Imaging input and output dynamics of neocortical networks in vivo: Exciting times ahead

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Many remarkable properties of neocortical networks are not detectable in the activities of single neurons. How the properties of single neurons and their synaptic connections combine to form networks that are capable of striking sensory processing and higher brain functions remains a major enigma. To understand how neuronal networks function, the activities of numerous single neurons must be studied simultaneously. The last few decades have seen the emergence of powerful functional imaging techniques that cover broad spatial and temporal scales (Fig. 1A), from single molecules to the intact human brain, which can be imaged noninvasively by techniques such as EEG, magnetoencephalography, and functional MRI. However, comprehensive understanding of neuronal computations requires spatial resolution at the level of single cells, and the speed of communication in these networks demands temporal resolution within milliseconds. As an additional imperative for understanding neuronal information processing, the inputs (synaptic potentials) must be distinguished from the outputs [action potentials (APs)]. Without such dissection, we cannot fully understand perception, higher brain functions, and behavior. Up to now, a comprehensive description of network input and output activity at the level of a single cortical neurons has not been possible. However, in a recent issue of PNAS, Kerr et al. (1) demonstrated that, by employing two-photon calcium imaging of bulk-labeled tissue, local input and output activities in the neocortex can be dissected in vivo. This approach should facilitate the exploration of basic mechanisms underlying neocortical development, function, and plasticity.

Several types of techniques have been used to study cortical networks function. Modern extracellular recording methods (2) enable us to obtain simultaneous measurements from multiple cells, but they suffer from poorly defined cell identities and a severe sampling problem and are incapable of resolving nonactive neurons. Optical imaging of voltage-sensitive dye (VSD) signals (3) has revealed spatiotemporal dynamics on the scale of cortical columns. However, it still lacks single-cell resolution, although in vitro imaging of individual processes within single cells has been demonstrated (4). Calcium imaging of electrical activity can be used to monitor activity in neuronal networks because APs and synaptic potentials promote calcium influx through voltage-dependent calcium channels, creating changes in intracellular calcium concentration in most neurons. Several research groups have made important contributions to the development of calcium imaging. First was the use of a naturally occurring substance, Acquarin, for optical imaging of calcium. Next, organic calcium indicators like Arsenazo (5) were injected into each neuron. A major breakthrough was providing an easy method for trapping probes in a large population of intact cells (e.g., ref. 6), which made it unnecessary to inject dye into each of the numerous single cells to be monitored. Instead, an ester derivative of the probe permeates the neuronal membrane, and the ester bond is cleaved by an intracellular enzyme; as a result, the charged probe is trapped and becomes an effective indicator. Next the heroic and successful efforts of Tsien (7) provided scientists with a large variety of such AM calcium probes. Another fundamental contribution was related to the resolution accomplished by optical imaging depending on the optics used. The laser scanning methodology coupled to two-photon imaging, developed by Denk et al. (8), provides superb spatial and temporal resolution in three-dimensional brain tissue. Yuste and Katz (9), using AM probes in young brain slices, pioneered imaging activities of many single cells. However, loading in adult slice and in vivo failed (10). With the aim of using AM probes also in adult neocortex in vivo, Stosiek et al. (11) recently developed the multicolor bolus loading technique for calcium-indicator loading of large cell populations in vivo. Ohki et al. (12) used this technique to provide a stunning view for the high precision of cortical maps for direction selectivity in cat visual cortex.

Kerr et al. (1) add a significant building block to this line of work by focusing on the temporal domain and the dissection of input and output activities, in vivo. The membrane-permeable calcium indicator was pressure-ejected through a glass pipette into layers 2 and 3 of the neocortex in anesthetized rats. Approximately 1 h later, all cells within a radius of several hundred microns were labeled (Fig. 1B and C). Notably, staining was observed not only in cell bodies but also in the neuropil. Because the loading technique was nonspecific, dissection of the various calcium-signal components was necessary. Using a red fluorescent dye (13) that identifies astrocytes in vivo to counterstain the astrocytic network (yellow cells in Fig. 1 B and C), they observed slow oscillations on a time scale of minutes in identified astrocytes. Neurons, in contrast, displayed spontaneous but infrequent calcium transients of short (<1 s) duration with rapid onset and exponential decay (Fig. 1F Right Inset) resembling AP-evoked calcium transients observed in vitro and in vivo (9, 10, 14).

To determine the reliability of spike detection, Kerr et al. (1) carried out cell-attached recordings. Spontaneous APs were then recorded extracellularly, while somatic calcium transients in the same cell were simultaneously measured optically. Strikingly, 97% of single APs and 100% of bursts were detected. The authors concluded that AP activity is reliably resolved with both single-cell and single-AP resolution. Thus, it was possible to optically detect the spike trains, representing output activity, in local neuronal circuits, providing an optical analog of multiple single-unit recordings but with the added advantage that AP activity can be assigned to all identified neurons within a cortical volume. For neuroscientists, this is a dream come true.

A significant contribution of Kerr et al. (1) was their exploration of the origin of the large fluorescence changes observed in the neuropil surrounding cell bodies. The time course of fluorescence intensity in regions not containing cell somata revealed large fluctuations, representing a bulk measurement of calcium signals in neuropil structures. To determine the origin of the neuropil signal, they combined in vivo two-photon calcium imaging with electrocorticograms (ECoG) and intracellular whole-cell recordings. In whole-cell recordings from individual cells, the membrane potential fluctuated between up and down states, as typically observed in neocortical neurons (15–17). This ongoing spontaneous activity was also apparent in

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the ECoG, which correlated with the intracellular recording. Finally, the neuropil fluorescence signals were well correlated with the ECoG, which occupied the lower two-thirds of the optical imaging “territory.” (B) Side projection of a two-photon image of OGB-1 loaded cells in the neocortex showing neurons (green) and astrocytes (red). (C) Higher magnification area of stained cell. (D) Pseudocolored representation of single cells shown in B depicting the fraction of up-states in which single neurons were active during a 90-s period (color scale). (E) Ongoing calcium transient from the neuropil; the OEG fluctuations (red) correlate with electrical ECoG signals (black). (F) Simultaneous calcium transients from the neuropil; the input (red) and the output from an identified neuron (green) recorded over several minutes. (Right Inset) Ongoing neuropil input signal and an output on expanded time scale.

References: