancy G. Forger[1] and **Geert J. de Vries**
Department of Psychology and Center for Neuroendocrine Studies, University of Massachusetts, Amherst MA 01003, USA

Nancy G. Forger: nforger@psych.umass.edu; Geert J. de Vries: devries@cns.umass.edu

Summary

Sex differences in the nervous system are found throughout the animal kingdom. Here, we discuss three prominent genetic models: nematodes, fruit flies, and mice. In all three, differential cell death is central to sexual differentiation and shared molecular mechanisms have been identified. Our knowledge of the precise function of neural sex differences lags behind. One fruitful approach to the "function" question is to contrast sexual differentiation in standard laboratory animals with differentiation in species exhibiting unique social and reproductive organizations. Advanced genetic strategies are also addressing this question in worms and flies, and may soon be applicable to vertebrates.

Introduction

In many animal species, males and females live in different social worlds, or at least play according to different social rules. Sex differences are commonly seen in courtship, communication, copulatory behaviors, and the processing of opposite-sex sensory cues [1,2,3]. Neural sex differences presumably allow for such behavioral differences and, indeed, are being found throughout the animal kingdom. This review compares sexual differentiation of the nervous system in three major genetic models of neural development: the nematode (*Caenorhabditis elegans*), the fruit fly (*Drosophila melanogaster*), and the laboratory mouse (*Mus musculus*).

In principle, neural sex differences could result from differences in any of the major neurodevelopmental events: neurogenesis, migration, neurite outgrowth, the differentiation of chemical phenotype, or cell death. Interestingly, however, in all three species(*C.elegans*, *D. melanogaster*, and *M. Musculus)*, cell death is the best-understood cellular mechanism underlying sexual differentiation of neural tissue. This suggests that cell death may be a highly efficient strategy for building a sex-specific nervous system. Alternatively, we may know so much about this mechanism because differences in cell number are often easier to detect than more subtle changes in connectivity or gene expression. In either case, as we describe below, recent studies have provided a fine-grained molecular analysis of how sex differences in cell number develop in worms, flies, and mice. Cell death has also been linked to neural sex differences in other animals, such as birds and frogs [4,5], although due to the

[1]Corresponding author.

more limited ability for genetic manipulation, our understanding of underlying molecular mechanisms is less complete for these species.

In contrast to the progress made in understanding the *mechanisms* of neuronal cell death, much less is known about the *function* of sex differences in neuron number. This is particularly true in mammals where neural circuits are extremely complex and sex differences tend to be quantitative rather than qualitative. One strategy for circumventing this problem is to study simpler nervous systems such as those of worms and flies, where cells can be individually identified and circuitry is better known. Another avenue for understanding the function of sex differences in mammals, especially those related to sociosexual behaviors, is to expand our world-view beyond laboratory rodents to include species with different social and reproductive strategies. A good example is recent studies on naked mole-rats, which are highly social, cooperatively breeding rodents that forego sexual differentiation of behavior or postpone it until well into adulthood[6,7]. Here, we first review the basics of sex determination and differentiation in *C. elegans*, *Drosophila* and rodents. Next, examples of sex differences in cell number in each species are discussed, along with what is known about underlying molecular mechanisms and the role in sociosexual behavior. We conclude with ongoing efforts to understand the meaning of neural sex differences.

Two sexes, three mechanisms?

Although *C. elegans*, *Drosophila* and mice all come in two sexes and rely on chromosomal sex determination, there are some important differences. The two sexes in *C.elegans* are males (XO) and hermaphrodites (XX; basically, modified females that produce and store sperm for self-fertilization), whereas fruit flies and mammals are male (XY) or female(XX). Sex determination in *C. elegans* depends on the ratio of X chromosomes to autosomes. In brief, the lower ratio in males leads to the expression of a secreted factor (her-1) that acts via a cell surface receptor (tra-2) to repress tra-1. Tra-1 is a master sexual regulator, which is active in hermaphrodites and blocks male cell fates (Table 1) [8].

Sex determination in *Drosophila* also depends on the ratio of X chromosomes to autosomes, but there is no known role for a secreted protein. Instead, the chromosomal sex of each cell determines the splicing within that cell of two key genes, *doublesex* and *fruitless*, which confer maleness or femaleness (Table 1) [9]. Thus, sexual differentiation is cell-autonomous. The alternative splicing of the *fruitless* gene is thought to be especially important for sex-specific cell fates in the nervous system, and the forced expression of male *fruitless* proteins can elicit male courtship behavior in otherwise normal female flies [10].

The predominant mechanism for sexual differentiation in mammals involves a gene on the Y chromosome that triggers testis development in males. Hormones produced from the testes (most notably, testosterone) then circulate throughout the body, differentiating the periphery and brain in a male direction [11*,12]. This occurs around the time of birth in mice and rats; thus, administering testosterone to genetic females perinatally masculinizes not only the body, but also many neural and behavioral features.

Although nematodes, fruit flies, and mice use different mechanisms to generate the sexes, there are important commonalities. In each case the sex determining molecular cascade relies on members of the DM(*Doublesex/mab-3*) family of transcription factors [13]. Moreover, there is growing evidence that in addition to hormones sex chromosomal genes exert direct effects on sexual differentiation of the brain and behavior in mammals [11*,14–16], bringing them closer to what is seen in worms and flies. Finally, in each species developmental cell death leads to important sex differences in neuron number. Neural development in all animals involves the overproduction of neurons, followed by a period of

pruning by apoptosis [17], and sexual differentiation apparently taps into this mechanism, as detailed below.

