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Similar Neural Pathways Control Foraging in Mosquitoes and Worms

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ABSTRACT Female *Aedes aegypti* mosquitoes bite human hosts to obtain a blood meal and, in the process, act as vectors for many disease-causing viruses, including the dengue, chikungunya, yellow fever, and Zika viruses. After a complete meal, the female mosquitoes lose attraction to their hosts for several days. New research shows that pharmacological activation of neuropeptide Y-like receptor (NPYLR) signaling elicits host aversion in female mosquitoes. This behavior of mosquitoes shows remarkable similarities to a bacterial-aversion behavior of the nematode *Caenorhabditis elegans*. Feeding on pathogenic bacteria causes bloating of the gut in *C. elegans* that leads to activation of NPYLR signaling and bacterial aversion. Several studies suggest that this newly discovered mechanism underlying foraging may be conserved across a large number of species. A better understanding of the regulation of NPYLR signaling pathways could provide molecular targets for the control of eating behaviors in different animals, including human-disease vectors.

KEYWORDS *A. aegypti*, *C. elegans*, NPR-1, aversion behavior, bacterial colonization, feeding, neuropeptide Y

The *Aedes aegypti* mosquito is the primary vector for many disease-causing viruses, including the dengue, chikungunya, yellow fever, and Zika viruses. The female mosquitoes require blood from a host for the development of eggs and to complete their full reproductive life cycle (1). In the absence of a blood meal, the mosquitoes fail to develop eggs and do not reproduce. Therefore, the female mosquitoes possess a strong host-seeking behavior for a blood meal that depends primarily on sensing carbon dioxide (CO_2) (1, 2). A single female goes through multiple blood-feeding and egg-laying cycles in her lifetime. Due to the cyclic nature of their feeding behavior, the female mosquitoes serve as effective carriers of disease-causing viruses from infected humans to healthy individuals. Thus, the suppression of the host-seeking behavior or elicitation of host aversion has the potential of being a practical method for reducing the spread of disease-causing viruses. In a recent study, Duvall et al. (3) reported that pharmacological activation of G protein-coupled neuropeptide Y (NPY)-related signaling elicits a host aversion behavior.

Extensive studies have been carried out to understand the blood-feeding behavior of mosquitoes, and they showed that host aversion consists of at least two phases: an early phase involving gut distension from a blood meal (4) and a sustained phase that lasts until the female lays her eggs (5, 6). Previous studies showed that a blood meal to repletion led to enhanced levels of neuropeptides in the hemolymph (7). Injection of hemolymph from blood-fed females or high doses of synthetic peptides that activate NPY-like receptors were sufficient to elicit host aversion in non-blood-fed females (6, 7). These findings suggested that a humoral response involving neuropeptides and activation of an NPY-related pathway plays a role in sustained host aversion in fully fed *A. aegypti* mosquitoes.

NPY-related signaling pathways are evolutionarily conserved and have been impli-

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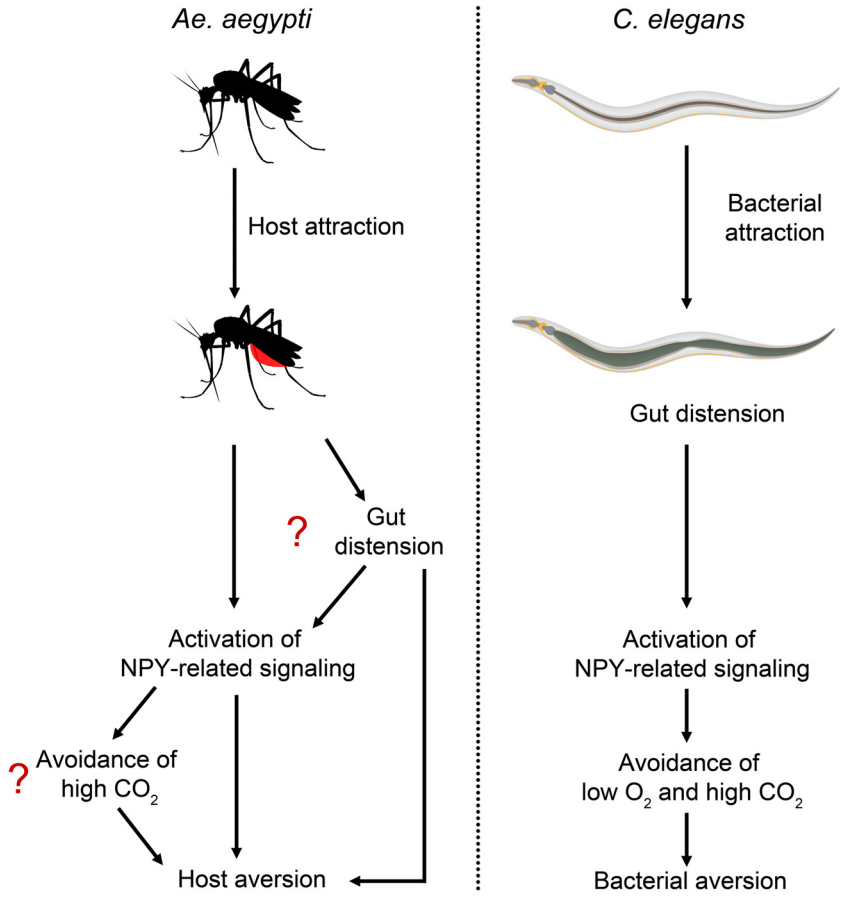

