Sandoz, G., Bichet, D., Cornet, V., Mori, Y., Felix, R., and De Waard, M. (2001). Eur. J. Neurosci. *14*, 987–997.

Soldatov, N.M., Oz, M., O'Brien, K.A., Abernethy, D.R., and Morad, M. (1998). J. Biol. Chem. *273*, 957–963.

Stotz, S.C., Jarvis, S.E., and Zamponi, G.W. (2003). J. Physiol., in press. Published online August 8, 2003.

Zuhlke, R.D., Pitt, G.S., Deisseroth, K., Tsien, R.W., and Reuter, H. (1999). Nature *399*, 159–162.

Toward a Molecular Description of Pheromone Perception

Successful sexual courtship depends on complex multimodal sensory integration. In this issue of *Neuron*, Bray and Amrein identify a male-specific gustatory receptor gene, *Gr68a*, expressed in leg chemosensory neurons. Genetic ablation of these *Gr68a* neurons suggests that they are intimately involved in perceiving female pheromone cues necessary for mating.

Drosophila fruit fly males, like males of most other species, must display an elaborate series of courtship behaviors in order to persuade females to mate (Figure 1). In a ritual that may seem uncannily familiar to humans, the male begins by locating and orienting toward the female. Next, the male taps the female on the abdomen with his foreleg and follows her if she is in motion. He extends and vibrates his wings in courtship song, then licks her genitalia with his proboscis and bends his abdomen in attempted copulation. The male may pause at any stage in the courtship behavior but will usually resume and persist in this sequence until copulation occurs. The initiation of courtship behavior is dependent upon multiple sensory stimuli. Visual and olfactory cues direct the orientation and following behaviors. Gustatory cues obtained while tapping and licking enable the male to assess the pheromone profile of his potential mate (reviewed in Hall, 1994; Greenspan and Ferveur, 2000). Integration of information from these complex stimuli is necessary for efficient courtship. Which peripheral neurons receive these sex-specific signals and how they are centrally integrated to produce stereotyped behaviors is poorly understood.

Fly courtship behavior appears to be innate, requiring neither previous experience nor learning to be perfected. This poses the intriguing question of how this behavior is genetically programmed. Thus far, investigations into genes influencing courtship behavior have focused on those that control sex differentiation during development. Mutations affecting several transcription factors that act early in the sex determination cascade (*doublesex, dissatisfaction, fruitless*) alter courtship behavior in adult flies. However, the tissue distribution of these genes is rather broad, suggesting a pleotropic effect on many target cells. Further, the downstream targets of these transcription factors are largely unknown, and the extent to which they influence sexual dimorphism in neural organization and wiring is unclear (reviewed in Hall, 1994; Greenspan and Ferveur, 2000). There is much to be learned about how the genetics controlling the organization of each sensory system allows information from external stimuli to be translated into behavior.

In other insect species, pheromones emitted by females are key elements in the process of attracting males into the proximity of receptive females for mating (Hildebrand, 1995). Studies of the neurophysiology and chemical ecology of these long-range moth pheromones have been extremely influential in our understanding of how the nervous system processes odor cues. However, it has not been possible to understand the molecular genetic basis for pheromone perception in these insects. It is therefore of interest to investigate the role of pheromones in *Drosophila*, a genetically tractable species. Cuticular pheromones have been shown to influence the stereotyped behavior exhibited by male *Drosophila* during courtship, acting either to stimulate his serenade toward a potential mate or to inhibit this activity toward other males, recently mated females, or females of other species. The major components of the cuticular pheromone profile are long chain hydrocarbons of limited volatility found predominantly on the abdomen. In *D. melanogaster*, the major female pheromones are 7, 11 heptacosadiene and 7, 11-nonacosadiene, which stimulate male courtship behavior. Males produce 7-tricosene, which inhibits courtship between males (Ferveur and Sureau, 1996). These and other cuticular hydrocarbons are present in varying ratios in different species, promoting courtship among members of the same species and potentially discouraging interspecies mating.

