

Box 51-1 Genetic and Neural Control of Mating Behavior in the Fruit Fly

In the presence of a female fruit fly, the adult male fly engages in a series of essentially stereotyped routines that usually culminate in copulation (Figure 51–6A). This elaborate male courtship ritual is encoded by a cascade of gene transcription within the brain and peripheral sensory organs that masculinizes the underlying neural circuitry.

Sex determination in the fly does not depend on gonadal hormones as it does in vertebrates. Instead, it occurs cell autonomously throughout the body. In other words, sexual differentiation of the brain and the rest of the body is independent of gonadal sex. The male-specific Y chromosome of fruit flies does not bear a sex-determining locus. Instead, sex is determined by the ratio of X chromosome number to autosome number (X:A). A ratio of 1 is determinative for female differentiation, whereas a ratio of 0.5 drives male differentiation.

The X:A ratio sets into motion a cascade of gene transcription and alternative splicing programs that leads to the expression of sex-specific splice forms of two genes, *doublesex* (*dsx*) and *fruitless* (*fru*). The *dsx* gene encodes a transcription factor that is essential for sexual differentiation of the nervous system and the rest of the body, with the sex-specific splice variants responsible for male- and female-typical development.

The *fru* gene encodes a set of putative transcription factors that are generated by multiple promoters and alternative splicing. In males, one particular mRNA (*fru*^M) is translated into functional proteins. In female flies, alternative splicing results in the absence of such proteins.

Males carrying a genetically modified *fru* allele that can only be spliced in the female-specific manner (*fru*^F) have essentially normal, *dsx*-dependent sexual differentiation. These *fru*^F males therefore resemble wild type males externally. However, the loss of *Fru*^M in these animals abolishes male courtship behavior directed toward

females. These data indicate that *Fru*^M is required for male courtship and copulation.

Conversely, transgenic female flies carrying a *fru*^M allele exhibit male mating behavior toward wild type females, indicating that *fru*^M is sufficient to inhibit female sexual responses and promote male mating.

Intriguingly, *fru*^F males do not court females and, like wild type females, do not reject mating attempts by wild type males or *fru*^M females. Similarly, *fru*^M females attempt to mate with both *fru*^M and wild type females. These data suggest that *fru*^M may also specify sexual partner preference, which in the case of wild type males would be directed to females.

In wild type females without *fru*^M, the neural pathways are wired such that these flies exhibit sexually receptive behaviors toward males. When groups of *fru*^F males (or *fru*^M females) are housed together, they court each other vigorously, often forming long chains of flies attempting copulation.

To build the circuitry underlying male courtship rituals, *fru*^M appears to initiate cell-autonomous male-typical differentiation of the neurons in which it is expressed. This leads to overt neuroanatomic dimorphism in cell number or projections of many classes of neurons (Figure 51–6B). Some neurons that express *fru*^M are not distributed in dimorphic patterns. In these neurons, *fru*^M may regulate the expression of particular classes of genes whose products drive a male-specific program of physiology and function.

Are neurons that express *fru*^M required for male courtship behavior? When synaptic transmission is genetically blocked in these neurons in adult males, all components of courtship behavior are abolished. Importantly, these males continue to exhibit normal movement, flight, and other behaviors in response to visual and olfactory stimuli. These findings demonstrate that *fru*^M appears to be expressed in a neural circuit that is essential for and dedicated to male fly courtship.

