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Wake Up and Smell the Pheromones

Odorant binding proteins (OBPs) are abundant proteins of unknown function expressed at high levels in insect and vertebrate chemosensory organs. In this issue of *Neuron*, Xu et al. show that *Drosophila* OBP76a is necessary for fruit flies to respond to the aggregation pheromone 11-*cis* vaccenyl acetate. The results suggest a mechanism by which this OBP is intimately involved in pheromone signal transduction.

Pheromones, substances released by one member of a species and detected by another, mediate an amazing diversity of animal behaviors. These chemical compounds are capable of eliciting fear, aggression, aggregation, sexuality, and recognition of kin and territory. Pheromone communication was first described in moths, which are capable of detecting a single molecule of female pheromone over an enormous distance. The first step in insect pheromone perception is the activation of pheromone-responsive chemosensory neurons. Although the functional properties of these neurons and the chemistry of the pheromones themselves have been under intense study for decades by many groups, the biochemical mechanisms by which pheromones selectively activate sensory neurons have remained obscure. Pheromone binding proteins were first described almost 25 years ago by Vogt and Riddiford (1981) as small secreted proteins that are present at high concentrations in the fluid bathing pheromone-sensitive olfactory sensory neurons (OSNs). These proteins bind pheromone in vitro, but their in vivo functional significance remains elusive. Large numbers of pheromone binding proteins and related odorant binding proteins (OBPs) have been identified in diverse insect species. A number of hypotheses have been advanced for their function, including partitioning hydrophobic pheromone from air to aqueous phase, concentrating or sequestering it, transporting pheromone to the OSN or inactivating it (Kaissling, 1998; Wojtasek and Leal, 1999). Recent work from Dean Smith's group represents a major breakthrough in our understanding of what role OBPs play in insect pheromone detection (Xu et al., 2005 [this issue of *Neuron*]).

Working with *Drosophila*, Xu et al. characterized the behavioral and electrophysiological phenotype of flies lacking odorant binding protein 76a (OBP76a; also known as LUSH). OBP76a is one of approximately 35 OBPs in the *Drosophila* genome and is expressed in a small subpopulation of T1 type OSNs in the adult antenna (Figure 1A). Using an extracellular recording technique that measures action potentials induced in OSNs, they find that *obp76a* mutant T1 neurons do not respond to 11-*cis* vaccenyl acetate (VA) (Figure 1B). VA is an aggregation pheromone produced by males that attracts both male and female flies (Figure 1D). Intriguingly, the mutant OSNs also show dramatically reduced spontaneous activity. Both normal spontaneous activity and VA responsivity are restored to the mutants by providing a wild-type copy of the *obp76a* gene. Infusing recombinant OBP76a protein directly into the T1 sensillum of mutant flies also restores VA sensitivity within 20 min of the OBP injection. This key experiment demonstrates that the T1 VA-sensitive neurons are present and viable in *obp76*a mutants, but lack only OBP76a to transduce the pheromone signal. Consistent with the dramatic electrophysiological phenotype, *obp76a* mutants fail to be attracted by synthetic VA or male flies that produce this pheromone (Figure 1C). In a final experiment, Xu et al. revisit the alcohol avoidance phenotype that gave the *obp76a* mutant its original name, *lush* (Kim et al., 1998). These mutants were originally found to be defective in avoiding high concentrations of ethanol and indeed their T2 sensilla fail to be inhibited by alcohols. Taken together, these experiments show that OBP76a is both absolutely required to detect the VA aggregation pheromone and modulates responses to high concentrations of alcohols.

Importantly, these results now implicate OBPs directly in pheromone signal transduction and suggest that these proteins do not act only to transport or inactivate stimuli. One particularly provocative hypothesis advanced by these authors is that pheromones act merely to stabilize a particular OBP conformation and that it is this OBP conformer that actually interacts with the membrane-associated pheromone receptor. The low rate of spontaneous activity in wild-type neurons could be ascribed to the stochastic isomerization of OBP in the absence of pheromone. In principle, it should then be possible to make a mutant OBP that is constitutively in the active conformation. If this hypothesis is supported by further experimentation, it would dramatically alter current thinking about pheromone signal transduction.

This work presents a number of interesting questions for future investigation. There are at least 35 other OBPs in the fly genome, and it will be of great interest to determine if OBPs play a crucial role in general odor detection in the fly. Recent work from John Carlson's lab suggests that odorant specificity is encoded by odorant receptors, which retain functional specificity even when moved to new OSNs, presumably bathed in different OBPs (Hallem et al., 2004). In addition, functional insect odorant receptors have been reconstituted in heterologous cells without OBPs (Wetzel et al., 2001; Sakurai et al., 2004). OBPs that interact with pheromones may therefore represent a special case of OBP function.

