Video: Fish Neurons Fire in Real-Time as It Stalks Prey

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By Carrie Arnold, *Science*NOW

Studying the links between brain and behavior may have just gotten easier. For the first time, neuroscientists have found a way to watch neurons fire in an independently moving animal. Though the study was done in fish, it may hold clues to how the human brain works.

"This technique will really help us understand how we make sense of the world and why we behave the way we do," says Martin Meyer, a neuroscientist at King's College London who was not involved in the work.

The study was carried out in zebrafish, a popular animal model because they're small and easy to breed. More important, zebrafish larvae are transparent, which gives scientists an advantage in identifying the neural circuits that make them tick. Yet, under a typical optical microscope, neurons that are active and firing look much the same as their quieter counterparts. To see what neurons are active and when, neuroscientists have therefore developed a variety of indicators and dyes. For example, when a neuron fires, it is flooded with calcium ions, which can cause some of the dyes to light up.

Still, the approach has limitations. Traditionally, Meyer explains, researchers would immobilize the head or entire body of a zebrafish larvae so that they could get a clearer picture of what was happening inside the brain. Even so, it was difficult to interpret neural activity for just a few neurons and over a short period of time. Researchers needed a better way to study the zebrafish brain in real time.

Enter Junichi Nakai of Saitama University's Brain Science Institute in Japan. He and colleagues selected a glowing marker known as green fluorescent protein (GFP) and linked it to a compound that would light up in the presence of large amounts of calcium. The researchers then inserted the DNA that codes for this marker into the zebrafish genome, tying it to a specific protein only found in neurons. This means that only actively firing neurons would fluoresce, and scientists could track neural activity without applying dye. Because the signal was stronger and clearer, researchers didn't have to immobilize the larvae.

To test the setup, Nakai and colleagues sent the genetically engineered zebrafish larvae hunting for food. When the larvae see a swimming single-celled animal called a paramecium, they engage in what animal behaviorists call a prey capture response: They turn their heads toward the paramecium, swim at it, and finally eat it.

Using their newly developed imaging system, Nakai and colleagues associated the sight of moving paramecium and prey capture behavior with the activation of a group of neurons in the optic tectum, the visual center of the zebrafish brain. The neurons pulsed in tandem with the movements of the paramecium—a sudden dart of the one-celled organism caused a bright flash of neural activity in the zebrafish tectum (see videos). The tectum went silent if the paramecium stilled. Only moving prey interested the larvae, the team reports today in *Current Biology*. These particular neurons, Nakai proposes, are part of a specific visual-motor pathway that links the sight of moving prey with swimming behavior.

"It's a good proof of principle study," Meyer says. "The most important thing is that they showed [the technique worked] on freely behaving fish."

All animals, from zebrafish to humans, contain an optic tectum, which coordinates eye movement and the organism's response to objects in their visual field. In humans, for example, the tectum helps us track a buzzing mosquito so that we can take a swat at it. This means that the tectal activity in these transparent larvae could have direct correlates in the brains of humans and other mammals, Nakai says. Scientists can also watch these responses over time and compare brain activity with different stimuli.

The neurons in the larvae continuously make new GFP, which allows ongoing detection of neural activity. "It means we can take the same measurements today, tomorrow, and the day after tomorrow," Nakai says. "This technique makes long-term measurement possible." He hopes the approach will allow scientists to associate a variety of specific behavior patterns with specific neural circuits. That, in turn, could improve the development of psychiatric drugs, as scientists will more easily be able to tell if a particular drug has the desired effects on the brain.

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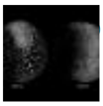
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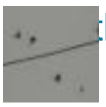
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