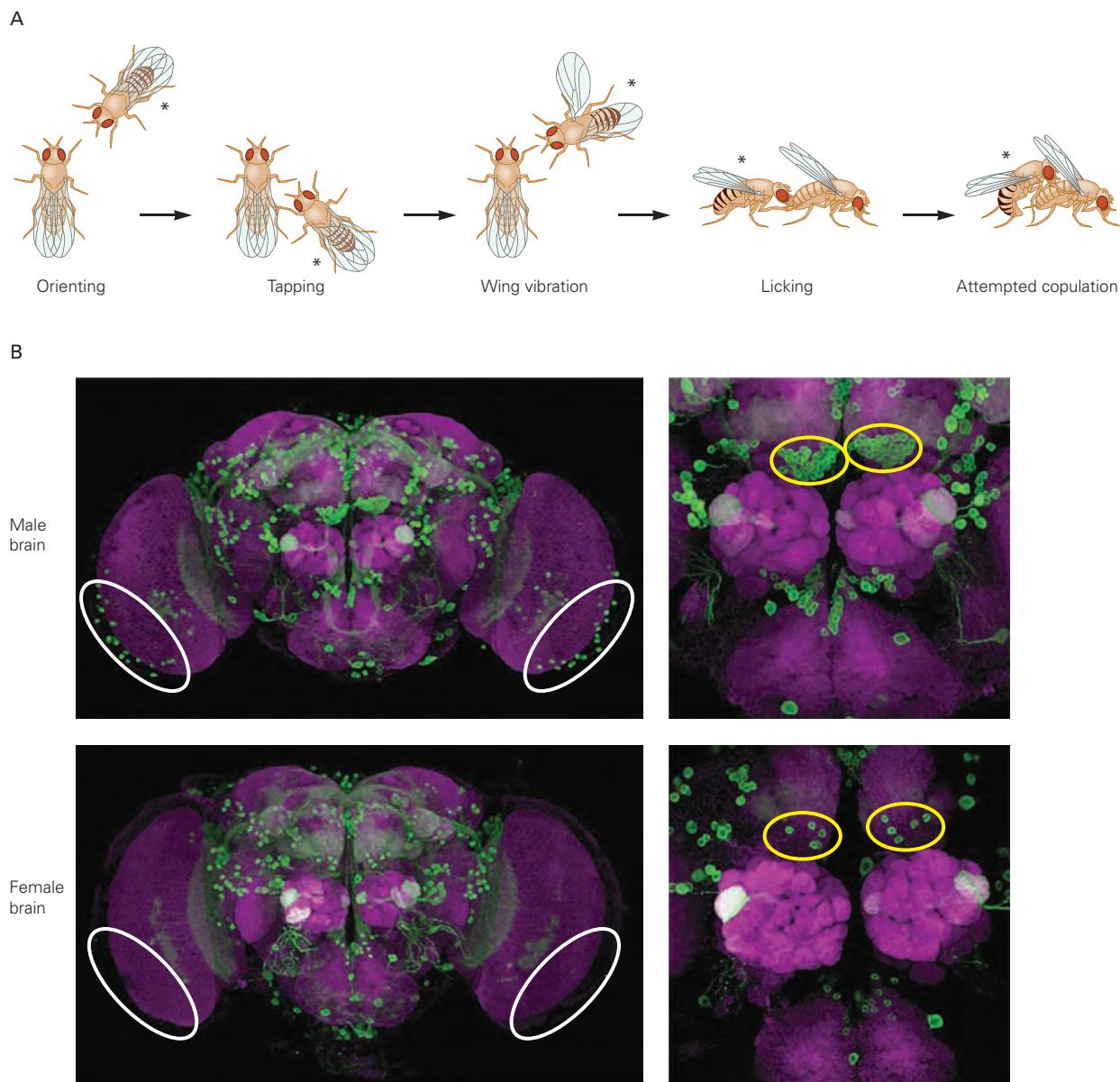


Figure 51–6 Control of male courtship in the fruit fly *Drosophila melanogaster*.

A. Male flies (labeled with asterisk) engage in a stereotyped sequence of behavioral routines that culminate in attempted copulation. The male fly orients toward the female and then taps her with his forelegs. This is followed by wing extension in the male and a species-specific pattern of wing vibrations that is commonly referred to as the fly courtship song. If the female fly is sexually receptive, she slows down and permits the male to lick her genitalia. The female then opens her vaginal plates in order to allow the male to initiate copulation. All steps in the male mating ritual require the expression of a sex-specific splice variant of the *fruitless* (*fru*) gene. (Adapted, with permission, from Greenspan and Ferveur 2000.)

B. The *fru* gene encodes a male-specific splice variant that is necessary and sufficient to drive most steps in the male fly courtship ritual. *Fru* expression is visualized using a fluorescent reporter protein (green) in transgenic flies. Neuronal clusters that express *Fru* are present in comparable numbers in the central nervous system of both male and female flies. However, there are regional sex differences in *Fru* expression. A cluster of *Fru*-expressing neurons is present in the male optic lobes (in the area within the white ellipses) but absent in the corresponding regions in the female brain. The two male antennal lobe regions (areas within yellow ellipses) contain about 30 neurons each, whereas each female region has only four to five neurons. (Adapted, with permission, from Kimura et al. 2005.)

