REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From – To)*
01-09-2019	Interim	Jan 1 2013-31 Dec 2018

4. TITLE AND SUBTITLE

Biophysics of Neuromodulation by Rapid Deposition of Energy
Annual report

5a. CONTRACT NUMBER
In-house

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
61102F

6. AUTHOR(S)

Christopher Valdez

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER
H0DK

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

711 HPW/RHDR

8. PERFORMING ORGANIZATION REPORT NUMBER

N/A

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Air Force Material Command, Air Force Research Laboratory, 711th Human Performance Wing, Human Effectiveness Directorate, Bioeffects Division, Radio Frequency Bioeffects Branch, (711 HPW/RHDR) , 4141 Petroleum Drive, JBSA Fort Sam Houston, Texas 78234-2644

10. SPONSOR/MONITOR'S ACRONYM(S)
711 HPW/RHD_
TSRL-2019-0156

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
AFRL-RH-FS-OP-2019-0003

12. DISTRIBUTION / AVAILABILITY STATEMENT

Distribution A. Approved for Public Release, TSRL-2019-0156, May 2019..

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

Neural, in vivo, opyohrnryivd

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			c. Valdez
Unclassified	Unclassified	Unclassified	SAR	49	**19b. TELEPHONE NUMBER** (include area code) N/A

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Biophysics of Neuromodulation by Rapid Deposition of Energy

Chris Valdez, Ph.D, DR-II
711 HPW/RHDR
6 May 2019

Air Force Research Laboratory

Airman Systems Directorate

- Human element of warfighter capability
- Integrate biological and engineering technologies to optimize and protect the Airman's capabilities

Bioeffects Divisions (RHD)

- Protect against and exploit the bioeffects of battlefield environment stressors

Radio Frequency Bioeffects (RHDR)

- Understanding the effects of directed energy weapons for more than 50 years

Outline

1) Introduction
2) IR Dosimetry
3) IR Bioeffects
    1) Past and Present
4) Future Direction

Central Question

What happens when directed energy interacts with biological material?

We are Interested in This Question Because…….

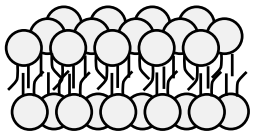
Input

- Different energy platforms
  - Infrared laser
  - Radio frequency
- Energy delivery parameters
  - High vs. low power
  - Penetration depth

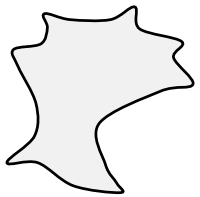
Output

- Damage
- Modulation
- Hardening
- Spectrum of biological effects
  - Chronic vs. transient

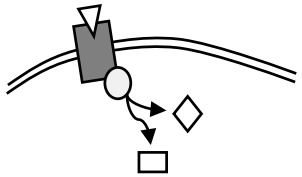
membrane order



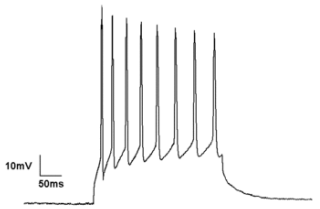
microtubules and F-actin



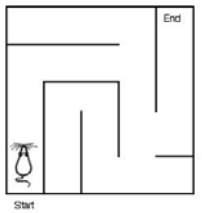
2nd messenger systems



electrophysiology



behavior



LABORATORY RESEARCH INITIATION REQUEST (LRIR) - RENEWAL

Executive Summary

Title: Biophysics of Neuromodulation by Rapid Deposition of Energy

Efforts at TSRL originated from an AFOSR proposal by Dr. Hope Beier
March 2018

Rapid Deposition of Energy

RF

Barrier to entry

- Laboratory transmitters are of a kind, N = 1

InFr

Reduced barrier to entry

- Laboratory-grade laser fibers are readily available
- May act as a *surrogate* for RF rapid deposition of thermal energy

Neural Modulation with IR Laser Pulses

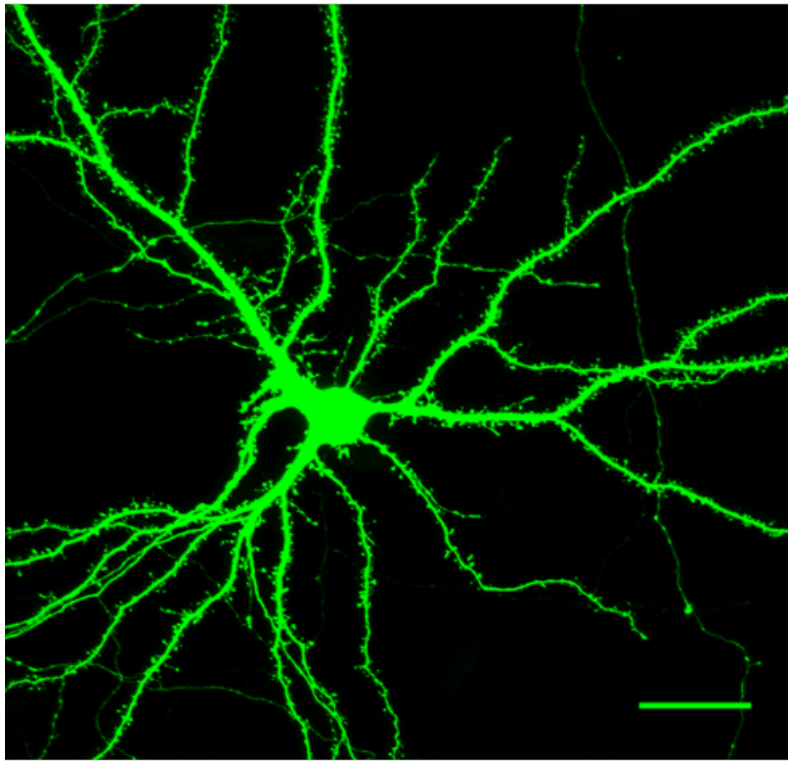
- Pulsed IR laser light used to modulate action potentials in neurons in response to a transient targeted deposition of energy.
- As opposed to other neural stimulation approaches (electrical, optogenetics), IR stimulation is:
  - Label-free
  - Contact-free
  - Artifact-free
  - Non-damaging

504 OPTICS LETTERS / Vol. 30, No. 5 / March 1, 2005

Optical stimulation of neural tissue *in vivo*

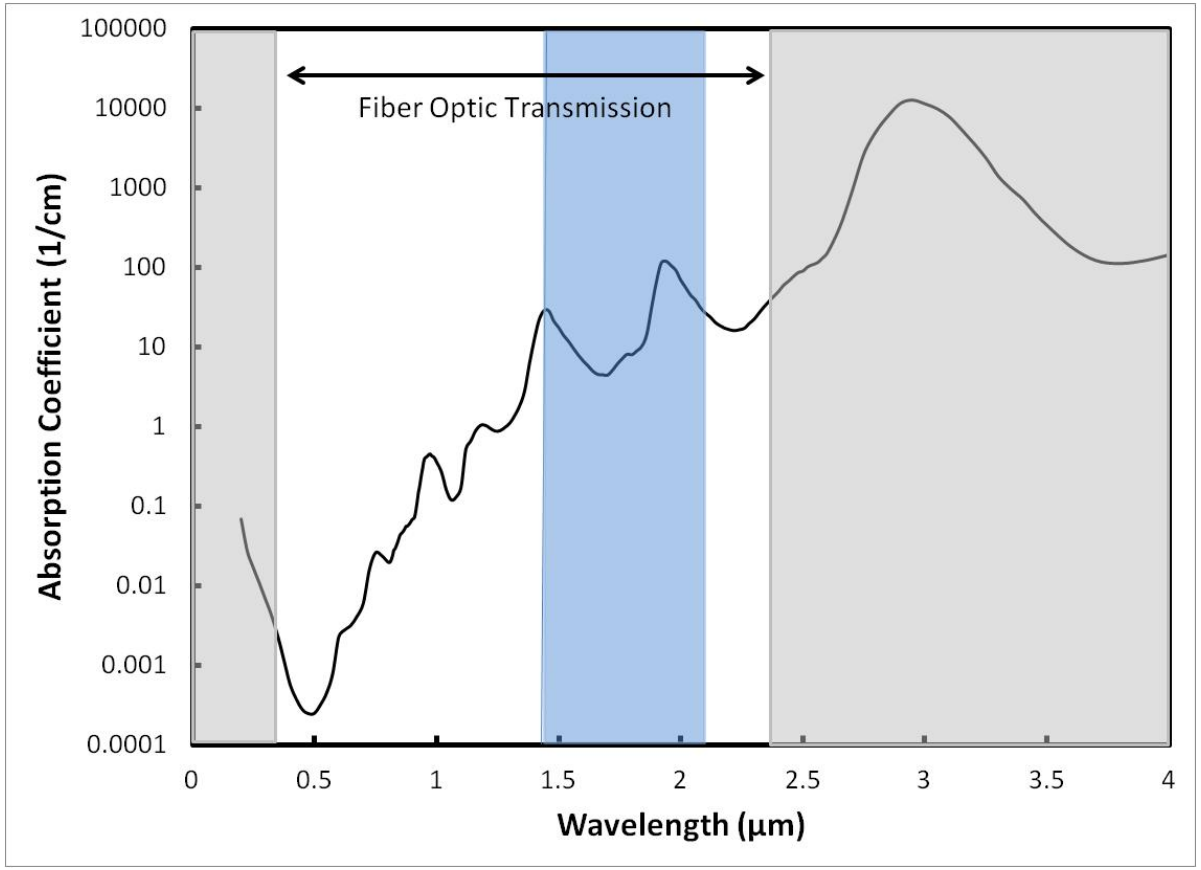
Jonathon Wells, Chris Kao, Karthik Mariappan, Jeffrey Albea, E. Duco Jansen, Peter Konrad, and Anita Mahadevan-Jansen

Departments of Biomedical Engineering and Neurosurgery, Vanderbilt University, 2201 West End Avenue, Nashville, Tennessee 37235



Effect Originates from High Absorption by Water

- IR energy highly absorbed by water
- Absorption of IR generates a fast thermal gradient

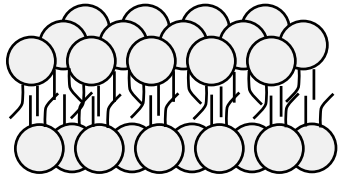


Hale and Querry, Appl. Opt 12:555 (1973)

Liljemalm, T. *Lasers Surg Med,* Jul 7 2013.

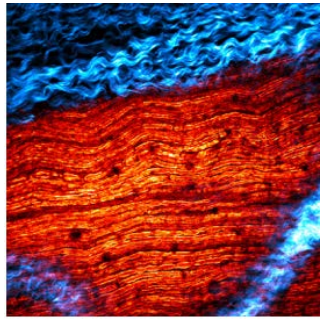
IR Impact on Biology

Lipid membrane dynamics
Photothermal mechanism



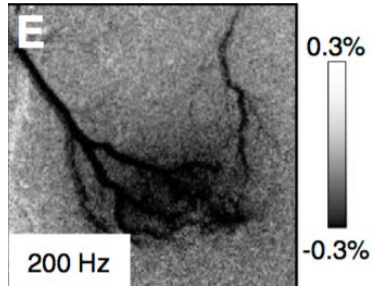
Moen, Biophy J, 2014

Peripheral nerve stimulation
refined spatial resolution compared to electrical stimulation



Wells, P. *J Neurosci Methods,* 2007
Tozburun, C. D. *Urology,* 2013.

Central nervous stimulation
Elicit intrinsic response in somatosensory cortex



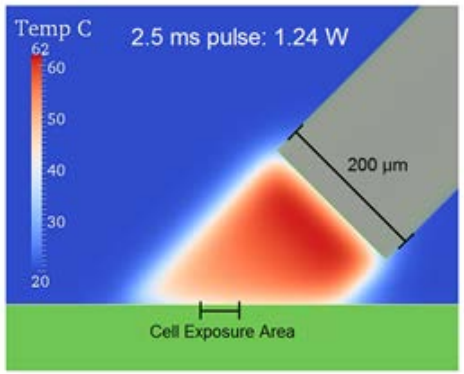
Wells, C. *Optics Lett,* 2005
Wells, *J Biomed Opt*, 2005
Cayce, NeuroImage, 2011

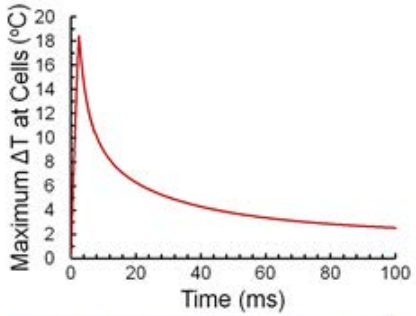
What biophysical properties are induced by Infrared exposure?

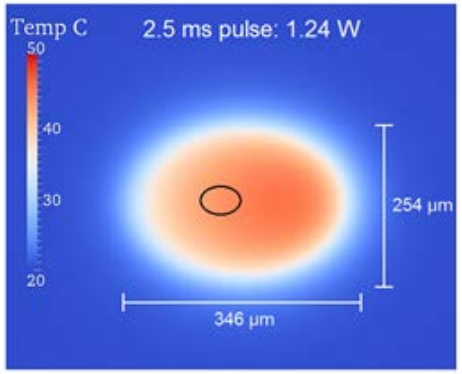
Thermal Gradient

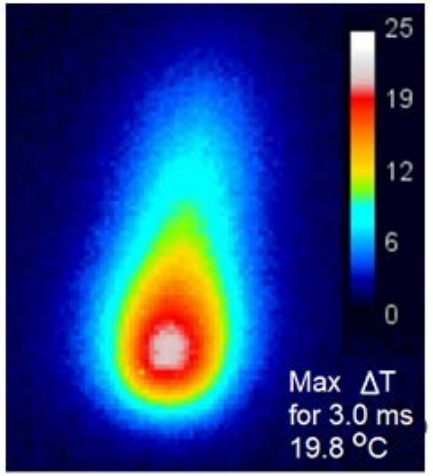
ms IR Laser Pulses :
$\Delta T \sim 7 \times 10^3$ °C/s

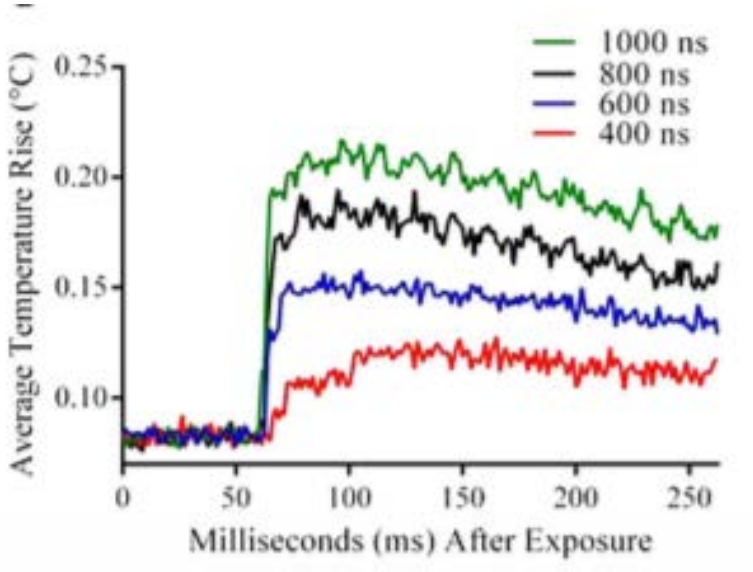
ns - µs Electrical Pulses:
$\Delta T > 2 \times 10^5$ °C/s









