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Supplemental Information

2-ns Electrostimulation of Ca^{2+} Influx into Chromaffin Cells: Rapid Modulation by Field Reversal

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Pulse exposure waveforms

A 2 ns bipolar pulse generator (FID GmbH FPG 10-1CN6V2, Burbach, Germany) was used to deliver unipolar and bipolar electric pulses. Pulse traces were captured during each experiment using a waveRunner 640Zi digital oscilloscope (Teledyne LeCroy, Chestnut Ridge, NY). The interphase interval of the bipolar pulses was varied from no time interval between the end of the first phase and the beginning of the second phase to up to 120 ns (longest interphase interval achievable on the pulser) (Figure S1). Interphase intervals of 10, 30, 60 and 120 ns were measured from peak-to-peak for repeatability.

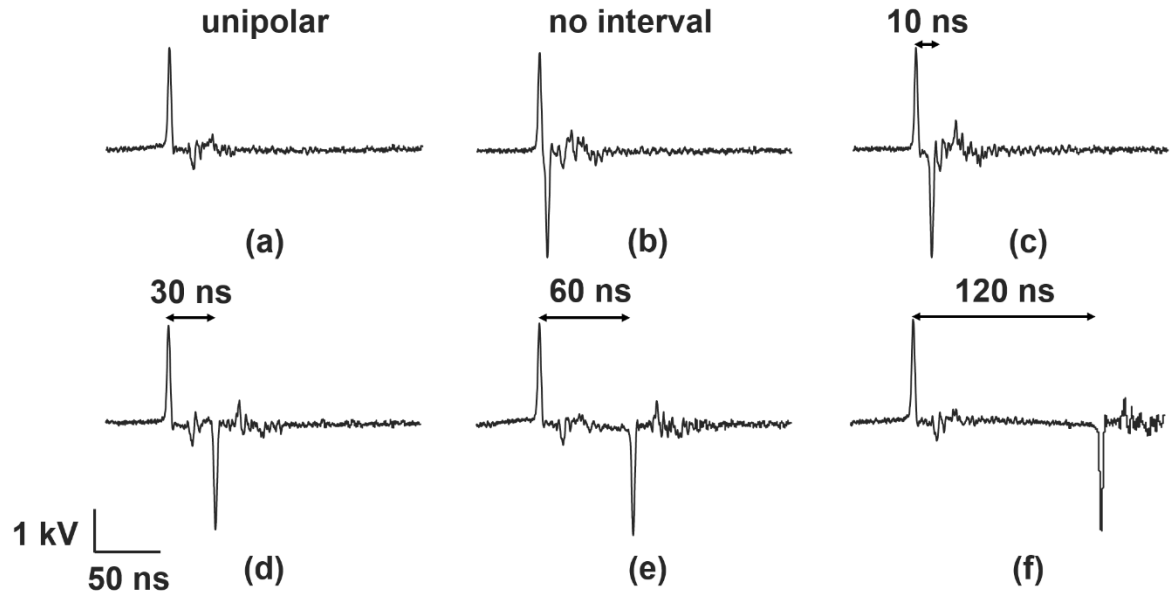

FIGURE S1 Representative oscilloscope traces of 2 ns unipolar and bipolar pulses having the same positive and negative phases but varying the interphase interval. (a) Unipolar pulse, (b) Bipolar pulse with no interphase interval, and (c) to (f) bipolar pulses with interphase intervals ranging from 10 to 120 ns, respectively, measured from peak to peak. In all plots, the amplitudes of the positive and negative phase are equal to ±1.8 kV. There is a small reflection of the pulse at the load so we accounted for that in the field calculation at the cells, which comes to the peak potential difference for the cells to see ~1.5 kV.

For experiments where the amplitude of the second (negative) phase of the bipolar pulse was changed, the second phase amplitude ratio was changed from 0.5:2 by superposition of a larger first phase amplitude and a (device-allowed) minimum amplitude second phase (Figure S2).

