NANOELECTROPULSE-INDUCED CHANGES IN CELL EXCITABILITY: A NEW APPROACH FOR NEUROMODULATION

Gale L. Craviso

Department of Pharmacology
University of Nevada Reno School of Medicine, Reno, NV

Normand Leblanc[1], Thomas W. Gould[2], Ji hwan Yoon[3], and Josette Zaklit[3]

[2]Department of Physiology and Cell Biology
University of Nevada Reno School of Medicine, Reno, NV

[3]Department of Electrical and Biomedical Engineering
College of Engineering, University of Nevada, Reno, Reno, NV

University of Nevada, Reno
School of Medicine

University of Nevada, Reno

Nanoelectropulse-induced changes in cell excitability – a new approach for neuromodulation

Objectives:

Develop practical applications of nanosecond electric pulses (NEP) to augment human performance.

- Remote electrostimulation of chromaffin cells in the medulla of the adrenal gland with single NEP or NEP trains to evoke a rapid, transient “adrenaline burst”.
- Determine the extent to which NEP affect other excitable elements in the adrenal medulla.
- Elucidate mechanisms of NEP interaction with adrenal chromaffin cells at the cellular level.

Technical Approach:

- Single cell level: amperometry and simultaneous Ca^{2+} imaging for real-time monitoring of NEP-evoked catecholamine release.
- Single cell level: Ca^{2+} and Na^{+} imaging in parallel with patch clamp electrophysiology to further elucidate how NEP interact with chromaffin cells.
- Tissue level: Ca^{2+} imaging to establish the efficacy and safety of NEP for chromaffin cell stimulation *in situ*.
- Modulating NEP signals in the RF range for remote delivery

Accomplishments:

- NEP directly activate chromaffin cells *in situ* without affecting other excitable elements in adrenal tissue.
- Identified novel role of L-type voltage-gated Ca^{2+} channels in a process that leads to Na^{+} influx into chromaffin cells following NEP exposure.
- Progress developing the capability to deliver NEP to intact adrenal gland tissue *in vivo*.

Publications: Yun et al., 2024; Two manuscripts submitted; Four manuscripts in various stages of preparation
NSF Career Grant awarded to Ji hwan Yoon
One patent application in process

DoD Benefit:

- Develop a patentable, wearable RF-based NEP delivery device for remote stimulation of chromaffin cells of the adrenal gland to:

INCREASE ALERTNESS
ENHANCE VISION AND HEARING
ENABLE PERSONNEL TO STAY AWAKE DURING SLEEP DEPRIVATION

- Contribute to advancing non-invasive approaches for focal and temporally precise control of physiological functions in general that could enhance human performance.

BACKGROUND

AFOSR HUMAN PERFORMANCE AND BIOSYSTEMS

- **Primary Goal:** Improve human performance capabilities by establishing ***new electric stimulation methods*** to augment human performance

THE ELECTROSTIMULATION APPROACH BEING EXPLORED

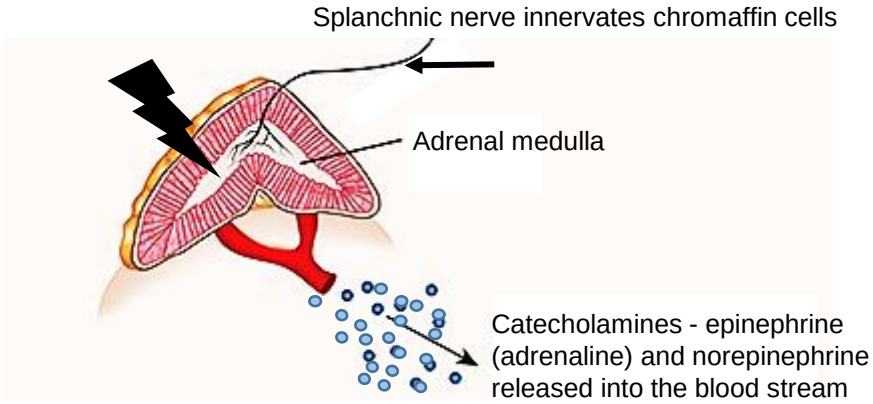
Nanosecond duration electric pulses (NEP)

- Have the ability to modulate/fine-tune cellular responses by altering the electric pulse duration and waveform
- Have the potential to be delivered to a targeted tissue *non-invasively* (remotely)

MAIN APPLICATION

- Evoking a *transient* “adrenaline burst” from adrenal chromaffin cells *on demand* (i.e., triggering a “fight or flight” response) *remotely*

TARGETING CHROMAFFIN CELLS OF THE ADRENAL GLAND



How would evoking a *transient* "*adrenaline burst" on demand be of benefit*:

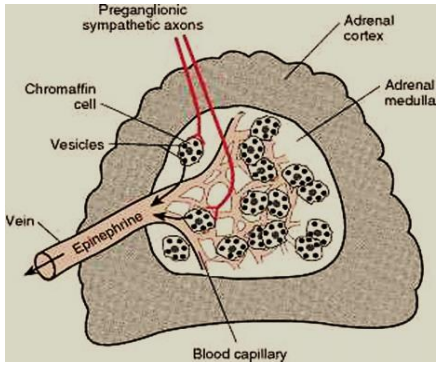
- alertness increased
- pupils dilate, improving vision
- hearing and other senses enhanced
- increased blood flow directed toward skeletal muscles causes a surge in energy and temporarily increases strength

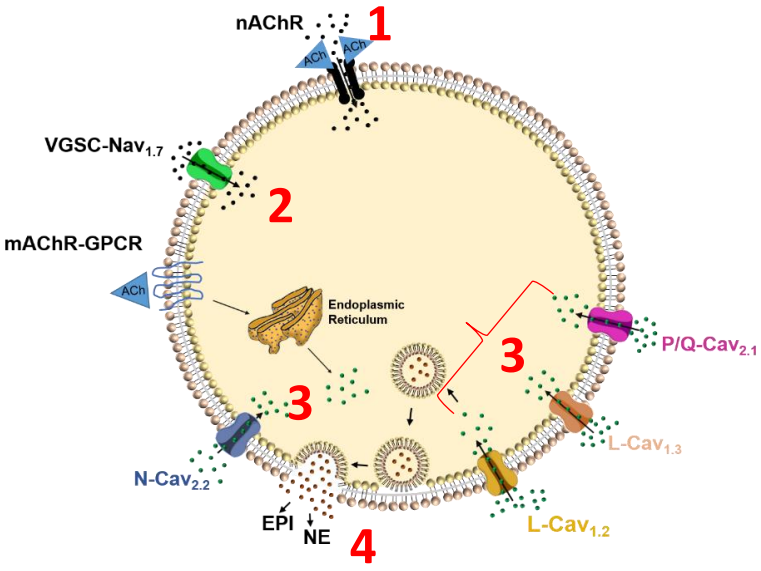
Another potential beneficial outcome of targeting the adrenal gland:

- Controlling the release of adrenal hormones with temporal precision may empower studies of stress biology and advance therapeutic approaches for stress-related mental illnesses (e.g., PTSD)

(Rosenfeld et al., Sci. Adv. 2020)