FIG 1 A blood meal to repletion and gut distension caused by bacterial colonization lead to activation of NPY-related signaling in female *A. aegypti* mosquitoes and *C. elegans*, respectively. The increased NPY-related signaling is responsible for host aversion behavior in mosquitoes and bacterial-aversion behavior in *C. elegans*.

cated in several biological processes, including foraging, neuronal excitability, stress response, and motivated feeding behavior (8). Small-molecule drugs that possess high affinity to NPY human receptors might bind to mosquito NPY-like receptors and serve as powerful tools to investigate the functional role of NPY-related signaling in host-seeking behavior. Duvall and coworkers (3) performed a targeted drug screen and found that agonists for NPY-related signaling elicit a host aversion behavior even in non-blood-fed mosquitoes. Conversely, an antagonist for the NPY-related signaling elicited host-seeking behavior even in blood-fed mosquitoes. From a screen of all 49 predicted *A. aegypti* peptide receptors, the authors identified NPY-like receptor 7 (NPYLR7) as the sole target of these drugs. In addition, the investigators identified 9 peptide ligands for NPYLR7, including several Phe-Met-Arg-Phe amides (FMRFamides). These results showed that modulating NPYLR7 activity might affect host-seeking behavior. To identify *A. aegypti*-specific agonists with no cross-reactivity to human NPY receptors, the authors challenged NPYLR7 with 265,211 unique small molecules. The investigators isolated six highly selective NPYLR7 agonists that inhibit attraction to humans in wild-type mosquitos but not in *NPYLR7* null mutants. Finally, the investigators showed that these drugs were capable of inhibiting biting and blood feeding on a live host and, therefore, suggested a novel approach to control infectious disease transmission by controlling mosquito behavior.

This newly described mechanism for induction of host aversion in mosquitoes shows striking similarities to a bacterial-aversion behavior in the model nematode *Caenorhabditis elegans* (Fig. 1). *C. elegans* lives in anthropomorphic environments rich

in rotting vegetation and decaying fruit, where it is in contact with soilborne microbes, including bacteria that can be used as a food source. A recent study shows that gut distension caused by bacterial ingestion activates a *C. elegans* NPY-like receptor signal, involving NPR-1 and its FMRF-like peptide ligands, leading to bacterial aversion (9). Inhibition of the NPR-1 signaling pathway elicits avoidance of low CO_2 and high O_2, while higher activity of the pathway induces avoidance of high CO_2 and low O_2 (10–12). Because bacterial lawns have high CO_2 and low O_2 due to bacterial metabolism, NPR-1 signaling integrates this information and helps in the elicitation of bacterial avoidance behavior in *C. elegans* (10, 13, 14). While mosquitoes seek hosts by their CO_2 levels and NPY-related signaling induces host aversion, it remains to be studied whether increased NPY-related signaling in blood-fed mosquitoes induces CO_2 avoidance by a mechanism similar to that of *C. elegans* (Fig. 1).

An important question that remains unanswered is how NPY-related signaling is activated in mosquitoes after a complete blood meal. In *C. elegans*, the activation of NPR-1 signaling was independent of colonization by live bacteria and depended on gut distension (9). Given the high similarities between the host aversion behavior of mosquitoes and bacterial-aversion behavior of *C. elegans*, it is possible that the NPY-related signaling is activated by similar mechanisms in mosquitoes. Indeed, gut distension is shown to be the reason for the early-phase induction of host aversion in mosquitoes. It will be interesting to study whether gut distension leads to the activation of the NPY-related signaling in mosquitoes.

Apart from mosquitoes and *C. elegans*, a variety of disparate species sense levels of CO_2 and/or O_2 in the environment and modulate their behavior accordingly (15). Other blood-feeding insects, such as black flies and tsetse flies, are attracted to CO_2 and use this signal to seek their human hosts. Similarly, the infective juveniles of the parasitic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* are attracted to CO_2 and use this cue for host seeking (16). CO_2 may also function as an alarm signal, as CO_2 emitted by stressed *Drosophila* acts as a signal for other *Drosophila* flies to flee (17). The hawkmoth *Manduca sexta* prefers flowers that emit a high level of CO_2, suggesting that CO_2 acts as a proximal signal for nectar (18). Given that CO_2 and/or O_2 levels in the environment modulate the behavior of different organisms, it will be important to study whether the molecular mechanisms of sensing CO_2 and O_2 are conserved across species. The sensing mechanism of CO_2 and O_2 are relatively well studied in the model organisms *C. elegans* and *Drosophila* (15). This knowledge might act as a primer to expedite the understanding of the underlying molecular mechanisms in disease vectors and parasites.

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