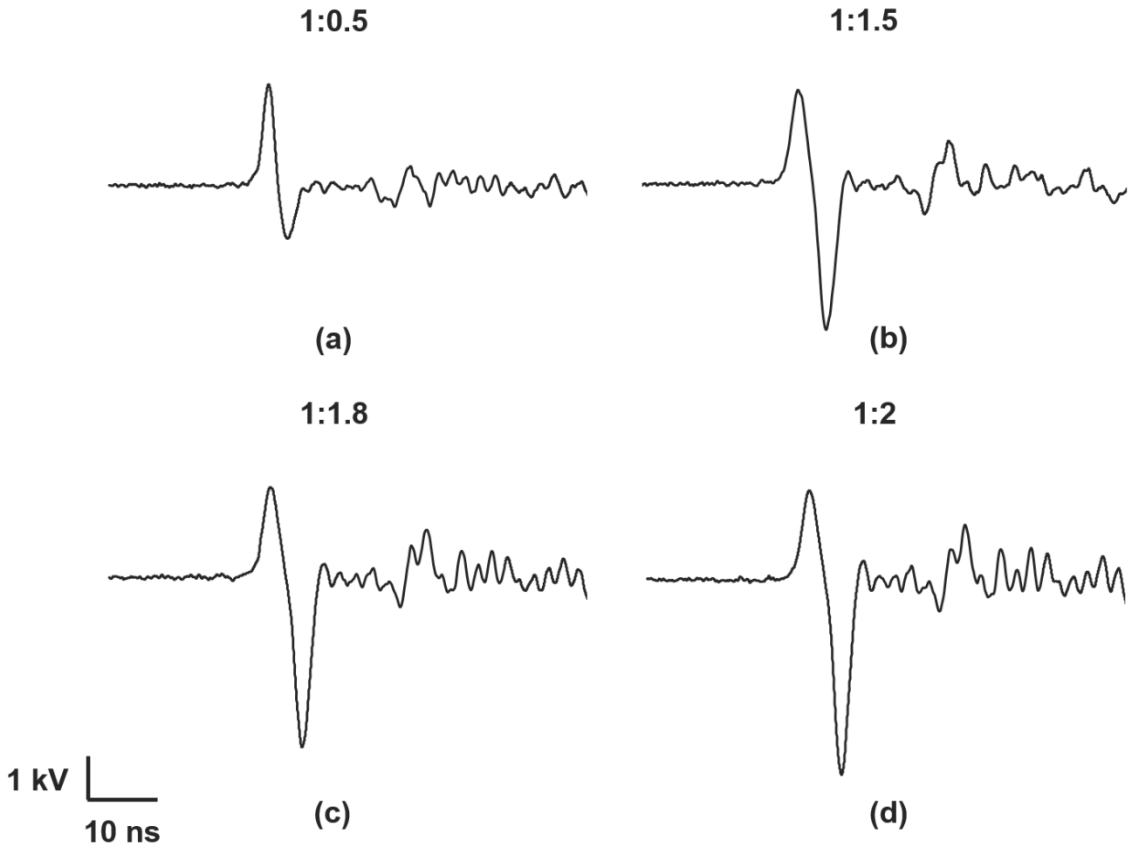

FIGURE S2 Representative oscilloscope traces of 2 ns unipolar and bipolar pulses with no interphase interval obtained by varying the amplitude of the second phase. (a) to (d) Bipolar pulses in which the positive phase was set to +1.8 kV and the negative pulse phase varied in amplitude from 0.9 kV to 3.6 kV, respectively. The second phase amplitude ratios (ratio of the amplitude of the second phase to that of the first phase) are indicated in each plot and varied from 0.5 to 2. The reflections were accounted for in the field calculations assuming the same amount as in the unipolar pulse.

Time course of the mean response of cells to unipolar and bipolar exposures with varying interphase intervals (cumulative results)

For experiments in which we varied the interphase interval from no delay between the positive and negative phase of the bipolar pulse to up to 120 ns, Figure S3 shows the cumulative results obtained from this study. These results represent the total number of cells exposed to the various pulses. Results shown in Figure 4 of the manuscript represent only a few number of cells tested. Continuous baseline fluorescence of the cells was monitored 60 s prior to stimulus application and continued for up to 5 min after the stimulus. Responses were monitored up until they returned to baseline.