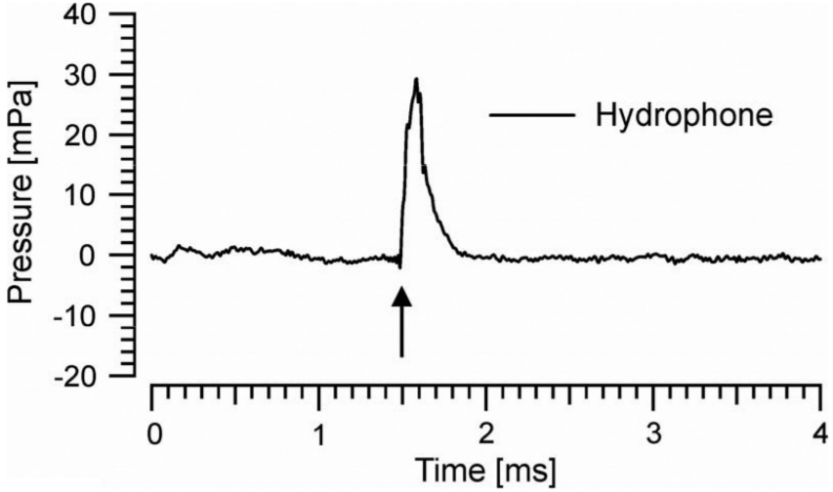


FLIR camera at 800 fps

Roth CC et al, Scientific Reports 2015 5:15063

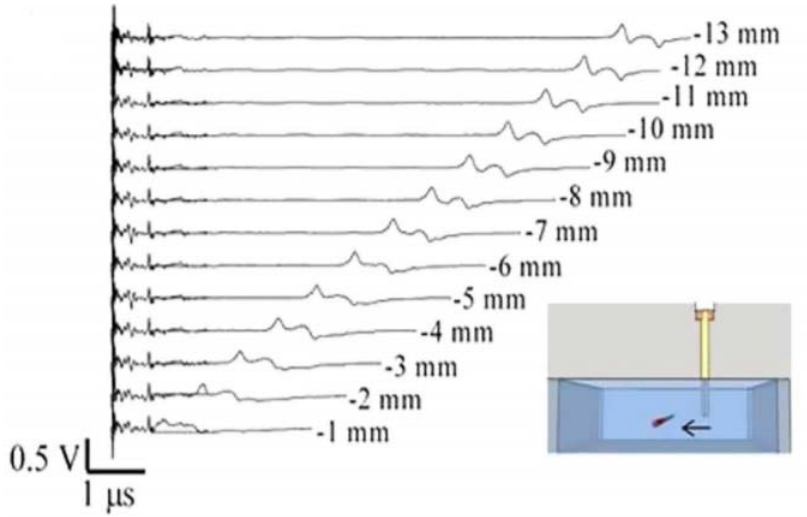
Pressure Transient Generation

100 µs, .35 J/cm^2 IR Laser Pulses: Cochlear stimulation



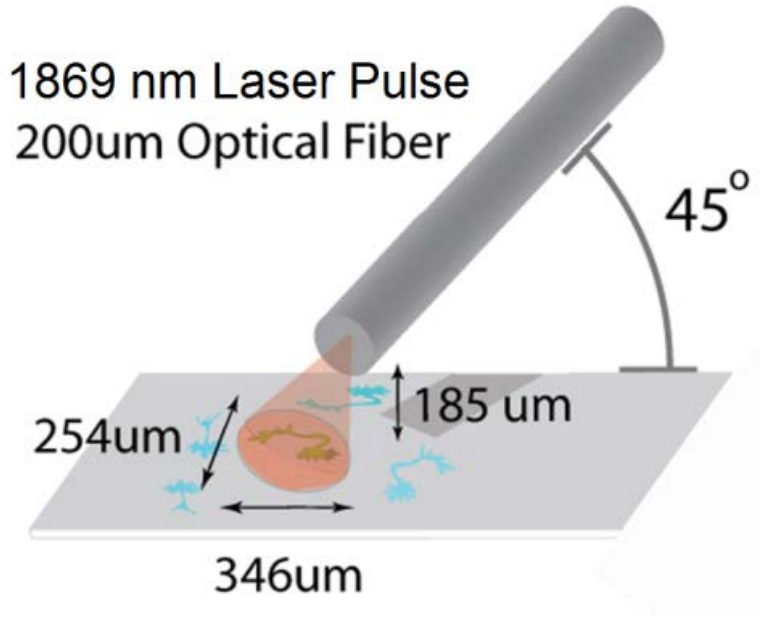
Teudt et al, IEEE Trans Biomed Eng. 2011; 58(6): 1648–1655

600 ns, 13.1 kV/cm Electrical Pulses: ~13 kPa

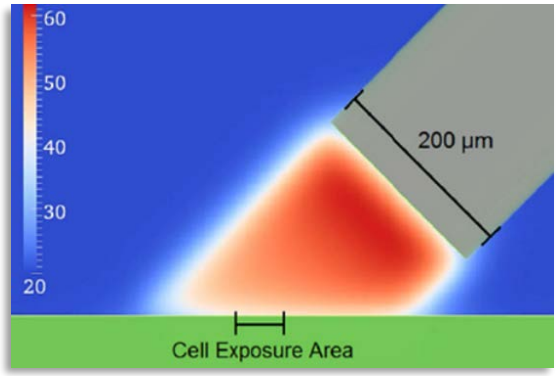


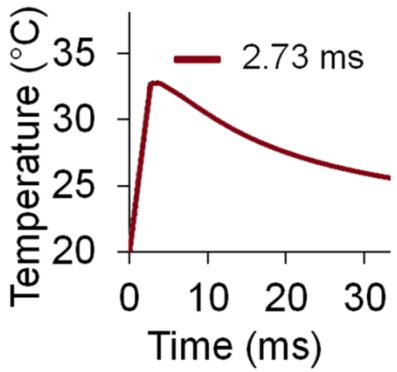
Roth CC et al, Scientific Reports 2015 5:15063

Infrared Laser Exposure



Pulse Width (ms)	Energy (mJ)	Radiant Exposure at Cells (J/cm^2)	ΔT (°C)
0.70	0.86	0.13	3.02
1.37	1.68	0.25	5.91
2.15	2.64	0.39	9.28
2.73	3.35	0.49	11.78
3.58	4.39	0.64	15.45
4.10	5.03	0.74	17.70
5.01	6.14	0.90	21.62
5.47	6.71	0.98	23.61
5.73	7.03	1.03	24.73





- 1- 3.5 ms single pulse exposure
- 1869 nm
- 1.1 – 4.5 mJ / pulse at fiber tip
- Delivered to cells by 200 µm fiber positioned near cells by micromanipulator

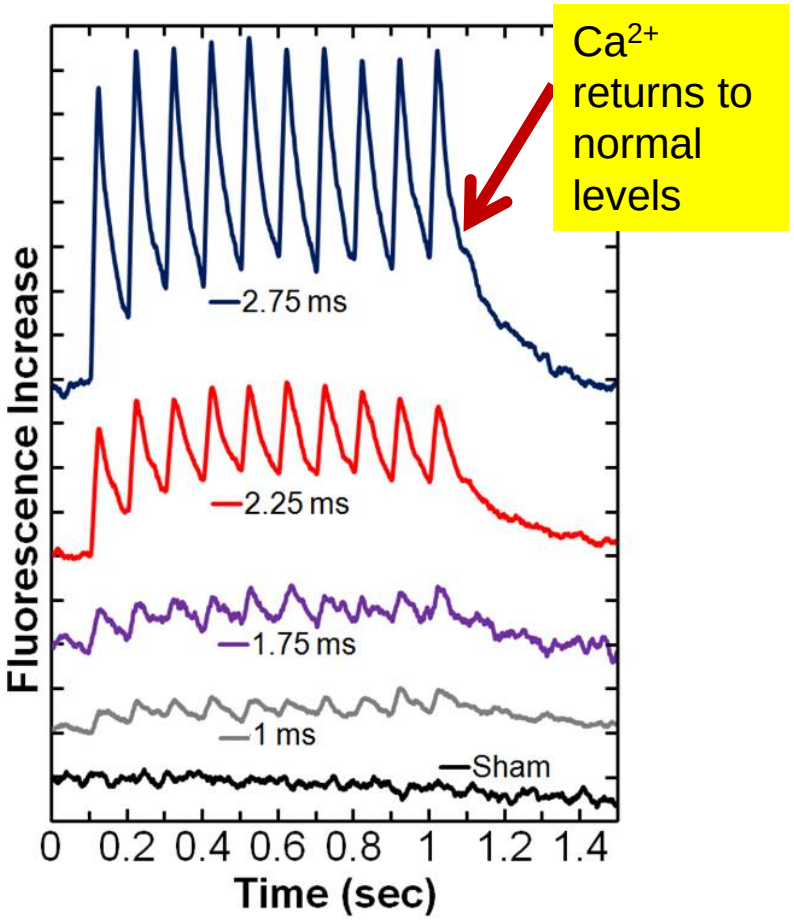
Results

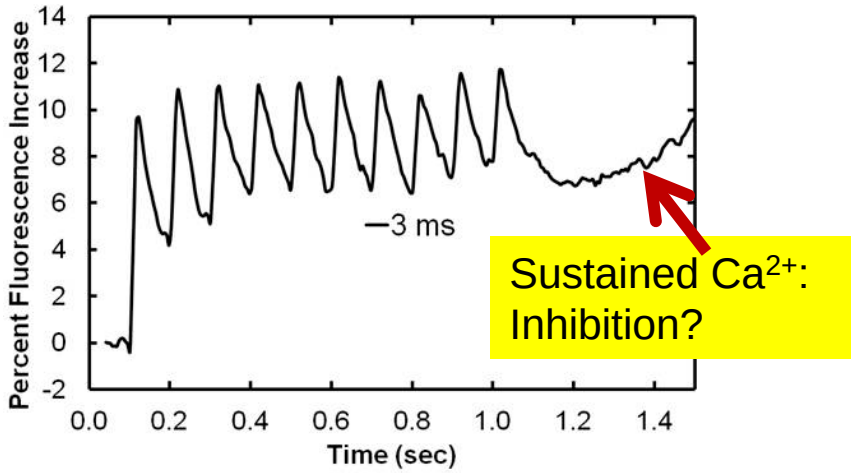
- IR delivery system can generate a 3.02 – 24.07 degree change in temperature from a single pulse (.07 – 5.73msec.)
- A single IR pulse (300msec.) can generate pressure transients
- IR-derived temperature transients exhibit an exponential decay

How does a IR exposure impact the cell?

Ca^{2+} Mobilization in Neurons- Too much or too little

Can infrared pulses trigger a calcium response in neurons? Is it repeatable and do cells recover?

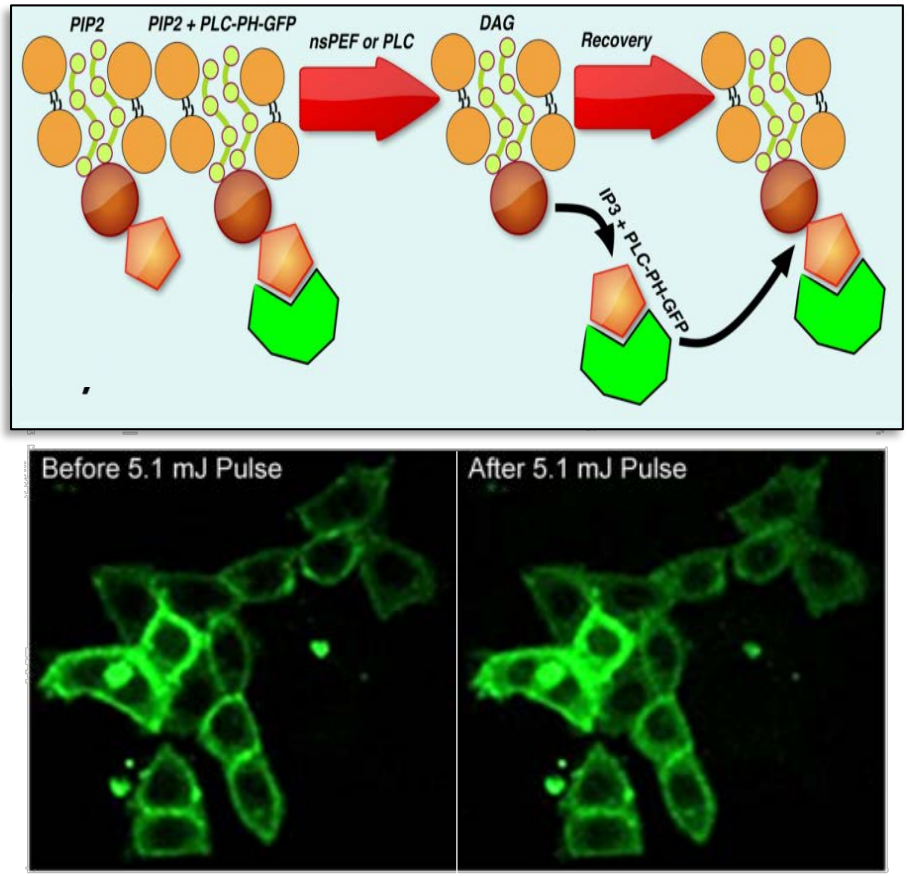


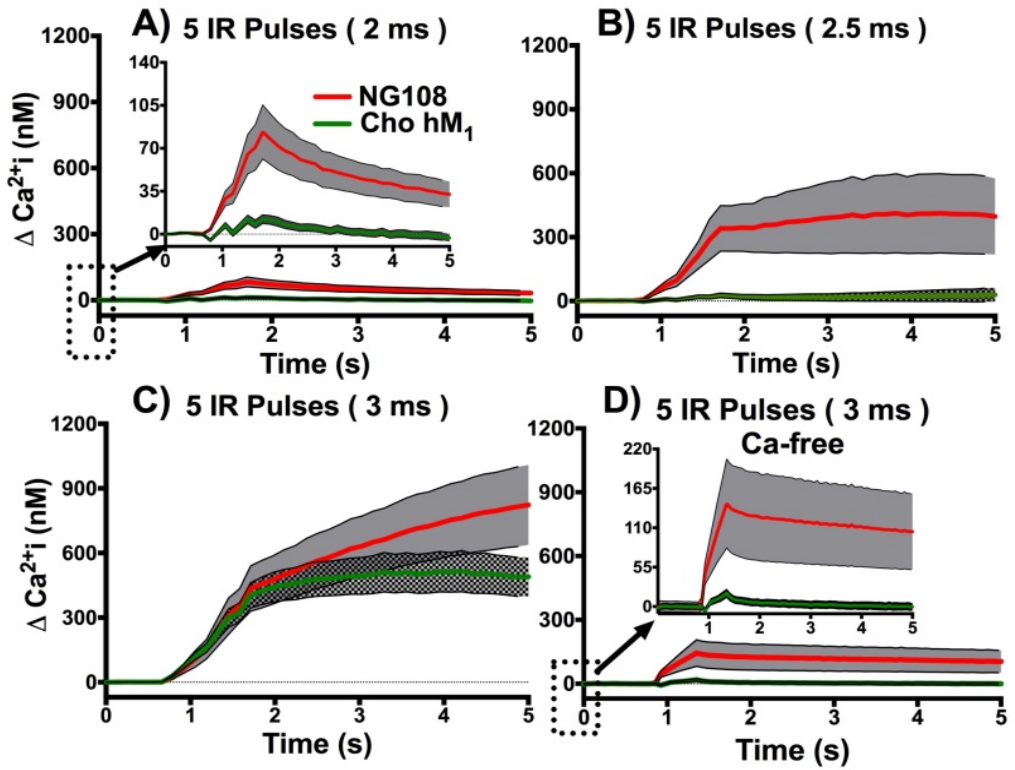


Goal: IR delivery that causes reversible and transient effects within the breadth of endogenous activity-dependent stimuli

