DEMONSTRATION OF AN OPTOGENETIC NEURONAL CONTROL INTERFACE

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ABSTRACT

Improved nerve interface approaches are sought for prosthesis control and sensory feedback as well as visceral organ study/modulation. Optical approaches that can read-in and read-out neural activity have advantages over electrode-based systems in terms of selectivity and non-invasiveness. To address limitations of existing nerve interface designs, we present an optical approach capable of reading activity from individual nerve fibers using activity-dependent calcium transients. Here we demonstrate the feasibility of using activity-dependent calcium transients to a control prosthetic hand. This work provides a proof-of-concept for an optogenetic nerve interface demonstrating as it does our ability to read-out signals at the axonal scale in real-time and apply it to a devices control.

INTRODUCTION

We are developing a Bidirectional Optogenetic Neural Interface to read-in and/or read-out action potentials from a nerve with the goal of creating a neural interface that is selective yet minimally invasive to the nerve. There are significant drawbacks to current nerve interface approaches. They either lack specificity - they use nerve cuff electrodes, such as the Flat Interface Nerve Electrode (FINE) Array**[1]** that must sit on the outside of the nerve and measure signals originating inside the nerve bundle, or they involve penetrating the nerve with needle electrodes - such as Longitudinal Intrafascicular Electrodes (LIFE)**[2]** or the Utah Slant Array**[3]**. Penetrating electrodes tend to be hard and rigid, resulting in a stiffness mismatch that causes irritation and necrosis, decreasing longevity. Instead of using electrical interfacing with the nerve, we will use light activated ion channels (opsins) and fluorescence protein Ca^{2+} or voltage indicators that allow stimulation and recording of action potentials of specific afferent or efferent neurons using viral vector transfection. Our Optogenetic Neuronal Interface is based on a fiber optic coupled miniature two-photon microscope with electrowetting adaptive optics **[4-7].**

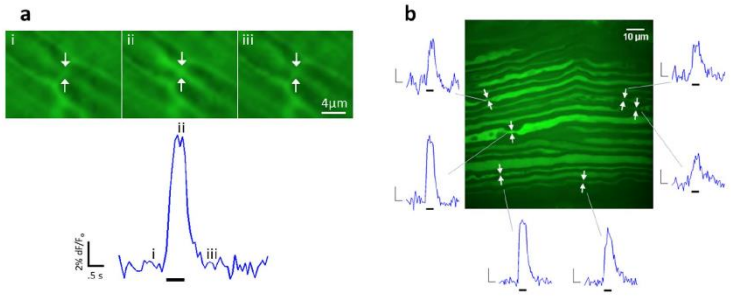


Figure 1: *Action potential elicited calcium signal detection in tibial nerve axon nodes of Ranvier [1].*

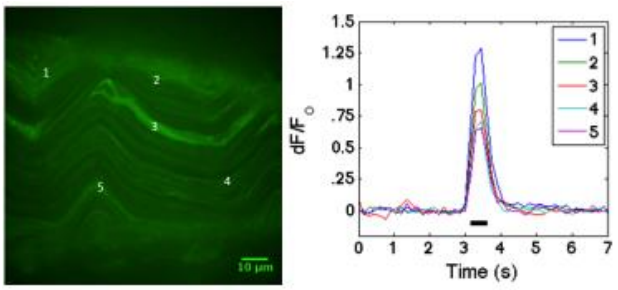


Figure 2: *Action potential elicited calcium signal detection in vagus nerve axons with GCaMP6f.*

The Bidirectional Optogenetic Neuronal Interface system is based on the principal of two-photon (TP) excitation**[8,9].** In TP excitation, a fluorophore is excited by short pulses of laser light. TP excitation offers intrinsic axial cross sectioning because the process only occurs at the focus of the objective lens. The technique offers resolutions of 175 nm lateral and 451 nm axial for 900 nm light focused with a 1.2 NA objective. This approach, when combined with a lateral scanning head, provides axon scale resolution that can be used to selectively interrogate an axon while excluding signals from the remaining tissue.

