Mosquitoes smell and avoid the insect repellent DEET

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The insect repellent DEET is effective against a variety of medically important pests, but its mode of action still draws considerable debate. The widely accepted hypothesis that DEET interferes with the detection of lactic acid has been challenged by demonstrated DEET-induced repellency in the absence of lactic acid. The most recent hypothesis suggests that DEET masks or jams the olfactory system by attenuating electrophysiological responses to 1-octen-3-ol. Our research shows that mosquitoes smell DEET directly and avoid it. We performed single-unit recordings from all functional ORNs on the antenna and maxillary palps of *Culex quinquefasciatus* and found an ORN in a short trichoid sensillum responding to DEET in a dose-dependent manner. The same ORN responded with higher sensitivity to terpenoid compounds. SPME and GC analysis showed that odorants were trapped in conventional stimulus cartridges upon addition of a DEET-impregnated filter paper strip thus leading to the observed reduced electrophysiological responses, as reported elsewhere. With a new stimulus delivery method releasing equal amounts of 1-octen-3-ol alone or in combination with DEET we found no difference in neuronal responses. When applied to human skin, DEET altered the chemical profile of emanations by a "fixative" effect that may also contribute to repellency. However, the main mode of action is the direct detection of DEET as indicated by the evidence that mosquitoes are endowed with DEET-detecting ORNs and corroborated by behavioral bioassays. In a sugar-feeding assay, both female and male mosquitoes avoided DEET. In addition, mosquitoes responding only to physical stimuli avoided DEET.

DEET mode of action | DEET-detecting neuron | fixative effect of DEET | mosquito olfaction | surface-landing bioassay

The insect repellent DEET, *N,N*-diethyl-3-methylbenzamide, has been used for >50 years, with 200 million people using it worldwide to reduce their risk of vector-borne diseases (1) but its mode of action has yet to be elucidated. The report that DEET modulates the physiological response of lactic acid-sensitive olfactory receptor neurons (ORNs) in the antennae of the yellow fever mosquito, *Aedes aegypti* (2), led to the hypothesis that DEET may interfere with and inhibit the response of the olfactory system to a normally attractive chemical signal (3). This notion of "jamming" the olfactory system has been substantiated, on the one hand, by behavioral observations indicating that lactic acid *per se* is a mosquito attractant and suggesting that DEET inhibits attraction to lactic acid (4, 5) and by the recent report on DEET attenuation of mosquito response to 1-octen-3-ol (6). However, repellency solely by inhibition of lactic acid detection was challenged by indoor (4) and field experiments (7) demonstrating the repellent effect of DEET with carbon dioxide as the only attractant. Intrigued by this controversy and puzzled by the dichotomy between sensory physiology and behavioral observations, we undertook a multidisciplinary approach aimed at unveiling the mode of action of DEET. We conducted a thorough survey of all functional ORNs housed in sensilla on the maxillary palps and antennae of the Southern house mosquito, *Culex quinquefasciatus*, investigated possible physiological interactions between DEET and odorants at the sensory level, and designed novel behavioral bioassays to study repellency in odorant-free environments. Here, we report the identification of an ORN housed in a trichoid sensillum on *C. quinquefasciatus* antennae that detects DEET in a dose-dependent manner. Meticulous investigations of possible physiological interaction between odorants and DEET at the sensory level clearly demonstrated that DEET attenuation of the olfactory system is a false positive because of reduced amounts of stimuli released when repellent and odorant are combined in the conventional stimulus delivery cartridges. Moreover, we provide convincing behavioral evidence suggesting that repellency of the Southern house mosquito is a matter of direct detection of DEET in the vapor phase.

Results and Discussion

Single Sensillum Recordings. Given the experimental evidence suggesting that interaction with lactic acid may not be essential for DEET repellency (4, 7), we aimed initially at revisiting the reported DEET-mediated inhibition of lactic acid-sensitive ORNs (2). First, we attempted to record from the lactic acid-sensitive groove peg sensilla on the antennae of the Southern house mosquito. Of 21 groove peg sensilla contacted from 7 females tested, we were able to record only weak excitatory responses from five sensilla and observed no significant differences between (*S*)- and racemic lactic acid (data not shown). The low sensitivity of the lactic acid-detecting ORNs and the low vapor phase of lactic acid required large amounts of stimulus (10 μg) thus defeating the purpose of comparing neuronal activity in the presence and absence of DEET. We also tested the yellow fever mosquito, *Aedes aegypti*, but preliminary recordings (data not shown) showed a similar low sensitivity of the peg sensilla to lactic acid, in line with both the high titer of lactic acid in human sweat (8) and the doses required for mosquito attraction (9). Given the technical difficulty of reexamining DEET effect on lactic acid detection and considering DEET repellency to mosquitoes attracted only to carbon dioxide (4, 7) we then examined the possible effect of DEET on CO_2 detection. We found no difference in neuronal responses, including spike frequencies and magnitude and kinetics of the receptor potential, when the carbon dioxide-sensitive ORN in the sensilla on the maxillary palps (10) were stimulated with CO_2 in the presence or absence of DEET [supporting information (SI) Fig. S1].