Beier, HT et al, J Neural Engineering 2014

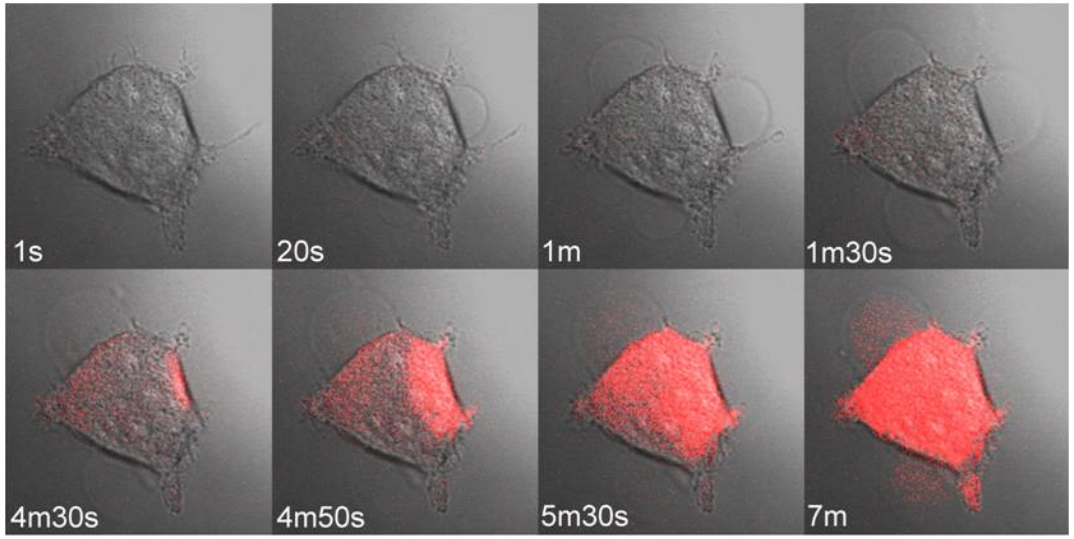
Activation of Intracellular Signaling Pathways



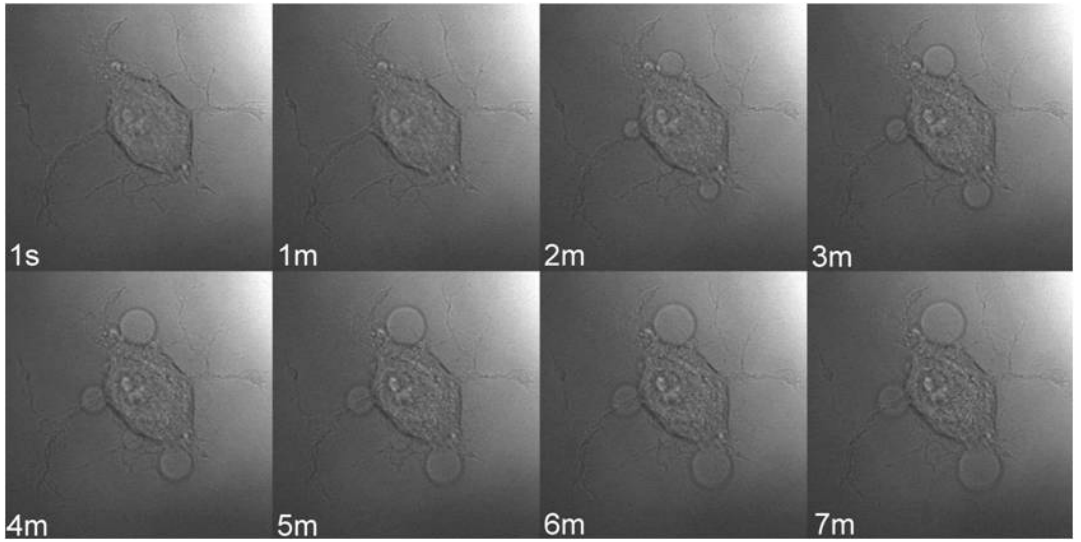


Blebbing from IR Stimulation

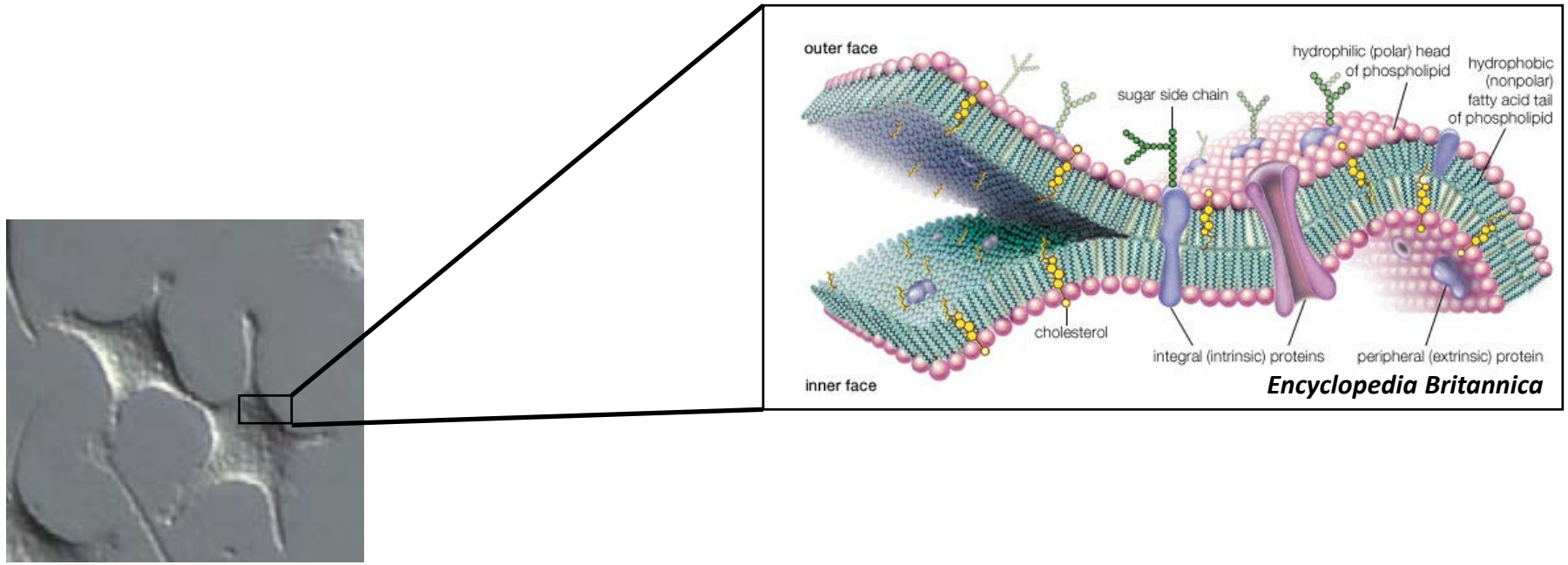
Blebbing with PI uptake
3.25 ms Exposure



Blebbing without PI uptake
2.75 ms Exposure



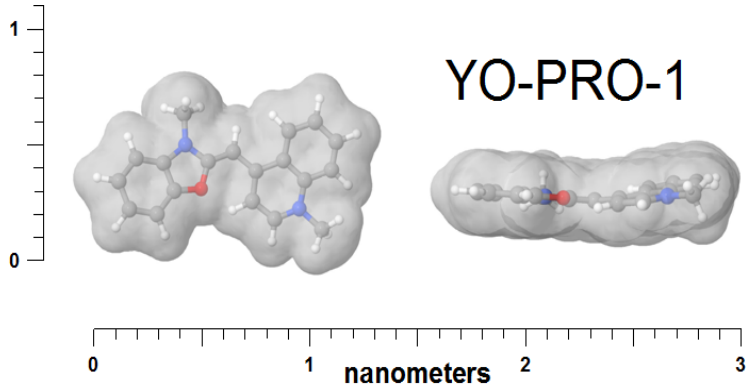
The Plasma Membrane is the Interface between IR and its Ability to Modulate Intracellular Cascades

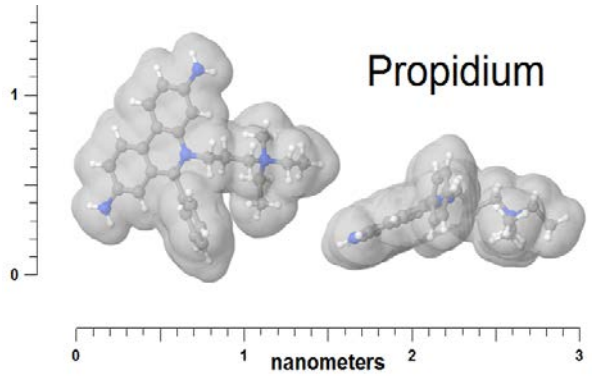


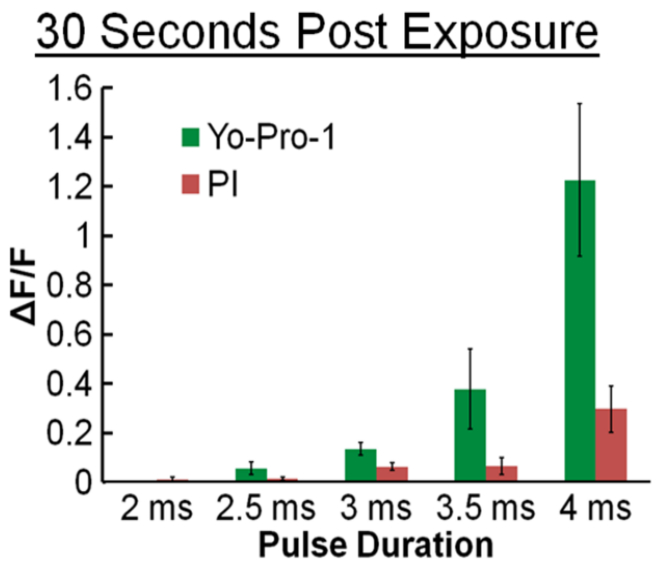
IR exposure modulates intracellular processes

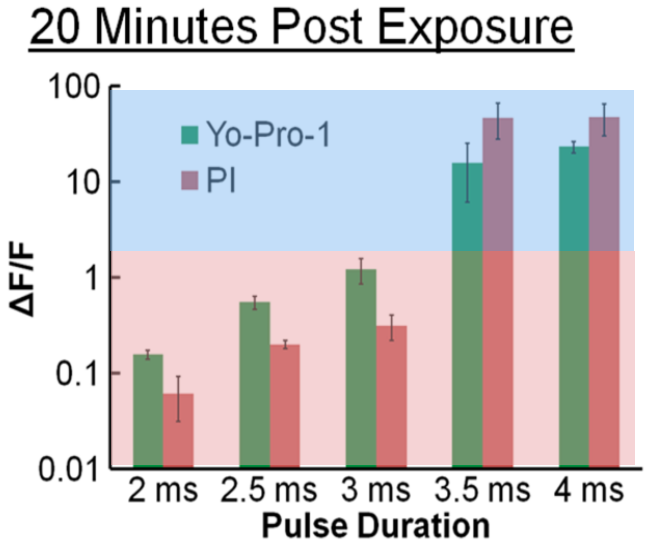
- Increase calcium influx
- Activate second messengers
- Cytoskeletal rearrangement

Membrane Permeabilization or Something Else?



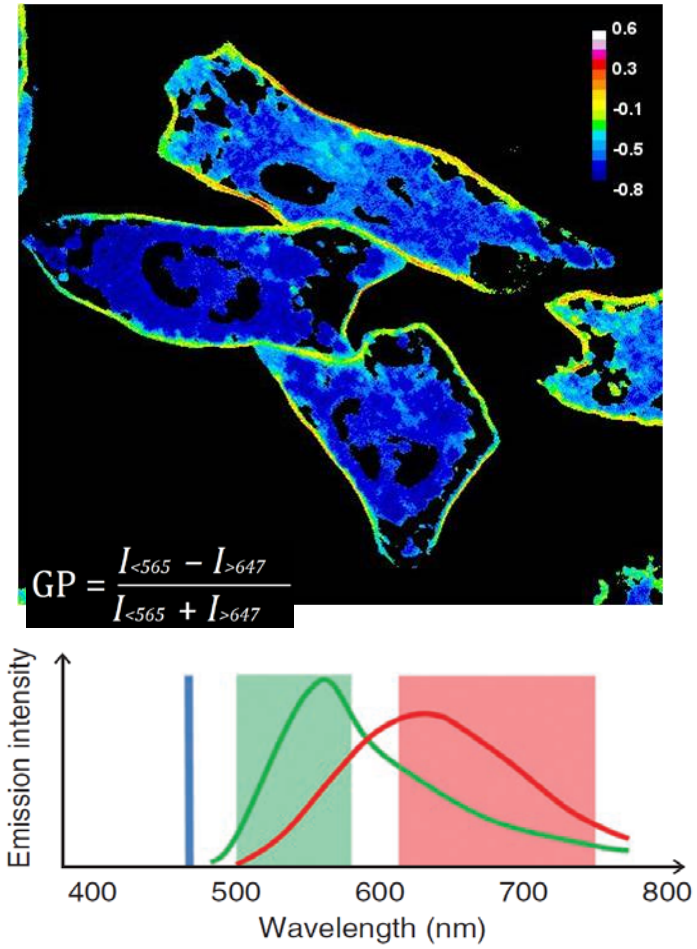






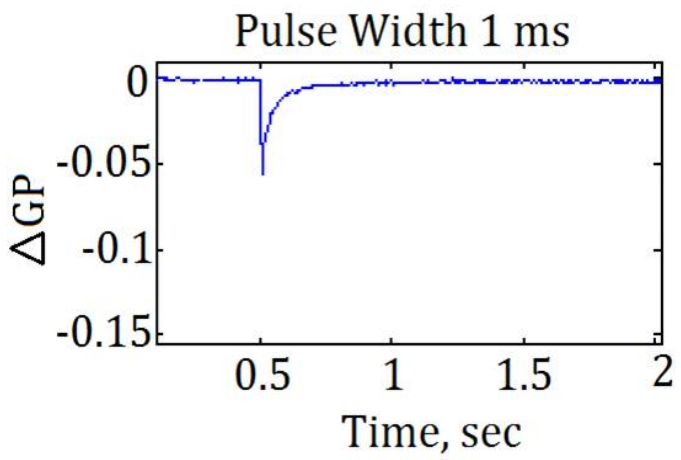
Polarity-Sensitive Fluorescent Probe

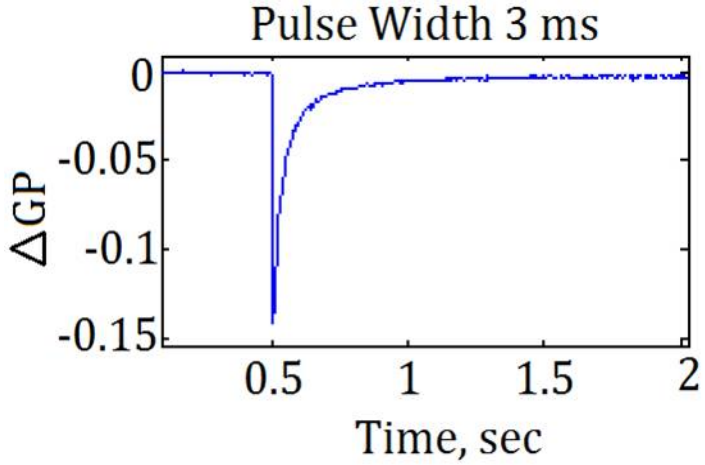
- Polarity-sensitive dye di-4-ANEPPDHQ
- Excitation maximum at 488nm (blue)
- Exhibits blue shift as membrane transitions from liquid-disordered (red) to liquid-ordered (green) state

