standing is still extremely incomplete and hampered by both technical and design limitations. For instance, all oxytocin administration studies to date have been performed in males, and oxytocin's influence on bonding and social behavior in women has not been investigated. Furthermore, it is not known whether intranasal application of vasopressin or oxytocin mimics physiologically relevant events or represents pharmacological artifacts.

Among genetic studies, convergent evidence supports a role for the *AVPR1A* locus in modulating human social behavior, but the link between genes, the brain, and behavior remains weak. For instance, *AVPR1A* polymorphism is associated with differences in amygdala activation and autism, but its correlation with gene expression has only been investigated in the hippocampus. Finally, only one study has investigated the distribution of oxytocin and vasopressin receptors within the human postmortem brain (*40*), and techniques have improved since its publication. Development of selective positron emission tomography ligands for both oxytocin and vasopressin receptors will allow for in vivo studies of receptor expression and shed new light on correlations between genetic polymorphisms, brain receptor variability, and social cognition in humans. Although these limitations hinder our understanding of these neuropeptide systems, they are largely not insurmountable.

Many diseases, such as depression and social phobia, display symptomatic altered or deficient social behavior. In severe instances, such as autism and schizophrenia, abnormal social behavior is extremely debilitating. Identifying the molecular underpinnings of these social deficits may yield important clues into their treatment. For example, peripheral infusion of oxytocin increased retention of social cognition via enhanced emotional understanding of speech intonation and, unexpectedly, decreased repetitive behaviors (*41*). As peptides do not efficiently cross the blood/brain barrier, it is unclear how peripheral infusion of oxytocin mediates these effects, but these results are nevertheless intriguing. Even within healthy populations, social support enhances our ability to deal with stress and recover from disease. Oxytocin administration enhances the stress-alleviating effects of social support (*42*). The therapeutic potential of manipulating the oxytocin system remains to be explored in clinical trials, and the development of potent, selective agonists that penetrate the blood/brain barrier would be an important advancement toward this goal.

An understanding of the neurobiology of social behavior raises important questions for society. Should salesmen be allowed to use airborne oxytocin agonists to manipulate trust toward their own benefit? Should marital therapists include oxytocin infusions along with behavioral therapies to salvage relationships? Will genetic testing be used to screen potential partners or prospective sons-in-law? These and other questions may become the topics of discussion for bioethicists as we gain more insights into the neurobiology and neurogenetics of oxytocin, vasopressin, and sociality.

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REVIEW

Wired for Sex: The Neurobiology of *Drosophila* Mating Decisions

Barry J. Dickson

Decisions about whom to mate with can sometimes be difficult, but making the right choice is critical for an animal's reproductive success. The ubiquitous fruit fly, *Drosophila*, is clearly very good at making these decisions. Upon encountering another fly, a male may or may not choose to court. He estimates his chances of success primarily on the basis of pheromone signals and previous courtship experience. The female decides whether to accept or reject the male, depending on her perception of his pheromone and acoustic signals, as well as her own readiness to mate. This simple and genetically tractable system provides an excellent model to explore the neurobiology of decision making.

Behavior unfolds as animals select specific actions on the basis of sensory input, internal physiological states, and individual experience. A major goal for neuroscience is to understand how information processing and storage in neural circuits guides such action selection, and thus behavior. Genetic approaches in model organisms greatly facilitate the identification, characterization, and manipulation of individual circuit elements and can thereby establish causal relationships linking cellular biochemistry, circuit function, and animal behavior.

The sex life of the fruit fly *Drosophila melanogaster* is an ideal subject for such a study. Males decide whom to court, and females decide with whom to mate. The world-wide distribution and abundance of *Drosophila*, and its success as a

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genetic model organism, attest to the fly's exceptional ability to get these decisions right. Whatever the underlying neural mechanisms are, there is no doubt that they are robust and adaptive. They are also accessible to both genetic and physiological investigation at the level of single identifiable neurons.

Sturtevant first described the fly's mating behaviors almost a century ago (*1*). Benzer and, in particular, Hall have led the genetic investigation of these behaviors over the past few decades (*2*). Increasingly, the fly's sex life is now also attracting the attention of neurobiologists. Building on these behavioral and genetic studies, researchers are probing the anatomy and function of the neural circuits that guide the mating decisions of *Drosophila*. It is still early days, but work on the fly's mating decisions has the potential to reveal fundamental mechanisms of action selection—to teach us how the brain maps sensory input, internal states, and individual experience onto moment-to-moment behavioral choices.

