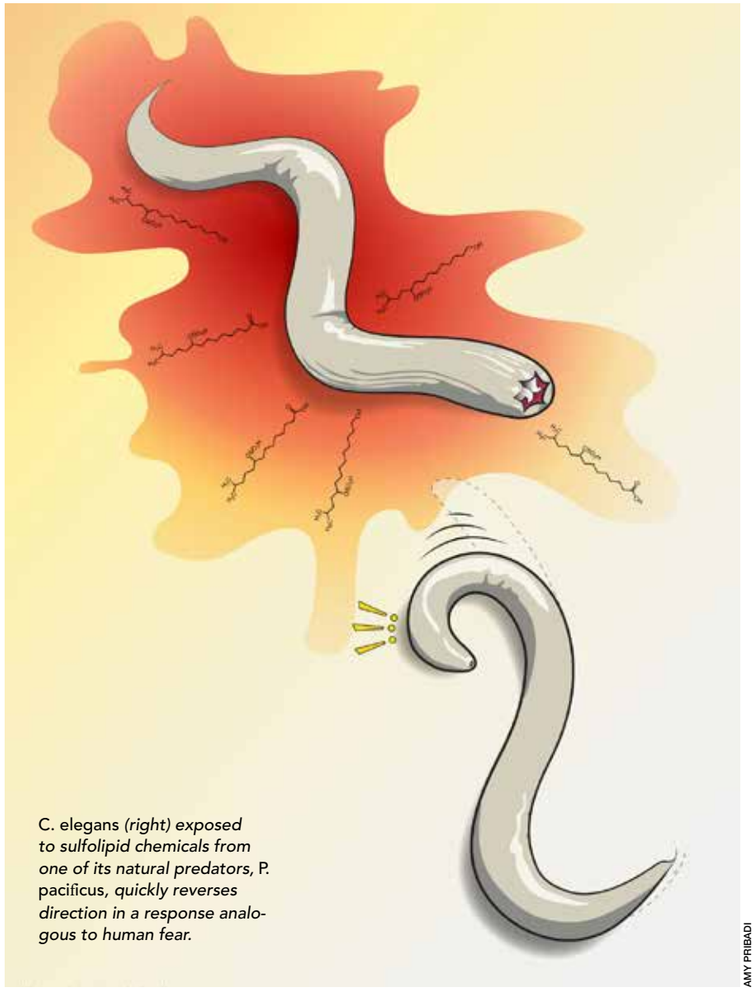
# The Chemistry of Fear

## A fear-like response in worms has parallels in human anxiety



C. elegans *(right) exposed to sulfolipid chemicals from one of its natural predators,* P. pacificus, *quickly reverses direction in a response analogous to human fear.*

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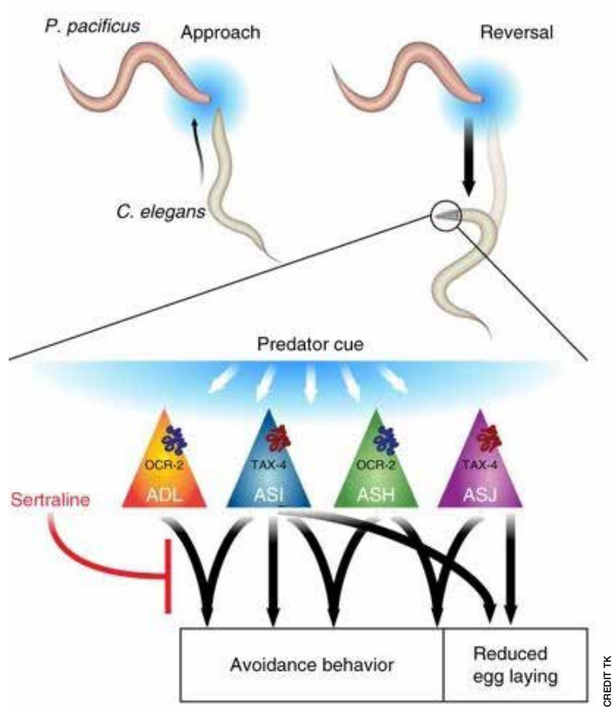
Animal survival depends on the ability to sense predators and generate appropriate behavioral and physiological changes. Such defensive response, including the commonly observed "flight or freezing," are often hard-wired into the genome of the prey—for example, mice reliably exhibit fear-like responses to cat odors despite not having encountered cats for hundreds of generations.

