Elucidating cellular and sub-cellular mechanisms of electromagnetic modulation of the nervous system

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SCHOOL OF ENGINEERING

Biophotonics Center

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Elucidating cellular and sub-cellular mechanisms of modulating the nervous system with infrared light

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Infrared Neural Stimulation

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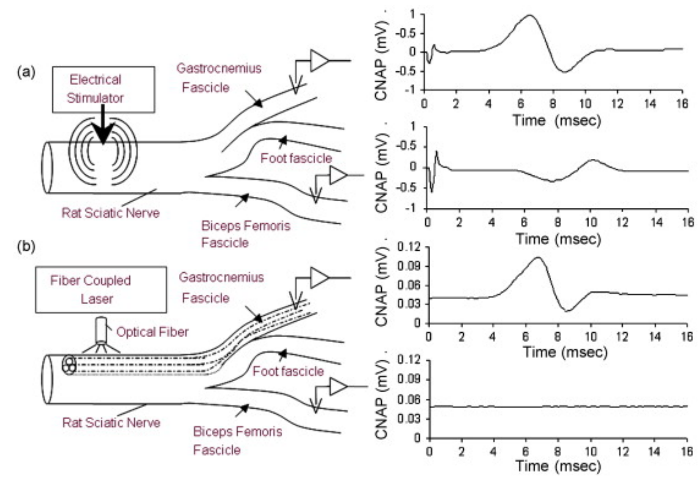
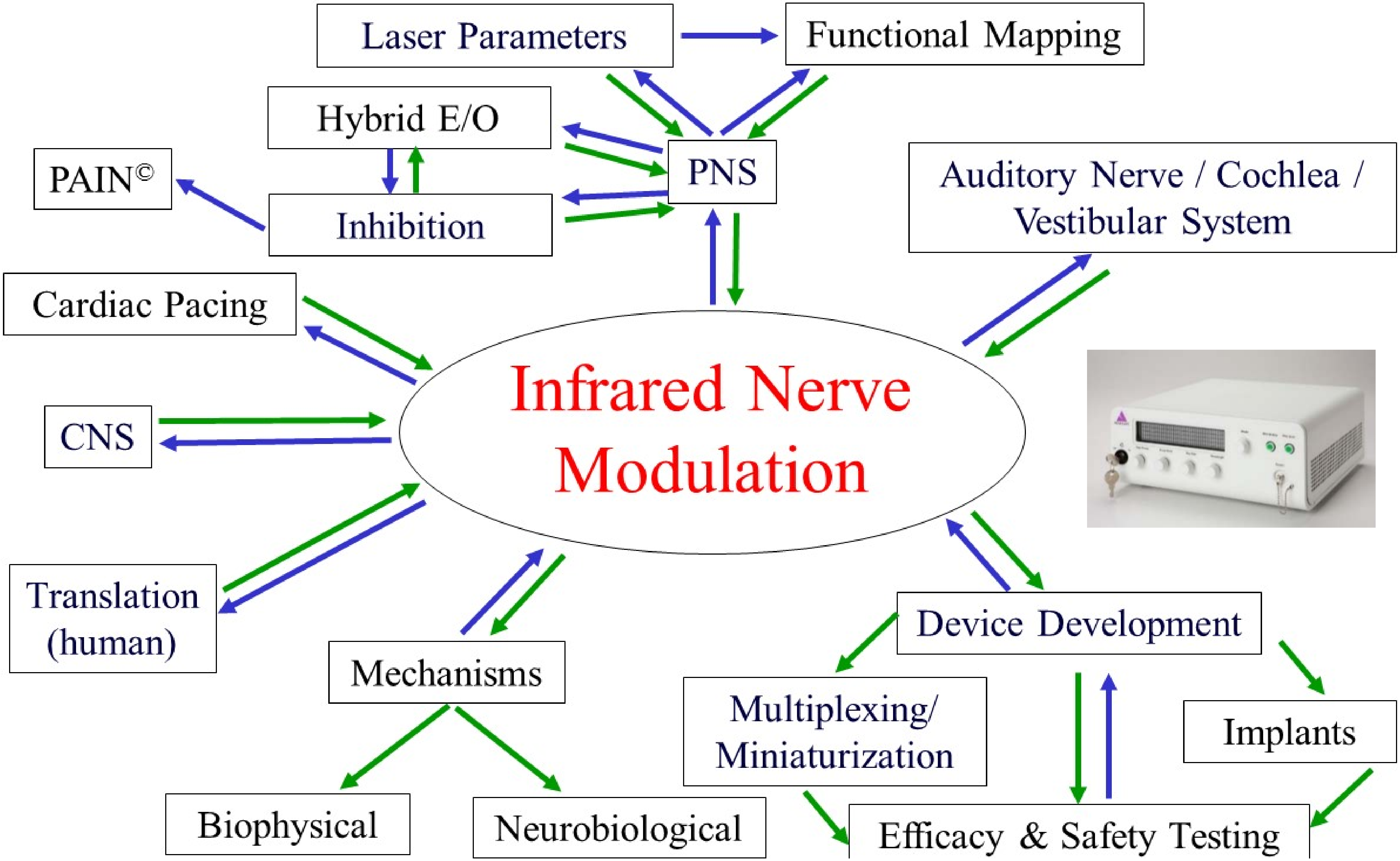
- Infrared Neural Stimulation (INS)
  - Direct induction of an evoked potential (EP/AP) in response to a transient targeted deposition of optical energy[1,2]
- Mechanism
  - Evokes neural activity via thermal gradient[3]
  - Transient change in membrane capacitance[4]



- Strengths[1,2]
  - Contact-free interface
  - High spatiotemporal precision
  - No stimulation artifact
- Demonstrated in various model systems
  - Peripheral nervous system (in vivo)
  - Central nervous system (in vivo and ion vitro
  - Cells
  - Cochlear system
  - Cardiac pacing

Development of INS



Mechanism?