The Male's Decision

Upon encountering another fly, a male must decide whether or not to court (Fig. 1). *Drosophila* males do not provide conspicuous nuptial gifts, and the courtship ritual itself may take only a few minutes. With such a modest investment, males are generally eager to try their luck. Nonetheless, there is a considerable reproductive benefit for males that can focus their efforts on those flies most likely to accept them: sexually mature female virgins of the same species. Evolution has endowed male flies with the innate ability to discriminate females from males and to court only females. Discriminating receptive from unreceptive females, however, is a skill acquired at least in part through trial-and-error learning.

Males rely primarily on chemical signals to detect suitable courtship objects, including both volatile pheromones detected by the olfactory system and nonvolatile pheromones detected by the gustatory system. If the male perceives pheromone signals predictive of mating success, he initiates an elaborate courtship ritual. A central component of this ritual is the courtship song produced by unilateral wing vibration. This song, or the visible wing extension that accompanies it, is an early and measurable readout of the male's decision to court. How then do pheromone signals, interpreted by a male brain, and in the context of previous courtship experience, guide the decision to sing?

Evaluating the evidence: Pheromone detection and processing. Many different pheromones have been shown to regulate *Drosophila* mating behaviors (*3*), but only for very few of these do we know the receptors and neurons that mediate pheromone detection. The best understood of these is the male pheromone *cis*-vaccenyl acetate (cVA), a volatile compound that modulates both male and female behavior. In males, detection of cVA suppresses courtship behavior, including the courtship song (*4*–*6*).

Flies detect odors through members of a large family of odorant receptors (ORs) that form heteromeric odor-gated ion channels (*7*–*10*). These receptors consist of a common Or83b subunit and a variable subunit that confers ligand specificity. The subunit that confers sensitivity to cVA is Or67d, expressed in a specific class of olfactory sensory neurons (OSNs) (Fig. 2A). Or67d is required in these neurons for cVA detection (*5*), and ectopic expression of Or67d in other OSNs renders them sensitive to cVA (*11*, *12*). Detection of cVA is facilitated by SNMP (sensory neuron membrane protein), a transmembrane protein of unknown function (*13*, *14*), and Lush, a secreted odorant binding protein (*15*). Lush binds cVA, and in doing so undergoes a conformational change (*16*). It is most likely this activated form of Lush, rather than cVA itself, that is the ligand for the Or67d:Or83b receptor.

OSNs of a specific class send axons to a discrete and stereotyped glomerulus in the antennal lobe, the insect analog of the mammalian olfactory bulb (*17*). These projections convert the peripheral map of odorant receptor activation into a spatial map of glomerular activation in the brain. This map is in turn conveyed to higher brain centers by the second-order olfactory projection neurons (PNs). Most of the OSN and PN circuitry has been mapped out at cellular resolution (*18*–*22*). The Or67d+ OSNs target a glomerulus called DA1 (*5*, *18*), where they faithfully pass the cVA signal on to the corresponding DA1 PNs (*23*, *24*) (Fig. 2A). The Or67d+ OSNs and DA1 PNs are both narrowly tuned to cVA, but the PNs are much more sensitive, presumably reflecting signal amplification due to the convergence of ~50 OSN inputs onto ~6 PNs. Both Or67d+ OSNs and DA1 PNs respond equally to cVA in males and females (*5*, *23*). However, the DA1 PNs form sex-specific arborizations in

