INVITED REVIEW

Optical read-out and modulation of peripheral nerve activity

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Abstract
Numerous clinical and research applications necessitate the ability to interface with peripheral nerve fibers to read and control relevant neural pathways. Visceral organ modulation and rehabilitative prosthesis are two areas which could benefit greatly from improved neural interfacing approaches. Therapeutic neural interfacing, or 'bioelectronic medicine', has potential to affect a broad range of disorders given that all the major organs of the viscera are neurally innervated. However, a better understanding of the neural pathways that underlie function and a means to precisely interface with these fibers are required. Existing peripheral nerve interfaces, consisting primarily of electrode-based designs, are unsuited for highly specific (individual axon) communication and/or are invasive to the tissue. Our laboratory has explored an optogenetic approach by which optically sensitive reporters and actuators are targeted to specific cell (axon) types. The nature of such an approach is laid out in this short perspective, along with associated technologies and challenges.

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Introduction
A suitable neural interface is sought for numerous applications, including prosthesis/rehabilitation, medical treatment, and better understanding of physiological systems. Advanced prosthetic hands for limb replacement have the ability to mechanically substitute the degrees of motion of the biological hand; yet there exists no nervous system interface that allows for their full control/articulation and sensing. In the viscera, there is broad effort to understand and control neural pathways to address disorders affecting these organs. The range of disorders that could potentially be studied and treated by interfacing with visceral nerve fibers is extensive. Vagus nerve stimulation (VNS) is known to elicit numerous clinically relevant modulatory effects; reduction of seizure frequency in intractable epilepsy (Gurbani et al., 2016) and attenuation of inflammation in rheumatoid arthritis (Koopman et al., 2016) are just two examples that have advanced to human treatment. The precise pathways that underlie function are poorly understood in many cases, however, and current therapeutic nerve stimulation is non-specific. These applications require the ability to decipher and modulate activity with a high degree of specificity (*i.e.*, at the fascicle or individual axon level) in order to read and affect highly targeted activity. Furthermore, the neural mapping of structure and function in many systems needs to be established in order to implement precise therapeutic and restorative therapies.

Electrode-based nerve interfaces are quite limited in this respect; a primary limitation is their poor neural specificity. Cuff electrodes, such as the Flat Interface Nerve Electrode (FINE) Array (Schiefer et al., 2013; Tan et al., 2015), have demonstrated useful fascicle-specific stimulation, yet read-out and read-in with individual axons across the nerve is infeasible. Other electrode designs, such as the Utah Slant Array (Normann et al., 2005), impale the nerve. This often leads to tissue necrosis and scar tissue, resulting in poor longevity of the interface, while still incapable of single axon resolution. Given the needs of these applications and the limitations of current electrical interfaces, a novel approach is desired that can read-out (sense) and read-in (stimulate or silence) neural activity to specific axons within a nerve, for both the mapping of clinically relevant circuits and their functional manipulation.

Optics-Based Nerve Interfacing
An optical (optogenetic) approach is a promising methodology to meet the goals of a functional nerve interface. Single axon communication is feasible since sub-micron resolution is easily achievable with optical systems. Optogenetic reporters can facilitate activity read-out by sensing either membrane voltage or intracellular calcium, while optogenetic actuators such as ChannelRhodopsin2 (ChR2 or its variants)

