Characterization and application of fluorescent indicators for imaging neuronal voltage computations

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***Short Abstract* — Neurons integrate inputs and generate outputs by regulating the voltage across their plasma membrane. Understanding these computations requires tools to monitor membrane potential (voltage) with high spatial and temporal resolution. Here, we quantitative characterize and apply a new generation of protein-based biosensors that report voltage dynamics as changes in brightness. We demonstrate that these genetically encoded voltage indicators (GEVIs) can report action potential with millisecond time-scale and subcellular spatial resolution in cultured neurons and brain slices with two-photon microscopy. We also show we can track action potential backpropagation through dendritic arbors, an important step towards studying how neuronal morphology tunes neuronal computations.**

***Keywords*— Fluorescence Microscopy, Neuronal computation, GEVI**

I. BACKGROUND

How the brain regulates behavior in response to environmental stimuli is a longstanding question in neuroscience. Neurons are generally thought to be the basic computational elements in the brain: they receive chemical signals called neurotransmitters at specialized structures named synapses, and integrate and transmit this information as voltage changes that propagate across spines, dendrites, the cell body, and the axon. Electrical activity at axonal termini can result in release of neurotransmitters, thereby activating downstream neurons. How individual neurons integrate multiple inputs remains poorly understood given the unavailability of tools to monitor voltage dynamics with sufficient spatiotemporal resolution [1].

Here, we describe advances in using protein-based indicators of voltage for monitoring computations in single neurons. Specifically, we propose to use Genetically Encoded Voltage Indicators (GEVIs) to quantitatively image membrane potential dynamics with subcellular resolution and millisecond-timescale resolution in genetically defined cell type [2-3]. We could potentially measure the membrane potential of tiny but critical structures of neurons such as an individual spine or the axon initial segment, which are typically inaccessible by other strategies. We also can record the membrane voltages of multiple locations simultaneously to stress how electrical signals change during propagation [4]. Voltage imaging also allows us to track long-term changes of activities of the same neuron to investigate how development, aging, and disease can alter the computation properties.

II. RESULTS

We have previously reported a voltage indicator ASAP1, a chimeric protein with a GFP variant inserted in an avian voltage sensitive domain (VSD). Voltage changes result in a conformational change in the VSD, thereby modulating the brightness of the coupled GFP [5]. Here, we report an improved voltage indicator and demonstrate that it increases detectability of action potentials, sub-threshold membrane potentials, and hyperpolarization events. We quantitatively characterize the performance of this new indicator in dissociated neurons and in brain slices under fluorescence microscopy. We further show that this indicator can track backpropagating action potentials in neuronal dendrites. We anticipate that further development of this methodology will enable quantitative modelling of computations by individual neurons.

III. CONCLUSION

We demonstrate that we can image voltage dynamics with high spatiotemporal resolution and show strong potential to understand the role of each neuronal structure in processing information.

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