Chemical messages from nonvolatile cuticular pheromones are believed to be detected by contact chemoreceptor neurons located on the male fly, but to date the identity of these neurons has been somewhat mysterious. Males have a greater number of taste bristles on their foretarsi than females (reviewed in Stocker, 1994), and stimuli received through foretarsi have been shown to be sufficient to induce courtship behavior when other sensory organs (antenna, palps, proboscis) are surgically ablated (Robertson, 1983). While observation suggests a role for these organs in pheromone detection during the contact behaviors of courtship, the identity of neurons receiving these chemical signals is poorly understood and the contribution of specific chemosensory receptor proteins has not been previously demonstrated. Several years ago, a large family of gustatory receptor (GRs) genes was identified from genome sequences and found to be expressed on several appendages, including labial palps of the proboscis, foretarsi, maxillary palps, wings, and female genitalia (Clyne et al., 2000; Dunipace et al., 2001; Scott et al., 2001). In this issue of *Neuron*, Bray and Amrein (2003) identify a subset of gustatory receptor neurons (GRNs) that are required for efficient courtship and demonstrate that the gustatory receptor expressed in these cells, *Gr68a*, is responsible for this contribution.

Using *Gr68a*-Gal4 lines to drive the expression of marker proteins β-galactosidase or green fluorescent protein, Bray and Amrein show that *Gr68a* is expressed exclusively in chemosensory neurons innervating taste bristles on male forelegs. Chromosomally female flies with mutations in the sex determination pathway genes

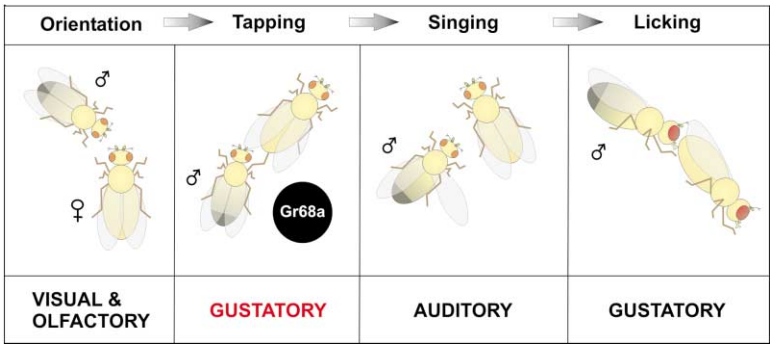

Figure 1. Stimuli Influencing *Drosophila* Courtship Behavior
Gr68a is expressed in male foretarsi and contributes to pheromone perception during the tapping stage of courtship.

tra or *dsx* show male expression patterns for *Gr68a*. The location and sexually dimorphic expression pattern of *Gr68a* as well as its regulation by genes in the sex determination cascade would be consistent with a role in pheromone detection during the contact behaviors of courtship.

To demonstrate the contribution of this subset of GRNs to courtship behavior, Bray and Amrein block synaptic transmission in *Gr68a* neurons with tetanus toxin light chain and observe effects on courtship behavior and mating success. Inactivation of *Gr68a* neurons reduces mating efficiency, implicating *Gr68a* in the reception of pheromone cues during courtship. A closer look at several stages of courtship behavior indicates a role for these neurons that begins early in the courtship sequence. The greatest differences in courtship behavior resulting from inactivation of *Gr68a* neurons are observed at the singing and licking stages, while the ability to orient and commence courtship activity does not seem to be greatly affected. These results are consistent with a functional role for *Gr68a* neurons in pheromone detection during the tapping stage of the courtship sequence.

Although most GRNs are thought to express only one GR, it is not known whether additional GRs are coexpressed with *Gr68a*. In order to attribute the reduction in courtship behavior to *Gr68a* specifically, the authors "knock down" the expression of *Gr68a* protein by inducing the expression of double stranded *Gr68a* RNA with the *Gr68a*-Gal4 driver. The initial reduction in mating success is less than that seen when the entire neuron is inactivated; however, the severity of the phenotype increases over time in a manner consistent with the dynamics of protein expression disrupted by the Gal4-RNAi method. Although these RNAi experiments are suggestive, only a genuine deletion of *Gr68a* would ultimately demonstrate that the function of the *Gr68a*-expressing neurons depends solely on this GR gene and not additional GR genes coexpressed in these neurons. Such a *Gr68a* null mutant would be important in future studies that seek to understand the pharmacology and signal transduction of GRs specific for identified pheromone components.

