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Cell Membrane Dynamics in Infrared Nerve Stimulation and Blocking

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14. ABSTRACT
In this grant, we explored and analyzed the underlying fundamental biophysical mechanisms and cell membrane dynamics associated with infrared nerve stimulation and inhibition. Short pulse single pulse interactions were studied to elucidate when excitatory and inhibitory effects occurred in the crayfish motor axon. With detailed intracellular recordings, we investigated infrared nerve inhibition effects of axonal action potentials and the downstream physiological outcomes. We found that stimulation and inhibition can be induced simultaneously by a single infrared light pulse and what biophysical mechanisms are associated with each regime. Temperature-sensitive potassium ion channels were identified to contribute to hyperpolarization that can reversibly and transiently suppress action potential firing. Cell membrane studies examined the passive axonal excitability and photothermal imaging allowed detailed characterization of the thermal diffusion dynamics associated with fibroblast cell models and their cell membrane. Conditions for suppressing action potential initiation and propagation and their downstream motor responses were derived. Overall, our research studies offered novel insights into the ion channel kinetics and cell membrane properties for infrared nerve inhibition and stimulation and provided a multi-modular approach with infrared nerve modulation, electrophysiology and photothermal microscopy.

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Cell Membrane Dynamics in Infrared Nerve Stimulation and Blocking

Pulsed infrared (IR) light has emerged as a new modality that can reliably modulate both neural and muscular activities with advantages over electrical modulation because it is contact-free, spatially selective and MRI compatible. While IR light modulation of excitable tissues has been demonstrated in a wide range of biological systems, in-depth neurophysiological analysis at the single cell level can reveal mechanisms associated with IR mediated excitatory and/or inhibitory effects. Detailed knowledge of the implicated biophysical processes can offer novel insights into the fundamentals of neuronal communication and is crucial to optimize optical technology so that specific physiological outcomes in complex neuronal networks can be induced for clinical translation. It has been demonstrated that spatiotemporal thermal transients induced by absorption of IR light in water and tissue can alter passive membrane properties (capacitance and resistance), ion channel kinetics and intracellular calcium dynamics. This can lead to cell depolarization and compound action potentials in IR nerve stimulation; however, the exact interplay of all the underlying phenomena are complex and still not yet fully understood for IR nerve stimulation and inhibition.

In this grant, we developed an IR nerve modulation and vibrational imaging platform to explore and analyze the associated biophysical mechanisms and cell membrane dynamics. With detailed intracellular recordings, we investigated IR nerve inhibition effects on axonal action potentials (APs), synaptic transmission and the downstream physiological outcomes. Optimizing the IR pulse duration, power and operating regime, we found that short single IR pulses can elicit excitatory and inhibitory effects in the crayfish motor axon. Overall, our research studies offered novel insights into ion channel kinetics and phenomena associated with IR nerve inhibition and stimulation and provided a multi-modular approach including IR nerve modulation, intracellular electrophysiology recordings and photothermal microscopy.

As a first aim, we explored IR light pulse induced inhibition dynamics with intracellular recordings with high temporal resolution. Axonal activities in the unmyelinated motor axon of the crayfish neuromuscular preparation were studied using 2 μm IR light pulses of varying durations, see Fig. 1. These findings can offer high-resolution analysis compared to the majority of previous studies that relied on extracellular measurements, such as single unit recordings, compound action potential measurements or imaging techniques to analyze the impact of IR illumination. The intracellular neuronal activities were monitored with two-electrode current clamp, while the IR-induced temperature changes were simultaneously measured by an open patch technique.