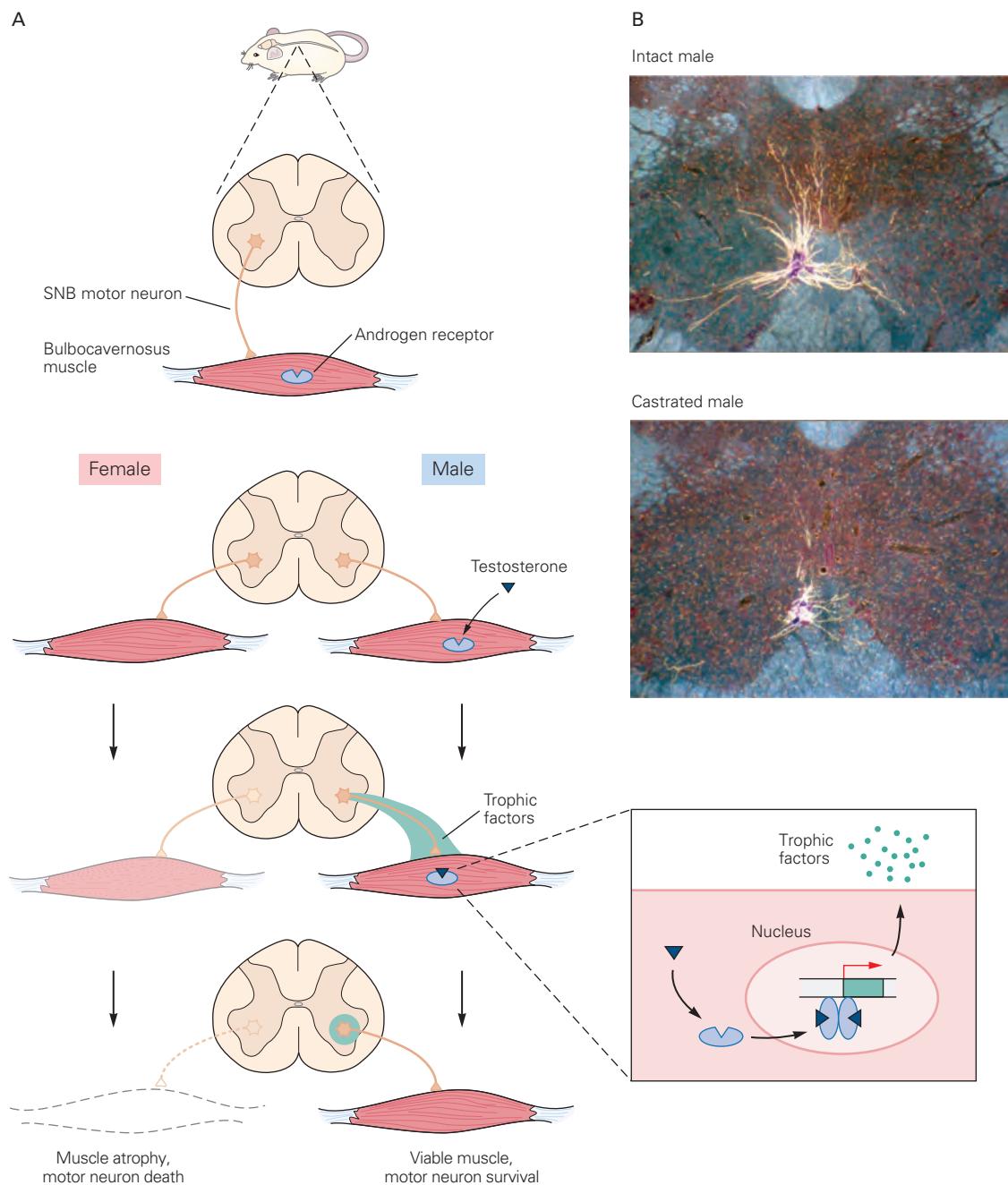
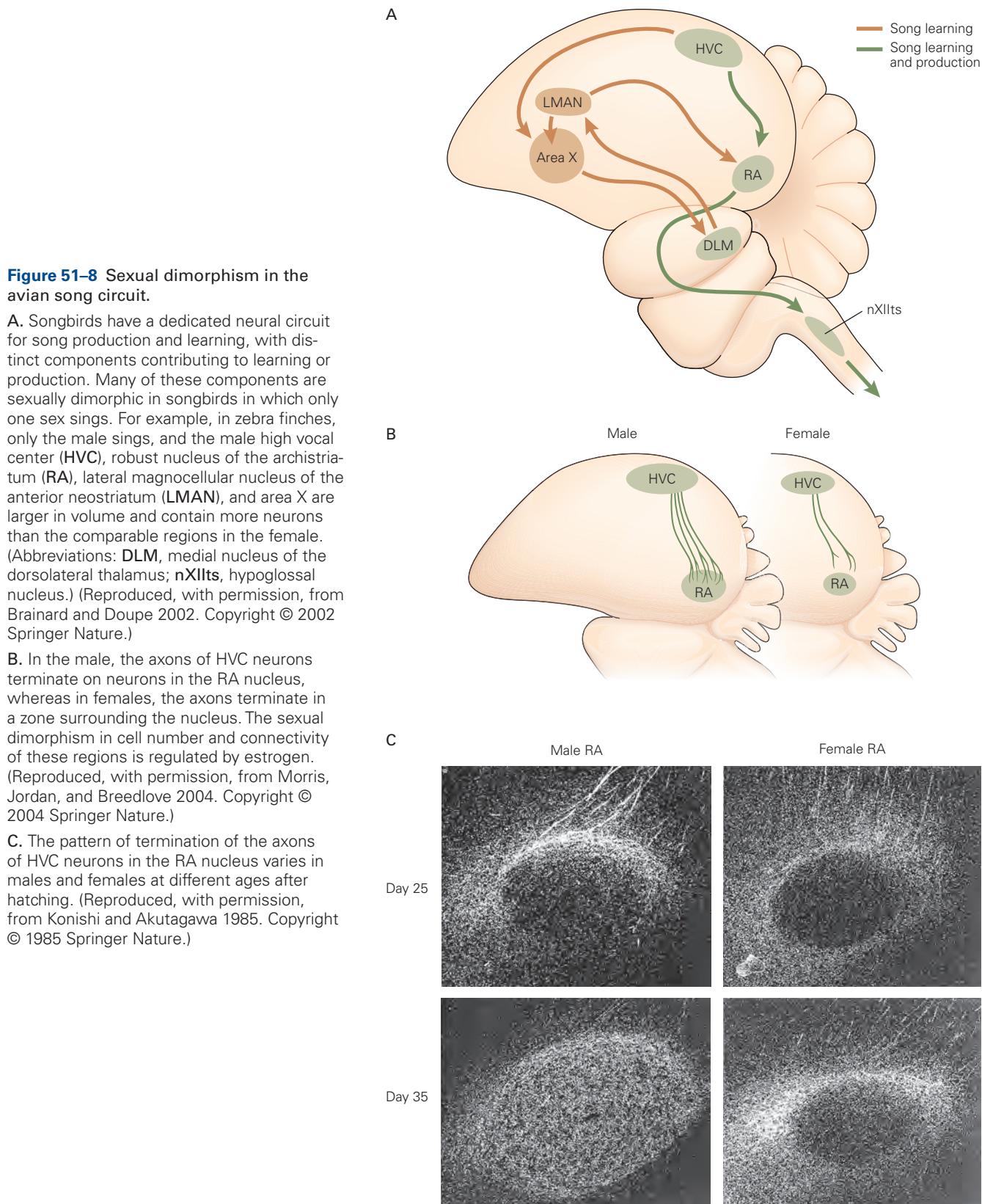