Finally, these results are among a spate of recent papers that support an important role for volatile pheromones in *Drosophila* social interactions. While fruit flies are not as spectacularly social as ant or bee societies—with their exuberant use of trail, alarm, and colony pheromones—flies may represent a powerful molecular genetic entry point to the problem of pheromone perception. Essentially nothing is known about the chemical ecology of volatile pheromones in *Drosophila*, including

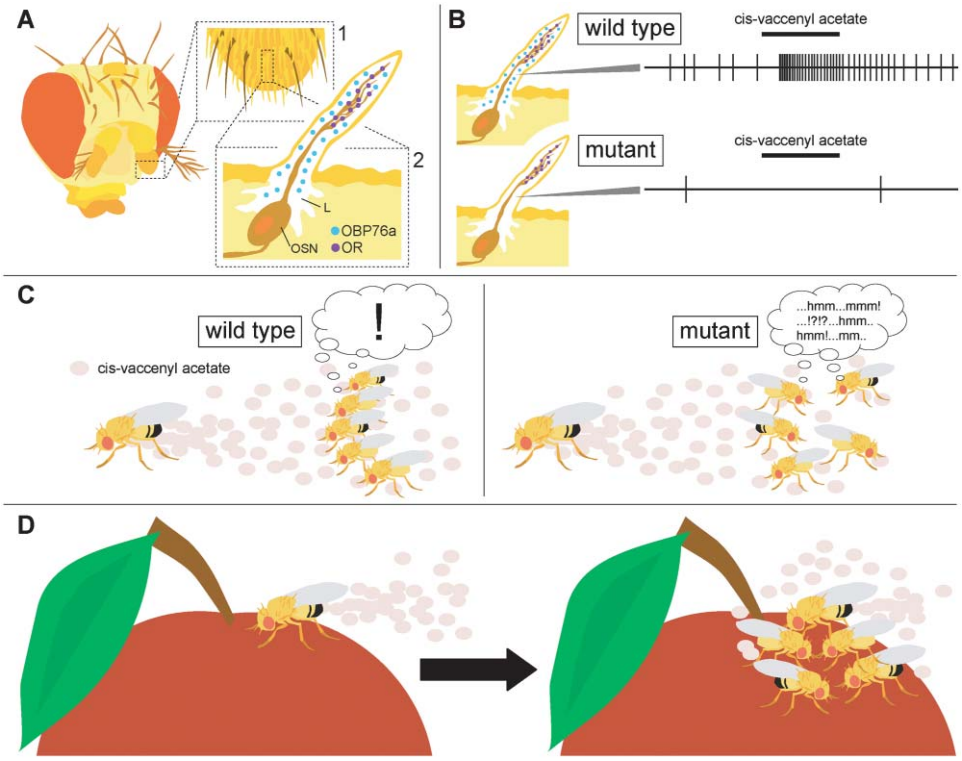

Figure 1. OBP76a Is Necessary for Flies to Detect the Aggregation Pheromone 11-*cis* Vaccenyl Acatate

(A) Schematic of the *Drosophila* olfactory system. The distal tip of the third antennal segment is densely covered with olfactory sensilla (inset 1). Transverse section through a T1 trichoid sensillum. *OSN*, olfactory sensory neuron; *L*, sensillum lymph; *OR*, odorant receptor; *OBP*, odorant binding protein (inset 2).

(B) Electrophysiological recordings from T1 OSNs in wild-type flies show responses to the pheromone 11-*cis* vaccenyl acetate that are absent in *lush* mutants. Mutant flies also show a drastic decrease (~400-fold) in spontaneous spiking activity.

(C) In wild-type flies, exposure to the male-produced pheromone elicits attractive behavior from both female and male flies (left), while attraction is abolished in both sexes of *lush* mutants (right).

(D) Under natural conditions, 11-*cis* vaccenyl acetate likely facilitates social aggregation on host fruits.

the chemistry of these compounds, the OBPs that interact with them, and the pheromone receptors and OSNs that are activated by them. Previous work on fly pheromones has been limited largely to studies of nonvolatile, cuticular hydrocarbons that are sensed by taste neurons on the leg and other appendages (Jallon, 1984; Bray and Amrein, 2003). However, clues that volatile *Drosophila* pheromones besides VA exist are slowly emerging. Suh et al. found that frightened flies emit dSO, a fear substance consisting of carbon dioxide and other unknown compounds that repels naive flies (Suh et al., 2004). Levine et al. found that an unknown volatile substance produced by flies acclimated to one time zone can disturb the sleep of flies in another time zone (Levine et al., 2002). It is also apparent from the trap assays in Xu et al. that males produce additional aggregation compounds besides VA and that females emit volatile attractants that act on males in an *obp76a*-independent manner. The nature of the chemicals that elicit these diverse behaviors—fear, sleep, aggregation—will be fascinating to discover. Also of interest will be further investigation into whether OBPs are central to perception of other pheromones, as *obp76a* has been shown here for VA. Identifying the pheromone receptors that recognize these special chemicals will be important. Recent work in the silk moth, *Bombyx mori*, suggests that the pheromone receptors in this animal are members of the odorant receptor gene family (Sakurai et al., 2004). Since *Drosophila* has a manageable number of such genes (62), it should only be a matter of time before more details emerge on how these powerful social cues are sensed.

