Chem. Senses 37: 295–298, 2012
doi:10.1093/chemse/bjs007
Advance Access publication February 14, 2012

A Flux Capacitor for Moth Pheromones

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Accepted January 25, 2012

Abstract

In this issue of *Chemical Senses*, Baker et al. propose a provocative and intriguing explanation for a commonly observed phenomenon in moth chemocommunication. Sex pheromones in moths typically consist of mixtures of long-chain unsaturated compounds in specific ratios. These ratios are correspondingly detected by male moths using separate olfactory sensory neurons for each pheromone component housed singly or multiply in long trichoid sensilla on the antennal surface. These neurons are often present in different proportions, typically with the neuron responding to the highest ratio component present in greatest abundance or with the largest dendritic diameter. In their article, Baker et al. postulate that these physical differences in neuron magnitudes arise to compensate for the higher molecular flux present with the most abundant pheromone components. Such a suggestion raises several questions concerning the physiological and behavioral nature of pheromone communication. Specifically, is the flux in a natural pheromone plume high enough to warrant increased flux detection for the most abundant components? Second, how can changes in neuronal number or size lead to increased flux detection? And finally, how would this increased flux detection be accomplished at molecular, cellular, and ultimately network scales? We address each of these questions and propose future experiments that could offer insight into the stimulating proposition raised by Baker et al.

Key words: channel capacity, dendrite size, insect pheromones, odorant flux ranges, odorant receptor neurons, olfactory sensilla

In the 17th century, Sir John Ray, an English naturalist and one of the progenitors of modern taxonomy, noted that a female peppered moth (*Biston betularia*) "came out from its chrysalis shut up in my cage; the windows were open in the room or closet where it was kept, and 2 male moths were caught by my wife who by a lucky chance went into the room in the night; they were attracted, as it seems to me, by the scent of the female and came from the outside" (Mickel 1973). To this day, the exquisite sensitivity and specificity of moth sex pheromone communication has fascinated the scientific world.

Moths exhibit a remarkable ability to both produce and detect a variety of specific chemical blends in precise ratios and concentrations that they use for sex attraction (Roelofs 1995). Male moths correspondingly possess distinct peripheral morphologies to accommodate these ratios, with separate olfactory receptor neurons for each pheromone component housed singly or multiply in long trichoid sensilla on the antennal surface. Additionally, these neurons are often present in drastically different proportions, generally with the neuron responding to the higher ratio component present in greatest abundance. In their review, "Working range of stimulus flux transduction determines dendrite size and relative number of pheromone component receptor neurons in moths," Baker et al. ponder the physiological and behavioral rationale for these morphological distinctions (Baker et al. 2012).

The proportional differences in pheromone-responsive neurons on the male moth antenna can manifest in 2 distinct, but not mutually exclusive, ways. Either male moths can possess higher numbers of sensilla housing major-component responsive neurons, such as in *Agrotis segetum* (Wu et al. 1999) or these neurons exhibit larger dendritic diameters when co-located with minor-component responding neurons, such as in *Ostrinia nubilalis* (Hansson et al. 1994; Olsson et al. 2010; Figure 1). Historically, the increased number and dendritic size of pheromone-specific neurons has been thought to correlate to a need for higher sensitivity. This appears logical at first, but Baker et al. note that a need for higher sensitivity should promote an increase in the number of receptor neurons responsive to "less" abundant pheromone compounds, whereas in many species, the opposite phenomenon is true.