Figure 51-7 Sexual dimorphism in the spinal nucleus of the bulbocavernosus muscle in the rat.

A. The spinal nucleus of the bulbocavernosus (**SNB**) is found in the male lumbar spinal cord but is greatly reduced in the female. The motor neurons of the nucleus are present in both sexes at birth, but the lack of circulating testosterone in females leads to death of the SNB neurons and their target muscles. It is thought that testosterone in the male circulation promotes the survival of the target muscles, which express the androgen receptor. In response to testosterone, the muscles provide trophic support to the innervating SNB neurons. This muscle-derived survival factor is likely to be ciliary neurotrophic factor or a related member of the cytokine family. Thus, testosterone acts on muscle cells to

control the sexual differentiation of SNB neurons. (Reproduced, with permission, from Morris, Jordan, and Breedlove 2004. Copyright © 2004 Springer Nature.)

B. Dendritic branching of SNB neurons is regulated by circulating testosterone in adult male rats. In males, the dendrites arborize extensively within the spinal cord (**upper photo**). The fact that the arbors are pruned in adult castrated male rats (**lower photo**) is evidence that this dendritic branching depends on androgens. The spinal cord is shown in transverse section, and the SNB neurons and their dendrites are labeled by a retrograde tracer injected into target muscles. (From Cooke and Woolley 2005. Reproduced, with permission from D. Sengelaub.)



Mating Behavior in Mammals Is Controlled by a Sexually Dimorphic Neural Circuit in the Hypothalamus

In many mammalian species, the preoptic region of the hypothalamus and a reciprocally connected region, the bed nucleus of the stria terminalis (BNST), play important roles in sexually dimorphic mating behaviors (Chapter 41; Figure 51–4). In male rodents and monkeys, these areas are activated during mating behavior; surgical lesions that ablate the preoptic region or the BNST result in deficits in male sexual behavior in male rodents and, in the case of preoptic lesions, disinhibit female-type sexual receptivity in males.

Both the preoptic hypothalamus and the BNST are sexually dimorphic, containing more neurons in males compared to females. The sexually dimorphic nucleus of the preoptic area (SDN-POA) also contains significantly more neurons in the male. A male-specific perinatal surge of testosterone promotes survival of neurons in the SDN-POA and BNST, whereas in females, these same cells gradually die off in the early postnatal period. This development is similar to that in the sexually dimorphic nuclei of the rodent spinal cord and the songbird brain, suggesting that androgen control is a common mechanism for production of sex differences in the size of neuronal populations.

Curiously, the ability of brain testosterone to promote the survival of neurons is likely to be exerted via aromatization into estrogen and subsequent activation of the estrogen receptors (see Figures 51–3 and 51–4). How then is the neonatal female brain shielded from the effects of circulating estrogen? In newborn females, there is very little estrogen in the circulation, and the small amount present is easily sequestered by binding to α -fetoprotein, a serum protein. This explains why female mice lacking α -fetoprotein exhibit male-typical behaviors and reduced female-typical sexual receptivity. In this case, then, structural sexual dimorphism does not result from differential effects of androgens and estrogens, but rather from sex differences in the level of hormone available to the target tissue.

Environmental Cues Regulate Sexually Dimorphic Behaviors

Sex-specific behaviors are usually initiated in response to sensory cues in the environment. There are many such cues, and different species use distinct sensory modalities to elicit similar responses. Courtship rituals can be triggered by species-specific vocalizations, visual signals, odors, and even, in the case of weakly

electric fish, by electric discharges. Recent genetic and molecular studies have led to significant insight into how sensory experience controls some of these behaviors in rodents. Here, we discuss two examples: the regulation of partner choice by pheromones and the regulation of maternal behavior by experience during infancy.

Pheromones Control Partner Choice in Mice

Many animals rely on their sense of smell to move about, obtain food, and avoid predators. They also rely on pheromones—chemicals that are produced by an animal to affect the behavior of another member of the species. In rodents, pheromones can trigger many sexually dimorphic behaviors, including mate choice and aggression.

