Species specificity of pheromone responses

Receptor neurons mediate behavioural responses in heliothine moths

by Thomas C. Baker

Different types of receptor neuron on male moth antennae are involved in the attraction of males to the sex pheromone blend emitted by females. Each type of receptor associated with attraction is usually tuned optimally to only one component of the (agonistic) conspecific pheromone blend and is housed in a long, hair-like sensillum trichodium (see page 19 of this issue for a classification of the different types of sensillum). Thousands of these sensilla festoon the antennae of male moths. Other neurons exist that are involved in antagonistic behavioural responses, such as cessation of upwind flight by males; these are tuned to respond only to components emitted by females of other species and are involved in reducing the attraction of males to blends containing these compounds, thereby minimizing mating mistakes with females of the wrong species.

In three different species of North American noctuid moth, in the group known as heliothines, we have found antennal neurons whose peak sensitivities correspond to those compounds that are important in causing both agonistic and antagonistic behavioural responses. These three species — *Helicoverpa zea*, *Heliothis virescens* and *Heliothis subflexa* — all have (Z)-11-hexadecenal (Z11–16:Ald) as the principal component of their sex pheromone blends, but emit different secondary components that help to give the blends their species-specificity. For instance, the secondary component in the pheromone blend of *Helicoverpa zea* is (Z)-9-hexadecenal (Z9–16:Ald)[1,2]. In *Heliothis virescens* the secondary component is (Z)-9-tetradecenal (Z9–14:Ald)[3,4], and in *Heliothis subflexa* the secondary components are (Z)-11-hexadecen-1-ol acetate (Z11–16:Ac) and (Z)-11-hexadecen-1-ol (Z11–16:OH)[5].

Using a technique of snipping the ends of these microscopic olfactory hairs with glass knives and recording the activities of the neurons housed inside, we found that approximately 70% of the 325 sensilla that we sampled on *Helicoverpa zea* male antennae contained a receptor neuron tuned only to the major component, Z11–16:Ald (Figure 1, type A)[6]. We discovered a second type of hair typical of approximately 20% of the sampled sensilla (Figure 1, type C) which houses both an agonistic as well as an antagonistic type of receptor neuron; this explained both the upwind flight attraction of *Helicoverpa zea* males to their own species' pheromone blend and their arrestment of upwind flight in response to the blends emitted by *Heliothis virescens* and *Heliothis subflexa*. The first type of receptor neuron in this class of hair is a large-spiking neuron that is sensitive to Z9–16:Ald, the secondary *Helicoverpa zea* pheromone component (Figure 1 and Figure 2c)[6], and is correlated with an agonistic behavioural response to pheromone. It is also responsive to Z9–14:Ald, the *Heliothis virescens* secondary pheromone component, at approximately the same dosage (Figure 2c). The sensitivity of this receptor neuron thus explains the attraction of *Helicoverpa zea* males that has been observed in response to two-component blends containing excessively small proportions of Z9–14:Ald added to Z11–16:Ald; Z9–14:Ald can substitute for the absence of Z9–16:Ald and cause attraction[7].

The second type of receptor neuron in this C-type sensillum (Figure 1) is a small-spiking neuron that is again responsive to Z9–14:Ald which, in the larger proportions that comprise the *Heliothis virescens* blend, acts as a strong antagonist to upwind flight[6-8]. However, this receptor neuron is actually more sensitive to two other strong behavioural antagonists, Z11–16:Ac and Z11–16:OH, emitted by *Heliothis subflexa* females[5] (Figure 2). Activation of this single, broadly tuned, antagonist receptor neuron could explain why *Helicoverpa zea* males will orient only to their conspecific females and not to either *Heliothis virescens* or *Heliothis subflexa*. Females of the latter