Sex differences in neurogenesis also have been reported [e.g., 18], but one may wonder why examples are not more common. In mammals, the answer at first appears straightforward. Most neurons are born well before the differentiating surges of testosterone. Moreover, a neuroblast born at a proliferative zone is not fully determined; its final destination and phenotype will depend on environmental cues, so it would be difficult to target cells to precise sexually dimorphic cell groups with a change in neurogenesis. These explanations may be misleading, however, when viewed through a wider lens: species such as worms and flies also rely on developmental cell death for neural sex differences, yet cell fate is more strictly determined and the overlap with hormones is not a consideration. Presumably, the sculpting of neuron number by cell death allows for more precision, or was easier to evolve, than the tweaking of cell number by neurogenesis. Once established, the mechanism of apoptosis was conserved, as the cell death cascades in worms, flies and mammals all converge on the activation of caspases, cysteine proteases that cleave key cellular proteins to cause the cell's demise [19]. Moreover, homologues of each of the members of the canonical cell death pathway in nematodes can be found in humans (20).

Sexual differentiation of neuron number

1. Roundworms

In *C.elegans*, the identity and number of cells that die is invariant between individuals. During development of the hermaphrodite, for example, exactly 131 cells die and many of these are neurons, or would become neurons if spared [21,22]. The adult nervous system consists of a series of ganglia containing exactly 294 neurons shared by both sexes, 8 hermaphrodite-specific neurons and about 90 male-specific neurons [23]. Only hermaphrodites have the so-called hermaphrodite-specific neurons (HSNs), a pair of bilaterally symmetric motoneurons that innervate vulval muscles required for egg laying behavior [24,25]. Other hermaphrodite-specific cells include the VC motoneurons that inhibit the vulval muscles [24]. The cephalic companion cells (CEMs) are specific to males. These four chemosensory neurons (together with cells of the core nervous system) are important for chemotaxis to hermaphrodite pheromones during courtship [26*]. Most other male-specific neurons also are implicated in sexually dimorphic functions, namely, the pheromonal or mechanical detection of hermaphrodites, and male-specific mating postures [23]. How sex differences in these other cells develop is less well known, however.

Painstaking visual observation of developing worms have revealed that HSNs and CEMs are born, migrate and to some extent differentiate normally before being eliminated in one sex by programmed cell death [22]. Subsequently four genes were identified that comprise the core cell death pathway in *C. elegans*: ced-3 (a caspase), ced-4 (homologous to the apoptosis adaptor molecule, Apaf1, in mammals), ced-9 (homologous to the mammalian pro-survival molecule Bcl-2), and egl-1 (homologous to pro-death members of the Bcl-2 family) [27]. Mutations in any of the four genes affect most of the 131 cells that die during development, including the sexually dimorphic HSNs. Interestingly, the pro-death egl-1 gene contains a binding site for the sexual regulator, tra-1 [28]. When tra-1 binds, egl-1 transcription is repressed, thereby allowing the survival of HSNs in hermaphrodites [28]. Thus, a direct link has been established from the sex-determining pathway to sexually dimorphic cell death of HSN neurons.

Recent work has also elucidated the molecular basis for the sex difference in CEM survival. The mechanism again involves tra-1, but in this case may be independent of the core cell death pathway. CEH-30 is an anti-apoptotic factor acting within CEM neurons of males

[29*,30*]. Hermaphrodites express tra-1, which binds and transcriptionally represses CEH-30, leading to CEM cell death. There is a mammalian homolog of CEH-30, Barhl1, and it can substitute for CEH-30 in protecting CEMs from death [29*]. Mutations of Barhl1 lead to increased cell death in some neural regions of mice [31], but whether this gene plays a role in sexually dimorphic mammalian cell death has not yet been examined.

2. Fruit flies

As noted above, alternative splicing of two key sex-determining genes – *doublesex* and *fruitless* -underlies sexual differentiation in *Drosophila*. Until recently there was thought to be a tidy dichotomy, with *doublesex* directing differentiation of the body and *fruitless* that of brain and behavior. This turns out to be an oversimplification, as several recent studies have demonstrated essential roles for the sex-specific splicing of *doublesex* as well as *fruitless* in sexual differentiation of neuroanatomy and behavior [32*–34*]. Male-specific fruitless proteins (Fru^M) are made in a few thousand neurons (about 3% of the total) of the nervous system [35]. Most Fru^M producing neurons in males have counterparts in females [36,37], but there are several important exceptions. For example, males have about 30 Fru^M -expressing interneurons in the cluster *fru*-mAL (medial cells above the antennal lobe), whereas females have only five [38]. This sex difference is apparently due to cell death because when the core *Drosophila* cell death genes (*hid*, *grim*, and *reaper)* are deleted, females have nearly as many mAL neurons as males [38]. The *fru*-mAL interneurons contribute to processing pheromonal signals that drive sexual behavior, so the sex-specific elimination of these cells may account for sex differences in the response to these cues.