MECHANISM BY WHICH CATECHOLAMINES ARE RELEASED FROM CHROMAFFIN CELLS





ACh: Acetylcholine

nAChR: nicotinic ACh receptor

mAChR-GPCR: muscarinic ACh receptor

VGSC-Nav: Voltage-Gated Na^+ Channel

Cav: Voltage-Gated Ca^{2+} Channel

NE: Norepinephrine

EPI: Epinephrine

Splanchnic nerve terminals release ACh

1. Activates primarily nAChR that are permeable to Na^+
2. Na^+ influx through nAChR causes membrane depolarization
3. Sequential opening of voltage-gated Na^+ and Ca^{2+} channels
4. Ca^{2+} influx leads to exocytosis that occurs within ***milliseconds*** of nAChR stimulation

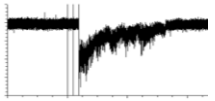
Main finding: Similar to nAChR activation, NEP can trigger Ca^{2+} influx via voltage-gated Ca^{2+} channels, resulting in the release of catecholamines

EXPERIMENTAL APPROACHES

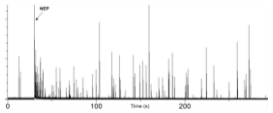
Cultured chromaffin cells (bovine and murine) exposed to NEP:

- Fluorescence imaging of changes in intracellular Ca^{2+} and Na^{+}

- Whole-cell patch clamp recordings of membrane currents

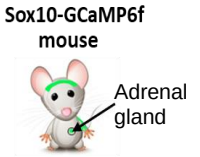


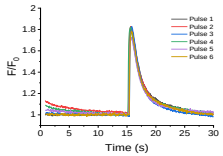
- Measurement of catecholamine release by amperometry



Chromaffin cells *in situ* (in murine adrenal slices) exposed to NEP:

- Fluorescence imaging of changes in intracellular Ca^{2+} - transgenic mice expressing the Ca^{2+} sensor GCaMP6f in chromaffin cells

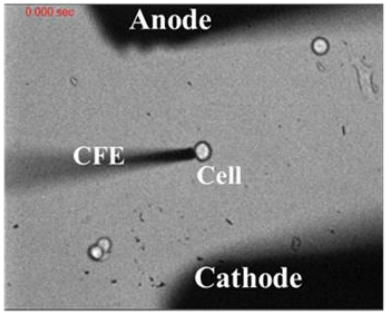


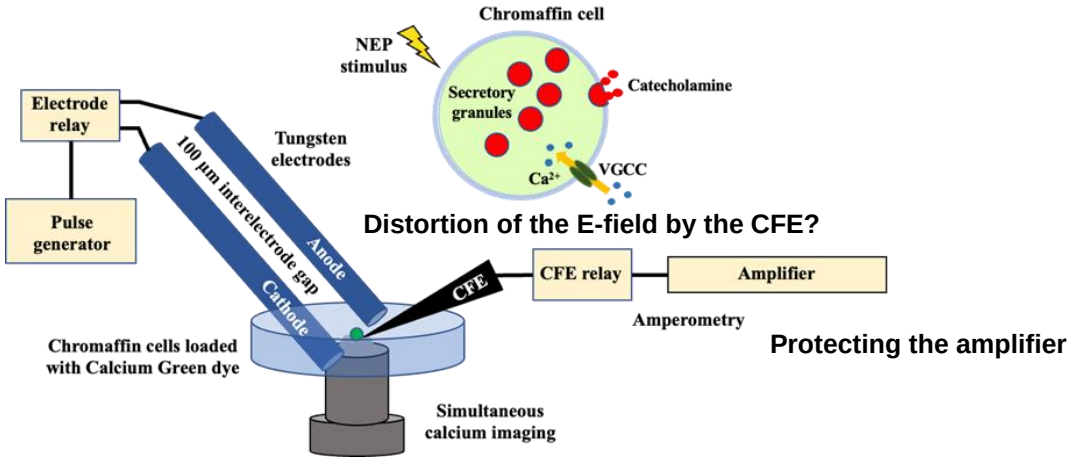


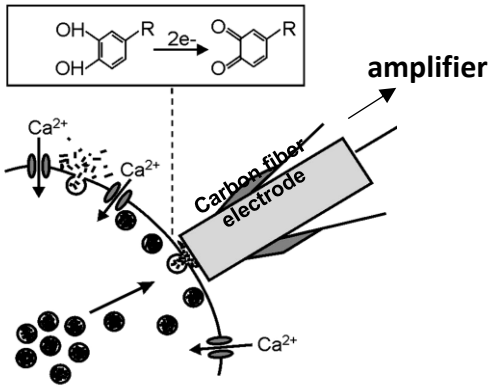
Satellite glial cells *in situ* (in murine adrenal slices) exposed to NEP:

- Fluorescence imaging of changes in intracellular Ca^{2+} - transgenic mice expressing GCaMP6f in satellite glial cells

NEP-EVOKED Ca^{2+}-DEPENDENT CATECHOLAMINE RELEASE IN REAL-TIME USING THE AMPEROMETRIC METHOD – AN "ADRENALINE BURST"

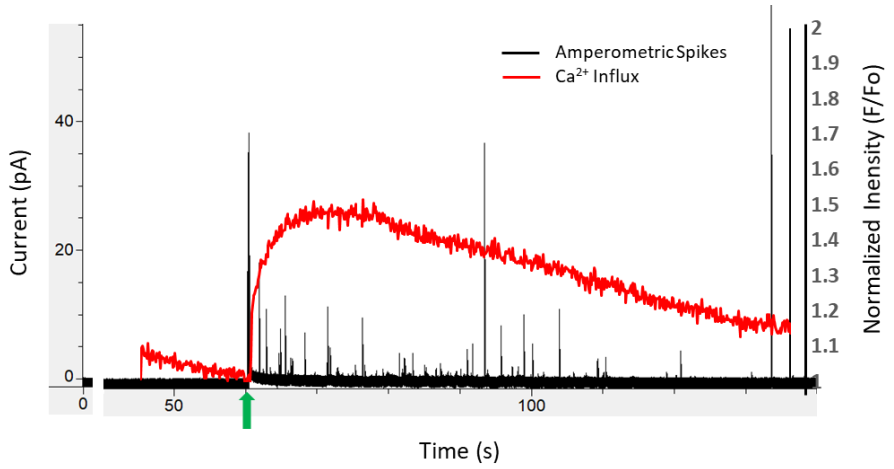






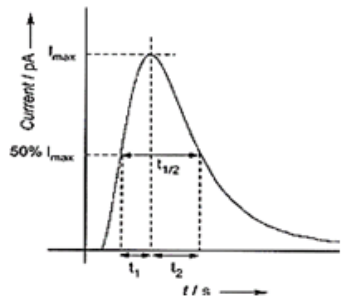
$$Q = nNF$$

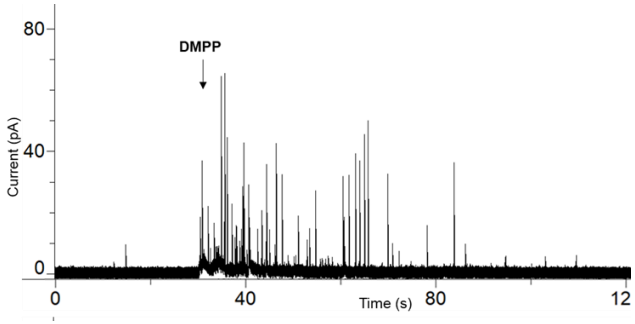
Q is the charge passed at the electrode surface
n is the number of moles of electrons transferred per mole of analyte oxidized
N is the number of moles of detected analyte
F is the Faraday constant (96,485 C/mol)