Pheromones are detected by neurons in two distinct sensory tissues in the vertebrate nose: the main olfactory epithelium (MOE) and the vomeronasal organ (VNO) (Figure 51–9A). It is thought that sensory neurons in the MOE detect volatile odors, whereas those in the VNO detect nonvolatile chemosensory cues. Removal of the olfactory bulb, the only synaptic target of neurons in the MOE and the VNO, abolishes mating as well as aggression in mice and other rodents. These and other studies indicate an essential role for olfactory stimulation in initiating mating and fighting.

Genetically engineered disruption of pheromone responsiveness in the MOE or VNO reveals that these sensory tissues have a surprisingly complex role in the mating behavior of mice. A functional MOE is essential to trigger male sexual behavior, and an intact VNO is required for sex discrimination and directing the male to mate with females.

Key to these experiments is the fact that olfactory neurons in the MOE and the VNO use different signal transduction cascades to convert olfactory input into electrical responses. The cation channel Trpc2 appears essential for pheromone-evoked signaling in VNO neurons; it is not expressed in MOE neurons, which use a different signal transduction apparatus. Thus, mice lacking the gene *trpc2* have a nonfunctional VNO and an intact MOE. Mating behavior directed to animals of the opposite sex appears unaltered in *trpc2* mutant males as well as females.

However, both male and female *trpc2* mutants often exhibit male sexual behavior with members of either sex. For example, *trpc2* mutant females mate with females in a manner seemingly indistinguishable from wild type males, except of course the females cannot ejaculate. These and other findings suggest that the VNO is used to discriminate among sexual partners.

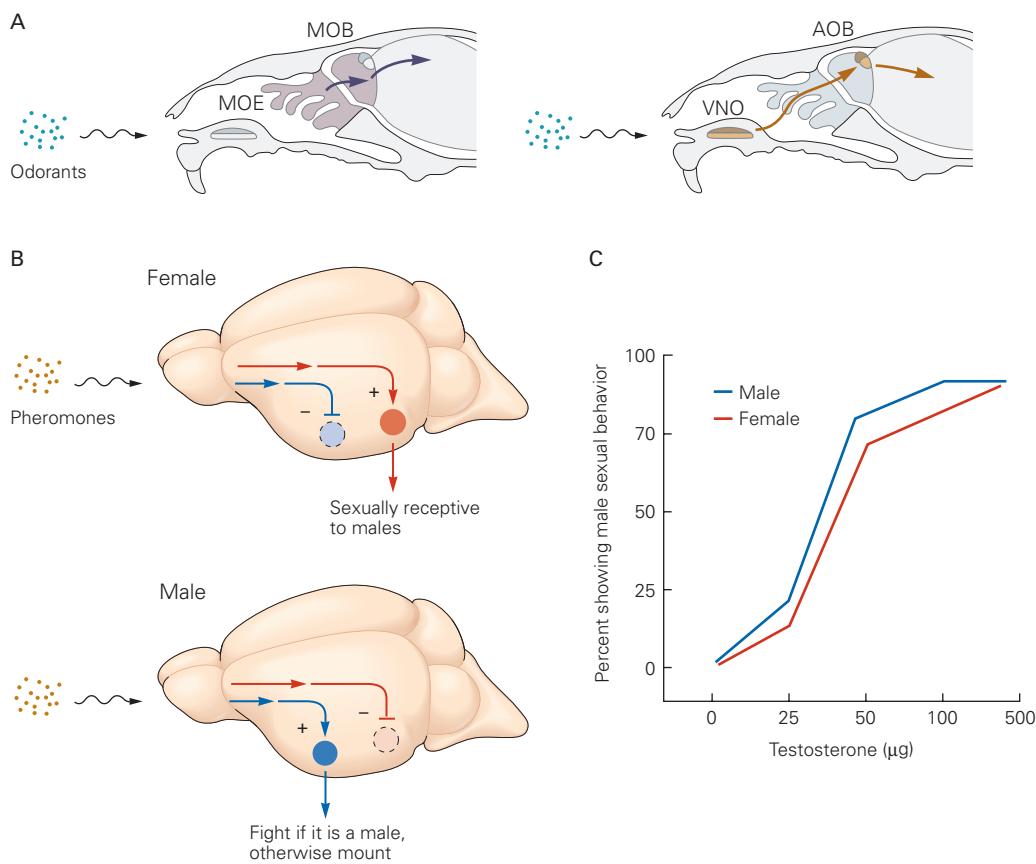


Figure 51-9 Pheromonal and hormonal control of sexually dimorphic behavior in mice.

A. Odorants are detected by sensory neurons in the main olfactory epithelium (MOE), which projects to the main olfactory bulb (MOB), and by neurons in the vomeronasal organ (VNO), which projects to the accessory olfactory bulb (AOB). Many of the central connections of the MOE and VNO pathway are anatomically segregated. (Adapted, with permission, from Dulac and Wagner 2006.)

B. Female mice possess the neural circuitry that can activate either male (blue) or female (red) mating behaviors. In wild type females, pheromones activate female mating behavior and inhibit male-type mating. By contrast, in males, pheromones activate a circuitry that will initiate fights with males and mating

with females. (Adapted, with permission, from Kimchi, Xu, and Dulac 2007.)