- Pulsed infrared light well characterized to evoke neural activity via thermal gradient[1]
- Cell physiological mechanisms:
  - Transient change in membrane capacitance[2]
    - "Non-excitable" wild-type frog oocytes and human HEK cells
    - Depolarization of lipid membrane
    - Represents universal mechanism
  - Direct activation of TRPV channels[3]
  - TRPV4 is implicated in the process
    - Heat sensitive cation channels (Ca^{2+} Na^{+})
    - Retinal and vestibular ganglion cells
  - Heat block for neural inhibition[4]
    - T-dependent changes in rate of activation of gating mechanisms for voltage gated ion-channels
    - Demonstrated to block action potential propagation in *Aplysia* buccal and rat sciatic nerves[5]

1-Wells, J.D. et al. *Biophys J*, vol. 93, pp. 2567-80, Oct 1 2007
2-Shapiro M.G. et al. . *Nat Commun*, vol. 3, p. 736, 2012.
3-Albert E.S. et al. *Journal of Neurophysiology*, vol. 107, pp. 3227-3234, 2012
4-Zongxia M et al. *Biomedical Engineering, IEEE Transactions on*, vol. 59, pp. 1758-1769, 2012.
5-Duke. AD. Doctor of Philosophy Dissertation, Biomedical Engineering, Vanderbilt University, 2012.

Roadmap

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- Using modeling to predict behavior
  - Optical, Thermal, Neurobiological
  - Varying optical parameters
  - Inhibition (or thermal block), Stimulation
- Using model systems to experimentally elucidate cell-light interactions, cell-cell interactions and within cell effects

Modeling to Understand Mechanism

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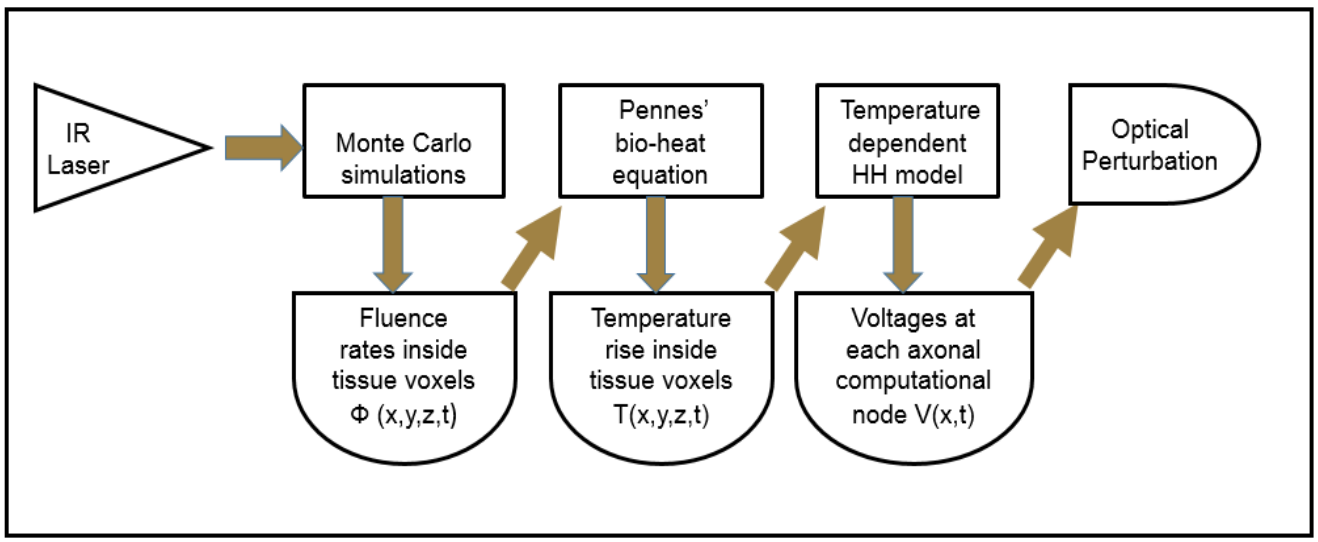
- Quantitative modeling makes testable predictions
- Modeling is an iterative process modified by experimental results
- Key model components:
  - How infrared photons interact with tissue
  - How heat moves through tissue
  - How an excitable axon or nerve cell responds to heat



Other Temperature-Dependent Features

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- Start with the Hodgkin-Huxley model
  - This model does not account for all the temperature dependent effects
- Additional temperature-dependent components include
  - Energy-dependent sodium/potassium pump
  - Resistance of the axonal cytoplasm, i.e., the axial resistance
  - The Q10 factor, which may vary for different gating processes and different temperature ranges
  - Peak conductances for the sodium and potassium voltage-dependent ion channels