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Toward Establishing a Therapeutic Window for rTMS by Theta Burst Stimulation

In this issue of *Neuron*, Huang et al. show that a version of the classic theta burst stimulation protocol used to induce LTP/LTD in brain slices can be adapted to a transcranial magnetic stimulation (TMS) protocol to rapidly produce long lasting (up to an hour), reversible effects on motor cortex physiology and behavior. These results may have important implications for the development of clinical applications of rTMS in the treatment of depression, epilepsy, Parkinson's, and other diseases.

While there is a vast literature of animal experiments exploring the mechanisms of long-term plasticity, very little is know about how these phenomena apply to the human brain. Repetive transcranial magnetic stimulation (rTMS), a noninvasive means of magnetically stimulating the brain through the intact scalp, has been put forward as a tool for probing this issue in humans. While some evidence for plasticity and changes in function extending beyond the immediate stimulation period have been observed, these effects have typically been small, variable, and short term. Clinically, rTMS has been proposed as a potential therapy for a variety of neurological and psychiatric diseases. But again, here the results have also been disappointing, and overall, progress in the application of rTMS has been hampered by concerns over safety, which for obvious reasons limit human studies to lower frequencies than have typically been used in animal studies.

The goal of the study by Huang et al. (2005 [this issue of *Neuron*]) was to test whether application of a theta burst paradigm (TBS)—low-intensity bursts of rTMA at 50 Hz—could produce evidence of plasticity in the motor cortex. Three different patterns of TBS (continuous, intermittent, and intermediate TBS) were applied to the primary motor cortex of subjects. The readouts for these experiments were EMG responses (motor evoked potentials [MEPS]) in a hand muscle, which were evoked using a single pulse of TMS. Continuous TBS (cTBS) resulted in suppression of the MEG response, while the responses were facilitated by intermittent TBS (iTBS). Depending on the number of pulses applied, these aftereffects could be observed for up to 1 hr. The authors went on to show that, depending on the length of the train of TBS applied and the pattern of activity, they could record different—either suppressive or facilitatory—aftereffects. The authors included a variety of controls to confirm that these effects of TBS were intrinsic to the motor cortex (for instance, spinal H reflexes were unaffected) and were not the result of direct effects of TBS on corticospinal circuits. The authors also observed behavioral effects, as measured by changes in reaction time after conditioning.

What is the significance of the present work? Huang et al. were able to use the relatively noninvasive technique of rTMS to obtain direction-specific LTP and LTD-like excitability aftereffects in the human motor cortex. Both quantitatively and qualitatively, these effects far outweigh the effects seen to date using other rTMS protocols. From the perspective of those interested in the mechanisms of LTP/LTD, these effects were obtained by mimicking the theta burst stimulation protocol widely used for the induction of LTP/LTD (Larson et al., 1986), and in fact, a similar dissociation of facilitation and inhibition for different lengths and patterns of trains has been observed in animal studies.

rTMS exerts its effects in the human brain basically by repetitive electrical brain stimulation. It may mimic any LTP or LTD stimulation sequence and is at present only technically limited by a maximum repetition rate of about 100 Hz and the magnetic field amplitude of about 1 to 2 Tesla that is available with modern stimulators. In comparison to the LTP/LTD literature, a major disadvantage of TMS as a mechanism for electrical stimulation of the brain is the poor spatial resolution. Some have even compared the effect of TMS on the brain as being the equivalent of a lightening bolt hitting a television set. Although the spatial precision can be somewhat increased by adapting the configuration of coils, the induced current flow affects at least several cubic centimeters of brain tissue and therefore is certainly far from the single or few neurons that are affected in LTP experiments that use electrical stimulation in animal slice preparations. Although spatial precision is clearly an issue, it may be possible to achieve some selectivity in human experiments by pairing associative stimulation using TMS with precisely timed somatosensory stimuli. Experiments using such protocols have also demonstrated impressive LTP/LTD-like aftereffects (Wolters et al., 2003).

The rTMS protocols that have been used so far and that were developed initially in Mark Hallett's lab at the NIH relied on the earliest LTP and LTD protocols, using regular stimulation frequencies: higher ($>$5 Hz) for excitation or lower ($<$1 Hz) for inhibition. Apart from relatively modest aftereffects, most experiments suffered from rather high interindividual variability. Also, recent experiments on cortical preconditioning challenge the use of these sometimes ambiguous regular stimulation frequencies. For example, stimulating the motor cortex first by a high rTMS stimulation frequency (Iyer et al., 2003)