All-optical electrophysiology in iPSC-derived neurons with synthetic NIR voltage reporter

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Abstract: We demonstrate a robust and reproducible protocol to achieve all-optical electrophysiology in iPCS-derived and primary cell cultures using synthetic voltage sensors and genetically encoded optogenetic actuators while minimizing cell death due to viral transfection. © 2020 The Author(s)

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1. Introdution

Traditionally, neuronal electrical properties have been evaluated using intracellular electrophysiology, which is low-throughput and labor intensive. Recent advances in optical microscopy and optogenetics offer a new experimental paradigm – "all-optical electrophysiology" – where optical voltage sensors and optogenetic (OG) actuators are combined for all optical stimulation and readout [1]. Applied to human-induced pluripotent stem cells (hiPSCs) [2], all-optical electrophysiology opens unprecedented opportunities for high-throughput phenotyping of neurons and neuronal networks possessing unique genetic background of individual patients. In prior studies, the voltage sensor and OG actuator were co-expressed using viral transfection [1]. However, for hiPSCs, transfection with large or multiple plasmids can often result in cell death. This can in turn bias the composition of the remaining neuronal networks towards cell types that are resistant against the transfection procedure. Moreover, this bias may differ across patient and control genotypes undermining the use of hiPSC-based models for biomedical research. Thus, we sought to establish an alternative protocol that uses synthetic voltage sensors, which can be easily and safely delivered to cell cultures mitigating the cell death problem. All-optical electrophysiology requires that the voltage sensor and OG actuator are spectrally orthogonal in order to minimize the crosstalk, i.e., avoiding excitation of the voltage sensor by light that controls the OG actuator and vice versa. Therefore, we chose BeRST (Berkeley Red Sensor of Transmembrane potential) [3] as the most red shifted among the available synthetic voltage indicators. BeRST requires no genetic manipulation, can be easily delivered to all cells and have high sensitivity for detection of single spikes [3]. We optimized our protocols of cell growth and imaging to enable robust, sensitive and reproducible voltage imaging. We further combined BeRST imaging with OG stimulation using an OG actuator CheRiff controlled by blue light, to enable all-optical electrophysiology. Further, we co-labeled our cells with a synthetic calcium indicator Oregon Green BAPTA-1 (OGB1) that facilitated diagnostics and screening for active cells prior to conducting all-optical electrophysiology.

2. Methods

Cell cultures: Human neurons were derived from hiPSC cultures as described in [4] and replated on Polyhornitine/Laminin-coated MatTek plates. Imaging was performed after 8 weeks of differentiation. Expression of OG actuator was carried out via lentiviral transfection of CheRiff-EGFP plasmid. Primary hippocampal cultures were prepared from postnatal day 0 rat pup brains as described in [5] and plated on Laminin coated MatTek plates. *Optical setup and recordings*: Cells were imaged on a custom-built epifluorescence inverted microscope (Fig. 1A) under constant perfusion with Tyrode's buffer (pH=7.4) at 28°C. Excitation of BeRST was achieved using a 500mW 635-nm CW laser (Opto Engine LLC) with an intensity at the sample of 25 W/cm^2. CheRiff was controlled using a 100-mW 473-nm CW laser (Cobolt 06-MLD) with fast analogue modulation (2 MHz). The same 473-nm laser was used for imaging of OGB1. Images were collected with a 40X oil objective

and imaged onto a sCMOS camera (Zyla 4.2 Plus, Andor). Oblique illumination was implemented to reduce background fluorescence due to out of focus debris and overlapping cells. OGB1 and BeRST data were collected at a frame rate of 50 Hz and 360 Hz, respectively. OG stimulation consisted of separate 60-sec train epochs while varying duration and frequency of individual blue laser pulses (Fig. 1G).

3. Results

Optimized cell growth protocol for high-quality voltage imaging: we optimized the protocol to generate well-adhered, uniformly distributed cells while reducing cell death and clustering. We first differentiated neurons on 10-cm dishes for 4-6 weeks until formation of dense networks. Then, we replated the neurons to imaging plates and allowed them to differentiate for another 2-4 weeks. We repeated the replating procedure 2 times including long (40 min) enzymatic dissociation with mechanical trituration to maximize the uniformity of cell distribution upon replating while maximizing cell viability .
Reliable voltage imaging in iPSC-derived neurons: we co-loaded cells with OGB1 (Fig. 1B) that allowed visualization of the cellular composition of studied cultures, quick diagnostics of cellular activity levels, and targeting of active cells for subsequent voltage imaging (Fig. 1C-F). We used primary hippocampal neuronal cultures to optimize data acquisition protocol for imaging and interrogation of neurons (Fig. 1C-F). Upon optimization of perfusion settings, chamber temperature, the imaging parameters, OG and pharmacological stimulation, we translated the same protocol to hiPSC-derived neurons. Combined with segmentation and data analysis techniques developed by others [1, 6], we observed robust and reliable recording of membrane potentials from human neurons (Fig. 1H-I).

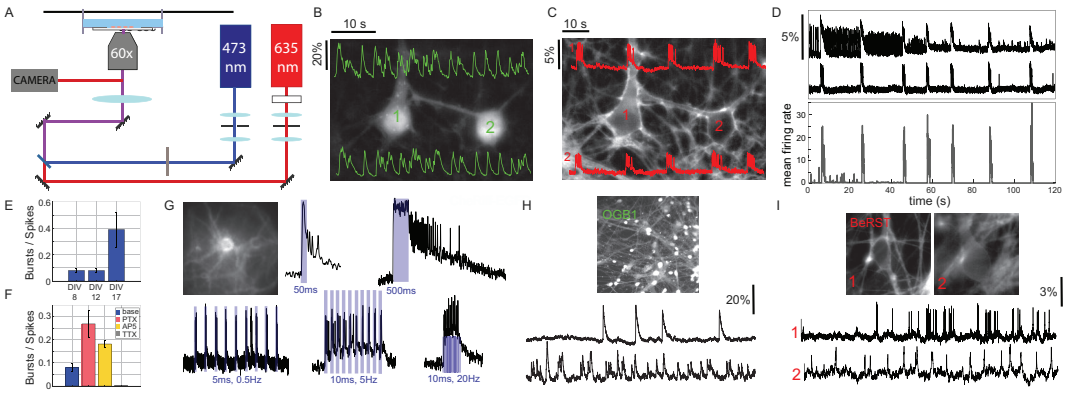

Fig. 1. **A.** Optical setup. **B.** OGB1 and **C.** BeRST staining and imaging in primary cultures. **D.** Network synchrony in primary neurons and mean firing rate computed on 11 cells from same FOV. **E.** Bursts to spike ratio in developing networks and **F.** under pharmacological stimulation. **G.** OG stimulation in primary neurons. **H.** Calcium and **I.** voltage imaging in iPSC-neurons.

4. Conclusions

We have developed a robust, sensitive and reproducible protocol for voltage/calcium imaging combined with OG and pharmacological stimulation in hiPSC-derived neurons based on synthetic activity probes. Our protocol mitigates cell death due to viral transfection and potential bias towards cell types that happen to be resistant to the transfection procedure.

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