Instead, Baker et al. suggest that the differences in pheromone receptor neuron magnitudes and/or dendritic sizes arise to compensate for the higher molecular flux present with the more abundant components. Insect antennae are

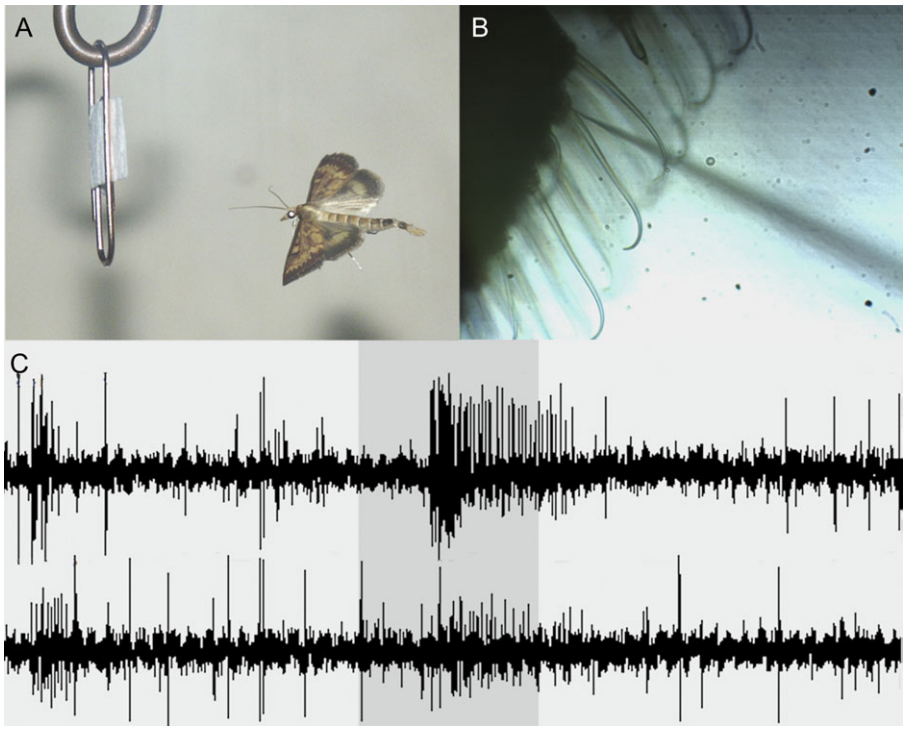

Figure 1 The European corn borer: Built for flux detection? (**A**) A male *Ostrinia nubilalis* approaching its pheromone blend in a flight tunnel (Photo: Béla Molnár). (**B**) Photomicrograph showing tungsten single sensillum electrophysiology of a single trichoid sensillum of *O. nubilalis* housing separate neurons responding to each pheromone component. (**C**) Trace (2.5 s) showing the response of colocalized pheromone-responsive neurons in an E-strain male responding to a 500 ms stimulus of *E* (top)- and *Z* (bottom)-tetradecenyl acetate. Stimulus timing indicated with gray bar. Note that the amplitude of the major-component responding neuron is larger, potentially eliciting greater flux detection as postulated by Baker et al.

flux detectors (see Kaissling 1998), meaning that they respond to instantaneous changes in concentration rather than the absolute concentration at any moment. Thus, Baker et al. posit that more and larger neurons (or those with longer and thicker dendrites or more pore openings in the sensillum cuticle) would allow detection of greater and broader changes in flux, as would be present for the most abundant component in a pheromone plume. A capacitance for flux, if you will.

This is a highly intriguing concept, and one that immediately raises several questions. First, is a capacitance for flux necessary? In other words, does the absolute flux in a natural pheromone plume reach high enough levels that a greater capacity for flux detection would be required? Although the filamentous nature of a plume is well known, the dynamics and maximal flux of pheromone plume release from individual females is not, particularly in terms of location and aggregation of females (cf. review in Cardé and Willis 2008). However, a study that recorded the response of *A. segetum* pheromone neurons in the flight tunnel found that maximum response levels of the neurons corresponded to arrestment of flight in the males at high blend concentrations (Valeur et al. 2000). Interestingly, this pheromone concentration closely corresponded to that achieved by the highest release rates of the females, suggesting that single females could indeed approach the maximal flux capacity of individual cells. However, more accurate ecological and chemical measurement of the kinetics of female pheromone release are needed to confirm this hypothesis.

