Comparing the effects of nanosecond electric pulses on excitable neural-type cells to those evoked by conventional electrical stimulation

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Introduction

- Conventional electric pulses (CEPs) are currently used in medical applications for neuromodulation, and they typically range from microseconds to milliseconds in duration. A disadvantage of CEPs is that electrodes must be implanted, often requiring invasive surgery (1). Alternatively, nanosecond electric pulses (NEPs) entail a nanosecond long pulse, which compared to CEPs, have the potential to be delivered non-invasively (2).
- We have been studying the effects of ultrashort (< 10 ns) NEPs on a well-characterized excitable cell model, bovine adrenal chromaffin cells. These cells resemble sympathetic neurons, which release the catecholamines epinephrine and norepinephrine, via the Ca^{2+}-dependent process of exocytosis. We have shown that a single 5 ns pulse triggers an immediate transient Ca^{2+} response solely due to Ca^{2+} influx via voltage-gated Ca^{2+} channels, and subsequent catecholamine release (3). This response resembles that evoked by the physiological stimulus, nicotinic receptor activation.
- The goal of this project is to compare the effects of NEPs (5 ns in duration) and CEPs (0.6 ms in duration) in evoking a Ca^{2+} response in bovine chromaffin cells using fluorescence imaging of Ca^{2+}.

Methods

Chromaffin Cell Dissociation

From culture, bovine chromaffin cell aggregates are dissociated into single cells, attached to a 35-mm glass bottom dish (Fig. 1), and loaded with a fluorescent calcium indicator (Calcium-Green-1-AM or Fluo-4-AM). The cells are put in a balanced salt solution (BSS) to be viewed on the stage of a Leica DMi8 inverted fluorescence microscope.

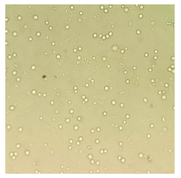

Fig. 1. Dissociated cells attached to a dish viewed at a 20X magnification.

Electrode Fabrication

Electrodes are fabricated by positioning two beveled 100-micron thick tungsten rods through two micropipette tips such that the rods are 100 microns apart. The free ends are soldered to a female BNC connector and the assembly is insulated with a strip of parafilm (Fig. 2).

Fig. 2. (Left): A complete custom-fabricated electrode, held in place by a micromanipulator.. (Right): 3X view of the tungsten rod tips of a custom-fabricated electrode.

Pulse Exposure

Each cell is exposed to one CEP or NEP using the electrode, which is held at forty microns above the cell using a Sutter Instruments MP-225 micromanipulator (Fig. 3). The cell is positioned in the center of the gap between tips of the electrode (Fig. 4). Each Ca^{2+} fluorescence sequence is captured with the Leica LAS-X software for a total of ninety seconds, with the pulse administered at fifteen seconds. A custom MATLAB program is used to compile pulse and fluorescence data and normalize the cell fluorescence intensity to 1.

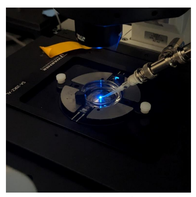

Fig. 3. A dish of dye-loaded cells ready for pulse exposure.

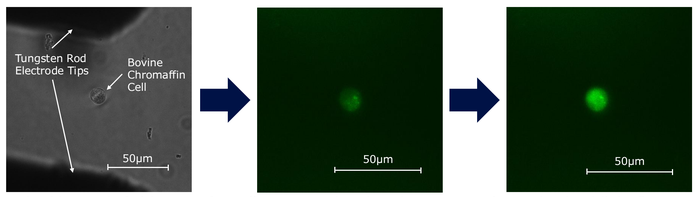

Fig. 4. (Left): Brightfield view of a cell positioned within the custom electrode. (Middle): fluorescence image of the cell at rest. (Right): The same cell at the peak of fluorescence, stimulated by an NEP.

