
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-Ga4 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.

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**Selected Reading**


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 pre-existing 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