Modified HH model

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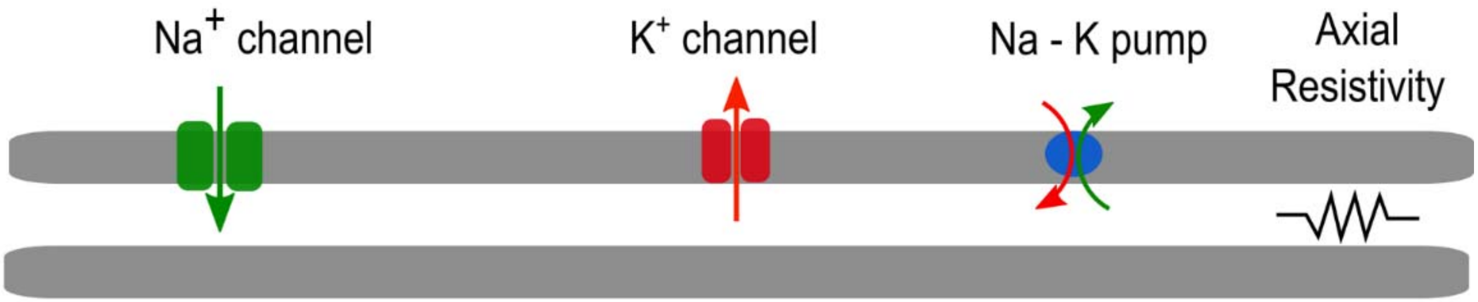

	gNa_{max}	Q_{10} m,h	gK_{max}	Q_{10} n	g_{pump}	ρ
HH	Constant	Constant	Constant	Constant	Absent	Constant
mHH	Variable, temperature dependent	Variable, temperature dependent	Variable, temperature dependent	Variable, temperature dependent	Present, temperature dependent	Variable, temperature dependent

Since then we have used this model to answer questions and optimize infrared inhibition

Inhibition of AP *Initiation*

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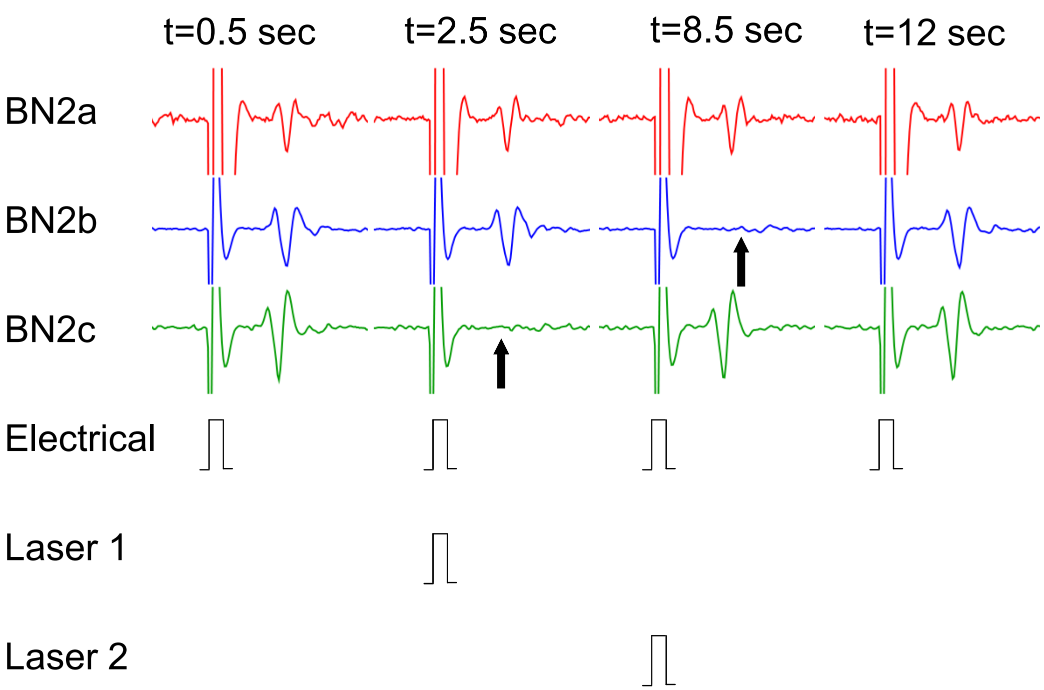

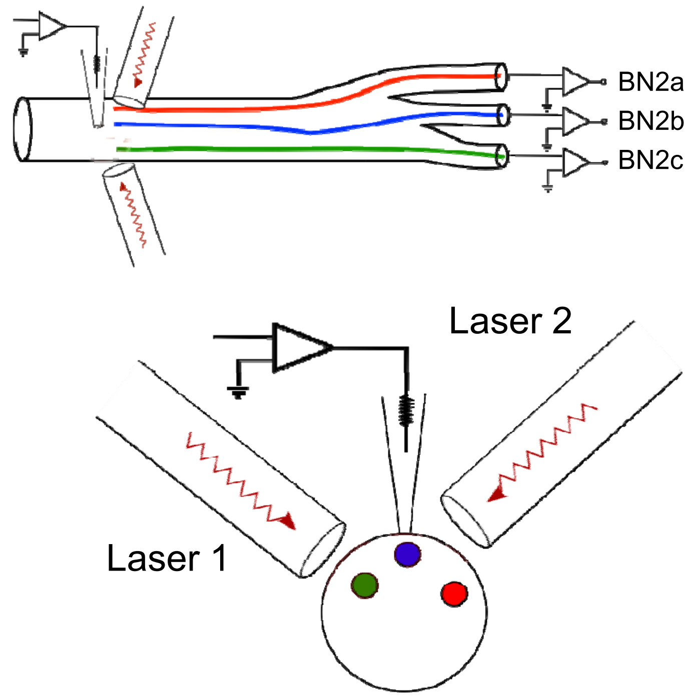

Infrared pulses reversibly and selectively inhibit electrically-initiated axonal activation

- Supra-threshold pulses synchronized with the two laser systems
- 2 Hz stimulation