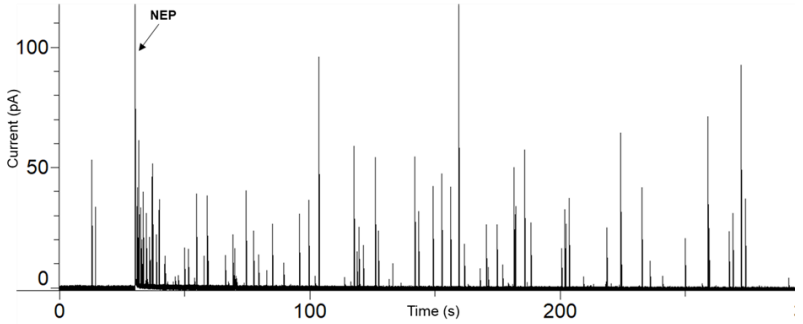


Plans For Next Year

- Finish the analysis of amperometric spikes to determine if the characteristics of each NEP-evoked event differs from that for nAChR-evoked events.
- Determine the length of time that exocytosis is triggered by an NEP versus nAChR activation
- Determine the extent to which NEP-evoked events occur with a delay.







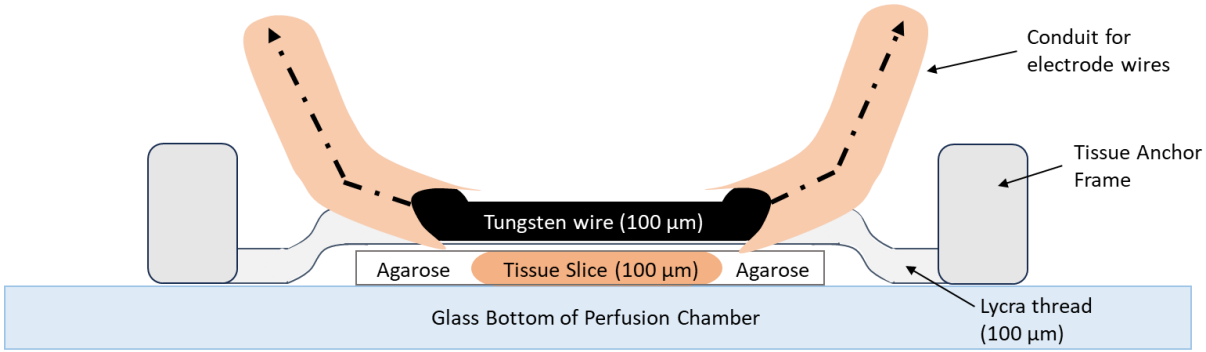
- Finish two manuscripts:
  - “Amperometric method for monitoring catecholamine release from adrenal chromaffin cells stimulated with high intensity nanosecond electric pulses” – near completion
  - “Amperometric characterization of the exocytotic response of adrenal chromaffin cells to stimulation by nanosecond electric pulses” – early stage of preparation

NEP-EVOKED Ca^{2+} RESPONSES IN CHROMAFFIN CELLS *IN SITU*

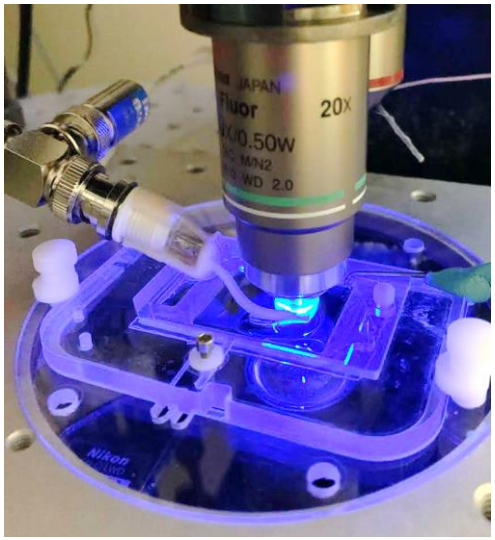
Monitor Ca^{2+} influx, the stimulus for evoking catecholamine release, in multiple chromaffin cells simultaneously in an adrenal slice with minimal photobleaching