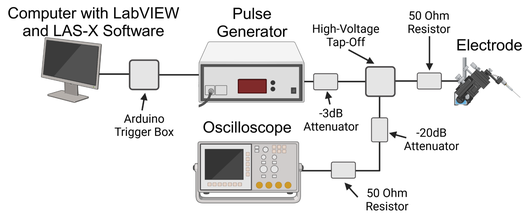

Fig. 5. Diagram of the NEP setup. The computer is connected to an Arduino trigger box, which is programmed to trigger the pulse generator fifteen seconds into the sequence. A high-voltage tap-off sends the pulse to the electrode and to an oscilloscope. The oscilloscope captures the pulse waveform, which is displayed in LabVIEW and saved to an excel file. Pulse Parameters: 5ns, 2kV. (Created in https://BioRender.com)

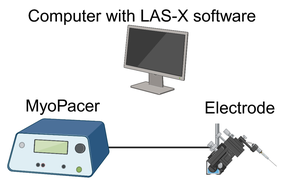

Fig. 6. Diagram of the CEP setup. The electrode is connected to the high voltage output of a MyoPacer Cell Stimulator. During the sequence, pulse is triggered manually at 15s. Pulse parameters: 0.6ms, 12V square waveform. (Created in https://BioRender.com)

Results

A single 5ns, 10MV/m pulse causes an immediate rise in intracellular Ca^{2+}

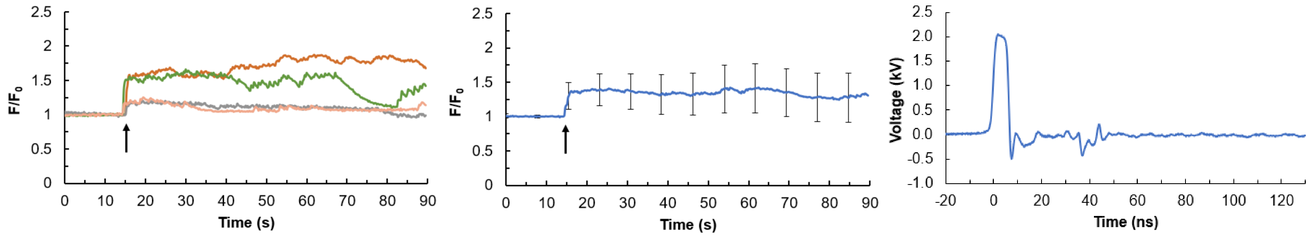

Fig. 7. (Left): Individual cell Ca^{2+} responses from a single dish. (Middle): Averaged response of the cells (n = 4, mean peak = 1.38 ± 0.20). (Right): 5ns, 2kV pulse trace used in the experiments.

Preliminary evidence shows that a 12V, 0.6ms CEP elicits a similar Ca^{2+} response in bovine adrenal chromaffin cells

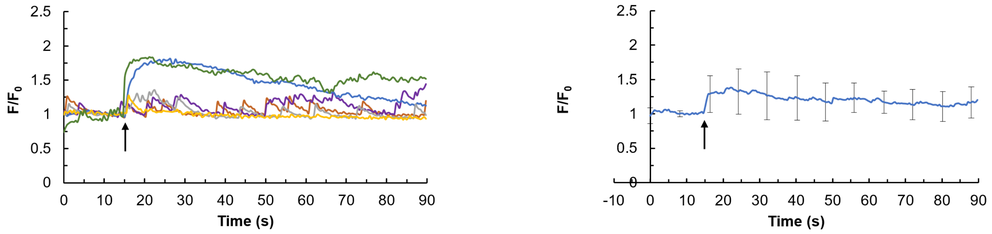

Fig. 8. (Left): Individual cell Ca^{2+} responses from two dishes. (Right): Averaged response of the cells (n = 6, mean peak = 1.32 ± 0.29).

Conclusions

- This semester I was able to successfully replicate the Ca^{2+} responses in bovine chromaffin cells elicited by a 5ns electric pulse, as was shown in previous research. Additionally, I successfully found parameters for a conventional electric pulse that stimulated a similar Ca^{2+} response in bovine chromaffin cells.
- Over the course of this project, I successfully learned a host of lab skills such as fluorescence microscopy, data analysis, working with cells, soldering, and chemical dilutions, to name a few. It was a true privilege to have been able to gain such invaluable research experience in my undergraduate career.
- This is an ongoing project by the Department of Electrical and Biomedical Engineering, and I am excited to continue working on NEP and CEP research. In the months to come I hope to explore the effects of bipolar NEPs and find more combinations of CEP parameters that elicit a Ca^{2+} response in bovine chromaffin cells.

References

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