Male *Drosophila* also have a cluster of about 20 neurons known as P1 that is completely absent in females [33*]. Differential cell death is again implicated in this difference because P1 cells are present in *hid/grim/reaper* mutant females. However, the P1 cluster still forms in males with an inactivating *fruitless* mutation and not in females expressing Fru^M proteins, indicating that in this case sexual differentiation is independent of *fruitless*. Many Fru^M cells co-express *doublesex* [34*] and, indeed, the female splice variant of the doublesex protein turns out to be responsible for the death of P1 cells [33*]. Importantly, mosaic females with a masculinized P1 cluster exhibit the initial steps of male courtship behavior, indicating that sexual differentiation of this single cell group can account for a prominent difference in sociosexual behavior.

3. Mice (and Rats)

In contrast to the situation in nematodes, in which lineage determines which cells will die, developmental neuronal cell death in vertebrates appears to be stochastic: a pool of essentially equal cells competes for trophic support, and one cannot predict *a priori* which will survive [17]. There are a number of well-studied sex differences in neuron number in rodents that depend on testosterone levels during critical periods in development and are due to cell death[Figure 1; reviewed in 39]. As in worms and flies, these have been linked to detection of opposite sex mates, copulatory behavior and “egg laying” (in this case, ovulation). For example, most nodes of the neural circuit from the accessory olfactory bulb to the hypothalamus that processes pheromonal cues are larger in male than in female rats [40].

In addition, male rats and mice have more neurons than females in the spinal nucleus of the bulbocavernosus (SNB) and principal nucleus of the bed nucleus of the stria terminalis (BNSTp), whereas the opposite pattern (female > male) is seen in the anteroventral periventricular nucleus of the hypothalamus (AVPV)[39]. SNB motoneurons innervate muscles that attach to the phallus and are active during copulation. The BNSTp, with over 20,000 cells and many cell types, is clearly multifunctional and has been linked to

pheromone processing, male sex behavior, gonadotrophin secretion, and the response to stressors[41]. AVPV is a region with direct projections to gonadotrophin releasing hormone cells that control ovulation [41]. In each case, the sex difference in cell number is due to testosterone acting during early development: testosterone treatment of females around the time of birth increases cell number in the SNB and BNSTp but reduces it in AVPV [39].

Similar to the strategy used in worms and flies, mice with mutations in cell death genes have been used to test the role of cell death in neural sex differences. Pro-survival and pro-death proteins of the Bcl-2 family critically regulate the death of developing neurons in mammals. The pro-death protein Bax, in particular, is required for most developmental neuronal cell death and apoptosis is nearly eliminated in many neural regions of *bax* knockout mice [42]. In mice with a targeted deletion of *bax*, cell number in the SNB, BNSTp and AVPV are increased and, importantly, sex differences in cell number are eliminated [43,44]. Sex differences in some behaviors (e.g. female sexual behavior and olfactory preference), also are eliminated in *bax* knockout mice [45; M Holmes, L Niel, D Monks, N Forger, unpublished].

The story becomes more complex when specific subtypes of neurons are examined, however. For example, the sex difference in vasopressin neurons in the BNSTp (male > female) is not eliminated in *bax* knockout mice, apparently because in this case testosterone directs the differentiation of neuronal phenotype [46]. Similarly, a small subset of neurons in AVPV is dopaminergic and females have about three times as many of these cells as do males [47]. Although this sex difference is due to the hormonal control of cell death [48], it is independent of Bax or Bcl-2 [43,49]. Neurons within AVPV that express kisspeptin, a peptide that stimulates GnRH secretion and regulates puberty, also are more numerous in females than in males and this sex difference is not altered by deletion of the *bax* gene [50]. The bulk of neurons in AVPV are GABAergic, and recent work demonstrates that sexual differentiation of these cells involves the tumor necrosis factor α-NF κB cell survival pathway as well as a *bax*-dependent mechanism[51*]. Thus, even within a single, relatively small nucleus such as AVPV, multiple sex differences in cell number due to different molecular mechanisms coexist. Interestingly, work on worms and flies contributed to what we know about *bcl-2*-realted and tumor necrosis factor α-NF κB cell survival pathways, and therefore differentiation of the mammalian brain.

Function of differential developmental cell death: What naked mole-rats have to tell us

Part of the problem in ascribing function to sex differences in the mammalian brain is that nuclei contain thousands of cells comprising multiple cell types (Figure 1). The BNSTp alone, for example, contains almost 100 times as many cells as in the entire *C. elegans* nervous system. In addition, sex differences in mammals typically are quantitative rather than absolute. Precisely what the “extra” cells in one sex buy the animal in terms of function is not well understood [52]. If the purpose of neural sex differences is to generate sex differences in behavior, then one might predict that species with minimal sex specific roles might lack sex differences. This seems to be true for naked mole-rats(*Heterocephalus glaber*). These small rodents live in underground colonies that include on average 70–90 and in some cases over 200 individuals [6,7]. Only a single female breeds and she mates with between one and three males. All other colony members are siblings or offspring of the breeders and are socially subordinate to the breeders. The subordinates provide pup care, foraging, and colony defense but never exhibit sexual behaviors. They also show no sex differences in behavior, and the genitalia, associated muscles, and nervous system are also remarkably monomorphic [53,54]. Even the sex difference in AVPV kisspeptin neurons, so prominent in other rodents, is absent in naked mole-rats [S Zhou, M Holmes, N Forger, B

Goldman, M Lovern, A Caraty, C Faulkes, CW Coen, abstract in Soc Neurosci Abstr 2010, 594:16]. Subordinates can become breeders, and this switch is accompanied by neural changes, including an increase in volume of several brain nuclei [54] and kisspeptin cell number [S Zhou abstract cited above]. These changes are seen in both sexes, however, so they correlate with breeding status and not with sex.