Peripheral nerve *read-out* of activity using calcium-sensitive fluorescent reporters: We have demonstrated read-out of genetically expressed activity-dependent calcium indicators, such as GCaMP6f, has been demonstrated in other work in vitro**[10]** [**Figure 1** & **Figure 2**]. We have also shown how a viral vector might be used as a mechanism for delivery of long-term optical protein expression in mouse neurons for optical read-out **[11]. Selective photo-stimulation (*read-in*) in nerve:** We have also demonstrated the ability to selectively read-in (or stimulate) to nerves optically [**Figure 3**].

Here we further demonstrate the feasibility of using optical approaches for prosthesis control by imaging the axonal fluorescence produced by action potentials travelling in an in vitro mouse nerve and using the change in image intensity to drive a prosthetic hand in real-time. This work provides a proof-of-concept for an optogenetic nerve interface demonstrating as it does our ability to read-out signals at the axonal scale in real-time and apply it to a devices control.

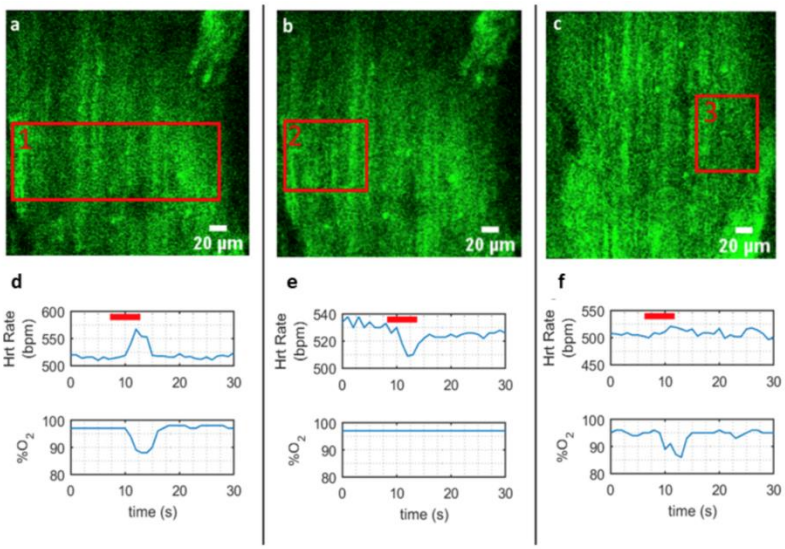
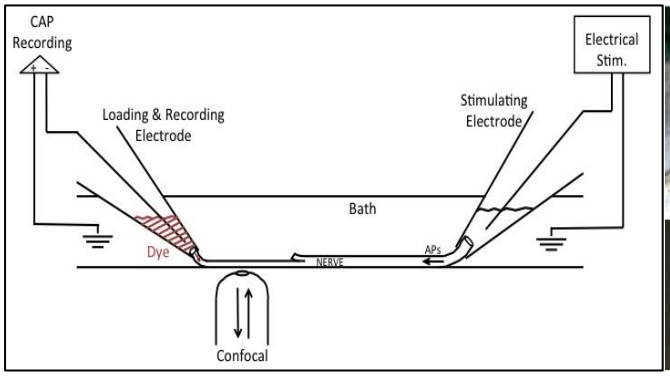


Figure 3: *Spatially selective photo-stimulation elicits differential vitals responses. (a-c) Regions (1-3) of 1040nm photo-stimulation within the cervical vagus nerve of an anesthetized ChAT-GCaMP6s mouse. (d-f) Corresponding vitals responses to photo-stimulation; region 1 elicits an increase in heart rate and a decrease in oxygen saturation; region 2 elicits a decrease in heart rate and no change in oxygen saturation; region 3 elicits a decrease in oxygen saturation. 1040nm stimulus was applied for 4 seconds with 20 ms pulses at 20 Hz.*