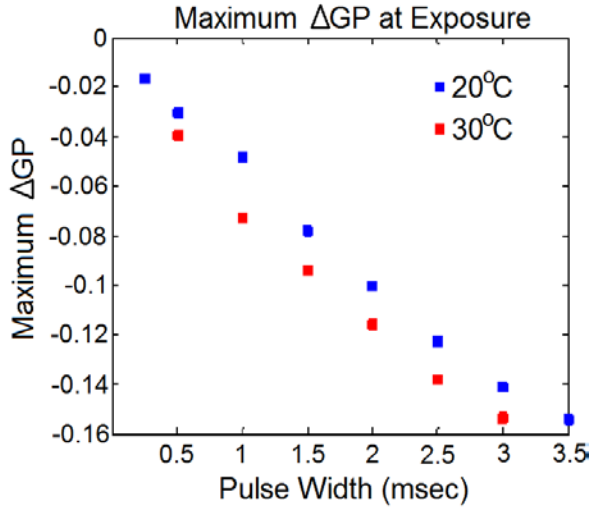


Owen, D. *et al.* Quantitative imaging of membrane lipid order in cells and organisms . *Nat. Protoc.* 7, 24-35 (2011)

Membrane Fluidity after Pulse



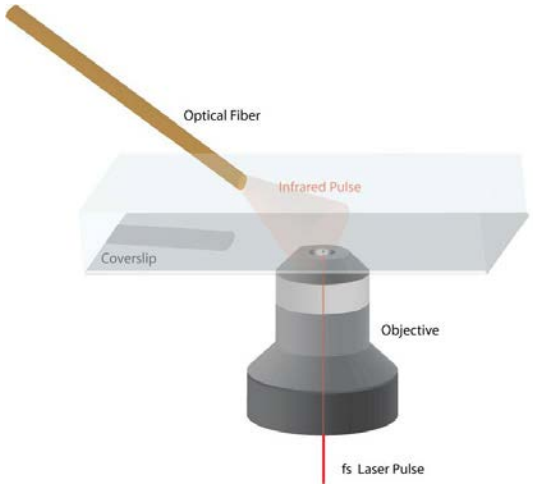


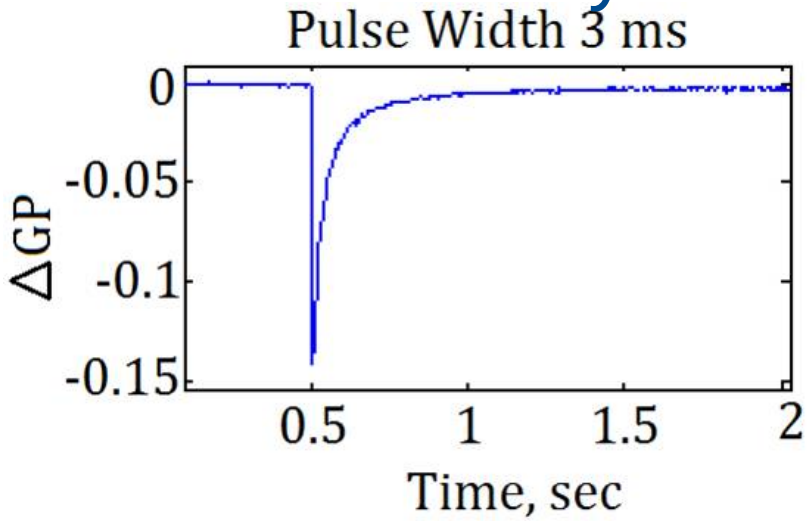


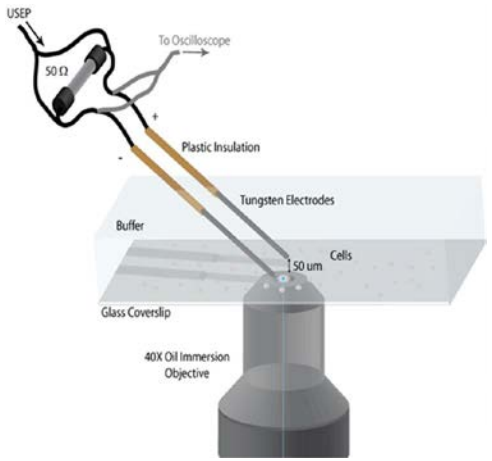
pulse width (msec)	max ΔGP	est. Δtemp
0.50	-0.0306	18
1.00	-0.0483	26
1.50	-0.0781	41
2.00	-0.1001	52

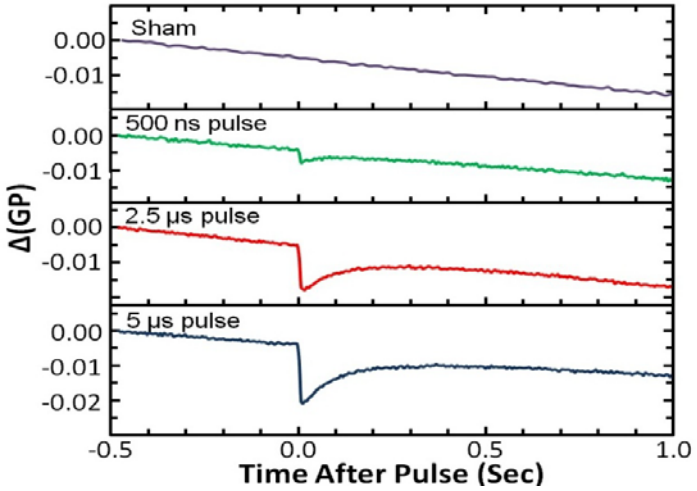
2 ms Pulse = ~15°C Rise has impact of 52°C ambient temperature rise

nsPEF and IR – Membrane Fluidity



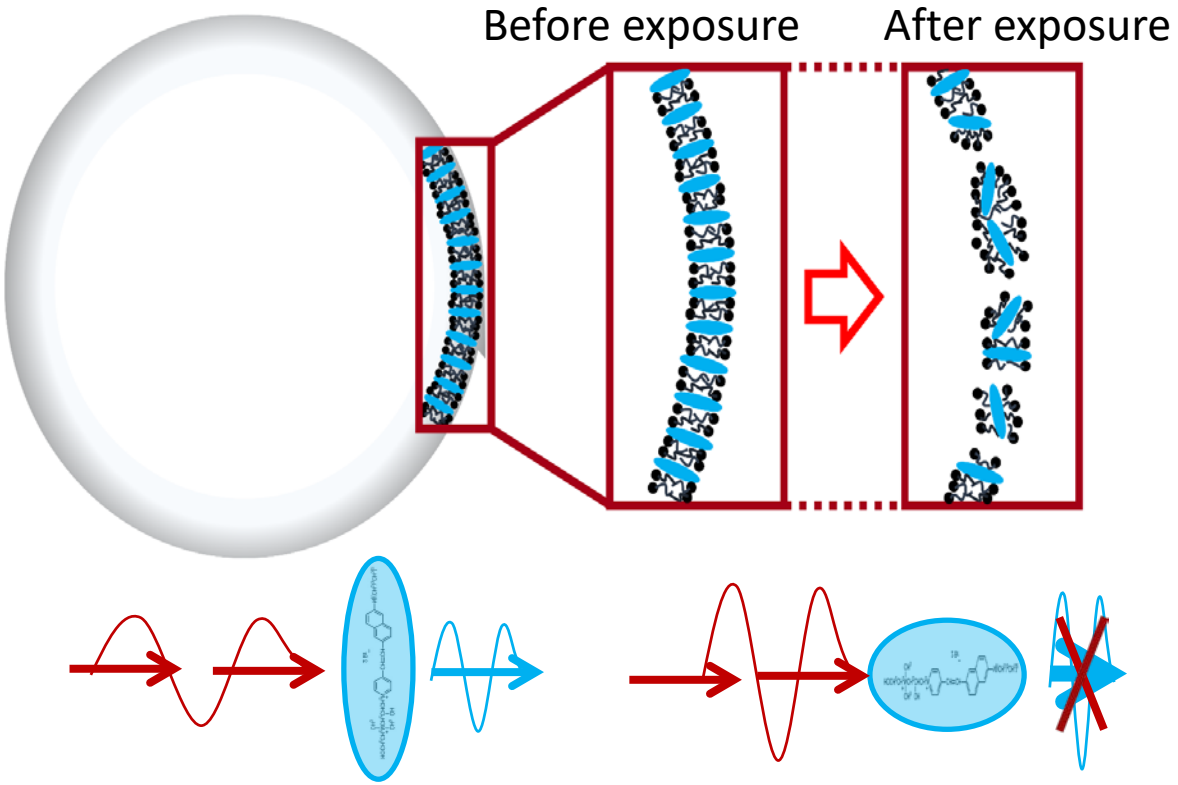






Does IR Exposure Induce Membrane Damage?

- Second harmonic generation (SHG) imaging
  - Di-4-ANEPPDHQ (DI-4) dye
    - Lipophilic dye to detect perturbations in the membrane
    - Generates nonlinear polarization induced by electronic disruption
    - Electromagnetic field of the incident laser bear
      - static electric field surrounding the probe
      - steady state molecular polarization of the probe



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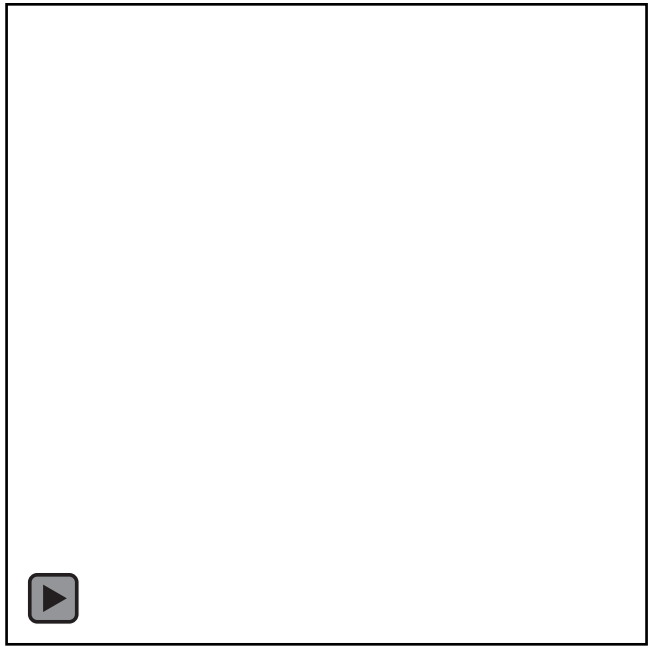
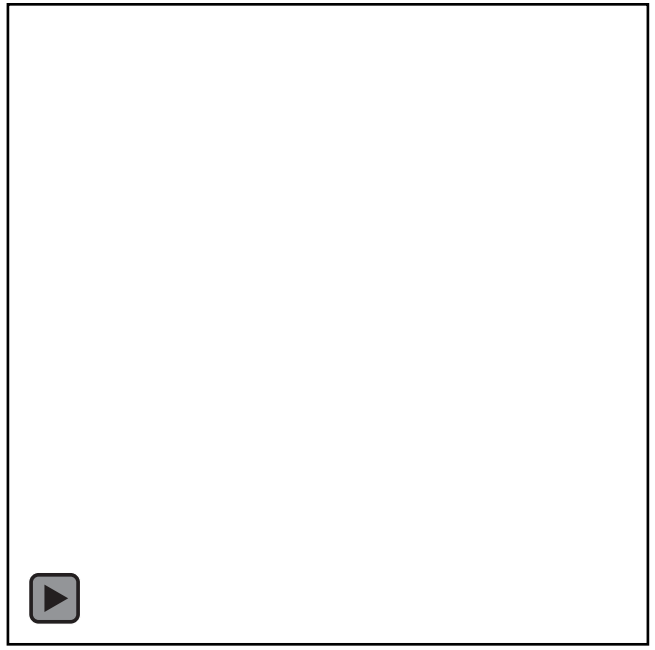
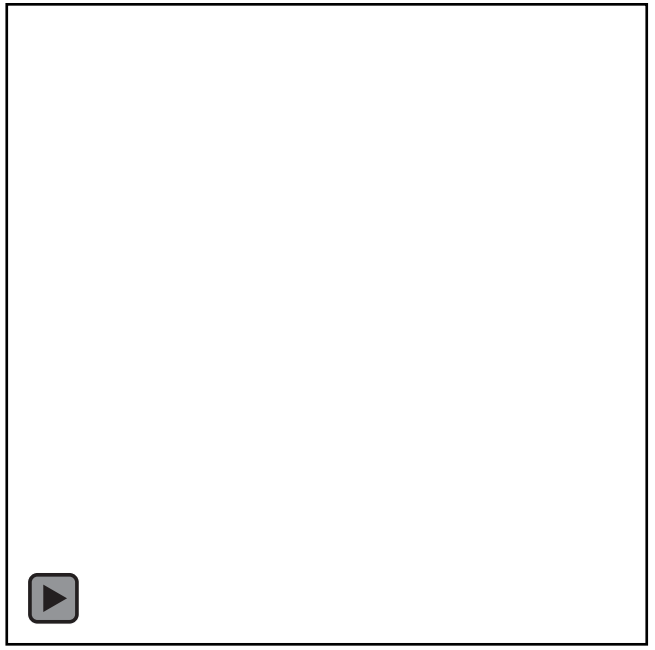
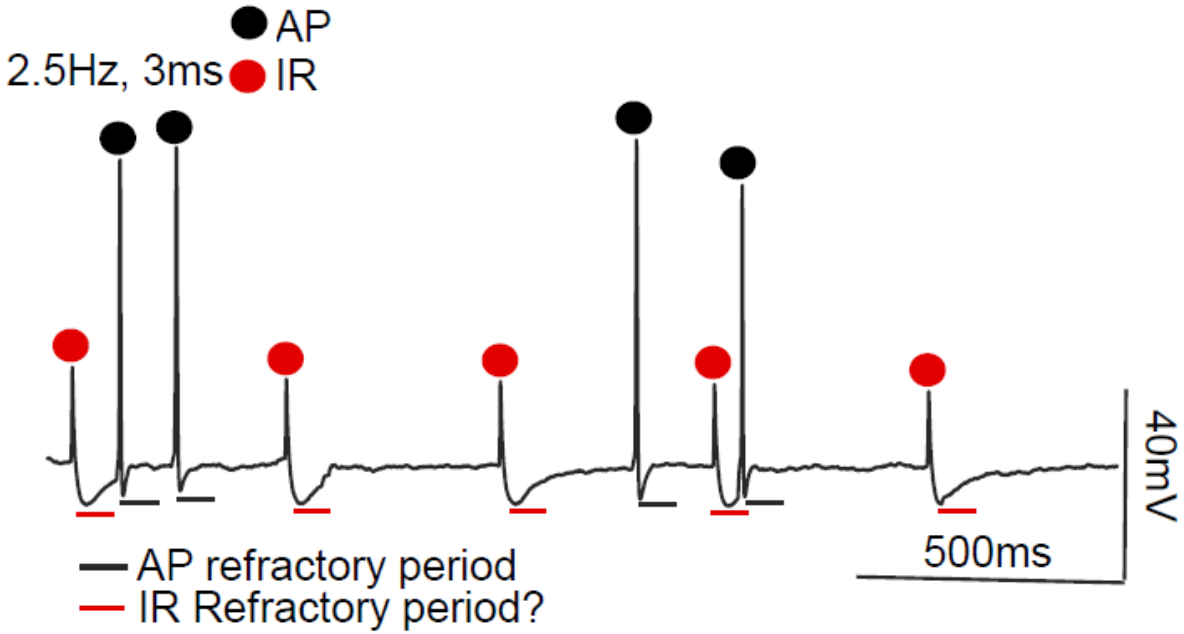


