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Combining Optical Imaging and Computational Modeling to Analyze Structure and Function of Living Neurons

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Abstract—**We are investigating the computational properties of principal neurons in the mammalian brain. To manage the small size and intricate structure of neuronal dendrites, we employ advanced optical imaging techniques in combination with automatic image reconstruction and computational modeling to study their complex spatio-temporal pattern of activity.**

I. Introduction

The mammalian brain contains some 10^{10} individual neurons that are highly inter-connected, each receiving input from somewhere between 10^2 and 10^5 other neurons. It is currently well-accepted that the information processing capability of the brain is not only based on the large number of neurons and their high connectivity, but also on the performance of individual neurons. Particularly, our view of the neuronal dendrite has changed from that of electric cables, passively conducting postsynaptic signals to the cell body, to that of active neuronal structures, capable of complex spatio-temporal electrochemical dynamics. Therefore, there exists a keen interest in understanding the information processing capability of a single neuron.

The structural and functional analysis of individual neurons is complicated by their intricate shapes, small sizes, and fast response times. Traditionally, structure was observed and documented with histological techniques that require fixing, staining and physical micro-sectioning of brain tissue. Function was monitored with micro-pipettes; for technical reasons, their access was mainly limited to the cell body or the proximal dendrite. Today, sophisticated optical imaging techniques are increasingly employed for studying neurons, replacing these invasive approaches. The strong light scattering properties of living brain tissue require fluorescence microscopes with shallow depth of focus that support an imaging approach called optical sectioning. A tissue volume is imaged as a series of two-dimensional datasets, from which a three-dimensional structure can be computationally reconstructed and analyzed. In addition to fluorescent structural labels, molecular probes have been developed with optical characteristics that depend on parameters of neural activity, such as the membrane potential or intracellular ion concentrations. Also,, various naturally occurring neurotransmitters have been molecularly engineered to remain "invisible" to their receptors until instantly activated by photonic energy. At present, advanced optical techniques utilizing confocal microscopy or multi-photon microscopy with fluorescent molecular probes and/or photo-release of receptor agonists for multi-site optical stimulation are the most versatile tools to study both structure and function of neurons in intact brain tissue.

Computational modeling is increasingly utilized to understand the relationship between neuronal structure and function. Specifically, the complex spatio-temporal activity pattern observed in neuronal dendrites make this analytical tool invaluable. Realistic models have to be constrained by the previously acquired structure of the studied neuron. Traditionally, modeling has been used to explain experimental observations, such as the generation of action potentials. Today, computational models are also employed to predict the function of complex neuronal entities which then can be experimentally tested.

Our goal is to understand the information processing capability of single neurons. To this end, we develop in our collaborating labs novel optical and computational techniques that we employ for analyzing living neurons by means of structural and functional imaging.

II. Methodology & Results

We have developed instrumentation and procedures that allow us to correlate structure and function of living neurons. At present, we are using tissue slices, acutely prepared from freshly dissected rat brains. Such brain slices are cut at a thickness of 300 – 400 µm, and contain many layers of intact and interconnected neurons that can be maintained and studied for several hours.

A. Structural Imaging

We have reconstructed the intricate three-dimensional structure of living neurons. This was achieved by systematic structural imaging of volumes of brain tissue. Individual pyramidal neurons in hippocampal brain slices were whole-

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cell patched at the cell body and diffusion-filled with a fluorescent label contained in the patch pipette, e.g. Alexa 594. Serial optical sections at small axial step sizes were obtained with a laser scanning microscope operated in either confocal or multi-photon mode. In order to achieve high-quality structures, images were taken at high lateral resolution. From such data sets, neurons were automatically reconstructed with a suite of custom image analysis software modules [1]. The raw data is first deconvolved with an experimental point spread function. Each stack is individually denoised by a novel frame-based denoising method. All the stacks are then automatically registered. The combined volume is segmented using a region growing algorithm, starting at the soma, followed by a skeletonization algorithm for centerline generation and radius estimation. This morphology data was then converted into a format appropriate to the simulation environment.

