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A natural variant and engineered mutation in a GPCR promote DEET resistance in *C. elegans*

Emily J. Dennis¹, May Dobosiewicz², Xin Jin^{2,6}, Laura B. Duvall¹, Philip S. Hartman³, Cornelia I. Bargmann^{2,4}, and Leslie B. Vosshall^{1,4,5}

¹Laboratory of Neurogenetics and Behaviour, The Rockefeller University, New York, NY 10065, USA

²Lulu and Anthony Wang Laboratory of Neural Circuits and Behaviour, The Rockefeller University, New York, NY 10065, USA

³Department of Biology, Texas Christian University, Fort Worth, TX 76129, USA

⁴Kavli Neural Systems Institute New York, NY 10065, USA

⁵Howard Hughes Medical Institute New York, NY 10065, USA

Abstract

DEET (*N,N*-diethyl-*meta*-toluamide) is a synthetic chemical identified by the United States Department of Agriculture in 1946 in a screen for repellents to protect soldiers from mosquito-borne diseases^{1,2}. Since its discovery, DEET has become the world's most widely used arthropod repellent, and is effective against invertebrates separated by millions of years of evolution, including biting flies³, honeybees⁴, ticks⁵, and land leeches³. In insects, DEET acts on the olfactory system^{4,6–12} and requires the olfactory receptor co-receptor *orco*^{7,9–11}, but exactly how it works remains controversial¹³. Here we show that the nematode *Caenorhabditis elegans* is sensitive to DEET, and use this genetically-tractable animal to study its mechanism of action. We found that DEET is not a volatile repellent, but interferes selectively with chemotaxis to a variety of attractant and repellent molecules. In a forward genetic screen for DEET-resistant animals, we identified a single G protein-coupled receptor, *str-217*, expressed in a single pair of DEET-responsive chemosensory neurons, ADL. Misexpression of *str-217* in another chemosensory neuron conferred responses to DEET. Both engineered *str-217* mutants and a wild isolate of *C. elegans* carrying a *str-217* deletion

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⁶Present address: Society of Fellows, Harvard University, Cambridge, MA 02138, USA

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Online Content Methods and Supplemental Data File 1 containing all raw data, statistical analysis, and strains used in the paper are available in the online version of the paper. References unique to the Methods appear only in the online paper.

Data Availability Statement All scripts and graphed data with the exception of raw video files are available in Supplemental Data File 1. Raw video files are available on request from the corresponding author.

Strains. Detailed genotypes of all *C. elegans* strains and their sources are in Supplemental Data File 1.

are DEET-resistant. We found that DEET can interfere with behaviour by inducing an increase in average pause length during locomotion, and show that this increase in pausing requires both *str-217* and ADL neurons. Finally, we demonstrated that ADL neurons are activated by DEET and that optogenetic activation of ADL increased average pause length. This is consistent with the “confusant” hypothesis, in which DEET is not a simple repellent but modulates multiple olfactory pathways to scramble behavioural responses^{10,11}. Our results suggest a consistent motif for the effectiveness of DEET across widely divergent taxa: an effect on multiple chemosensory neurons to disrupt the pairing between odorant stimulus and behavioural response.

We explored whether and how *C. elegans* nematodes respond to DEET during chemotaxis^{14,15} (Fig. 1a), and to test three competing hypotheses for the mechanism of DEET: “smell-and-repel” —olfactory detection that triggers avoidance^{4,8,12}, “masking” — blocking olfactory pathways that mediate attraction^{6–8}, and “confusant” —modulation of multiple olfactory sensory neurons to scramble the perception of an otherwise attractive stimulus^{10,11}.

We first tested the smell-and-repel hypothesis. DEET was not repellent alone when presented as a volatile point source (Fig. 1b), similar to *Drosophila melanogaster* flies⁷ and *Aedes aegypti* mosquitoes¹¹, but in contrast to a report in *Culex quinquefasciatus*⁸. To ask if DEET masks responses to attractive odorants^{6,7} or directly inhibits their volatility⁸, we presented DEET with the attractant isoamyl alcohol and found that DEET had no effect on attraction (Fig. 1c). In considering alternate ways to present DEET, we mixed low concentrations of DEET uniformly into chemotaxis agar (Fig. 1d). DEET-agar reduced chemotaxis to isoamyl alcohol in a dose-dependent manner (Fig. 1e). We tested three additional attractants, butanone, diacetyl, and pyrazine, and the volatile repellent 2-nonanone. DEET decreased attraction to butanone and avoidance of 2-nonanone, indicating that it affects responses to both positive and negative chemosensory stimuli (Fig. 1f). DEET also affected chemotaxis to diacetyl, but not pyrazine. Notably, both are sensed by the AWA sensory neuron (Fig. 1f). This is similar to *D. melanogaster*, where DEET can disrupt responses to some odorants and not others, even in a single chemosensory neuron¹⁰.