The relative lack of sex differences in naked mole-rats presumably is related to their social structure. Sex differences in cell number, in particular, are generally "permanent," and may not be a good strategy for generating the sex-specific social and reproductive behaviors required of the very small percentage of animals that will ever become breeders. In support of this argument, a solitary mole-rat species is more "traditionally" dimorphic [55]. Naked mole-rat breeders may rely on more subtle neural sex differences for sex-specific roles, such as changes in gene expression or synapse number. Indeed, the only sex difference found thus far in the naked mole-rat brain concerns the expression of androgen receptors [56]. Analagously, in *Astatotilapia burtoni*, a chiclid fish with a social structure that involves dominant and subordinate individuals with different reproductive strategies, social status changes are associated with altered neural expression of receptors for sex steroids, kisspeptin, and GnRH [57–59].

As in naked mole-rats, some functions that differ between male and female mice may not depend on changes in gross neural morphology. While under normal conditions only male mice display male sexual behavior, females exhibit high levels of this behavior if gonadectomized in adulthood and treated with testosterone or following a single gene deletion of a vomeronasal receptor [60–62]. The most parsimonious explanation is that the basic circuitry does not differ much between males and females whereas sensitivity to elicit these behaviors does. Although differential sensitivity could be based on differences in cell number, it could also be due to the differentiation of synapse number or neuronal and glial gene expression [12].

Future Strategies

A time-honored approach to the problem of ascribing functions to neural sex differences is to turn to simpler systems. In mammals, it can be argued that we know more about the function of the difference in the SNB, a simple neuromuscular system, than any other neural sex difference [63], although even in this case the afferent inputs are not completely known. The lower complexity of flies and worms combined with a rich arsenal of genetic methods make their "brains" more accessible to functional analysis. Optogenetics has recently been used to directly compare the neural circuitry in male and female flies at both functional and anatomical levels. For example, by using light-activated ion channels to stimulate *fru*-expressing neurons in the relevant ganglia, it was demonstrated that flies of both sexes have the basic circuitry for male courtship song, although it normally lies dormant in females [64*]. A combined genetic and optical approach also recently identified sex specific axonal projections that may explain how the activation of a single olfactory receptor on the same set of sensory neurons can lead to very different behavioral outcomes in male and female flies [65*]. With these [66,67] and other [68–69] genetic tools now being developed for mammalian behavioral systems, there is good hope that our understanding of the function of sexually dimorphic cell types in mammals will soon increase as well.

Acknowledgments

Work from the authors' laboratories is supported by grants from the National Institutes of Health R01-MH068482 and R01-MH047538 and the National Science Foundation IOS-0642050 and IBN 9421658.

