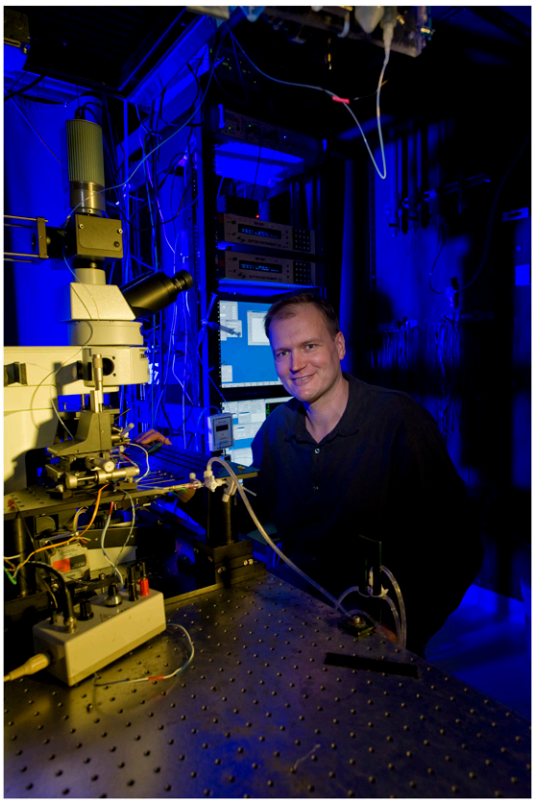
QnAs with Karel Svoboda

Prashant Nair, *Science Writer*

When Karel Svoboda describes his decades-long efforts to visualize the brain with ever-greater precision, the unmistakable impression is that of a man driven by an insatiable urge to expand the frontiers of technology in the service of science. Since the mid-1990s, when he and his colleagues developed transformative imaging techniques to peer at brain cells with unprecedented clarity, Svoboda, a neuroscientist at the Howard Hughes Medical Institute's Janelia Research Campus in Virginia, has expanded the toolbox for unraveling the mysteries of the brain. Svoboda is perhaps best known for using a technique called two-photon microscopy to detect cellular and molecular changes that occur at synapses—the hubs of neuronal communication—during learning and short-term memory. For his pathbreaking techniques and insights, Svoboda, a member of the National Academy of Sciences, earned the Academy's 2017 Pradel Research Award. Svoboda recently discussed his work with PNAS.

PNAS: What are the advantages of two-photon microscopy over standard fluorescence microscopy for imaging neurons in intact brain tissues?

Svoboda: Fluorescence microscopy, in which fluorescent molecules are added to a specimen using histologic or genetic methods to visualize target structures, offers high sensitivity; it is possible to visualize a few fluorescent molecules against a background of billions of nonfluorescent molecules in cells. But fluorescence microscopy was largely limited to imaging thin specimens, such as cell cultures or tissue sections that are a few micrometers thick. This is because of the phenomenon of light scattering. A glass of milk is opaque because as soon as light enters the milk, it gets randomly scattered and becomes diffuse, destroying contrast. Similarly, scattering destroys contrast in fluorescent microscopy in tissues. To understand the dynamics of biomolecules in intact tissues, we needed a technique that could overcome light scattering. Two-photon microscopy uses infrared light, which gets scattered much less than visible light, to excite fluorophores. More importantly, two-photon microscopy allows us to collect fluorescent signal from specimens much more efficiently than conventional fluorescence microscopy. Together, these two attributes make it a suitable technique for imaging intact tissues with high sensitivity, specificity, resolution, and contrast.



Karel Svoboda. Image courtesy of Robert Merhaut Photography.

PNAS: Two-photon microscopy was developed at Bell Labs in New Jersey in an effort led by German physicist Winfried Denk and others. What was your role in the development of this tool?

Svoboda: The history of two-photon microscopy is decades old, but Winfried Denk, working with Watt Webb and Jim Strickler at Cornell University, laid the groundwork. What happened at Bell Labs was that Denk realized that the technique could be used to image scattering tissues at high resolution. I was a postdoc with Denk and David Tank in the mid-1990s and took advantage of the technique to study the biology of synapses in intact tissues, a feat that had not been possible until then. Today, using the technique, we routinely investigate neuronal structure and dynamics in intact brains.

PNAS: This was before the realization that green fluorescent protein (GFP) can be used as an imaging

www.pnas.org/cgi/doi/10.1073/pnas.1710344114

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tool in mammalian cells. How did the advent of GFP influence two-photon microscopy?

Svoboda: GFP was discovered long before then, but it was cloned and demonstrated to be fluorescent by itself only in 1994. For a few years, there was little work with GFP to study the brain. I went to Cold Spring Harbor Laboratories [in New York] to learn how to combine two-photon microscopy with emerging molecular genetic methods to unravel the cell biology of neurons in the intact brain.

