DEET repels ORNery mosquitoes

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DEET ([*N-N*]-diethyl-*m*-toluamide) is widely used around the world as a repellent for mosquitoes and other biting insects. It was originally identified by a structure–activity study using synthetic compounds (1). Recently, a number of new compounds with similar activity [e.g., picaridine (2)] have been identified, but DEET remains the gold standard. Despite a tremendous number of studies (3), however, a plausible and evidence-based mechanism for DEET's action has remained elusive. In a recent study, Ditzen *et al.* (4) wrote, "Here we show that DEET blocks electrophysiological responses to olfactory sensory neurons to attractive odors in *Anopheles gambiae* and *Drosophila melanogaster*" (Fig. 1*A*). However, in this issue of PNAS, Syed and Leal (5) present a new mechanism that demonstrates that mosquitoes detect DEET by means of olfaction and that this is the direct cause of their avoidance behavior (Fig. 1*B*).

Various classes of compounds are termed "insect repellents." Some, such as pyrethroids (e.g., the recently developed metofluthrin) and DDT, work by insecticidal effect, whereby sublethal toxicity causes the insect either to be ineffective in its attack or to escape from the region of insecticide application. Repellency, however, should relate to a behavioral effect caused by perception at the peripheral sensory nervous system, causing the insect to not bite and to leave the prospective host, with true behavioral repellency involving avoidance of the source of the repellent material, whether placed on the prospective host or near it. Until recently, two main classes of behavioral insect repellents were known: (*i*) those, like DEET and picaridine, that have been obtained through testing synthetic compounds for repellency in one of the forms discussed above; and (*ii*) those that include a wide range of plant essential oil components, often with strong aromas as perceived by humans, that act against blood-seeking insects presumably by suggesting a strong plant ecosystem. For best effect, DEET requires good skin coverage, but even with that, the insects can remain in an irritating cloud around the protected individual. (This is particularly evident with the Scottish biting midge, *Culicoides impunctatus*.) The alternative, plant-derived compounds are highly volatile and can repel the insects from the region of the host but usually require frequent application. In addition, some insects apparently can still detect the host by means of a highly sensitive and selective olfactory-based host-location capability. More recently, successful attempts have been made to identify experimental repellents derived from species closely related to the host range but not naturally acting as hosts (6, 7). There is also considerable promise in repellents active against the malaria mosquito (*An. gambiae*), the yellow fever mosquito (*Aedes aegypti*), and *C. impunctatus* that have been identified from odors collected by air entrainment of humans who show little or no attractiveness to these insects and from cases where attractiveness is naturally masked by the specific compounds giving rise to this repellency (ref. 8 and J.G.L., N. J. Seal, J. I. Cook, N. M. Stanczyk, M.A.B., S. J. Clark, S. A. Gezan, L. J. Wadhams, and J.A.P., unpublished data). Nonetheless, the repellent value of DEET and other compounds derived from structure–activity studies far outweighs other currently available repellents, so understanding the mechanism by which DEET acts remains of paramount importance.

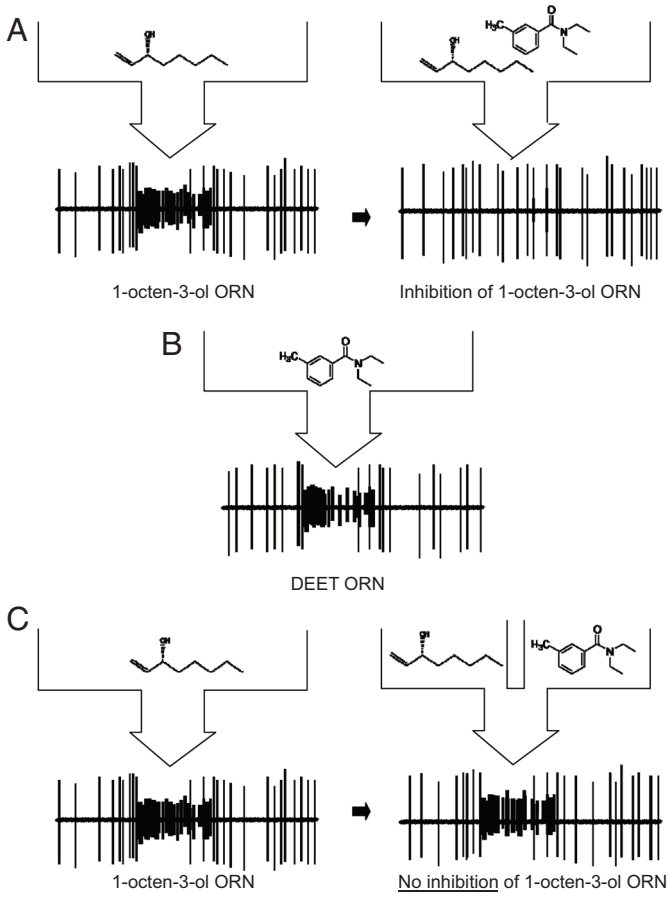

Fig. 1. Depiction of mosquito olfactory receptor neuron (ORN) responses. (*A*) ORN response to the attractant 1-octen-3-ol, and to 1-octen-3-ol plus DEET, introduced into the airflow from a single odor cartridge, giving the impression that DEET causes ORN inhibition, as stated by Ditzen *et al.* (4). (*B* and *C*) However, according to Syed and Leal (5), a separate DEET ORN is responsible for repellency (*B*), and, when 1-octen-3-ol and DEET are introduced into the airflow from two different odor cartridges, no ORN inhibition occurs (*C*).

Syed and Leal (5) demonstrate the existence in some insects of specific ol-

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factory receptor neurons (ORNs) that respond to DEET. These ORNs, which the authors found to be located on the antennae and maxillary palps of the Southern house mosquito, *Culex quinquefasciatus*, are associated with short, horn-like sensory organs called "trichoid sensilla." The ORNs respond in a dose-dependent manner to DEET, and this response is associated with behavioral avoidance, thereby demonstrating true behavioral repellency. It is unlikely that a positive response would have evolved to this compound. Therefore, it is possible that the ORNs responding to DEET have evolved to respond to naturally occurring compounds that have repellent properties conveying ecological benefit. Indeed, Syed and Leal show that certain plant-derived terpenoids (thujone, eucalyptol, and linalool) cause electrophysiological responses at concentrations lower by an order of magnitude. Thus it would appear that DEET, with its relatively low volatility, can remain on human skin for some time and release slowly, by evaporation, to stimulate the ORNs responding to natural essential oil repellent components, to which the DEET receptor (possibly as a consequence of the unnatural origin DEET) is an order of magnitude less sensitive.

Understanding the mechanism by which DEET acts remains of paramount importance.

Syed and Leal (5) go on to show that when DEET is presented experimentally together with known attractants (e.g., 1-octen-3-ol, for which there is a separate and specific, although sometimes colocated, ORN) in the same odor cartridge, the availability of the attractant is physically reduced, which gives the impression that the insect odorant receptor is inhibited by DEET. Because this phenomenon was not accounted for in the controls used by Ditzen *et al.* (4), their proposed mechanism can be questioned, at least for the time being. However, there are quantitative differences between the electrophysiological effects shown by Ditzen *et al*. and the "fixitive" effect shown by Syed and Leal, and these need to be resolved. Nevertheless, we now have evidence of specific responses by ORNs to DEET and confirmation that these responses relate to avoidance behavior.

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