References and recommended reading

1. Kelly D, Bass A. this issue.
2. Wilczynski W. this issue.
3. Stowers L. this issue.
4. Kirn JR, DeVoogd TJ. Genesis and death of vocal control neurons during sexual differentiation in the zebra finch. J Neurosci 1989;9:3176–87. [PubMed: 2795159]
5. Kay JN, Hannigan P, Kelley DB. Trophic effects of androgen: development and hormonal regulation of neuron number in a sexually dimorphic vocal motor nucleus. J Neurobiol 1999;40:375–85. [PubMed: 10440737]
6. Bennet, NC.; Faulkes, CG. Social organization in African mole-rats. In: Bennet, NC.; Faulkes, CG., editors. African Mole-Rats: Ecology and Eusociality. Cambridge University Press; 2000. p. 87-126.
7. Lacey, EA.; Sherman, PW. Social organization of naked mole-rat colonies: evidence for division of labor. In: Sherman, PW.; Jarvis, JUM.; Alexander, RD., editors. The Biology of the Naked Mole-rat. Princeton University Press; 1991. p. 275-336.
8. Zarkower, D. C. elegans Research Community. WormBook. WormBook; 2006. Somatic sex determination. http://www.wormbook.org
9. Billeter J-C, Rideout EJ, Dornan AJ, Goodwin SF. Control of male sexual behavior in *Drosophila* by the sex determination pathway. Curr Biol 2006a;16:R766–R776. [PubMed: 16950103]
10. Demir E, Dickson BJ. *fruitless* splicing specifies male courtship behavior in *Drosophila*. Cell 2005;121:785–794. [PubMed: 15935764]
*11. Arnold AP. The organizational-activational hypothesis as the foundation for a unified theory of sexual differentiation of all mammalian tissues. Horm Behav 2009;55:570–578. A clear and provocative treatise that unifies the field of vertebrate sexual differentiation by carefully considering the roles of hormones, sex chromosomes and their interactions. [PubMed: 19446073]
12. McCarthy, MM.; de Vriesm, GJ.; Forger, NG. Sexual Differentiation of the Brain: Mode, Mechanisms, and Meaning. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, S.; Rubin, RT., editors. Hormones, Brain and Behavior. 2. Academic Press; 2009. p. 1707-1744.
13. Haag, ES. The C. elegans Research Community. WormBook. WormBook; 2005. The evolution of nematode sex determination: *C. elegans* as a reference point for comparative biology. http://www.wormbook.org
14. De Vries GJ, Rissman EF, Simerly RB, Yang LY, Scordalakes EM, Auger CJ, Swain A, Lovell-Badge R, Burgoyne PS, Arnold AP. A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits. J Neurosci 2002;22:9005–9014. [PubMed: 12388607]
15. Dewing P, Chiang CW, Sinchak K, Sim H, Fernagut PO, Kelly S, Chesselet MF, Micevych PE, Albrecht KH, Harley VR, Vilain E. Direct regulation of adult brain function by the malespecific factor SRY. Curr Biol 2006;16:415–420. [PubMed: 16488877]
16. Chen X, Grisham W, Arnold AP. X chromosome number causes sex differences in gene expression in adult mouse striatum. Eur J Neurosci 2009;29:768–776. [PubMed: 19250439]
17. Buss RR, Sun W, Oppenheim RW. Adaptive roles of programmed cell death during nervous system development. Ann Rev Neurosci 2006;29:1–35. [PubMed: 16776578]
18. Zhang JM, Konkle AT, Zup SL, McCarthy MM. Impact of sex and hormones on new cells in the developing rat hippocampus: a novel source of sex dimorphism? Eur J Neurosci 2008;27:791–800. [PubMed: 18333959]
19. Hengartner MO. The biochemistry of apoptosis. Nature 2000;407:770–776. [PubMed: 11048727]
20. Putcha GV, Johnson EM. 'Men are but worms:' Neuronal cell death in C. elegans and vertebrates. Cell Death Diff 2004;11:38–48.
21. Sulston JE, Horvitz HR. Post-embryonic lineages of the nematode, *Caenorhabditis elegans*. Dev Biol 1977;56:110–156. [PubMed: 838129]
22. Sulston JE, Schierenberg E, White JG, Thomson JN. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev Biol 1983;100:64–119. [PubMed: 6684600]

23. Portman DS. Genetic control of sex differences in *C. elegans* neurobiology and behavior. Adv Gen 2007;59:1–37.

24. White JG, Southgate E, Thomson JN, Brenner S. The structure of the nervous system of the nematode *Caenorhabditis elegans*. Phis Trans R Soc Lond B 1986;314:1–340.

25. Trent C, Tsuing N, Horvitz HR. Egg-laying defective mutants of the nematode Caenorhabditis elegans. Genetics 1983;104:619–647. [PubMed: 11813735]

*26. White JQ, Nicholas TJ, Gritton J, Truong L, Davidson ER, Jorgensen EM. The sensory circuitry for sexual attraction in *C. elegans* males. Curr Biol 2007;17:1847–1857. Uses ablation experiments to demonstrate that the male-specific CEM neurons are required for chemotaxis to hermaphrodite pheromones. However, the requirement can be overcome if ablations are performed prior to maturation, demonstrating that non-dimorphic cells of the “core” nervous system can compensate and are also critically involved in this behavior. [PubMed: 17964166]

27. Conradt B, Horvitz HR. The C. elegans protein EGL-1 is required for programmed cell death and interacts with the Bcl-2-like protein CED-9. Cell 1998;93:519–529. [PubMed: 9604928]

28. Conradt B, Horvitz HR. The TRA-1A sex determination protein of C. elegans regulates sexually dimorphic cell deaths by repressing the egl-1 cell death activator gene. Cell 1999;98:317–327. [PubMed: 10458607]

*29. Schwartz HT, Horvitz HR. The C. elegans protein CEH-30 protects male-specific neurons from apoptosis independently of the Bcl-2 homolog CED-9. Genes Dev 2007;21:3181–3194. [PubMed: 18056428]

*30. Peden E, Kimberly E, Gengyo-Ando K, Mitani S, Xue D. Control of sex-specific apoptosis in C. elegans by the BarH homeodomain protein CEH-30 and the transcriptional repressor UNC-37/Groucho. Genes Dev 2007;21:3195–3207. This reference, together with [21] demonstrates a novel molecular mechanism for sex-specific sparing of CEM neurons in C. elegans that may be independent of the canonical cell death pathway. Evidence is presented that the Bar homeodomain transcription factor CEH-30 is expressed in CEM neurons of males and prevents their death. Moreover, Tra-1 binding sites are identified in the CEH-30 gene, suggesting a mechanism by which the terminal regulator of sexual fate controls CEH-30 expression. [PubMed: 18056429]

31. Li S, Qiu F, Xu A, Price SM, Xiang M. Barhl1 regulates migration and survival of cerebellar granule cells by controlling expression of the neurotrophin-3 gene. J Neurosci 2004;24:3104–3114. [PubMed: 15044550]

*32. Rideout EJ, Billeter JC, Goodwin SF. The sex-determination genes fruitless and doublesex specify a neural substrate required for courtship song. Curr Biol 2007;17:1473–1478. [PubMed: 17716899]

