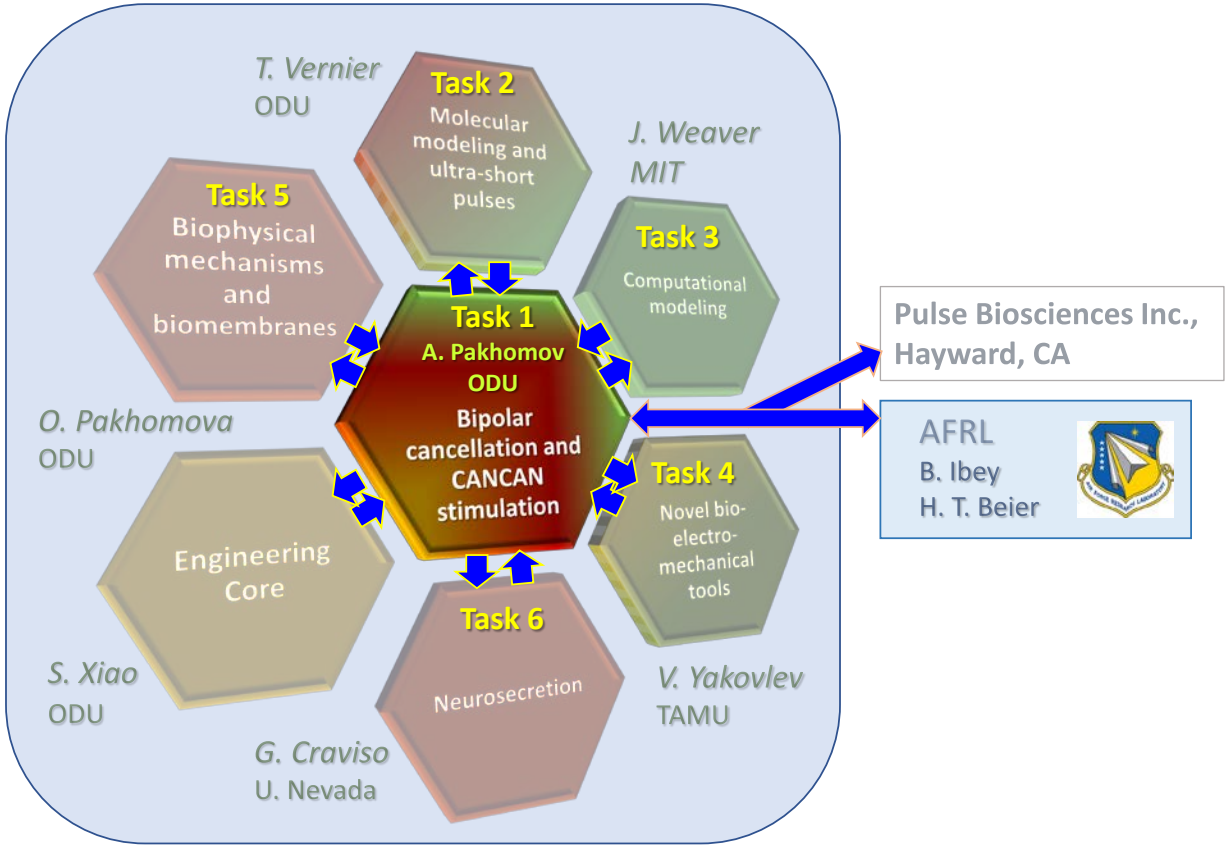
Universality of Bipolar Cancellation for Nanoporation and Nanoelectropulse Stimulation

Andrei Pakhomov

More Task 1 topics

(MURI Objective: "Comprehensive understanding of nsPEF effects…")

- Neuronal excitation by nsPEF
- Selective susceptibility to nsPEF
- Facilitation of nsPEF effects
  (combined effects, delayed hypersensitivity…)
- "Twin pulses"
- Repair of nsPEF injury
- Mechanisms, mechanisms, and mechanisms…



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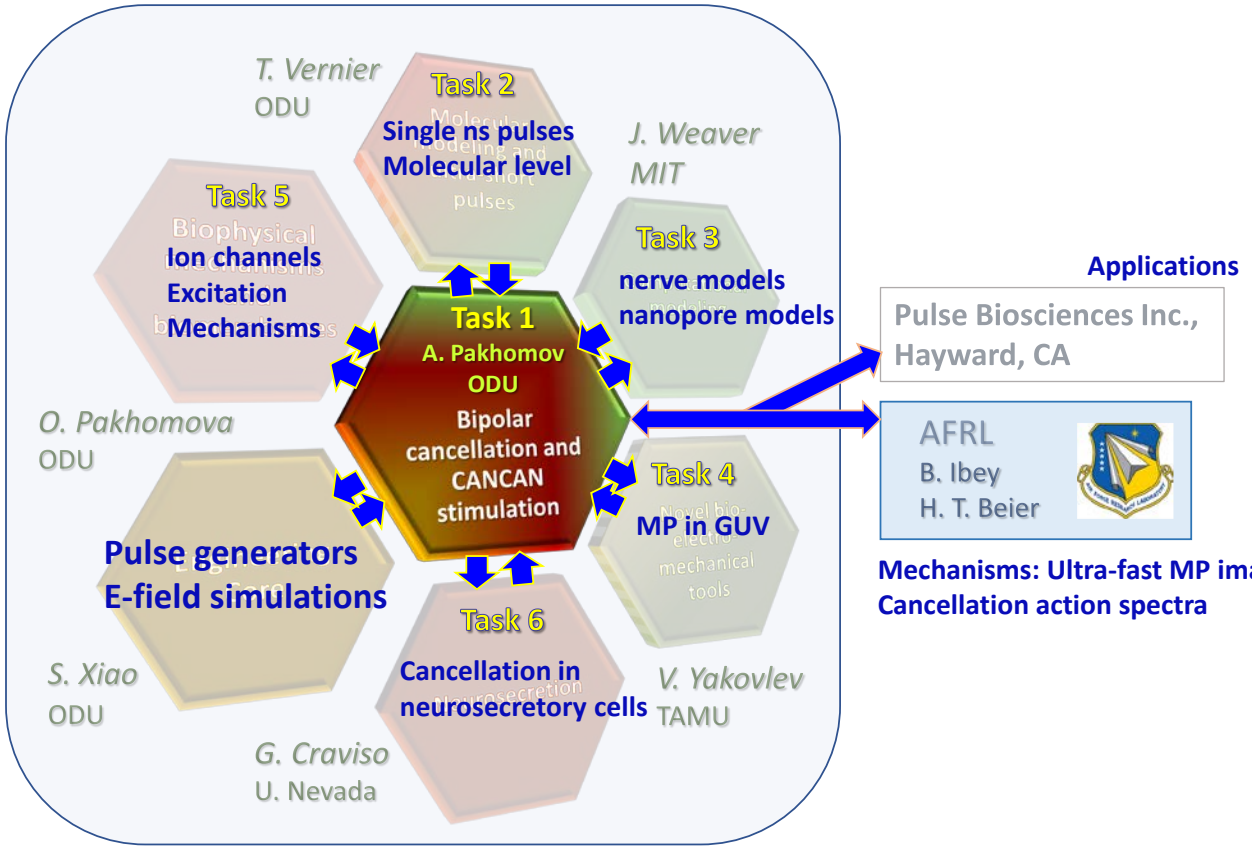


Frank Reidy Research Center for Bioelectrics
Old Dominion University, Norfolk, VA
CBE

Membrane Permeabilization by "Long" Bipolar Pulses (BP, µs-ms)

Tekle, E., R. D. Astumian, et al. (1991). Proc Natl Acad Sci U S A **88**(10): 4230-4234.