Next, we conducted comprehensive single unit recordings from all olfactory receptor neurons (ORNs) housed in maxillary palps and antennal sensilla to determine whether the Southern house mosquito is endowed with DEET-detecting ORNs. The maxillary palps have only one morphological and physiological type of olfactory sensilla, the peg sensilla on the fourth segment (10), whereas the antennae are covered with a variety of olfactory sensilla, including four morphologically different types of trichoids, in addition to the groove peg sensilla. We recorded from >100 peg sensilla on the maxillary palps of 60 mosquitoes and found that no ORN responded to DEET. We focused our first recording from the antennae on A-2 type trichoid sensilla,

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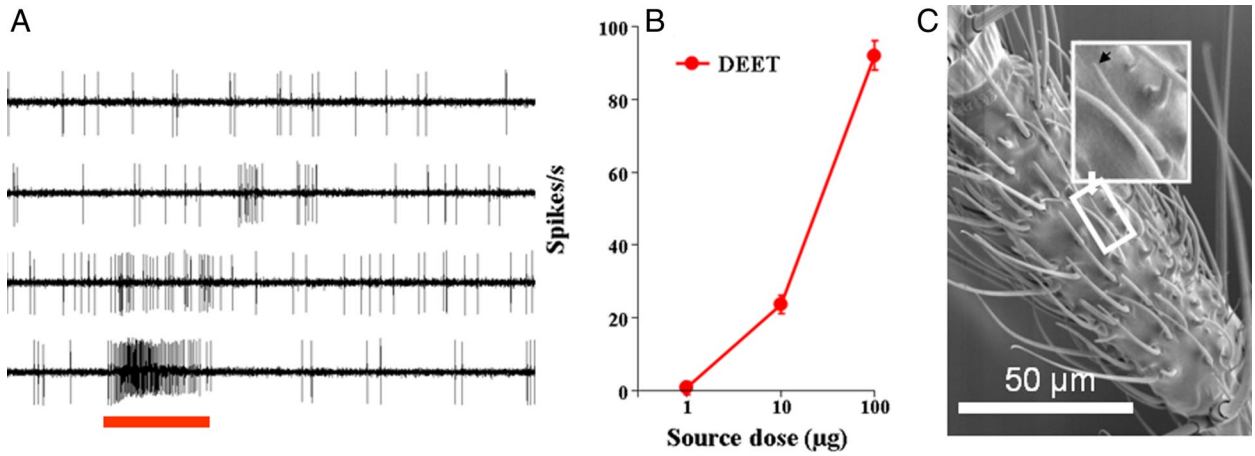

Fig. 1. Excitatory responses from an ORN housed in a trichoid sensillum upon stimulation with increasing doses of DEET. (*A*) Single sensillum recordings with control, 1, 10, and 100 μg of DEET (top to bottom). (Scale bar: 500 ms.) (*B*) Dose-response curve ($n = 5$). (*C*) Scanning electronmicrograph of the second segment of the antenna displaying various types of olfactory sensilla. The white arrow in the micrograph and the black arrow in the *Inset* highlight the type of short trichoid sensillum housing ORN sensitive to DEET and other terpenoids.

previously reported to be sensitive to high doses of DEET (4, 11). Despite several trials, we observed no DEET-induced response, but we avoided challenging our preparations with extreme doses (50 mg) of DEET as previously used (4) to minimize contamination of the single sensillum recording unit. We limited our highest doses to 100 μg, which seemed reasonable given the strong electroantennographic responses obtained with 10-μg dose (12). Our first encounter with a DEET-sensitive ORN showed a very clear neuronal response when a 100-μg DEET-laden cartridge was introduced in the air stream flowing over the antennae. The headspace vapor phase alone diffusing from the cartridge before puffing was enough to stimulate the ORN; the neuronal response was very clear when the stimulus was puffed on the preparation (Fig. S2). Subsequent recordings from different individuals showed that this DEET-sensitive ORN responded in a dose-dependent manner and with a threshold of 1 μg (Fig. 1 *A* and *B*). This DEET-sensitive ORN is housed in a short trichoid sensillum (Fig. 1*C*). Of the two ORNs housed in these sensilla, DEET was detected by a larger spike amplitude ORN, whereas a shorter spike amplitude neuron was sensitive to 1-octen-3-ol (Fig. 2). The DEET-sensitive ORN was also stimulated in a dose-dependent manner by terpenoid compounds, such as thujone, eucalyptol, and linalool (Fig. 2), which have already been reported as repellents (13–15).

False Positive Inhibition of 1-Octen-3-ol-Detecting Neurons. Given the colocalization of ORNs sensitive to DEET and 1-octen-3-ol, we investigated whether this insect repellent interferes with the detection of this odorant. 1-Octen-3-ol is detected with remarkably high sensitivity by ORNs housed in peg sensilla on the maxillary palps (10), but its ecological significance is yet to be elucidated. It is known, however, that 1-octen-3-ol is an attractant for various other species of mosquitoes (16). Because ORNs stimulated by 1-octen-3-ol in maxillary palps are more sensitive than those in antennae, we investigate possible effect of DEET on the detection of 1-octen-3-ol by the maxillary palps. The responses of 1-octen-3-ol-detecting ORNs decreased dramatically when a DEET-laden filter paper was placed in the stimulus cartridge in addition to the filter paper delivering the odorant (Fig. 3). There was significant decrease in the response to 1-octen-3-ol plus DEET compared with 1-octen-3-ol alone at all tested doses of 1-octen-3-ol, but the lower the dose the greater the reduction in response (0.1 ng, 29.4 ± 6.3 vs. 0 spikes/s; 100% reduction in response; 1 ng, 144.67 ± 15.3 vs. 15 ± 4.4 spikes/s, 89.6%; 10 ng, 191.5 ± 13.9 vs. 98.5 ± 11.6 spikes/s, 48.6%). Similar results have been recently reported for the malaria mosquito, *Anopheles gambiae* (6). In marked contrast to previously observed interactions of semiochemicals at the periphery (17, 18), we noticed no apparent changes in receptor potential and dynamics of neuronal response, except for a reduction in spike frequency (Fig.