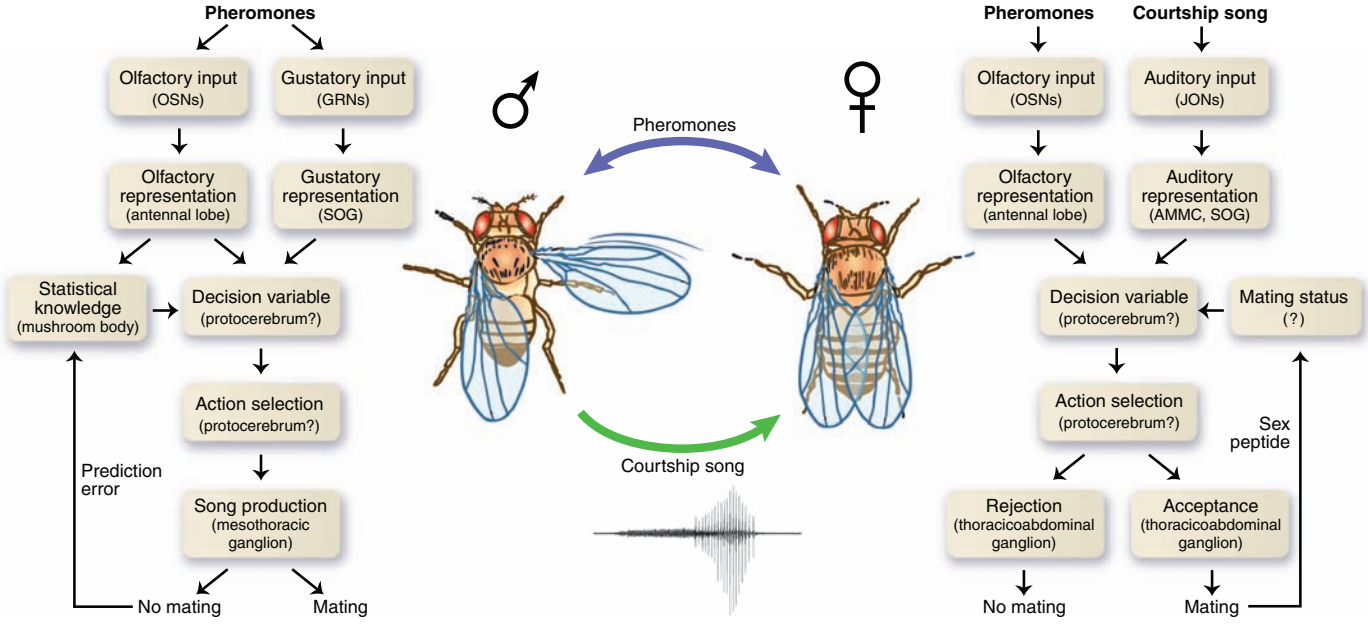

Fig. 1. Mating decisions. Elements of *Drosophila* male (**left**) and female (**right**) mating decisions. Parentheses indicate the relevant neurons or regions. OSNs, olfactory receptor neurons; GRNs, gustatory receptor neurons; JONs, Johnston's organ neurons; AMMC, antennal mechanosensory and motor center; SOG, suboesophageal ganglion. The decision variable reflects the likelihood of mating, constructed from sensory representations, acquired knowledge, and, for the female, current mating status. The decision variable guides a binary choice: for the male, to sing or not; for the female, to accept or reject the male.

the protocerebrum, hinting that they may feed the cVA signal into circuits that process it differently in males and females (*23*).

DA1 is one of three glomeruli that are larger in males than in females (*25*, *26*), analogous to the dimorphic glomeruli that receive pheromone input in the moth antennal lobe (*27*). The two other dimorphic glomeruli in *Drosophila* are VL2a and VA1v. The OSNs that innervate VL2a have not been identified, but VA1v has long been known as the target of OSNs that express the receptor subunit Or47b (*17*). Or47b confers sensitivity to fly odors produced by both sexes (*12*), and genetic perturbation of these neurons delays the onset of male courtship (*28*). This suggests that the Or47b/VA1v pathway may convey a pheromone signal that stimulates courtship toward either sex. This unidentified pheromone might, for example, be a species-specific stimulatory cue.

A simple model, then, is that sex and species might be encoded by the combined activity of the Or67d/DA1 and Or47b/VA1v pathways: *Drosophila melanogaster* female pheromones would activate the Or47b/VA1 pathway alone, whereas male pheromones would activate both pathways. Where in the brain would these signals be integrated? Current evidence suggests that pheromones, unlike fruit odors, are conveyed through the antennal lobe with little if any cross-channel processing (*24*). In the protocerebrum, however, the DA1 and VA1v PNs converge in a discrete region of the lateral horn that is spatially segregated from the region targeted by PNs that respond to fruit odors (*20*). This region is sexually dimorphic and may be the site at which the two pheromone signals are integrated to compute a sex-specific "decision variable" (*29*) that guides subsequent action selection (Fig. 1).

