Introduction: DEET (*N,N*-diethyl-*meta*-toluamide) is a chemical and a potent repellent of disease vectors such as mosquitoes and ticks. Discovered in a screen of over 7,000 molecules, DEET was developed for the U.S. Army for application on human skin in 1946. Although DEET is the world's most widely used insect repellent, the neurobiological mechanisms of how DEET mediates avoidance remain controversial. Better understanding of the processes underlying DEET's effectiveness would lead to the development of safer and more effective insect repellents.

Background & preliminary data: Previous research shows that DEET's mechanism of action is multimodal[1,2,3]. It acts through mosquitoes' sense of smell, evidenced by animals lacking *orco*[2], a necessary subunit of insect olfactory receptors, and through bitter-sensitive receptors in the labellum[3] (Fig. A). Through behavioral assays and video analysis, we have shown that mosquitoes sense and are repelled by DEET on contact through their legs (tarsi)[4] (Fig. A) Although the olfactory mechanisms of DEET are better understood, little is known about how DEET repels on contact.

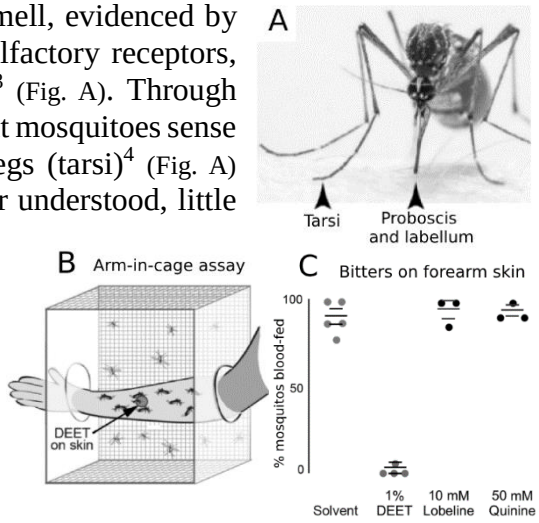

Figure | A. Female *Aedes aegypti* mosquito feeding on a human arm. **B.** DEET-treated arm with 25mm circle of accessible skin. **C.** Blood-feeding with indicated compounds applied to human arm.

Our results demonstrate that when mosquito tarsi come into contact with DEET, the mosquitoes will not blood feed from that surface (Fig. C). Previous work suggests that DEET avoidance acts through bitter receptors in the labellum[3] (Fig. A). We found that high concentrations of bitters (such as quinine and lobeline), which are sufficient to prevent feeding when contacted by the labellum[3], are ineffective at mediating avoidance through tarsal contact when applied to human skin or an artificial surface (Fig. B & C). My preliminary work suggests that DEET repels mosquitoes on contact through an avoidance pathway more strongly than or independent of bitter taste sensation, and that this deters them from blood-feeding. But nothing is known about the cellular or molecular mechanisms by which the tarsi detect DEET.

For my PhD thesis work, I propose to decipher this contact-mediated pathway to elucidate the biological mechanism of action for DEET in mosquito tarsi. Using genetic tools recently developed in the mosquito, I will locate DEET-sensitive cells in the tarsi and identify receptor(s) that mediate DEET avoidance.

Aim 1: Which neurons in *Aedes aegypti* tarsi respond to DEET? To investigate DEET contact repellency further, I will use *in vivo* calcium imaging to compare neural activity responses to different chemical compounds in sensory cells within the mosquito tarsi. I will use an *Ae. aegypti* pan-neuronal promoter to express the genetically-encoded calcium sensor, GCaMP6s, in all tarsi sensory neurons. Using a recent protocol from my lab[5], I will present chemical solutions over intact tarsi while recording neural activity with a two-photon microscope and analyze the location and response dynamics of activated sensory cells. To determine if neurons in the tarsi are specialized to encode different chemical cues, I will compare responses within these tarsi sensory cells to DEET, sweet compounds, and bitter compounds. In behavioral assays, *Aedes aegypti* respond very differently to bitter, sweet, and DEET cues, therefore I hypothesize that chemosensory cells in the tarsi have different population response patterns to these different chemical cues. This may be in the form of different cells reacting to different compounds, or discrimination of chemical cues encoded through differences in population-level activity. This work will advance our understanding of the peripheral sensory response that chemical cues elicit in *Ae. aegypti* tarsi.

