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Insight into how insects sense and process pain and other negative stimuli

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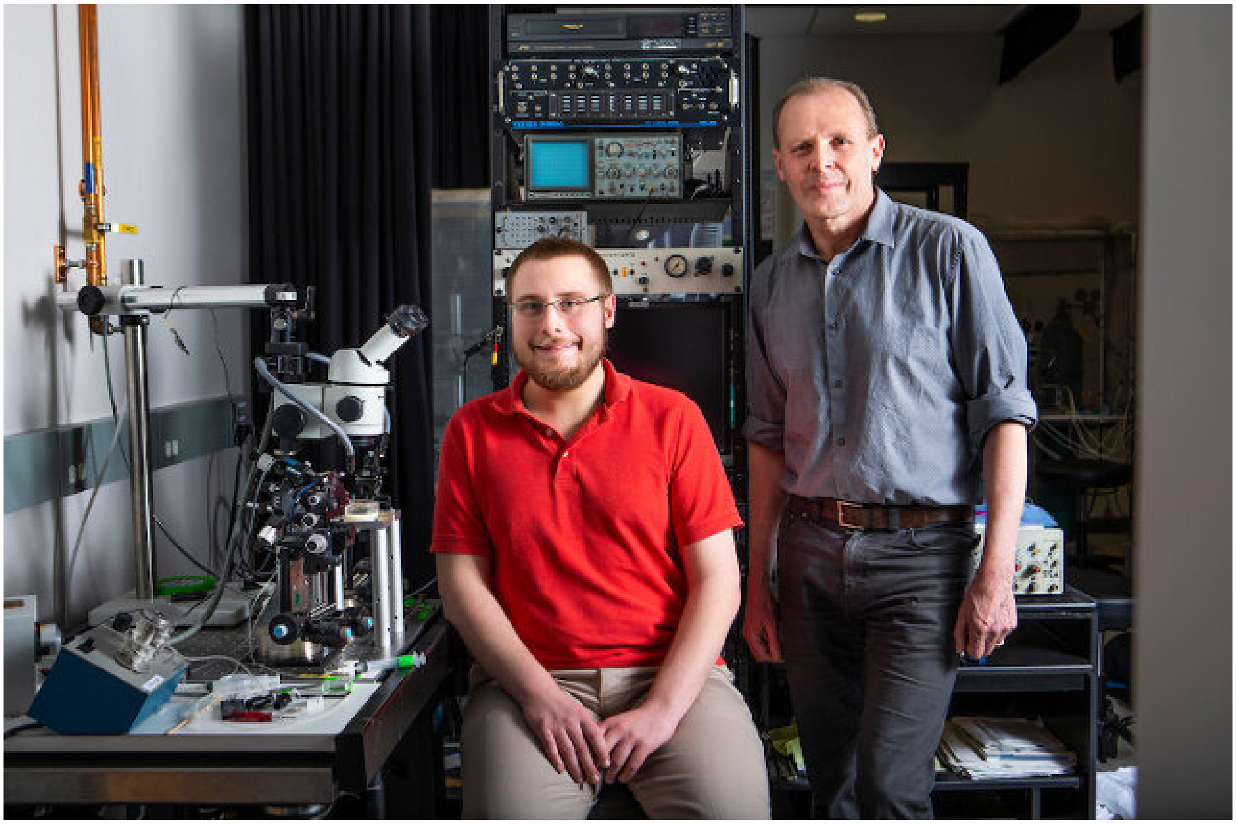

“These findings are an important milestone for developing the tobacco hornworm as a new model system to help us understand the neural mechanisms of nociception and pain,” said Barry Trimmer, right, here with Daniel Caron in February. Credit: Anna Miller

Scientists know that most organisms react to things that cause them pain, but they know more about some species than others. Take the fruit fly—it's a favorite species to do all sort of research on, from genetics to, yes, how they detect pain.

But there is little known about how other insects sense harmful stimuli. Scientists at Tufts, including an undergraduate biology major, recently discovered that tobacco hornworm caterpillars, which range throughout the Americas, can sense and respond to different noxious stimuli using a single cellular mechanism. The researchers published a paper about the findings in the *Journal of Experimental Biology* in January.

Daniel Caron—a senior honors thesis student who worked in the lab of Barry Trimmer, the Henry Bromfield Pearson Professor of Natural Science—first set out to test whether the caterpillars respond differently when either struck with infrared lasers or prodded with narrow stainless steel rods.

Caron, with the help of Martha Rimniceanu, watched as the tobacco hornworm caterpillars quickly and precisely contorted their bodies around and touched where they felt pain, responding similarly to the heat from the lasers and the pressure from the rods.

Caron was curious why the caterpillars respond so similarly to two very different sensations. The team pursued a hunch that cells under the caterpillar's skin might be able to detect both types of heat and pressure, just like a group of cells in fruit flies that react to changes in pressure, temperature, and light.

Trimmer recalls Caron's passion for the project. "Like all good scientists, Dan built on others' work, in this case the pioneering senior thesis work of Martha, and carried out thoughtful and meticulous experiments to characterize these neurons," he said.

In order to study how the caterpillars' cells respond to heat and pressure, Caron had to learn a technique to examine the activity of caterpillars' miniscule cells. He spent an entire semester learning how to attach tiny glass electrodes to cells, to be able to record their electrical activity. After months of frustration, he successfully recorded the activity of cells under the caterpillar's skin while simultaneously poking the skin around the cells with either lasers or metal rods.

As predicted, the same cells could respond to both heat and mechanical pressure—just like a group of cells in fruit flies.

To make sure that he really had looked at responses from just a single cell rather than a group of similar cells, Caron prodded the area again with his lasers and rods, but now at a much faster rate. That tested whether a singular cell would respond to both types of noxious stimuli, rather than two similar cells responding separately to each stimulus. If it was a single cell, the activity of that cell should decrease over time as it got used to the repeated stimulation.

At first, Caron could not see any change in how the cells responded, so he tried striking the cells at a very rapid rate.

His perseverance once again led to a novel discovery. In these cases, the cells under the skin didn't just decrease their responsiveness to both types of stimuli; sometimes the cells didn't respond at all.

To ensure that this change wasn't from simply having damaged the cells, Caron made sure that the cells eventually reacted again to both heat and pressure. Not only did this confirm that he recorded from single cells, but it also is the first piece of evidence of a cellular "depression" in insects in response to harmful repeated poking.

Since the fruit fly and the tobacco hornworm are separated by more than

260 million years of evolution, this finding suggests that the cellular mechanism underlying how pain is sensed might be highly conserved across species. This means that other species might also have similar mechanisms for sensing pain.

Trimmer concurs. "These findings are an important milestone for developing the tobacco hornworm as a new model system to help us understand the neural mechanisms of nociception and pain," he said. Caron's discovery of the similarities between tobacco hornworms and fruit flies could inform future research on pain and nociception in other animals, including humans.

"This shows how Tufts undergraduates are making significant contributions to science," said Trimmer. "I am proud of Dan and Martha's hard work and intellectual accomplishments."

More information: Daniel P. Caron et al. Nociceptive neurons respond to multimodal stimuli in Manduca sexta, *The Journal of Experimental Biology* (2020). DOI: 10.1242/jeb.218859

Provided by Tufts University

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