

Ultrafast Voltage Imaging of Single Neurons at Ten Kilohertz in Behaving Mice

Eric Lowet, Sheng Xiao, Jerome Mertz, Xue Han

Department of Biomedical Engineering, Boston University, Boston, MA, USA
elowet@mailfence.com, shengx@bu.edu, jmertz@bu.edu, xuehan@bu.edu

Abstract: We performed five and ten kilohertz imaging of individual hippocampal neurons expressing SomArchon using a high-speed sCMOS camera. The ultrafast imaging speed allows for detailed characterization of action potential waveforms in hippocampal neurons. © 2021 The Author(s)

1. Introduction

Neuronal action potentials (APs) occur on the millisecond time scale to support temporally precise neural computation in the brain. Since the classical studies of sodium and potassium ion channel conductance in AP generation by Hodgkin-Huxley in the 1950s, many voltage-gated ion channel subtypes with distinct conductance kinetics have been shown to influence AP waveforms in mammalian neurons [1]. Thus far, detailed characterizations of AP waveforms have been performed via patch clamp recordings, mainly in *in vitro* preparations. These *in vitro* studies have associated certain AP waveform features with neuronal morphology and genetic identity, which provided the experimental evidence for classifying neuron subtypes or activity states using extracellularly recorded spikes. Extracellular recordings, though can be easily deployed in the brain, detect extracellular current flow at the recording site, where the transmembrane current flow through the neuron of interest is masked by current sources from nearby neurons and passing through neuronal processes. Thus, extracellular recording techniques lack the precision to reliably resolve AP waveforms. Recent progress in the development of high performance genetically encoded voltage indicators provides an exciting opportunity to record membrane voltage in the brain. In particular, the fully genetically encoded voltage indicator SomArchon allows for fluorescence imaging of individual APs from genetically defined neurons in behaving mice [2]. SomArchon exhibits rapid voltage responses with an activation time constant of 0.6ms and an inactivation time constant of 1.1ms, which account for 88% of the total amplitude upon 100mV depolarization voltage steps [2]. The fast temporal kinetics of SomArchon in principle supports the detection of AP waveforms, if imaging is performed at ultrafast speed. However, voltage imaging in the brain has been limited to a sampling rate of 1 kHz so far, due to the lack of high-speed sampling optical system.

To evaluate whether voltage imaging can resolve AP waveforms at a single spike level, we performed ultrafast imaging of SomArchon expressing CA1 neurons at 5 kHz and 10 kHz. We characterized the width of individual APs within CA1 complex spikes. Complex spikes are the occurrence of multiple APs with short inter-spike-intervals (ISIs) during large, long-lasting membrane depolarizations. The short ISIs between APs within a complex spike have allowed their identification as spike bursting using extracellular recordings, and extracellular recording studies have associated CA1 spike bursting with unique aspects of learning and memory [3]. However, recent *in vivo* patch clamp studies demonstrated that some APs have long ISIs of tens of milliseconds [4, 5], challenging the identification of complex spikes as extracellularly recorded spike bursting that rely on ISIs alone.

2. Methods and Results

We integrated a low noise, ultrafast sCMOS camera (Kinetix, Teledyne photometrics) into a custom widefield microscope designed for SomArchon voltage imaging. Animal preparation and imaging condition were similar to that described in our previous study [2], where individual CA1 neurons were imaged with a 40× 0.8NA objective, while mice were awake head-fixed freely locomoting. We performed SomArchon voltage imaging at 5 kHz and 10 kHz with the sCMOS camera at the 8-bit mode (10 kHz: n = 432 spikes in 2 neurons from 1 mouse, 5 kHz: n = 229 spikes from 3 neurons in 3 mice). We quantified the full-width-at-half-maximum (FWHM), defined as the time interval above 50% of the maximum spike amplitude. At 10 kHz sampling rate, we found that single AP, those occur outside of complex spikes, had a FWHM of 0.88±0.02 ms (Fig.1f). The first APs within the complex spike had similar FWHM as single APs (1th: 0.95±0.08ms), but the subsequent APs exhibit sequential increase in FWHMs and were significantly wider than single APs (2nd: 1.0±0.05ms, 3rd: 1.05±0.1 ms, 4th: 1.31±0.1ms and >4th: 1.75±0.11 ms, all p<0.05, t-test). Similar FWHMs were also obtained when imaged at 5 kHz (Fig.1g). The

sequential widening of APs within a complex spike observed here is consistent with that obtained via patch clamp recordings in awake rats and mice [4,5].

To test whether FWHMs difference can be detected with 1 kHz sampling rate, we downsampled SomArchon fluorescence traces to 1 kHz, and found that FWHM of most APs within complex spikes did not differ from single APs (1.67 ± 0.07 ms), except later APs (Fig. 1h, $>4^{\text{th}}$ AP, 3 ± 0.6 ms, $p < 0.05$, t-test). Additionally, AP spike-to-baseline ratio (SBR) decreased at higher sampling rates (SBR, 1 kHz = 9.15 ± 0.16 , 5 kHz = 8.3 ± 0.15 , 10 kHz = 7.16 ± 0.15). The reduction in SBR at higher sampling rate is consistent with that observed previously with voltage sensitive dyes [6], as lower sampling rate provides better SBR by avoiding multiple camera readout.

