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THEORETICAL PRINCIPLES UNDERLYING OPTICAL STIMULATION OF MYELINATED AXONS EXPRESSING CHANNELRHODOPSIN-2

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Abstract

Numerous clinical conditions can be treated by neuromodulation of the peripheral nervous system (PNS). Typical electrical PNS therapies activate large diameter axons at lower electrical stimulus thresholds than small diameter axons. However, recent animal experiments with peripheral optogenetic neural stimulation (PONS) of myelinated axons expressing channelrhodopsin-2 (ChR2) have shown that this technique activates small diameter axons at lower irradiances than large diameter axons. We hypothesized that the small-to-large diameter recruitment order primarily arises from the internodal spacing relationship of myelinated axons. Small diameter axons have shorter distances between their nodes of Ranvier, which increases the number of nodes of Ranvier directly illuminated relative to larger diameter axons. We constructed “light-axon” PONS models that included multi-compartment, double cable, myelinated axon models embedded with ChR2 membrane dynamics, coupled with a model of blue light dynamics in the tissue medium from a range of different light sources. The light-axon models enabled direct calculation of threshold irradiance for different diameter axons. Our simulations demonstrate that illumination of multiple nodal sections reduces the threshold irradiance and enhances the small-to-large diameter recruitment order. In addition to addressing biophysical questions, our light-axon model system could also be useful in guiding the engineering design of optical stimulation technology that could maximize the efficiency and selectivity of PONS.

Keywords

optogenetic; node of Ranvier; action potential; ChR2

INTRODUCTION

Optogenetic neural stimulation is a powerful technique for providing insight into the function of the nervous system (Deisseroth, 2011). Recently, the optogenetic toolbox has

CONFLICT OF INTEREST

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been applied in the peripheral nervous system (Llewellyn et al., 2010; Sharp and Fromherz, 2011). Peripheral optogenetic neural stimulation (PONS) of the motor system preferentially activates small diameter axons which are typically connected to fatigue-resistant motor units (Llewellyn et al., 2010). In contrast, traditional electrical stimulation strategies preferentially activate large diameter axons that typically innervate fatigue-prone motor units (Singh et al., 2000). The biophysical basis for the large-to-small diameter recruitment order of myelinated axons via electrical stimulation is primarily due to the internodal spacing relationship (Nilsson and Berthold, 1988). When an extracellular electrical stimulus is applied to the axon, the larger internodal spacing of larger diameter axons generates a larger second difference of the extracellular voltage distribution at nodes of Ranvier (McNeal, 1976). This results in a larger transmembrane driving force in larger diameter axons for a given level of current injection from the stimulating electrode (Rattay, 1989).

The biophysical mechanism(s) of the small-to-large diameter recruitment order for optogenetic stimulation of motor axons are less clear. The goal of this study was to generate computational models of PONS to characterize the basic features of action potential initiation in myelinated axons expressing channelrhodopsin-2 (ChR2). We hypothesized that the small-to-large diameter recruitment order in PONS also primarily arises from the internodal spacing relationship, but for the opposite reason as in electrical stimulation. Specifically, when a light stimulus is applied to the axon, the smaller internodal spacing of smaller diameter axons increases the probability of illuminating higher numbers of nodes of Ranvier. We explored this hypothesis with a computational model, which followed the general optogenetic stimulation modeling methodology of Foutz et al. (2012). We then compared optical and electrical stimulation of a range of different diameter myelinated axons to identify basic principles that dictate action potential initiation. Finally, we directly compared our simulation predictions to the experimental work of Llewellyn et al. (2010) which first characterized the small-to-large diameter recruitment order of PONS.

EXPERIMENTAL PROCEDURES

Our PONS model system was created in NEURON (v 7.1) using Python (2.7.1) (Hines et al., 2009).

Light density distribution model

Our light density distribution model was previously described in Foutz et al. (2012), which evolved from Aravanis et al. (2007), and was parameterized to match the light transmission data of Gradinaru et al. (2009) (Fig. 1A–C). The parameters of the light density distribution model are listed in Table 1. All given irradiance values correspond to the irradiance immediately exiting the surface of the light source.