The male brain. Singing is a male-specific action. Females either do not select this action or cannot execute it. Such sex differences in neural function appear to be hard-wired during development (*30*). Sex in flies is primarily determined by the sex-specific splicing of two genes, *fruitless* (*fru*) and *doublesex* (*dsx*), both of which encode putative transcription factors (*31*–*33*). The expression and function of *fru*'s sex-specific transcripts is confined to the nervous system (*32*–*38*), whereas *dsx* acts in both neuronal and non-neuronal tissues (*39*). There is little overt dimorphism in the central nervous system, but numerous fine sex differences have been reported that depend on either *fru*, *dsx*, or both. A general rule may be that *dsx* controls neuroblast proliferation to produce initial differences in neuronal number (*40*, *41*), whereas *fru* acts in postmitotic neurons to regulate their survival or arborization patterns (*23*, *26*, *42*–*44*). The latter includes, for example, the dimorphic axonal arborizations of the DA1 PNs (*23*).

The sex differences sculpted by *fru*, but not those contributed by *dsx*, account for male-specific singing. Males that lack the male-specific fru^M isoforms do not sing (*33*, *38*), whereas those that lack dsx^M still do (*45*, *46*). Conversely, females forced to express fru^M sing (*47*), whereas those that express dsx^M do not (*45*, *48*). These observations justify the intense research focus on the set of neurons that express *fru* in efforts to understand how, in males, pheromone detection elicits singing.

There are ~2000 *fru*-expressing neurons in both sexes, including sensory, central, and motor neurons (*26*, *36*, *49*) (Fig. 2B). Among these are the OSNs and PNs that detect and process pheromones (*23*, *26*, *49*), motor neurons that regulate wing vibrations (*40*), and central neurons that contribute to the intervening neural processing (*43*, *50*). If the synaptic activity of all the *fru* neurons is blocked, all aspects of male courtship are suppressed, including song (*26*, *49*).

Classic studies of genetic sex mosaics (gynandromorphs) revealed that only a specific region in the dorsal protocerebrum must be genetically male for a fly to sing (*51*, *52*). The *fru* neurons in this region are therefore strong candidates to trigger courtship song. With modern genetic methods, this gynandromorph approach has now been extended to cellular resolution. If a small set of ~20 *fru* neurons in the dorsal brain, called the *fru*-P1 neurons, are masculinized in an otherwise normal female, then the female sings to other females (*43*). However, other neurons must also participate in the decision to sing, as many mosaic females without male *fru*-P1 neurons also sang in this study, and not all of those with male *fru*-P1 neurons did (*43*). Nonetheless, these findings suggest that the *fru*-P1 neurons are a critical element of the decision-making circuitry that triggers singing.

One implication of these gynandromorph studies is that the female thorax has the ability to sing, even though this action is never selected in a normal female. Indeed, direct optical stimulation of the thoracic *fru* neurons in headless flies ("flyPods") induces both males and females to sing (*50*). Evidently, a brief and artificial activation of thoracic *fru* neurons can kick-start local song-generating circuits present in both sexes. The same treatment in intact flies does not elicit robust singing, possibly because uniform activation of *fru* neurons in the brain generates conflicting inhibitory and stimulatory signals. Normally, these signals might be generated in a sex-specific manner in the brain *fru* neurons, including perhaps the *fru*-P1 neurons.

The song produced by female flyPods is not a perfect rendition of the normal male courtship song. It is, however, significantly improved by expressing fru^M in all the *fru* neurons (*50*). This implies that fru^M contributes to the sexual differentiation of the circuits that produce the song as well as those that call them in to action. Correct male-specific differentiation of these song circuits also requires dsx^M, because the song is aberrant in flies that express fru^M but lack dsx^M, regardless of whether they are male (*46*) or female (*40*).

