

# Enter the matrix

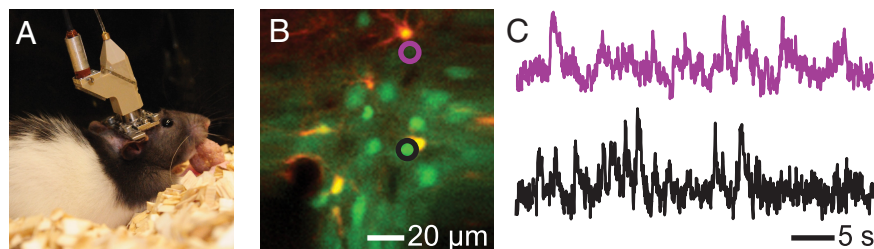
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Understanding the computations the brain performs to guide behavior largely relies on electrical recordings of action potentials generated by neurons in awake, behaving animals. Despite their widespread usage, the high density of neurons in cortex poses a significant limitation on the number of neighboring cells whose signals can be resolved even with state-of-the-art electrode-based methods. Thus current methods can only go so far in understanding neuronal processing. Optical methods to image the activity of groups of neurons promise to ameliorate many of the problems faced by electrical recordings, but typically these methods are restricted in their range of application. Traditional systems are too bulky to mount on freely moving animals and have had only qualified success in monitoring action potentials with sufficient temporal precision. In this issue of PNAS, Sawinski et al. (1) successfully use two-photon microscopy to image cortical neurons in freely moving rats and resolve the Ca<sup>2+</sup> transients that accompany action potentials in populations of up to tens of neurons (Fig. 1). The current technology represents a critical step forward in the use of optical tools to assess the spiking activity of cells in the brain—at least those in the upper layers of cortex. The success of Sawinski et al. (1) appears to remove a significant barrier to the more widespread use of imaging to study the neuronal mechanisms of behavior.

The cortical neuropil contains a dazzling diversity of highly interconnected cell types (2). Most cortical neurons are pyramidal cells, but the computations performed by these cells critically depend on the activity of interlinked networks of both pyramidal and interneurons. Each pyramidal cell synapses onto thousands of other cells. A complete picture, therefore, depends on identified recordings from populations of all subclasses of neurons to characterize their activity patterns.

Electrical recordings offer a far from complete view of the working brain. Electrodes naturally favor action potentials that can be isolated from large, active pyramidal cells because patterns of current flow in these neurons generate extensive, distinctive electric fields. As a result, it is likely that the activity of an overwhelming majority of neurons is not measured. Increasing the density of ex-



**Fig. 1.** The use of the fiber optic microscope. (A) The fiberscope is shown mounted on a rat. (B) Image that shows both neurons (green) and astrocytes (orange) in upper layers of the visual cortex. (C) Calcium transients in two different neurons (purple and black circles in B) in the awake animal during a visual task are shown.

tracellular recordings, e.g., through the use of multisite silicon probes (3), allows recordings from more neurons in a volume. Yet other major challenges remain. In particular, the recordings do not localize the neurons in space very well, so each action potential may obscure the activity of other neurons. Thus, isolating the firing of many simultaneously active, nearby cells from electrical recordings remains effectively out of reach.

The idea of using a fiber tip as a light source for scanning microscopy dates back to the pioneering work of Delaney et al. (4), who built a portable confocal microscope. Unfortunately, the severe scattering of most biological tissue and the need to collect only ballistic photons from the emitted light precluded the ability to resolve cell structure  $>20\ \mu\text{m}$  below the surface. The barrier to deep imaging was broken with the advent of two-photon microscopy (5), which allows fluorescent objects to be imaged as long as scattering of the excitation light occurs on a longer scale than the confocal length; this constraint is typically met in brain tissue (6, 7). Once two-photon microscopy was achieved *in vivo* (8, 9), the next step was to move it to the free-ranging animal. This challenge was met by Helmchen et al. (10). Despite the passage of nearly a decade since the publication of that ground-breaking paper, and the realization of ever more compact and sophisticated head-mounted fiberscopes (11–13), recording with portable two-photon microscopes has been concerned primarily with anatomical features and blood flow. The new work from Sawinski et al. (1) marks a transition toward the beginning of neurological discovery with this technology.

Of major importance in the new work is that Sawinski et al. (1) performed measurements of internal calcium signals in individual cells while animals were free to locomote and head-rear as part of a visual sensory task (1). Impressive as this accomplishment is, issues remain. The interpretation of calcium transients in terms of action potentials depends on statistical inference, much like the assignment of electrical spikes to single units (14). Further, while the optical access associated with the fiberscope is noninvasive, the need to stain neurons with an organic calcium indicator is very much an invasive process (15). These technical hurdles are likely to diminish in complexity as endogenously expressed calcium indicators improve in sensitivity (16, 17) and animals with appropriate cell-specific expression become more readily available (18). While imaging through the full depth of rat cortex is likely to be possible, albeit with special laser sources (7, 19), an additional limitation of this new technology is that there appears to be little prospect for imaging deeper structures without surgical removal of overlying brain areas (20, 21). Difficulties aside, the unassailable advantage of the fiberscope is that one can inventory the position of all cells within a field through the use of a colabel or intrinsic fluorescence and thus assess the fraction of neurons that respond to a given set of features. This yields a space–time matrix of neuronal activity that allows the sparse-

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ness (22) and synchrony (23) of neuronal coding to be assessed.

A typical comment about this new technology is that it is complicated and thus will only be under the purview of a few technical aficionados. Yet many complicated devices, ranging from MP3 players to miniature digital cam-

eras, are consumer items. It is likely that the miniature fiberscope and related instruments (24) will fall into this mold. With the advent of autotuning femtosecond lasers and efficient interconnects for fiber optics, all that may be needed to use a fiberscope is a steady hand to prepare a craniotomy

and, until genetically expressed calcium indicators improve, to inject dye into the parenchyma (15). Our bet is that the article by Sawinski et al. (1) will serve as the tipping point for in vivo imaging studies that will reveal the spatiotemporal structure, and thus the grammar, of neuronal computation.

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