We first focused on studying the action potential (AP) waveforms and how their shape and timing was altered by IR light illumination to link recorded AP properties to passive membrane and ion channel kinetics. Locally initiated APs evoked by current stimulation could be reversibly blocked or suppressed in amplitude and duration by IR light pulses of 20 ms and 500 ms duration (7-13 mW average power, corresponding to locally induced temperature rises up to 8 – 12 °C). Close to the threshold level of depolarization, the IR light pulses could completely inhibit the AP initiation, see Fig. 1(b). For suprathreshold electrical stimulation, the APs were reduced in amplitude and duration and the firing frequency decreased by about 20 % by the IR light pulse. While the AP amplitude and duration were reduced similarly during IR illumination, we reported for the first time that the recovery of the AP duration after the IR light pulse was slower than that of the AP amplitude. The ratio of the decay time constant between the duration and amplitude

recovery was 3.8. The input resistance (consisting of the membrane and axoplasmic resistance) was found to have decreased by 8.8% with the application of the IR light pulses. Overall, this study [1,2] showed that the interplay of temperature-dependent sodium (Na^+) channel inactivation and potassium (K^+) channel activation kinetics combined with membrane resistance changes contributes to the IR light induced inhibition of axonal action potentials.

Building on these results regarding the IR light response mechanism of individual axons, we aimed to investigate how neural function and the corresponding physiological outputs are modulated in complex neuronal networks. Specifically, we were interested how temperature-sensitive targets not only individually but collectively respond to IR light pulses. While the IR light induced modulation of axonal excitability has been studied in more detail, we focused on exploring the function of a neuron as a whole, including its intrinsic excitability, integration of synaptic inputs, and downstream synaptic outputs with pulsed IR light illumination. We investigated the neuromuscular transmission in the crayfish neuromuscular junction [3], which was recorded with intracellular electrodes in muscle cells or macro-patch pipettes on terminal bouton clusters.

For this second aim, locally initiated and propagating APs were evoked, either locally by two-electrode current clamp or at a distance. The IR light pulses completely and reversibly terminated the locally initiated APs firing at low frequencies, which resulted in a block of the synaptic transmission, see Fig. 1(c). However, for propagating APs and locally initiated APs (cf. Figs. 1(a), (d)) firing at high frequencies, the IR light pulses only suppressed, but did not block the amplitude and duration of the APs. The waveform of the suppressed APs of the motor axons could recover after emerging from the localized IR light illumination site, leaving the muscular and synaptic responses unaltered, see Figs. 1(e), (f). This result offered an important insight into the neuromuscular transmission processes. It can help choose targets for IR light application (e.g. distance to muscle when suppressing APs) to achieve a net dominant functional output for a given experimental condition and available optical parameters. When IR light pulses are applied to complex neuronal networks such as mammalian cortexes, the IR light inhibition of locally initiated APs could serve as a basic model for understanding the impact of IR light on the neuronal soma and on the axon initial segment. Assuming neurons in a network are under various levels of synaptic drive, our results suggest that neurons under weak excitatory drive will be inhibited disproportionally compared to those under strong excitatory drive. Since the function of a neuronal network is mainly defined by the frequency and the number of APs, our results [3] offer novel insights how IR light may modulate and bias the output of a network.

Next, we investigated biophysical mechanisms and IR pulse operation regimes for IR mediated stimulation and inhibition. We found that stimulation and inhibition can be induced simultaneously by a single IR light pulse [4]. Temperature-sensitive potassium ion channels were identified to contribute to a hyperpolarization that can reversibly and transiently suppress action potential firing. A single and brief IR light pulse induced a membrane depolarization, whose amplitude depends on the pulse duration and total energy deposition. This membrane depolarization facilitated AP generation when the axon was near firing threshold. For IR pulses with higher energy levels and longer pulse durations (few milliseconds), the depolarization was followed by a membrane hyperpolarization, which could inhibit action potentials. Pharmacological tests suggest a heat sensitive TREK type channel may underlie the hyperpolarization in the crayfish motor axon. Together with short pulse train studies, these findings not only offer insights into the cellular processes associated with IR nerve modulation but also provide guidelines how to optimize the IR light pulse parameters.