The emerging picture is that *fru* contributes to the sexual differentiation of neural circuits at all levels—sensory processing, action selection,

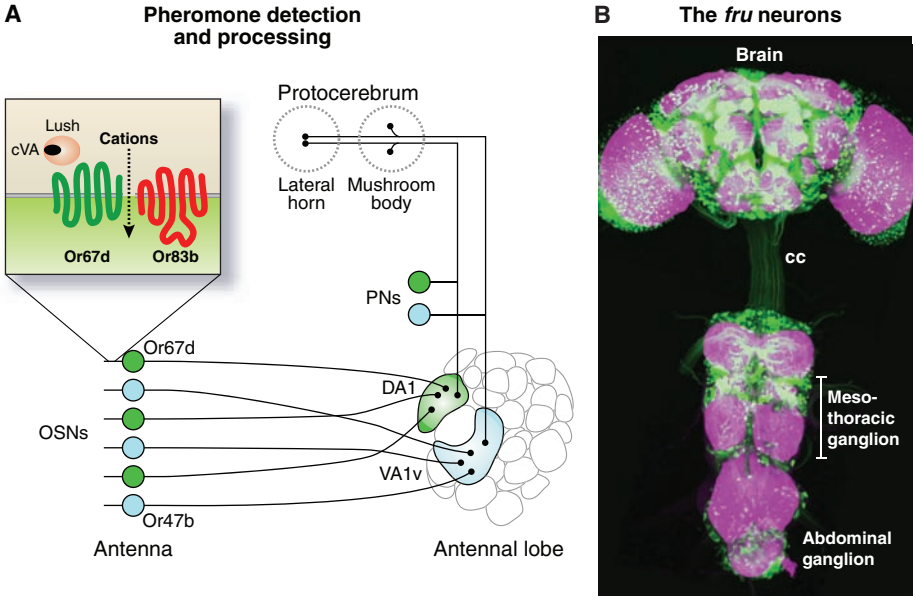

Fig. 2. Wired for sex. (**A**) cVA detection and processing in Or67d+ OSNs and DA1 PNs. (**B**) *fru*+ neurons in the CNS. Confocal image of the brain and ventral nerve cord of a fru^{GAL4} *UAS-PA-GFP* male, stained with nc82 to reveal synapses (magenta). Green fluorescent protein (GFP) fluorescence is shown in green. cc, cervical connective.

IMAGE PROVIDED BY ANNE VON PHILIPSBORN.

and action execution. Pronounced sex differences in behavior are due, however, to the differences that *fru* sculpts in circuits that select between alternative actions. In contrast, sensory and motor circuits may be largely common to both sexes but fine-tuned by *fru* and *dsx* for optimal performance according to the particular requirements of each sex. A consequence of this design is that perturbations that subvert or bypass the action selection circuits may readily cause one sex to inappropriately but poorly perform behaviors characteristic of the other.

Learning to predict mating success. Males and females differ reliably in their pheromone profiles, and evolution has encoded instructions for discriminating between them into the genome—instructions that are used to build hard-wired brain circuits. Obtaining a high rate of courtship success, though, also requires males to discriminate sexually mature virgins from immature females, unreceptive females that have recently mated, and females of other species. Because female pheromone profiles can vary substantially with time and place (*53*, *54*), the optimal classification scheme is not something that can easily be hard-wired into the brain. Innate mechanisms can implement a useful rule of thumb, but the optimal strategy for each location must be learned through experience. At least some circuit elements must remain plastic in order to record this experience. In this case, evolution has written into the genome the instructions for solving the classification problem, not the solution itself.

Evidence for this form of learning has come from experiments showing that males that experience courtship rejection by mated females are less inclined to subsequently court other mated females (*55*, *56*). Similarly, *Drosophila melanogaster* males experienced at courting *Drosophila simulans* females show suppressed courtship of other *simulans* females (*57*). In both cases, courtship of receptive *melanogaster* virgins is undiminished, indicating that the experienced male is indeed better able to discriminate receptive from nonreceptive females. This form of learning through trial-and-error interactions with the local environment is well modeled by classical reinforcement learning schemes (*58*).

The neural mechanisms that underlie this learning are still largely unknown. Dopamine neurons are strong candidates to convey reinforcement signals that report unexpected rejection and might be used to update circuits that compute or use the pheromone-based decision variable. The pheromone signals mediated by the Or67d/DA1 and Or47b/VA1v olfactory pathways are candidates for such experience-dependent modulation. The male pheromone cVA is transferred to females during mating (*59*, *60*) and could potentially be used to discriminate mated females from virgins. Indeed, cVA has been proposed to contribute to courtship learning, albeit not as a conditioned stimulus but rather as an unconditioned stimulus that suppresses the male's subsequent response to attractive female pheromones (*60*). This study, however, examined a general suppression of courtship toward all types of female that is sometimes observed when decapitated animals are used as test objects, not the selective suppression of courtship toward mated females that is observed with live animals (*55*, *56*, *61*, *62*).