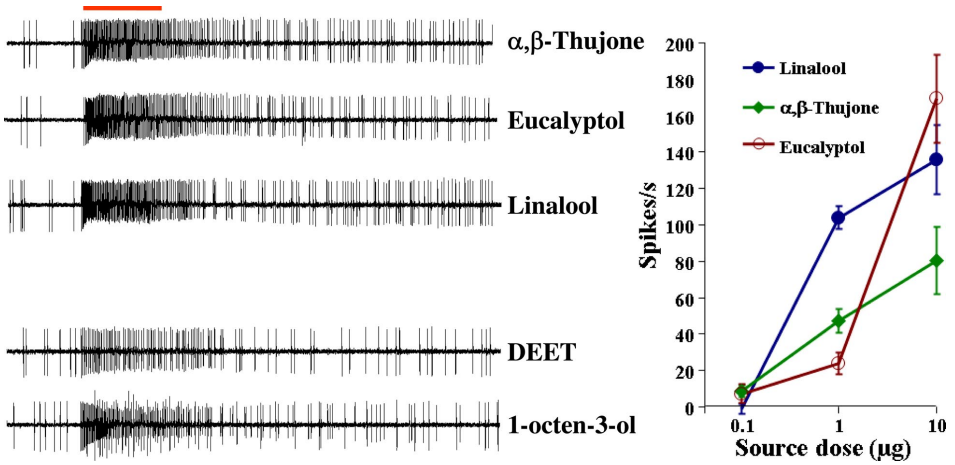

Fig. 2. Excitatory responses from DEET sensitive ORN to 10-μg dose of terpenoids (*Upper* traces) and 100 μg of DEET (*Lower* trace). Response of the second ORN, colocated in the DEET-detecting sensilla to 10 μg of 1-octen-3-ol. Note the larger spike amplitude of the DEET-sensitive ORN and the smaller spike amplitude of the neuron responding to 1-octen-3-ol. (Scale bar: 500 ms.) Dose-response curves indicating lower threshold and higher sensitivity of the DEET-detecting ORN to some terpenoid compounds compared with DEET (see Fig. 1*B*).

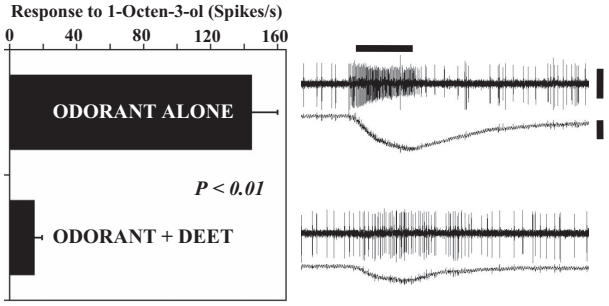

Fig. 3. Responses to 1-octen-3-ol from ORNs in peg sensilla on the maxillary palps. The spike frequency decreased dramatically when 1-octen-3-ol was delivered from the same stimulus cartridge along with DEET. (*Left*) Significant decrease in spike frequency ($P < 0.01$, $n = 5$; Mean± SEM). (*Right*) Electrophysiological recordings depicting a typical response pattern. *Upper* and *Lower* traces in each recording are action potential and DC coupled sensillum potential, respectively. (Scale bars: spikes, 1 mV; DC, 4 mV; horizontal bar, 500 ms.)

3). All these observations suggesting that the ORNs were essentially being subjected to lower doses of odorants prompted us to quantify the odorant delivered from the stimulus cartridge. We analyzed by gas chromatography (GC) the amounts of 1-octen-3-ol collected by solid phase microextraction (SPME) directly from the tip of the stimulus cartridge. When a second filter paper loaded with DEET was placed in the cartridge along with the filter paper impregnated with the odorant, we observed a dramatic decrease in the amount of 1-octen-3-ol released from the stimulus cartridge compared with 1-octen-3-ol alone (Fig. 4). We then concluded that the reduced neuronal activity observed by us (Fig. 3) and others (6) is merely an experimental artifact because of decreased amounts of odorant delivered from the stimulus cartridge upon addition of a DEET strip. Although the "DEET-induced reduction" in 1-octen-3-ol responses were recorded from different mosquito species, the Southern house mosquito (Fig. 3) and malaria mosquito (6), the experimental artifact was unrelated to mosquito physiology but rather because of trapping of odorants in the conventional stimulus delivery system used in both cases. To examine further possible DEET-induced modulation of ORN response to odorants, we changed the design of the stimulus delivery (*i*) to compartmentalize DEET and odorant, (*ii*) to avoid excessive back pressure and possible condensation and consequently trapping of odorants on the DEET-laden filter paper, and (*iii*) to allow simultaneous delivery of both compounds (Fig. 5*B*). In this new set-up, SPME-GC analysis indicated no significant difference in the

