Researchers describe a new way to visualize force-sensing neurons

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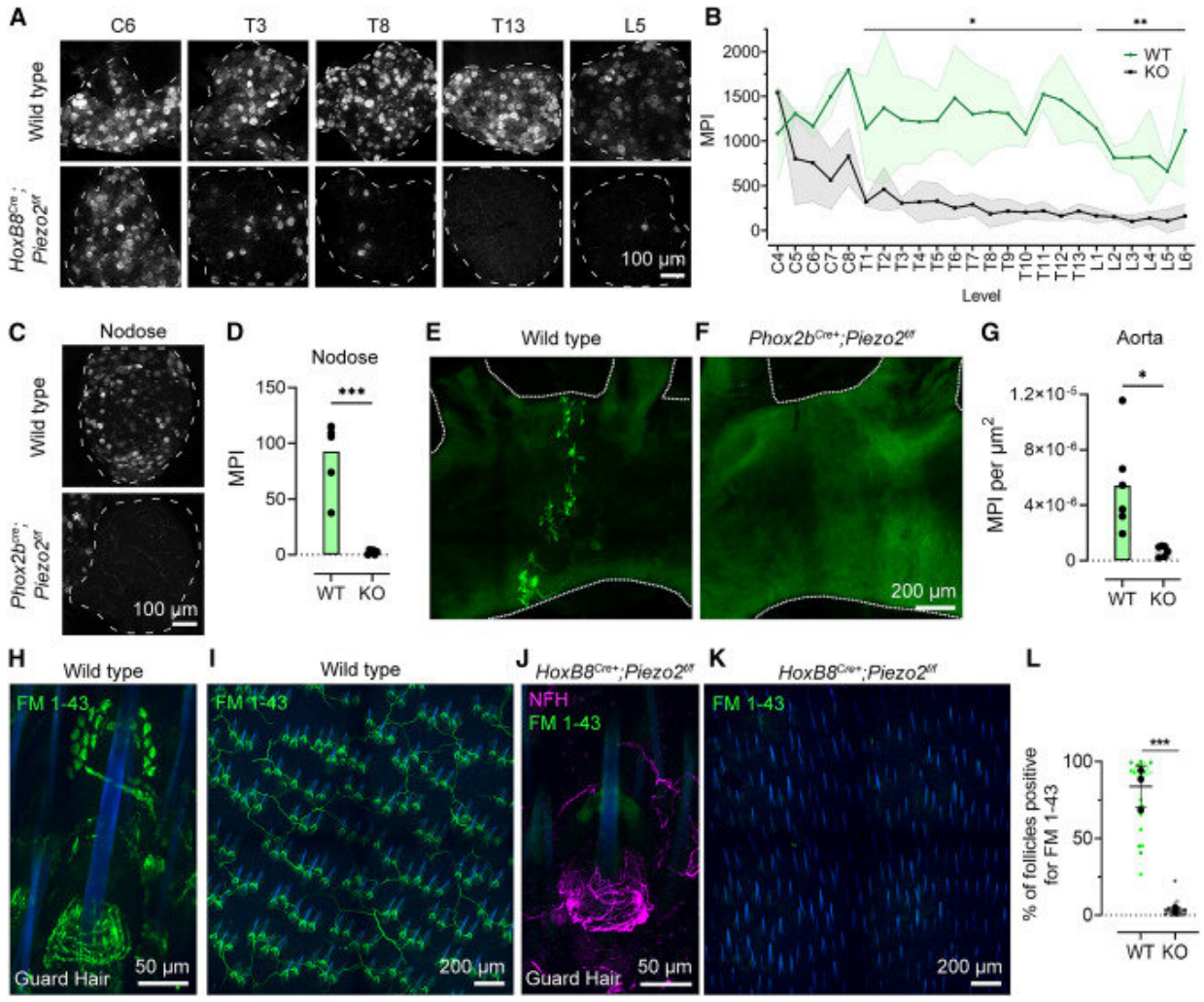

The majority of FM 1-43 sensory neuron uptake depends on Piezo2 expression. Credit: *Neuron* (2023). DOI: 10.1016/j.neuron.2023.05.015

A recent study by researchers at Texas Children's Hospital, Baylor College of Medicine, and Scripps Research Institute has discovered fluorescent dye FM 1-43 as an effective and versatile tool to visualize PIEZO2 ion channel activity in mechanosensory neurons.