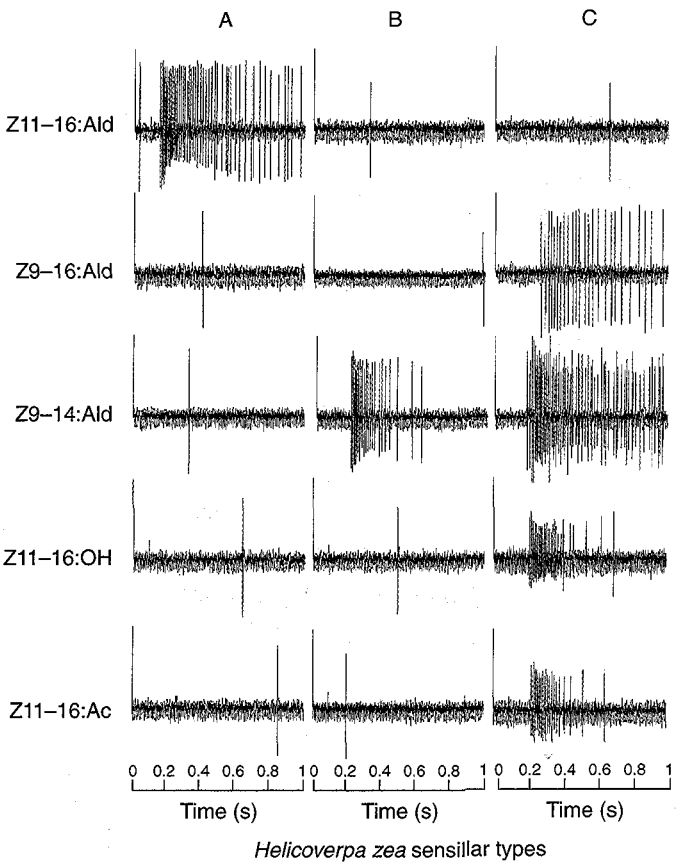

Figure 1. Sensillar types A, B and C on *Helicoverpa zea* male antennae contain neurons tuned to different pheromone-related components. Type A always contains a large-spiking neuron related to attraction (agonism) tuned to Z11–16:Ald. Type B contains neurons tuned only to Z9–14:Ald, which is a component emitted by *Heliothis virescens* (not *Helicoverpa zea*) females. Type C contains a large-spiking agonistic-related neuron tuned equally to the *Helicoverpa zea* pheromone component Z9–16:Ald and to Z9–14:Ald. Type C also contains a small-spiking neuron related to antagonistic behavioural response that is tuned to Z11–16:Ac and Z11–16:OH, but also has some sensitivity to Z9–14:Ald (From ref. 6).

two species also emit the *Helicoverpa zea* pheromone components Z11–16:Ald and Z9–16:Ald, but any firing by agonistic *Helicoverpa zea* receptor neurons tuned to these components would conceivably be counteracted by the activities of the antagonistic neuron responding to any of these three compounds. This antagonist type of receptor neuron may also explain why a blend of Z11–16:Ald and a small amount of Z9–14:Ald is never as attractive to *Helicoverpa zea* males as the conspecific blend. Enhanced specificity for the conspecific blend arises because the antagonist receptor neuron is never stimulated to fire by Z9–16:Ald — only the agonist neuron is stimulated, even when large proportions of Z9–16:Ald are added to Z11–16:Ald (Figure 3). However, when Z9–14:Ald is used instead of Z9–16:Ald, and the proportion of Z9–14:Ald becomes too great, the threshold of the antagonist receptor neurons is exceeded (Figure 2d and Figure 3), and the upwind flight response begins to be suppressed by the firing of this neuron[6-8].

It is interesting that both the agonist- and antagonist-types of receptors neuron in *Helicoverpa zea* are more broadly tuned to, i.e. more accepting of, a wider range of compounds than is usually the case in moth sex-pheromone communication systems. Indeed, after quantitative measurements were made of the amounts of these compounds actually emitted from the odour cartridges used for stimulation in this study, we found that the agonist-sensitive receptor neuron is equally responsive to Z9–14:Ald (a non-pheromone component) and Z9–16:Ald, a rare occurrence for a pheromone-component-tuned receptor neuron (Figure 2c)[6]. The antagonist-sensitive receptor neuron was found to be tuned with a slightly lower threshold to Z11–16:Ac, with nearly equal sensitivity to Z11–16:OH (Figure 2d). This receptor neuron's threshold for Z9–14:Ald was significantly higher, but the amounts of this compound emitted by *Heliothis virescens* in its blend are also greater, so that even with its lower sensitivity, the *Helicoverpa zea* receptor neuron should be sufficiently sensitive to respond antagonistically to Z9–14:Ald found in *Heliothis virescens* pheromone plumes.