PNAS: What were your initial findings on brain structure and function?

Svoboda: We used two-photon microscopy to image GFP-labeled neurons in the brains of mice over time and in response to experience. One finding to emerge from these studies was that the overall structure of neurons—the dendrites on the input side and axons on the output side—is remarkably stable. Each dendrite makes connections only with the axons that are within reach, and the partners that are within reach do not change. By contrast, on the micrometer scale, protrusions called dendritic spines and axonal boutons appear and disappear. This changes the connections between neurons as a function of experience and learning. In other words, we were able to watch the experience-dependent rewiring of the brain in real time. It would have been impossible to visualize synaptic turnover of this kind without a high-resolution, high-sensitivity technique like two-photon microscopy; each dendritic spine is only a couple of hundred attoliters [10^{-18} liters] in volume and holds no more than a dozen GFP molecules.

PNAS: The potential of two-photon microscopy was not fully realized until the development of the now-ubiquitous calcium-sensing imaging probes at Janelia Farm. Can you elaborate?

Svoboda: The first in vivo experiment with calcium imaging and two-photon microscopy was done in 1996 using a synthetic calcium indicator. This was a backbreaking experiment in which we used a micrometer-scale glass needle to deliver fluorescent molecules, in this case synthesized in a chemistry lab. These molecules glow when calcium levels are high. When we recorded action potentials from the neurons using the same needle/fiber, we found sawtooth-patterned fluorescence elevations. Neurons communicate through action potentials, so being able to visualize such spiking is crucial to understanding their dynamics. We and others realized that action potentials could be detected using calcium imaging. But our method was inefficient. Roger Tsien [feted for his pioneering work in developing molecular imaging tools] had begun developing genetically encoded, calcium-dependent proteins that could be targeted to cells using the methods of molecular biology and genetics. At Janelia, with key contributions from the [Loren] Looger and [Eric] Schreiter labs, as well as the GENIE project, we honed those techniques to develop calcium-sensing fluorescent proteins, called GCaMP proteins, specifically for recording action potentials in neurons. These sensors are now in use at thousands of labs worldwide.

PNAS: Do you envision a future in which two-photon microscopy is used to image structures in the intact human brain?

Svoboda: We are learning how to introduce fluorescent proteins into the brain in a manner that's less and less invasive. To my knowledge, however, there isn't really any concerted effort to try to use these tools in people. That said, you could make the argument that calcium imaging combined with two-photon microscopy may be less invasive to image the human brain than some electrophysiological assays used nowadays on patients with epilepsy, for example. The use of next-generation optical tools in a clinical setting is conceivable within the next couple of decades.

PNAS: In *eLife* last year, you reported the development of an advanced two-photon mesoscope for neural imaging (1). In what way does this instrument represent an advance over its predecessor?

Svoboda: With the high signal intensity afforded by the GCaMP proteins, we can record a large number of neurons in multiple brain regions. But for studying neuronal activity and dynamics in this GCaMP-enabled way, we need microscopes with a large field of view as well as high resolution. Conventional microscopy is aimed at visualizing tiny structures at high resolution, but within a small field of view. From a physics perspective, field of view is not really a limiting factor for resolution. However, most microscope objectives offer a small field of view because optical manufacturers have a number of constraints when they design objectives. We designed an objective for our two-photon microscope with a field of view around 25 times larger than that of previous microscopes, while compromising on some of these constraints (we can correct for these constraints using other methods). This microscope maintains the requisite subcellular resolution needed to record activity from individual neurons. That said, I can't emphasize enough that the development of molecular tools like GCaMP is the main driver of these innovations; the hardware engineering, impressive and complex as it is, simply follows suit.

PNAS: What impact is your custom-designed instrument likely to have on neuroscience?

Svoboda: We were worried that the impact would be limited. Often, it takes years or decades for novel technologies such as this to be widely adopted. Our mission at Janelia is partly to develop technologies

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for other researchers. So we invited 13 labs from three continents to come to Janelia and learn how to clone the microscope. They have done this successfully and are now using the microscope for all kinds of imaging applications, such as imaging brain activity during navigation, mapping activity for brain connectome work, etc.

PNAS: Would you care to share your thoughts on receiving the Pradel research award?

Svoboda: I am honored to receive the award, and am particularly thrilled about the fact that it comes with a generous dollop of funds that must be earmarked for research.

1 Sonfroniew NJ, Flickinger D, King J, Svoboda K (2016) A large field of view two-photon mesoscope with subcellular resolution for in vivo imaging. *eLife* 5:e14472.

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