A likely site for any experience-dependent modulation of pheromone responses is the mushroom body, a well-studied center for olfactory learning in insects (*63*). This protocerebral brain region receives input from both olfactory and reinforcement pathways. A specific class of mushroom body neurons—the γ neurons—express *fru*, and two separate lines of evidence have implicated these neurons in courtship learning. First, disrupting *fru* function in γ neurons blocks courtship suppression in short-term learning paradigms (*49*), although this has only been tested in assays for general rather than selective courtship suppression. Second, selective long-term courtship suppression requires the CPEB protein Orb2 specifically in γ neurons during or shortly after training (*61*). If mushroom body γ neurons are indeed the site for plasticity in pheromone processing, then we still need to identify the missing circuit elements that would integrate this signal with the lateral horn pathway so that past experience can guide future action.

The Female's Decision

Once the male decides to court, whether mating actually occurs is largely a matter of female choice (Fig. 1). The female decides on the basis of her assessment of her suitor's quality and on her own readiness to mate. If she decides to accept the male, she slows down and opens her vaginal plate for copulation. If not, she rejects the male by extruding her ovipositor in his direction, or simply flying away. How does the female select between these alternative actions, guided by stimuli from the male and her own internal state?

Assessing male quality. Female mating is in part stimulated by male pheromones, including cVA acting through the Or67d receptor (*5*). The most potent signal from the male, however, is his courtship song (*64*, *65*). Mute males (with clipped wings) have very little chance of courtship success (*1*, *66*), as do males that produce a poor song (*67*). The courtship success of mute males is, however, greatly improved by playback of a prerecorded song from a high-quality male (*66*, *68*). The song alone even induces lone females to slow down their movement (*69*, *70*), just as receptive females normally do in the presence of a singing male. The critical component of the courtship song is a series of short pulses, typically spaced about 35 ms apart (*68*, *71*, *72*). This interpulse interval is species-specific (*72*) and is a key factor in species recognition (*73*, *74*).

Flies detect sound through rotational movements of the antenna that are induced by the vibration of air particles (*75*). These movements activate exquisitely sensitive stretch receptor neurons in Johnston's organ, the fly's "ear," capable of detecting displacements of just a few nanometers (*76*). Just as the distinct classes of OSN in the olfactory system are each specialized to detect specific kinds of odorant, distinct classes of Johnston's organ neurons (JONs) may respond to distinct mechanical stimuli. Some may detect courtship song, but others may respond to vibrational signals important for other forms of acoustic communication (*77*), for flight control (*78*), or in gravity-sensing (*79*). In this regard, it is interesting to note that distinct subsets of JONs project to distinct regions in the brain (*80*), hinting at a functional segregation of mechanical signals analogous to the segregation of food odors and pheromones in the olfactory pathways. Many JONs are *fru*+ (*26*, *49*) and may have specific roles in the detection or processing of courtship song analogous to the specialization of *fru*+ OSNs for pheromone detection.

Female receptivity. How the female responds to these male signals depends on her own readiness to mate. Young virgins do not mate, nor do females that have recently mated. Adult females reach sexual maturity only when they are 1 to 2 days old (*81*). Immature virgins are, however, still attractive to males, who court them vigorously. This experience may provide females with an opportunity to learn about the quality of local males (*82*), just as males may use this experience to learn about local female pheromone profiles (*55*).

Once they mate, females store sperm for extended periods and use it efficiently (*83*). Unless they encounter a second male of substantially higher quality, there is little to be gained by mating again. It is also in the first male's interest that a female, once inseminated, does not readily mate again. This common interest has led to the evolution of a mechanism that renders females unreceptive after an initial mating, typically lasting until her sperm supply is depleted (*81*). Male seminal fluid contains a small peptide, the sex peptide (SP), that binds tightly to sperm (*84*, *85*). SP induces a suite of postmating responses in the female, including her reluctance to mate again. Females that mate to mutant males lacking SP readily mate again (*86*, *87*). Conversely, direct injection of SP into virgin females renders them unreceptive (*84*).

