Photoswitches shed light on spontaneous free swimming in zebrafish (w/ Video)

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A new way to select and switch on one cell type in an organism using light has helped answer a long-standing question about the function of one class of enigmatic nerve cells in the spinal cord.

Through targeted insertion of light-sensitive switches into these cells in awake zebrafish larvae, University of California, Berkeley, and UC San Francisco scientists have found that these mysterious cells trigger burst swimming - the periodic tail twitching typical of larvae.

While the finding could have implications for humans, because mammals have similar cells protruding into the spinal fluid, the discovery highlights the power of new techniques that employ photoswitches - light-gated ion channels - and gene targeting to non-invasively turn on small populations of cells as easily as flipping a light switch.

Claire Wyart, post-doctoral fellow at UC Berkeley's Department of Molecular and Cell Biology, and UCSF post-doctoral fellow Filippo Del Bene are the joint first authors of a paper describing these results that appears in the Sept. 17 issue of the journal *Nature*.

"With these optogenetic tools, we can activate single neurons in awake behaving animals and directly demonstrate the consequence of neuron activation on behavior," said Wyart. "This 'optogenetic' approach enabled us to learn something important about spinal circuits."

Wyart said that the strategy used here could be generalized to study all types of neurons, such as those in the smell, vision, touch and hearing centers of the brain.

"Optogenetics opens up a new and extremely exciting area of study, singling out one type of cell and finding out what it's doing," she said.

"This is a new way to do neuroscience," said coauthor Herwig Baier, professor of physiology at UCSF. "Instead of sticking electrodes into the brain to record and monitor activity in the nervous system, what we are doing is manipulating the function of neurons noninvasively with light, the gentlest way to make a manipulation."

"With these optically sensitive channels, it becomes possible to play back to the nervous system its normal innate activity and see what behavior results," added co-author Ehud Isacoff, UC Berkeley professor of molecular and cell biology.

Other coauthors of the Nature paper, in addition to senior authors Isacoff and Baier, are former UC Berkeley chemist Dirk Trauner, now at the University of Munich; Erica Warp, a graduate student in Isacoff's UC Berkeley lab; and Ethan Scott from Baier's UCSF laboratory. Scott is now at the University of Queensland in Brisbane, Australia.

Trauner, along with Isacoff and Richard Kramer, UC Berkeley professors of molecular and cell biology, worked for more than six years to perfect the technique of inserting optical switches into cells, and they formed the Nanomedicine Development Center for Optical Control of Biological Function to spearhead applications. One of the long-term goals of the joint UC Berkeley-Lawrence Berkeley National Laboratory center, which is funded by the National Institutes of Health, is to insert photoswitches into retinal cells to restore vision.

So far they have succeeded in engineering light-sensitive potassium ion channels and glutamate receptors to turn neurons on when zapped by ultraviolet light and turn the neurons off when zapped by green light, or vice versa. The researchers achieve this by attaching to the channel a chemical, called azobenzene, that changes shape when hit by light, opening or closing the ion channel. When the potassium channel opens, potassium ions flow through it and inhibit the cell; when the glutamate receptor channel opens, sodium, potassium and calcium ions flow through it and excite the cell.

Much of the early optogenetic work has confirmed results suggested by other approaches. Wyart and her UCSF and UC Berkeley colleagues have now applied the technique to search for a behaviorally relevant cell and found, to their surprise, a previously unknown function for the Kolmer-Agduhr (KA) cells in the spinal cord. The KA cells aren't standard relay neurons with dendrites and axons, but sensory neurons with cilia - small, movable hairs - that protrude into the spinal fluid, plus long axons extending up the spinal cord. They evidently sense something, but what, the researchers wondered.

Wyart and Isacoff teamed up with Baier's laboratory, where Del Bene and Scott produced 10 strains of zebrafish with photoswitches inserted in specific spinal cord nerve cell populations. When Wyart shined light on the fish with photoswitches in their KA neurons, the fish waggled their tails in a manner that exactly mirrored spontaneous slow forward swimming. Placing the transparent zebrafish larvae under a microscope, Wyart used a Digital Micromirror Device (DMD) to strongly focus light onto a small number of KA neurons, successfully switching on only a few KA cells at a time. She found that she had to switch on about 10 of the KA neurons to trigger swimming.

Knocking out these cells greatly reduced burst swimming, but did not eliminate it, suggesting that the KA neurons may be lowering the

threshold for triggering reflex swimming.

"It came as a great surprise that these neurons played a role in locomotion at all," said Isacoff. "There is an apparent homologue of the KA neuron in mammals, so this may be a general modulatory principle for vertebrate locomotion, although it may change from positive drive early in development to negative drive later."

Earlier studies in lampreys by Sten Grillner, a professor in Stockholm at the Karolinska Institute's Nobel Institute for Neurophysiology, showed that nerve cells - including KA neurons - using GABA (gamma aminobutyric acid) as an inhibitory neurotransmitter were important modulators of swimming. The current study narrows this down to one kind of GABAergic neuron: the KA neuron.

Wyart continues to explore the role of KA neurons, but hopes to exploit the new optogenetic and gene targeting techniques to discover the roles of other types of neurons in the spinal cord.

Other researchers have developed an alternative optogenetic approach - inserting the gene for a light-sensitive ion channel isolated from algae - that also shows promise for directly showing the behavior triggered by activating cells. In fact, it is easier to use, though not as flexible as the approach developed by Isacoff, Trauner and Kramer, Baier said.

"Optogenetic targeting is a powerful approach, and we have really only started the work," he added. "We still have to learn how the KA neurons are connected to drive the muscles. Really, there is no way we could have done this experiment other than with optogenetics."

Source: University of California - Berkeley (news : web)