Studies of both vertebrates and invertebrates indicate that signaling between predators and prey usually involves multiple senses, including sight, hearing, and most frequently smell. Considerable progress has been made in identifying the sensory neurons that detect predator-released odors. In mice, the chemosensory neurons in the olfactory system—vomeronasal organ (VNO), Gruenberg ganglion, and main olfactory epithelium—have been shown to trigger defensive behavior through detection of signals from cat urine and fox feces. These neurons project to higher brain regions, where predator odor information is processed, to generate defensive behavior. While neural circuits that detect odors vary between individuals, those that sense predator-released odors appear to be the same for members of the same species. However, the precise identities of the participating neurons, their connections, and the nature of the circuit computations driving this invariant defensive behavior have remained elusive.

As we reported in the March 19, 2018 issue of *Nature Communications*, we approached these questions by analyzing the response of the nematode *Caenorhabditis elegans* to a predatory nematode *Pristionchus pacificus*. Nematodes are microscopic worms and are the most numerous multicellular animals on Earth. These two nematodes likely shared a common ancestor around 350 million years ago. Recent studies have shown that the somewhat larger *P. pacificus* is a facultative predator; it will kill and consume *C. elegans* if its own nervous system is under crowded and/or starvation conditions.

*C. elegans*, with its fully mapped neural network, comprising just 302 neurons connected by identified synapses and powerful genetic tools, is ideally suited for molecular and circuit-level analysis of complex behavior. Combining chemical and genetic methods, we dissected the signaling circuits underlying *C. elegans*' responses to *P. pacificus*. We found that a novel class of small molecules—sulfolipids, or fatty acids—excreted by *P. pacificus* trigger defensive responses in *C. elegans*. These *P. pacificus*-derived chemical signals, or predator cue, are detected by *C. elegans* by multiple sensory neurons and processed via signaling pathways.

We further found that *C. elegans* exposed to predator cue did not lay eggs for many minutes following exposure, even when placed on food (bacterial lawn), suggesting that predator cue-induced stress affects egg-laying behavior. Consistent with this idea, previous studies have shown that *C. elegans* retain eggs in the gonad when exposed to environmental stressors. To test our hypothesis, prey were exposed to predator cue for thirty minutes, and egg-laying was monitored for many hours following cue removal. Animals exposed to predator cue laid significantly fewer eggs than controls during the initial sixty minutes following cue removal. During the next hour, these animals laid more eggs than controls, suggesting that predator cue transiently modified egg-laying behavior, but not egg production. Collectively, these results indicate that starving *P. pacificus* release a potent, non-volatile factor (predator cue) that elicits multiple prey responses, namely urgent escape behavior followed by up to one hour of reduced egg laying.

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C. elegans *detects predator cue using sensory circuits consisting of neurons ASI, ASJ, ASH, and ADL that use pathways, or CNG and TRP ion channels, to generate rapid avoidance. In contrast, CNG channels act in ASI and ASJ neurons to reduce egg laying over many minutes. The human anti-anxiety drug sertraline (brand name "Zoloft") reduces both avoidance and egg-laying behavior downstream of these sensory neurons.*

To identify signaling pathways regulating responses to predator cue, we screened a variety of human anti-anxiety drugs since these compounds have previously been shown to reduce predator-induced defense responses in prey. In this screen, wild-type animals were pre-treated with different compounds for thirty minutes before testing their responses to predator cue. In a pilot screen of thirty compounds, we found that pre-treating prey with a selective serotonin reuptake inhibitor, sertraline (brand name 'Zoloft') reduced avoidance to predator cue and purified sulfolipids. Sertraline also reduced avoidance responses to fructose, and, to a lesser extent, copper sulfate, suggesting that the drug affects some, but not all repellent circuits. Suppression of avoidance behavior by sertraline was dependent on drug concentrations and lasted at least thirty minutes after the drug was removed.

In addition, we found that Zoloft acted on *C. elegans* GABA (γ-aminobutyric acid) signaling in a neuron that affects the worm's sleep. Whether this is the case in humans is not yet known, but points to a potential pathway to understand why Zoloft works in some people and not others. The research eventually could lead to a change in how these drugs are prescribed. We hope the findings from this paper will contribute to the field by providing a broader picture of some of these signaling activities. Our findings suggest that fear and anxiety are ancient and evolved much earlier than we originally thought. The pathways, nerves, circuits and genes that we'll now be able to study in *C. elegans* should inform us about this process in humans.

This article was adapted from "Predator-secreted sulfolipids induce defensive responses in *C. elegans*," by Zheng Liu, Maro J. Kariya, Christopher D. Chute, Amy K. Pribadi, Sarah G. Leinwand, Ada Tong1, Kevin P. Curran, Neelanjan Bose, Frank C. Schroeder, Jagan Srinivasan, and Sreekanth H. Chalasani, *Nature Communications*, published 19 March 2018, © 2018 The Authors, Distributed under a Creative Commons Attribution 4.0 International License.

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