Such flexibility in response by both the agonist- and antagonist-sensitive receptor neurons could create the potential for interesting shifts in blend discrimination by males. These broadly tuned neurons may have been one of the ways in which reproductive character displacement in pheromone blends could have occurred — in *Helicoverpa zea*, for example, as an adaptive response to the presence of *Heliothis virescens*. Other examples of broadly tuned, more flexible, receptor systems can be found in moths such as *Yponomeuta rorellus* as an apparent, adaptive, reproductive blend in the presence of other *Yponomeuta* species inhabiting the same geographic regions at the same time of year[9]. In *Y. rorellus*, a broadly tuned agonist-sensitive type of receptor neuron and antagonist-sensitive receptor neurons are both present. The presence of such accommodating

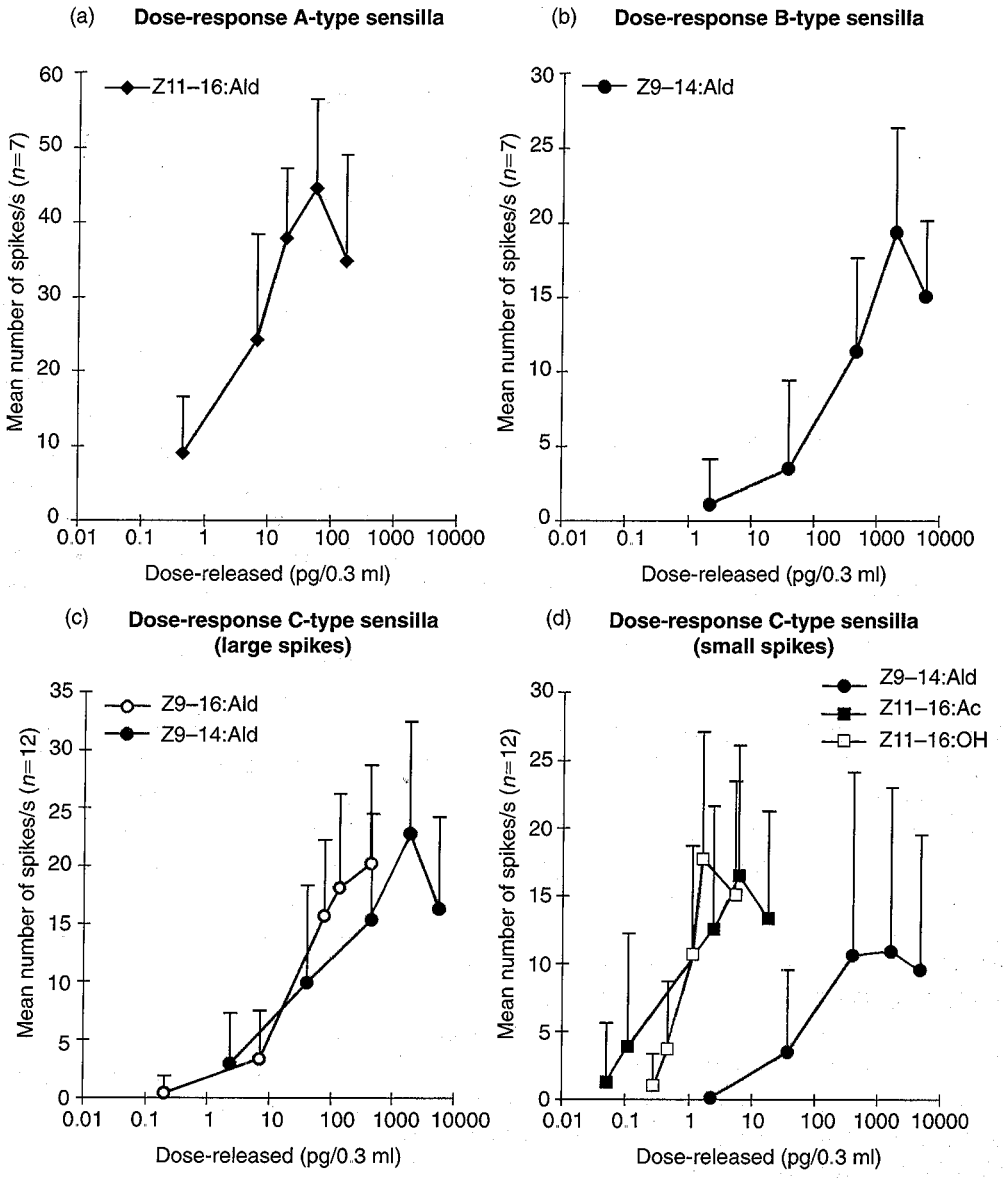

Figure 2. Sensitivities of the neurons in the three different sensillar types to their respective optimal odourants. Note that in sensillar type C, the large-spiking agonist-related neuron (c) is equally sensitive to Z9–16:Ald and Z9–14:Ald and that the small-spiking neuron (d) is optimally sensitive to Z11–16:Ac and Z11–16:OH, both of which are known behavioural antagonists. This neuron also responds to Z9–14:Ald, but any behavioural antagonism would come at a slightly higher airborne concentration than attraction, due to stimulation of the large-spiking neuron in the same way (see ref. 6).

pheromone-component receptors on male antennae may, fortuitously, allow the males to accept mutant females that express an altered blend, and allow the mutation to be carried along in the population without the need for a simultaneous, complementary, component-specific mutation to occur in the receptor system in the male population.