In the female, SP activates a specific receptor, SPR, a G protein-coupled receptor that activates the adenosine 3′,5′-monophosphate signaling pathway (*88*). Females lacking SPR remain receptive even after an initial mating or SP injection. SPR is broadly expressed in the nervous system, but its function is both necessary and sufficient within the *fru* neurons (*88*). Evidently, SP regulates female receptivity by modulating the properties of some subset of the *fru* neurons.

The female brain. Circuits somewhere in the female brain must integrate sensory inputs from the olfactory system, auditory system, and reproductive tract to decide between the alternative actions of accepting or rejecting the male (Fig. 1). These circuits remain largely uncharted. Classic gynandromorph studies have mapped a region of the dorsal brain that must be genetically female for a mosaic animal to be receptive (*89*), but modern methods for creating genetic mosaics have not yet been exploited to extend this analysis to cellular resolution.

It is possible that *fru* neurons in the brain are involved in female mating decisions, just as they are in male mating decisions. Silencing synaptic transmission of the *fru* neurons in females inhibits female receptivity (*90*), as does masculinizing them with fru^{M} (*47*). Similarly, although fru^{F} males (which produce female rather than male *fru* transcripts) are not particularly attractive to other males, they do show features of female behavior when they are occasionally courted (*91*). These fru^{F} males also behave like females when tested for aggression (*92*). Collectively, these findings strongly implicate at least some of the *fru* neurons in female behavior. However, none of these effects has yet been mapped to a specific subset of the *fru* neurons, and so it is still too early to tell whether they reflect the perturbed function of *fru* neurons in sensory, central, or motor processing. *fru* neurons clearly are involved in the sensory input relevant for female decision making, including *fru*+ pheromone-sensing neurons. The critical question, however, is whether *fru* neurons also contribute to the female decision-making circuits, and if so, whether these are the same as or different from those circuits that make mating decisions in the male. The classical gynandromorph studies suggest that they might be distinct (*89*).

Perspective

I have presented here our current understanding of the neural mechanisms that guide the mating decisions of male and female fruit flies. This picture contains many obvious gaps, and an urgent goal is to trace out the relevant neural circuits more completely and at cellular resolution. With the powerful genetic methods now available for circuit dissection (*93*, *94*), this should not take too long. Indeed, the precision with which individual circuit elements can be identified and manipulated is advancing much more rapidly than the methods for analyzing circuit function. Explaining behavior may be the ultimate goal, but behavior itself is a noisy and distal readout of circuit function. The behavioral output of genetically perturbed neural circuits will not always be meaningful, but the physiological properties of specific circuit elements may well be changed in highly informative ways.

This work should gradually reveal how chemical and auditory cues are detected and processed in the fly's brain, how these signals are interpreted in the context of internal physiological states and past experience, and how this information is used to make decisions that are fundamental to the animal's reproductive success. Will this teach us anything about information processing and storage in even more complex brains? Mating behaviors and their key regulatory genes evolve rapidly, and we cannot expect to extend these findings across vast evolutionary distances by homology. Neural networks may, however, be built by assembling simple and common modules into complex neuronal architectures (*95*, *96*). Similar architectures may be used to solve similar computational problems, even if the molecular mechanisms differ. The mammalian and insect olfactory systems, for example, use molecularly distinct receptor families to detect odors, and these odors trigger very different sets of behaviors. Yet, surprisingly, they process olfactory information in very similar ways (*97*). There may be only a limited set of efficient neural solutions to complex behavioral problems, including difficult decisions such as choosing a mate. Studying this process in the fly holds the promise of revealing how the computations performed by defined neural circuits can guide decision making and behavior, and how these computations emerge from the biochemical properties of the constituent neurons and their connections.

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PERSPECTIVE

Searching for Genes Underlying Behavior: Lessons from Circadian Rhythms

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The success of forward genetic (from phenotype to gene) approaches to uncover genes that drive the molecular mechanism of circadian clocks and control circadian behavior has been unprecedented. Links among genes, cells, neural circuits, and circadian behavior have been uncovered in the *Drosophila* and mammalian systems, demonstrating the feasibility of finding single genes that have major effects on behavior. Why was this approach so successful in the elucidation of circadian rhythms? This article explores the answers to this question and describes how the methods used successfully for identifying the molecular basis of circadian rhythms can be applied to other behaviors such as anxiety, addiction, and learning and memory.