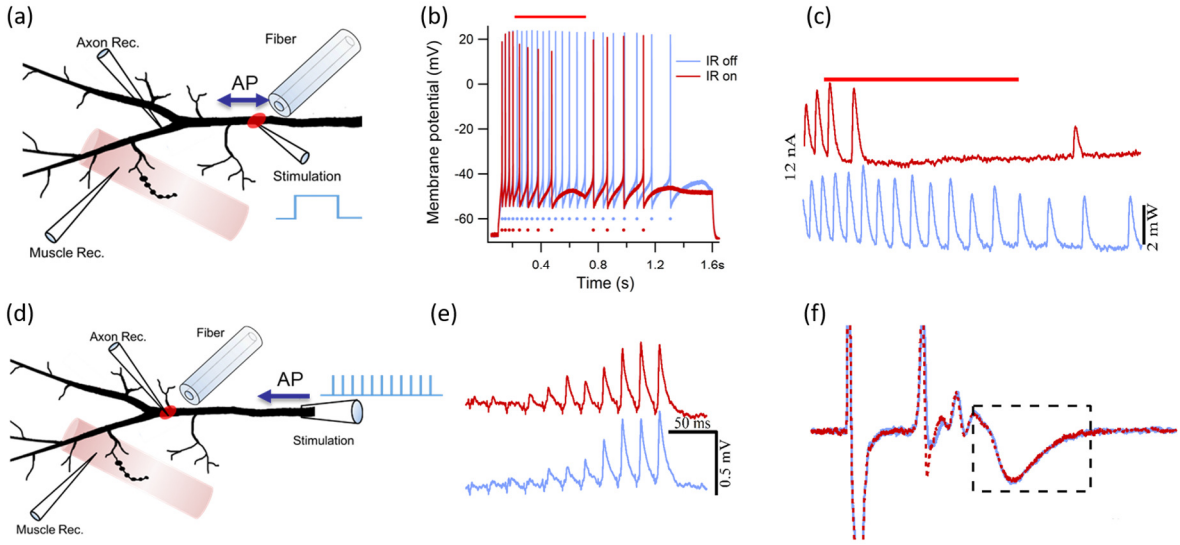

Figure 1: Infrared nerve inhibition and synaptic transmission studies on the crayfish motor axon: (a) Setup of IR nerve inhibition experiments. (b) Locally initiated action potentials are completely inhibited by IR light pulses (red, control without IR light in blue), (c) leading to blocking of the downstream inhibitory postsynaptic potentials. The red bars indicate the timing and duration of the IR light pulse. (d) Setup for propagating APs that are locally suppressed in their amplitude and duration by IR light pulses. (e) The excitatory postsynaptic potentials and (f) the endplate currents show no difference (red traces with IR light), indicating that the suppressed propagating APs recovered.

While changes in neuronal waveforms were detected with electrophysiology and the combination of pharmacological studies and fluorescence imaging offered additional insights into underlying dynamics in the conducted studies, structural and chemical conformation changes could not be monitored. Thus, to identify chemical and molecular changes present in tissues and cells, we developed Vibrational Infrared Photothermal and Phase Signal (VIPPS) imaging as a label-free, non-destructive, contactless microscopy technique, see Fig. 2. This method enables sub-cellular imaging at sub-diffraction limited spatial resolution and we demonstrated direct cross-registration of our images with fluorescence microscopy for the first time, cf. Figs. 2(b) – (g).

Our system addresses one of the existing challenges in vibrational spectroscopy and microscopy of resolving features with overlapping absorption profiles by differentiating features based on different thermal diffusion properties. So far, most mid-IR imaging of cells at the subcellular level has been performed on isolated cells grown directly on the imaging windows. Due to similar absorption properties between intracellular and extracellular matrix proteins, there are currently few IR studies undertaken on tissue engineered models, in which cells are grown in two and three dimensional extracellular matrices. We demonstrate for the first time high contrast imaging of engineered tissue models consisting of fibroblast cells grown in a protein rich extracellular matrix composed of collagen [5]. Nano-sized cell membranes could be localized to well below the optical diffraction limit since the membranes were found to act as thermal barriers with changes in the rate of heat transport near the cell plasma and nuclear membrane, see Figs. 2 (h), (i). Further, by studying the Amide I band, we could identify regions of protein accumulation with different secondary structural conformations. VIPPS therefore offers a powerful approach not only for chemical and molecular analysis but also to investigate the previously not accessible cell development in engineered fibroblast tissue models.