Faurie, C., E. Phez, et al. (2004). Biochimica Et Biophysica Acta-Biomembranes **1665**(1-2): 92-100

Kotnik, T., G. Pucihar, et al. (2003). Biochim Biophys Acta **1614**(2): 193-200

Arena, C. B., M. B. Sano, et al. (2011). Biomed Eng Online **10**: 102 (1-2 µs)

Kotnik, T., D. Miklavcic, et al. (1998). Bioelectrochemistry and Bioenergetics **45**(1): 3-16.

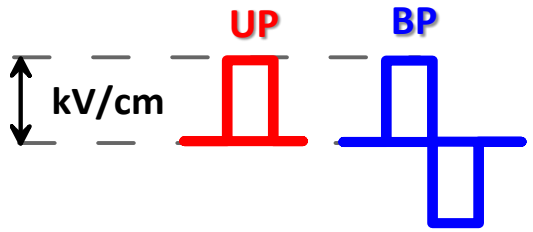
Kotnik, T., L. M. Mir, et al. (2001). Bioelectrochemistry **54**(1): 83-90

Faurie, C., M. Rebersek, et al. (2010). Journal of Gene Medicine **12**(1): 117-125.

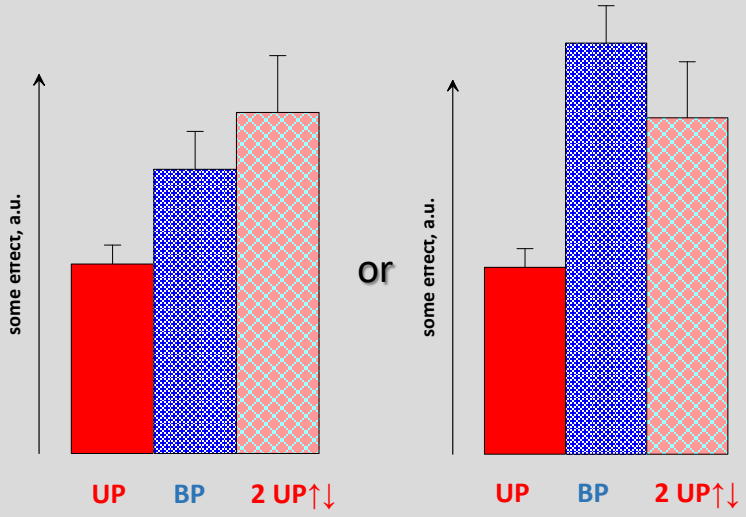
Arena, C. B., M. B. Sano, et al. (2011). IEEE Trans Biomed Eng **58**(5): 1474-1482 (1-2 µs)

With minor exceptions, BP were more efficient than unipolar pulses (UP), or equally efficient

"… compared the efficiency of triangular, sine, and rectangular bipolar pulses …
… results can be explained on the basis of the ***time during which the pulse amplitude exceeds a certain critical value****"*

