New imaging technique sheds light on adult zebrafish brain

April 30 2020

Cornell scientists have developed a new technique for imaging a zebrafish's brain at all stages of its development, which could have implications for the study of human brain disorders, including autism.

Zebrafish are translucent when young, making them good models for live imaging, but they become opaque with age, which has prevented researchers from seeing into a live adult brain.

Now, an interdisciplinary team from Cornell Neurotech has developed a microscopy tool to use with adult zebrafish engineered with calcium sensors that light up to reveal when neurons are activated.

Since all vertebrate brains are fundamentally similar, the approach allows scientists to learn basic principles of brain structure and function that apply to all vertebrates, including humans.

"All vertebrate brains are, to a first approximation, the same, with nearly all brain regions [present] in nearly every vertebrate," said Joseph Fetcho, professor of neurobiology and behavior and director of Cornell Neurotech in the College of Arts and Sciences. "This is not surprising because they all, even the simplest ones, have to do the same things to survive and reproduce."

Fetcho is a co-senior author of the study, "Deep Three-Photon Imaging of the Brain in Intact Adult Zebrafish," which published April 27 in *Nature Methods*. The other senior author is Chris Xu, professor of applied engineering and physics in the College of Engineering and the Mong Family Foundation Director of Cornell Neurotech-Engineering.

When nerve cells activate, they flood with calcium. The fish used in Fetcho's studies are engineered with a protein that binds to the calcium in nerve cells. The protein also fluoresces when excited by laser light with a 480-nanometer wavelength, and the fluorescing cells can be imaged with a microscope.

The problem: When delivering that pulse of light, a single 480nm photon aimed through the top of the fish's head will excite other fluorescent proteins in the beam's path, blurring the image. The new technique works by delivering 1400nm wavelength photons to a focal point in the brain. This way, each individual photon has a wavelength that's too long to excite intermediary proteins, but three photons together will carry enough energy to excite a fluorescent protein when light is concentrated at the focal point.

The laser then scans repeatedly along a line in the brain. By repeatedly imaging, parallel lines add up to a two-dimensional cross section of a brain region. By repeating this process at different depths, the researchers attain a three-dimensional image of brain structures.

With the new tool, Fetcho said, researchers may now use fish that are engineered to develop a version of autism and other disorders, and watch how the disease progesses as the fish ages. These fish models could also be used to test potential treatments to see if they improve function, and how brain structure and function change if a condition improves.

"This is a step ... toward cures for some of the devastating brain disorders faced by humans," Fetcho said.

Xu's lab developed the imaging technology, while Fetcho's lab worked on the neurobiology and behavioral aspects of the study. Co-author Andrew Bass, the Horace White Professor of Neurobiology and Behavior in A&S, also provided a different fish model that is still in development, for a smaller relative of zebrafish, called Danionella dracula, which would be easier to image.

More information: Dawnis M. Chow et al, Deep three-photon imaging of the brain in intact adult zebrafish, *Nature Methods* (2020). DOI: 10.1038/s41592-020-0819-7

Provided by Cornell University