Inhibition of AP *Propagation*

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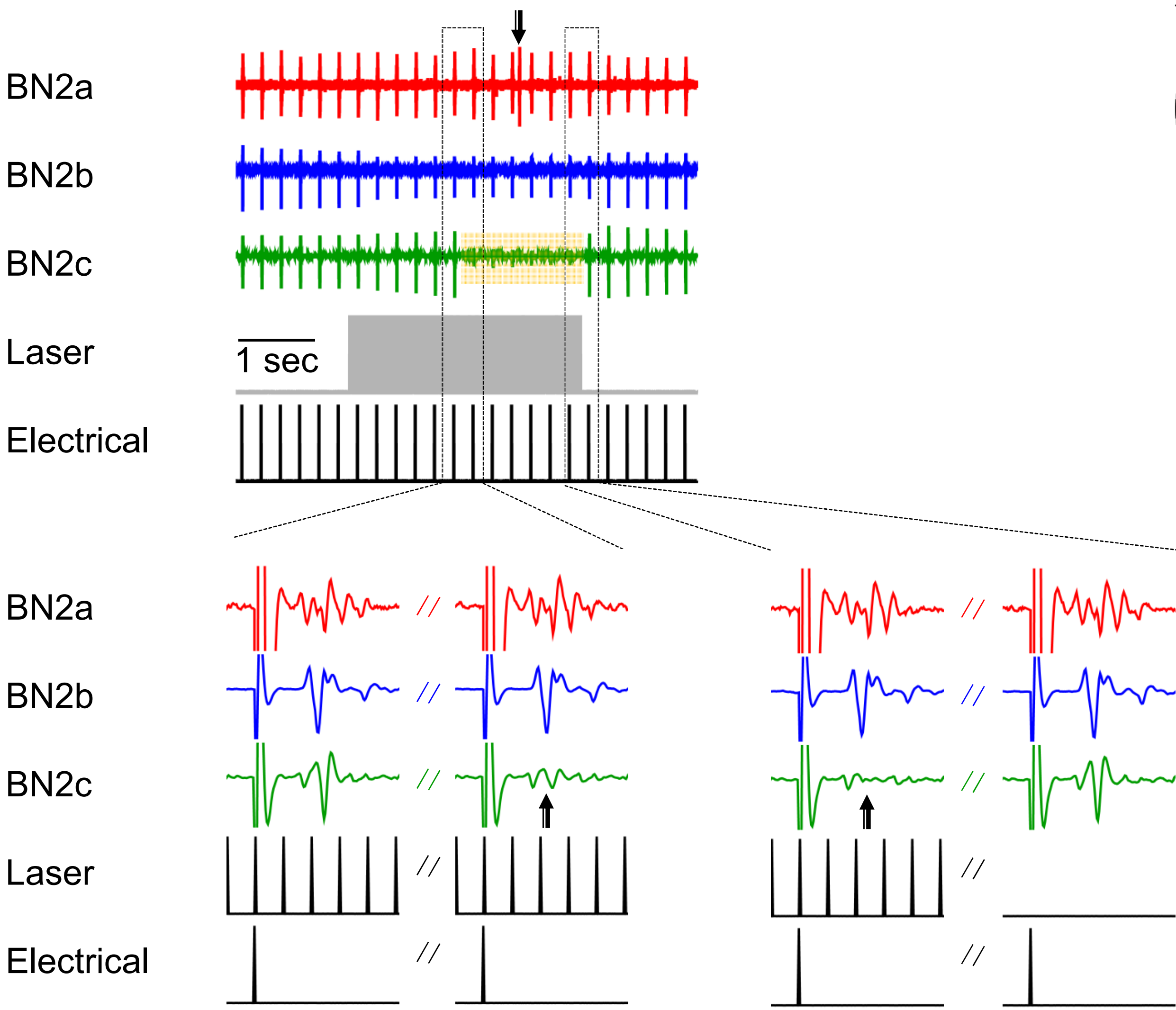

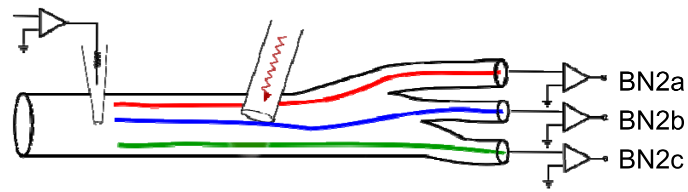

Infrared pulses reversibly inhibit action potential propagation

- Delayed effect
- Some effects on other branches
- Blocking strength increases with duration of laser exposure

Summary

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- Infrared light can safely and effectively activate peripheral and central neural tissues
- Infrared light reversibly blocks initiation and propagation of nerve conduction
  - Spatial precision and safe blocking is attainable with optimized light delivery
- We could combine electrical and optical energies for improved safety
- We now have a computational model that can help us understand infrared inhibition and optimize it

Optimizing Inhibition

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- Infrared Neural Inhibition have not been optimized
  - Optimization may lead to lower temperature rises
  - New irradiation geometries must be tested

- We hypothesize that the temperature rise at inhibition threshold can be reduced by distributing the heating over a greater area

- mHH predicts a reduction in temperature when we use 2 fibers instead of 1 fiber to deliver infrared light
  - Distributed deliovery

1) Hodgkin AL, Katz B. *J Physiol*. 1949;9:240-249. 2) Mou Z et al. *IEEE Trans Biomed Eng*. doi:10.1109/TBME.2012.2194146. 3) Duke AR et al. *Sci Rep*. 2013. doi:10.1038/srep02600.
4) Lothet, EH et al. *Scientific Reports.* June 2017. 3275(7) doi:10.1038/s41598-017-03374-9DO