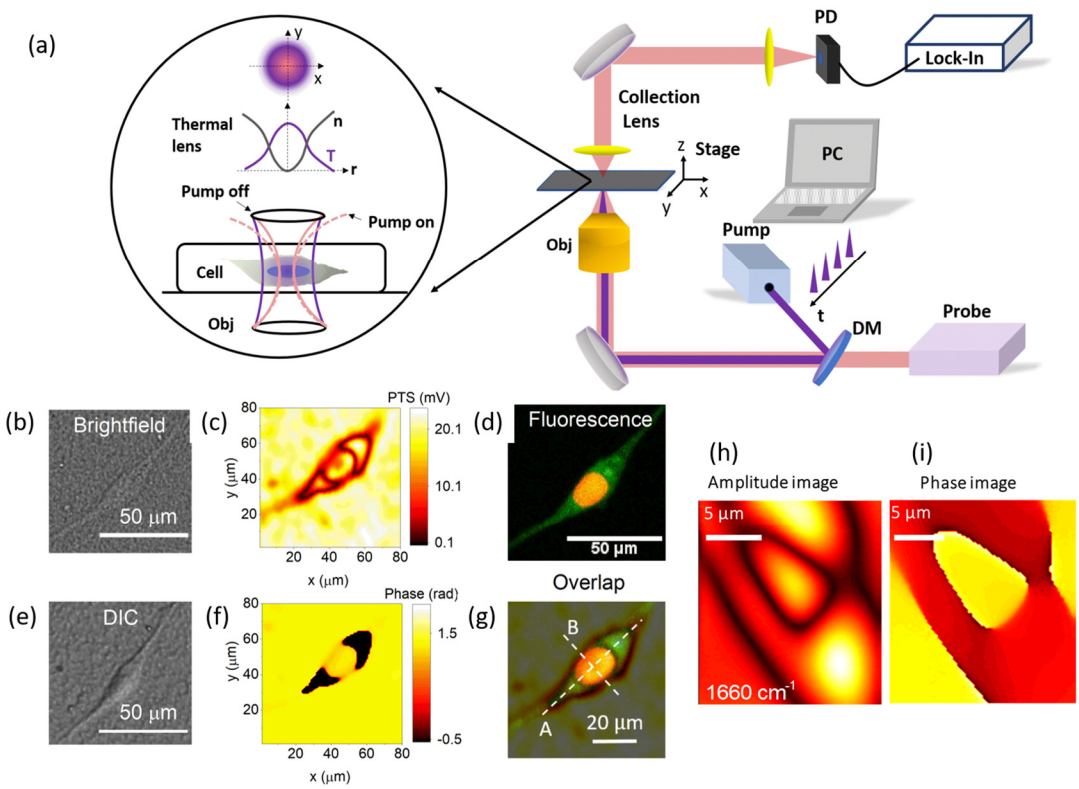

Figure 2: (a) Setup of VIPPS microscope. The pump and probe beam are combined in a dichroic mirror (DM) and collinearly focused onto the sample. The modulated forward scatter of the probe laser is detected in a heterodyne measurement on a photodetector (PD) with a lock-in amplifier (Lock-In) and separated into a photothermal amplitude and phase signal. The zoom-in illustrates the thermal lens generation in the sample due to the absorbed pump beam. (b) – (g) Cross registration of VIPPS imaging of collagen embedded fibroblast cells with other imaging modalities. Brightfield image of (b) labeled mouse fibroblast cell. Photothermal signal (PTS) images of (c) labeled cell at a wavenumber of 1660 cm^{-1}. (d) Fluorescence image with GFP in green and nucleus RFP in red. (e) DIC image. (f) Photothermal phase image. (g) Overlap between photothermal and fluorescence image. Unlabeled human fibroblast cell amplitude (h) and phase (i) image, clearly showing the position of the cell and nucleus membrane as a dark line in (h) with high corresponding contrast in the phase (i).

Publications

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