METHODS

Nerve Preparation: The sciatic nerve and its tibial nerve branch are excised from adult wild type mice and loaded from the tibial end with a synthetic calcium indicator (2 mM Calcium Green-1 Dextran, ex/em = 506/531 nm) dissolved in a buffer containing 130 mM KCl and 30 mM MOPS, pH 7.2 in *accordance with Supplementary Figure 1, Fontaine et al, 2017* ***[11]*** **(Figure 4) .** The tibial end is freshly cut in a zero-calcium buffer to ensure open axon cylinders before being suctioned into a tight-fit electrode with the dye buffer to facilitate longitudinal axonal dye-loading via diffusion and/or axoplasmic transport. The suction electrode on the tibial nerve also serves to record electrical activity within the nerve. The sciatic end of the nerve is drawn into a suction electrode for electrical stimulation of compound action potentials (CAPs). All experiments were performed in accordance with our Institutional Animal Care and Use Committee (IACUC) regulations and approved protocol.



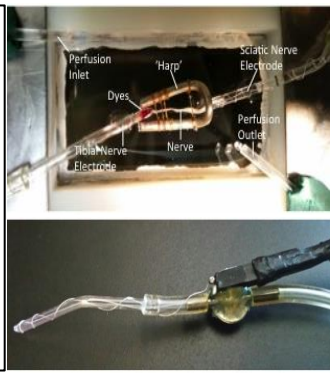


Figure 4: *Nerve dye-loading, electrophysiology & imaging configuration*

Electrophysiology: CAPs are generated and recorded throughout the experiment using 50 µs square pulses to confirm and monitor nerve viability. The stimulation voltage threshold for maximum CAP amplitude is determined. CAP amplitudes were monitored throughout the duration of the incubation period, to confirm stable nerve health.

Optical Imaging/Recording: Dye labeled axons were imaged in a region of nerve near the tibial recording electrode. The nerve was gently pressed to the optical glass of the chamber with low-tension silk strings attached to a small weight for imaging on an inverted microscope. Placement of the small 'harp-like' device did not affect the CAP. Fluorescence imaging was performed on a spinning disk confocal microscope (Intelligent Imaging Innovations, Marianas). A 515nm laser line was used to excite the Calcium Green-1. Pixels were binned (2x2) to improve the frame read-out time for fast imaging. To record calcium transients, time-lapse images were acquired at 12-20Hz (motor update rate), during which the nerve was stimulated by an electrical stimulator triggered via TTL pulses from the microscope. Fluorescence was imaged onto an EMCCD camera (Photometrics Evolve) through a 525/50nm emission filter. Images were collected with a 63X, 1.4NA oil-immersion objective lens. Photobleaching of the signal was kept minimal by the reduction of laser power and exposure, and any mild decay due to photobleaching was not removed.

***Prosthetic Hand Modification*:** The electronics in the original Bebionic v2 hand (RSL Steeper, UK) **(Figure 5)** were replaced with a custom motor controller system (Sigenics Inc., Chicago, IL) and included a central Arduino controller board and six

satellite boards referred to as 'penny boards' (as they were the size of a penny). Each penny board was connected by a four-wire I2C bus with each board associated with an individual finger for finger flexion/extension with two for the thumb to drive flexion/ extension and abduction/ adduction. For velocity control motor commands indicating the speed and direction of motion for the driven finger were sent from a Matlab script to the Arduino (SparkFun Electronics, Boulder, CO) which converted the serial commands into I2C commands. Position encoder values from the prosthetic finger motor were recorded simultaneously and converted to joint angle measurements post hoc. For the Bebionic the fingers can flex from 0-95º and run at a max speed of about 2 rads/sec. For position control, desired finger position is sent over the I2C bus to the motor controller and a local on-board PID loop handles positioning of the finger.

