Surprising new role for calcium in sensing pain

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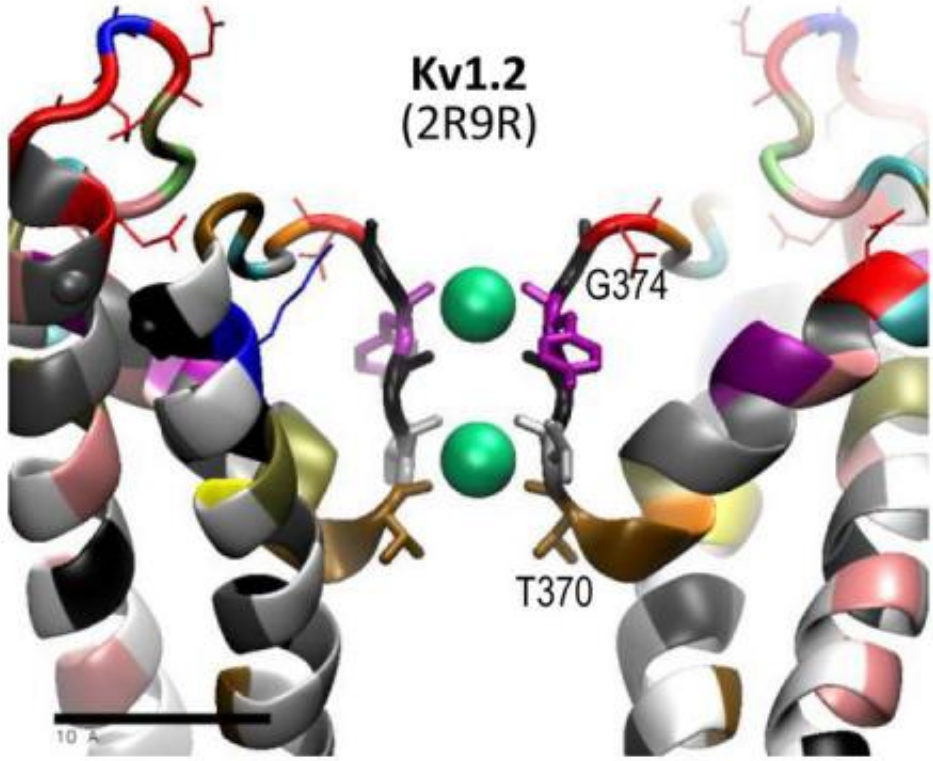

A consensus shape for the calcium ion channel in the worm's pain receptor nerve that was reached by computer modeling. Credit: Damian van Rossum and Andriy Anishkin, Duke University

When you accidentally touch a hot oven, you rapidly pull your hand away. Although scientists know the basic neural circuits involved in

sensing and responding to such painful stimuli, they are still sorting out the molecular players.

Duke researchers have made a surprising discovery about the role of a key molecule involved in pain in worms, and have built a structural model of the molecule. These discoveries, described Sept. 2 in *Nature Communications*, may help direct new strategies to treat pain in people.

In humans and other mammals, a family of molecules called TRP ion channels plays a crucial role in nerve cells that directly sense painful stimuli. Researchers are now blocking these channels in clinical trials to evaluate this as a possible treatment for various types of pain.

The roundworm Caenorhabditis elegans also expresses TRP channels—one of which is called OSM-9—in its single head pain-sensing neuron (which is similar to the pain-sensing nerve cells for the human face). OSM-9 is not only vital for detecting danger signals in the tiny worms, but is also a functional match to TRPV4, a mammalian TRP channel involved in sensing pain.

In the new study, researchers created a series of genetic mutant worms in which parts of the OSM-9 channel were disabled or replaced and then tested the engineered worms' reactions to overly salty solution, which is normally aversive and painful.

Specifically, the mutant worms had alterations in the pore of the OSM-9 channels in their pain-sensing neuron, which gets fired up upon channel activation to allow calcium and sodium to flow into the neuron. That, in turn, was thought to switch on the neural circuit that encodes rapid withdrawal behavior—like pulling the finger from the stove.

"People strongly believed that calcium entering the cell through the TRP channel is everything in terms of cellular activation," said lead author

Wolfgang Liedtke, M.D., Ph.D., an associate professor of neurology, anesthesiology and neurobiology at Duke University School of Medicine and an attending physician in the Duke Pain Clinics, where he sees patients with chronic head-neck and face-pain.

With then-graduate student Amanda Lindy, "we wanted to systemically mutagenize the OSM-9 pore and see what we could find in the live animal, in its pain behavior," Liedtke said.

To the group's surprise, changing various bits of OSM-9's pore did not change most of the mutant worms' reactions to the salty solution. However, these mutations did affect the flow of calcium into the cell. The disconnect they saw suggested the calcium was not playing a direct role in the worms' avoidance of danger signals.

Calcium has been thought to be indispensable for pain behavior—not only in worms' channels but in pain-related TRP channels in mammals. So results from the engineered OSM-9 mutant worms will change a central concept for the understanding of pain, Liedtke said.

To see whether calcium might instead play a role in the worms' ability to adapt to repeated painful stimuli, the group then repeatedly exposed pore-mutant worms to the aversive and pain stimuli.

After the tenth trial, a normal worm becomes less sensitive to high salt. But one mutant worm with a minimal change to one specific part of its OSM-9 pore—altered so that calcium no longer entered but sodium did—was just as sensitive on the tenth trial as on the first.

The results confirmed that calcium flow through the channel makes the worms more adaptable to painful stimuli; it helps them cope with the onslaught by desensitizing them. This could well represent a survival advantage, Liedtke said.

To put the findings into a structural context, Liedtke collaborated with computational protein scientists Damian van Rossum and Andriy Anishkin from Penn State University, who built a structural model of OSM-9 that was based on established structures of several of the channel's relatives, including the recently resolved structure of TRPV1, the molecule that senses pain caused by heat and hot chili peppers.

The team was then able to visualize the key parts of the OSM-9 pore in the context of the entire channel. They understood better how the pore holds its shape and allows sodium and calcium to pass.

Liedtke said that understanding this structure could be a great help in designing compounds that will not completely block the channel but will just prevent calcium from entering the cell. Although calcium helps desensitize worms to painful stimuli in the near term, it might set up chronic, pathological pain circuits in the long term, Liedtke said.

So, as a next step, the group plans to assess the longer-term effects calcium flow has in pain neurons. For example, calcium could change the expression of particular genes in the sensory neuron. And such gene expression changes could underlie chronic, pathologic pain.

"We assume, and so far the evidence is quite good, that chronic, pathological pain has to do with people's genetic switches in their sensory system set in the wrong way, long term. That's something our new worm model will now allow us to approach rationally by experimentation," Liedtke said.

More information: "TRPV-channel-mediated calcium-transients in nociceptor neurons are dispensable for avoidance behavior," Amanda S. Lindy, Puja K. Parekh, Richard Zhu, Patrick Kanju, Sree V. Chintapalli, Volodymyr Tsvilovskyy, Randen L. Patterson, Andriy Anishkin, Damian B. van Rossum, Wolfgang B. Liedtke. *Nature Communications*, Sept. 2,

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