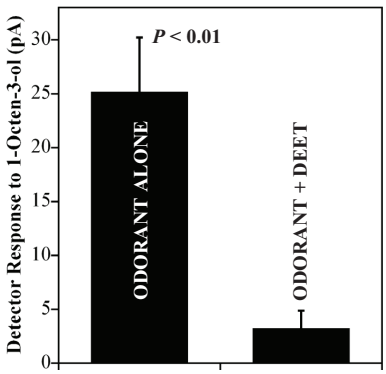

Fig. 4. Decrease in the amounts of 1-octen-3-ol collected by SPME at the tip of stimulus cartridges (Pasteur pipettes) and analyzed by GC. The pipettes containing equal amounts of 1-octen-3-ol (100 ng) loaded on filter paper strips. The DEET cartridge contained a second filter paper strip impregnated with 5 μl of pure DEET.

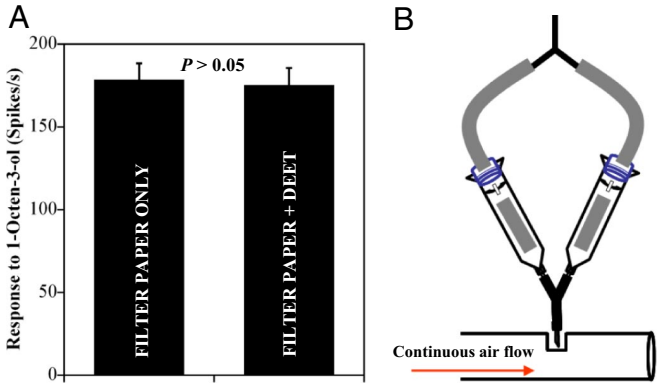

Fig. 5. Neuronal responses recorded from an ORN in the maxillary palps of *C. quinquefasciatus* sensitive to 1-octen-3-ol. (*A*) Responses to stimulus (10 ng) in the presence and absence of DEET were indistinguishable. (*B*) Filter paper strips loaded with 1-octen-3-ol and DEET (or blank) were placed on separated syringes and puffed simultaneously. The compensatory flow cartridges are omitted from this diagram for clarity.

amounts of 1-octen-3-ol collected from stimulus cartridge with odorant plus filter paper alone compared with cartridges containing both odorant and DEET (data not shown). Similarly, there were no significant differences in neuronal responses when the 1-octen-3-ol-detecting ORNs were stimulated with this odorant alone or in combination with DEET (Fig. 5*A*). We, therefore, confirmed that the observed "DEET-induced inhibition" of 1-octen-3-ol, recorded by us (Fig. 3) and others (6) from the maxillary palps of mosquitoes, was merely a false positive due to trapping of odorants in the Pasteur pipet when a second, DEET-laden filter paper is added to the cartridge.

Fixative Effect of DEET. Based on our experimental observation that DEET trapped the odorant in the conventional stimulus cartridge we hypothesized that when applied to human skin DEET might suppress the release of physiologically relevant compounds. By trapping airborne volatile collections with SPME and comparing the GC profiles of an arm treated with DEET versus an untreated arm (same subject), we observed a significant decrease in the amounts of the major compounds released from the skin, namely, 6-methyl-5-hepten-2-one, octanal, nonanal, decanal, and geranyl acetone (Fig. 6) on the DEET-treated arm. Detailed analysis of human skin emanations and their relationship to mosquito attraction have been already reported (19–23), but our aim was to determine whether DEET affects the release of physiologically relevant compounds. Some of the major human-derived compounds suppressed by DEET have been previously reported as attractants or repellents (19–23) and most of them are indeed electrophysiologically active (data not shown). Of notice, the skin of the human subject was exhaustively washed with warm water before the experiments and none of the compounds detected were observed in the soap regularly used by the human subject (Fig. S3). The suppression of volatile compounds released from the skin resembles the fixative effect widely used in perfumery. In our case it leads to an alteration of the host chemical profile and a possible interference with attraction. In this context, it may be considered a "masking" effect (21, 24, 25) on the release rather than on the reception of chemical signals.

Sugar-Feeding Bioassay. We developed an odorant-free bioassay to investigate whether mosquitoes are repelled by direct detection of DEET. Two lines of evidence suggest that mosquitoes are repelled by smelling DEET. First, experimental evidence refuted the hypothesis that DEET interferes with and reduces the detection of lactic acid (7), carbon dioxide, 1-octen-3-ol, and