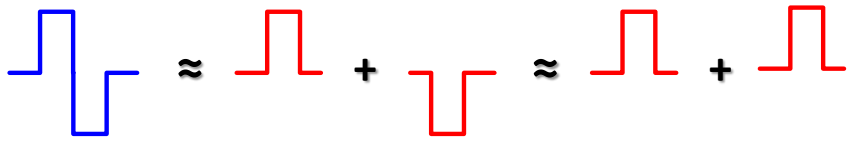


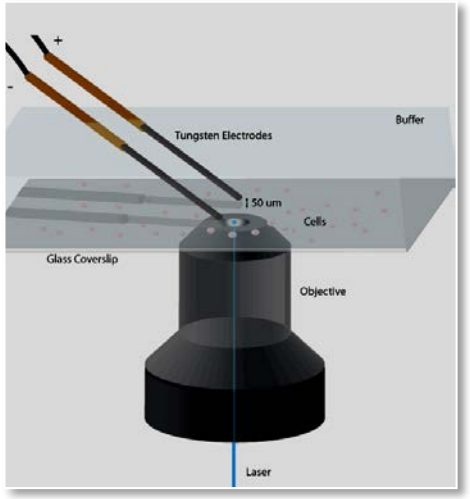
µs-ms pulses



or

BP ≈ 2 UP↑↓ ≈ 2 UP ↑↑

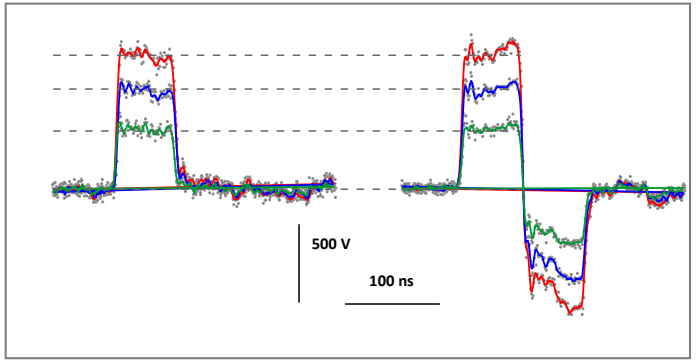


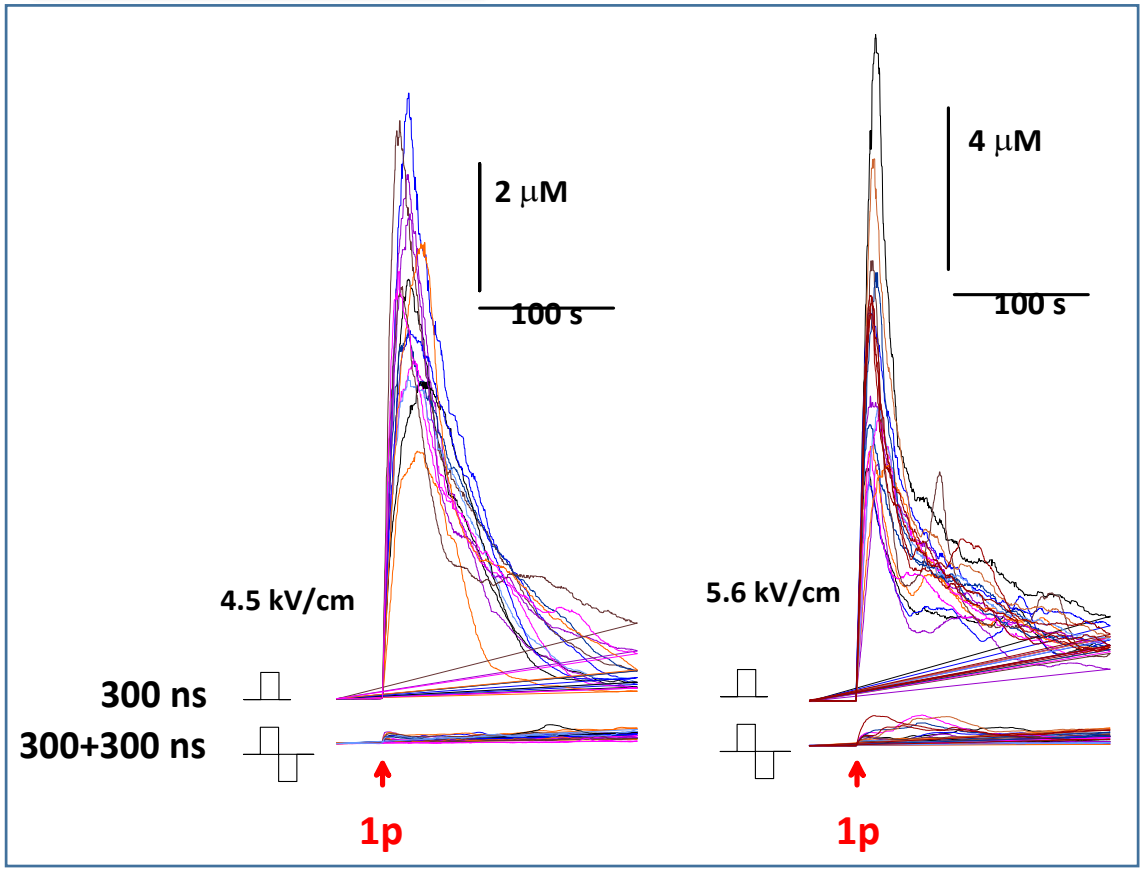


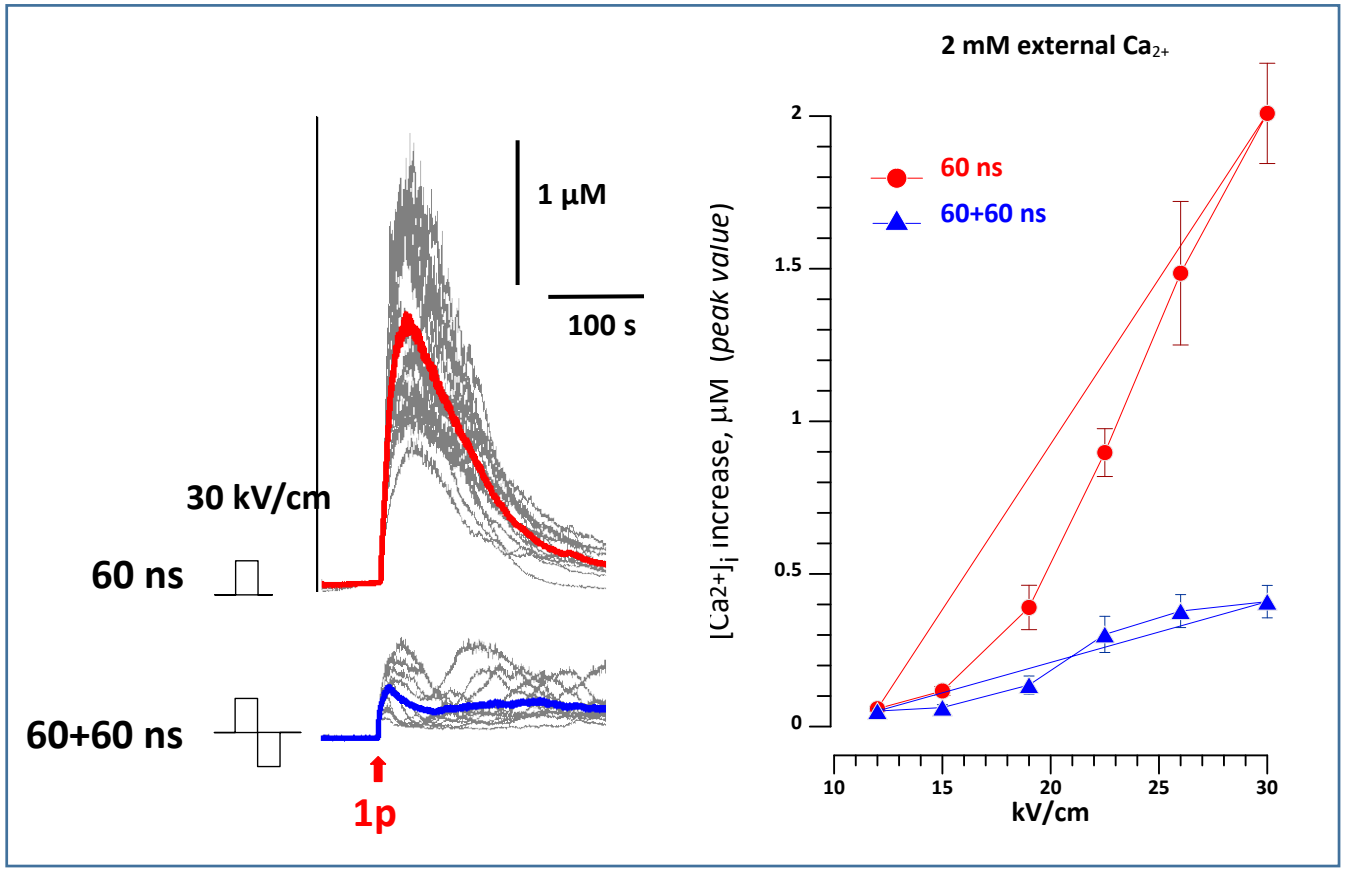
Bipolar Cancellation by nsPEF

Ca^{2+} activation

CHO cells, single pulse,
microscope-based exposure set-up
different pulse durations and amplitudes





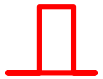


Pakhomov et al., *Cancellation of cellular responses to nanoelectroporation by reversing the stimulus polarity*. Cell Mol Life Sci. 2014;71(22):4431-41.