C. Testosterone activates male sexual behavior in male and female mice. The data are from a study in which the gonads of male and female mice were surgically removed in adulthood. None of the animals exhibited male sexual behavior with wild type females following surgery. After administration of testosterone, mating behavior was restored in castrated males, and females displayed male sexual behavior. This effect was dose-dependent; at the highest dose, male and female mice exhibited comparable levels of male-type mating behavior toward wild type females. (Adapted, with permission, from Edwards and Burge 1971. Copyright © 1971 Springer Nature.)

When the VNO is inactivated, animals can no longer distinguish between males and females, and mutants therefore exhibit male sexual behavior toward members of both sexes. Similarly, adult wild type females treated with testosterone also exhibit male sexual behavior toward females (Figure 51-9C).

One implication of these studies is that female mice possess the neural circuitry for male sexual behavior (Figure 51-9B). Activation of this neural circuit is inhibited in wild type females by sensory input from

the VNO and by the lack of testosterone. Removal of the VNO or administration of testosterone activates male sexual behavior in females. Male pattern mating behavior has been observed in females of many species, indicating that the findings in mice are likely to be of general relevance. Thus, neural pathways for male sexual behavior appear to be present in both sexes. Similarly, the female-typical behavior of male rats following hypothalamic lesions suggests that the neural pathway for female sexual behavior also exists in the

male brain. In such cases, it is the differential regulation of these circuits that underlies the sexually dimorphic expression of male and female sexual behaviors.

Early Experience Modifies Later Maternal Behavior

The preoptic area of the hypothalamus and the BNST are also important for another set of sexually dimorphic behaviors in females. Nursing rodents are good mothers, building a nest for their litter, crouching over the pups to keep them warm, and returning the pups to the nest when they happen to crawl away. Surgical lesioning or experimental stimulation of the preoptic region abolishes or activates these maternal behaviors, respectively.

Studies of these behaviors have shed light on variations among individual females and how these

differences exert lifelong effects on behavior of the offspring. Female lab rats exhibit distinct, stable forms of maternal care: Some lick and groom (LG) their pups frequently (high-LG mothers), whereas others lick and groom less frequently (low-LG mothers). Female offspring of high-LG mothers display high-LG activity when they themselves become mothers compared to female offspring of low-LG mothers (Figure 51–10). Moreover, pups of high-LG mothers show less anxiety-like behaviors in stressful conditions than do the pups of low-LG mothers.

These results suggested that levels of licking and grooming behavior and stress responses are genetically determined. However, studies by Michael Meaney and his colleagues provide an alternative explanation. When female rat pups are transferred from

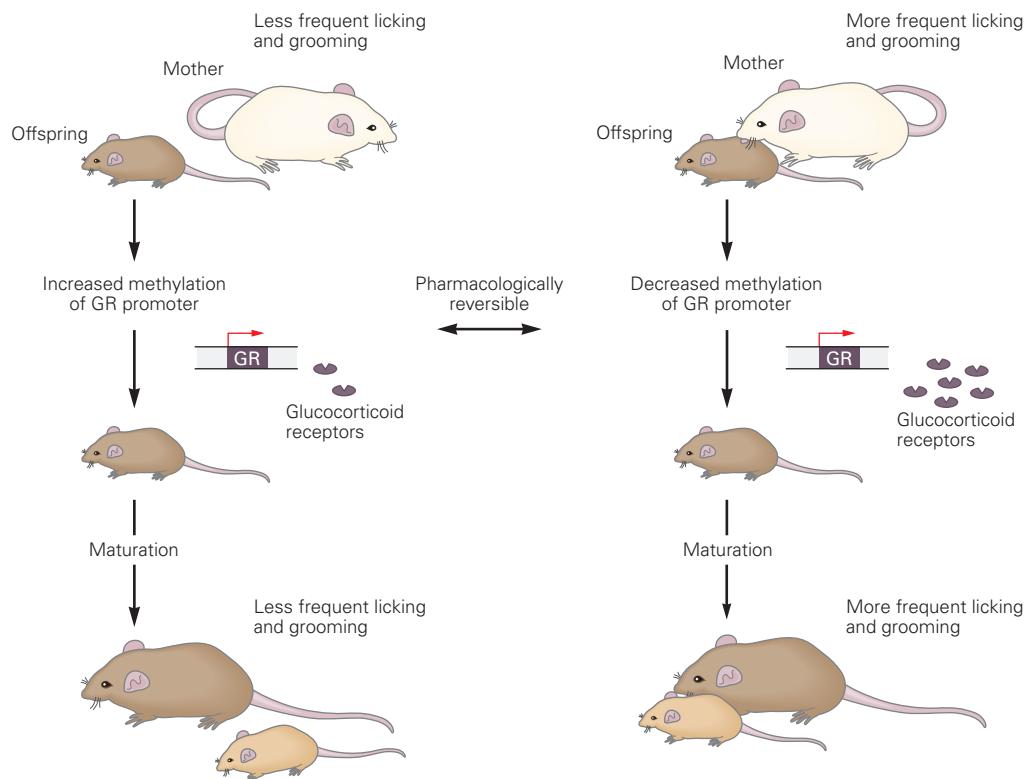


Figure 51–10 Epigenetic regulation of maternal behavior in rats. In a common lab rat strain, different mothers lick and groom their pups at low or high frequencies, resulting in distinct epigenetic modifications at the glucocorticoid receptor (GR) promoter. Mothers that lick and groom at high frequency raise progeny with low levels of DNA methylation at the GR promoter, resulting in higher levels of GR expression in the hippocampus. Females raised by these mothers exhibit higher frequencies of licking and grooming behavior