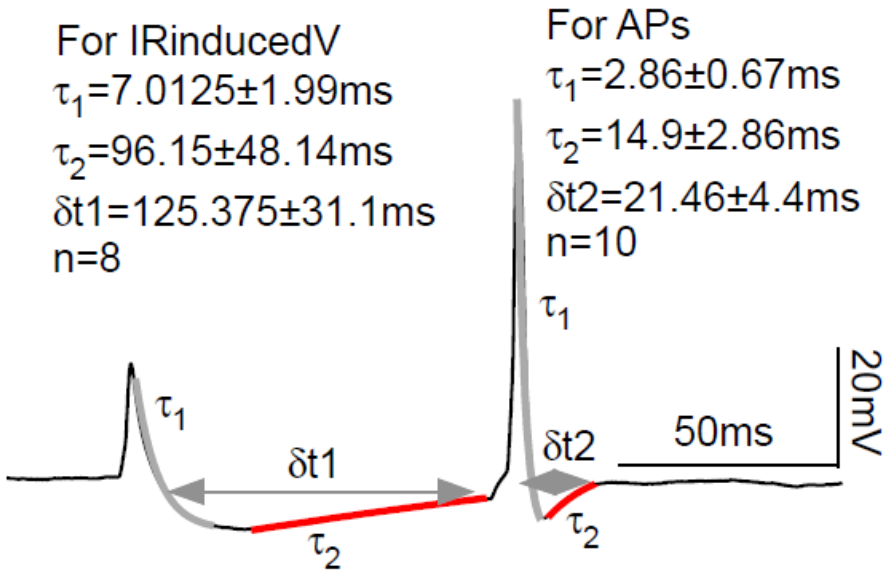




- Fundamentally different behaviors
- Could indicate more complex channel-mediated dynamics

Alteration of Membrane Potential

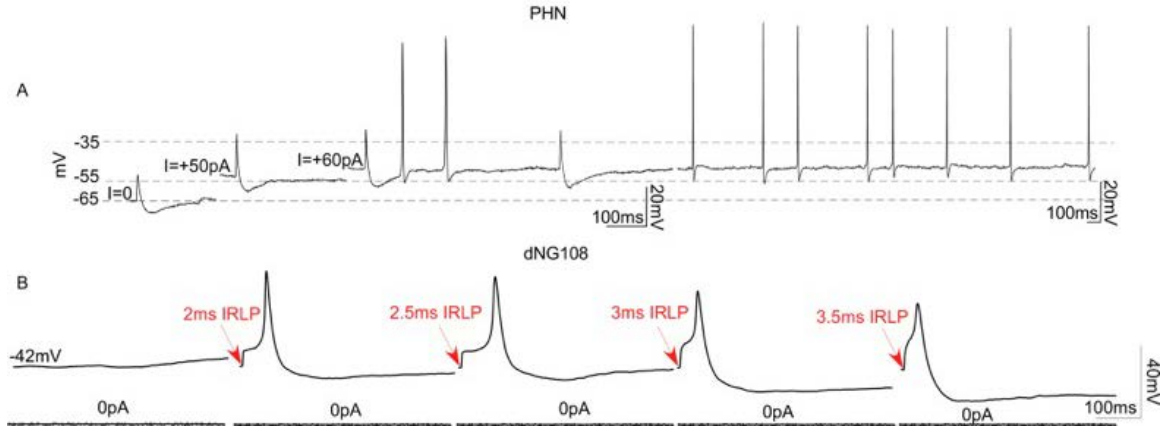


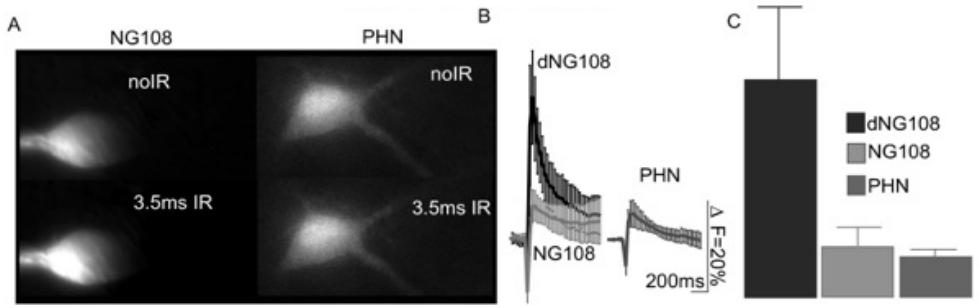


Membrane Proteomic Profile Impacts IR-induced Stimulation

NG108 cells
- motor neuron-like
- synapse on muscle
- highly binary input

Primary hippocampal neuron
- learning and memory
- highly modulated inputs
- synapse on another neuron





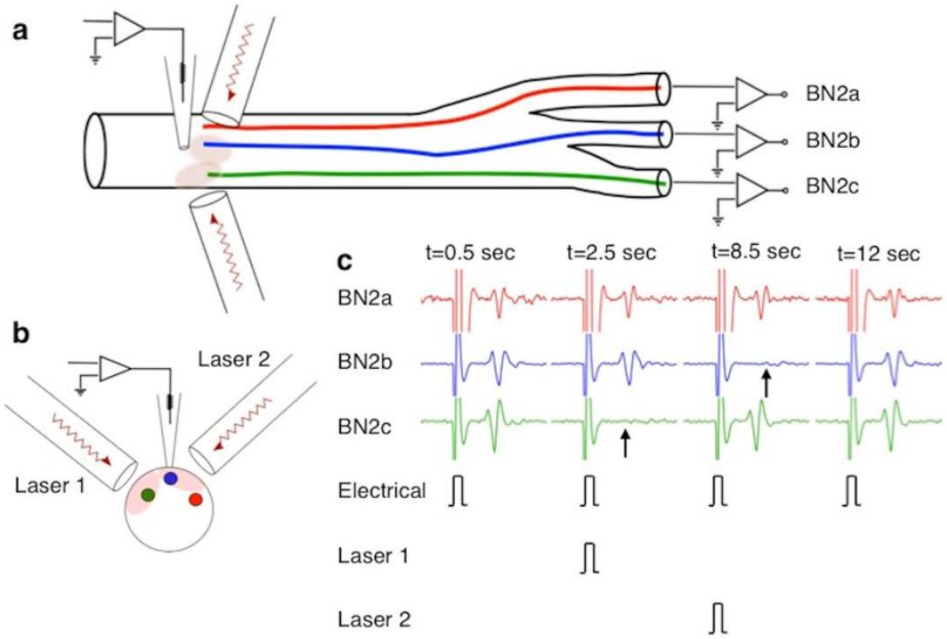
Too Much or Too Little: Infrared Stimulation Can Inhibit AP

- Synchronizing the electrical stimulus with IR eliminates an AP
- Each laser eliminated different electrically-evoked response

AP elimination reliably occurred when IR delivered
- 10 ms prior to start of the electric pulse
- 0.25 ms after the electric pulse

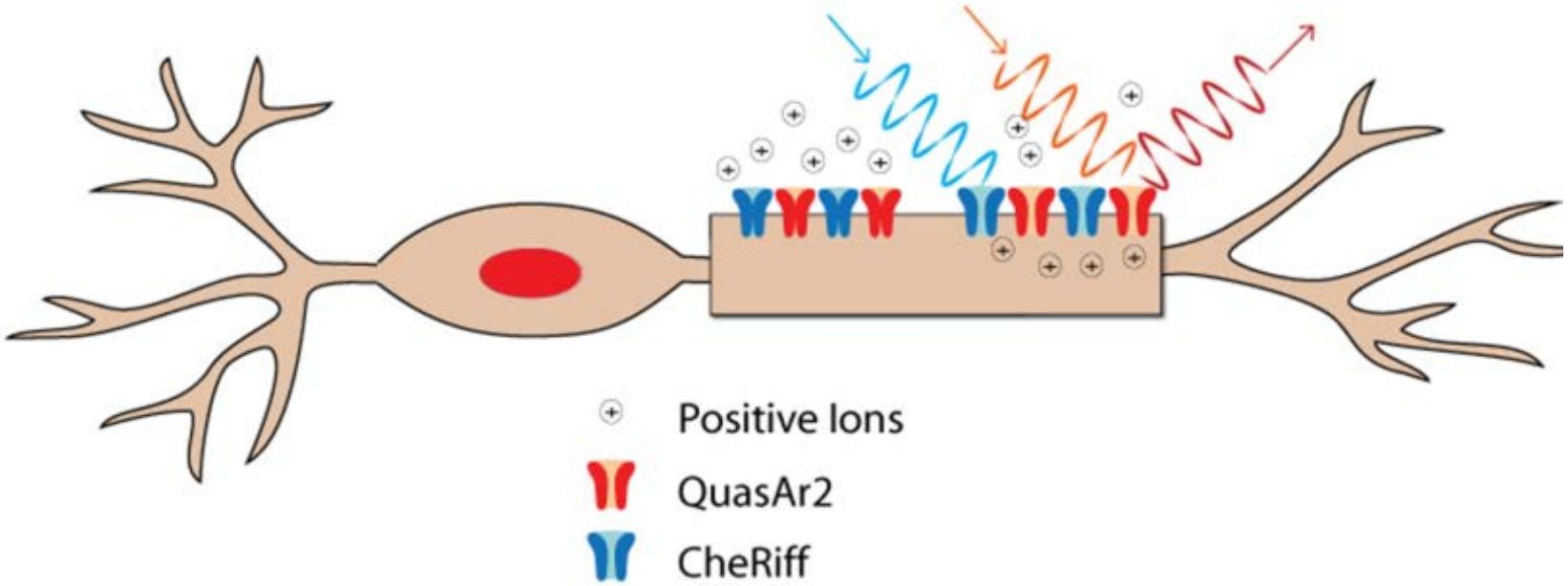
Figure 1 : Infrared inhibition of AP initiation.

From: Transient and selective suppression of neural activity with infrared light



Duke et. al., Scientific Reports 2013

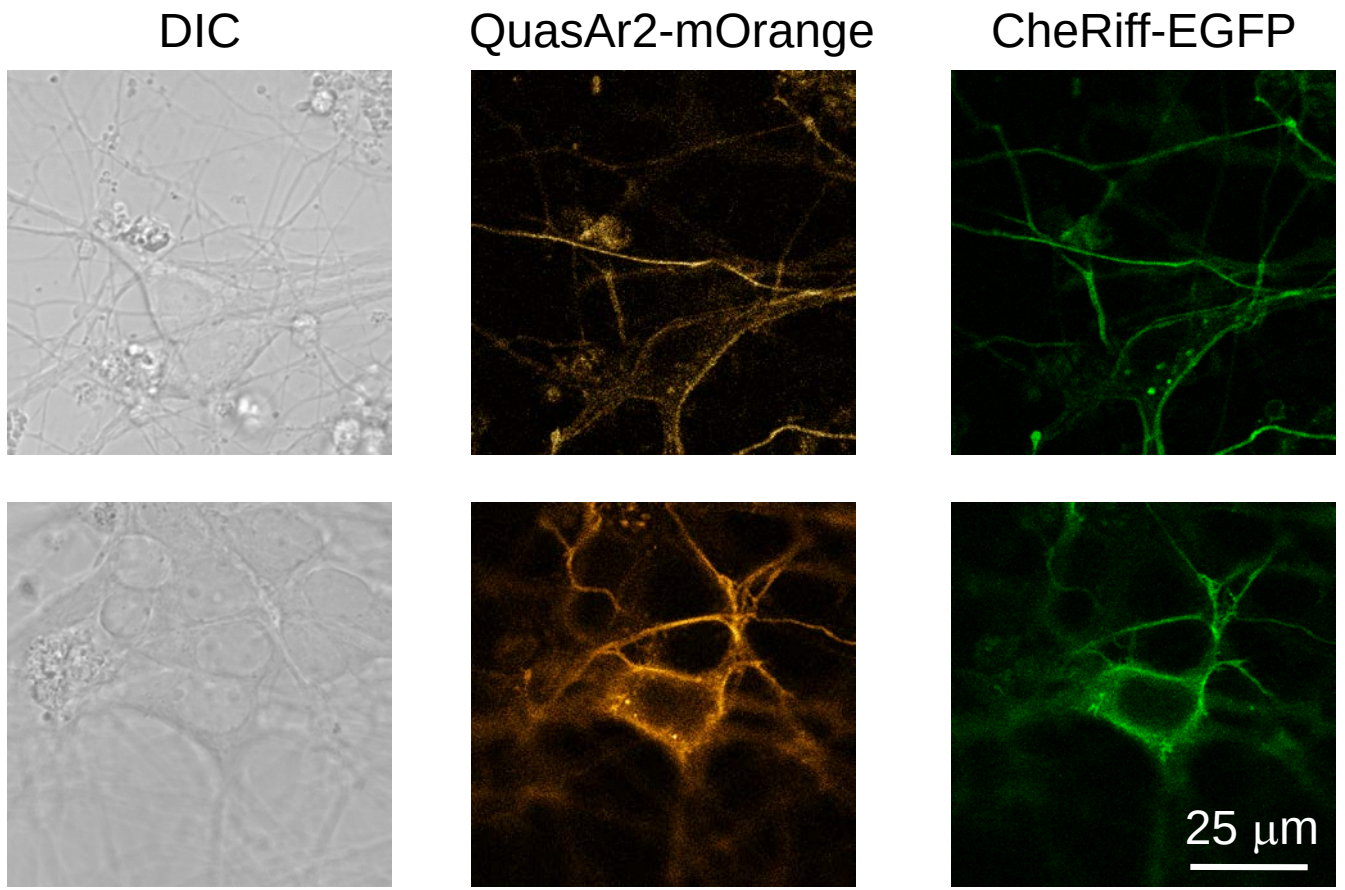
All Optical-Electrophysiology



Blue Light Stimulates Neurons
Red Signal Reports Action Potentials

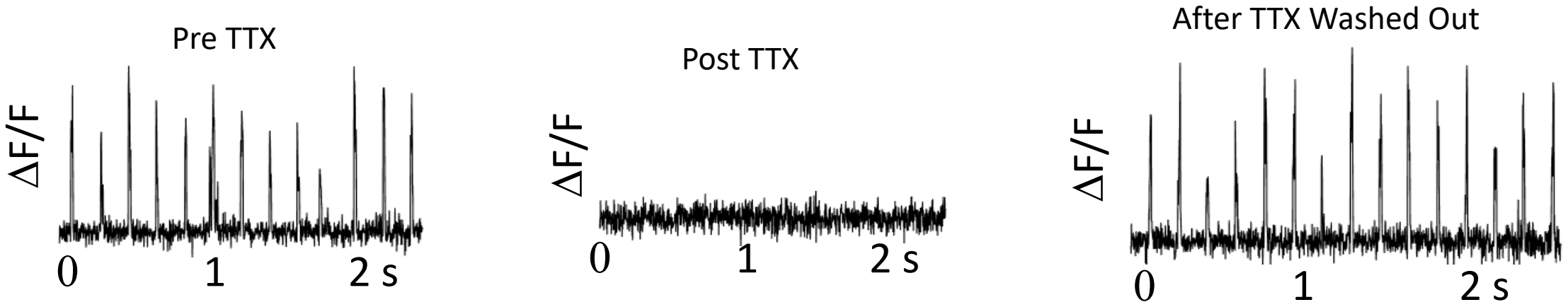
Validation of Optopatch2 Transfection

- mOrange and EGFP images confirm successful transfection of Optopatch2



Confirmation of Blue Light Induced Action Potentials

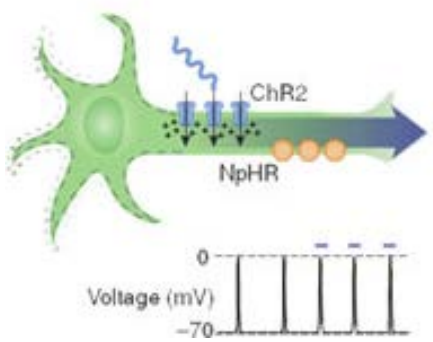
Blue light pulsed at 10 Hz



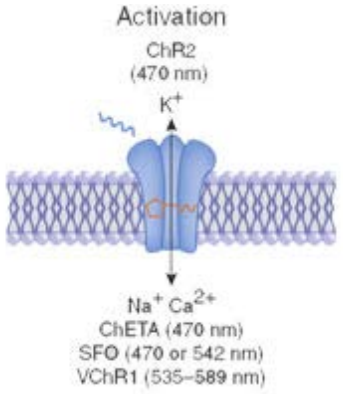
- Tetrodotoxin (TTX) inhibits action potentials
- TTX experiment confirms blue light induction of action potentials