Aim 2: Is DEET-sensitivity conferred by different RNA expression patterns? In *C. elegans*, a subset of neurons express a G-protein coupled receptor, *str-217*, that is necessary for DEET behavioral response[6]. To determine if sensory cells *Ae. aegypti* tarsi express a similar specialized receptor, I will perform RNA expression analysis on separate populations of cells responsive to DEET, bitters, and sugars. Depending on the location and pattern of cells identified in Aim 1, I will develop methods for dissociating sensory cells from mosquito tarsi using either laser-capture microdissection (LCM) or fluorescent activated cell sorting (FACS) on photoactivatable GFP to precisely isolate and harvest these separate chemosensitive cell populations. Collaborating with the Rockefeller University genomics core, I will use single-cell RNA sequencing (sc-RNAseq) to compare RNA expression patterns of DEET-responsive cell populations to tarsal cells responsive to bitters and sweet compounds. I expect cell populations that respond to different tastants to differentially express a number of proteins and receptors. Through this Aim, I will develop a method for in-depth investigation of mosquito tarsal RNA expression while creating a list of candidate molecules that may be sufficient or necessary for DEET sensitivity.

Aim 3: Can candidate genes for DEET-sensitivity be validated through genetic knock-out? To identify the functional relevance of genes identified in Aim 2, I will use CRISPR-Cas9-based gene editing[2] to create knock-out animals for candidate genes that may confer DEET-responsiveness. I will then use the behavioral screen in Figure B to determine if the mutant animal has become DEET-insensitive, or partially insensitive. Ultimately, this work would result in the first identification of a receptor in mosquito tarsi that mediates avoidance behavior upon contact.

Intellectual merit: This work has the potential to advance the field of chemosensation and solve a long-standing mystery in neurobiology. Although DEET is highly effective in repelling a wide range of evolutionarily divergent invertebrates, the mechanism of DEET avoidance is still controversial 70 years after its discovery. Uncovering the mechanism of DEET avoidance promises to elucidate new principles underlying how chemosensation is encoded and subsequently translated into behavior.

Broader impacts: Mosquitoes and ticks that blood-feed on human hosts can transmit pathogens that cause a number of devastating diseases, threatening hundreds of millions of lives yearly. Identifying biological processes that mediate avoidance of blood-feeding, such as those underlying the mechanism of DEET, may lead to the development of more effective insect repellents that could last longer at lower doses and reduce the exposure of human populations to dangerous vector-borne diseases.

In addition, because the general public has experience with insect repellents, DEET presents itself as a very tractable example for public and youth engagement with the sciences. I am excited to continue my efforts in science communication to inspire young potential scientists with such an accessible topic. I will participate again this year in Rockefeller University's Science Saturday, an annual science festival for over 1000 children in grades K–8 and their families, where I will host a demonstration around DEET to illustrate basic principles of chemosensation. I will also teach a class on chemosensation at the Rockefeller Summer Neuroscience Program, a graduate student-led course for disadvantaged high school students from New York City public schools. I am eager to share my passion for sensory perception and chemosensation, through a topic that children will already be familiar with. Using my research project on DEET, I hope to help students realize that their personal observations can be of scientific value, potentially inspiring them to pursue their own interests in science.

References: 1. M. Ditzen, *et al., Science* 319, 1838-1842 (2008). **2.** M. DeGennaro, *et al., Nature* 498, 487-491 (2013). **3.** Y. Lee, *et al., Neuron* 67, 555-561 (2010).). **4.** E.J. Dennis, **O.V. Goldman**, L.B. Vosshall, *in revision at Current Biology*. **5.** B.J. Matthews, M.A. Younger, *et al., bioRxiv* (2018). **6.** E.J. Dennis, *et al., Nature* 562, 119–123 (2018).