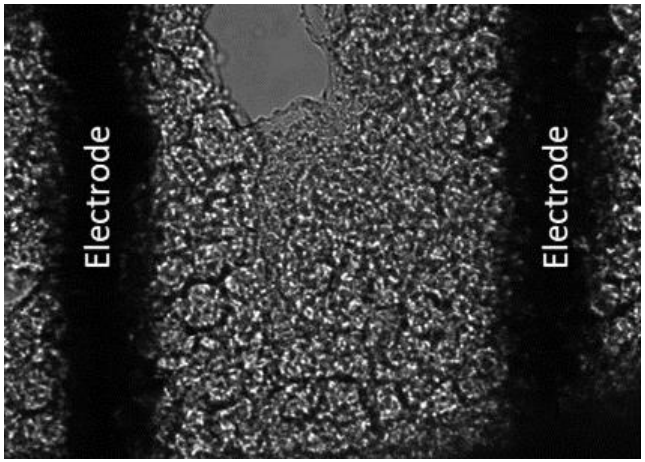
Sox10-GCaMP6f mouse

Adrenal slice



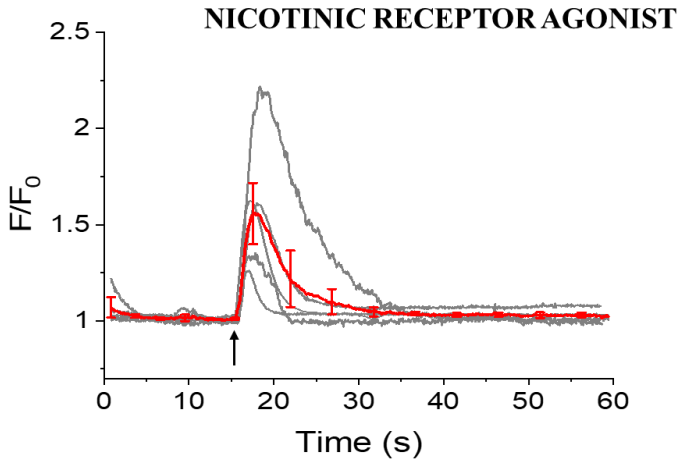
NEP delivery to tissue slice

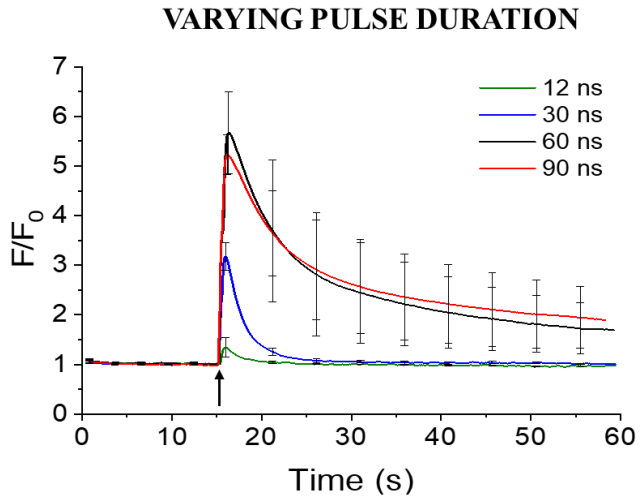


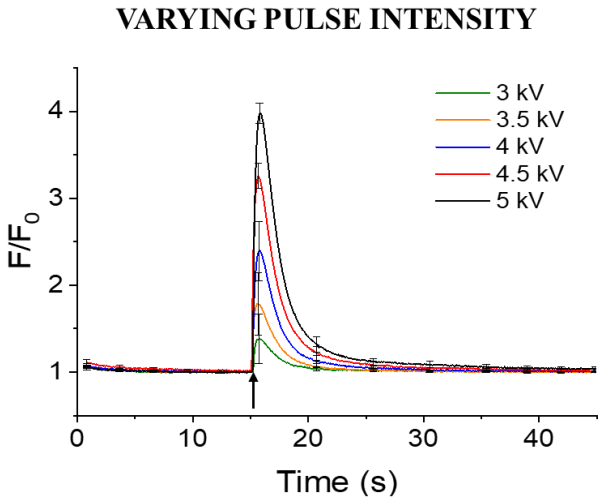


NEP-EVOKED Ca^{2+} RESPONSES IN CHROMAFFIN CELLS *IN SITU*

Pulse duration, not pulse intensity, is important for determining the duration of the Ca^{2+} response

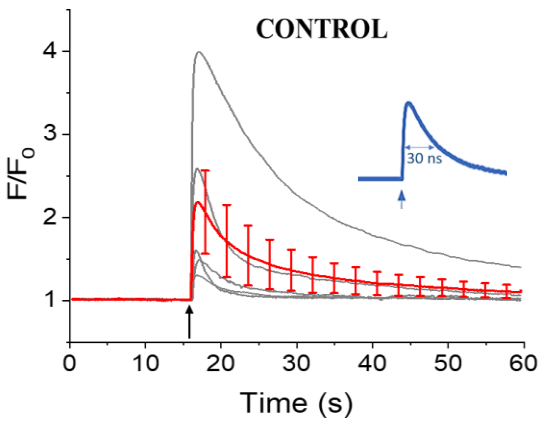


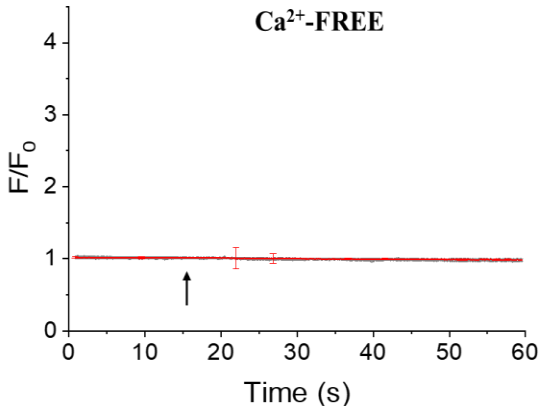


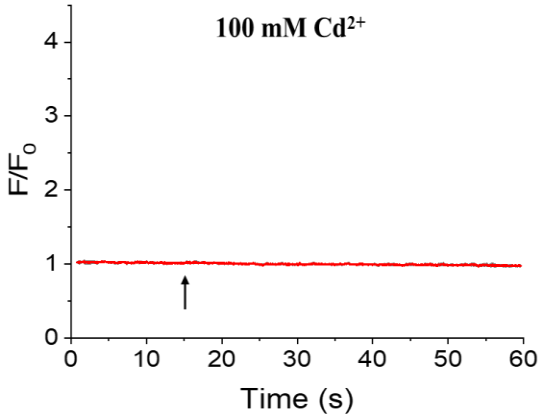


NEP-EVOKED Ca^{2+} RESPONSES IN CHROMAFFIN CELLS *IN SITU*

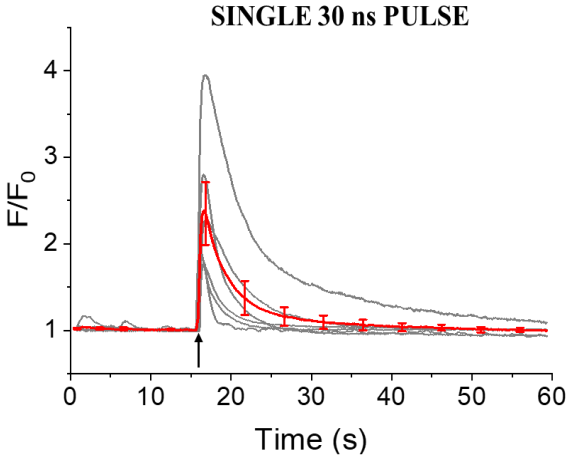
The rise in Ca^{2+} is due to Ca^{2+} influx via voltage-gated Ca^{2+} channels

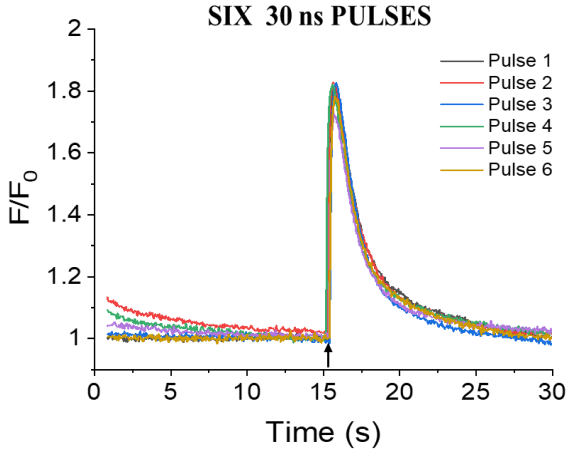






Ca^{2+} influx can be repeatedly stimulated to a similar degree by multiple pulses