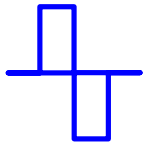
Bipolar Cancellation by nsPEF

Cell killing

U937 and CHO cells, multiple pulses, cuvette exposure

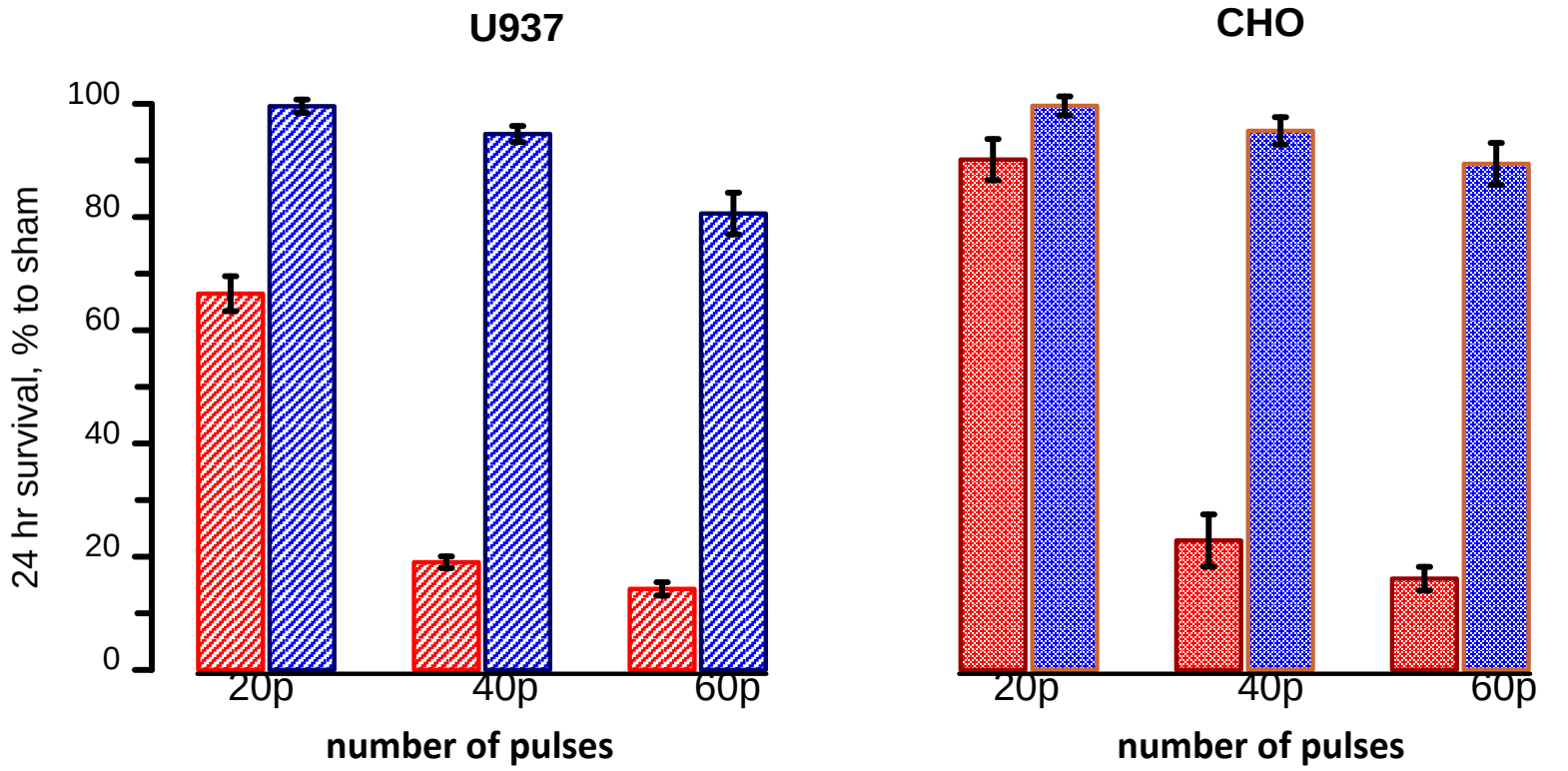


60 ns, 1 Hz, 40 kV/cm



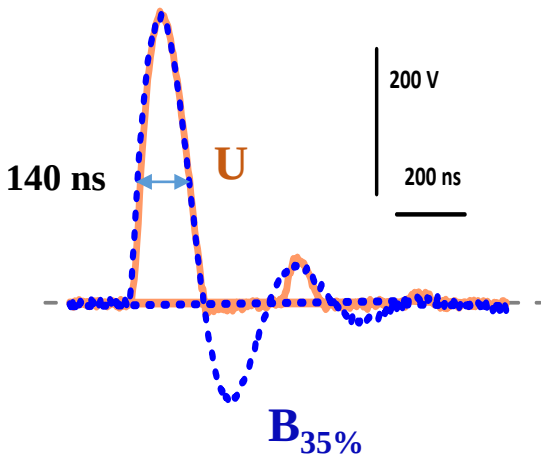
60+60 ns, 1 Hz, 40 kV/cm
(80 kV/cm peak-to-peak)

MTT assay, mean +/- s.e., n=5-7



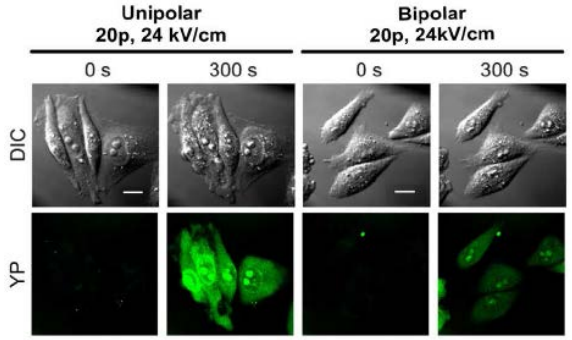
Pakhomov et al., *Cancellation of cellular responses to nanoelectroporation by reversing the stimulus polarity*.
Cell Mol Life Sci. 2014;71(22):4431-41.

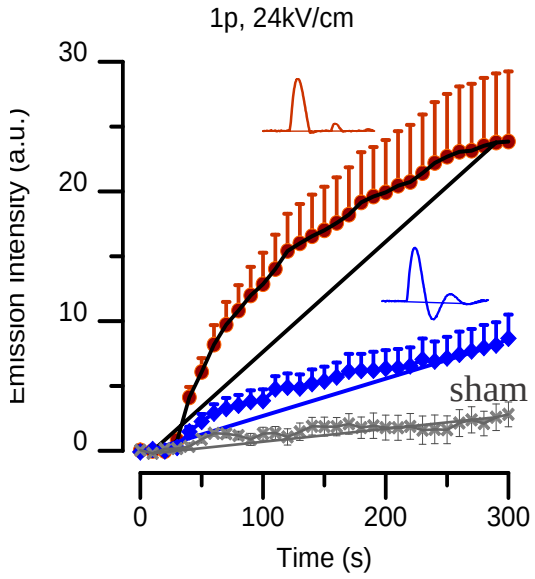
Bipolar Cancellation by nanosecond electric pulse oscillations (NEFO)