Second, do more and larger neurons in fact lead to better "flux capacitance"? This question is somewhat more difficult to address. In addition to the electrophysiological and neuroanatomical studies suggested by Baker et al., a key experiment would be to alter numbers or sizes of pheromone-specific neurons on a male antenna and assess his performance in a plume. This could be possible by ablation of specific antennal sections in male moths with a heterogeneous distribution of pheromone sensillum types (as suggested for *A. segetum*; Wu et al. 1999; or for main component type C neurons in *O. nubilalis*; Hallberg et al. 1994). Alternatively, genetic manipulation that removes or reduces certain sensillum types (such as the *atonal* and *amos* mutants in *Drosophila*; see Benton et al. 2009) or that heterologously expresses receptors in neurons present in the "wrong" sensilla (such as the famous "empty neuron"; Dobritsa et al. 2003), could alter the topology of the antenna to observe its effects on plume tracking and flux detection. Unfortunately, there is only one transgenic moth currently available for olfactory studies, *Bombyx mori* (Tamura et al. 2000; 2007), and it requires only a single receptor neuron type to exhibit the full behavioral response to the pheromone (Sakurai et al. 2011). With the abundance of genomes becoming available, we hope that additional transgenic techniques will soon be

developed for other moth species to allow testing of the hypotheses of Baker et al.

Finally, if answers to the first 2 questions are yes, then how is higher flux capacitance accomplished by increasing the numbers or sizes of neurons? As Baker et al. point out, increasing the number of neurons on the antenna would logically increase the capacity to detect flux as there would be more neurons present that could continue to detect high levels of a component even if others had become saturated (i.e., strength in numbers). Yet, the larger dendritic size is more puzzling. Unfortunately, we still do not know if larger dendritic sizes lead to higher receptor expression, although this could potentially be tested with coupled antibody staining of the ubiquitous coreceptor Orco (Larsson et al. 2004) and electron microscopy (as for sensory neuron membrane proteins [SNMPs]; Rogers et al. 2001). Additionally, other aspects of the pheromone response (discussed by Baker et al.) including binding proteins, degrading enzymes, SNMPs, etc., should also be considered as important to the flux capacity as heterologous expression of pheromone receptors in nonnatal neurons has shown varying impacts on their sensitivity and kinetics (Syed et al. 2010). These experiments should be accompanied by dynamical modeling of pheromone receptor neurons (see discussion of Kaissling 2009 in Baker at al. as well as Gu and Rospars 2011) to assess the interaction and kinetics of these parameters. Lastly, it is still not clear how these peripheral differences are retained through the multisynaptic sensorimotor sequence leading to behavior. In the macroglomerular complex (MGC), the first synapse for pheromone neurons in the insect brain, there is a tremendous convergence of roughly 86000 pheromone receptor neurons from the antenna onto 35–40 outgoing projection neurons (as in *Manduca sexta*; Homberg et al. 1989). Coupled recordings of peripheral and central neurons (e.g., Vickers et al. 2001; Jarriault et al. 2010) and particularly multiunit recordings at both sites (as has been performed in the MGC; Christensen et al. 2000) could help elucidate the transformation of these neuronal ratios in the central nervous system.

The Baker et al. review offers a wellspring of intriguing questions concerning pheromone detection for future research. Despite being the most well-studied system for chemical communication, Baker et al. reveal how little we still understand pheromone communication in moths. Suddenly, the old story becomes new again, and like Michael Corleone (Godfather III), "Just when I thought I was out . . . they pull me back in."

Funding

Funding provided by the Max Planck Society.

Acknowledgements

The authors wish to thank Drs Andreas Reinecke and Ewald Groβe-Wilde for fruitful discussions concerning this topic and Béla Molnár for the photograph of *O. nubilalis*.

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