Experimental validation

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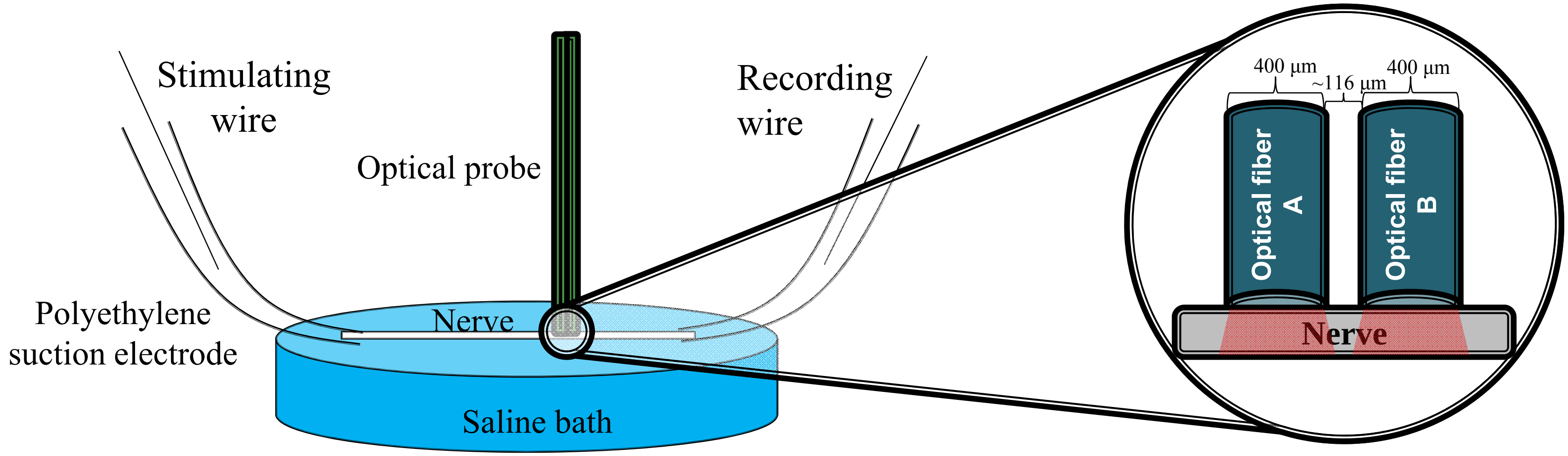
- *Aplysia* pleural abdominal nerves (n=6) were dissected out and placed in a saline bath
- Suction electrodes were applied to each end
- Dual-fiber optical probe placed in between electrodes



Jeremy B. Ford, Michael W. Jenkins, Hillel J. Chiel, E. Duco Jansen, "Reducing peak temperatures during infrared inhibition of neural potentials," Proc. SPIE 10052, Optogenetics and Optical Manipulation, 100520B (28 February 2017);

Summary

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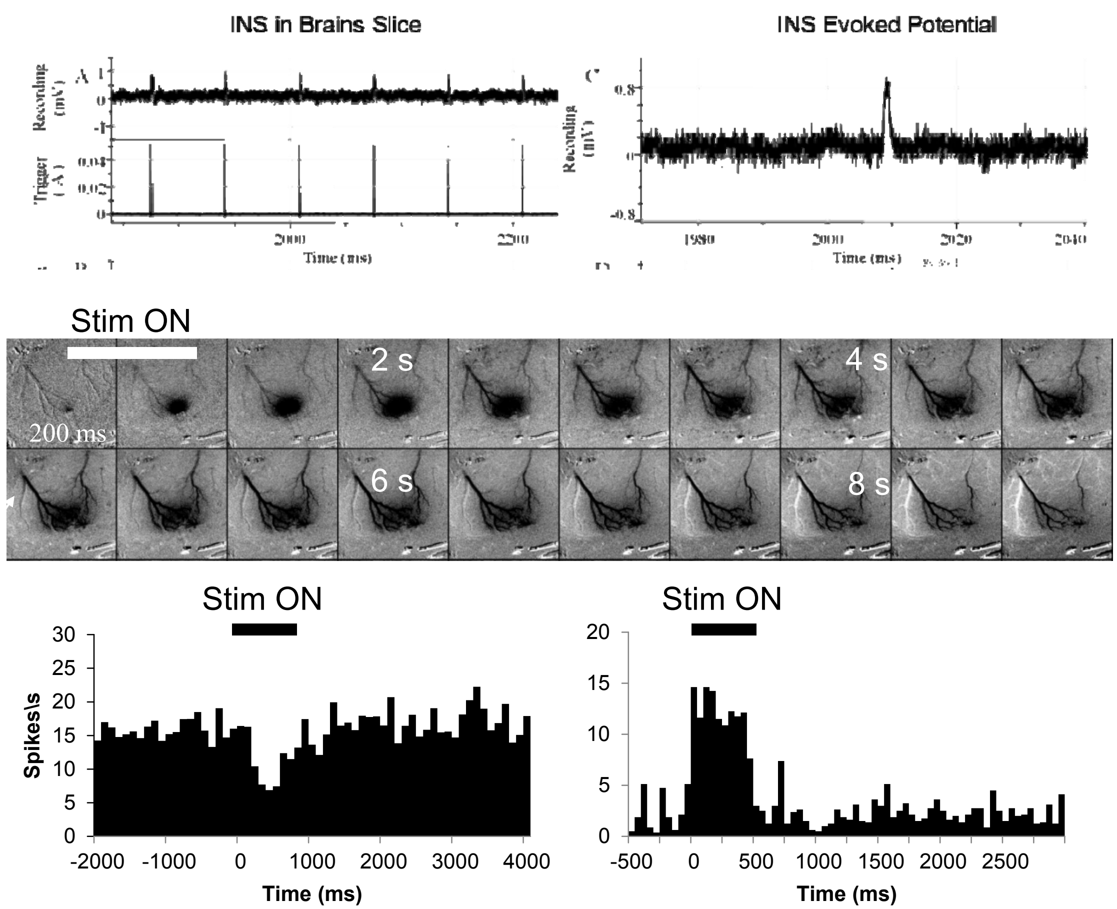
- Radiant exposures per irradiating fiber can be reduced by increasing the number of fibers along the nerve
- This correlates to a reduction in peak temperature rise and a lower probability of damage
- This reduction relies on an increase in the length of heated axon, or block width
- Application of this principle can help optimize application of light for clinical therapy