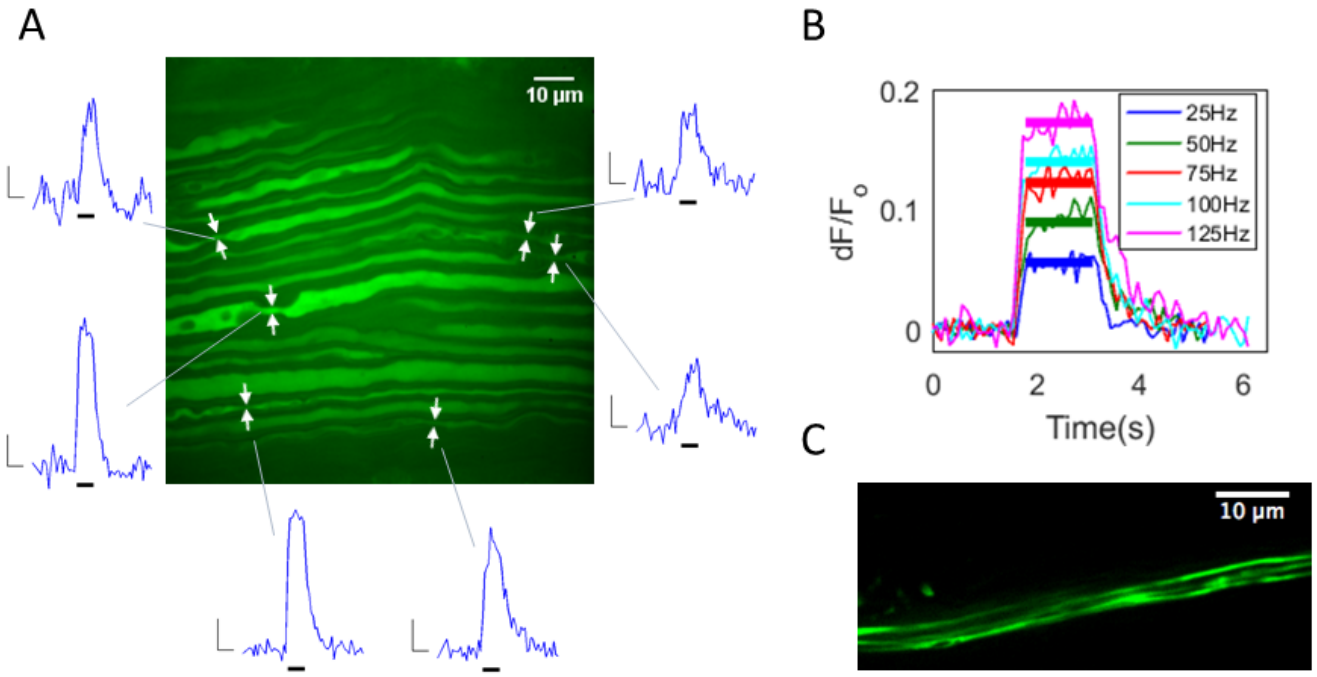

Figure 1 Imaging of neural activity with calcium sensitive fluorescent sensors.
(A) Field of mouse tibial nerve axons loaded with the synthetic calcium indicator Calcium Green-1 Dextran. At least six nodes of Ranvier yield a calcium-coupled fluorescence change in response to a 1 s train of action potentials (100 Hz). Signal amplitudes among the six nodes range from 11–24%. Black bar indicates action potential stimulus. Inset scale bars: 1 second and 5% signal change. (B) Frequency-modulated calcium fluorescence traces from a peripheral nerve axon node of Ranvier with bars indicating mean steady-state amplitude. (Data from panels A & B are from Fontaine et al., 2017.) (C) Image of the genetically expressed calcium indicator GCaMP6f transduced in axons of the peripheral nerve by intramuscular injection of an adeno-associated viral (AAV) vector.

and HaloRhodopsin can stimulate or silence activity respectively in a light-driven fashion. Current voltage-sensitive fluorescent proteins are not suitable for *in vivo* application due to insufficient signal strength. However, calcium sensors such as the genetically encoded calcium indicator GCaMP6 have been shown to provide substantial signal in response to action potentials *in vivo* (Chen et al., 2013; Badura et al., 2014). Activity-dependent calcium-fluorescence transients have recently been demonstrated in peripheral nerve axons ex *vivo*, where signal dynamics were characterized with respect to action potential parameters using a synthetic calcium indicator molecule (Fontaine et al., 2017). In the peripheral nerve, myelinated axons produce a calcium signal at the node of Ranvier in response to activity (**Figure 1A**), while smaller diameter fibers exhibit relatively non-localized calcium transients along segments of axon. We have measured calcium signals with both synthetic (Calcium Green-1 dextran) and genetically encoded (GCaMP6f, GCaMP6s) sensors in individual axons at the single action potential level in the intact *ex vivo* peripheral nerve. The amplitude and duration of elicited calcium signals are well correlated to the underlying neural activity: signal amplitude is graded in proportion to the frequency and number of action potentials in a burst/train (**Figure 1B**), and signals persist for the duration of an action potential train (Fontaine et al., 2017). Both nodal signals from larger myelinated axon nodes, and non-localized signals in small-diameter axons have also been recorded in the vagus nerve using the genetically encoded calcium indicators GCaMP6f and GCaMP6s (our unpublished data).

This work has demonstrated the potential of using activity-dependent calcium transients as a read-out of neural activity in individual axons. Optical read-in has been demonstrated in the *in vivo* rodent peripheral nerve in prior studies which incorporated blue light activation of genetically targeted axonal ChR2 for activation of motor units (Llewellyn et al., 2010; Towne et al., 2013).