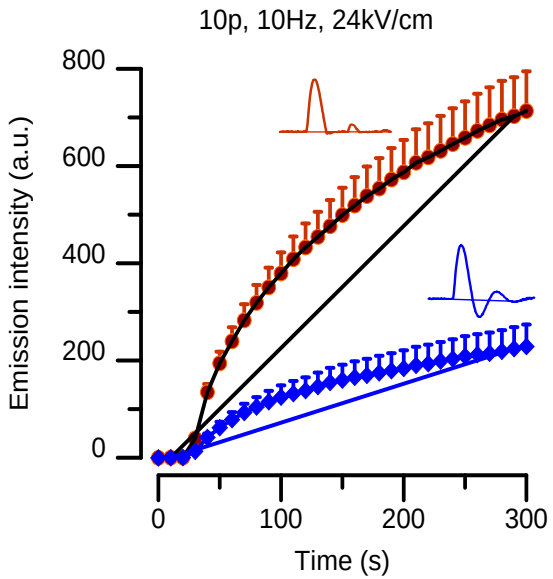


YO-PRO-1 uptake

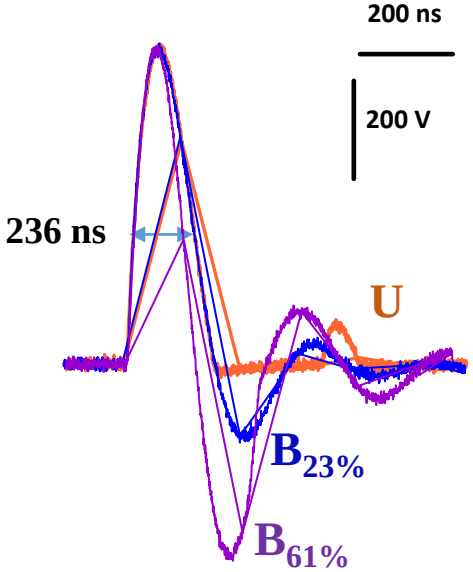
CHO cells, single and multiple pulses

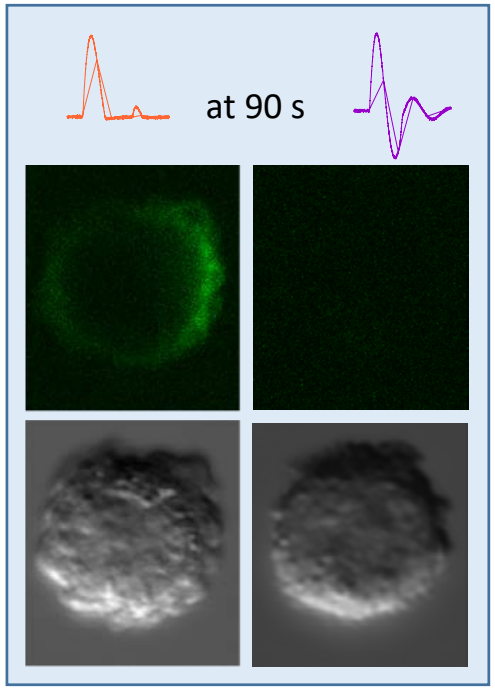






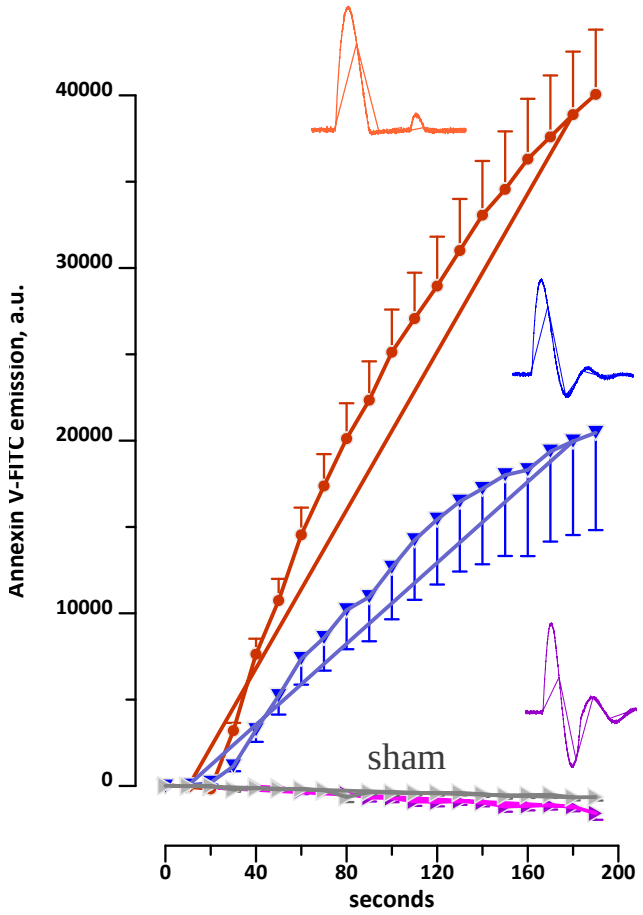
Gianulis et al., *Electroporation of mammalian cells by nanosecond electric field oscillations and its inhibition by the electric field reversal*. Sci Rep. 2015;5:13818.