*33. Kimura K-I, Hachiya T, Koganezawa M, Tazawa T, Yamamoto D. Fruitless and Doublesex coordinate to generate male-specific neurons that can initiate courtship. Neuron 2008;59:759–769. [PubMed: 18786359]

*34. 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;13:458–466. [24], [25] and [26] demonstrate that sexual differentiation of the brain and behavior in Drosophila requires the sex-specific splicing of both fruitless and doublesex. Ref. [24] uses females constitutively expressing male-specific isoforms of fruitless to disentangle the roles of doublesex from those of fruitless. Kimura et al. [25] find a cluster of male-specific neurons, P1, and demonstrate that the loss of these neurons in females is due to the actions of the female splice variant of the Doublesex protein. [26] comprehensively characterizes doublesex expression throughout development and identifies independent roles for fruitless and doublesex in the specification of neural sex differences. This paper also is of note for demonstrating that disruption of doublesex disrupts female as well asmale pre-and post-mating behaviors; in contrast to abundant work on males, the neural underpinnings of female mating behavior have rarely been examined in this field. [PubMed: 20305646]

35. Lee G, Foss M, Goodwin SF, Carlo T, Taylor BJ, Hall JC. Spatial, temporal, and sexually dimorphic expression patterns of the fruitless gene in the Drosophila central nervous system. J Neurobiol 2000;43:404–426. [PubMed: 10861565]

36. Stockinger P, Kvitsiani D, Rotkopf S, Tirian L, Dickson BJ. Neural circuitry that governs Drosophila male courtship behavior. Cell 2005;121:795–807. [PubMed: 15935765]
37. 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;436:395–400. [PubMed: 15959468]
38. Kimura K-I, Ote M, Tazawa T, Yamamoto D. Fruitless specifies sexually dimorphic neural circuitry in the Drosophila brain. Nature 2005;438:229–233. [PubMed: 16281036]
39. Forger NG. Control of cell number in the sexually dimorphic brain and spinal cord. J Neuroendocrinol 2009;21:393–399. [PubMed: 19207822]
40. Guillamón A, Segovia S. Sex differences in the vomeronasal system. Brain Res Bull 1997;44:377–382. [PubMed: 9370202]
41. De Vries, GJ.; Simerly, RB. Anatomy, development, and function of sexually dimorphic neural circuits in the mammalian brain. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Moss, RL.; Rubin, RT., editors. Hormones, Brain, and Behavior. Academic Press; 2001. p. 137-191.
42. White FA, Keller-Peck CR, Knudson CM, Korsmeyer SJ, Snider WD. Widespread elimination of naturally occurring neuronal death in Bax-deficient mice. J Neurosci 1998;18:1428–1439. [PubMed: 9454852]
43. Forger NG, Rosen GJ, Waters EM, Jacob D, Simerly RB, de Vries GJ. Deletion of bax eliminates sex differences in the mouse forebrain. Proc Natl Acad Sci USA 2004;101:13666–13671. [PubMed: 15342910]
44. Jacob DA, Bengston CL, Forger NG. Effects of Bax gene deletion on muscle and motoneuron degeneration in a sexually dimorphic neuromuscular system. J Neurosci 2005;25:5638–5644. [PubMed: 15944391]
45. Jyotika J, McCutcheon J, Laroche J, Blaustein JD, Forger NG. Deletion of the Bax gene disrupts sexual behavior and modestly impairs motor function in mice. Dev Neurobiol 2007;67:1511–1519. [PubMed: 17525992]
46. De Vries GJ, Jardon D, Reza M, Rosen GJ, Immerman E, Forger NG. Sexual differentiation of vasopressin innervation of the brain: cell death versus phenotypic differentiation. Endocrinol 2008;149:4632–4637.
47. Simerly RB, Zee MC, Pendleton JW, Lubahn DB, Korach KS. Estrogen receptor dependent sexual differentiation of dopaminergic neurons in the preoptic region of the mouse. Proc Natl Acad Sci USA 1997;94:14077–14082. [PubMed: 9391155]
48. Waters EM, Simerly RB. Estrogen induces caspase-dependent cell death during hypothalamic development. J Neurosci 2009;29:9714–9718. [PubMed: 19657024]
49. Zup SL, Carrier H, Waters EM, Tabor A, Bengston L, Rosen GJ, Simerly RB, Forger NG. Overexpression of bcl-2 reduces sex differences in neuron number in the brain and spinal cord. J Neurosci 2003;23:2357–2362. [PubMed: 12657695]
50. Semaan SJ, Murray EK, Poling MC, Dhamija S, Forger NG, Kauffman AS. BAX-dependent and -independent regulation of Kiss1 neuron development in mice. Endocrinol. 2010 in press.
*51. Krishnan S, Intlekofer KA, Aggison LK, Petersen SL. Central role of TRAF-interacting protein in a new model of brain sexual differentiation. Proc Natl Acad Sci USA 2009;106:16692–16697. This paper identifies a novel cell death pathway in the mammalian brain that contributes to a female-biased sex difference. More neurons normally die in the AVPV of neonatal male rats than in females. The authors demonstrate that TRIP [tumor necrosis factor (TNF) receptor associated factor 2 inhibiting protein]is expressed at higher levels in the AVPV of males and link this to repression of the TNFα-NF κB cell survival pathway. Male AVPV also expresses higher levels of the pro-death gene bax, but this appeared independent of TRIP. [PubMed: 19805359]
52. De Vries GJ, Södersten P. Sex differences in the brain: The relation between structure and function. Horm Behav 2009;55:589–596. [PubMed: 19446075]
53. Holmes MM, Goldman BD, Goldman SL, Seney ML, Forger NG. Neuroendocrinology and sexual differentiation in eusocial mammals. Front Neuroendocrinol 2009;30:519–533. [PubMed: 19416733]

