Tracking neuronal activity in the living brain

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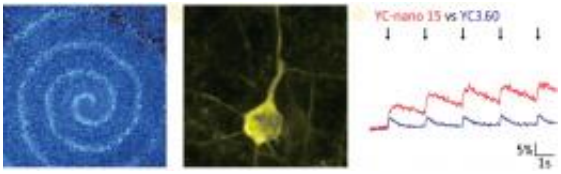

Figure 1: The fluorescent indicator YC-Nano reveals waves of calcium flux (left) corresponding to signals generated by Dictyostelium cells as they undergo aggregation and action potentials in mouse cortical pyramidal cells (center) with a better signal to noise ratio (right). Credit: 2010 Katsuhiko Mikoshiba & Takeharu Nagai

Refinements to a fluorescent calcium ion indicator give scientists a powerful tool for tracking neuronal activity in the living brain

As electrical signals travel along chains of neurons, each cell undergoes a dramatic shift in its internal calcium ion (Ca^2+) concentration because specialized channels allow ions to flood into the cytoplasm. This shift provides a valuable indicator for tracking neural activity in real time, so scientists have developed several fluorescent protein-based Ca^2+ indicators that are genetically encoded and can therefore be expressed directly in cells of interest.

Generally these indicators do not perform as well in live animals as *in vitro*. Takeharu Nagai of Hokkaido University and Katsuhiko Mikoshiba of the RIKEN Brain Science Institute in Wako suspected that indicators

with higher affinity for Ca^{2+} might work better. However, their approach was risky. “It was generally believed that extremely high-affinity Ca^{2+} indicators would result in low cell viability due to disturbed $Ca^{2}+$ homeostasis, and show no signal changes due to saturation by resting Ca^{2+},” say Nagai and Mikoshiba. “From this point of view, our attempt was totally against common sense.”

Nevertheless, the indicators, dubbed YC-Nano, developed by Nagai and his colleagues proved to be a remarkable success. The indicators were derived from yellow cameleon (YC), a genetically encoded indicator consisting of two fluorescent proteins, a ‘donor’ and an ‘acceptor’, connected by a Ca^{2+}-binding domain. In the presence of $Ca^{2}+$, the structure of YC rearranges such that the two come close together in a manner that allows energy from the excited donor to induce a readily detectable signal from the acceptor; in the absence of Ca^{2+}, only a minimal signal is produced.

The researchers introduced various modifications that lengthened the Ca^{2+}-binding segment between the two fluorescent domains, introducing additional flexibility that considerably improved indicator sensitivity. The best-performing versions exhibited five-fold greater $Ca^{2}+$ affinity than YC and a high dynamic range. “We were quite surprised that we managed to systematically produce a series of indicator variants with different affinity by a very simple protein engineering trick,” says Nagai.

YC-Nano accurately tracked the complex patterns of Ca^{2+} activation seen in the aggregating process of social amoeba *Dictyostelium*, revealing propagating waves throughout the aggregates in a rotating spiral. These indicators also performed well in monitoring neuronal activity in the brains of mice, and Mikoshiba foresees numerous experimental applications in the near future. “Since YC-Nano can be stably expressed in specific types of neurons for a long range of time,” he says, “we expect to perform chronic in vivo imaging and analyze the modifications

of neuronal network activities underlying learning, development or diseases of the brain."

More information: Horikawa, K., et al. Spontaneous network activity visualized by ultrasensitive Ca^2+ indicators, yellow Cameleon-Nano. Nature Methods 7, 729–732 (2010).

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