We have also found neurons on the antennae of *Heliothis virescens* and *Heliothis subflexa* that appear to correspond to these two species' behavioural agonists and antagonists (A.A. Cossé, J.L. Todd, N.J. Vickers and T.C. Baker, unpublished work). In the heliothine moths, an interesting pattern is emerging that is consistent with what is known about neurons on other moth species' antennae. It is interesting that, in nearly every case, neurons tuned to the compounds known to be antagonistic are co-compartmentalized within the same hair as the neurons tuned to known agonists. There appears to be a behavioural reason for this that has to do with a previously under-appreciated aspect of olfactory resolution in insects recently emphasized[10]. We found that *Helicoverpa zea* males flying upwind in a wind tunnel could resolve filaments of pheromone and antagonist, Z11–16:Ac, separated by as little as 1 mm in space and 0.001 s in time (Figure 4). Such fine-grained resolution should require two differentially tuned types of receptor neuron to be as closely juxtaposi-

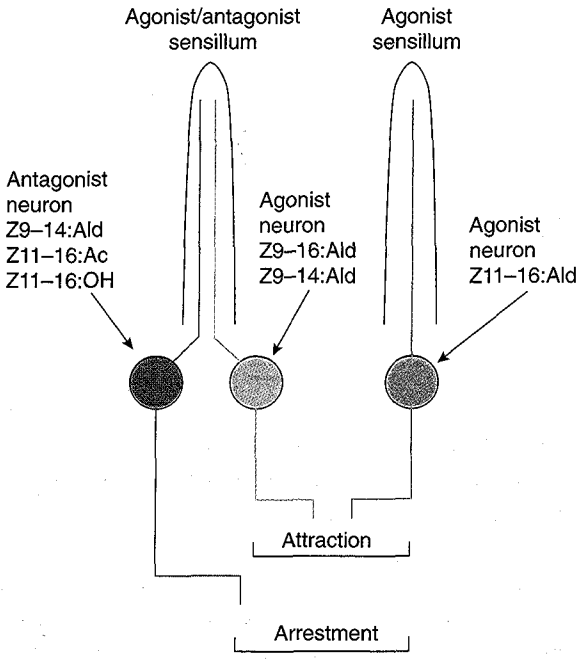

Figure 3. A depiction of the relationship of sensillar types A (right) and C (left) to upwind flight attraction (agonism) and arrestment (antagonism) in *Helicoverpa zea* males. Attraction occurs when the pheromone components Z11–16:Ald and Z9–16:Ald stimulate the respective neurons that are tuned to them. If the small-spiking neuron in sensillar type C is stimulated, upwind flight is reduced by the increasing amount of activity in this neuron until arrestment of flight predominates.

