Moth uses fine tuning for odour resolution

Male moths, when responding to their species' blend of sex pheromones, cease their upwind flight when additional compounds are added to the mixture. Often these behavioural antagonists are the pheromone components of sympatric species that emit similar pheromone blends, and thus may function to prevent mating with females of the wrong species. Antagonists must be emitted from the same point source as the pheromone blend to be optimally effective\(^1\), suggesting a fine discrimination between the occurrence of pheromone and antagonist.

Here we report that males of a North American noctuid moth species, *Helicoverpa zea*, can distinguish strands of pheromone from those of a behavioural antagonist separated by no more than 1 mm and, at most, 0.001 seconds.

The pheromone blend for *H. zea* is a 20:1 ratio of two components: (Z)-11-hexadecenal (Z11-16:Al) and (Z)-9-hexadecenal (Z9-16:Al). We found that male *H. zea* readily flew upwind and touched a Pasteur pipette source emitting this blend when it was pulsed at 5 s\(^{-1}\) (with 0.02 s pulse duration; Fig. 1, treatments 1 and 3).

Significantly fewer males acted in this way when the antagonist (Z)-11-hexadecen-1-ol acetate (Z11-16:Ac) (ref. 5) was pulsed at the same frequency from the same pipette, the antagonist being added to a second filter paper at a loading of 50% of that of the pheromone (Fig. 1, treatment 7).

However, weaker antagonism resulted from placing this same amount of antagonist on a filter paper in a separate pipette whose tip was stationed only 1 mm either upwind or downwind of the tip of the pheromone-emitting pipette. Emission from this pipette was pulsed simultaneously with the pheromone (Fig. 1, treatment 5). Several other wind-tunnel experiments using similar protocols resulted in similar outcomes (results not shown).

Responses to other treatments suggested that the lack of optimal suppression of upwind flight to the non-coincident pulses was due to incomplete mixing of the strands of antagonist with those of the pheromone. The same two pipettes used in the pulsed treatment were made to emit continuously, allowing the plumes' two strands of antagonist and pheromone to mix more completely than during pulses as they travelled down the tunnel. Using this set-up, there was significantly greater antagonism of upwind flight (Fig. 1, treatment 6) than during pulsing (Fig. 1, treatment 5).

The intermittency of the signal was not in itself the cause of reduced antagonism. When the antagonist was loaded in the same pipette as the pheromone and pulsed (Fig. 1, treatment 7), there was as much antagonism as when the continuous regime was used. This was the case regardless of whether or not continuous emission occurred with the antagonist in the same pipette (Fig. 1, treatment 8) or in a separate pipette from the pheromone (Fig. 1, treatment 6).

Measurements of the ratios of pheromone to antagonist emitted from the pipette tips\(^6\) showed that the ratio during separate emissions was 198:1, and during co-emission from the same pipette was 225:1. It should be stressed that the incomplete mixing during pulsing would only separate the strands by 1 mm at most, or by 0.003 s if the male moth were stationary in the 40 cm s\(^{-1}\) wind. Given the males' 90 cm s\(^{-1}\) airspeed generated by the moth flying mainly, but not entirely, straight upwind, this temporal separation would be 0.001 s at most.

We propose that the olfactory processing system responsible for this remarkably fine olfactory resolution may arise from the organization of antennal neurons. In Lepidoptera, including *H. zea* (ref. 6), antennal neurons tuned to a known antagonist are almost always co-compartmentalized within a single receptor hairs with a neuron tuned to a pheromone component\(^7\). Only by sampling the air at the same point in space and time can a system integrating the inputs of two functionally different sensors, tuned to different compounds, determine whether there is complete spatial and temporal coincidence in the arrival of two odors.

Such fine resolution could have evolved owing to its importance in mate finding. Males who could detect and respond to pure strands of pheromone, regardless of any imperfect mixing with antagonists, would have been at a selective mating advantage\(^8\).

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