INS in the cortex

- Infrared neural stimulation (INS) has been applied as a label-free, high spatial resolution stimulation strategy.
- Possible application of INS in the CNS
  - Brain mapping
  - Epileptic suppression
  - Parkinson's treatment
- Multiple cellular mechanisms are transduced into neural activity.
  - neurotransmitter, GABAergic[1]
  - Ion channels[2]
  - electrical capacitance of the cell membrane[3]

1. Feng, H.-J. et al., Neurosci.2010
2. Albert et al., Neurophysiol.2012
3. Shapiro et al., Nat. Commun.2012
4. Fitzgerald, M.J., et al. Clincal neuroanatomy and neuroscience,2012

INS in the cortex



- Feasibility shown in rat thalamocortical brain slices[1]
- Demonstrated INS evokes intrinsic imaging signal similar to signals evoked by natural stimulation[2,3]
- Related INS evoked intrinsic imaging response to neural activity
  - Inhibition[2]
  - Excitation[3]
- Demonstrated no functional damage associated with INS[2,3]

1- Cayce, J.M., *et al.* Infrared Neural Stimulation of Thalamocortical Brain Slices. *STQE, IEEE Journal of* **16**, 565-572 (2010).
2- Cayce J, Friedman R, et al (2011) "Pulsed infrared light alters neural activity in rat somatosensory cortex *in vivo.*" Neuroimage 57(1):155-166.
3- Cayce J, et al (2012) "Functional Characterization of infrared neural stimulation in non-human primate cortex" BiOS Photonics West, San Francisco CA

INS induces propagating calcium wave

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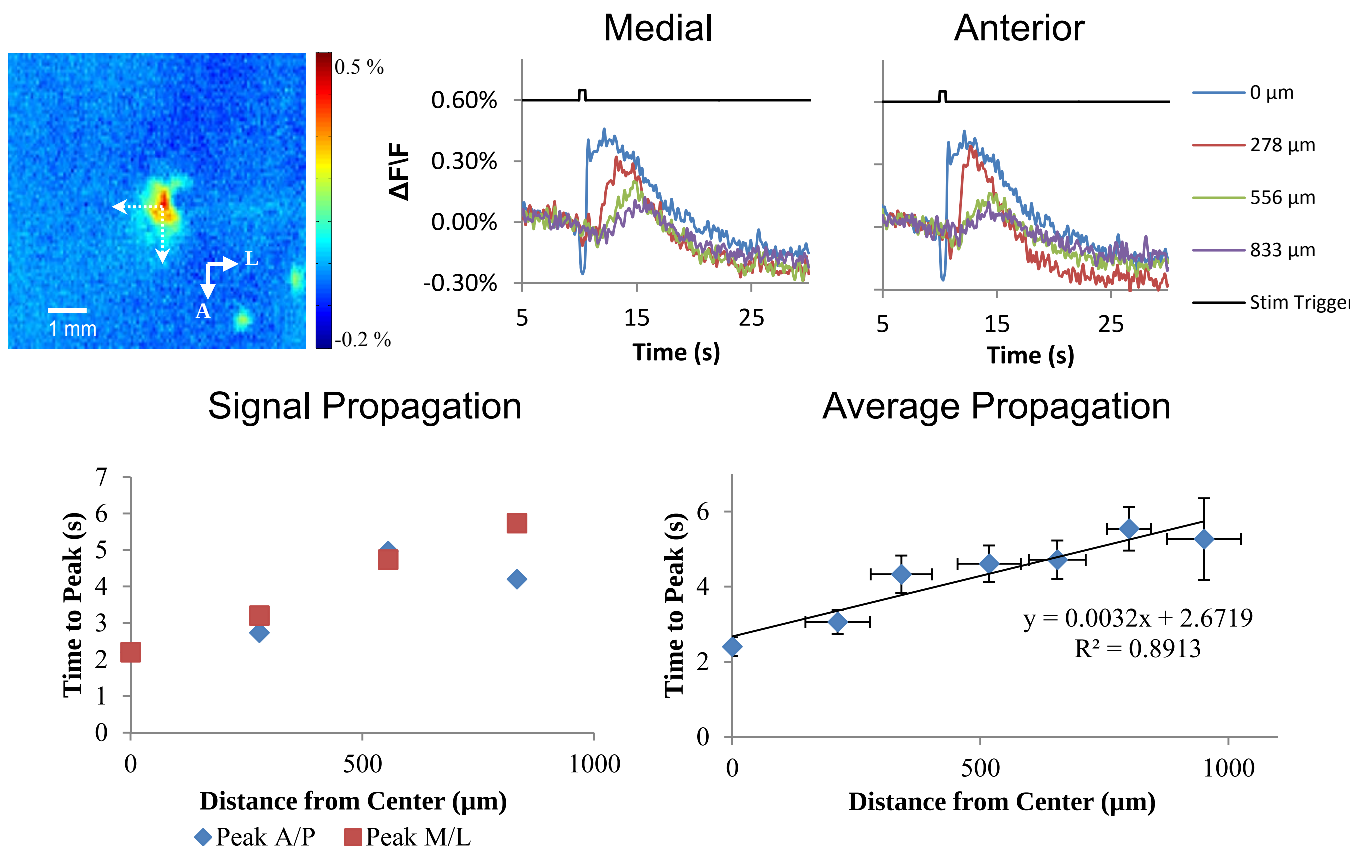


