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Invertebrate Neuroethology: Food Play and Sex

How do animals perceive their environment and make appropriate behavioral choices based on those perceptions? New data have uncovered a novel sensory pathway that promotes *Drosophila* male courtship behavior in response to food.

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"Food and sex, those are my two passions. It's only natural to combine them."
— George Costanza, Seinfeld, 'The Blood'.

From bacteria to humans, all organisms obtain vital information from chemosensory signals in their environment. The integration of multiple environmental chemical cues conveying information about essential resources (such as food), potential dangers, and appropriate mating partners act to guide behavioral choices. Males and females are particularly dependent upon detection and intake of food resources for survival and reproductive success. There are a variety of examples in the literature illustrating how sexual behavioral interactions are influenced by food. For instance, in some species of spiders, the male woos the female by offering a food gift during courtship. If she accepts the 'nuptial gift', the male proceeds towards copulation [1]. Similar behaviors are reported in a range of insect and bird species [2,3]. A notable example is the female cricket, which feeds on secretions exuded from the male abdominal tergal glands during mating [4].

The fruit fly *Drosophila melanogaster* offers an excellent system to explore the mechanisms by which external cues are sensed and integrated to execute complex behaviors [5]. Reproductive success requires *Drosophila* flies to locate mates and find an appropriate place to lay fertilized eggs [6]. Food would appear to be an ideal meeting point to gather, mate and produce progeny. Yang *et al.* [7] have previously reported a link between food and egg-laying site choice. However, a role for food in courtship behaviors has been less clear. An exciting new study by Grosjean *et al.* [8] has now identified a link between the intensity of male sexual behavior in *Drosophila* and the presence of food. Furthermore, their findings imply that both pheromonal and food sensory information are integrated to promote copulation.

Courtship behaviors primarily depend upon olfactory cues (volatile pheromones) and gustatory cues (contact pheromones) allowing *Drosophila* males to recognize and evaluate potential mates by assessing their sex, species and reproductive state [9]. These male sexual behaviors are largely specified by neuronal circuitry expressing the male-specific transcription factor Fruitless (Fru^M). From sensory information to motor output, these neurons contribute to the assessment of potential mates and the execution of male courtship [10]. Known volatile sex pheromones are sensed at close-range by odorant receptors expressed in *fru^M* olfactory sensory neurons in the antenna, the fly olfactory organ [11]. The axons of these sensory neurons project into glomeruli within the antennal lobe in the brain, the equivalent of the mammalian olfactory bulb, where they synapse with second order neurons that propagate olfactory information to higher brain centers [12]. Recently, a novel family of olfactory receptors called the ionotropic receptors was identified in *Drosophila*. These receptors are expressed in a complementary fashion to that of odorant receptors [13]. In this follow up study, Grosjean *et al.* [8] noticed that ionotropic receptor 84a (IR84a) is expressed in the ciliated dendrites of *fru^M*-expressing antennal sensory neurons that innervate VL2a, one of the glomeruli known to be larger in males [14] (Figure 1).

Since *fru^M* neurons have previously been shown to be involved in mate recognition through pheromone sensing [15], the team investigated whether *Ir84a* neurons are tuned to recognize odors produced by male or female flies. Electrophysiological recordings of *Ir84a* neurons showed that this is not the case. A large screen of structurally diverse odors led to the identification of phenylacetaldehyde and phenylacetic acid as close-range volatile ligands for the IR84a receptor. These chemical compounds are found in natural drosophilid food sources, such as overripe bananas and the prickly-pear cactus *Opuntia ficus-indica*. These fruits are also common egg-laying sites for female *Drosophila*. Generation of a mutant null allele by inserting the yeast transcriptional activator GAL4 into the *Ir84a* locus (*Ir84a^{Gal4}*) allowed Benton's group to verify that the response to phenylacetaldehyde and phenylacetic acid was indeed abolished in *Ir84a^{Gal4}* homozygous mutant neurons. Moreover, this phenotype was rescued by restoring the function of IR84a in these neurons. The data presented in this study show unambiguously that expression of *Ir84a* in the olfactory neurons allows the fly to respond to discrete odors elicited by compounds found in natural fruit fly substrates that serve for feeding, breeding and egg-laying.