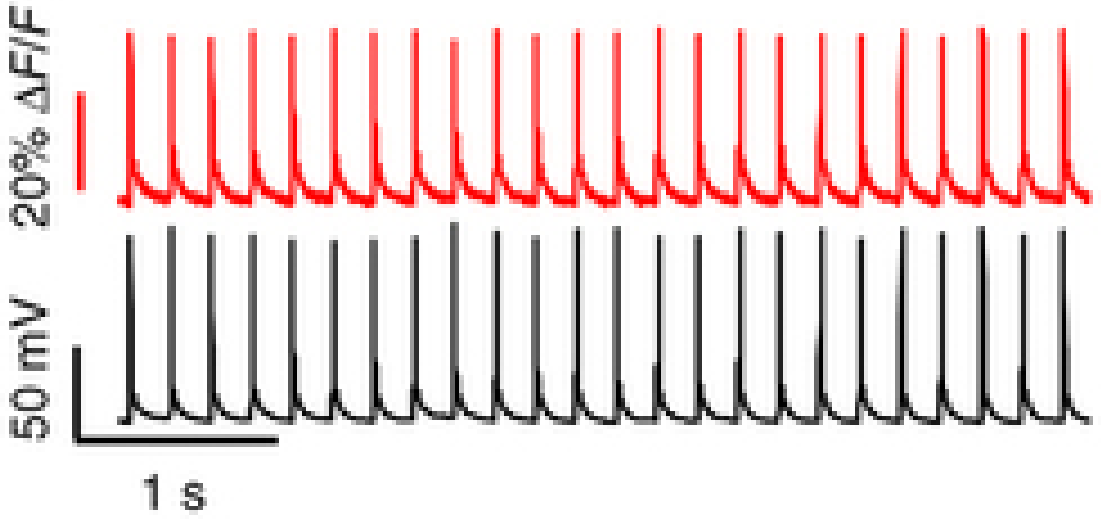
All Optical Stimulation, Reporting and Inhibition of Action Potentials

Channel rhodopsin
activated by light



Archaerhodopins
voltage-sensitive fluorescence report of AP



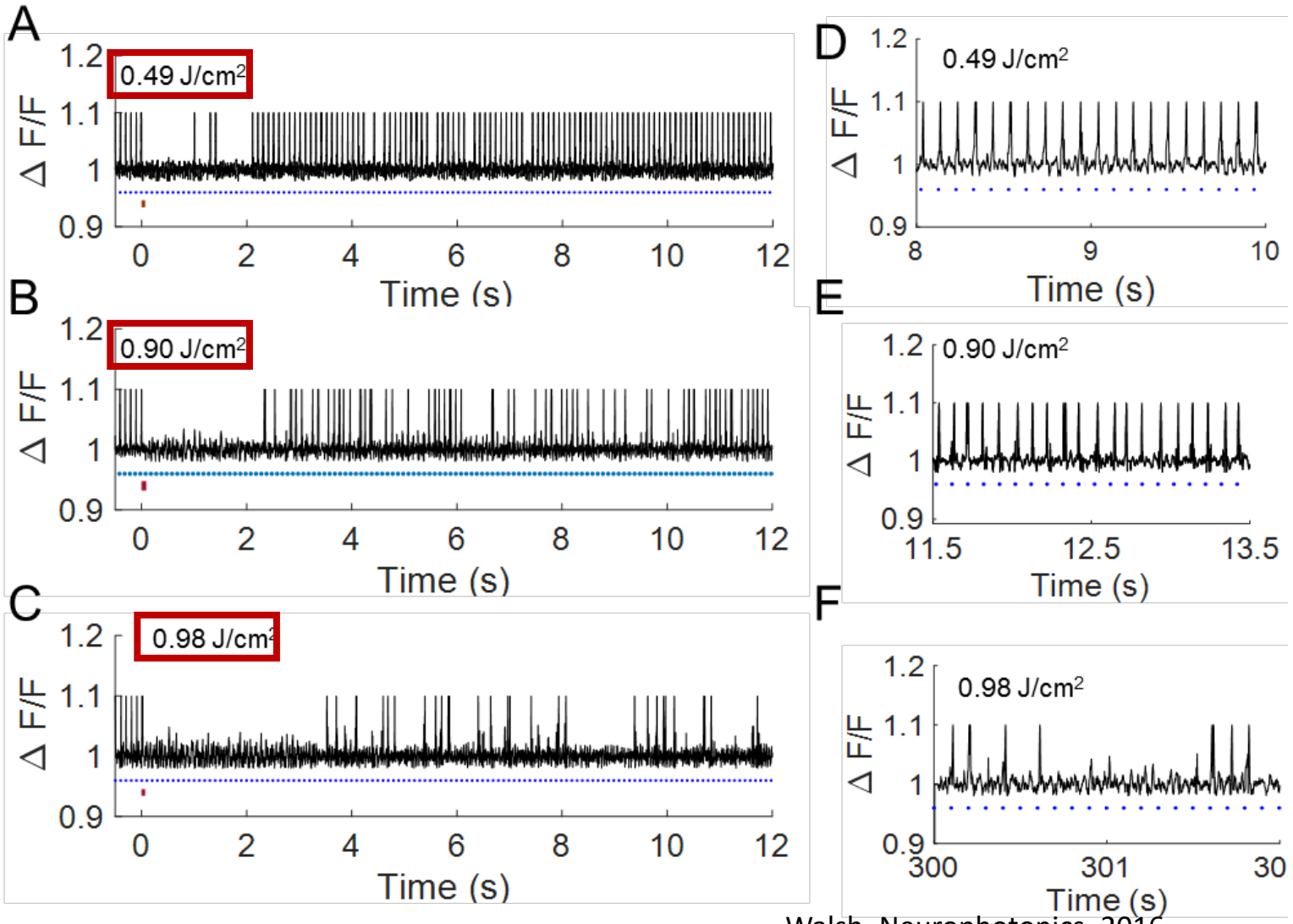


DRH296: FCK-Optopatch2 was a gift from Adam Cohen (Addgene plasmid # 51694)

Hochbaum, et al. Nat Methods, 2015 Pastrana. Nat Methods, 2011

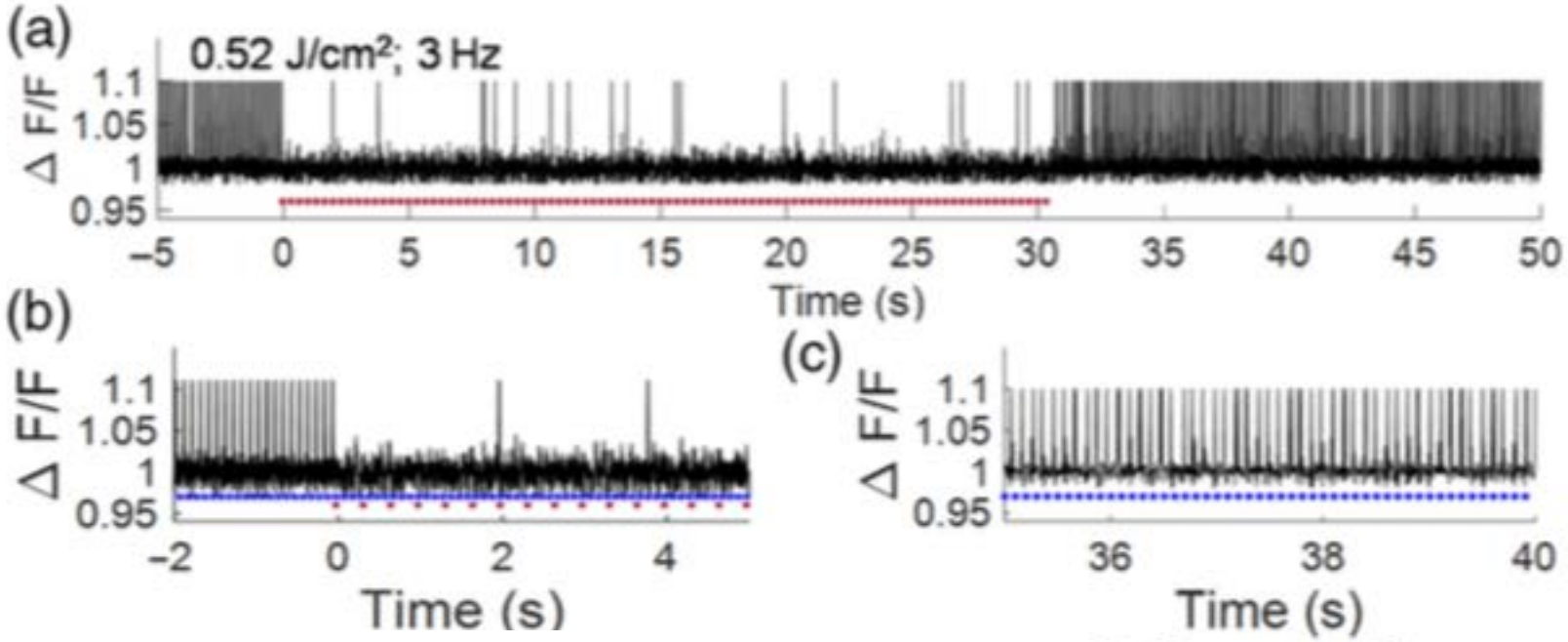
Short IR Laser Pulse – AP Block

- Short IR pulses cause a transient AP block
- AP recovery is dependent on applied energy



Walsh, Neurophotonics, 2016

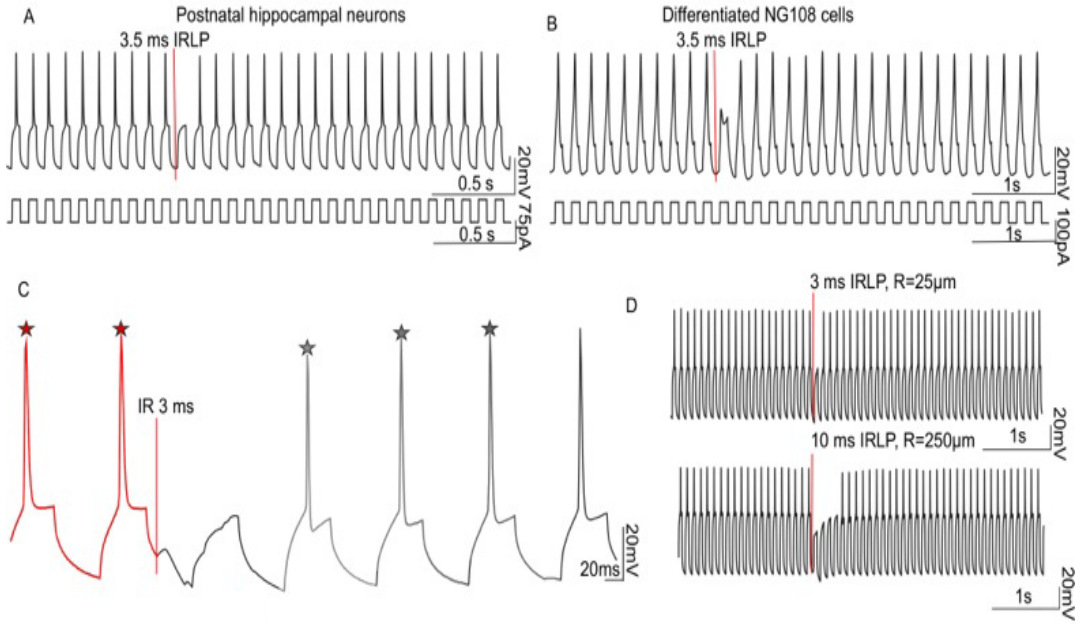
Sustained AP Spike Frequency is Modulated by Pulsed High Power IR Delivery



Walsh, Neurophotonics, 2016

AP Inhibition From Innate Neuronal Signaling Properties

- AP induced by current delivery
- Delivered to
  - hippocampal neurons
  - NG108 cells

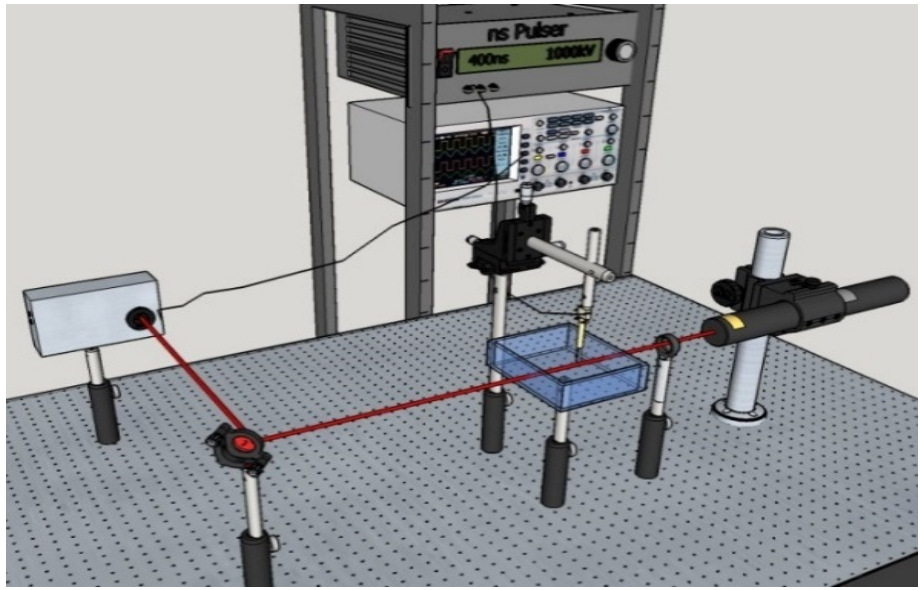


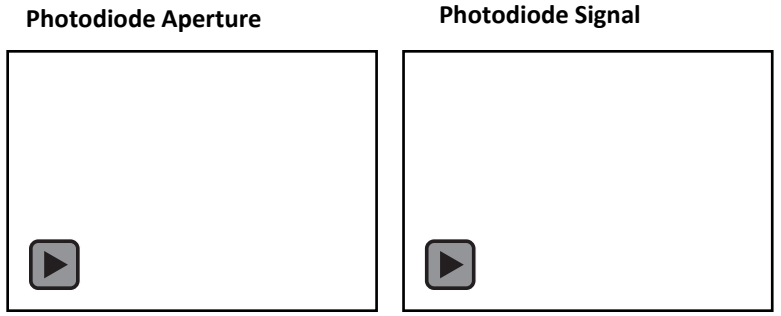
Results

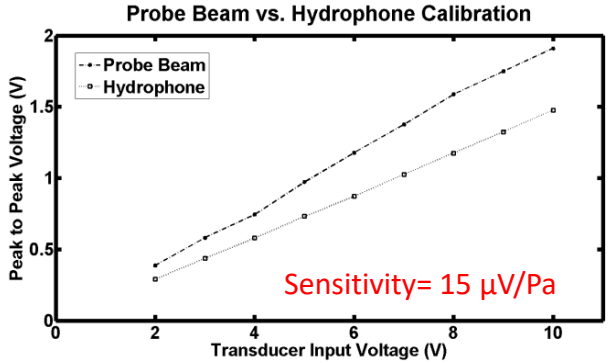
- A single IR pulse generates distinct calcium transients
  - Intracellular calcium stores are released in response to IR exposure
- IR pulse exposure may modulate cytoskeleton dynamics
- Plasma membrane fluidity dynamics are altered by IR exposure, but pore formation is not detected (unlike nsEP)
- IR-generated graded potentials exhibit a longer refractory period compared to control stimulation
- The unique composition of membrane proteins impacts the amplitude of IR-induced activity
- IR-induced AP inhibition is detected from optogenetic and current induced stimulation