Calcium wave propagates away from stimulation location

- Suggests network connectivity
  - Neuron and Astrocyte involvment

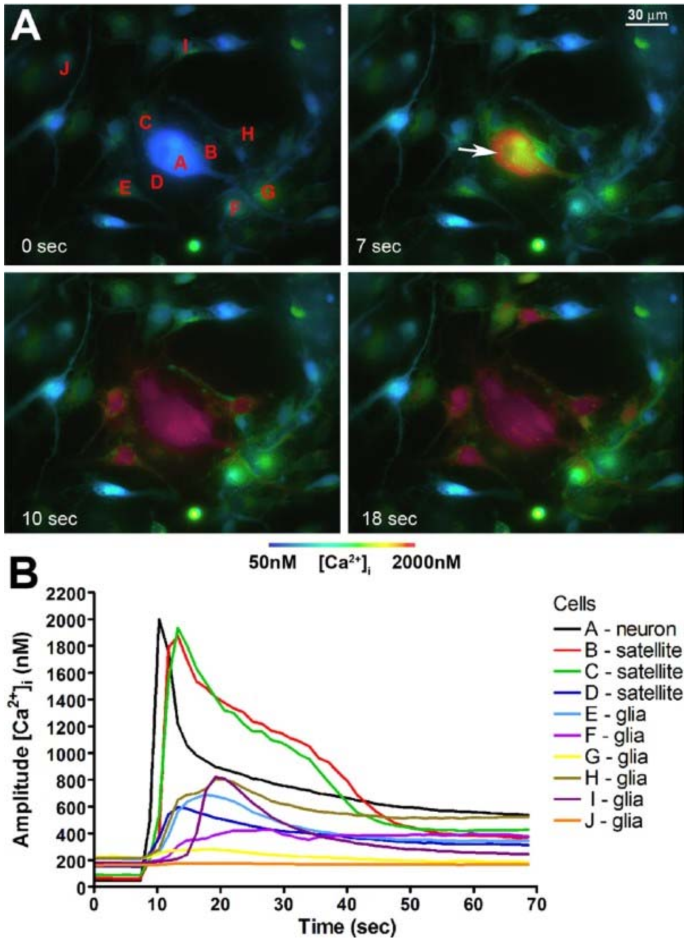
Hypothesis for calcium signal

- Calcium signal evoked by INS exhibits fast and slow components
  - Fast component = Neuronal
  - Slow component = Astrocyte

- Neuronal
  - Dendritic tree projects to layers I, II, III
  - Dendrites have a long response to single stimulus[1]
  - Interneurons highly concentrated in layer I

- Astrocyte Activity
  - High concentration in layer I
  - Spontaneous calcium signal has similar time course to INS induced wave[2]

Image Timecourse



1 - Murayama, M. and Larkum M. E. PNAS 2009
2 - Takata et al. PLoS One 2008

Suadicani et al. Neuron Glia Biology 2009

2-Photon imaging of INS evoked calcium signals

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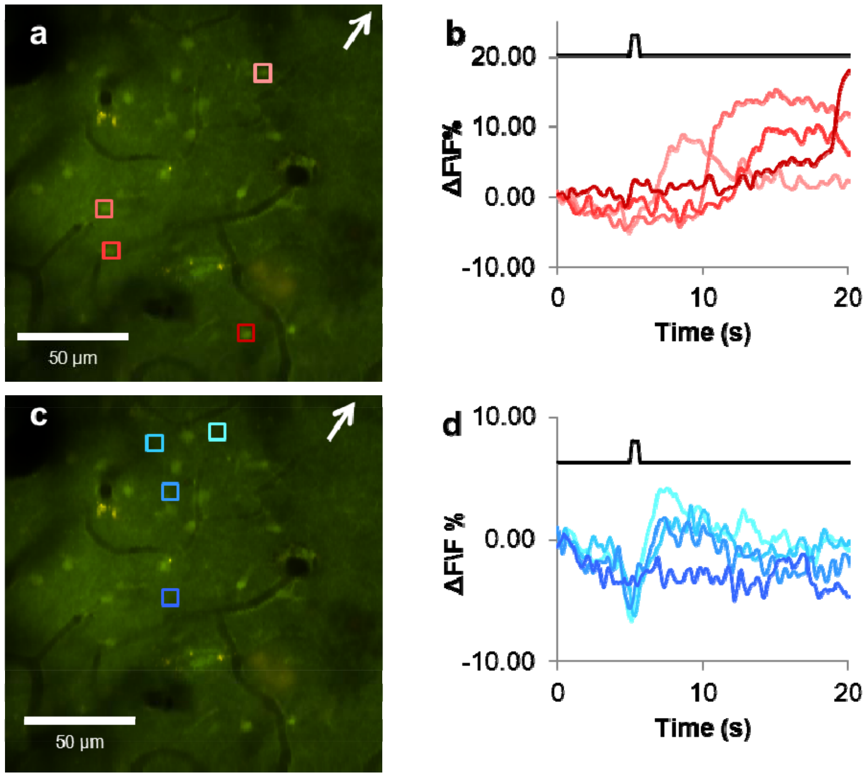
Astrocyte Response

Neuropil Response



Calcium signal propagates through astrocyte network

- Average velocity: 23.3±4.35 µm/s
- Within previously reported values (10 – 60 µm/s)[1]

Local response in neuropil supports activation of apical dendrites

1- Kuga et al. J of Neuro 2010

Summary I

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- Infrared neural stimulation evokes complex calcium signals in rat somatosensory cortex
  - Fast and slow component signal
  - Wave-like propagation
- Identified that INS modulates glutametergic neural activity and astrocyte calcium signaling
  - Signal heavily influenced by astrocytes
  - Glutametergic activity likely from apical dendrites
  - 2-Photon imaging indicates INS evokes calcium signaling in astrocytes and local neuropil response
- There is a need to tease out the cell-cell interactions and identify what happens at the sub-cellular level

MANTIS

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- The innovative features of MANTIS
- Multi-modal Advanced Non-linear and Thermal Imaging System are
  - Inclusion of 5 modalities in a single platform (CARS, SRS, TPEF, SHG and Thermal)
  - Designed to function in backscattered mode (upright microscope)
  - Planned for in vivo and in vitro preparations
  - Capable of introducing neural activation/inhibition (electrically and optically) during these measurements
  - Co-registration of images from the 5 modalities to allow correlation of the varied information.

