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RESEARCH NEWS

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Neuroscience: Watching and Learning from Flies on the March

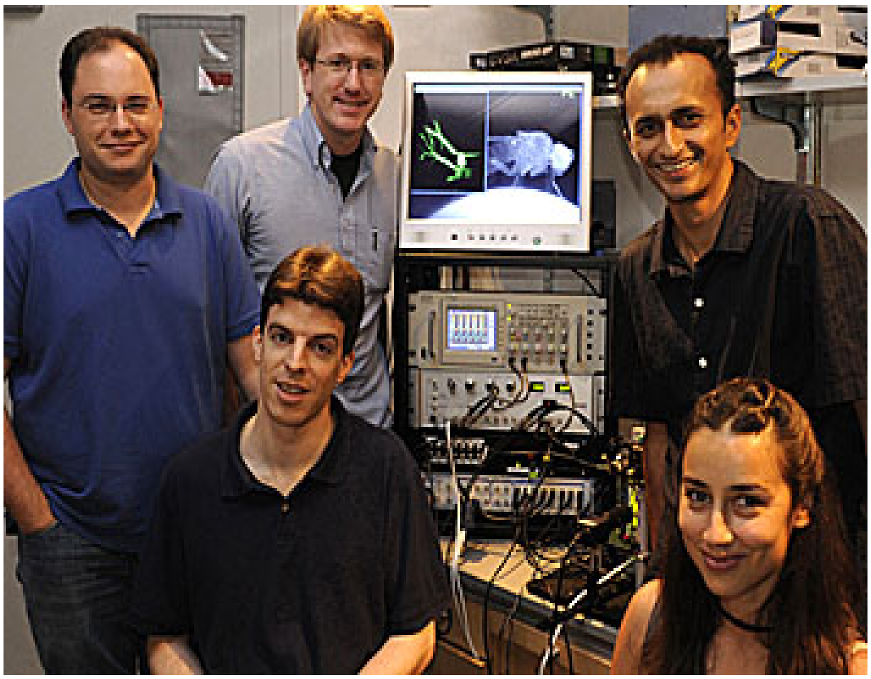

Image Title: Members of the research team that developed the "Drosophila-on-a-Ball" system. Standing (l-r): Michael Reiser, Gus Lott, Vivek Jayaraman. Sitting (l-r): Johannes Seelig and Eugenia Chiappe. Other contributors (not pictured here) include: Nir Dutta, Jason Osborne. - Reed George

Using a microscope, a floating foam ball, and rock-steady hands, Janelia Farm researchers have developed a way to measure the brain activity of a fruit fly while it is walking. The new technique literally creates a window into the tiny tangle of neurons in the insect's brain, so that researchers can watch as those cells work together to let the fly move and respond to changes in its environment.

Established methods had allowed researchers to image in real time as neurons in the brains of larger species, such as rats and mice, signaled to one another during behavior. The new technique, developed in the lab of Vivek Jayaraman, a group leader at the Howard Hughes Medical Institute's Janelia Farm Research Campus, is the first to do so in the tiny brain of *Drosophila melanogaster*. Vivek and his collaborators demonstrated this sophisticated system in a study published July 24, 2010 in *Current Biology*. In that work, the researchers report that they used the technique to learn that neurons in the fly's optic lobes—regions of the brain that process vision—are tuned differently to the speed of external motion when the insect is walking and when it is stationary.

This new approach, described in a paper published online in the journal *Nature Methods* on June 6, 2010, allows scientists to ask complicated questions about brain function—such as how the same neural circuits can both trigger and be affected by behavioral responses to a fast-changing environment—using a superbly simple but very powerful animal model. "The hope is to build a more mechanistic and complete picture of the relationship between circuit activity and behavior," notes Vivek. "We want to make the fly more suitable for this kind of systems neuroscience research."

Researchers in the field of systems neuroscience examine how networks of neurons work together to enable perception and behavior. There are many advantages to using fruit flies over larger animals for these kinds of studies. Flies display relatively complex behaviors—from serenading their mates to learning to avoid shapes and odors associated with uncomfortable heat—using just 100,000 neurons, compared with many millions in the mouse. What's more, thanks to enormous progress in genetic engineering, scientists can choose from thousands of fly strains in which different types of brain cells have been labeled. "In the fly, we have the ability to march through cell type by cell type, and look at what's going on at every layer of the circuit during an interesting behavior," Vivek says.

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- Vivek Jayaraman

Still, *Drosophila*'s size makes it tricky to work with. Broadly speaking, there are two ways to measure electrical activity in the fly brain. Researchers can use electrophysiology, in which they insert an electrode into the brain to pick up electrical spikes from a particular cell.

The less invasive and complementary method is called two-photon calcium imaging. The technique takes advantage of the fact that as a neuron fires, its internal concentration of calcium rises sharply. When a calcium-sensitive fluorescent dye is infused into the brain, a surge in neuronal activity causes an increase in fluorescence. Specially designed two-photon microscopes can then pick up the light signals when neurons fire.

Both of these techniques typically require the fly to be immobilized. This isn't a big problem for researchers studying the set of sensory neurons in the brain that fire somewhat automatically in response to environmental stimuli, such as a beam of light or a sound wave. But for probing deeper levels of brain processing, in which circuits integrate and respond to changing signals from many brain regions and the environment, it is important to study animals as they actively perceive and respond to incoming information. Vivek's team, which includes postdoctoral fellows Johannes Seelig and Eugenia Chiappe, and Janelia Farm collaborators Michael Reiser, a fellow, and Gus Lott, an instrumentation design and fabrication engineer, tweaked the two-photon imaging set-up so that it could be used while a fly is walking.

The system works like this: while keeping the fly near a chilled surface to temporarily knock it out, researchers glue the neck and thorax of the insect to a tiny holder on a movable chamber. They then gently remove the skin covering its head, leaving the brain exposed, and place the chamber inside a two-photon microscope. The fly's legs are left to walk freely atop a tiny foam ball that's floating on top of a column of air. Once in place, the fly watches frames of visual stimuli on an LED monitor. Using an optical tracker machine that monitors the three-dimensional movement of the ball driven by the fly's legs, researchers can measure the fly's precise walking movements and correlate them with the stimulus- and behavior-related brain activity picked up by the microscope.

In their first biological study using this set-up, the team investigated how the flies' brains respond to objects moving at different speeds. As it turns out, optic lobe neurons that respond to visual motion respond more strongly to faster moving objects when the insect is walking than they do when the fly is standing still.

This makes ecological sense: The faster an animal walks, the faster its visual surroundings move relative to its eyes. If a fly is stationary, it doesn't need to be as alert to fast visual motion as when it is walking or flying when the consequences of misinterpreting such motion could be far greater. "To have a very sensitive system takes a lot of energy. So, for situations where you don't need to be that sensitive, you're better off not wasting that energy," Chiappe says.

Because the apparatus is easily modified to allow the fly's wings to move freely, it could also be used to study flying behavior, the researchers say.

The papers are the culmination of three years of painstaking work with the flies. "There were so many small details, right up till the end—it required really steady hands," Seelig says.

The researchers will post detailed instructions, high-quality photographs, and videos of their technique on an open-source website, http://www.flyfizz.org, so that, with commercially available or easily manufactured equipment and a bit of practice, any other lab could set it up. "We really made an effort to make it simple and accessible to as many people as possible," Seelig says.