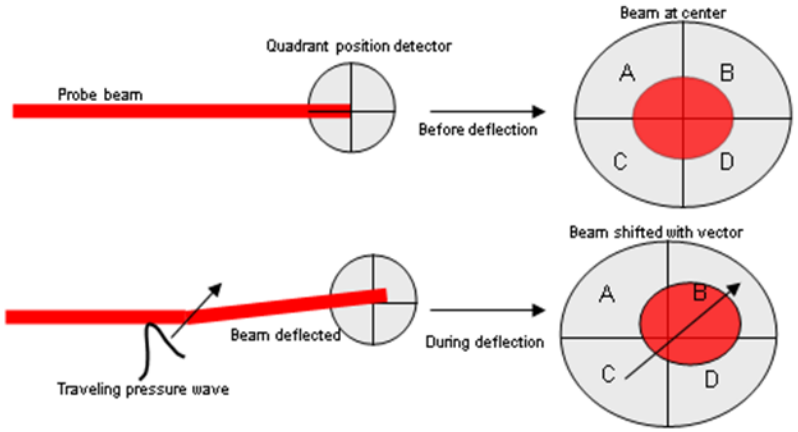
Given the broad experimental findings, how do we increase the physiological context of our work?

IR Dosimetry: Probe Beam Deflection Technique (PBDT)









First Use Case at TSRL: Sub-microsecond Electric Pulses (ex. nsEP)

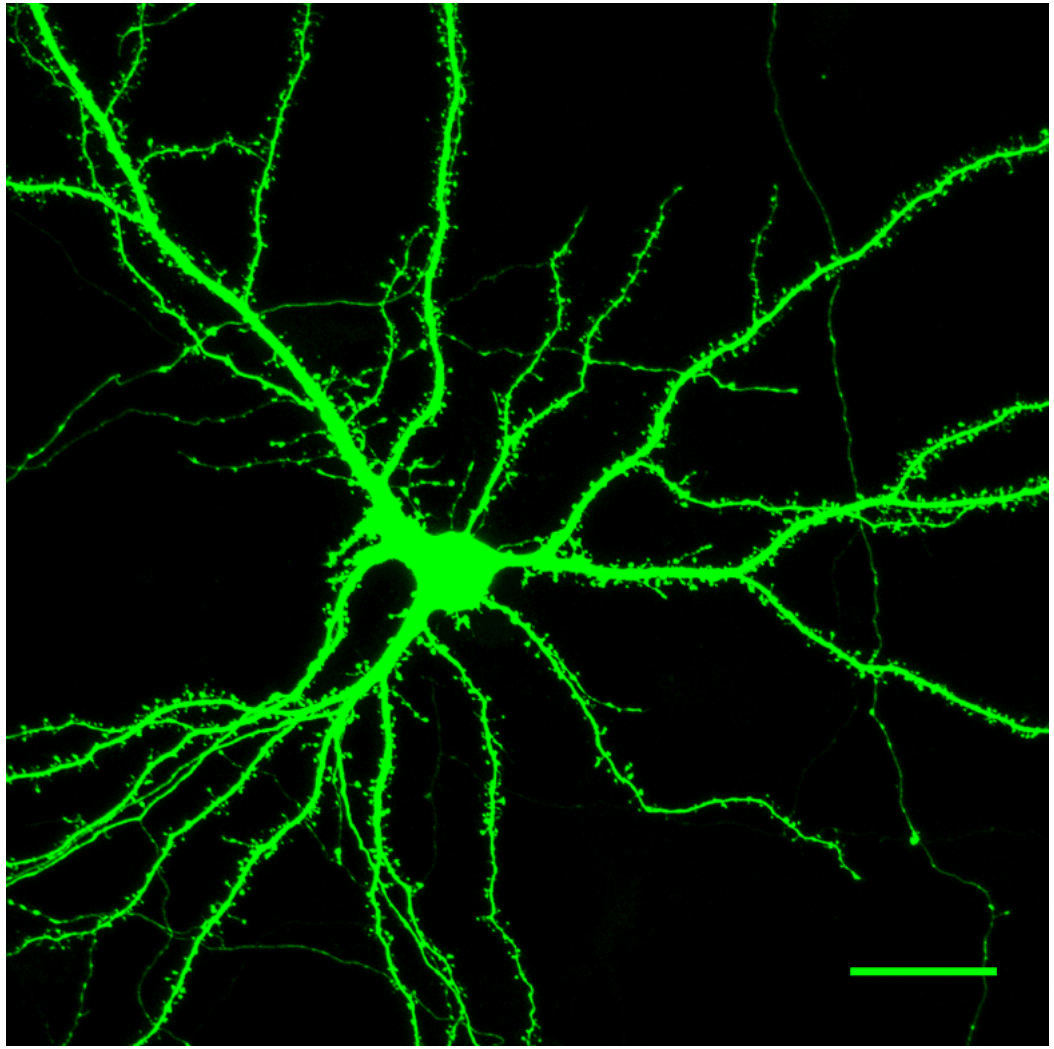
US Patent Application #US201715815963 PBDT Microscope

US Patent Application #US2017_____ Off axis Artifact reduction

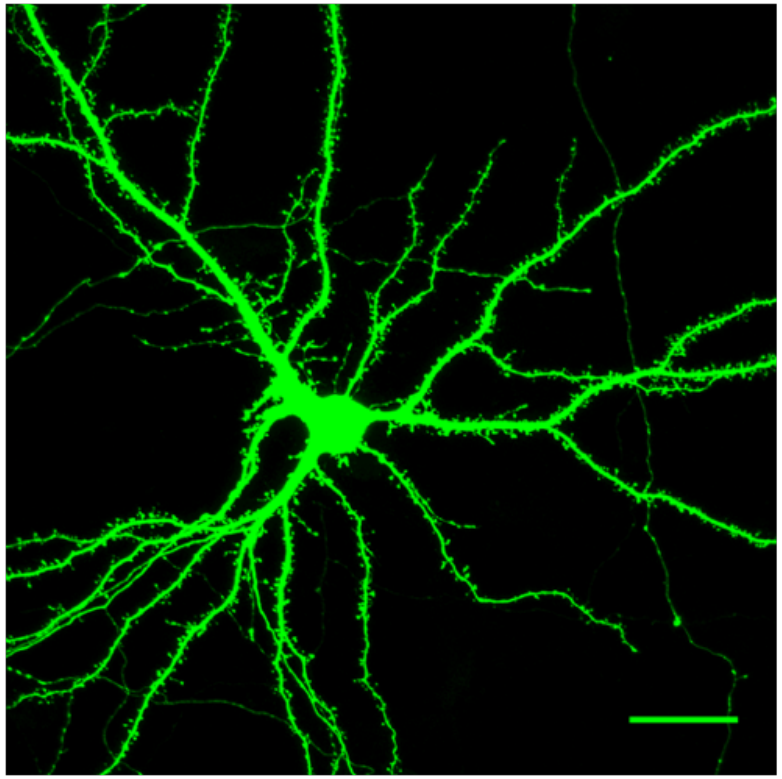
Ronald A Barnes, Caleb C Roth, Hope T. Beier, Gary Noojin, Christopher Valdez, Joel Bixler, Erick Moen, Mehdi Shadaram, and Bennett L. Ibey, "Probe beam deflection optical imaging of thermal and mechanical phenomena resulting from nanosecond electric pulse (nsEP) exposure in-vitro," Opt. Express 25, 6621-6643 (2017)

Roth CC, Barnes Jr. RA, Ibey BL, et al. Characterization of Pressure Transients Generated by Nanosecond Electrical Pulse (nsEP) Exposure. *Scientific Reports*. 2015;5:15063. doi:10.1038/srep15063.

Barnes, R. A., S. Maswadi, et al. (2014). "Probe beam deflection technique as acoustic emission directionality sensor with photoacoustic emission source." Applied optics **53**(3): 511-519 (2014).

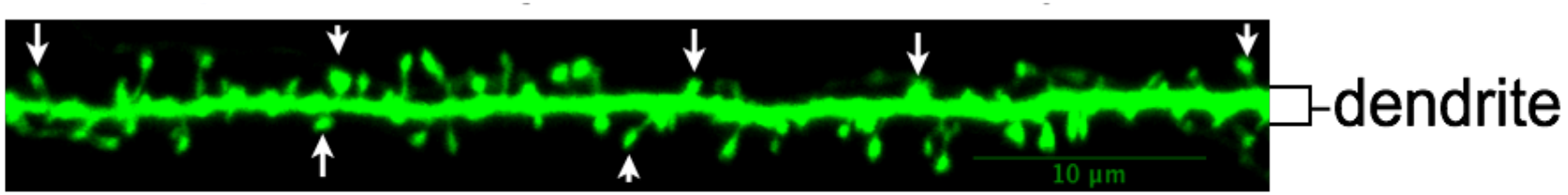


Dendritic Arborization



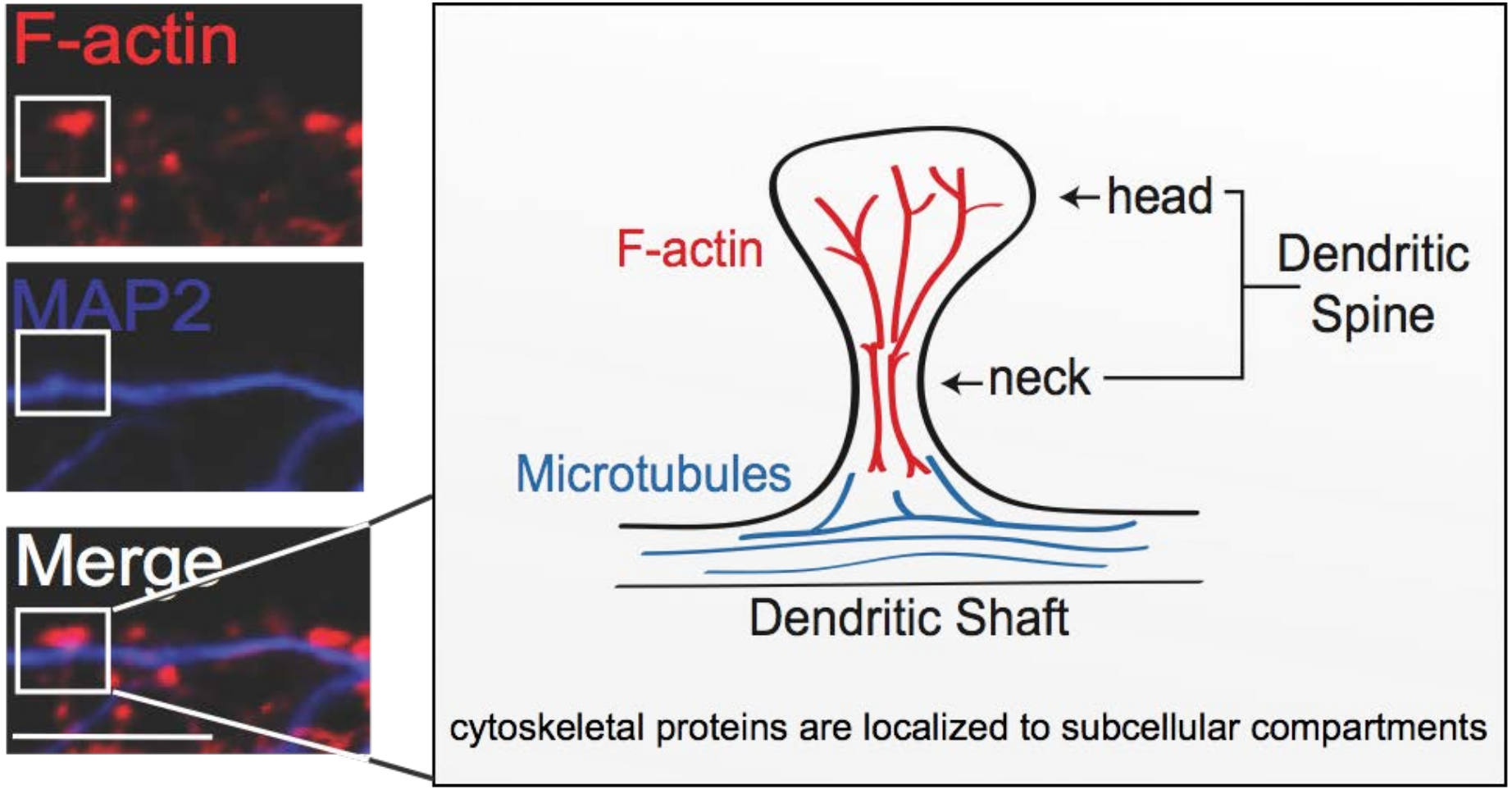
1. Promote cell-to-cell contact during development
2. Establish dendritic receptive field
3. Alter action potential propagation in mature circuits