Current Status

- MANTIS based biochemical imaging of NG108s and other neuronal cells are in progress
- Role of the other types of cells in the brain needs to be studied
- Last Biophysics review meeting triggered a conversation with
  - Valentina Benfenati at CNR about astrocytes
  - Mark Hutchinson at CNBP about microglia

CNR Bologna

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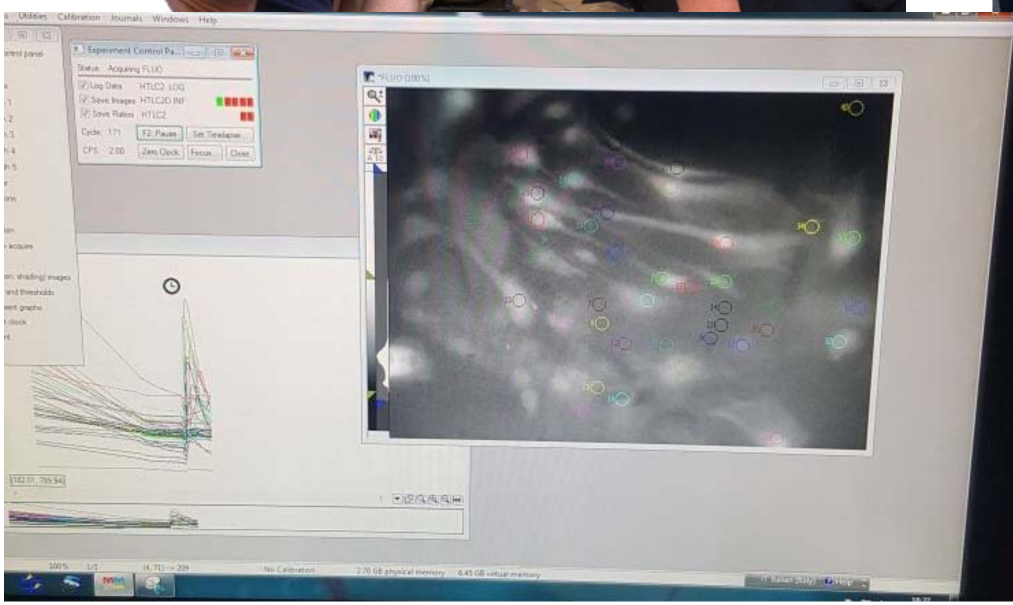
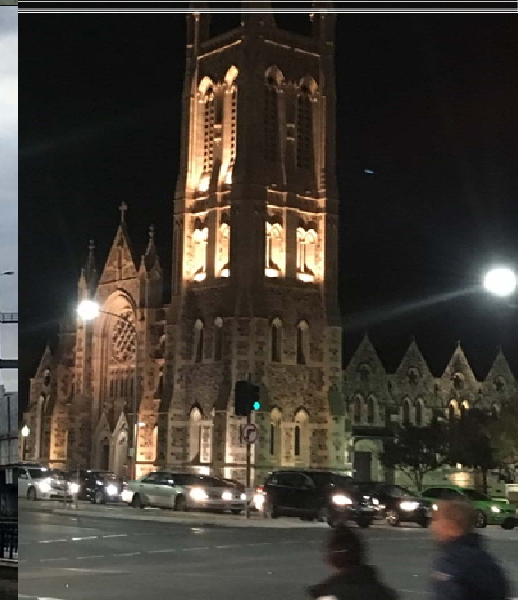


CNBP (Adelaide and Melbourne)



Shedding light on brain microdomains

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International
Multi and interdisciplenary
Multiscale

VANDERBILT UNIVERSITY

<u>Prof.Anita Mahadevan-Jansen (Biophotonics)</u>

Consiglio Nazionale delle Ricerche

<u>Valentina Benfenati (Neuroscience (Astrocytes)</u>

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?

Centre *for* Nanoscale BioPhotonics
ARC CENTRE OF EXCELLENCE

<u>Prof. Mark Hutchinson (Neuroscience and Microglia)</u>

Vision

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- To build a 3 pillar approach - (a) Nanotechnology & Materials, (b) Neuroscience & Neurobiology and (c) Photonics
- Goal is to enhance our understanding of the fundamental processes in the bio-electric system (such as the brain) at multiple scales - from whole body in vivo down to sub-atomic, vibrational scales.
  - Interface with cells for electrical and optical recordings
  - Develop 2D to 3D model systems that mimics in vivo brain structure and function
  - Modulate the system so that new underpinnings of communication and processes are elucidates
  - Image brain/cellular processes down to sub-atomic scales
  - Understand the role of key molecules in brain processes
  - Improve our understanding of cell-material interactions in the context of brain function
  - Understand the modification in behavior and function with disease/disorder

Future Directions

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- Jeff Brooker and Brain Mehl
- Mohit Ganguly, Jeremy Ford, Wilson Adams, Logan Jenkins, Manqing Wang

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Questions?