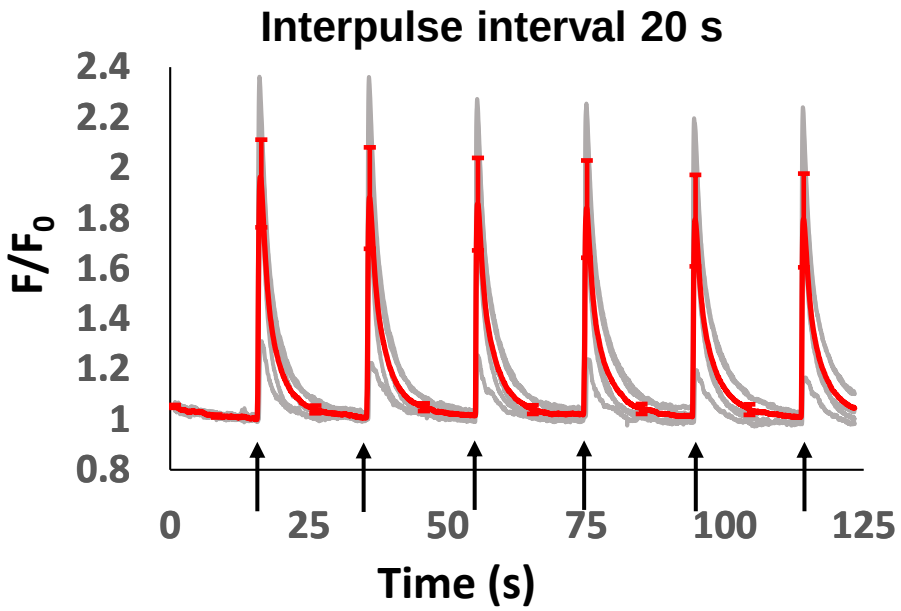


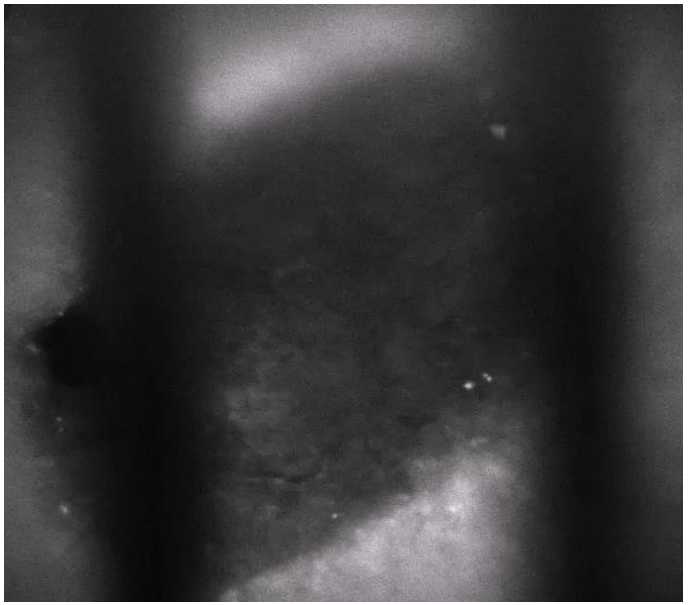


A pulse delivered once every minute

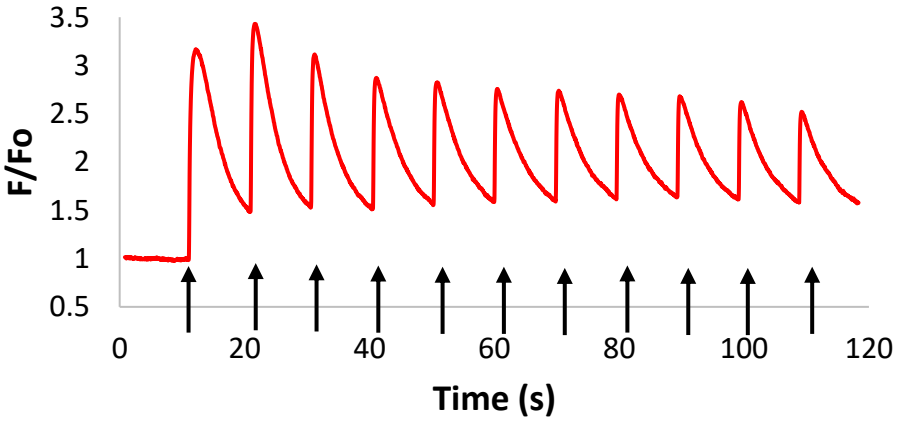
NEP-EVOKED Ca^{2+} RESPONSES IN CHROMAFFIN CELLS *IN SITU*

Shortest interpulse interval to achieve transient Ca^{2+} responses to a pulse train is 20-30 s



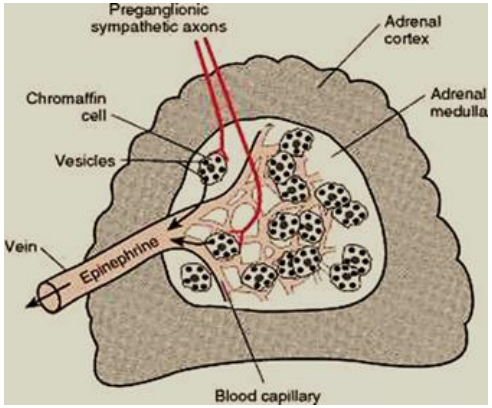


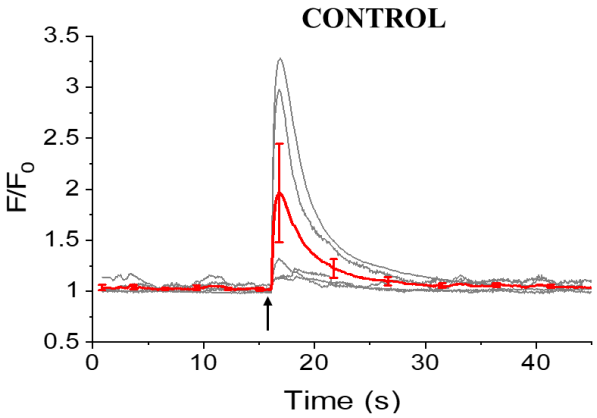
Interpulse interval now 10 s

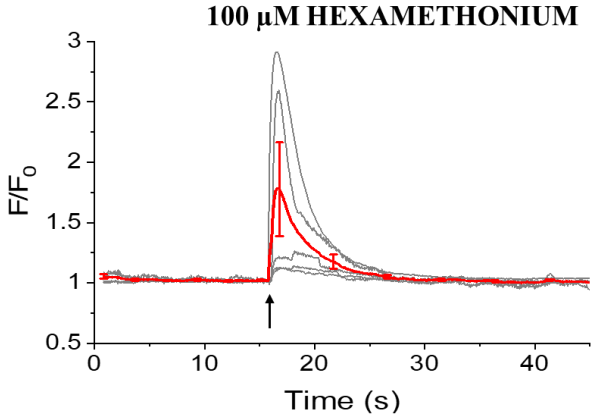


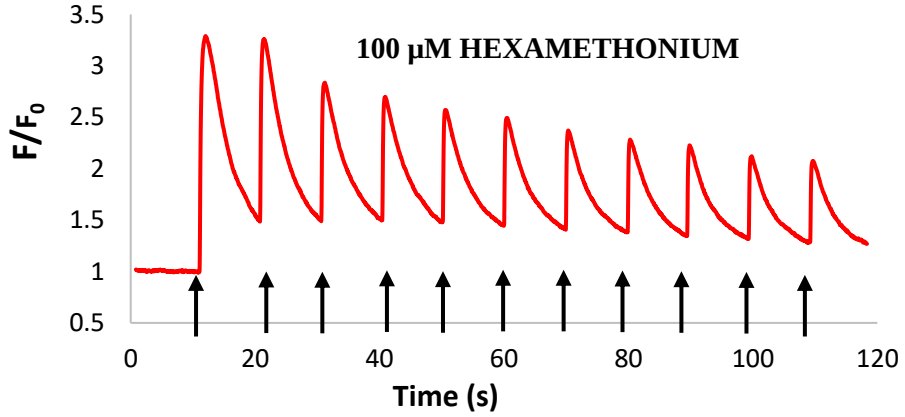
NEP-EVOKED Ca^{2+} RESPONSES IN CHROMAFFIN CELLS *IN SITU*

Due to the DIRECT activation of chromaffin cells and not due to the NEP triggering the release of acetylcholine from splanchnic nerve terminals innervating the cells – SPECIFICITY





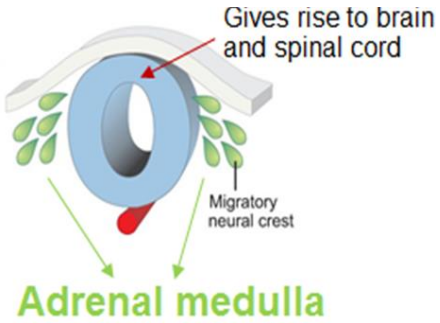




What about other "excitable" elements in the tissue?