In the 1970s, Seymour Benzer and his colleagues uncovered a remarkable number of genes that underlie neural and behavioral functions. They treated the fruit fly *Drosophila* with mutagens and systematically screened them for behavioral abnormalities (*1*, *2*). The discovery, in one of these screens, of flies with mutations in the *period* gene—which show longer or shorter cycles of the flies' endogenous 24-hour clock—by Konopka and Benzer (*3*) remains the exemplar for genetic dissection of behavior (*4*). Why was the search for circadian mutants so successful, and why were unbiased approaches to gene discovery so important?

Today, we understand the molecular mechanism of the circadian clock in a number of model organisms ranging from bacteria to humans (*5*, *6*). In retrospect, it is clear that the genes regulating circadian rhythms would not have been easily uncovered without the use of forward genetic screens (*2*). In each model organism (*Drosophila*, *Neurospora*, cyanobacteria, *Arabidopsis*, and mouse), previously unknown pathways were identified by the cloning of circadian mutants (*5*). Even today, with the benefit of complete genome sequences, the function of most of these "clock genes" would have been difficult to work out without those screens because our preconceived notions of the properties of a clock gene were largely incorrect. For example, a long history of anatomical and physiological experiments in mammals [beginning with the localization of the central clock, the suprachiasmatic nucleus (SCN), in 1972 (*7*)] indicated that clock genes should be tissue-specific and restricted to the SCN. In addition, it was assumed that clock genes would be transcribed in a circadian pattern. Both of these assumptions were incorrect, at least in part. The *Clock* and *Period* genes are expressed ubiquitously, and *Period*, but not *Clock*, is expressed in a circadian pattern (*8*–*10*). We now realize that clock genes are really housekeeping genes and are integral to the most basic functions of cells, and that virtually all cells in the body contain cell-autonomous circadian oscillators (*11*–*13*).

The genetic screens for circadian mutants were successful because we have a deep understanding of circadian phenotypes and robust assays. Of the measurable parameters of circadian rhythms (period, phase, and amplitude), the choice of circadian period as a primary phenotype has proven to be key. Circadian period length is a fundamental aspect of the clock that can be easily and accurately measured by 24/7 automated monitoring (*14*). Parameters such as amplitude are inherently ambiguous because they can be influenced by processes downstream of the circadian oscillatory system (that is, by output pathways). Phase measures can also be ambiguous because phase can be influenced by changes in input pathways that entrain the oscillator. Under steady-state conditions, period length (even when measured at the behavioral level) is directly correlated with the period of the underlying circadian pacemaker system (*15*) and thus is a very sensitive measure of the rate-limiting molecular steps in the circadian pathway.

Another key to success has been the accuracy with which circadian period length can be measured. The onset of rhythms in activity—in particular, wheel-running by rodents—is a remarkably precise phenotype (*14*). The inbred mouse strain C57BL/6J, for example, shows an average period length for circadian wheel-running in constant darkness of 23.7 hours with a standard deviation (SD) of 0.17 hours, or 10 min (*16*). This is a relative standard deviation (RSD = SD/mean) of only 0.72% (Fig. 1A and table S1). [The RSD of circadian rhythms of individual mice is even lower, about 0.2%, which is second in precision only to the neural oscillator driving the electric organs in fish (*17*).] Thus, in genetic screens, more than 99% of C57BL/6J mice have circadian periods between 23.2 and 24.2 hours (which represents ±3 SD from the mean); and any mutagenized mouse with a period outside this range is likely to be a mutant. (The precision of circadian rhythms in mice is strain-dependent, and C57BL/6J is one of the most precise for period length.) Indeed, phenotype-driven genetic screens based on period length have been the most successful for the discovery and functional assessment of circadian clock genes (*2*).

How can one apply what we have learned from circadian clock genetics to discover genes underlying other complex behaviors in the mouse? Over the past decade, my colleagues and I have systematically applied forward genetic screens in the mouse using the point mutagen *N*-ethyl-*N*-nitrosourea (ENU) to find mutants that affect learning and memory, anxiety, locomotion, vision, and response to psychostimulant drugs (*18*). To select appropriate screens, we looked for behaviors in which the phenotype was well established and for which we had an understanding of the neural loci and circuitry underlying the behavior. We required that the behavioral assay be amenable to automated data acquisition. The phenotypic screen also had to be scalable to achieve a throughput of more than 200 mice screened per week, so that ~10,000 mice could

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