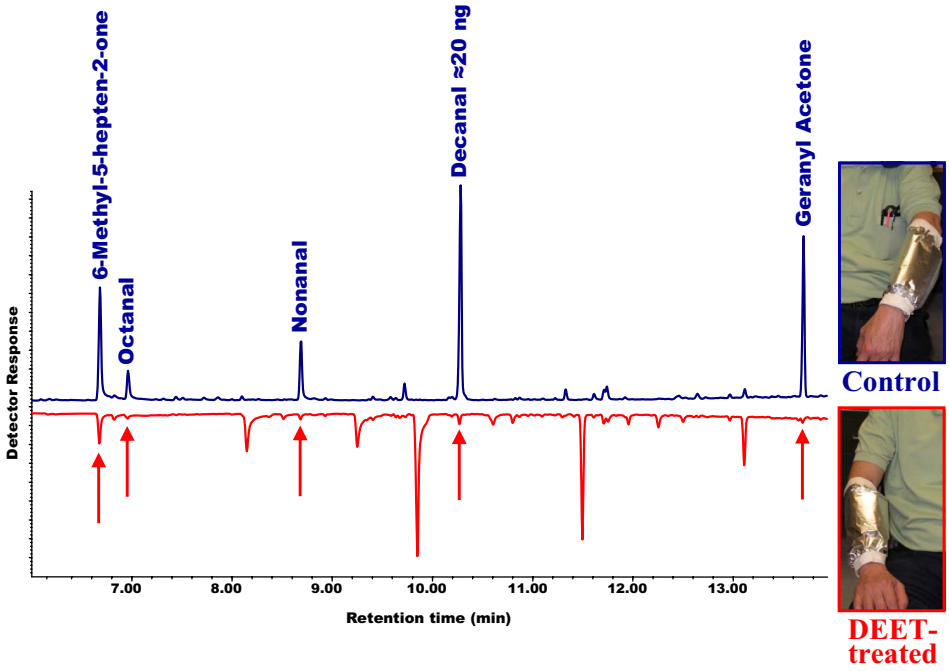

Fig. 6. GC-MS traces of human-derived compounds extracted by SPME. Skin emanations were collected simultaneously from one arm treated with DEET (*Lower*, red trace) and an untreated arm *(Upper*, blue trace) of the same subject. Arrows indicate suppression in the amounts of five physiologically relevant compounds from the arm treated with DEET. The other peaks in the GC-MS trace from the treatment (*Lower*) are impurities from DEET, whereas an overshooting peak at 15.5 min for DEET was omitted.

other odorants (see above). Second, we have identified DEET-sensitive ORN. In our apparently odorant-free, feeding bioassay, there was no significant difference between mosquitoes landing in the two Petri dishes treated with solvent only ($P = 0.5$, Mann–Whitney test) (Fig. 7), thus indicating no bias in the arena. When one of the filter paper cylinders was treated with DEET, mosquitoes avoided the repellent-treated arena and landed significantly more in the control arena (solvent only) ($P < 0.01$) (Fig. 7). To land on the sugar-treated cotton rolls, mosquitoes did not have to make any direct contact with DEET, but they had to pass through a curtain formed by vapors of DEET released from the filter paper cylinder. Landings on the side devoid of DEET (control) were very distinct from the few landings observed on the DEET side. Mosquitoes landing on the vicinity of a DEET-treated filter paper cylinder departed shortly after entering to the proximity of the DEET-impregnated area. These results clearly indicate that interactions with lactic acid are not necessary for DEET-induced repellency thus corroborating previous experimental evidence (4, 7). In addition, these results strongly suggest that no interactions with odorants in general are necessary for DEET-induced repellency. Interestingly, these sugar-feeding assays showed that males also avoided DEET (12.9 ± 3.5% of responding adults, $n = 5$). Although repelling males is a by-product of the properties of this chemical intended for determent of biting female mosquitoes (26), it does demonstrate that repellency is not sex specific, but rather a common behavior in adults. Although it is reasonable to assume that no interaction occurred between DEET and odorant(s), it is very difficult, if not impossible, to unambiguously demonstrate that no volatile compounds were released from these sugar solutions. Even nonvolatile compounds like sucrose may be contaminated with trace amounts of volatile compounds (24), but it is unlikely that these trace "contaminants" would attract mosquitoes. However, DEET could interfere with detection of water vapors (27). Therefore, we designed an additional bioassay, the surface landing (SL) assay, which was based entirely on physical stimuli as cues for mosquito orientation and landing.

Surface-Landing Bioassay. Adult mosquitoes were quite active in this paradigm. Soon after the lights were turned off they started flying inside the cage, landing on and probing the Dudley tubes (Fig. S4). In some trials we observed as much as 88% of total mosquitoes landing on Dudley tubes on the DEET-free side within 10 minutes. Of 50 female mosquitoes tested in each trial ($n = 5$), an average of 29.4 mosquitoes landed on the control (DEET-free), compared with 3.6 landings on the DEET side (Fig. 8 *A* and *B* and Movie S1). We noticed that individual landings on the DEET-free control side were longer and almost every mosquito that landed on the tube, probed the surface as if in an attempt to feed, whereas DEET-induced repellency resulted in lower landings and shorter residence time on the tube, but we limited behavioral quantification to number of landings. Throughout these experiments, we observed only one mosquito landing on a DEET-treated paper ring; the other mosquitoes observed on the repellent side landed on the tip of the arm away from the paper cylinder or in the space between the tube and the paper cylinder. This SL bioassay designed to be devoid of any chemostimuli allowed us to address many long-standing questions regarding the mode of action of DEET. First, it does not support the hypothesis that DEET interferes with the reception of other chemical signals masking the chemical signature of an otherwise attractive subject/object (4–6). The simplest explanation for mosquito avoidance of Dudley tubes treated with DEET is a direct detection of this insect repellent. Secondly, our results do not support the hypothesis that DEET somehow works by interfering with the ability of mosquito to sense water vapor (27).