In *D. melanogaster* and *Ae. aegypti*, DEET inhibits attraction to complex natural odour blends^{7,11}. In *C. elegans*, DEET eliminated chemotaxis to the food odour of OP50 *E. coli* bacteria (Fig. 1g). Remarkably, supplementing bacterial odour with pyrazine, but not isoamyl alcohol, restored chemotaxis (Fig. 1g–i). Therefore, DEET chemosensory interference is odour-selective, and affects responses to both attractive and repellent stimuli.

Previous work to identify the genetic basis of DEET-sensation using forward genetics yielded an X-linked DEET-insensitive *D. melanogaster* mutant¹⁶, and a dominant genetic basis for mosquito DEET-insensitivity¹⁷, but neither study identified the underlying genes. Reverse genetic experiments in *D. melanogaster* and three mosquito species identified *orco* as required for DEET-sensitivity^{7,9–12}, but this gene is insect-specific¹⁸, raising the question of what pathways are used in non-insect invertebrates. We therefore carried out a forward genetic screen for *C. elegans* mutants capable of chemotaxing toward isoamyl alcohol on DEET-agar plates (Fig. 2a). Of five DEET-resistant animals, three produced offspring that

chemotaxed toward isoamyl alcohol on DEET-agar plates (Fig.2b). We used whole genome sequencing to identify candidate mutations in two strains, and focused on *LBV003*, which maps to *str-217*, a predicted G protein-coupled receptor (Fig.2c, d). In the course of mapping *str-217*, we discovered that a divergent strain of *C. elegans* isolated in Hawaii, CB4856 (Hawaiian), is naturally DEET-resistant (Fig.2e) and contains a deletion in *str-217* (*str-217^{HW}*), leading to a predicted frame shift and early stop codon (Fig.2c,d, Supplemental Data File 1). This is not a unique phenomenon: 119 of 247 sequenced strains in the *C. elegans* Natural Diversity Resource (CeNDR)¹⁹ contained predicted changes in STR-217 compared to N2 wild-type (Supplemental Data File 1). We next tested three near-isogenic lines with a single, homozygous genomic segment of Hawaiian chromosome V introgressed into a wild-type background²⁰ (Fig.2e). Only the ewIR74 line contains *str-217^{HW}* and was DEET-resistant (Fig.2e). Expressing a rescue/reporter strain (Fig.2f) in *LBV003* (Fig.2g) or *ewIR74* (Fig.2h) rendered both fully sensitive to DEET (Fig.2g,h).

In insects, DEET interacts directly with chemosensory neurons^{7,9–12}. To investigate if DEET modulates primary sensory detection of isoamyl alcohol in *C. elegans*, we used *in vivo* calcium imaging to monitor AWC responses with and without DEET. AWC is the primary sensor of isoamyl alcohol²¹. AWC responded to the addition of DEET with a rapid increase in calcium that decreased to baseline over the course of 11 min of chronic DEET stimulation (Fig.3a). In the presence of DEET, AWC responses to isoamyl alcohol decreased in magnitude, but there were no significant differences in AWC activity between wild-type and *str-217^{-/-}*, a predicted null mutant produced by CRISPR-Cas9 genome-editing (Fig.3a–c). The polymodal nociceptive neurons ASH also responded to DEET (Fig.3d,e), but animals lacking ASH are fully DEET-sensitive (Fig.3f) This suggests that DEET-resistance caused by *str-217* requires other neurons.

To identify such neurons, we determined where *str-217* is expressed by examining our *str-217* rescue/reporter strain, and found GFP expression in a single pair of ADL chemosensory neurons (Fig.3g). This was unexpected because ADL is not required for chemotaxis to isoamyl alcohol²², suggesting an indirect role in DEET chemosensory interference. To confirm that *str-217* is required in ADL, we expressed *str-217* under control of another ADL promoter (*srh-220*) in *str-217^{-/-}* animals. ADL-specific rescue of *str-217* rendered this mutant sensitive to DEET (Fig.3h). To ask if ADL is required for DEET-sensitivity, we inhibited chemical synaptic transmission by expressing the tetanus toxin light chain in ADL^{23,24}. These animals showed similar DEET-resistance as *str-217* mutants (Fig. 3i), demonstrating that ADL neurons contribute to DEET-sensitivity.