Despite the lack of response of the receptor to fly-derived stimuli, the

authors were still intrigued by the anatomical overlap between *Ir84a*-expressing neurons and the *fru^M* circuitry and decided to explore the function of IR84a in mating behaviors. They found that *Ir84a* homozygous mutant males displayed greatly reduced courtship activity when paired with a virgin female. A set of tests performed using courtship chambers perfumed with, or without, phenylacetic acid fragrance showed that the presence of the odorant doubled male courtship activity. As expected, this increase in courtship activity was absent from the poorly courting *Ir84a^Gal4* homozygous mutant males. Thus, IR84a activation in response to food odors enhances courtship.

No sexual dimorphism was found either in the number of *Ir84a*-expressing sensory neurons or their projections to the antennal lobe. Electrophysiological recordings of *Ir84a* neurons in response to phenylacetic acid were similar for males and females. However, Grosjean *et al.* [8] found that removing the function of IR84a in females had no gross effect on mating. It remains to be determined whether the IR84a olfactory pathway regulates other aspects of female behavior. It is known that mating triggers behavioral and physiological changes in *Drosophila* females, including an increase in egg-laying [16]. Furthermore, *Drosophila* females engage in active probing of the environment, apparently to evaluate the quality of the egg-laying sites, before depositing their eggs [7]. Future studies may connect IR84a function with female post-mating responses.

How do *Ir84a* neurons convey sensory information from glomeruli to higher order processing centers? The authors used a combination of neuronal staining, axonal tracing and brain registering to produce a plausible neuroanatomical map. This allowed them to map first order IR84a neurons to second order projection neurons. Projection neurons innervating VL2a, and therefore carrying IR84a sensory information to higher brain centers, do not appear to bundle with projection neurons of the general food odor pathways. Instead, they seem to be mostly interdigitated with projection neurons of pheromonal pathways that target
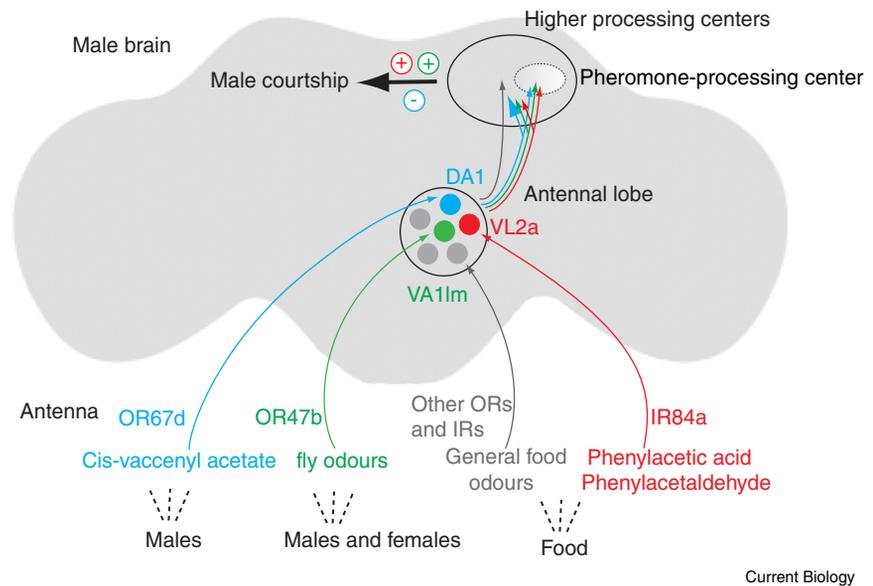
Figure 1. Proposed model for the perception and integration of olfactory sensory signals that regulate *Drosophila* male courtship.

Olfactory sensory neurons in the antenna detect odors and send axons to glomeruli in the olfactory center, the antennal lobe, in the brain. VL2a glomerulus receives information from *Ir84a*-expressing neurons, which respond to odors derived from host food/oviposition substrates. DA1 and VA1 lm glomeruli receive pheromonal information from *Or67d*-expressing neurons (which respond to the male pheromone 11-cis-vaccenyl acetate, cVA) [11,15] and *Or47b*-expressing neurons (which respond to unidentified female and male-derived odors) [18,19], respectively. Olfactory information is then propagated through projection neurons to higher brain centers, such as the mushroom body and lateral horn. OR67d/DA1 connectivity was previously shown to target a specific [8] area in the lateral horn associated with pheromone processing [11,20]. Grosjean *et al.* generated a 'map' for the IR84a/VL2a pathway by tracing and registering a collection of brain images obtained from various sources. According to their model, VL2a projection neurons are segregated from projection neurons responding to general food odour pathways but they are anatomically interconnected with the VA1 lm/DA1 pheromone pathways and target a specific area in the lateral horn involved in pheromone processing. Note that only half of the male brain is shown in the schematic.

a region of the lateral horn specialized in pheromonal processing. Such an anatomical configuration suggests — though does not prove — that the IR84a/VL2a sensory pathway is an independent food pathway that may be integrated together with the OR67d/DA1 and OR47b/VA1lm pheromone pathways to promote mating (Figure 1).