In summary, we have demonstrated that DEET (*i*) is detected by specific ORNs on the antennae of *Cx quinquefasciatus*, (*ii*) induces avoidance in sugar-seeking male and female mosquitoes, and (*iii*) causes reduced landing of females in the vicinity of an attractive, warm, and black surface. Together these results lead us to clearly conclude that the mosquitoes smell and avoid DEET.

Materials and Methods

Insects. *C. quinquefasciatus* used in this study were from a laboratory colony originating from adult mosquitoes collected in Merced, CA in the 1950s and maintained under lab conditions at the Kearney Agricultural Center, University of California, as previously described (10). For electrophysiological and behavioral experiments, we used exclusively sugar-fed only, 5–10-day-old adults maintained at high humidity and 14:10 h light/dark photoperiod. *Aedes aegypti* sugar-fed females were provided from a laboratory colony originated

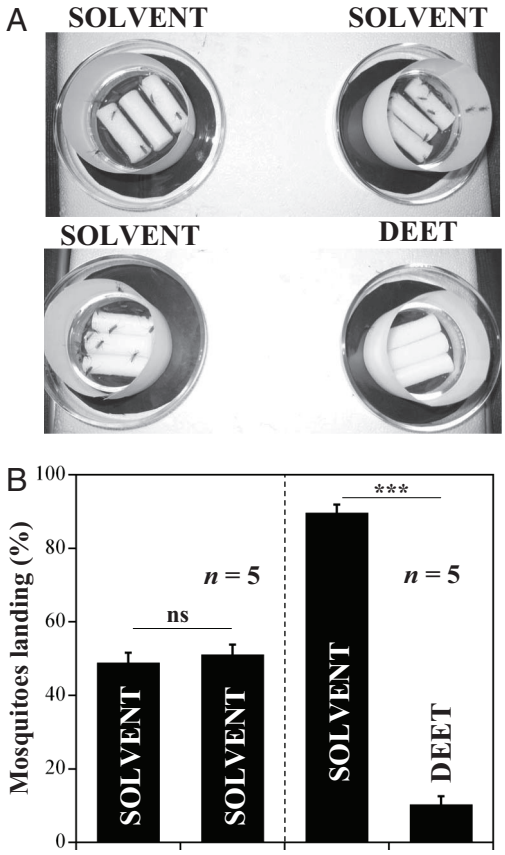

Fig. 7. DEET repellency in the absence of attractants. (*A*) Snapshots of the sugar-feeding bioassay. Although mosquitoes landed equally on solvent Petri dishes *(Upper)*, they avoided landing on the side impregnated with DEET (*Lower*). (*B*) Mosquito landings were significantly higher in Petri dishes treated with solvent only compared with those treated with DEET, whereas there was no significant difference between the two sides of the arena.

from mosquitoes collected in Mae Sot Province, Thailand, and maintained at the Mosquito Research Laboratory, University of California, Davis, CA.

Chemicals. Racemic 1-octen-3-ol and linalool were kindly provided by Bedoukian Research Inc. Technical grade $\alpha+\beta$-Thujone, eucalyptol (99.5%), (*S*)- and racemic lactic acid (90% pure), and dichloromethane (DCM, HPLC grade) were purchased from Fluka, and DEET (97% pure) was from Aldrich. Chemicals were diluted in DCM, wt/vol, to make a stock solution of 100 μg/μl and decadic dilutions were made. For bioassays, DEET was delivered in DCM but ethanol solutions were applied to human skin, whereas no solvent was used to transfer neat DEET to filter papers for stimulus cartridge. Carbon dioxide stimuli were generated from 5% CO_2/95% O_2 medical grade cylinder purchased from Airgas (Woodland, CA). The concentrations of CO_2 and the doses of other compounds specified throughout this paper are those at the point of stimulus release.

Chemical Analysis. Gas chromatography (GC) and GC-mass spectrometry (GC-MS) were done on a 6890 Series GC and a 5973 Network Mass Selective Detector (Agilent Technologies), respectively. Both instruments were equipped with the same type of capillary column (HP-5MS, 30 m x 0.25 mm; 0.25 μm; Agilent Technologies). The temperature program for the stand alone GC started at 100°C for 1 min, increased to 160°C at a rate of 12°C/min, followed by an increase at 25°C/min to 250°C, and held at this final temperature for 10 min. The GC coupled to the mass spectrum was operated at 70°C for 2 min and increased to 200°C at a rate of 10°C/min. Both GCs were operated under splitless mode with the injection port at 250°C and a postrun for 10 min at 290°C.