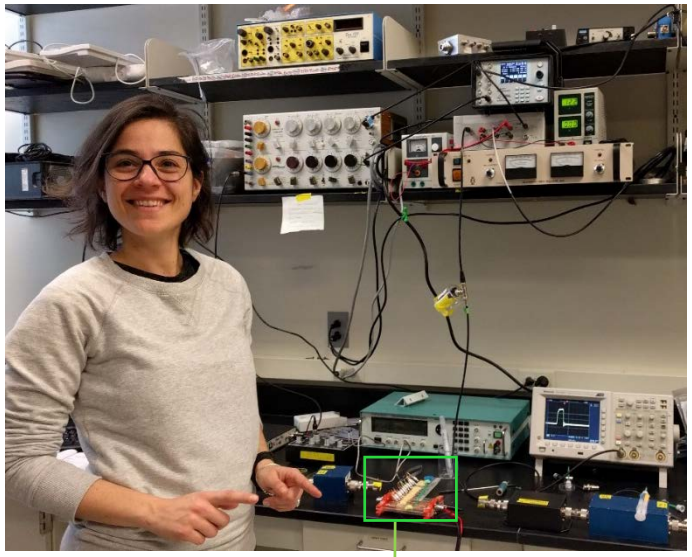


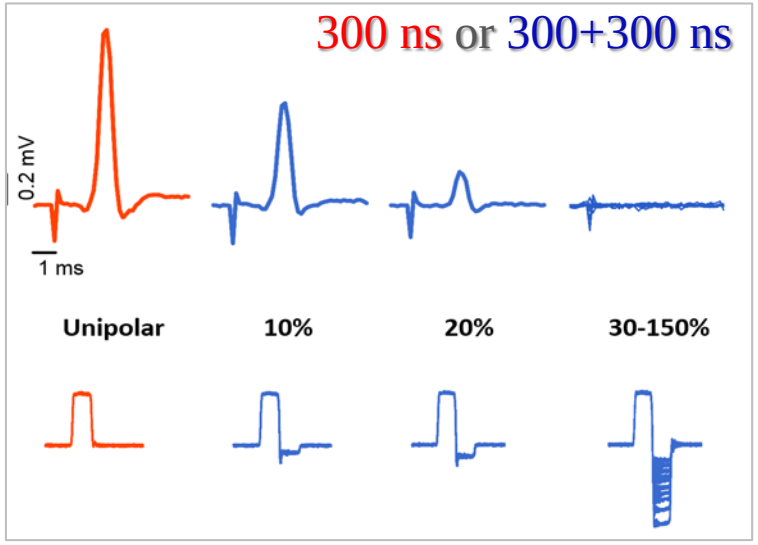
PS externalization

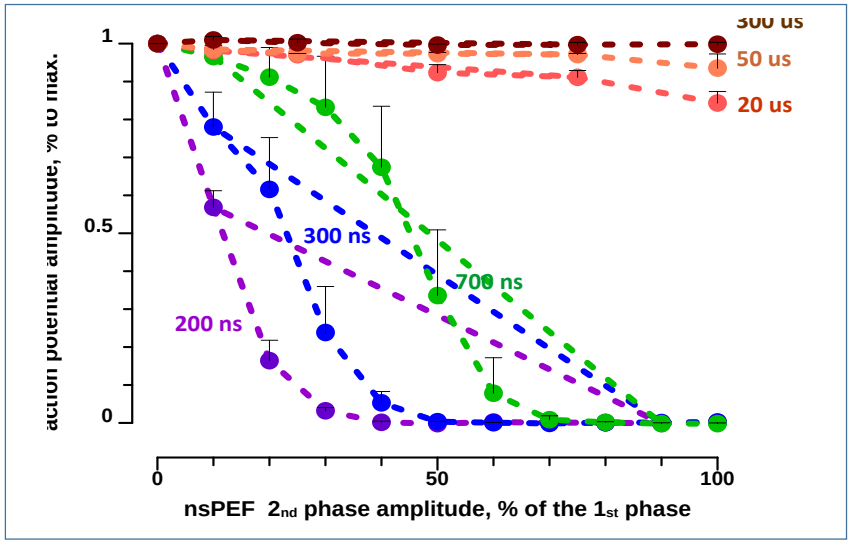
U937 cells, 50p, 10 kV/cm, 5 Hz

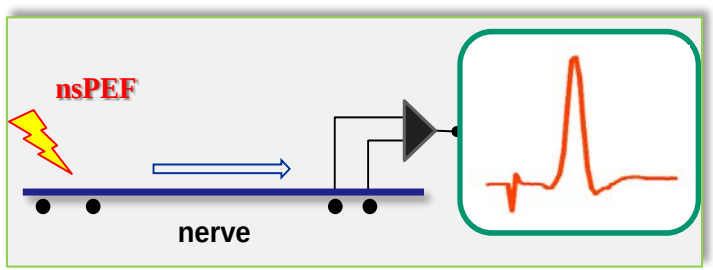


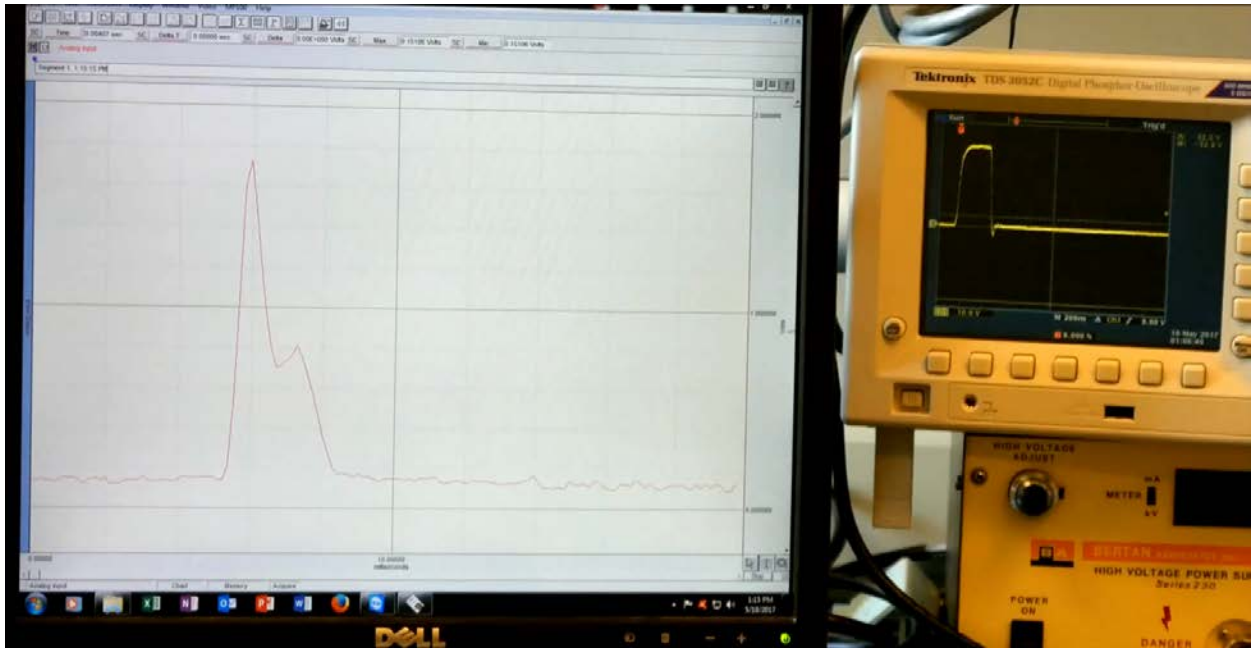
Bipolar Cancellation of Electrostimulation **of nerve fibers**







