NEUROSCIENCE

Detecting acetylcholine

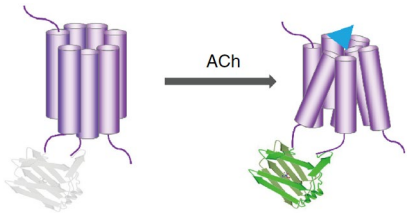
A genetically encoded sensor based on G-protein-coupled receptors can detect the neurotransmitter acetylcholine in vitro and in vivo.

Acetylcholine functions in both the central and the peripheral nervous system. It can act either as a neurotransmitter or as a neuromodulator. Despite the importance of cholinergic neurotransmission, the tools used to study these processes have their limitations. "Probes, especially genetically encoded probes, to detect neurotransmitters and modulators are a bottleneck for us to understand neuronal communication," says Yulong Li from Peking University.

Together with his team, Li tries to overcome this bottleneck by developing genetically encoded sensors for a variety of neurotransmitters and neuromodulators. The researchers recently published a dopamine sensor and now report an acetylcholine sensor, GACh, that is based on a similar design. Li was inspired to use G-protein-coupled receptors (GPCRs) as a scaffold to mimic how neurotransmitters are sensed in nature. In the case of the acetylcholine sensor, the researchers inserted circularly permuted GFP into an intracellular loop of different muscarinic receptors, a class of acetylcholine receptors. Acetylcholine binding triggers a conformational change in the GPCR, which can then be read out through a change in the fluorescence of the inserted GFP.

Working from this idea, the researchers tried to generate sensors for different neuromodulators such as norepinephrine, acetylcholine and dopamine. "Acetylcholine is the one that worked the best at the beginning," says Li. To develop additional sensors, they had to determine the optimal insertion site for the fluorescent protein through trial and error. In particular, the insertion sites for the dopamine and acetylcholine sensors are different.

Li points out that his team's sensor has advantages over established methods to detect acetylcholine. GACh is genetically encoded, which allows for transgenic expression in specific cell types, and the gene is small enough to be packaged into adeno-associated viruses. "That really lowers the bar for people in the field to use this tool," says Li. Alternative methods include microdialysis and fast-scan cyclic voltammetry. However, microdialysis is relatively slow, and voltammetry is hampered by the fact that acetylcholine is not easily oxidized. Neither of these approaches reaches cell-type specificity. "Our way is one of the most convenient ways to achieve cell-type specificity, chemical specificity, and very good spatial and temporal resolution," says Li.



Design principle of the acetylcholine (ACh) sensor GACh. Adapted with permission from Jing et al. (2018).

Li and his colleagues demonstrated the utility of the GACh sensor in a variety of different contexts. In addition to the initial characterization in non-neuronal and neuronal cell culture, the team monitored acetylcholine neurotransmission in cultured and acute brain slices, as well as in pancreas and adrenal gland preparations, and verified the sensor's properties in response to pharmacological manipulation. Moreover, the researchers imaged cholinergic neurotransmission in the olfactory system of transgenic *Drosophila* exposed to different odors. Finally, the team used two-photon microscopy to monitor acetylcholine release in the visual cortex of awake mice upon visual stimulation.

Although the team has already shown that the acetylcholine sensor can be used in vivo, the demonstrations so far have required averaging over multiple trials. Li and his team are already working on improving the existing sensor, with the goal of increasing the change in fluorescence upon acetylcholine binding. The team also continues to develop additional neurotransmitter sensors and is interested in expanding the color palette toward red and near-infrared sensors. ❐

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Research papers

Jing, M. et al. A genetically encoded fluorescent acetylcholine indicator for *in vitro* and *in vivo* studies. *Nat. Biotechnol.* **36**, 726–737 (2018).