To determine how pheromonal and food-related olfactory inputs are integrated to regulate male courtship behavior, it will be necessary to identify specific VL2a projection neurons that respond to phenylacetaldehyde or phenylacetic acid via IR84a, and establish their synaptic connections with third-order neurons in the brain. This may also reveal sexual dimorphisms in downstream circuitry that could account for the sex-specific copulatory behaviors reported in this study.

In a complementary study, Silbering *et al.* [17] found that, in isolation, phenylacetaldehyde and phenylacetic acid odors are mainly repulsive to flies. Yet, in the context of a mating partner, they attract a male to a female, increasing the chances of copulatory success on the appropriate food source. It follows that odor-evoked behaviors depend on the environmental milieu, the social context, and the animal's internal state. Future challenges will be to determine how animals prioritize a specific behavior when exposed to conflicting signals, and how experience-dependent exposure to such signals modifies behaviors over the lifespan of the fly.

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Spatial Representation: Maps in a Temporal Void

It has been suggested that the matrix-like firing structure of entorhinal grid cells is caused by interference between membrane oscillations at slightly different theta frequencies. A recent report suggests that grid signals can be generated in the absence of theta oscillations.

Lisa M. Giocomo and Edvard I. Moser

Spatial representation in mammals is thought to depend on place-specific neurons in the hippocampus and entorhinal cortex. 'Place cells' in the hippocampus fire in a single location within the environment, reflecting the encoding of complex relationships between self-motion and external landmarks [1]. Place cells receive input from grid cells in the entorhinal cortex – place-modulated neurons whose firing locations define a periodic triangular array covering the entirety of the animal's environment [2,3]. Grid cells are thought to provide the metrics for spatial representation and navigation [3,4].

The mechanism generating the periodic grid map remains one of the biggest unresolved questions. One class of computational models suggests that the spatial periodicity of grid cells is derived from the theta rhythm, a prominent hippocampal-entorhinal network oscillation in the 6–10 Hz frequency range [5,6]. In these models, a periodic spatial signal is formed by interference between a velocity modulated oscillation and a baseline oscillation at slightly different theta frequencies [7–9]. Implicit to the 'oscillatory interference' models is a breakdown of grid patterns in the absence of theta oscillations. Recent work suggests that some species may navigate at least partially without theta activity. While nearly continuous theta oscillations are a prominent characteristic of electro-encephalogram (EEG) signals during foraging in rodents, theta waves appear more intermittently in other species, including bats [10] and humans [11]. The sporadic nature of the theta activity is maintained during movement in bats [10]. In a recent paper, Yartsev and colleagues [12] take advantage of this species variability to determine whether grid cells can exist in the absence of theta oscillations.

Yartsev *et al.* [12] recorded single-unit activity from the hippocampus and medial entorhinal cortex of Egyptian fruit bats as the animals crawled around a large enclosure in search of food. Under these conditions, place cells and grid cells could both be identified. Grid cells showed all the cardinal features of rodent grid cells, including a hexagonal firing lattice, co-localization of cells with similar grid orientation and grid spacing, offsets in firing phase of co-localized neurons, an increase in scale along the dorsal to ventral axis, and velocity modulation of the firing rate. The medial entorhinal cortex of bats also contained the same functional cell types as in rodents; pure grid cells, conjunctive grid-head direction cells, pure head direction cells and border cells. These results are significant for two reasons. First, the data show that grid cells are present in a species that is phylogenetically distant from rodents [13] (Figure 1). In spite of this distance, the cytoarchitecture of the hippocampal and entorhinal cortices of bats and rodents is very similar. The formation of grid structure may depend on the common architectural features. A second and more important point is the observation of grid structure in the apparent absence of theta activity. Theta oscillations were present only in one-second long bursts with an average interval between the bursts of 37 seconds in the entorhinal cortex. Between the bursts, theta oscillations