The study, published in *Neuron*, was led by Dr. Kara Marshall, assistant professor at Baylor College of Medicine and investigator at the Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, with Dr. Ardem Patapoutian, Nobel Laureate and professor at Scripps Research Institute.

Mechanosensation is the ability to detect dynamic mechanical stimuli like pressure, stretch, and shear stress, and it is crucial for the function of many physiological processes. Sensory neurons gather mechanical stimuli from the environment and internal organs to convey this information to the brain.

In 2010, a landmark study from the Patapoutian lab led to the discovery of PIEZO1 and PIEZO2 as essential components of distinct mechanically-activated ion channels. Subsequent studies by this group and others have demonstrated the critical role these channels play in sensing and responding to external and internal mechanical stimuli such as touch, proprioception, and bladder stretch sensation. The broad expression of these receptors in sensory neurons suggests it may have yet undiscovered roles in many organ systems.

"There are no known endogenous chemical agonists for PIEZO2, so identifying where PIEZO2 is expressed is an important clue that a specific cell or tissue may detect and respond to mechanical forces," said Dr. Marshall, who is also a McNair Scholar.

"However, one of the challenges in understanding mechanosensation is our inability to specifically identify mechanosensory neurons.

Unfortunately, antibodies against PIEZO2 or a PIEZO2-GFP knock-in mouse have had limited efficacy for in vivo localization of PIEZO2—a technical hurdle that the scientists in the field have struggled with for years and one that has slowed down further progress."

FM 1-43 as a specific in vivo marker of PIEZO2 expression

In their quest to find an in vivo visualization tool for mechanosensory neurons, this research team turned to fluorescent styryl dye FM 1-43, which partitions into lipid membranes and has long been used to visualize intracellular trafficking pathways in various tissues both in vitro and in vivo.

In addition to its well-documented role in cellular trafficking, FM 1-43 has been proposed to enter cells through a separate mechanism: via sensory ion channels. This dye results in vivid cellular labeling via several sensory ion channels in vitro, and was observed to label many sensory neurons in vivo.

To test how much of FM 1-43 labeling was specifically due to PIEZO2-expressing mechanosensory neurons in animals, the team generated transgenic mice that lacked PIEZO2 in certain tissues during development. To their absolute surprise, they found that in these animals the FM 1-43 labeling was significantly reduced, suggesting that most labeling in the sensory nervous system depended on PIEZO2 activity and was not a result of non-specific affinity for many ion channels, as believed previously.

Based on these and other experiments, the authors concluded that although FM 1-43 is non-specific in vitro, it specifically labels PIEZO2-dependent mechanosensory neurons in vivo. The mechanisms

underlying these differences in FM 1-43 labeling remain to be explored.

FM 1-43 as a versatile in vivo marker for PIEZO2-mediated activity of mechanosensory neurons

Furthermore, the researchers also observed that PIEZO2-dependent FM 1-43 labeling overlapped closely with neurons that had high levels of PIEZO2 activity, demonstrating its utility not only as an in vivo marker of PIEZO2 expression but also its activity.

Importantly, the investigators used this tool to identify a previously undescribed tree-like neuron type in the urethra. The activity-dependence of the dye allowed them to demonstrate that these neurons are engaged during urination. Little is known about the important sensory neurons in the lower urinary tract, so this demonstrates the utility of FM 1-43 as a tool to find novel mechanosensory cell types and begin to define their function.

"FM 1-43 robustly labeled PIEZO2 channels in vivo in cultured cells, and in a wide range of species including mice, bats, and moles, highlighting the ease and versatility of this technique," Dr. Patapoutian said.

"To our knowledge, this is the only example of a tool that allows for permanent localization of the activity of a specific ion channel. Taken together, we expect FM 1-43 dyes will aid researchers in confirming and better defining the many functions of PIEZO2 ion channels and lead to novel insights about the physiological roles and mechanisms involved in mechanosensation in different organ systems."

More information: Kara L Marshall, Labeling PIEZO2 activity in the

peripheral nervous system, *Neuron* (2023). DOI: 10.1016/j.neuron.2023.05.015.
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