The results showed that a 2 ns bipolar pulse with no interphase interval completely cancels the response elicited by the unipolar pulse. A 10 ns interphase interval elicited a Ca^{2+} transient that was reduced significantly in amplitude compared to that elicited by the unipolar pulse. Increasing the interphase interval to 30 ns elicited Ca^{2+} responses that increased in amplitude to reach a value similar to the one evoked by the unipolar pulse.

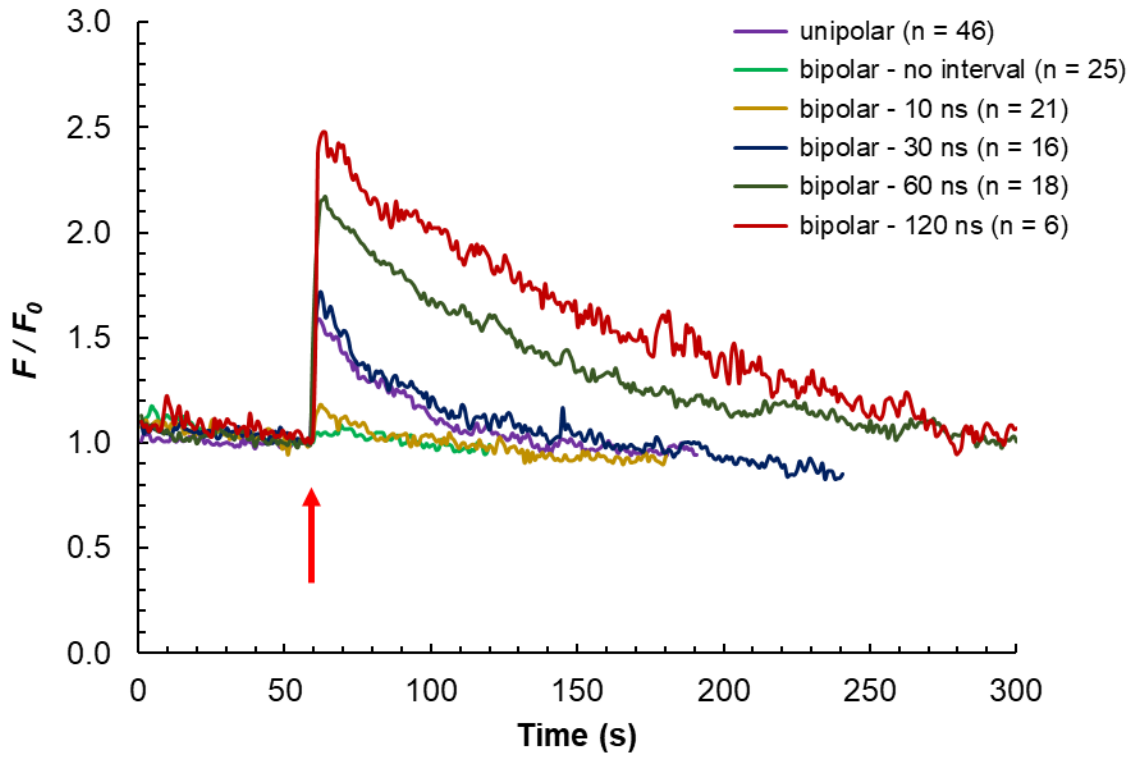

FIGURE S3 Cumulative results of Ca^{2+} responses obtained for different interphase intervals of a 2 ns bipolar pulse. Results are plotted as the averaged fluorescence traces for cells exposed to a 2 ns unipolar pulse or a 2 ns bipolar pulse with various interphase intervals. Representative results are shown in Figure 4 of the manuscript, and these results represent the total number of experiments performed. The number of cells tested are: n = 46 for the unipolar pulse (light blue trace), n = 25 (dark blue trace), 21 (orange trace),

16 (green trace), 18 (red trace) and 6 (purple trace) cells for bipolar pulses with no interphase interval, 10, 30, 60 and 120 ns interphase intervals, respectively. The arrow indicates the time of pulse delivery.