Requisite Methods and Challenges

Fiber-coupled optical device

Imaging of activity within the *in vivo* nerve will require miniaturized fiber-coupled microscopes (FCMs) capable of delivering/detecting light between a laser and neural targets. The incorporation of a high-density optical fiber bundle enables lateral resolution for imaging at the distal end of the optical fiber, and an electro-wetting lens (Terrab et al., 2015) can facilitate

rapid axial scanning with no moving parts, to achieve three-dimensional imaging. Such devices are in development, including a system by Ozbay et al. (2015), which has demonstrated three-dimensional two-photon imaging in the *in vivo* mouse brain (manuscript under review). Imaging activity across numerous neuronal processes in this manner is not trivial due to the challenge of exciting and detecting optical signals with meaningful brightness and resolution through the device. To be functionally useful, the system needs to collect enough signal, while scanning over a sufficient volume of tissue/axons, at an appropriate speed. It is likely that sensors such as GCaMP will continue to be improved in the future with enhancements in sensitivity and dynamic range, rendering optical signals of activity even more robust. The continued development of red-shifted sensors and actuators (Klapoetke et al., 2014; Dana et al., 2016) may also enable expanded multi-wavelength systems.

The optical read-in to single axons is also technically challenging, but read-in to a population or subset of axons is relatively less challenging. A single optical fiber can be used to deliver light for broad illumination of the nerve and achieve specificity determined by the genetic targeting of the actuator, and by targeting spectrally separate opsins to different axonal populations. (The cross-sectional portion of nerve that can be reached with sufficient power is dependent on the size of the nerve and the optical penetration). In both cases, a nerve 'cuff' could position the distal end of the FCM or optical fiber to abut the neural tissue.

Adeno-associated viral vectors

The genetically encoded protein that serves as the sensor or effector must be delivered to the cell type of interest. Adeno-associated viruses (AAVs) have become a widely used vector for gene delivery, with numerous AAV based gene therapies currently in clinical trials, and one approved by the European Medicines Agency (Naso et al., 2017). AAV particles, lacking viral DNA and loaded with genes of interest, can provide a safe and effective method for gene delivery, with relatively limited immunogenic and mutagenic concerns. AAVs are poorly immunogenic compared with other viruses, yet potential responses to its viral components and the delivered transgene/protein are challenges that need to be addressed. AAVs can be tailored for specific applications through serotype and promoter selection, as well as consideration of serotype specific transduction/transport properties. Many serotypes undergo axonal and trans-synaptic transport, thus enabling a myriad of potential transduction strategies. AAVs have been employed in numerous studies to transduce neurons and neuronal processes of the central and peripheral nervous systems with optogenetic proteins (Andrasfalvy et al., 2010; Kravitz et al., 2010; Towne et al., 2013; Christensen et al., 2016; Williams et al., 2016). Using intramuscular injection (anterior tibialis muscle) and retrograde transport, our group delivered GCaMP6f to axons of the common peroneal nerve (**Figure 1C**) and detected robust action potential-elicited calcium transients in these axons (Anderson et al., 2017). Other studies have demonstrated the functional delivery of ChR2 to peripheral axons (Towne et al., 2013; Williams et al., 2016). Although transgene delivery would impose a significant regulatory hurdle for clinical use, the AAV-mediated targeting of optically-sensitive reporters and actuators to functionally pertinent axons may be clinically achievable.

Discussion

An optical approach to nerve interfacing has the potential to open up new avenues of study and therapy. Established studies demonstrate the feasibility to both read activity in axons of the PNS with calcium-sensitive reporters and actuate them with light activated opsins. Bioelectronic medicines, which strive to modulate therapeutically relevant processes by intervening with their neural pathways (Birmingham et al., 2014), may benefit greatly from optogenetic techniques. The methods described in this paper for axon-specific communication could be used to both identify axon subtypes that are pertinent to given functions, and subsequently monitor and/or modulate them. A closed loop system could activate or silence genetically identifiable axons of an organ in response to a real-time reading of molecular markers in the blood (glucose, for example) or neural activity itself. Given the frequency-modulated nature of activity-dependent calcium signals, neural frequency or 'strength' in a given pathway can be inferred. Once such a fluorescence signal is detected from an axon, it can be used in a variety of ways, including as a command signal to a prosthetic actuator. Motor axon interrogation in the limb may be useful for the control of artificial hands/limbs (Fontaine et al., 2018), provided that the interface can monitor a sufficient number of units at an appropriate speed. Sensory feedback may also be possible with the induction of activity in proprioceptive or mechanoreceptive afferents.

With the current and developing palette of reporters and actuators, and the capability of specific genetic targeting, there are numerous ways in which an optogenetic system could be employed in the peripheral nervous system for the study of neural pathways, as

well as interventional therapies such as physiological system modulation and device control.

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