WA1

Combining two-photon imaging with electrophysiology in vivo: from the synapse to the network

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Understanding how information is represented and processed in the mammalian neocortex requires measurement of spatiotemporal activity patterns in identified networks of neurons in vivo. What will be required is the ability to be simultaneously record both input and output of cortical microcircuits with single-cell and single-spike resolution. Recently, two-photon laser scanning microscopy (2PLSM) has provided a viewing window into the in vivo brain. The advantages of multi-photon laser excitation combined with in vivo bulk labeling techniques have been exploited to image both cellular and subcellular structures within the mammalian brain on time scales ranging from milliseconds to weeks. Bulk loading of brain tissue with Acetoxymethyl (AM) ester derivatives of calcium indicators has become a potentially powerful tool in the quest to understand encoding of information in neuronal populations. Several issues arise with the use of this technique: 1) all tissue and structures are labeled with these dyes requiring specific counterstaining with either genetically encoded labels or additional dyes such as astrocyte specific sulforhodamine 101. 2) because of sparse neuronal action-potential (AP) firing in many cortical areas, it is therefore necessary to ensure the detection of single AP evoked calcium signals. In addition, there is a compromise between the spatial/temporal resolution and signal to noise ratio of signal detection. Combining these imaging techniques with simultaneous targeted electrophysiological recordings allows for the probing of neuronal circuits as well as the calibration of neuronal electrical signals with imaging data. Here I will present work that combines both 2PLSM imaging of on-going neuronal population activity and various electrophysiological techniques to simultaneously record neuronal output activity from local neuronal populations with single cell and single AP resolution. In addition, because the neuropil is also loaded using this loading technique, I will also present data showing that the ongoing neuropil signal represents axonal activity and reflects a volume averaged input signal to the local circuit.

I will describe how both targeted cell attached and whole-cell recording techniques were used to establish that AP activity is reliably resolved with single-cell and single-AP resolution in bulk-loaded neurons. This made it possible to optically extract AP patterns, representing "output" activity, in local neuronal circuits. These results revealed that spatial organization of active neurons was not stable but displayed considerable heterogeneity over the time course of minutes. This heterogeneity indicated that spontaneous activity does not emerge exclusively in a particular subset of neurons but rather is generated by a continually changing subpopulation of neurons. Spontaneous calcium signals in the neuropil were tightly correlated to electrocorticogram and intracellular membrane potentials of neurons embedded within the local network. This optical encephalogram (OEG) represents bulk calcium signals in axonal structures, and provides a measure of local input activity. Because input–output relationships are of key importance for the understanding of signal processing in neuronal circuits, this optical approach should enable the study of input–output transformations during sensory input and how they are affected by varying levels of background activity such as they occur during different behavioral states.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

WA2

Prediction of neuron firing rate from calcium imaging: a single cell approach

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One important challenge in neuroscience is to examine the spiking activity of neuron assembly in a relevant physiological model. Of particular interest is the early olfactory circuit and the opportunity that such a well-defined circuit offers to decipher basic rules of sensory processing. Direct extra-cellular electrical measurements using multi-electrodes and spike-sorting algorithms have been used, mainly in insects, to examine odor-evoked neuronal activity. Such a technique allows simultaneous recordings of spatially compact groups of neurons with an excellent time resolution ($< 10\,\mu s$), but without any cellular resolution, i.e. neurons are not individually identified. The understanding of some unresolved coding issues in olfaction, for example the manner in which odor representations are modified in space and time as they proceed from sensory neurons to their main target population will require recordings of neurons that are both large in number and that have been well-identified. Calcium imaging, using two-photon scanning microscopy has been proposed to examine such odor-evoked population activity and used in in vivo studies on insects and mammals. Nevertheless, the extraction of spiking activity from neuronal cytoplasmic calcium variation is not straightforward, since calcium signals are not coupled exclusively to spiking. We investigated this issue in locust projection neurons (PNs) by combining simultaneous intra-dendritic voltage recording and two-photon dendritic calcium imaging in vivo during odor stimulation in intact, non-anesthetized animals. Our goal was to determine whether dendritic calcium signals might be used to estimate a single PN spike output with reasonable accuracy in vivo.

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WA3

In vivo voltage-sensitive dye imaging of population dynamics and its selective disruption by targeted viral injections

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Determining the spatial and temporal dynamics of sensory and motor networks in the intact system is necessary for a complete understanding of the functional architecture of the mammalian brain. Imaging of voltage-sensitive dye signals applied *in vivo* offer excellent spatial and temporal resolution of spontaneous and evoked responses and permits one to monitor the activity of large networks during cortical processing. Using the rat barrel cortex as a model system I will present a method for imaging the subthreshold activity of a population of neurons located in layers 2/3 and show the spatiotemporal characteristics of whisker-evoked responses. Combining voltage-sensitive dye imaging with simultaneous whole-cell patch clamp recording allows investigation of the correlation between the measured voltage-sensitive dye signals and membrane potential activity, particularly specific correlations with sub- and suprathreshold activity. These recordings have provided insights into the exact nature of the voltage-sensitive dye imaging signals. Further insights into the function of cortical networks will come with combination of recordings of the activity of networks of neurons with selective manipulation of cellular activity by *in vivo* infection of populations of neurons with viral constructs. To this end we have used both intrinsic optical signals and mapping of extracellular electrical activity to deliver lenti viral vectors into defined cortical columns representing specific individual whiskers. These targeted lenti viral injections allow identification of the anatomy of large numbers of cells involved in the generation of the recorded voltage-sensitive dye signals. Furthermore it allows the potential for specific manipulation of cellular properties with the objective of influencing selective populations of cortical neurons at specific stations in the sensory processing pathway in the cortex.

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