Since both *str-217* and ADL contribute to DEET-sensitivity, we used calcium imaging to ask if ADL responds to DEET, and if this requires *str-217* (Fig.4a). Both wild-type and *str-217^{-/-}* mutants carrying a rescue plasmid, but not *str-217^{-/-}* mutants, showed ADL calcium responses to DEET, albeit with some variability in response (Fig.4b–d). To exclude the possibility that DEET activates ADL indirectly, we measured ADL calcium responses in *unc-13* and *unc-31* mutants, which are deficient in synaptic vesicle fusion²⁵ or dense-core vesicle fusion²⁶, respectively. ADL responses to DEET were normal in both strains (Fig.4f–h). A known ADL agonist, the pheromone C9²³, activated ADL in wild-type, *str-217^{-/-}*

mutant, and rescued animals (Fig.4j–l), suggesting that the lack of DEET responsiveness in *str-217* is selective.

We then asked if *str-217* is sufficient to confer DEET responses. While DEET did not activate HEK293T cells expressing *str-217* (Supplemental Data File), *in vivo* misexpression of *str-217* in AWB significantly increased the DEET-sensitivity of these sensory neurons (Fig.4m–o). This suggests either that *str-217* encodes a DEET receptor or cooperates with other proteins to increase DEET-sensitivity.

We next explored how ADL activity can interfere with chemotaxis, by tracking the position and posture of individual animals on DEET-agar or solvent-agar plates (Fig.5a–c). Wild-type, but not *str-217*^{−/−} mutants (Fig.5d), showed dramatic increases in average pause length on DEET-agar. Although *str-217*^{−/−} mutants are resistant to DEET compared to wild-type, their chemotaxis is not fully DEET-resistant (Fig.3h), and many *str-217*^{−/−} animals never reached the odorant source (Fig.5e). Additionally, prolonging the assays did not significantly increase performance in wild-type animals (Fig.5f). We suspect that DEET has additional effects on chemotaxis in addition to increasing pause duration.

To test if the increase in pausing on DEET occurs only during chemotaxis, we tracked wild-type, *str-217*^{−/−} mutant (Fig.5g), and *ADL::Tetanus toxin* (Fig.5h) animals on DEET-agar and solvent-agar plates without odorants. Only wild-type animals had a higher average pause length on DEET-agar (Fig.5g–h), and this was not seen during pyrazine chemotaxis (Fig.5i).

To test if ADL activity alone is sufficient to increase average pause length, we optogenetically activated the light-sensitive ion channel ReaChR²⁷ in ADL in wild-type animals and observed an increase in average pause length (Fig.5j,k). We conclude that ADL mediates the increase in average pause length seen on DEET-agar, and speculate that increase in long pauses is one mechanism by which DEET interferes with chemotaxis.

Here we add the nematode *C. elegans* to known DEET-sensitive animals and uncover a new neuronal mechanism for a DEET-induced behaviour. This work opens up *C. elegans* as a system to test new repellents *in vivo* and to discover additional genes and neurons that respond to DEET. The molecular mechanism by which the *str-217* mutation renders ADL DEET-insensitive and worms DEET-resistant remains to be understood. *str-217* is an orphan G protein-coupled receptor that is evolutionarily unrelated to DEET-sensitive insect odorant receptors. Our results are consistent with the hypothesis that *str-217* is a DEET receptor, or that it interacts with additional proteins to make ADL DEET-sensitive. Interestingly, pyrazine chemotaxis is not disrupted by DEET, consistent with our model that DEET is not a simple repellent, but a modulator of behaviour that interferes with chemotaxis to some but not all odorants. The *str-217*-dependent mechanism of action of DEET in nematodes is reminiscent of the “confusant” hypothesis in insects. In insects, DEET alters responses of individual olfactory sensory neurons to attractive odorants^{7,10}, thereby interfering with behavioural attraction. In *C. elegans*, DEET inhibits attraction to some odorants by activating neurons that induce competing behaviours like pausing. We speculate that its promiscuity in interacting with multiple molecules and chemosensory neurons across vast evolutionary scales is the key to the broad effectiveness of DEET.

Methods

Nematode culture and strains.

C. elegans strains were maintained at room temperature (22–24°C) on nematode growth medium (NGM) plates (51.3 mM NaCl, 1.7% agar, 0.25% peptone, 1 mM CaCl₂, 12.9 μM cholesterol, 1mM MgSO₄, 25mM KPO₄, pH 6) seeded with *Escherichia coli* (OP50 strain) bacteria as a food source^{28,29}. Bristol N2 was used as the wild-type strain. The CB4856 (Hawaiian) strain, harbouring WBVar02076179 (*str-217^{HW}*) (<http://www.wormbase.org/db/get?name=WBVar02076179;class=variation>) and Hawaiian recombinant inbred strains for chromosome V were previously generated²⁰. Generation of extra-chromosomal array transgenes was carried out using standard procedures³⁰, and included the transgene injected at 50 ng/μL, the fluorescent co-injection marker *Pelt-2::GFP* at 5 ng/μl (with the exception of LBV004 and LBV009, which did not include a co-injection marker), and an empty vector for a total DNA concentration of 100 ng/μl. CRISPR-Cas9-mediated mutagenesis of *str-217* was performed as described, using *rol-6* as a co-CRISPR marker³¹. The resulting *str-217* mutant strain [LBV004 *str-217(ejd001)*] results in a predicted frame-shift in the first exon [indel: insertion (AAAAAAA), deletion (CTGCTCCA), final sequence GCGTCGAAAAAAATTCAG; insertion is underlined]. The *str-217* rescue construct (*Pstr-217::str-217::SL2::GFP*) used a 1112 nucleotide length fragment 56 nucleotides upstream 5' of the translation start of *str-217*.

Microscopy and image analysis.

L2-adult stage hermaphrodites were mounted on 1% agarose pads with 10 mM sodium azide (CID 6331859, Sigma-Aldrich, catalogue #S2002) in M9 solution (22 mM KH₂PO₄, 42mM Na₂HPO₄, 85.6 mM NaCl, 1μM MgSO₄, pH 6). Images were acquired with an Axio Observer Z1 LSM 780 with Apotome a 63X objective (Zeiss), and were processed using ImageJ.

Chemotaxis assays.

Chemotaxis was tested as described¹⁵, on square plates containing 10 mL of chemotaxis agar (1.6% agar in chemotaxis buffer: 5 mM phosphate buffer pH 6.0, 1 mM CaCl₂, 1 mM MgSO₄)³². Additions of either ethanol (solvent-agar) or 50% DEET (CID: 4284, Sigma-Aldrich, catalogue #D100951) in ethanol (DEET-agar) were added after agar cooled to <44±C and just before pouring. A total volume of 300 μL ethanol or DEET in ethanol was added to each 100 mL of agar mixture for all experiments except Figure 1b and c, Figure 5j and k. Plates were poured on the day of each experiment, and dried with lids off for 4 hours prior to the start of the assay. 1 μL 1 M sodium azide was added to two spots on either side of the plate just before beginning the experiment to immobilize animals that reached the odorant or ethanol sources. Three days prior to all chemotaxis experiments, 4–6 L4 animals were transferred onto NGM plates seeded with *E. coli* (OP50 strain). The offspring of these 4–6 animals were then washed off of the plates and washed twice with S-Basal buffer (1 mM NaCl, 5.74 mM K₂HPO₄, 7.35 mM KH₂PO₄, 5 μg/mL cholesterol at pH 6–6.2)²⁸ to remove younger animals, and once with chemotaxis buffer. Immediately before the start of the experiment, two 1 μL drops of odorant diluted in ethanol, or ethanol solvent control, were spotted on each side of the plate on top of the sodium azide spots. 100–300 animals were

then placed into the centre of the plate in a small bubble of liquid. The excess liquid surrounding the animals was then removed using a Kimwipe. Odorants diluted in ethanol were used in this study at these concentrations unless otherwise noted: 1:1000 isoamyl alcohol (CID: 31260, Sigma-Aldrich, catalogue #W205702), 1:1000 butanone (CID: 6569, Sigma-Aldrich, catalogue #360473), 10 mg/μL pyrazine (CID: 9261, Sigma-Aldrich, catalogue #W401501), 1:10 2-nonanone (CID: 13187, Sigma-Aldrich, catalogue #W2787513). For bacterial chemotaxis assays, 20 μL of either LB media, or OP50 bacterial suspension grown overnight and diluted in LB media to 1.0 OD at 600nm was applied instead of or in addition to odorants. Assays were carried out for 60–90 min at room temperature (22–24°C) between 1pm – 8pm EST with the exception of Figure 5f, which were quantified after either 55–65 minutes (1 hour), 115–125 minutes (2 hours), 175–185 minutes (3 hours), or 235–245 minutes (4 hours). Plates were scored as soon as possible, either immediately or, if a large number of plates was being scored on the same day, plates were moved to 4°C to immobilize animals until they could be scored. The assay was quantified by counting animals that had left the origin in the centre of the plate, moving to either side of the plate (#Odorant, #Control) or just above or below the origin (#Other), and calculating a chemotaxis index as $[\#Odorant - \#Control] / [\#Odorant + \#Control + \#Other]$. A trial was discarded if fewer than 50 animals or more than 250 animals contributed to the chemotaxis index and participated in the assay.

Mutant screen.