with their own pups. Mothers that lick and groom at low frequency raise progeny with high DNA methylation levels at the GR promoter and lower levels of hippocampal GR expression. Females nursed by these mothers subsequently exhibit similar low levels of licking and grooming of their pups. Pharmacological reversal of the epigenetic modifications at the GR promoter results in a corresponding change in both GR expression and maternal behavior. (Adapted from Sapolsky 2004.)

their mother to a foster mother at birth, their maternal behavior and stress responses as adults resemble those of their foster mother rather than those of their biological mother. Thus, experience in infancy can lead to lifelong behavioral patterns. Because these patterns impact maternal behavior, their influence can endure over many generations.

How does brief and early experience lead to such long-lasting changes? One mechanism involves a covalent modification of the genome. Stress responses are coordinated by glucocorticoids acting on glucocorticoid receptors in the hippocampus. Throughout life, tactile stimulation, including grooming, leads to transcriptional activation of the glucocorticoid receptor gene, which ultimately leads to reduced release of hypothalamic hormones that trigger stress responses. Tactile stimulation during early life also regulates the glucocorticoid receptor gene in a second way. A key site in the glucocorticoid receptor gene is methylated by the enzyme DNA methyltransferase, leading to gene inactivation. Initially, gene methylation occurs in all pups, but pups reared by high-LG mothers are selectively demethylated. Thus, in animals reared by high-LG mothers, the effects of adult experience are potentiated. This is an example of epigenetic modification by which genes can be turned on or off more or less permanently. These animals exhibit blunted behavioral responses to stressful stimuli later in life.

What are the biological links between early experience and behavioral variation? A peptide hormone, oxytocin, plays a major role. Classic work showed that oxytocin regulates provision of milk by the mother, which occurs via reflex ejection in response to suckling (milk let-down). Oxytocin is synthesized by neurons in the hypothalamus and released into the general circulation through their projections in the posterior pituitary. It elicits smooth muscle contraction in the mammary gland, resulting in milk ejection. Oxytocin release from the pituitary is controlled by suckling, which provides a sensory stimulus that is conveyed to the hypothalamus by spinal afferent nerves.

Oxytocin and a related polypeptide hormone vasopressin also play important roles in regulating maternal bonding and other social behaviors (Chapter 2). In these cases, experience appears to modulate behaviors by affecting both release of oxytocin and levels of the oxytocin receptor in specific brain areas. In both rats and voles, individual differences in the care females provide their offspring correlate with variations in oxytocin receptor level in specific brain areas. Especially noteworthy is that oxytocin receptor levels in several regions are higher in female offspring reared by high-LG mothers than in female progeny of low-

LG-mothers. Thus, sensory stimulation may affect activity of these polypeptide hormone systems, which in turn regulate maternal and other social behaviors.

A Set of Core Mechanisms Underlies Many Sexual Dimorphisms in the Brain and Spinal Cord

In the previous few sections, we described neural circuits that regulate several sexually dimorphic behaviors. Can we discern any common themes?

A variety of sexually dimorphic neural circuits, or wiring diagrams, can in principle generate sex differences in behavior (Figure 51–11). Although it is challenging to trace the chain of causality from genetic factors to dimorphic circuits to sex-specific behaviors, there are a few general possibilities. In one, a neural circuit, from sensory input to motor output, might be unique to one sex. In fact, this alternative is seldom encountered. Most behaviors are shared between the sexes, and even behaviors such as feeding, maternal retrieval of a pup by the scruff of its neck, or biting (during territorial scuffles between males) all call upon similar jaw movements. Consistent with this commonality, it appears that most sexual dimorphisms in behavior arise from sex differences in key neuronal populations within common circuits. The activity and connectivity of these populations alter behavioral output in a male- or female-typical manner.

Estrogen can act not only during development but also in adults to periodically reconfigure presynaptic connectivity within a hypothalamic neural circuit, ensuring that female mice only mate when they are ovulating and fertile. These studies paint a picture of dynamic neural circuits in the female brain: Wiring diagrams are plastic and responsive to hormonal changes across the estrous cycle, which is analogous to the menstrual cycle in humans. Similarly, estrogen also exerts cycle-related effects on dendritic spine plasticity in other brain regions, although the behavioral consequences in these instances are less well understood.

