

A *Drosophila* OBP Required for Pheromone Signaling

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Pest insects have a profound impact on agriculture and human health. Substantial global losses of crops, stored agricultural products, timber, and livestock can be attributed to damage and destruction by insects (1). Blood-feeding insects such as mosquitoes, flies, and ticks transmit devastating infectious diseases, including malaria. Insect-borne diseases account for millions of deaths annually, and insect-associated illnesses surpass 300 million annual reported cases (2). The toxicity of pesticides and the emergence of pesticide resistance limit their utility. Finding alternative means to control insect pests remains a challenge. Here, I explore a new approach that aims to control species-specific pheromone signaling and thereby modulate insect behavior.

Pheromones are chemical signals emitted by an animal to influence the behavior, physiology, or development of other animals of the same species. In insects, pheromones elicit stereotypic behaviors including mating, reproduction, egg-laying, and aggregation. Understanding the mechanism of pheromone perception could give us the ability to manipulate insect behavior and develop sustainable methods of pest control.

The prevailing theory of pheromone perception is that pheromone-responsive chemosensory neurons are activated directly by pheromone molecules. Indeed, recent work with moth receptors indicates that they can be directly stimulated by pheromone (3). However, the pheromone concentrations required to activate heterologously expressed receptors are millions of times the concentrations known to activate insect systems (4, 5). What other components contribute to pheromone sensitivity?

Odorant binding proteins (OBPs) are small, soluble proteins specifically expressed in both olfactory and gustatory systems of terrestrial animals. OBPs are secreted by nonneuronal support cells into the fluid bathing the neuronal dendrites. Members of this class bind directly to odorant

ligands. Although the first OBP was identified almost 25 years ago by Vogt and Riddiford (6) as a pheromone-binding protein, the *in vivo* functional significance of these proteins remains elusive. A number of hypotheses have been advanced, including partitioning hydrophobic pheromone from air to aqueous phase, concentrating or sequestering ligands, transporting pheromone to the neuron, or inactivating pheromone (7, 8).

To determine the role of an OBP *in vivo*, I examined the *Drosophila* mutant, *lush* (9), to observe the odorant receptor's neuronal activities and its effects on insect behavior. LUSH (also called OBP76a) is an OBP expressed exclusively in the chemosensory system in both males and females in approximately 150 olfactory hairs (the trichoid sensilla). Trichoid sensilla serve as specialized olfactory structures for pheromone perception in other insects (10). Using single-sensillum record-

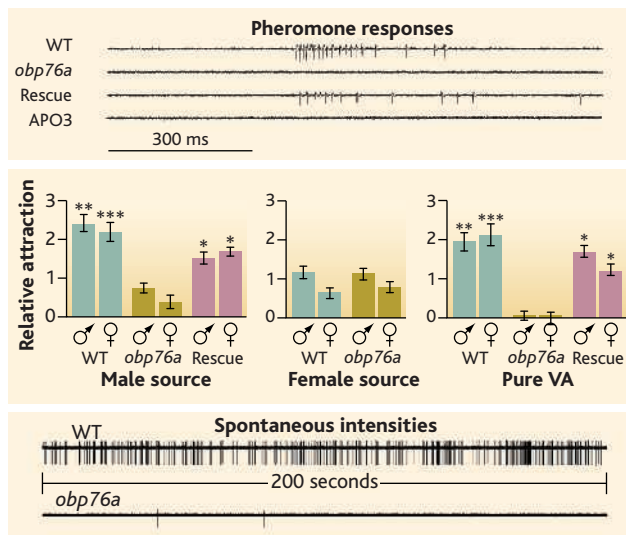
ing techniques (11), I assayed the electrical activity of trichoid olfactory neurons from control and *lush* mutant animals to determine if the mutants showed defects in sensitivity to the only known *Drosophila* volatile pheromone (male-specific), cis-vaccenyl acetate (VA) (12–14). My work showed that trichoid sensilla from *lush* mutants are completely defective for VA-evoked

responses (see the figure, top panel), revealing that the binding protein is required for VA sensitivity (15). Expression of a wild-type *lush* transgene in the mutants restores LUSH expression (9) and VA sensitivity (see the figure, top panel). Other OBPs, like the moth pheromone binding protein APO3,

failed to restore VA sensitivity in the *lush* mutant background, despite its presence in the sensillum lymph in the transgenic animals. Therefore, there is a specific requirement for extracellular LUSH protein in VA sensitivity.

Having established that *lush* mutants are defective for detection of VA, I investigated whether this deficit influences behavior in response to VA pheromone. Because VA is thought to function as an aggregation pheromone that attracts both male and female flies (12), I carried out odorant trap assays to test whether *Drosophila* males and females are attracted to VA-producing males or pure VA and whether

lush mutants are defective for responses to these cues (9, 16). The results show that wild-type male and female flies are equally attracted to wild-type male flies as a source of VA pheromone placed in odor traps. Behavioral attraction of *lush* mutant flies to wild-type males is significantly reduced compared with that of control flies (see the figure, middle panel). When female flies, which do not make VA, were used as bait, I found no difference in this attraction between wild-type and *lush* mutants (see the figure, middle panel). When pure VA was used as bait, both wild-type



Insensitivity of *lush* mutants to VA. (Top) Representative traces of VA-responsive neurons (concentration of VA = 1%). **(Middle)** Behavioral responses of flies to different sources of VA. **(Bottom)** Comparison of spontaneous firing rates of trichoid neurons between wild-type and mutant flies.

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males and females were attracted to it, but mutants lacking LUSH protein were completely defective for attraction (see the figure, middle panel). Accordingly, OBP LUSH is absolutely required for pheromone perception *in vivo*.

Surprisingly, in addition to the insensitivity to VA, I also noted that the spontaneous activity of trichoid neurons was nearly abolished in the *lush* mutants. Instead of one spike per second, the spontaneous activity in VA-sensitive neurons from *lush* mutants was approximately one spike every 430 ± 55 s, which corresponds to about a 400-fold reduction in spontaneous activity (see the figure, bottom panel). I demonstrated that this defect in neuronal activity in the absence of pheromone is due to loss of LUSH protein and, importantly, recombinant LUSH protein added directly into adult *lush* mutant trichoid sensilla through the recording pipette restored spontaneous as well as VA-evoked responses within 5 min.

These data suggest that LUSH is not a simple ligand transporter, but instead may function as an adapter linking the pheromone molecules to neuronal activation, possibly by direct interaction with the receptor or as a coactivator with pheromone. If true, inhibitors of OBPs that prevent pheromone binding might have potential as novel blockers of pheromone signaling.

To determine the feasibility of this approach, I synthesized a series of VA antagonists to target LUSH. The preliminary data show that one of the antagonists can specifically inhibit VA-evoked action potential firing in the wild-type. Conversely, this antagonist induces an excitatory action in the *lush* mutant, but not in the wild-type, suggesting that the inhibitory effect could be LUSH dependent.

In summary, my work provides new insight into pheromone signal transduction and points to new approaches to pest and disease-vector control.

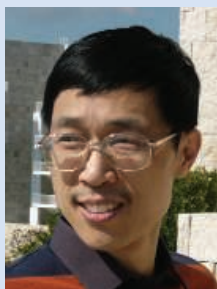
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2005 Grand Prize Winner

The author of the prize-winning essay, Pingxi Xu, was born and grew up in the northern province of Jiangsu, China. His first career was as a pediatrician, but after years of pediatric practice, Dr. Xu became interested in exploring basic science. To this end, he returned to university in Xian, China, and in 1988 earned a Master's degree in biochemistry and in 1999 a Ph.D. in neurobiology. In 2000 he joined Dr. Dean Smith's lab at the University of Texas Southwestern Medical Center in Dallas. Here he worked hard at understanding the molecular basis of pheromone signaling in *Drosophila*. Dr. Xu's goal is to apply this knowledge to control mosquito breeding by interrupting their perception of pheromones. Although his focus has moved from babies to bugs, his goal remains essentially the same: to improve human health by preventing the occurrence and spread of disease.



Finalists



Justin Blau, for his essay "How Flies Time: Circadian Clocks in *Drosophila*." Dr. Blau was born and raised in London, England. He received his undergraduate degree from Cambridge University in 1991, and his Ph.D. from the Imperial Cancer Research Fund, where he studied basic mechanisms of eukaryotic transcription with David Bentley. After graduating in 1996, he joined Mike Young's lab at the Rockefeller

University in New York to study how clock genes drive daily (circadian) rhythms of behavior in *Drosophila*. Dr. Blau started his own lab at New York University in 2000, where he continues to investigate how genes and neurons interact to drive this fundamental behavior.

Paul Frankland, for his essay "Networking to Remember: The Cortex and Remote Memory." Dr. Frankland grew up in Folkestone, England. He studied psychology at the University of

Sheffield and completed his Ph.D. work in neuroscience in the lab of Dr. John Yeomans at the University of Toronto. After graduating in 1996, he went on to conduct his postdoctoral work in the lab of Dr. Alcino Silva, first at Cold Spring Harbor Laboratory in New York and then at the University of California, Los Angeles (UCLA). At UCLA he used genetically engineered mice to model normal cognitive function, as well as cognitive dysfunction associated with various inherited diseases. In 2003, he started his own lab at the Hospital for Sick Children in Toronto. A focus in his lab is on understanding how enduring, or remote, memories are organized in the brain.



Johanna Montgomery, for her essay "Synapses in a State: A Molecular Mechanism to Encode Synaptic History and Future Synapse Function." Dr. Montgomery was born and raised in New Zealand. She graduated from the University of Otago in 1999 with a Ph.D. in physiology. During her Ph.D. studies, Dr. Montgomery completed the Neurobiology Course at The Marine Biological Laboratory in Woods Hole, Massachusetts. She began postdoctoral work in the laboratory of Dr. Daniel Madison at Stanford University, where she used paired whole-cell recording techniques to reveal distinct mechanisms of synapse plasticity. She then pursued further postdoctoral training with Dr. Craig Garner at Stanford University to examine the molecular aspects of synapse function. Last year, Dr. Montgomery returned to New Zealand to establish the Synaptic Function Research Group at the University of Auckland, where she is focusing on the molecular and physiological mechanisms of synapse function and plasticity.



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