About 100 wild-type (Bristol N2) L4 animals were mutagenized in M9 solution with 50 mM ethyl methanesulfonate (CID: 6113, Sigma-Aldrich, catalogue #M0880) for 4 hours with rotation at room temperature. Mutagenized animals were picked to separate 9 cm NGM agar plates seeded with *E. coli* (OP50 strain) and cultivated at 20±C. ~5,000 F2 animals were screened for DEET resistance on 20.3 cm casserole dishes (ASIN B000LNS4NQ, model number 81932OBL11). Five animals across three assays were more than ~2 cm closer to the odorant source than the rest of the animals on the plate and were defined as DEET-resistant. This phenotype was heritable in three strains, and each strain was backcrossed to OS1917 for 4 generations. Whole-genome sequencing³³ was used to map the mutations to regions containing transversions presumably introduced by the EMS, parental alleles of the N2 strain used for mutagenesis, and missing alleles of the wild-type strain OS1917 used for backcrossing^{34,35}. LBV003 mapped to a 5 Mb region on chromosome V, which was further mapped to *str-217*. LBV002 mapped to a 6.8 Mb region on chromosome V, which was further narrowed down to a likely candidate gene, *nstp-3(ejd002)*. In LBV002, *nstp-3(ejd002)* contains a T>G transversion of the 141st nucleotide in the CDS, which is predicted to produce a Phe48Val substitution in this predicted sugar:proton symporter. We were unable to map the DEET-resistant mutation(s) in LBV001.

str-217 heterologous expression in mammalian tissue culture cells.

HEK-293T cells were maintained using standard protocols in a Thermo Scientific FORMA Series II water-jacketed CO₂ incubator. HEK-293T cells were obtained directly from Invitrogen. Cells were transiently transfected with 1 μg each of pME18s plasmid expressing *GCaMP6s*, *Gqα15*, and *str-217* using Lipofectamine 2000 (CID: 100984821, Invitrogen, catalogue #1168019). Control cells excluded *str-217*, but were transfected with the other two

plasmids. Transfected cells were seeded into 384 well plates at a density of 2×10^6 cells/ml, and incubated overnight in FluoroBrite DMEM media (ThermoFisher Scientific) supplemented with foetal bovine serum (Invitrogen, catalogue #10082139) at 37°C and 5% CO₂. Cells were imaged in reading buffer [Hanks's Balanced Salt Solution (GIBCO) + 20 mM HEPES (Sigma-Aldrich)] using GFP-channel fluorescence of a Hamamatsu FDSS-6000 kinetic plate reader at The Rockefeller University High-Throughput Screening Resource Centre. DEET was prepared at 3X final concentration in reading buffer in a 384-well plate (Greiner Bio-one) from a 46% (2 M) stock solution in DMSO (Sigma-Aldrich). Plates were imaged every 1 s for 5 min. 10 μ L of DEET solution in reading buffer or vehicle (reading buffer + DMSO) was added to each well containing cells in 20 μ L of media after 30 s of baseline fluorescence recording. The final concentration of vehicle DMSO was matched to the DEET additions, with a maximum DMSO concentration of 7.8%. Fluorescence was normalized to baseline, and responses were calculated as max ratio (maximum fluorescence level/baseline fluorescence level) (Supplemental Data File 1).

ADL calcium imaging.

Calcium imaging and data analysis were performed as described³⁶, using single young adult hermaphrodites immobilized in a custom-fabricated $3 \times 3 \times 3$ mm polydimethylsiloxane (PDMS) imaging chip. *GCaMP5k* was expressed in ADL neurons under control of the *sre-1* promoter²³ and was crossed into *str-217*^{-/-} and the *str-217*^{-/-} rescue strain. Imaging of *unc-13* and *unc-31* mutant strains was performed by crossing ADL::*GCaMP5k* expressing animals to the *unc*- strains and selecting for fluorescent, uncoordinated animals. Animals were acclimated to the imaging room overnight on *E.coli* (OP50 strain) seeded plates. All stimuli were prepared the day of each experiment, and were diluted in ethanol to 1000X the desired concentration before being further diluted 1:1000 in S-Basal buffer. Young adult animals were paralyzed briefly in (–)-tetramisole hydrochloride (CID: 27944, Sigma-Aldrich, catalogue #L9756) at 1 mM for 2–5 min before transfer into the chip to paralyze body wall muscles to keep animals stationary during imaging. All animals were pre-exposed to light (470+/- 40 nm) for 100 s before recording to attenuate the light response of ADL³⁷. Experiments consisted of the following stimulation protocol: 20 s S-Basal buffer, followed by 3 repetitions of 20 s DEET (0.15% DEET and 0.15% ethanol in S-Basal) and then 20 s S-basal buffer. ADL responses desensitize rapidly³¹, so only the first of the three DEET pulses was analysed.