Single Sensillum Recordings. Recordings from antennae were essentially performed as described earlier for palps (10). Sensilla were identified by comparing the antennal preparations with scanning electron micrographs made at the UC Davis campus facility. The olfactory sensilla in *Culex* are morphologically comparable to those in *Aedes aegypti* (28), and they can be classified into trichoid (sharp-tipped, A1 and blunt-tipped A2), and grooved pegs (A3) on antenna, and the peg sensilla on palps. To identify DEET-sensitive ORNs, we recorded from at least 15 sensilla of each type. The stimulation protocol was slightly modified. We used either borosilicate glass Pasteur pipettes (Fisher Scientific) or 5 ml of Micro-Mate glass syringes (Popper & Sons, Inc.) instead of the polypropylene syringes used in previous studies (10). Although preliminary trials indicate that neuronal responses obtained with polypropylene syringes were comparable to those recorded with glass syringes, we avoided using plastic syringes because of possible interactions of DEET with plastic. We also designed a stimulation method to simultaneously puff DEET and 1-octen-3-ol from two separate stimulus cartridges. Here, the stimulus flow from a flow controller (Syntech stimulus controller, CD-02/E) was split in two lines by adding a ''Y'' splitter, with one line connected to the 1-octen-3-ol cartridge and the other connected to a DEET or blank cartridge. The outlets of the two syringes were connected to a ''Y'' splitter with a needle that fed the merged flow into the main air stream. Similar arrangement was made to deliver the compensatory flow from the stimulus controller to the main airflow. Humidified air at 20 ml/s was continuously blown over the preparations to which a stimulus pulse was added, resulting in ≈10x dilution.

Solid Phase Microextraction (SPME) Analysis of Odorant Released from Cartridge. To measure the effect of DEET on stimulus delivery, the amounts of 1-octen-3-ol released from Pasteur pipettes loaded with one or two strips were compared. One filter paper strip (4 × 50 mm) was impregnated with 1-octen-3-ol and the second one was loaded with 5 μl of pure DEET. We used a SPME gray fiber (StableFlex, divinylbenzene/Carboxen on polydimethylsiloxane coating; 50/30 μm coating; Supleco) to collect and quantify the amounts of 1-octen-3-ol delivered from stimulus cartridge. To determine whether DEET would interfere with the adsorption of 1-octen-3-ol on the fiber, we first compared the amounts of this odorant collected with a clean syringe and a syringe exposed to pure DEET for 5 min before odorant collections. Having observed no significant difference in the amounts of 1-octen-3-ol collected with fresh vs. DEET-exposed fibers we proceeded with quantification of volatiles delivered by stimulus cartridges. Each pipette was connected to a stimulus controller (CS5 model, Syntech) and 10 puffs of 2 s each were delivered with a 1 s gap between each puff. The gray SPME fiber was exposed in the pipette tip during the puffs and the SPME fiber was immediately retracted after the last puff and injected into a stand alone GC (see above). Seven repetitions of each treatment were performed.

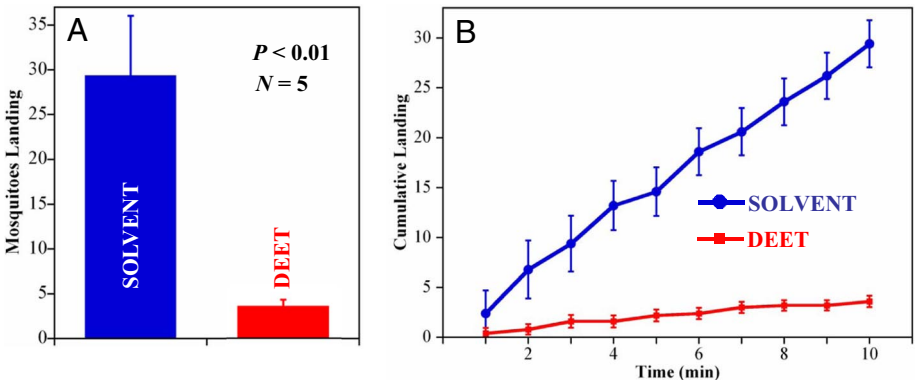

Fig. 8. DEET-induced repellency of mosquitoes responding to physical stimuli. Females landed preferentially on the solvent-treated side of this two-choice assay. (*A*) Total landings for 10 min. (*B*) Cumulative landing over time.

Collection of Human-Derived Volatile Chemicals. The human subject, a 54-year-old Latino, very attractive to mosquitoes, washed his forearm exhaustively with warm water in preparation for the experiments. A gray SPME fiber (StableFlex, divinylbenzene/Carboxen on polydimethylsiloxane coating; 50/30 μm coating; Supelco) was attached to his forearm by a Johnson & Johnson cloth tape. The tip of the exposed SPME fiber was protected from direct contact with the skin by enclosing it in a borosilicate glass tube (8 cm; outer diameter, 5 mm). The glass tube was highly perforated to allow chemicals to pass through and be adsorbed on the fiber. The forearm was wrapped in a cylinder made of aluminum foil. The cylinder was carefully slipped over the arm without disturbing the SPME fiber and both ends were loosely attached to the skin with cloth tape. The other forearm of the subject was finely sprayed with 2 ml of 20% DEET in ethanol using Kontes Chromatography TLC Reagent Sprayer (Fisher Scientific) to generate $\approx$1 mg of DEET/cm^2, a commonly used dosage in evaluation of repellents (29, 30). After letting it dry for $\approx$5 min another gray SPME fiber was attached and the arm wrapped with an aluminum cylinder. Because of DEET spray, the control arm was always the first to be prepared. After an hour, the aluminum cylinders were removed and the fibers mounted on SPME syringes and analyzed by GC-MS. As a control, we also analyzed by GC-MS headspace volatiles collected by SPME from the soap (Dove Beauty Bar, Unilever) regularly used by the human subject.