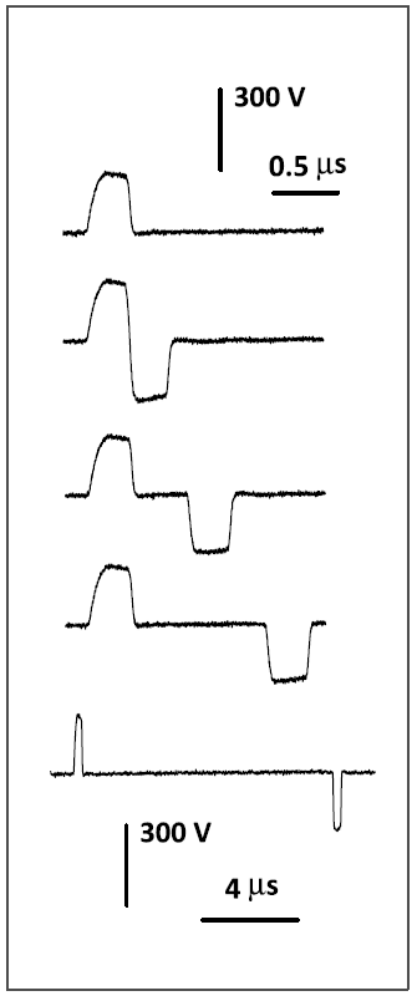


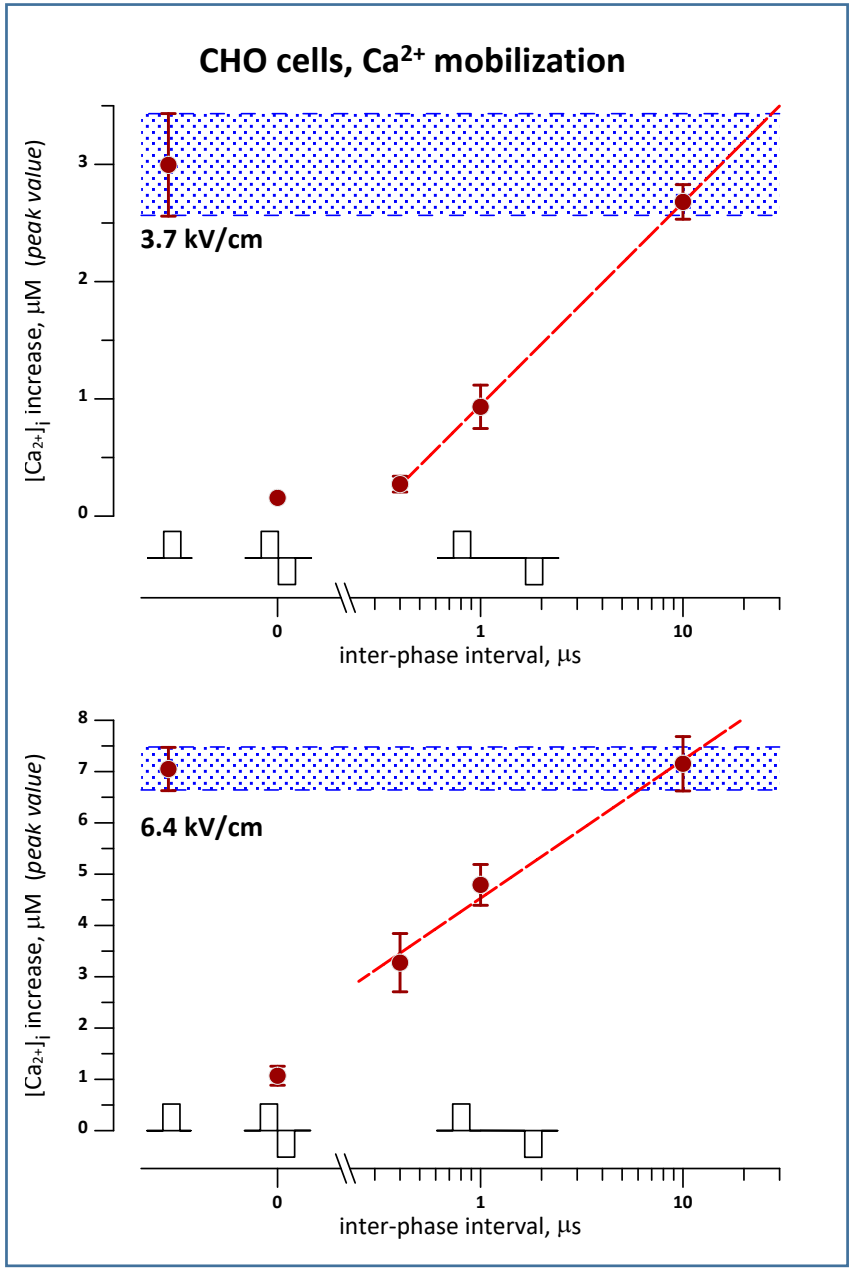


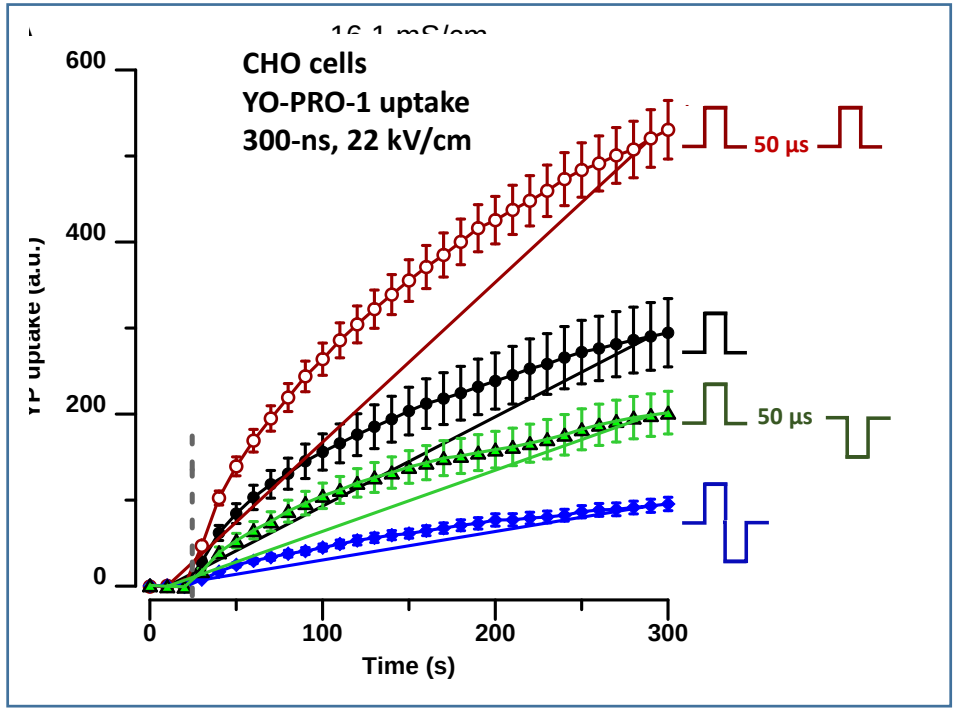
Synergy with **Task 3**:
nerve excitation and conduction modeling

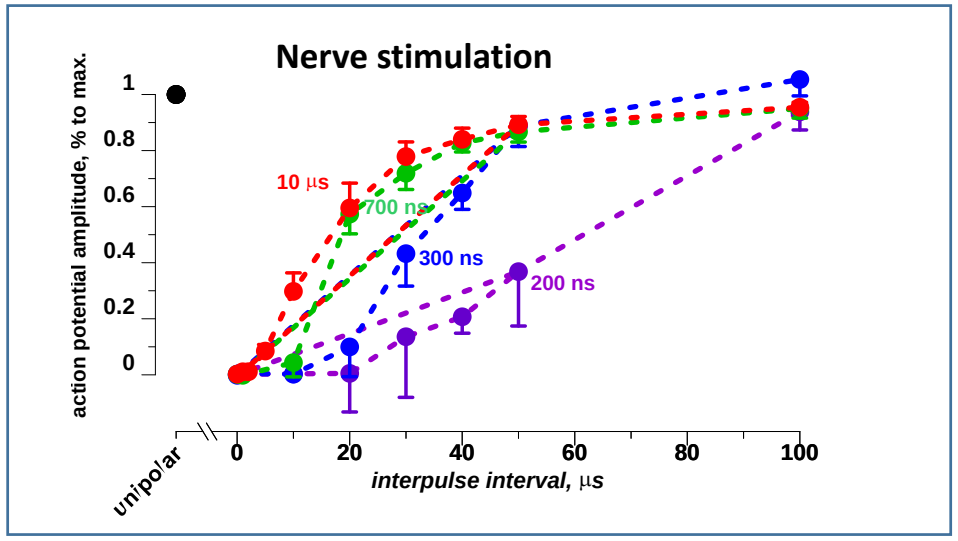
Separation of two phases into two unipolar nsPEF of opposite polarities

cancellation can be observed with up to 10-50 µs separation

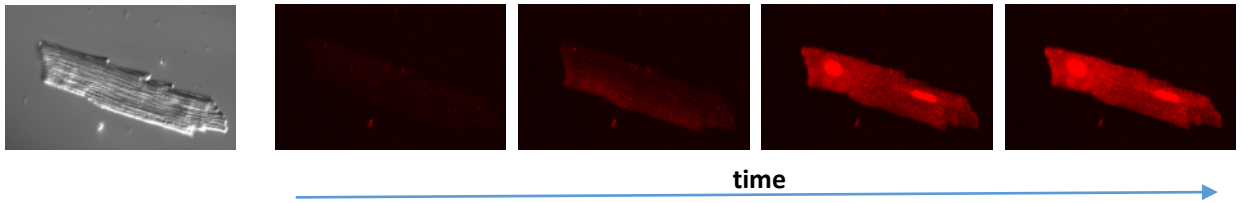


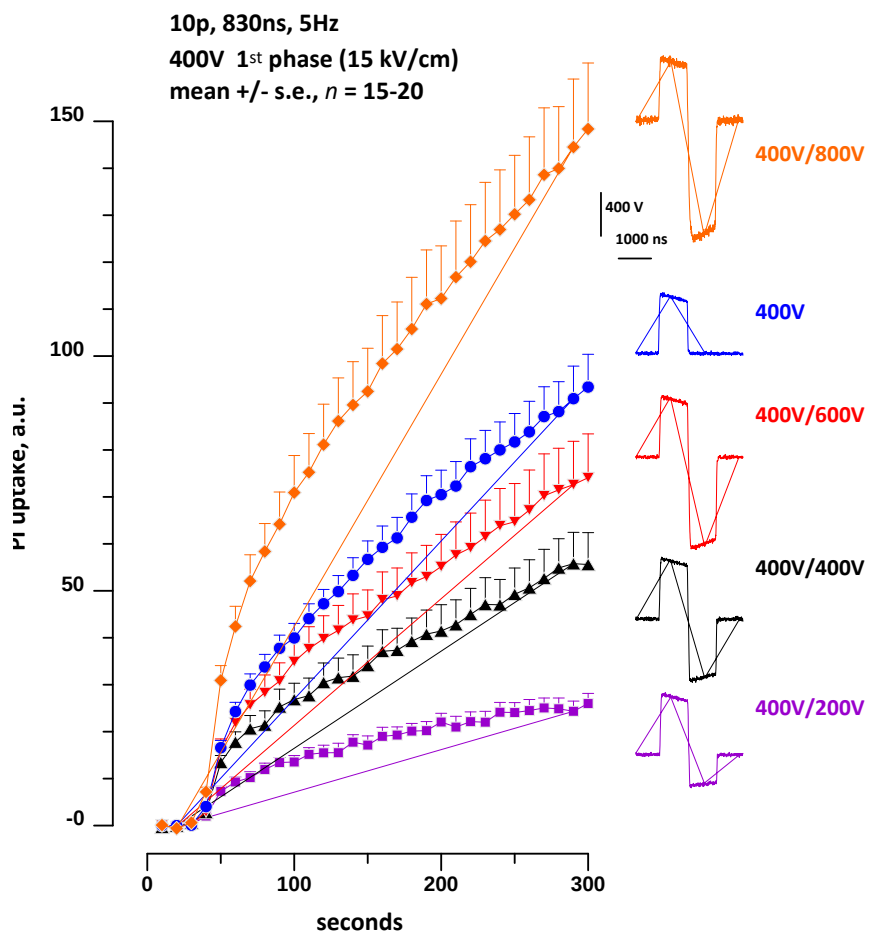




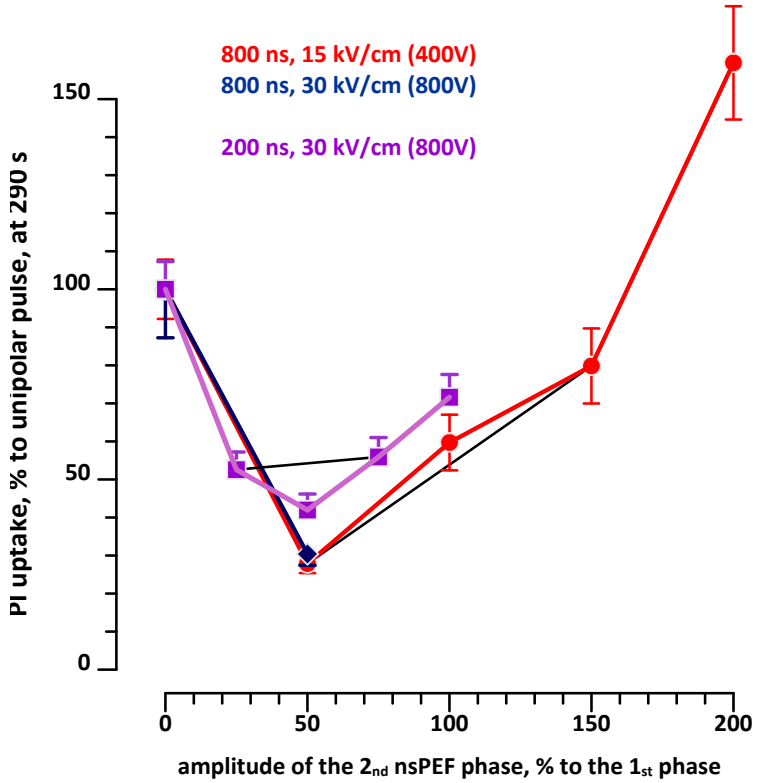


Can we get a stronger cancellation by varying the 2nd phase amplitude?