All GCaMP signals were recorded with Metamorph Software (Molecular Devices) and an iXon3 DU-897 EMCCD camera (Andor) at 10 frames/s using a 40x objective on an upright Zeiss Axioskop 2 microscope. Custom ImageJ scripts¹⁵ were used to track cells and quantify fluorescence. In Figure 4b, f, and j, all frames in 20 s before the DEET pulse were averaged and divided by the average of the frames during the 20 s DEET or C9 pulse to calculate F/F_0 . In Figure 4c, g, and k, traces were bleach-corrected using a custom MATLAB script and then the 5% of frames with the lowest values were averaged to create F_0 . F/F_0 was calculated by $(F - F_0)/F_0$ and then divided by the maximum value to obtain $F/F_{\max}$ ³⁸. The average value during the stimulus was calculated for each animal and plotted as a single dot in Figure 4d, h, and l. The heatmap traces in Figure 4c, g, and k were also smoothed by 5 frames, such that each data point n is the running average of $n-2$, $n-1$, n , $n+1$, and $n+2$.

AWC, ASH, and AWB calcium imaging.

Calcium imaging of freely moving worms and subsequent data analysis were performed as described³⁸, using a 3 mm² microfluidic PDMS device with two arenas that enabled simultaneous imaging of two genotypes with approximately 10 animals each. For AWC imaging, we used an integrated line (CX17256) expressing *GCaMP5a* in AWC^{ON} neurons under control of the *str-2* promoter crossed into *str-217*^{-/-} animals. Adult hermaphrodites were first paralyzed for 80–100 min in 1 mM (–)-tetramisole hydrochloride and then transferred to the arenas in S-Basal buffer. The stimulus protocol was as follows: In S-Basal, three pulses of 60 s in buffer and 30 s isoamyl alcohol, followed by 120 s in buffer. Next, the animals were switched to S-Basal with 0.15% ethanol (solvent buffer) and three pulses of 60 s in buffer and 30 s in isoamyl alcohol in solvent buffer followed by 120 s in solvent buffer before a switch to S-Basal with 0.15% ethanol and 0.15% DEET (DEET buffer). In DEET buffer, animals were given 6 pulses of 60 s in DEET buffer and then 30 s in isoamyl alcohol in DEET buffer, followed by 120 s in DEET buffer before switching to solvent buffer. In solvent buffer, the animals received three pulses of 60 s in buffer and 30 s in isoamyl alcohol in solvent buffer followed by 120 s in solvent buffer before a switch to S-Basal. In S-Basal, the animals received three pulses of 60 s in buffer and 30 s isoamyl alcohol, followed by 60 s in buffer.

Each experiment was repeated 3–4 times over 2–3 days and pooled by strain for analysis (wild-type: 31 animals, 4 experiments, 3 days; *str-217*^{-/-}: 23 animals, 3 experiments, 2 days). Images were acquired at 10 frames/s at 5X magnification (Hamamatsu Orca Flash 4 sCMOS), with 10 ms pulsed illumination every 100 ms (Sola, Lumencor; 470/40 nm excitation). Fluorescence levels were analysed using a custom ImageJ script that integrates and background-subtracts fluorescence levels of the AWC neuron cell body (6×6 pixel region of interest). Traces were normalized by subtracting and then dividing by the baseline fluorescence, defined as the average fluorescence of the last 2 s of the first three isoamyl alcohol pulses. The traces in Figure 3a–b were also smoothed by 5 frames, such that each data point *n* is the running average of *n-2*, *n-1*, *n*, *n+1*, and *n+2*. The response magnitudes in Figure 3c were calculated by taking the mean of the last 2 s of an isoamyl alcohol pulse, subtracting the mean of the 2 s before the isoamyl alcohol pulse (F0), and dividing by this F0. The response magnitudes were calculated for the 5th (0.15% ethanol in S-Basal buffer) and 8th (0.15% DEET and 0.15% ethanol in S-Basal buffer isoamyl alcohol pulses).

ASH calcium imaging was performed similarly with the following exceptions. For ASH imaging, we used a strain (CX10979) expressing *GCaMP3* in ASH neurons under control of the *sra-6* promoter. The stimulus protocol used was as follows: 60 s in S-Basal, 60 s in 0.15% ethanol in S-Basal buffer, 60 s in S-Basal, 60 s in 0.15% DEET in S-Basal, and finally 60 s in S-Basal buffer. Each experiment was repeated over 2 days and pooled for analysis (wild-type: 15 animals in 2 experiments on 2 different days).