The irradiance of photons per unit area at a given location in the tissue medium was calculated by multiplying the initial source irradiance, I_0 , by the transmittance from that given location to the center of the optical source (Foutz et al., 2012). The transmittance was defined by several factors: (1) a unimodal Gaussian cross-sectional light density distribution (Weik, 1989), (2) a conical spreading of unfocused light along the source-to-axon depth axis related to the numerical aperture of the optical source and index of refraction of the tissue,

and (3) a Kubelka–Munk approximation of the scattering and absorbance of light in the tissue (Kubelka and Munk, 1931). The distance from the optical source to the Chr2 channels was approximated as the distance from the center of the optical source to the center of each compartment of the axon model with Chr2 channels (Foutz et al., 2012).

We created several light density distribution models to evaluate the impact of light source design on the small-to-large diameter recruitment order of PONS. The first model was parameterized to represent a 200- μm -diameter fiber optic (Fig. 1A). The second model was parameterized to simulate a single LED representative of the individual LEDs used in Llewellyn et al. (2010) (Fig. 1B). The third model was a ring of 4 LEDs that surrounded the axons, and the fourth model was intended to represent the 16 LED cuff (four 4 LED rings in series) used in the experiments of Llewellyn et al. (2010) (Fig. 1C). When simulating illumination from the LED cuff, the sciatic nerve model (see below) was assumed to be 360 μm in diameter (Llewellyn et al., 2010). The surface of each light source in the cuff was assumed to be located 350 μm from the edge of the sciatic nerve boundary as approximated from Fig. S1 of Llewellyn et al. (2010).

Chr2 model

Chr2 was modeled as a nonspecific, voltage-sensitive cation channel with four distinct channel states within two functional categories: open states (O1 and O2) and closed states (C1 and C2) (Nikolic et al., 2009; Grossman et al., 2011) (Fig. 1D). When not referenced specifically in the text, the Chr2 model parameters are listed in Table 2. Due to the unidirectional K_r term, the Chr2 model assumes all channel states will eventually accumulate in the C1 channel state; thus the initial channel state conditions were $C1=1$, $C2=O1=O2=0$. A voltage-dependent term with empirical constants to better fit experimental results was included (Nikolic et al., 2009; Grossman et al., 2011). This model also takes into consideration the existence of two exponential decay rates for the Chr2 transmembrane current after a light pulse is terminated (Grossman et al., 2011).

Axon model

Each axon model was a multi-compartment myelinated, concentric, double cable axon with a resting potential of -75 mV, run at a temperature of 37 degrees C. The morphologic parameters of the axon models and electrical parameters of the endogenous ion channels were derived from the previously published MRG axon models (McIntyre et al., 2002). These models were originally described at discrete fiber diameters; however, in Fig. 7 we linearly interpolated axon model parameters between existing MRG diameters to create a more representative range of smaller diameter axons in the mouse sciatic nerve, and a more continuous recruitment curve (Table 3). All other figures use only originally described MRG axon diameters.

The four-state Chr2 model was added to the axonal membrane of the MRG model (Fig. 1E). All quantified measurements presented in the results, unless otherwise noted, were from the central node of Ranvier, which was defined as the axon compartment directly underneath the center of the optical source and was the reliable location of action potential generation from optical stimulation. The length of the axon model (e.g. 5620- μm and 27,000- μm for 3-

μm and 12.8- μm fibers, respectively) was defined to be substantially longer than the area of light induced ChR2 activation ($\sim 4500 \mu\text{m}$ for the 16 LED cuff light density distribution model).

Electrical stimulation

Simulations of cathodic electrical extracellular stimulation were generated by a point source electrode placed in an infinite homogenous anisotropic medium (longitudinal resistivity = 300 $\Omega\text{-cm}$, transverse resistivity = 1200 $\Omega\text{-cm}$) (McIntyre et al., 2002). The electrode was placed directly above the central node of the myelinated axon. The point source electrode example represents an electrical stimulation strategy most analogous to optical stimulation with the fiber optic.