ADRENAL MEDULLARY SATELLITE GLIAL CELLS

NEURAL CREST



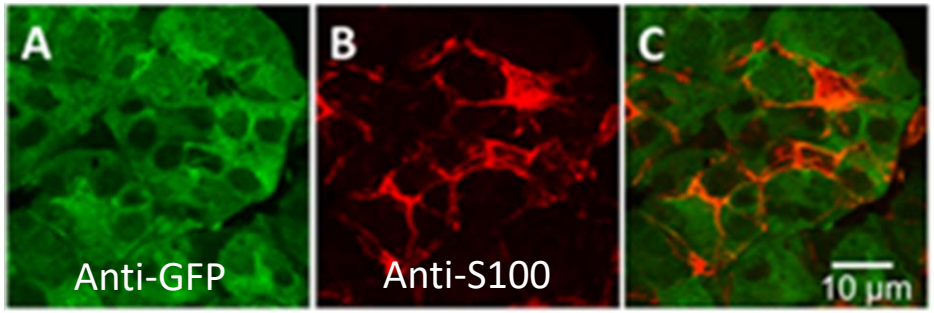
Sox10-GCaMP6f mouse

Chromaffin cells
Satellite glial cells

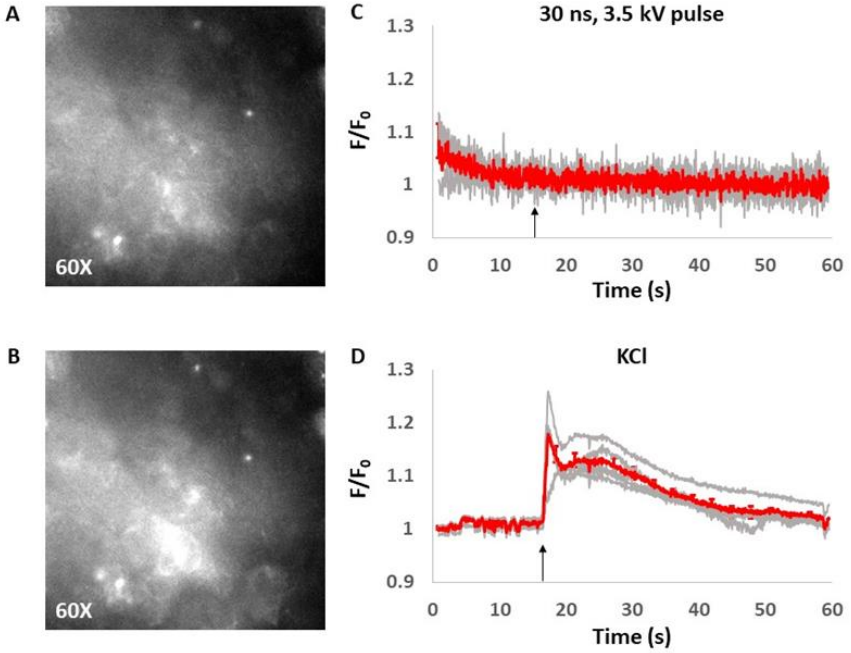
Kir4.1-GCaMP6f mouse

Satellite glial cells

SOX10-GCaMP6f mouse



Kir4.1-GCaMP6f mouse



Plans For Next Year

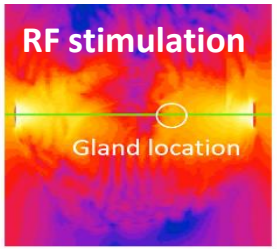
- Identify the specific voltage-gated Ca^{2+} channels involved in the stimulation.
  - So far we have only used Cd^{2+}.

- Determine the **specificity** of responses of chromaffin cells *in situ* to NEP versus conventional electric stimulation (micro- and milli-second duration electric pulses).
  - Published studies of others report that stimulating chromaffin cells *in situ* with conventional pulses show that splanchnic nerve terminals get activated more easily than chromaffin cells.

- Complete a manuscript that is in preparation.

ULTIMATE RESEARCH GOAL: NON-INVASIVE MODULATION OF CATECHOLAMINE RELEASE

Work toward strategies that minimize distortion/attenuation of the stimulus when delivered to the tissue target



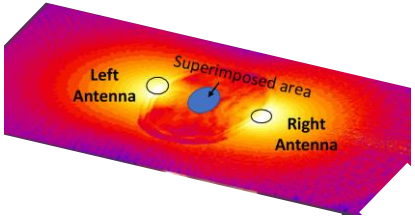
- NEP signal gets distorted inside the body (simulations)
- Bet way to get an NEP to a target site - use radiofrequency (RF) pulses that penetrate deeper into the body

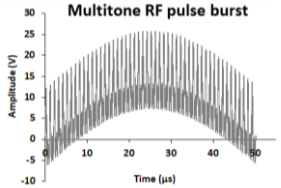
For this purpose different types of RF pulses are being investigated:

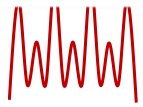
- One is a multitone RF pulse in which stimulation of a target site occurs when the two signals (low-frequency + high-frequency) combine.