AWB calcium imaging was performed similarly to AWC imaging with the following exceptions. For AWB imaging, we used a control strain (CX17428) expressing *GCaMP5a* in AWB neurons under the *str-1* promoter and a test strain (CX17660) expressing *GCaMP5a* under the *str-1* promoter as well as expressing *str-217* in AWB neurons under the *str-1* promoter. Adult hermaphrodites were first similarly paralyzed in 1 mM (–)-tetramisole

hydrochloride for 80–100 minutes, but the first 65–75 minutes was in S-Basal buffer and the last 15 minutes was 1 mM (–)-tetramisole hydrochloride in ethanol-buffer. The stimulus protocol used was as follows: 60 s in 0.15% ethanol in S-Basal buffer, 10 s in 0.15% DEET and 0.15% ethanol in S-Basal buffer, and 60 s in 0.15% ethanol in S-Basal buffer. A 4×4 pixel region of interest was used during tracking of the neurons. Baseline fluorescence was defined as the median fluorescence of the 10 s preceding the DEET pulse. Response and peak magnitudes were calculated using traces smoothed by 5 frames and identifying the maximum value within the DEET pulse. Five sets of experiments were conducted over 3 days for a total of 41 wild-type animals and 49 animals expressing *str-217*.

In Figure 4n, traces were bleach corrected using a custom MATLAB script and then the 5% of frames with the lowest values were averaged to create F_0 . F/F_0 (%) was calculated by $(F - F_0)/F_0^{38}$. The heatmap traces in Figure 4n were also smoothed by 5 frames, such that each data point n is the running average of $n-2$, $n-1$, n , $n+1$, and $n+2$. Peak F/F_0 (%) in Figure 4o reflects the maximum value of F/F_0 (%) during the DEET pulse.

Chemotaxis tracking and analysis.

8–20 adult hermaphrodites were first transferred to an empty NGM plate and then 4–15 were transferred to an assay plate to minimize bacterial transfer. Animals were then placed in the centre on either a 0.15% DEET-agar or solvent-agar plate, and their movement was recorded for 60 min at 3 frames/s with 6.6 MP PL-B781F CMOS camera (PxeLINK) and Streampix software. Assays were carried out at room temperature, between 12–8pm EST, and lit from below. Worm trajectories were extracted by a custom Matlab (MathWorks) script¹⁵, and discontinuous tracks were then manually linked. Tracks were discarded if the animal moved less than two body lengths from its origin over the course of the 60 min trial. If an animal came within 1 cm of the isoamyl alcohol stimulus, the track was truncated to remove information from animals immobilized at the odorant source because of the addition of sodium azide.

ADL optogenetic stimulation.

L4 animals expressing an *Psrh-220::ReaChR*²⁷ array or array-negative animals from the same plate were raised overnight in the dark on an NGM plate freshly seeded with 100 μ L of 10X concentrated *E. coli* (OP50 strain) with or without 50 μ M all-*trans* retinal (CID: 720648, Sigma-Aldrich, catalogue #R2500), which is required for ReaChR-induced activity. The next day, adult hermaphrodites were first transferred to an empty NGM plate and then 4–15 animals were transferred to the 10 cm circular assay plate to minimize bacterial transfer. Videos were recorded for 26 min at 3 frames/s with a 1.3 MP PL-A741 camera (PxeLINK) and Streampix software. Blue light pulses were delivered with an LED (455 nm, 45 μ W/mm², Mightex) controlled with a custom Matlab script^{15,39}. Animals were exposed to normal light for 120 s, before exposure to 6 repetitions of blue light (10 Hz strobing) for 120 s, and 120 s of recovery (LED OFF). Worm trajectories were extracted by a custom Matlab script³⁹. Pausing events were extracted, and all pauses ≥ 3 frames (1 s) were used for further analysis. Pauses were classified as “ON” if any frame included light illumination. A pause that began just before illumination began, but remained paused while the illumination occurred, was considered an ON pause, as well as any pauses that began in the during light

illumination considered ON. All other pauses were classified as “OFF” pauses. In the analysis in Figure 5j, we took an average pause length for all ON pauses and all OFF pauses for each animal and pooled all of the animals on each plate. To control for any baseline differences between animals and experiment-to-experiment variation, we examined the increase in average pause length in Figure 5k.

Phylogenetic Analysis of str-217 in wild isolates.

Data were obtained from CeNDR36¹⁹ and plotted in R. Only predicted deletions in exons or missense changes of high confidence were included (Supplemental Data File 1).

Statistical Analysis.

R v3.3.2 was used for all statistical analysis. Inclusion and exclusion criteria were pre-established for all experiments, and plate positions were pseudo-randomized in behaviour experiments. Additionally, qqPlots were evaluated before performing ANOVAs. For the analysis of optogenetic experiments, a Levene’s Test identified heteroskedasticity in these data that was addressed with a boxcox translation. AWC imaging data were similarly boxcox translated and transformed to adjust for the rightward skew.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1 | DEET interferes with wild-type *C. elegans* chemotaxis

a, Chemotaxis assay. **b-c**, Chemotaxis with point source stimuli of DEET without (**b**) or with isoamyl alcohol (**c**). **d**, Chemotaxis assay on DEET-agar plates. **e**, Chemotaxis dose-response to isoamyl alcohol. **f**, Chemotaxis to indicated odors. **g**, Chemotaxis to bacterial food source with indicated odors. **h-i**, Chemotaxis dose-response to isoamyl alcohol (**h**) and pyrazine (**i**). In **b-c**, and **e-i**, each dot represents a chemotaxis index of a single population assay. Data labelled with different letters indicate significant differences [mean $\pm$ s.e.m.; $p < 0.05$, two-sided t-test (**b-c**) or one-way (**e**) or two-way (**f-i**) ANOVA and Tukey's Post-hoc test]. See Supplementary Data File 1 for sample sizes and details of statistical analyses in all figures.

