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Understanding the input-output transfer function of cortical neurons

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ABSTRACT

We aim to understand how brain circuits, learn, memorize or process information. To achieve this aim, we follow a bottom-up approach by focusing on single neurons from rat brains and study how different synaptic inputs of a single neuron translate to an output or an action potential. We have custom-built a unique two-photon laser microscope that incorporates a holographic projector, which transforms the incident laser into multiple foci at the sample volume. The hologram is programmable so we can position the different foci anywhere around the neuron in 3D. Each focus can be used to trigger a synaptic input or used as an optical probe to record the activity of the neuron. We can therefore stimulate and probe the activity from multiple locations within the neuron's dendritic tree using light. For triggering inputs, a focal stimulation represents a synaptic input via two-photon photolysis of caged neurotransmitters. For recording, a laser focus excites a calcium indicator that changes in fluorescence whenever the neuron is active. Using these techniques, we have now identified a novel function of a specific set of dendrites that can have a significant role in learning and memory. The set of dendrites we are probing are currently unexplored due to their very thin morphology. We were able to observe unique properties that allow these dendrites to be more receptive to inputs whenever the neuron fires a series of action potentials. Hence, they have a functional role in the brain's capacity to learn and memorize.

1. INTRODUCTION

To understand how the brain works, it is crucial to characterize the input-output transfer function of individual neurons in the brain. Neurons are the fundamental units that receive and transmit information through neuronal interconnects, otherwise known as synapses. When a neuron receives a synaptic input, ion-channels allow ions to flow into the neuron and sets a voltage transient or otherwise known as an excitatory post-synaptic potential (EPSP). Synaptic inputs occur at different regions of the neuron's dendritic tree, which extends spatially in three dimensions (3D). Moreover, the inputs come in particular temporal sequences. Thus, each neuron receives multiple synaptic inputs in four dimensions (4D). As a neuron processes these inputs, the integration of multiple EPSPs sets the neuron to fire an action potential (AP); thereby propagating its processed information to other neurons in the network. The flow of ions in and out of the cell during an EPSP and/or AP changes ion concentrations inside the cell particularly that of Ca^{2+}. Monitoring the changes in Ca^{2+} concentration at different regions across the dendritic tree can hence be correlated with neuronal activity.

Laser-based techniques to manipulate and monitor neuronal network activity have initiated major breakthroughs in neuroscience[1,2]. Apart from static imaging of neurons, laser-based stimulation and recording are now established techniques for studying neuronal function. Laser-scanning systems have been used for quasi-simultaneous multi-site stimulation along the dendritic arbour of a neuron, by rapidly switching the focus from one site to another. Temporal patterns or sequences of light stimulation on post-synaptic neurons can be implemented, which mimic correlated firing patterns from pre-synaptic neurons. Moreover, it is also important to provide a spatial map of the neuron's response across different regions of its dendritic tree. Monitoring the changes in Ca^{2+} concentration is achieved optically via functional Ca^{2+} imaging, which makes use of a laser to excite a fluorescent Ca^{2+} indicator. Changes in the indicator's fluorescence intensity are correlated with the changes in the intracellular Ca^{2+} concentration. Such technique is now widely used for analyzing neuronal circuit dynamics.

To bring about laser induced stimulation of synaptic inputs and optical recording, we have developed a holographic approach to produce multiple foci targeted onto the neuron. Each focus allows simultaneous multi-site two-photon (2P) stimulation in 3D-space [3,4] as well as arbitrary time gated stimulation thereby enabling a full 4D photostimulation of synapses [5]. Spatiotemporal stimulation of dendritic branches provides for an accurate physiological emulation of neuronal communications. On the other hand, to monitor the changes in Ca^{2+} concentration along the dendrites, we use the same holographic approach to perform simultaneous multi-site 2P excitation of a Ca^{2+} indicator loaded into the

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neuron[6,7]. Fluorescence from localised regions of the various foci can be simultaneously recorded using a highly-sensitive camera (e.g. electron-multiplying charge coupled device, EMCCD). Our holographic 2P microscope facilitates the ability to track the neuron's response in either stimulation or recording mode and therefore provides for an efficient way to characterise the input-output transfer function of neurons in the brain.

2. RESULTS

Using our holographic 2P microscope, we have identified a novel function of a specific set of dendrites that can have a significant role in learning and memory. We analyzed the input-output characteristics of cortical pyramidal neurons, which have complex dendritic branches and are known to propagate dendritic spikes due to active conductances. Dendritic spikes are regenerative activation of ion channels that nonlinearly intensifies the membrane potential and influences the integration of synaptic inputs. Specifically, we studied layer 5 pyramidal neurons (L5PN) in the cortex and focused on their oblique dendrites, which start to branch out along the apical trunk. These set of dendrites are currently unexplored due to their very thin morphology making them difficult to be probed via conventional electrophysiology. Using the h2p microscope in stimulation mode, we performed simultaneous stimulation by uncaging caged excitatory neurotransmitters (e.g. glutamate) to analyze the impact of coincident synaptic inputs onto distal tuft and proximal apical oblique dendrites. We studied the roles of apical obliques in gating distally initiated synaptic currents as they propagate to the soma [3].

Next, using the h2P microscope in optical recording mode, we show the generation of dendritic spikes by monitoring the Ca^{2+} intake along the dendrites following the injection of a series of current pulses via the patch electrode. The current injection produces back-propagating action potentials (bAPs) inducing Ca^{2+} transients at the oblique dendrites. The changes in Ca^{2+} response was then plotted against frequency of the bAP train. We swept the frequency and plotted the peak amplitude of the Ca^{2+} transient as a function of frequency. While some oblique dendrites showed a flat or linear increase in the amplitude of the Ca^{2+} transient, certain oblique dendrites exhibited a step-increase at f=70Hz indicating that a dendritic spike has been generated. We initially tested 100 L5PNs from rat somatosensory cortex and 34% of the cells exhibited a dendritic spike at frequencies $50 < f < 70$ Hz. Our initial results confirm the existence of dendritic spikes in apical obliques of L5PNs.

3. CONCLUSION

Understanding the roles of various dendritic domains especially their capacity to generate dendritic spikes can have profound implications to learning and memory as well as in the processing of sensory information. Our bottom-up approach to understand neuronal function from the level of dendrites and single neurons is a crucial step towards achieving the ultimate goal of decoding the brain.

4. ACKNOWLEDGEMENTS

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