Simulated EMG

Our theoretical predictions of motor axon activation were translated into simulated muscle fiber activation using a symmetric branching model. Estimates of the number of muscle fiber(s) innervated by an axon, or fiber number (FN), were quantified using 2^n , where n corresponds to the number of axonal branches (Morgan and Proske, 2001) (Table 3). In our simulations, $\text{FN} = 0.021D^{3.53}$, where D corresponded to axon diameter in μm . The branching model assumes that axons continue to branch singularly with a cross-sectional area ratio of 1.37 (children-to-parent) until a terminal diameter of 3 μm is reached (Morgan and Proske, 2001). Based on this axon branching model, the activity of larger diameter axons has a more substantial effect on the resultant EMG signal.

Activating function

Previous work on electrical stimulation of axons has determined that membrane polarization is related to the second spatial derivative of the extracellular potential distribution generated along each axon ($\nabla^2 V_e / x^2$) (McNeal, 1976; Rattay, 1989). This value has been used to define the electrical activating function which provides an explanation for the large-to-small recruitment order of myelinated axons with electrical stimulation. PONS produces membrane polarization related to the light activated ChR2 current per membrane surface area (i_{ChR2}). This current density term represents a somewhat analogous optical activation function and it is voltage, space, and time dependent:

$$i_{\text{ChR2}} = (g_{\text{O1}} O1(x, t) + g_{\text{O2}} O2(x, t))^* (V - E_{\text{CAT}})^* p_{\text{ChR2}} \quad (1)$$

As displayed in Fig. 3, we evaluated this optical activation function at the cessation of the light pulse at all nodal locations along the axon.

Sciatic nerve model

We created a simplified theoretical representation of the mouse sciatic nerve with populations of axon models that could be stimulated by our light source models (Figs. 1 and 7). Simulations were performed with axons oriented perpendicular to the optical source at varying source-to-axon depths. We compared axon recruitment when using either our

simulated representation of the 16 LED cuff used in the Llewellyn et al. (2010) experiments or an alternative 4 LED ring.

The sciatic nerve, Ω , was modeled as two independent sub-populations of axons: Ω_S and Ω_L , that could be thought of as innervating either the Soleus (SOL) or the Lateral Gastrocnemius (LG) muscles, respectively. The Ω_S and Ω_L axonal sub-populations were characterized by different probabilistic distributions across varying axonal diameters, $p(D|\Omega)$, and depths within the nerve, $p(Y|\Omega)$. The probability distributions of this population model were reproduced from the histological results of Llewellyn et al. (2010). The Ω_L axons on average were of larger axonal diameters and located more toward the periphery of the sciatic nerve bundle. The Ω_S axons on average were of smaller axonal diameters and located deeper in the sciatic nerve bundle.

The overall model system enabled us to quantify the threshold source irradiance necessary for action potential initiation in individual axons of the sciatic nerve model. These thresholds were determined for a range of light pulse durations, axon diameters, and optical stimulation methodologies. The location of the nodes of Ranvier relative to the light distribution model is important in determining optical activation. Placement of a node directly under the light source provides the minimal irradiance necessary to generate an action potential. In turn, the data presented in Figs. 2–6 were generated with the central node of Ranvier of the axon model (i.e. node 10) perfectly aligned with the center of the light source. For the 16 LED cuff in Figs. 2–6, the central node was aligned to the center of one of the middle 4 LED rings (Fig. 3A).

Nodes of Ranvier of different axons within a nerve are not necessarily perfectly aligned with each other, especially when comparing axons of different diameters because of their different internodal spacing. Therefore, to create a population model capable of reproducing EMG signals, nodes of Ranvier were at variable locations within the different light distribution models used in Fig. 7. The central node of each axon was uniformly distributed along the nerve from -500 to $+500$ μm of the center of the light source.