Another recurring theme in the developing brain is that masculinization is controlled by estrogen during the organizational phase. This control has profound enduring effects on social behaviors in adult life. Testosterone (which is aromatized into estrogen) or estrogen treatment of neonatal rodent females masculinizes the brain. As adults, these females are no longer sexually receptive to males, and in fact display male-typical social interactions, albeit at reduced intensity. Providing testosterone to these females, to mimic adult levels of testosterone in males, boosts the intensity of social behaviors, including territorial aggression (the propensity of animals to fight over territory or mates),

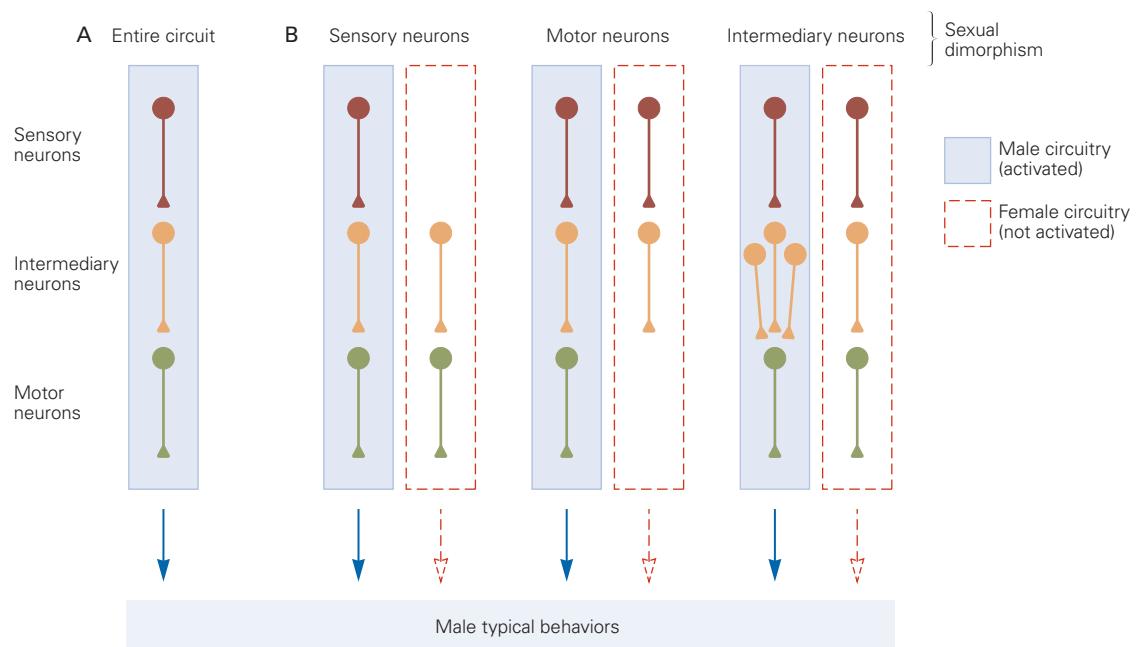


Figure 51–11 Possible circuit configurations that underlie sex differences in behavior. Neural circuit diagrams can be configured to generate sex differences in behaviors. Although it is possible to envision a neural circuit entirely exclusive to one or the other sex, most behaviors are shared between the sexes, and the current consensus is that sex differences in behavior or physiology reflect sexual dimorphisms in key

neuronal populations embedded within an otherwise shared neural circuit. Such sexual dimorphisms have been found at the level of sensory neurons, motor neurons (as discussed for spinal nucleus of the bulbocavernosus neurons), or neurons interposed between sensory and motor pathways (such as the BNST and the sexually dimorphic nucleus of the preoptic area).

to male-typical levels. Thus, the perinatal surge of testosterone acts largely via aromatization into estrogens to masculinize the brain, whereas in adult life, both testosterone and estrogen facilitate the display of male-typical social interactions (Figure 51–12A).

These findings imply that male mice lacking androgen receptor exclusively in the nervous system should not only have male genitalia but also exhibit male patterns of social behavior, albeit at reduced intensity. This has in fact been borne out nicely by genetic engineering studies in mice; such mutant male mice indeed appear indistinguishable externally from control males, but they exhibit male-type sexual and aggressive behaviors with diminished intensity. However, there is growing evidence that the developmental control of masculinization of the brain by estrogen has shifted during evolution such that testosterone may be the predominant masculinizing agent in primates, including humans.

How do the actions of the limited number of sex hormones modulate the display of a large array of complex social interactions such as courtship vocalizations (similar to songbirds, many animals, including mice, vocalize as part of their mating ritual), sexual

behavior, marking (the propensity of animals of many species to claim territory with pheromones secreted in bodily fluids), and aggression? As described earlier in this chapter, sex hormones bind to cognate receptors to modulate gene expression in target cells. These steroids are available at different times, amounts, and places in the brain of the two sexes. Accordingly, sex hormone-regulated genes are expressed in sexually dimorphic patterns that are also different for different brain regions. These genes regulate differentiation and adult function of neural circuits along male- or female-typical lines (Figure 51–12B).

Experimental inactivation of such sex hormone-regulated genes reveals that individual genes influence only a subset of the sexually dimorphic social interactions without altering the entire behavioral program of males and females. Thus, an additional emerging theme is that sex hormones control differentiation and function of neural circuits in a modular manner, with different sex hormone-regulated genes acting in distinct neuronal populations to regulate separate aspects of male- or female-typical behaviors. In short, there is no single neuronal population that governs