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RESEARCH NEWS

Sniffing out Ca^{2+} signaling in olfactory cilia

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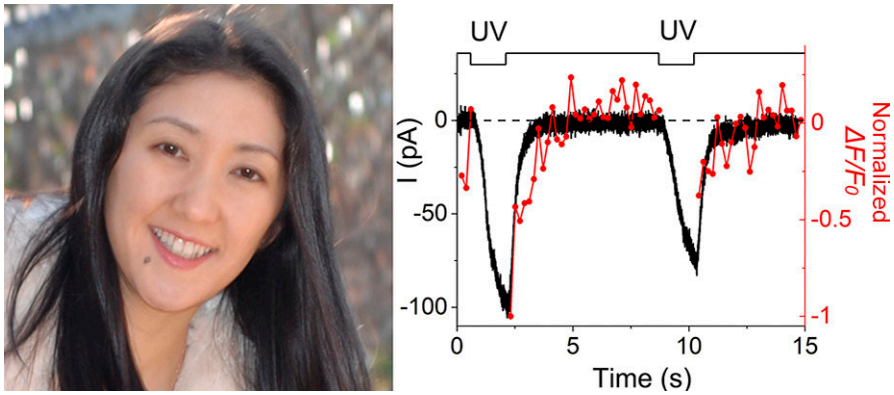
***JGP* study (Takeuchi and Kurahashi. 2023. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.202213165) reveals that segregation of signals within sensory cilia allows Ca^{2+} to play opposing roles in olfactory signal transduction.**

Our sense of smell begins in the sensory cilia that protrude from the surface of olfactory receptor cells (ORCs), where the chemical signal of odorant molecules is converted into an electrical signal that can be transmitted to the brain. The binding of molecules to odorant receptors in the ciliary membrane triggers production of the second messenger cAMP, which, in turn, binds and activates CNG ion channels, leading to an influx of Ca^{2+} ions into the cilium (1). In this issue of *JGP*, Takeuchi and Kurahashi successfully image Ca^{2+} dynamics within the long, thin cilia of ORCs, allowing them to explain how Ca^{2+} plays contrasting roles in subsequent signal transduction events (2).

After entering through CNG channels, Ca^{2+} amplifies the olfactory signal by activating Cl^- channels to fully depolarize the ORC (3). However, in a feedback process known as olfactory adaptation, Ca^{2+}, via the Ca^{2+}-binding protein calmodulin, suppresses the activity of CNG channels to reduce the signals generated by lingering smells (4, 5). "At first glance, one might think that these two processes cancel each other out, but the actual cellular response is not like that," says Hiroko Takeuchi, an associate professor at Osaka University. "We wanted to investigate how Ca^{2+} plays both excitatory and inhibitory roles within a submicron area of the cell."

The thin structure of ORC sensory cilia has made it difficult to analyze Ca^{2+}'s divergent effects on olfactory signaling. But Takeuchi, together with Professor Takashi Kurahashi, devised a method to simultaneously measure Ca^{2+} dynamics and membrane currents in the same cilium (2). Isolated ORCs are patch-clamped in a whole-cell configuration and the recording pipette is used to introduce a fluorescent Ca^{2+}-sensitive dye and caged cAMP to the ciliary cytoplasm. UV photolysis with a laser scanning microscope can then be used to uncage the cAMP and activate CNG channels in a small region of the cilium, triggering Ca^{2+} influx and the generation of membrane currents.



Hiroko Takeuchi (left) and Takashi Kurahashi reveal that Ca^{2+}'s opposing functions in signal amplification and suppression are segregated within the sensory cilia of olfactory receptor cells. Simultaneous measurements of intraciliary Ca^{2+} dynamics (red trace) and membrane current (black trace) shows that Ca^{2+} levels return to baseline after an initial UV-triggered stimulus, yet the response to a second stimulus is still reduced due to Ca^{2+}-mediated olfactory adaptation.

When the UV stimulus ceased, intraciliary Ca^{2+} levels returned to baseline in <2 s, showing a similar time course to the decline in membrane current (including Ca^{2+}-activated Cl^- components). But this immediate return to basal Ca^{2+} levels was surprising, because Ca^{2+}-dependent olfactory adaptation is known to persist for >10 s (4). Indeed, when Takeuchi and Kurahashi subjected cilia to two pulses of UV stimulation, the second pulse elicited a smaller current response (indicating adaptation) even though Ca^{2+} levels returned to baseline in the intervening period.

One explanation for this segregation of Ca^{2+}-dependent signal boosting and Ca^{2+}-mediated adaptation could be that the presence of the Ca^{2+} buffer EGTA in the recording pipette alters the dynamics of ciliary Ca^{2+} in some unexpected way. "However, removing EGTA did not change the result, which indicates that the same segregation phenomenon happens in native cilia," Takeuchi says.

Takeuchi explains that, after the initial influx of Ca^{2+} through CNG channels, the ability of Ca^{2+}-activated Cl^- channels to boost the signal is most likely regulated by free Ca^{2+} in the ciliary cytoplasm. But, when cytoplasmic Ca^{2+} levels return to baseline, some Ca^{2+} remains bound to calmodulin, which mediates olfactory adaptation by binding to CNG channels.

Having established a method to simultaneously measure intraciliary Ca^{2+} and

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membrane current, the researchers now hope to gain a more quantitative understanding of Ca^{2+} dynamics in sensory cilia and how it influences this crucial first stage in our sense of smell.

References

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