Sugar-Feeding Behavioral Bioassay. This bioassay was performed in 30 $\times$ 30 $\times$ 30 cm cages made of aluminum rods and plastic connectors draped with dark green netting cage covers (BioQuip). A white Styrofoam sheet ($\approx$30 $\times$ 30 cm) was placed at the bottom of the cage covering the entire base. Two black paper circles (diameter, 10 cm) carved out of a thick rough nonreflective black paper sheet were placed on the Styrofoam and separate from each other by 10 cm. Two large glass Petri dishes (100 $\times$ 15 mm) were placed on the top of the black circles. Smaller glass Petri dishes (60 $\times$ 15 mm) were loaded with three dental cotton rolls (3.8 cm; Sullivan Dental Products Inc.) soaked with 8 ml of freshly prepared 10% sucrose (Aldrich) solution and placed inside the larger Petri dishes. DEET was delivered from paper cylinders (diameter $\approx$6.1 cm; height, 4.5 cm) made of Whatman chromatography paper (Grade 1; Whatman International Ltd., England) and placed over the smaller Petri dishes. Two hundred microliters of 100 μg/μl solution of DEET in DCM was applied uniformly on the top periphery (height, $\approx$ 10 mm) of a filter paper to form a ring of DEET-impregnated cylinder of $\approx$1 mg/cm^2, a dosage commonly used in efficacy tests of repellents (29, 30). Controls were prepared by impregnating the cylinders with 200 μl of DCM. Treatment and control cylinders were left in a fume hood for 5 min to let the solvent evaporate and then inserted snugly around the smaller Petri dishes using separate pairs of forceps to avoid cross contamination. Choice assays (solvent vs. solvent or solvent vs. DEET) were conducted from 6:30 to 8:30 p.m. (PST). Twenty to 50 adult mosquitoes, 5–15-day-old, were used per trial. Three days before the assays, mosquitoes were removed from unlimited access to a 10% sucrose solution (10). Observations began soon after the placement of Petri dishes inside the cage, and the total numbers of adults landing on the sugar source were counted for 10 min. Nearly all mosquitoes fed until fully engorged, but a small number ($\approx$2%) flew away before repletion during the behavioral observations. Thus, the total number of landings may have included a small percentage of adults that eventually returned for further feeding.

Surface-Landing Bioassay. This bioassay was designed to mimic a human arm without any odors or humidity. Two Dudley bubbling tubes (overall length 17.5 cm, capacity 50 ml; Fisher Scientific) were roughened on the exterior by sand paper. The two Dudley tubes were connected via ''Y'' connectors and flexible tubing (outer diameter, 12.7 mm) to a circulating water bath (Lauda Ecoline Staredition, RE 106; Lauda GMBH & Company, Lauda, Germany), which was maintained at 38°C. A black reducible paint (Palmer Acrylic Paint, Raven 173203; Palmer Paint Products, Inc.) was added to the circulating water to give a black appearance to the tubes. The behavioral arena was isolated from the circulating water. The two Dudley tubes were held 15 cm apart horizontally by metal clamps and inserted through two holes cut in a 30 $\times$ 30 $\times$ 4 cm Styrofoam sheet. A red paper sheet was inserted through the tubes so as to rest on the surface of a Styrofoam sheet. The tubes were introduced into a mosquito cage (30 $\times$ 30 $\times$ 30 cm) having two equivalent holes on one side. $\approx$6.3 cm of each tube's length was exposed inside the arena. A digital video camera recorder equipped with an IR light (Digital Handycam, DCR-PC101, Sony) was connected to the opposite side of the cage through a small hole. With the red paper background and IR illumination, we could clearly observe individual mosquito behavior in the video recordings. The influence of DEET on mosquito landings was evaluated by surrounding one Dudley tube with a DEET-treated paper ring and the other with solvent (DCM) alone. Paper cylinders (height, $\approx$2 cm; diameter, $\approx$3.8 cm) were prepared from chromatography papers (Watman). Two hundred microliters of a 10% DEET solution in DCM was applied uniformly along the rim of the cylinder to form a DEET-impregnated ring. The control was treated only with DCM. The paper rings were positioned closer to the Styrofoam plate, surrounding the Dudley tubes and held by insect pins (BioQuip). The solvent or DEET-treated edges were placed away from the Styrofoam plate. The paper rings were handled by separate forceps for DEET or solvent. Before and after each trial, Dudley tubes were washed with running hot water for 10 min and air dried. To avoid exposing the Dudley tubes to human odors, all handlings were done with paper napkins, with no bare human hands contacting the tubes throughout the experiments. Experiments were conducted in the dark from 6:30 to 8:30 p.m. (PST). Added to the observation cages were fifty adult female mosquitoes collected from a rearing cage holding 5–10-day-old adults where they were given ad libitum access to 10% sucrose solution. The cage was closed immediately and the human observer left the room. Treatments were altered between each trial from left to right so as to preclude any possible positional effects. Data presented represent the result of five independent trials.

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