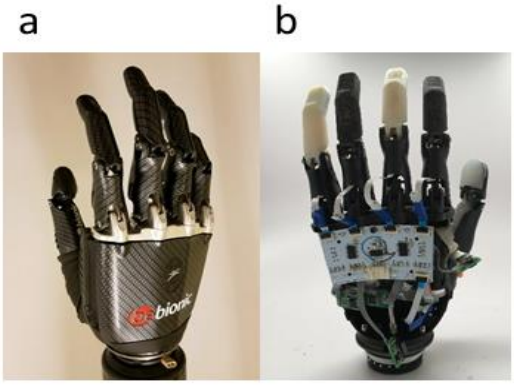


Figure 5*: (a) Commercially available Bebionic v2 hand (b) Modified Bebionic hand used for finger actuation experiments. Custom electronics were installed in order to control individual motors within the prosthesis. The Bebioinc has motor encoders that can measure finger position and be used in closed -loop control..*

Control Interface and Method: A standard laptop computer running SlideBook 6.0 software (Intelligent Imaging Innovations) took the raw time-lapse images from the microscope and sent them to a custom Matlab program (Mathworks, MA) which calculated the intensity of the region-of-interest (ROI) on the selected axon and based on the computed value sent commands to the motor controllers of the prosthetic hand via a serial link. A setup function in the Matlab script established the serial communication between the computer and the prosthetic hand. A second function received the time-lapse captures from SlideBook and translated the image data into an optical signal by averaging nodal ROI pixel intensities in each frame. The change-in-intensity is the control signal-of-interest. We see a baseline intensity for zero firing rate and a 15-18% for a firing rate of 125Hz. Since baseline is not constant, we set a threshold of 2%. This gives us our command signal range: for 0-125hz we expect a 2-18% dF/F which should map to 0-100% of our command signal for the motor. Initially we mapped the optical signal to the prosthetic finger velocity in an open-loop velocity control paradigm that is standard-of-care [**12**]. The hand was set up in a "cookie-crusher" configuration so single-site control could be used. In this case when the amplitude of the signal rises above the optical signal threshold the finger was driven in flexion at a speed proportional to the change-in-intensity. Velocity gains were adjusted to achieve a full range of motion.

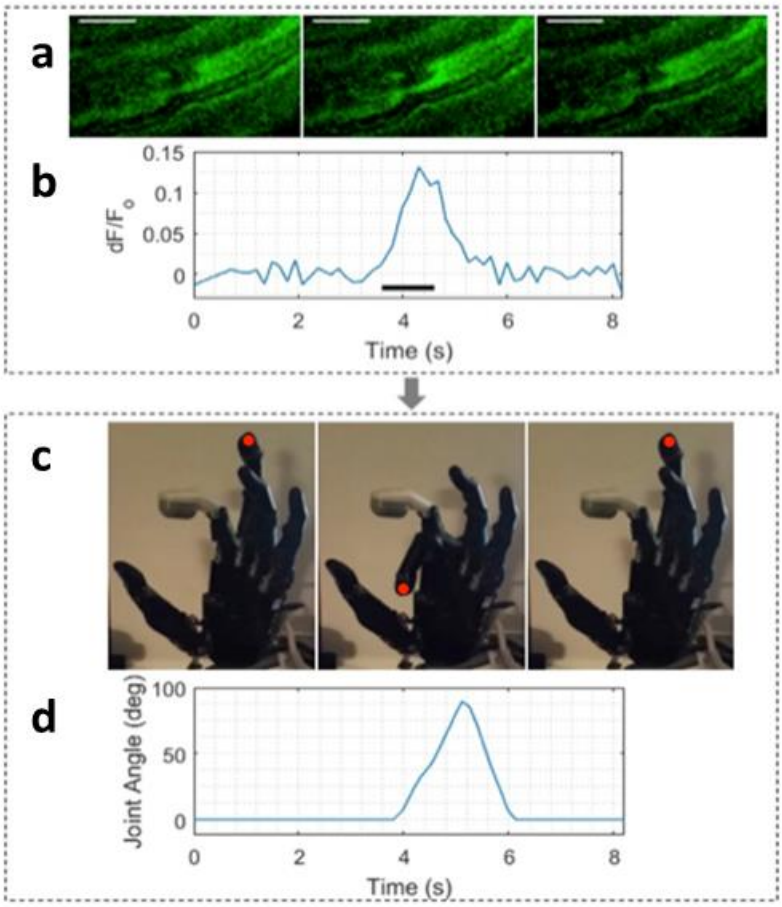


Figure 6*: Real-time prosthetic digit actuation by action potential evoked calcium fluorescence signal in a peripheral nerve axon. (a) Confocal images of a CalciumGreen-1-Dextran-loaded axon node-of-Ranvier used to control finger actuation, shown before, during and after the activity-induced fluorescent signal (scale bar 10μm). (b) Quantitative trace of the calcium-fluorescence signal in response to the 1s, 100Hz train of action potentials (black bar). (c) Prosthetic hand's middle finger flexes and extends under control of the optical signal from panel b. Virtual red dot denotes the tip of the middle finger driven in the experiment. (d) Corresponding finger joint angle illustrates digit flexion occurring during supra-threshold optical control signal.*