B. *Functional Imaging*

To monitor their function, live neurons were filled with a fluorescent indicator, e.g. Oregon Green BAPTA. This Ca^{2+} indicator was also included in the patch pipette, together with the fluorescent label used for structural imaging. In order to overcome the limits of temporal resolution inherent to commercially available laser scanning microscopes, we have developed imaging instrumentation that employ an inertia-free scanning mechanism. These scanners utilize diffraction rather than reflection for steering a laser beam. Their function is based on sound waves in the radio frequency range that travel through an acousto-optic medium, e.g. TeO_2, creating a diffraction grating that deflect a laser beam depending on the acoustic frequency. We have employed such acousto-optic deflectors (AODs) for fast multi-site optical recording with non-confocal [2], [3] and confocal laser scanning microscopy [4], [5].

The latest system combines the advantage of high spatio-temporal resolution with multi-photon excitation, i.e. small excitation volume and deep tissue penetration. As a prerequisite, we developed means to compensate for dispersion effects of ultra-fast laser pluses, as they are needed for multi-photon excitation [6]. This Random Access Multi-Photon (RAMP) microscope allows multi-site functional imaging at more than 50,000 sites/second, where the location and number of sites are user-selected, e.g. 50 sites at 1,000 frames/second [7]. At present, we are significantly increasing the versatility of this instrument by expanding its capability to scan a volume without axially translating the objective lens, which has been the temporal bottleneck in volume imaging [8]. Although this three-dimensional scanning scheme requires 4 AODs instead of the 2 AODs that are needed for two-dimensional scanning, the scan rate remains unaffected. Therefore, this novel scanning scheme allows unprecedented sampling rates within a volume, supporting fast multi-site functional imaging from complex three-dimensional structures such as neuronal dendrites.

C. *Optical Multi-site Stimulation*

To study the input/output relationship of individual pyramidal neurons, their dendrites must receive physiologically relevant input patterns. We have implemented an AOD-based laser scanner that supports multi-site optical stimulation by fast local photo-release of neurotransmitter, i.e. caged glutamate [9]. We have recently converted this stimulation scheme from single-photon to multi-photon excitation, in order to avoid the intense tissue light scattering in the near ultraviolet (NUV) and take advantage of the possible tight focusing in the near infrared (NIR). This has allowed us to deliver glutamate in highly a localized manner, resulting in selective activation of single postsynaptic sites residing on individual dendritic spines [10]. The speed of our laser scanner supports the simultaneous activation of tens of such sites in order to study dendritic information processing. If requirements are relaxed to allow non-simultaneous activation, the scanner is capable of activating hundreds of sites.

D. *Computational Modeling*

The accuracy of the cylinder dimensions is highly critical for the validity of the simulation results. Errors of 10% in the radial dimension of dendrites lead to amplitude errors of 14% in the simplest neuronal responses to synaptic input (data not shown). These errors could be amplified in regimes where the neuron's behavior is known to be highly non-linear. Current manual reconstruction techniques based on wide-field illumination are limited in that many dendritic details have a size accessible only to the resolution capabilities of scanning light microscopy.

To analyze neuronal function, reconstructions obtained from the structural imaging data were used to construct the morphologies of compartmental models in the NEURON simulation environment [11]. For this purpose, reconstructed neurons were automatically converted into a segmented cylinder representation [1].

II. Discussion

We are taking a novel approach to combine optical imaging and computational modeling in the analysis of neuronal structure and function. In most previous attempts, the structural imaging and reconstruction of neurons were undertaken after the functional imaging had been performed. In fact, most reconstructions were done on fixed rather than on live neurons. Our long-term objective is to utilize the predictive nature of realistic computational models to optimize experimental protocols. Eventually, online reconstruction of the structure and simulation of the function of live neurons can lead to guided imaging experiments with significantly increased collection rates of meaningful experimental data.

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