Electrophysiological studies have shown that gustatory neurons respond to sugar, water, and salt (reviewed in Matsunami and Amrein, 2003). Trehalose, a carbohydrate that is a major component of *Drosophila* food, is currently the only ligand identified for a specific GR, *Gr5a* (Duhanukar et al., 2001). While Bray and Amrein are not able to assign a specific ligand to *Gr68a*, their findings are significant in that they expand the profile of potential GR substrates to include pheromones. By comparing courtship behavior levels toward flies with male or female pheromone profiles, Bray and Amrein find that inactivating *Gr68a* neurons reduces courtship toward females or toward males expressing female pheromones but has no effect on male-male courtship. This implies that *Gr68a* neurons detect stimulatory pheromones from females and that inhibitory pheromones produced by males are most likely detected through other receptors.

In addition to identifying a cellular and molecular component in the reception of courtship stimuli, this study contributes information that will be useful in elucidating the logic of the gustatory coding. Current estimates indicate that there are at least 70 GRs, which could potentially permit the detection of a very large and diverse set of taste ligands (Clyne et al., 2000; Dunipace et al., 2001; Scott et al., 2001). Interestingly, a subset of GR genes is expressed in olfactory neurons and may participate in the recognition of specialized volatile odorants not detected by conventional odorant receptors. In adult flies, GRNs project to the subesophageal ganglion (SOG) and the ventral ganglion, but current knowledge of the molecular logic of the coding of taste information is limited. Understanding the range of ligands for GRs and identifying ligands for specific GRs will eventually demonstrate how chemosensory input is organized on a primary level, providing the foundation for understanding the dynamics of taste recognition in *Drosophila*. On a broader level, *Drosophila* provides a nice system for studying the integration of multiple sensory inputs that the average male fly must process to succeed in the mating game: the appearance, smell, and taste of a receptive female fly.

Lauren Giarratani and Leslie B. Vosshall
Laboratory of Neurogenetics and Behavior
The Rockefeller University
1230 York Avenue, Box 63
New York, New York 10021

Selected Reading

Bray, S., and Amrein, H. (2003). Neuron *39*, this issue, 1019–1029.

Clyne, P.J., Warr, C.G., and Carlson, J.R. (2000). Science *287*, 1830–1834.

Duhanukar, A., Foster, K., van der Goes van Naters, W.M., and Carlson, J.R. (2001). Nat. Neurosci. *4*, 1182–1186.

Dunipace, L., Meister, S., McNealy, C., and Amrein, H. (2001). Curr. Biol. *11*, 822–835.

Ferveur, J.-F., and Sureau, A. (1996). Proc. R. Soc. Lond. B Biol. Sci. *263*, 967–973.

Greenspan, R., and Ferveur, J.F. (2000). Annu. Rev. Genet. *34*, 205–232.

Hall, J. (1994). Science *264*, 1702–1714.

Hildebrand, J.G. (1995). Proc. Natl. Acad. Sci. USA *92*, 67–74.

Matsunami, H., and Amrein, H. (2003). Genome Biol. *4*, 220.

Robertson, H.M. (1983). Experientia *39*, 333–335.

Scott, K., Brady, R., Jr., Cravchik, A., Morozov, P., Rzhetsky, A., Zuker, C., and Axel, R. (2001). Cell *104*, 661–673.

Stocker, R.F. (1994). Cell Tissue Res. *275*, 3–26.

The High and Low of Visual Awareness

What specific network of neural activity mediates awareness? In this issue of *Neuron*, Wilke et al. report psychophysical results showing that perturbations of early topographic visual areas can lead to all-or-none image disappearance, demonstrating the importance and versatility of low-level visual processing in controlling visual awareness.

Suppose a bright red disc appears near fixation. What does it take to see it? When this question is restated in terms of what neural activity is essential for seeing the disc, the answer becomes elusive. Certainly, the light pattern from the red disc must sufficiently stimulate retinal cells so that their activity is raised significantly above baseline. What else is required?

Cells in the primary visual cortex (V1) may be particularly important—individuals with V1 lesions assert that they do not see anything when objects stimulate retinal regions corresponding to the lesioned parts of V1. Some inputs bypass V1, sending direct signals to higher visual areas such as V3, V4, IT (inferotemporal cortex), and MT (middle temporal area). Though these connections may allow V1 lesioned individuals to respond appropriately to "unseen" stimuli and perform above chance on forced-choice pattern discriminations ("blindsight"), they do not support normal experiences of seeing (see Lamme, 2001, for a review).

Sufficient activation of V1 might thus be necessary for visual awareness. V1 lesions substantially decrease activity in the ventral visual pathway (e.g., V2, V4, and IT) thought to mediate object perception. Thus, activation of higher areas through connections bypassing V1 might be too weak to enter visual awareness. Would sufficient activation of higher visual areas support awareness in the absence of V1 activity? Ideally, an isolated contribution from each area should be assessed by brain stimulation studies in which normal feedforward inputs to each area are simulated without concurrent activation of lower areas. At present, neuropsychological results (from humans and monkeys with lesions in different brain areas) suggest that no single visual area is both necessary and sufficient for visual awareness (see Lamme et al., 2000, for a review).

An alternative to seeking "the locus" of visual awareness is to characterize visual awareness as a global state of neural activation. Intuitively, it seems that attended stimuli that induce strong feedforward activations in multiple visual areas should enter awareness. Apparently, when the sensory activation of the visual system is diminished by reducing image contrast, the pattern becomes less perceptually salient and eventually disappears at sufficiently low contrasts. Interestingly, however, recent results by Wilke et al. (in this issue of *Neuron*) and Bonneh et al. (2001) suggest that (1) strong retinal stimulation does not necessarily translate to visibility and (2) substantial contributions to awareness come from low-level codings that are dissociated from activation strengths.

Bonneh et al. demonstrated that attended salient shapes spontaneously and intermittently disappeared, for several seconds at a time, when presented against a background of moving dots (which never overlapped the shapes)—termed motion-induced blindness (MIB). Rather than gradual dimming and brightening, disappearances and appearances of the shapes were all-or-none. Crucially, reducing the luminance contrast of the shapes, thereby reducing neural responses to the shapes in low-level visual areas, did not increase their disappearance. Disappearance of multiple shapes was also influenced by similarity-based grouping. Bonneh et al. thus concluded that MIB was mediated by neural suppression in high-level visual areas. They attributed the potency of moving dots in extinguishing high-contrast shapes to "sensory dissociation" induced within the visual inputs (due to the co-existence of static and dynamic patterns), which shifted the visual system into an all-or-none competition mode. They noted that this idea was consistent with binocular rivalry, in which sensory dissociation induced by presenting a dissimilar image to each eye causes the percept to alternate exclusively between the two images (see Blake and Logothetis, 2001, for a review).

Wilke et al. discovered that when a target shape was presented first and then followed (a fraction of a second to seconds later) by the addition of background dots, the onset of the background dots caused the attended salient shape to disappear. Though an onset of moving dots was the most potent in extinguishing the target, an onset of static dots or a color change of the preexisting dots were also effective. Because a disappearance could be attributed to the stimulus manipulation in each trial, this new paradigm, which Wilke et al. termed "generalized flash suppression (GFS)," allowed detailed psychophysical investigations of the stimulus factors that influenced perceptual disappearance of a salient shape. Whereas the properties of MIB implicated suppression in high-level processing as the primary cause of pattern disappearance, Wilke et al. demonstrated that subtle perturbations of early topographic visual areas contributed substantially to the disappearance.

For example, presenting the target in both eyes rather than in only one eye reduced target disappearance, whereas presenting the background dots in both eyes increased target disappearance. Apparent contrast should