54. Holmes MM, Rosen GJ, Jordan CL, de Vries GJ, Goldman BD, Forger NG. Social control of brain morphology in a eusocial mammal. Proc Natl Acad Sci USA 2007;104:10548–10552. [PubMed: 17556547]

55. Seney ML, Kelly DA, Goldman BD, Sumbera R, Forger NG. Social structure predicts genital morphology in African mole-rats. PLoS One 2009;4:e7477. [PubMed: 19829697]

56. Holmes MM, Goldman BD, Forger NG. Social status and sex independently influence androgen receptor expression in the eusocial naked mole-rat brain. Horm Behav 2008;54:278–285. [PubMed: 18455726]

57. Burmeister SS, Kailasanath V, Fernald RD. Social dominance regulates androgen and estrogen receptor gene expression. Horm Behav 2007;51:164–170. [PubMed: 17081541]

58. Maruska KP, Fernald RD. Reproductive status regulates expression of sex steroid and GnRH receptors in the olfactory bulb. Behav Brain Res 2010;213:208–217. [PubMed: 20466023]

59. Grone BP, Maruska KP, Korzan WJ, Fernald RD. Social status regulates kisspeptin receptor mRNA in the brain of Astatotilapia burtoni. Gen Comp Endocrinol. Aug 3; [epub ahead of print].

60. Edwards DA, Burge KG. Early androgen treatment and male and female sexual behavior in mice. Horm Behav 1971;2:49–58.

61. Wersinger SR, Sannen K, Villalba C, Lubahn DB, Rissman EF, De Vries GJ. Masculine sexual behavior is disrupted in male and female mice lacking a functional estrogen receptor alpha gene. Horm Behav 1997;32:176–183. [PubMed: 9454668]

62. Kimchi T, Xu J, Dulac C. A functional circuit underlying male sexual behaviour in the female mouse brain. Nature 2007;448:1009–1014. [PubMed: 17676034]

63. Sengelaub DR, Forger NG. The spinal nucleus of the bulbocavernosus: firsts in androgen-dependent neural sex differences. Horm Behav 2008;53:596–612. [PubMed: 18191128]

*64. Clyne JD, Miesenböck G. Sex-specific control and tuning of the pattern generator for courtship song in Drosophila. Cell 2008;133:354–363. The authors use light-activated ion channels to stimulate neurons in the abdominal-thoracic ganglia that express fruitless and generate courtship song. Males produce this song by rapidly oscillating the wing closest to the courted females. This paper demonstrates that females have the basic circuitry for song, but it usually lies dormant. When stimulated, females generate a song, albeit one that is slightly “out of tune.”. [PubMed: 18423205]

*65. Datta SR, Vasconcelos ML, Ruta V, Luo S, Wong A, Demir E, Flores J, Balonze K, Dickson BJ, Axel R. The Drosophila pheromone cVA activates a sexually dimorphic neural circuit. Nature 2008;452:473–477. Previous work showed that the cVA pheromone acts through the same sensory neurons to elicit different behaviors in males and females. Here, the authors use a two-photon laser scanning microscope to photoactivate green fluorescent protein expressed in the relevant sensory cells. This allows them to trace axonal projection at high resolution, and to find a sex difference in projection pattern that may explain the sex difference in behavior. Expressing FruM ectopically in females causes formation of the male-like axonal projection. [PubMed: 18305480]

66. Gradinaru V, Mogri M, Thompson KR, Henderson JM, Deisseroth K. Optical deconstruction of Parkinsonian neural circuitry. Science 2009;324:354–359. [PubMed: 19299587]

67. Tsai H-C, Zhang F, Adamantidis A, Stuber GD, Bonci A, de Lecea L, Deisseroth K. Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. Science 2009;324:1080–1084. [PubMed: 19389999]

68. Raskin K, de Gendt K, Duittoz A, Liere P, Verhoeven G, Tronche F, Mhaouty-Kodja S. Conditional inactivation of androgen receptor gene in the nervous system: effects on male behavioral and neuroendocrine responses. J Neurosci 2009;29:4461–4470. [PubMed: 19357272]

69. Juntti SA, Tollkuhn J, Wu MV, Fraser EJ, Soderborg T, Tan S, Honda S, Harada N, Shah NM. The androgen receptor governs the execution, but not programming, of male sexual and territorial behaviors. Neuron 2010;66:260–272. [PubMed: 20435002]