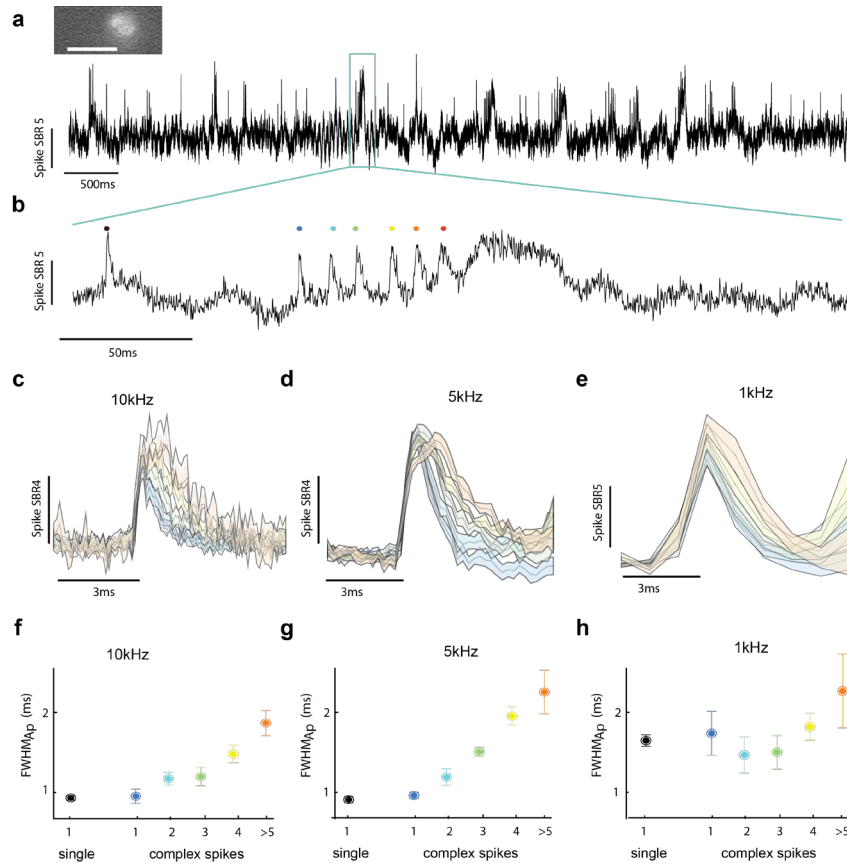


Figure 1. (a) An example 5 second long SomArchon fluorescence recording at 10kHz from a CA1 neuron in an awake animal. Top, SomArchon fluorescence image of the neuron recorded. Scale bar, 25μm. Bottom, SomArchon fluorescence trace. (b) Zoom in of the SomArchon fluorescence trace during the period highlighted by the light blue box in (a). Individual APs were marked by colored dots. The black dot marked a single AP outside of complex spikes, and the colored blue-to-red dots marked APs within a complex spike. (c-e) SomArchon fluorescence aligned to the peak of APs within complex spikes recorded at 10 kHz (c), 5 kHz (d), and 1 kHz (e). The colors correspond to the sequence of APs, with blue being the 1st AP, light blue being the 2nd, green being the 3rd, yellow being the 4th, and orange being the 5th and more. (f-h) Quantification of FWHM for single APs and APs in complex spikes at 10 kHz sampling rate (f), 5 kHz (g) and 1 kHz (h).

3. Conclusion and Discussions:

We demonstrated action potential waveform characterization using SomArchon voltage imaging at 5 kHz and 10 kHz, using an ultrafast sCMOS camera. We detected sequential widening of APs within CA1 complex spikes in the brains of behaving mice, a feature that cannot be resolved via extracellular recording techniques. These results demonstrate a new capability of voltage imaging in resolving AP waveforms in awake mice, highlighting the exciting possibilities of studying the functional significance of AP waveforms during behavior.

4. References

1. Bean BP. The action potential in mammalian central neurons. *Nature Reviews Neuroscience*. Nature Publishing Group; 2007. pp. 451–465. doi:10.1038/nrn2148
2. Piatkevich KD, Bensussen S, Tseng H an, Shroff SN, Lopez-Huerta VG, Park D, et al. Population imaging of neural activity in awake behaving mice. *Nature*. 2019; doi:10.1038/s41586-019-1641-1
3. Sjöström PJ, Nelson SB. Spike timing, calcium signals and synaptic plasticity. *Current Opinion in Neurobiology*. Elsevier Ltd; 2002. pp. 305–314. doi:10.1016/S0959-4388(02)00325-2
4. Epsztein J, Brecht M, Lee AK. Intracellular Determinants of Hippocampal CA1 Place and Silent Cell Activity in a Novel Environment. *Neuron*. Cell Press; 2011;70: 109–120. doi:10.1016/j.neuron.2011.03.006
5. Harvey CD, Collman F, Dombeck DA, Tank DW. Intracellular dynamics of hippocampal place cells during virtual navigation. *Nature*. Nature Publishing Group; 2009;461: 941–946. doi:10.1038/nature08499
6. Quicke P, Song C, McKimm EJ, Milosevic MM, Howe CL, Neil M, et al. Single-Neuron Level One-Photon Voltage Imaging With Sparsely Targeted Genetically Encoded Voltage Indicators. *Front Cell Neurosci*. Frontiers Media S.A.; 2019;13: 39. doi:10.3389/fncel.2019.00039