Multitone RF pulses

Low-frequency pulse



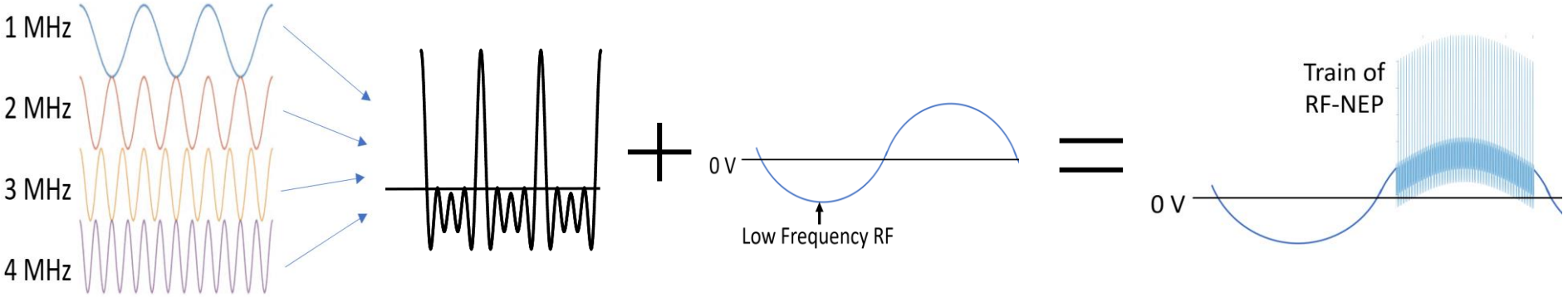




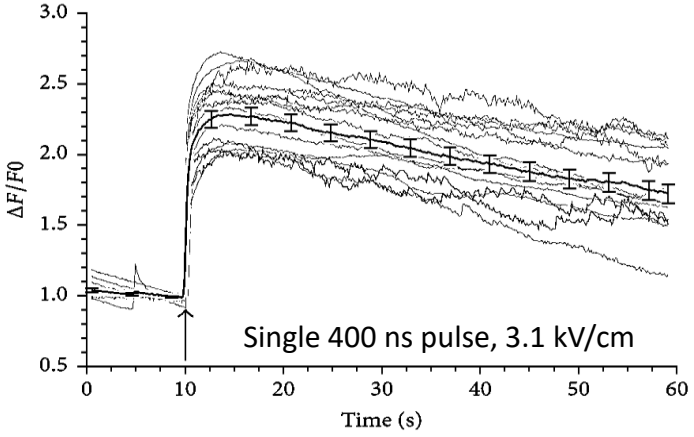
High-frequency pulse

ULTIMATE RESEARCH GOAL: NON-INVASIVE MODULATION OF CATECHOLAMINE RELEASE

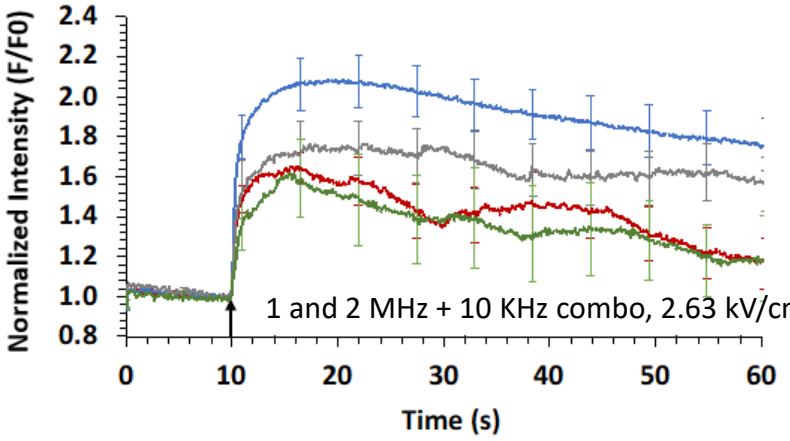
Multitone RF NEP: uses MHz compression to deliver multiple subthreshold NEPs at high repetition rates



Ca^{2+} response of chromaffin cells to multitone low-intensity RF NEP



Bagalkot et al., Biomed. Res. Int., 2018

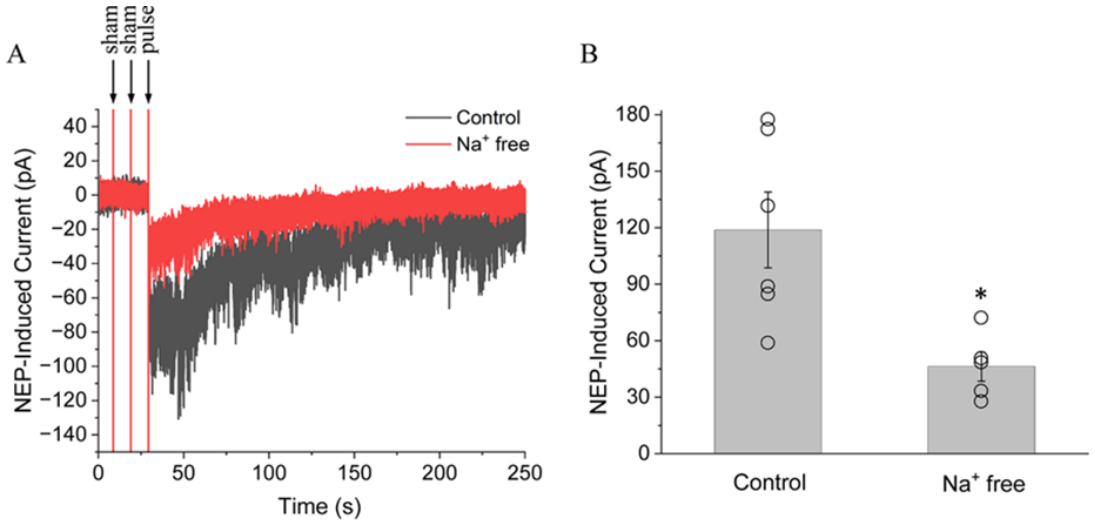


Plans For Next Year

- Determine how chromaffin cells in adrenal gland slices respond to Multitone RF NEP
  - Devise a setup for testing, including antennas for delivery
  - With a recently obtained Dragonfly Nanodimension 3D circuit printer, fabricate both rigid and flexible patch antennas or antenna arrays in arbitrary shapes, as the dimensions and shapes of the antennas are dependent on the frequency and bandwidth of the multitone RF-based signals that will be tested.
- Determine how the parameters of these stimuli must be adjusted as the distance between the pulse delivery electrode and tissue target increases, which would serve as a first proof-of-concept for remote delivery.