Dendritic Spines

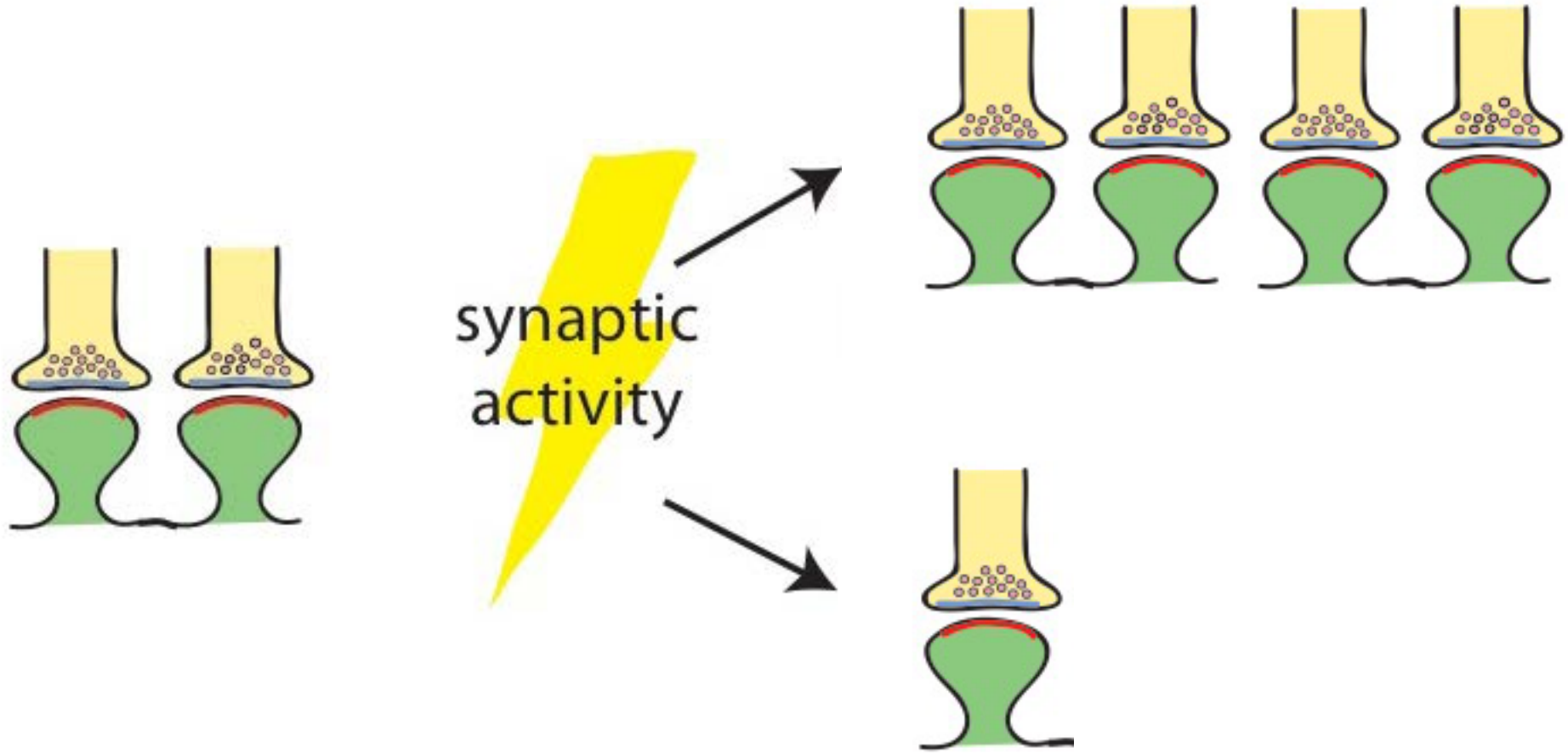


1. Densely populated with synaptic proteins
   - AMPA and NMDA receptors
   - 1:1 synapse to dendritic spine ratio
2. Provides input specificity
   - isolate receptor trafficking
   - amplify secondary messenger systems at activated synapses

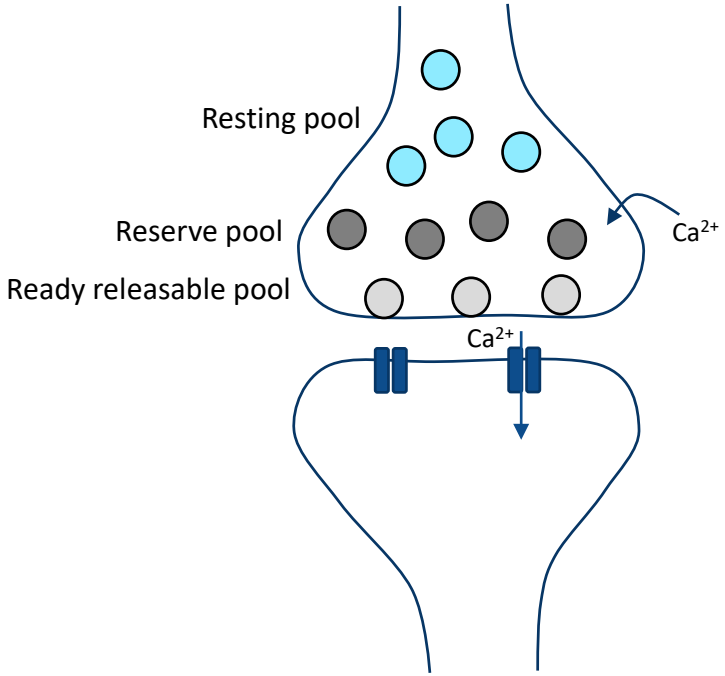
Cytoskeletal Proteins Are Enriched in the Dendritic Spine

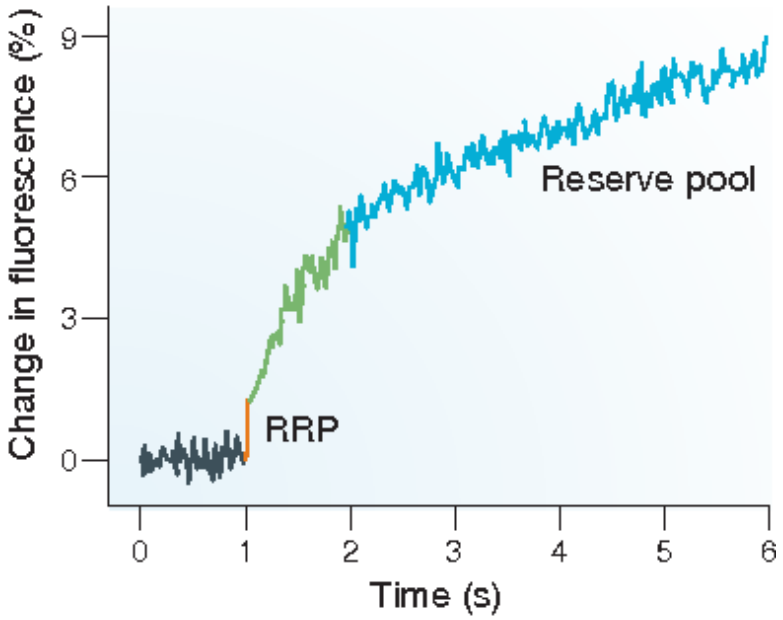


The Synapse is the Center for Structural and Functional Plasticity that Mediated Neuronal Function

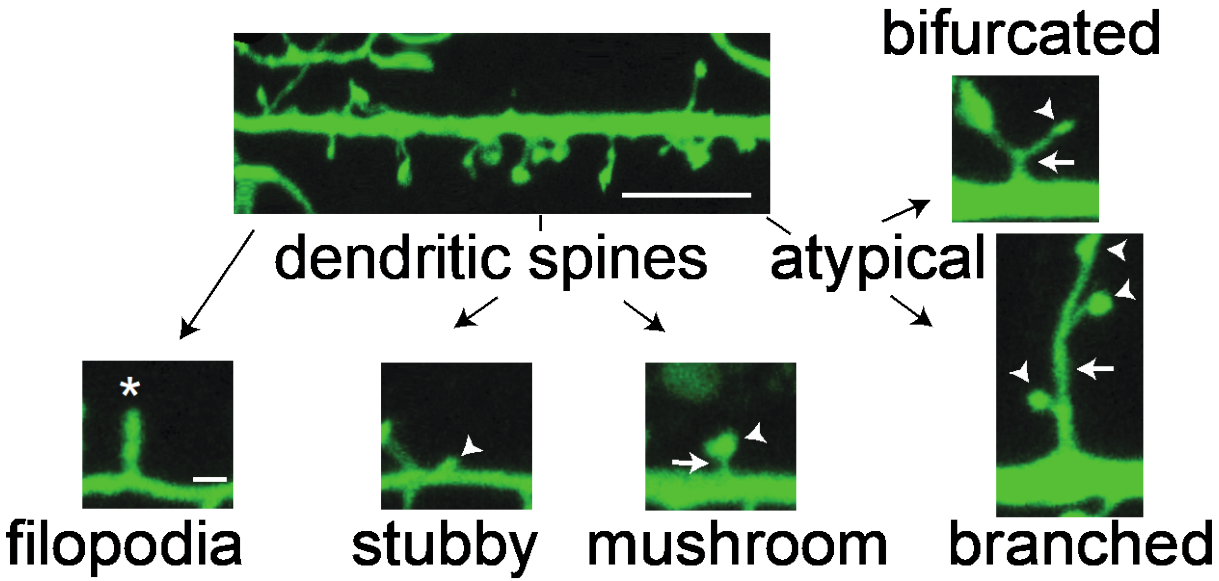


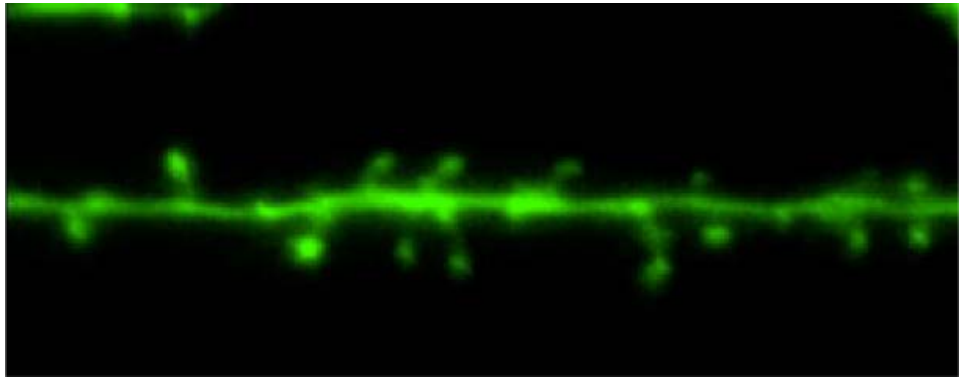
How does IR Modulate Calcium Dynamics at the Synapse?





Evaluate if IR Energy Modulates Synaptic Structural Plasticity

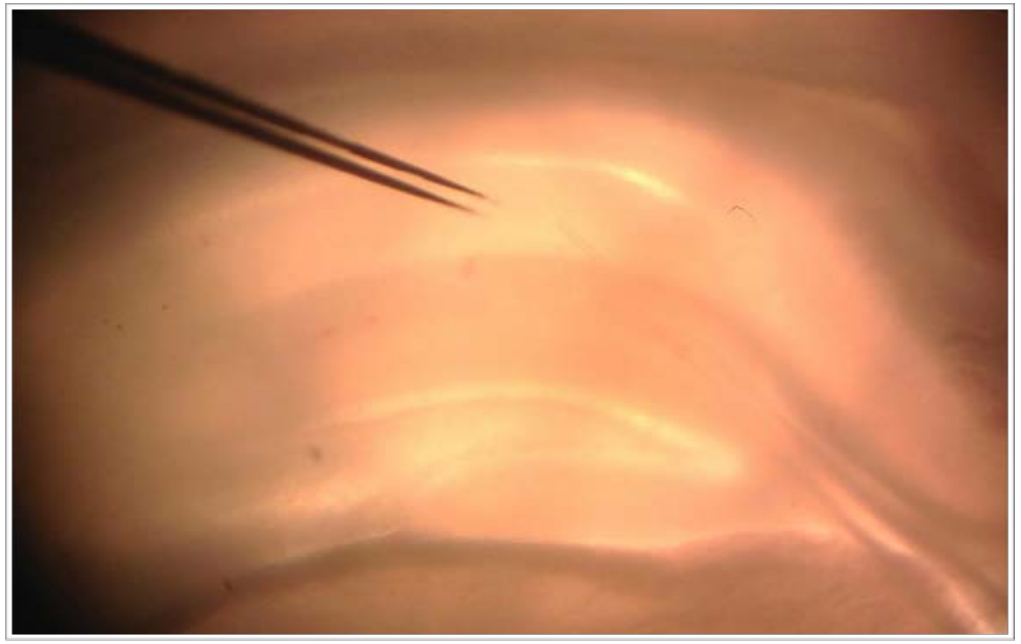




Does IR Energy Modulate Circuit Level Synaptic Transmission?

Long-term potentiation

- induced by high-frequency train of stimuli
- causes a persistent strengthening of synaptic connections
- major cellular mechanism that underlies learning and memory



Conclusions

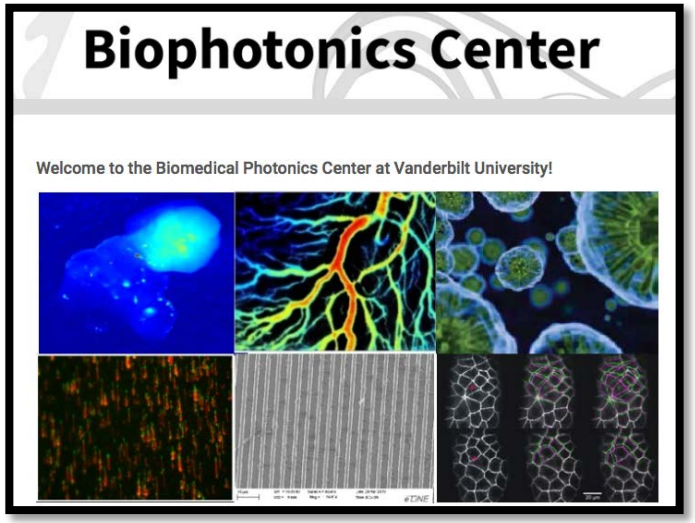
- Infrared exposure can generate distinct temperature transients, which can be finely-tuned for broad applications
- TSRL has matched the broader academic field in our experimental utility and basic scientific understanding of infrared neuromodulation
- Infrared exposure to primary neurons can potentiate and inhibit neuronal activity
- We have demonstrated a role of IR-induced neuromodulation on the membrane, intracellular, and cytoskeletal level in mammalian cells

Future Directions

- Expand on the physiological context of IR induced neuromodulation
- Develop deeper understanding of IR dosimetry during experimentation
- Identify the synaptic site of calcium activity as a result of IR exposure
- Determine if neuronal architecture is modulate by IR energy at dendritic arbors and spines
- Measure the impact of IR exposure on field excitatory postsynaptic potentials

Acknowledgements

Infrared Directed Energy Modifies Neuronal Network Activity
Funding: AFOSR LRIR
PI: Chris Valdez, Ph.D.
Co-PIs: Dr. Ronald Barnes Jr., Dr. Joel Bixler, Dr. Ibtissam Echchgadda, and Dr. Bennett Ibey
711 HPW/RHDR, Bioeffects and Mechanisms CRA & DE Simulation and Modeling CRA



How does IR-induced rapid disposition of energy alter neuronal physiology?

Why IR stimulation?
- Fast disposition of energy
- High fidelity IR pulse design
- Distinctly operable with real-time microscopy
- Surrogate for RF directed energy applications

How is IR delivery quantified?

Energy type

Fast thermal gradient

Applied

Probe Beam Deflection
- nanosecond time resolution of thermal disposition
- Energy quantification at fiber tip vs. cell floor


Dr. Ronald Barnes Jr.


Lt. Jennica Egan


Dr. Robert Thomas


Mr. Chad Oian


Mr. Jason Payne

In Silico

COMSOL Multiphysics

What bioeffects?

How are bioeffects measured?


Dr. Joel Bixler


Ms. Maria Troyanova

Real-time microscopy

Electrophysiology

Histology

Proteomics

- Pre and post synaptic calcium
- Live monitoring of neuronal cytoskeletal dynamics
- Action potentials and field EPSP
- Synaptic mechanism (PLC)


Dr. Chris Valdez


Dr. Gleb Tolstykh


Dr. Bennett Ibey


Dr. Ibtissam Echchgadda


Ms. Anna Sedelnikova

Questions?