The probability of activation for an axonal sub-population, χ , was uniquely determined for each combination of axonal diameter and input irradiance at locations within the nerve characteristic of that subpopulation. The percent of axons activated within an entire sub-population for a given irradiance was determined as a summation across all axon diameters of the probability of axonal diameter with the probability of activation:

$$PA_{\text{SOL}}(I) = \sum_D p(D|\text{SOL}) \chi(I|\text{SOL}, D) \quad (2)$$

$$PA_{\text{LG}}(I) = \sum_D p(D|\text{LG}) \chi(I|\text{LG}, D) \quad (3)$$

The resultant EMG signal of the axons activated within an entire axonal sub-population was then determined as a summation across all axon diameters of the probability of axonal diameter with the probability of activation and the fiber number, FN (i.e. the number of

muscle fibers innervated for the axonal diameter of interest as determined by the branching model).

$$\text{EMG}_{\text{SOL}}(I) = \sum_D p(D|\text{SOL}) \text{FN}(D) \chi(I|\text{SOL}, D) \quad (4)$$

$$\text{EMG}_{\text{LG}}(I) = \sum_D p(D|\text{LG}) \text{FN}(D) \chi(I|\text{LG}, D) \quad (5)$$

These equations assume that the conditional probabilities for an axon at varying depths within the nerve and axonal diameters were independent within axonal sub-populations. The conditional probabilities were reproduced from Llewellyn et al. (2010), as described above, and all probability data were imported into MATLAB and interpolated to allow for finer discretization. The probability of each discrete axonal diameter was determined by integrating the probability distribution within the domain equally spaced between diameters as seen in Table 3.

Experimental measurements of absolute ChR2 density in neural membrane are lacking; however, an arbitrary base value is necessary to explore the theoretical effects of optical stimulation. Therefore, we defined a base ChR2 density using the 16 LED cuff to approximate the experimental results from Llewellyn et al. (2010). Their experimental results showed that the threshold irradiance capable of generating 50% of the peak SOL EMG response was 6.63 mW/mm² (Fig. 7D, E). We reproduced this response in our model by defining the ChR2 density per axon membrane surface area as 4.41E12 channels/cm² and this value was used for all simulations presented.

RESULTS

A light-axon model system was used to evaluate irradiance thresholds for action potential generation in myelinated axons of varying diameters. We created several stimulation representations including: 16 LED cuff, 4 LED ring, singular LED, singular fiber optic, and a point source cathodic electrode. We then compared our model predictions to experimental measurements of muscle recruitment during optical stimulation with 0.5-ms pulses applied to the Thy1-ChR2 transgenic mouse sciatic nerve with a 16 LED cuff (Llewellyn et al., 2010).

It is important to note that the 16 LED cuff and 4 LED ring generate much lower threshold irradiance values compared to the singular optical sources (LED and fiber optic). In turn, some of the irradiance values used in Figs. 2–6 are well outside the range that would be considered normal or safe for *in vivo* optical stimulation. These results are presented to provide a generalized theoretical basis for comparison across the different stimulation modalities. Fig. 7 is then intended to provide an experimentally relevant comparison.

Action potential initiation

Action potential initiation from optical stimulation originated in the node of Ranvier directly below the center of the optical source. Fig. 2 shows an example action potential generated

by a 0.5-ms pulse of blue light from a fiber optic. This very short pulse duration was used in the original Llewellyn et al. (2010) experiments. However, it should be noted that this pulse duration is much shorter than typically used in optogenetic studies, and is short compared to the ChR2 time constant (1.3 ms) (Nikolic et al., 2009). As such, higher threshold irradiance is required to increase the activation rate constants, particularly K_{a1} , to a maximum at the cessation of the light pulse. The activation rate constants then slowly decrease back to zero after the light pulse dependent upon the empirical decay constants and ChR2 time constant. The increase in activation rate constants elicits a voltage-independent change in the ChR2 states, particularly an increase in the highly-conductive, short-lived O1 state from the equilibrium C1 state. The increase in ChR2 conductance results in a voltage-dependent negative transmembrane current, which causes depolarization of the membrane potential to a threshold point (~ -55 mV for the MRG model). The sodium channels then take over responsibility for generating an action potential spike. Interestingly, the temporal dynamics of ChR2 based depolarization are primarily defined by the time constants of the off, or dark, state when using such short optical pulse durations. This is demonstrated by the ChR2 conductance reaching a peak after cessation of the stimulus.