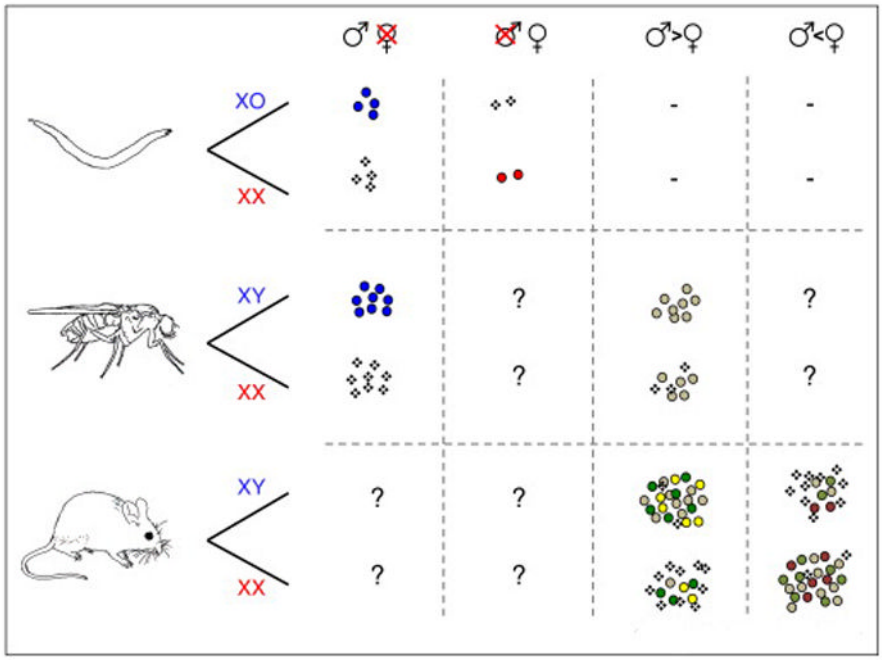

Figure 1.

Sexual differentiation of neuron number in worms, flies and mice. The columns represent four different possibilities: male-specific cell groups (cells completely absent in females), female-specific (absent in males), as well as male-or female-biased (greater cell numbers in one sex). In worms, sex differences in neuronal cell number are absolute. Male-specific neurons (e.g., CEMs) die in XX hermaphrodites (first column; healthy cells represented by colored circles and dead cells by stippling) while female-specific neurons (e.g. HSNs) die in XO males (second column). Flies provide examples of male-specific neurons (first column; e.g. P1 cluster) as well as quantitative sex differences favoring males (third column; e.g. mAL cluster). Compared to the vast literature on male behavior, much less is known about the neural substrate of female behavior; we are not aware of examples of female specific or female-biased cell groups in flies (question marks in columns two and four). In mice, sex differences are not absolute and cell numbers are vastly greater. Mice have no known examples of male-or female-specific neurons (columns one and two), unless one considers the subset of spinal motoneurons in the SNB that innervate only the bulbocavernosus muscle. There are examples of male-biased (e.g., BNSTp) and female-biased (e.g. AVPV) sex differences (columns three and four); in both cases, cell groups are comprised of many different phenotypes (different colored cells in columns three and four). Sex differences may be seen in overall cell number, in specific cell types, or both. As discussed in the text, the magnitude of the differences and the molecular mechanisms underlying sex differences in cell number can vary for different cell phenotypes within single sexually dimorphic nuclei.

Table 1

Sex determination mechanisms, examples of sexual dimorphisms in the nervous system, and core cell death pathways in nematodes (*Caenorhabditis elegans*), fruit flies (*Drosophila melanogaster*) and mice (*Mus musculus*).

	C. elegans	*Drosophila*	*Mus musculus*
Sexes	Male (XO), Hermaphrodite (XX)	Male (XY), Female (XX)	Male (XY), Female (XX)
Sex determining and differentiating mechanisms	X chromosome to autosome ratio determines production of a secreted protein (Her-1) that in turn regulates the activity of Tra-2 and Tra-1.	X chromosome to autosome ratio determines Sxl and Tra production leading to the alternate splicing of *fruitless* and *doublesex*.	Testis-determining gene on the Y chromosome causes formation of testes leading to the secretion of hormones that act throughout the body. Also direct sex chromosome effects.
Neural sex differences	**HSN** (egg-laying), **CEM, RN** (chemo- and mechanosensory detection of hermaphrodite)**, CP** motoneurons (male copulatoy behavior).	**fru-mAL** (pheromone processing), **P1** (courtship initiation), **MIND** (innervate male-specific muscle)	**SNB** (innvervate male-specific muscle), **BNSTp** (pheromone processing, sex behavior, stress), **AVPV** (control of ovulation).
Canonical cell death genes	egl 1/ced 9/ced 4/ced 3	hid/grim/reaper	Pro-survival and pro-death members of the Bcl-2 family

Abbreviations: AVPV, anteroventral periventricular nucleus; BNSTp, principal nucleus of the bed nucleus of the stria terminalis; CEM, cephalic companion neurons; fru-mAL, fruitless expressing neurons medially located above antennal lobe; HSN, hermaphrodite-specific neurons, MIND, muscle of Lawrence-inducing motoneuron; P1, fruitless expressing neurons in posterior region-1, RN, ray sensory neurons; SNB, spinal nucleus of the bulbocavernosus; Tra, transformer; Sxl, sex lethal.