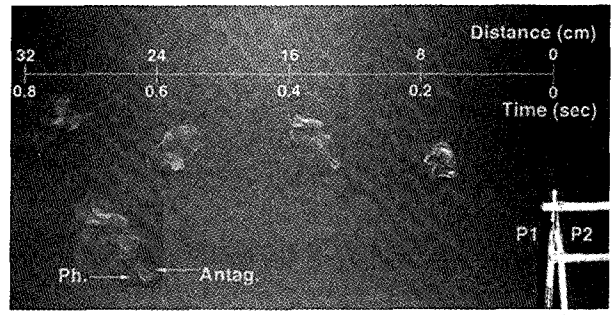

Figure 4. Smoke visualization of how two strands of odour would look emerging from pipette tips (P1 and P2) stationed only 1 mm apart, a distance over which *Helicoverpa zea* can discriminate strands of pheromone from strands of antagonist. Abbreviation used: Ph., pheromone; Antag., antagonist (see ref. 6).

tioned as possible to optimize the detection of possible non-coincidental arrival of the odour strands to which each neuron is tuned[10]. In fact, in *Helicoverpa zea*, the neurons tuned to Z9–16:Ald and Z11–16:Ac are, in fact, co-compartmentalized within the same olfactory hairs wherever they occur on the antenna[6].

For these three species of heliothine moth that co-exist across much of their North American ranges, such fine-grained resolution could have evolved from its importance to mate-finding. Strands of two different odourants that are not perfectly coincident every time they arrive can only have originated from two different point sources (pheromone glands from two different females) at some points upwind[10]. Only strands of pheromone and antagonist that are perfectly coincident every time can have originated from one female upwind, a female of the wrong species. Males which have the ability to detect and respond to pure strands of pheromone, regardless of any imperfect co-mingling of the pheromone with strands of antagonists from other females' plumes, will have been at a selective mating advantage in their evolutionary past. The way the receptor neurons on *Helicoverpa zea* antennae, as well as those on the antennae of other species, are compartmentalized in receptor hairs may give us a glimpse into the pressures that have shaped males' abilities to find mates while minimizing mistaken orientations to, and mating mistakes with, females of the wrong species[10] ●

Supported by USDA/NRICGP grant 95-37-301-1805 to T.C. Baker and T.A. Christensen

References

1. Klun, J.A., Plimmer, L.R., Bierl-Leonhardt, B.A., et al. (1980) J. Chem. Ecol. **6**, 165–175
2. Vetter, R.S. and Baker, T.C. (1984) J. Chem. Ecol. **10**, 193–202
3. Roelofs, W.L., Hill, A.S., Cardé, R.T. and Baker, T.C. (1974) Life Sci. **14**, 1555–1562
4. Klun, J.A., Bierl-Leonhardt, B.A., Plimmer, J.R., et al. (1980) J. Chem. Ecol. **6**, 177–183
5. Teal, P.E.A., Heath, R.R., Tumlinson, J.H., et al. (1981) J. Chem. Ecol. **7**, 1011–1022
6. Cossé, A.A., Todd, J.L. and Baker, T.C. (1998) J. Comp. Physiol. **A182**, 697–711
7. Vickers, N.J., Christensen, T.A., Mustaparta, H. and Baker, T.C. (1991) J. Comp. Physiol. **A169**, 275–280
8. Fadamiro, H. and Baker, T.C. (1997) Physiol. Entomol. **22**, 316–324
9. Löfstedt, C., Hansson, B.S., Dykerman, H.J. and Herrebout, W.M. (1990) Physiol. Entomol. **15**, 47–54
10. Baker, T.C, Fadamiro, H. and Cossé, A.A. (1998) Nature (London) **393**, 530

Thomas C. Baker is a Professor and Chair of the Department of Entomology at Iowa State University, Ames, Iowa, USA. He received his BSc and MSc from Cornell University, and his PhD from Michigan State. His major research interest is in the neuroethology of pheromone-mediated flight in moths, as well as developing pheromones and other semiochemicals for use in agricultural and urban environments.