RESULTS

An axon which fluoresced in response to the simulated motor command was selected. The calcium response originated at the center of the selected node-of-Ranvier and propagated bi-directionally into the internodal region of the axon. The nodal region, which was used for the motor command signal, showed approximately

12% change (dF/F) in fluorescence intensity. This signal amplitude was comparable to that achieved in prior work for an action potential pulse train frequency of 100 Hz [12]. Since an open-loop velocity control paradigm was employed, the digit was driven in flexion for the duration of the supra-threshold optical signal at a rate proportional to the signal intensity (about 1.5 rads/sec). Upon cessation of the command signal the finger is driven in extension at max speed (2 rads/sec) until the hand is fully open, per the cookie-crusher paradigm (**Figure 6).**

Proportional Control was demonstrated using previously recorded signals collected for a range of action potential pulse trains frequencies which were then used post-hoc to drive fingers in a position-control paradigm. As characterized in earlier work **[10]** average fluorescence amplitudes of sustained stimulus are linearly modulated by the action potential pulse train frequency. Such graded signals therefore encode intensity of the motor command. The fingers flexed to a position proportional to the intensity change produced by action potential pulse train frequency which was modulated between 25-125Hz (**Figure 7).**

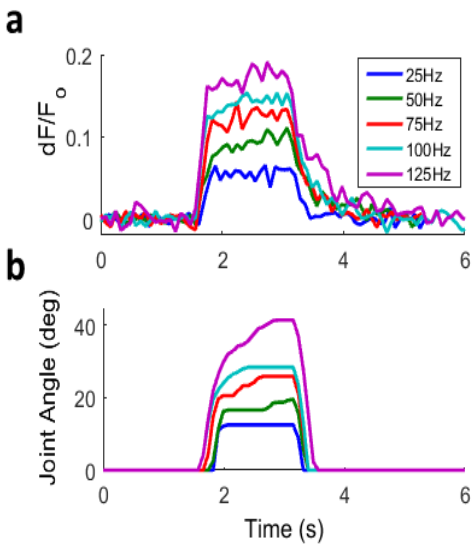


Figure 7: *Motor flexion of the prosthetic digit is graded by the action potential pulse train frequency of the optical calcium signal. (a) Graded calcium-fluorescence transients in an axon node-of-Ranvier in response to a range of action potential frequencies. (b) Resulting finger joint angles of the prosthetic finger as driven with control signals from panel a.*

While previous studies have optically stimulated peripheral nerve axons for functional modulation of motor units [13-15] using the light-activated ChannelRhodopsin2 (ChR2) there is an absence of literature describing the use of optically obtained signals from peripheral axon activity for device control. However, the range of action potential frequencies used to drive the prosthesis in this study is within a physiologically relevant range since action potential pulse train frequency typically varies between 15-500Hz (in the non-refractory range). The control signal was derived from a 1 second, 100 Hz action potential burst would likely correspond to a low-side motor command. The present experiments demonstrate the potential for read-out and control using an *ex vivo* model. In other work [11] we have demonstrated that similar signals (dF/F) can be obtained using a genetically encoded calcium indicator, GCaMP, with a retro-viral (rAAV) delivery.

CONCLUSIONS

Proof-of-concept for an optogenetic nerve interface is demonstrated by showing our ability to read-out signals at the axonal scale in real-time and apply it to the control of a prosthetic hand. Optical signals generated by frequency modulated action potentials in an axon were transduced to provide proportional prosthetic finger actuation.

ACKNOWLEDGEMENTS

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