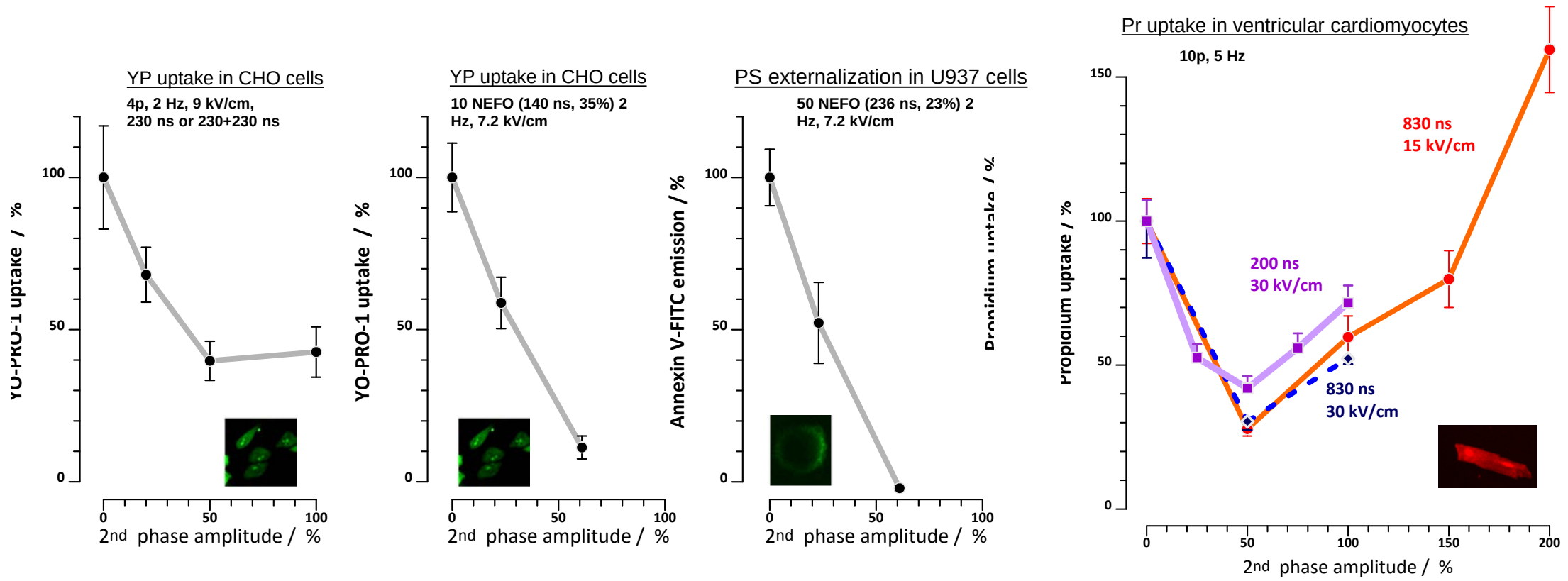
Propidium uptake in primary ventricular cardiomyocytes, mouse



- **the smallest 2nd phase (50%) caused the strongest cancellation!**
- too much of the 2nd phase made the effect stronger than unipolar
- the degree of cancellation was the same for 15 and 30 kV/cm!
- the degree of cancellation was (almost) the same for 200- and 830-ns pulses
  (in contrast to nerve excitation results)

Maximum cancellation is typically achieved when the 2nd phase is at 50-60% of the 1st one

- because larger 2nd phase may have the effect of its own



Max. Cancellation at 50%:

- vital for Tasks 2,3,4, and 6; for engineering of CANCAN pulsers; and for CANCAN trials in Task 1 (summary talk)

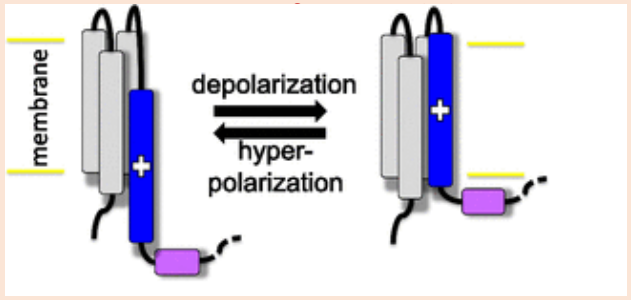
Mechanism(s) of Bipolar Cancellation

1. Assisted membrane discharge
2. Reversal of electrophoretic Ca^{2+} uptake
3. **A two-step effect of nsPEF, with reversible first step**

cancellation of nerve excitation

Most logical mechanism

the first step is opening of voltage gated Na^+ channels, which is slow (10s of µs) and can be halted by polarity reversal, thus preventing action potential



cancellation of nanoelectroporation

a) *oxidation-reduction mechanism*
reversible membrane oxidation by nsPEF, followed by irreversible oxidation; polarity reversal causes reduction and prevents irreversible oxidation

b) *"foot-in-the-door" mechanism*
nsPEF opens transient pores, which are stabilized into "long-lasting pores" by insertion of some macromolecules. Polarity reversal pushes the molecules out and prevents pore stabilization

c) *re-organization of membrane proteins which takes time and delays the onset of the permeable state*

d) *??*

http://www.pnas.org/content/112/1/124/F1.expansion.html

Mechanism(s) of Bipolar Cancellation *(with **Task 5**)*

1. Assisted membrane discharge
2. Reversal of electrophoretic Ca^{2+} uptake
3. **A two-step effect of nsPEF, with reversible first step**

A Synthetic Theory of Nanoporation and Bipolar Cancellation

1. Nanopores = ion channels
(non-voltage gated, but voltage-sensitive)

2. Larger pores = damaged channels

cancellation of nerve excitation

Most logical mechanism

the first step is opening of voltage gated Na^+ channels, which is slow (10s of µs) and can be halted by polarity reversal, thus preventing action potential

experiments confirm model predictions!

Reilly JP, Diamant AM. *Neuroelectric mechanisms applied to low frequency electric and magnetic field exposure guidelines--part II: non sinusoidal waveforms*. Health physics 2002;83(3):356-365.

cancellation of nanoelectroporation

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d) *??*

Take-home notes

1. Excitation and membrane damage by nsPEF can be cancelled by the polarity reversal, (= by a second nsPEF of the opposite polarity)
2. Bipolar cancellation is universal for diverse cells and endpoints, but is restricted to nsPEF
3. Bipolar cancellation "tapers out" within 10-50 µs
4. Developed action spectra for pulse width, shape, and intensity
5. Mechanism of cancellation:
   - *has yet to be proven*
   - "Synthetic theory" may overhaul the entire field
6. Applications: CANCAN and more (selective effects)
7. Extensive impact on all MURI Tasks