Figure 2 | *str-217* mutants are DEET-resistant.

a, Forward genetic screen schematic (DEET-resistant mutants circled). **b**, Chemotaxis of indicated strains. **c**, *str-217* genomic locus. **d**, STR-217 protein snake plots in indicated strains. **e**, Chromosome V schematic (left) and chemotaxis (right) in indicated strains. **f**, *str-217* rescue/reporter construct. **g-h**, Chemotaxis of indicated strains. In **b**, **e**, and **g-h** each dot represents a chemotaxis index of a single population assay. Data labelled with different letters indicate significant differences (mean $\pm$ s.e.m, $p < 0.05$ ANOVA and Tukey's Post-hoc test in **e**, and two-sided t-test in **b** and **g-h**).

Figure 3 | Several *C. elegans* neurons respond to DEET, but ADL neurons are required for DEET-sensitivity.

a, Average traces of GCaMP activity in AWC^{ON} cells of indicated genotype over a 36 min experiment. **b**, A subset of traces from (a) re-plotted separately. **c**, Response magnitudes of data in b. **d**, GCaMP activity in ASH neurons to indicated stimuli. **e**, Quantification of (d). **f**, Chemotaxis of indicated strains to isoamyl alcohol. **g**, *str-217* rescue/reporter GFP expression in a single ADL neuron (scale bar: 10 μ m). **h-i**, Isoamyl alcohol chemotaxis of indicated strains. In a-b, d, each trace represents mean (dark line) and s.e.m. (lighter area) GCaMP response of all animals of each genotype. In c and e, each dot represents response of a single animal. In f and h-i, each dot represents a chemotaxis index of a single population assay [mean $\pm$ s.e.m., $p < 0.05$, two-way (b, e) or one-way (h) ANOVA with Tukey's Post-hoc test; paired t-test in d and g].

Figure 4 | *str-217* is necessary for ADL responses to DEET and can confer DEET sensitivity to AWB.

a, e, i, and m, Calcium imaging schematics. **b, f, and j,** Pseudocolored images of ADL responding to 0.15% DEET (b, f) or 100 nM C9 (j) in animals of indicated genotype (fold increase in mean fluorescence 20 s during first stimulus pulse/mean of 20 s before the stimulus pulse). Arrows point to corresponding animal in following panels. **c, g and k,** Heat maps of GCaMP activity in response to DEET (c, g) or C9 (k). Each row represents activity in one animal. **d, h, and l,** Mean normalized responses of the data in c, g, and k during DEET (d, h) or C9 (l) entire pulse. **n,** Heat maps of AWB calcium imaging response to 0.15% DEET, each row represents imaging from one animal cropped to show the 10 s before, during, and after the DEET pulse. **o,** Quantification of (n). In d, h, l, and o each dot represents a single neuron in a single animal. Data labelled with different letters indicate significant differences [mean $\pm$ s.e.m. $p < 0.05$, one-way ANOVA and Tukey's Post-hoc test (d, h, l); two-sided t-test (o)]. The rescue construct (c) is the same as in Fig. 3g-h.

Figure 5 | DEET increases average pause length by activating *str-217* and ADL.

a-b, Top: example trajectories of single wild-type isoamyl alcohol chemotaxis on solvent-agar (a) or DEET-agar (b). Each dot depicts the x, y position every 10 s. Bottom: raster plots of paused frames for animal above. **c,** Example pauses from (b). Images were extracted and converted to silhouettes every 6 s and superimposed. **d,** Average pause length of indicated stimuli and genotypes. **e,** Top: Location of animals in (d) after 60 minutes. Bottom: Histogram of animal location from e. **f,** Wild-type DEET chemotaxis at indicated times. **g-i,** Average pause length of indicated stimuli and genotypes. **j,** Average pause length of indicated genotypes and LED status. Lines connect experimental pairs. **k,** Difference in average pause length for each experimental pair in j. In d, f-k each dot represents a single experimental plate. Data labelled with different letters indicate significant differences [mean $\pm$ s.e.m. $p < 0.05$, one-way (f) or two-way ANOVA (d,g-k) and Tukey's Post-hoc test].