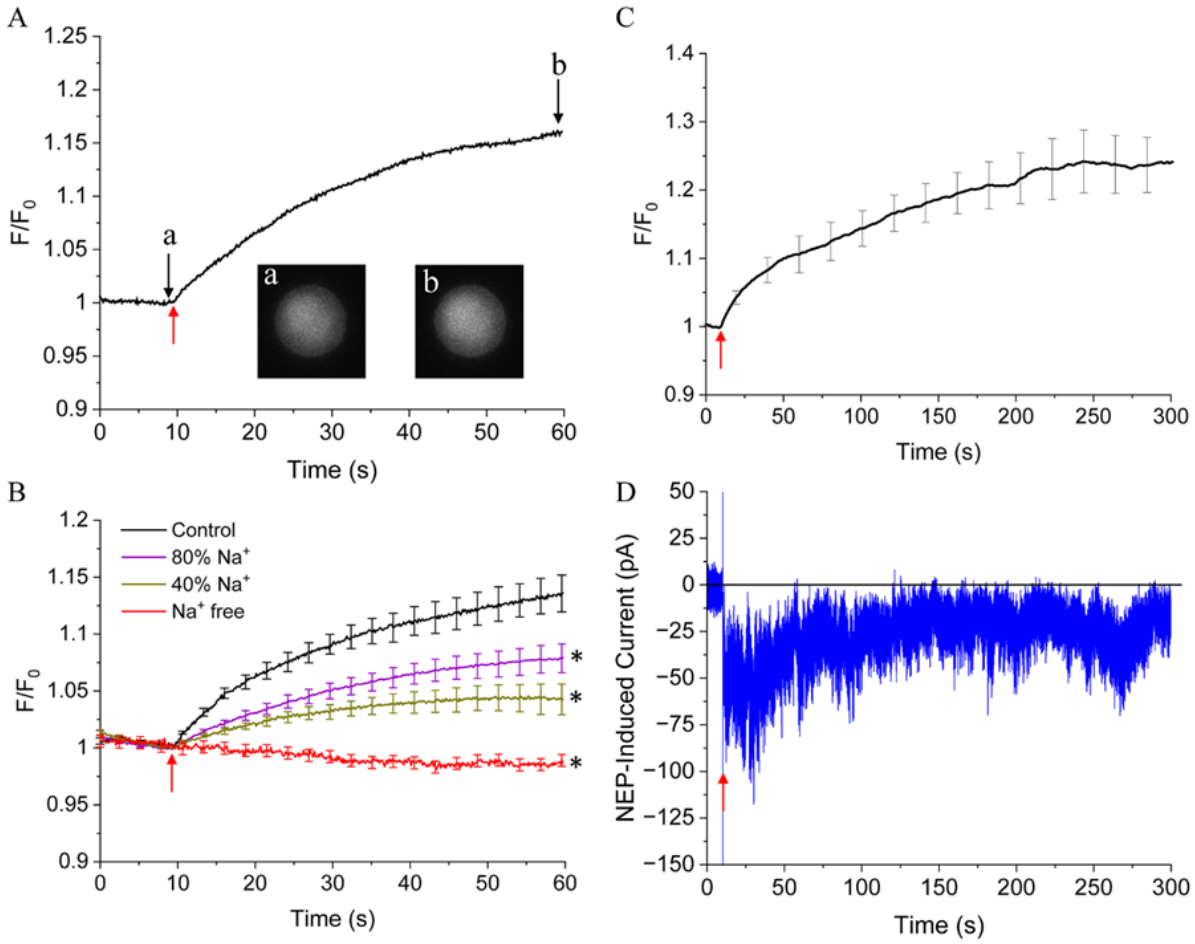
IDENTIFYING AND ELUCIDATING NOVEL EFFECTS OF NEP ON CHROMAFFIN CELLS

- Whole-cell patch clamp has consistently shown that a 5 ns, 5 MV/m pulse evokes an inward current carried mainly by Na^+ (Yoon et al., 2016; Yang et al., 2022).
- The inward current has characteristics consistent with those of non-selective cation channels, with ~75% of the current being attributed to activation of TRPC4/5 channels and NALCN (determined by the use of specific blockers of each).



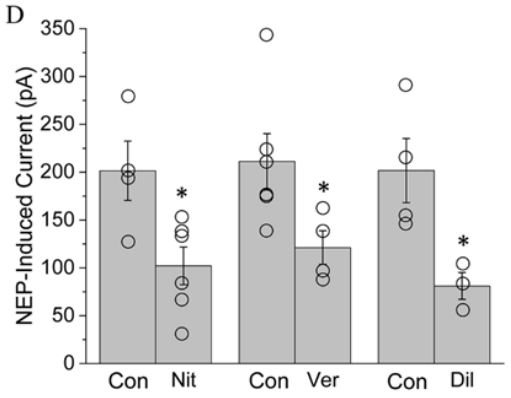
- Can Na^+ fluorescence imaging serve as a complementary approach to further investigate NEP-evoked Na^+ entry into chromaffin cells under less invasive and hence more physiological conditions?

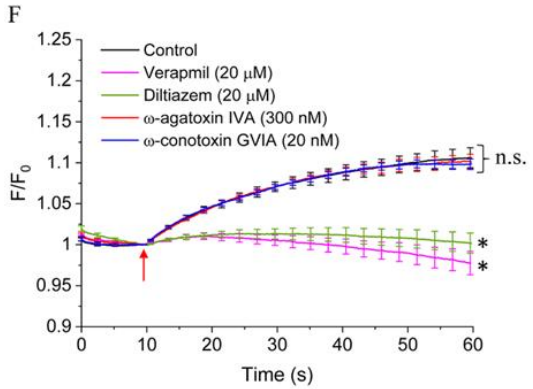
Na^+ IMAGING USING THE FLUORESCENT Na^+ INDICATOR ING-2 - DEMONSTRATES Na^+ INFLUX

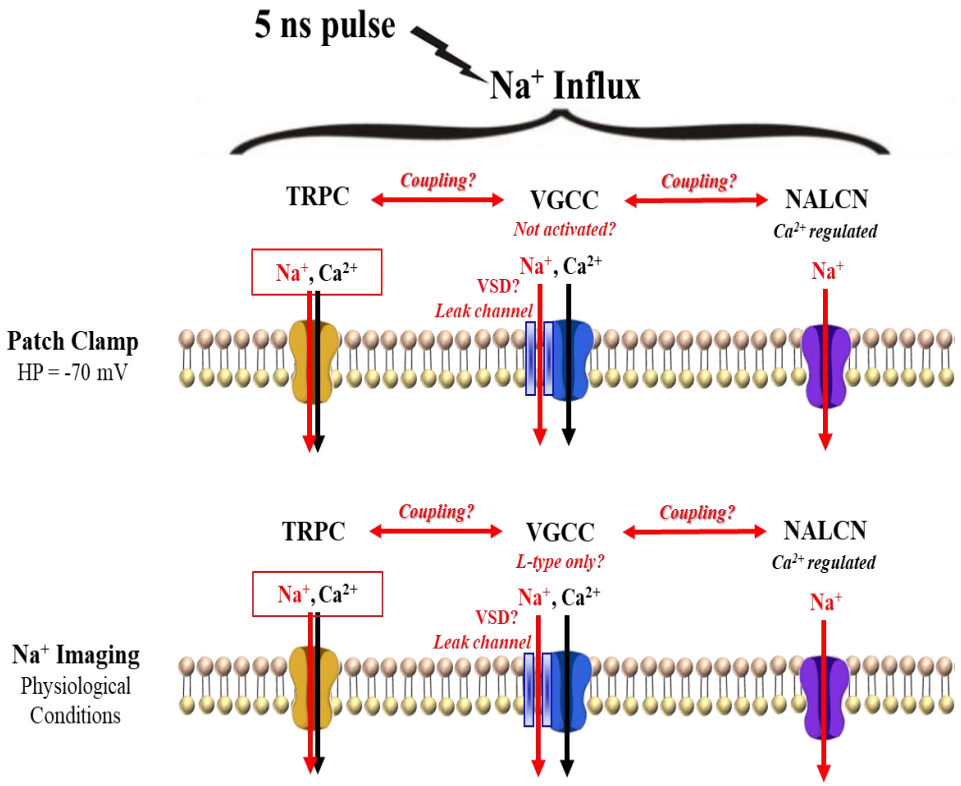


TRPC4/5 channel and NALCN inhibitors block the responses

BOTH RESPONSES ARE BLOCKED BY INHIBITORS OF L-TYPE VOLTAGE-GATED Ca^{2+} CHANNELS







Plans For Next Year

Elucidate the underlying mechanism!

ACKNOWLEDGEMENTS

Dr. Patrick Bradshaw

AFOSR Grant FA9550-14-1-0018

Research Asst Professors

Lisha Yang, Ph.D.
Nicole Procacci, Ph.D.

Undergraduate Students

Dea Lika*
Malaya Sankar
Steve Shin

York Meats, Fallon, NV

Graduate Students

Ciara Viola
Anithakrithi Balaji*
Vasilii Mansurov
Emily Bobrowsky
Jose Moreno Duran
Sung Hae Yun
Kyung Eun You

Thank you for your attention!