Optical activating function

We defined an optical activating function as the transmembrane ChR2 current per membrane surface area. Unlike electrical stimulation where the second difference of the voltage distribution along the axon can be calculated as a static value, the optical activating function is voltage-dependent. This results in a time-varying relationship that cannot be determined analytically. The optical activating function is initially zero at all nodes prior to the light stimulus, reaches a maximum just prior to cessation of the light pulse, and is non-zero after cessation of the light pulse due to the time delay associated with the decay of the open states to closed states (Fig. 2). In Fig. 3 we quantified the optical activating function at the cessation of the light stimulus when using irradiance values that were just subthreshold for action potential initiation. These results highlight the importance of light source representation, and the number of nodes directly illuminated, in defining axon polarization. The more focal the light source the more similar the optical activating functions are for the different diameter axons (Fig. 3C). However, as the light source becomes more distributed in comparison to the internodal spacing, the polarization is spread across multiple nodes (Fig. 3A, B). This decreases the peak i_{ChR2} value necessary for action potential initiation. This effect is especially pronounced in smaller diameter axons because of their shorter internodal spacing.

Strength–duration relationships

The effect of pulse duration modulation on threshold irradiance for axons of different diameters is shown in the strength–duration (SD) relationships of Fig. 4. With increasing optical pulse duration, the threshold irradiance necessary to initiate an action potential reaches an asymptote, referred to as the rheobase irradiance. These results are similar to the electrical SD relationships; however, the chronaxie is much longer with optical stimulation of ChR2 compared to electrical stimulation. For example, the chronaxie for optical stimulation with the 16 LED cuff, single LED, and single fiber optic models were approximately 5, 3.5, and 3 ms for a 5.7- μm axon and source-to-axon depth of 530 μm . That

same axon stimulated with an electrical point source electrode had a chronaxie of approximately 0.2 ms (Fig. 4).

The SD results also illustrate the different axon diameter-dependent recruitment orders for optical vs. electrical stimulation. Optical stimulation with any light distribution model, and perfect alignment of the light source with a node of Ranvier, exhibits a small-to-large diameter recruitment order (Fig. 4A–C). In contrast, electrical stimulation exhibits a large-to-small diameter recruitment order (Fig. 4D). Interestingly, as the light source becomes more distributed, the relative difference in threshold amplitude between different axon diameters is more pronounced across all, but particularly at longer, pulse durations.

Intensity–distance relationships

Fig. 5 compares the threshold irradiance of the different stimulation modalities while varying the source-to-axon distance. The different light source models all required larger threshold irradiance for action potential initiation in larger diameter axons (i.e. small-to-large recruitment order) especially at larger source-to-axon distances. However, use of a more distributed light source had a dramatic effect on the threshold irradiance. Fiber optic stimulation required approximately an order of magnitude higher irradiance than the 16 LED cuff, and this effect was accentuated at larger source-to-axon distances.

Relative contribution of internodal spacing

The myelinated axon has multiple anatomic and physiological parameters that vary as a function of axon diameter, including internodal spacing (Nilsson and BIRTHOLD, 1988). We propose that internodal spacing is an important parameter in dictating the threshold irradiance. Fig. 6 shows the effect of increasing or decreasing the internodal spacing on threshold irradiance for a range of different diameter axons. The simulations indicate that the general trend of small-to-large diameter recruitment for optical stimulation and large-to-small diameter recruitment for electrical stimulation remains intact even when axons of different diameters have the same internodal spacing. However, the relative threshold difference between different axon diameters is dependent on the light source used to stimulate them. For example, in the 16 LED cuff (Fig. 6A), decreasing internodal spacing increases the number of nodes illuminated and decreases threshold irradiance. Further, in cases where the internodal spacing is less than the light spread such that only one node is illuminated, decreasing internodal spacing does not decrease threshold irradiance. Rather, in these cases, decreasing internodal spacing results in a decrease of axial resistivity, increasing the spread of current from the single illuminated node, and conversely increasing the threshold irradiance. This is seen in the singular LED model with internodal spacing greater than 1000 μm (Fig. 6B) or in the fiber optic model at all internodal spacings (Fig. 6C). However, even when only one node is effectively illuminated, the general trend of small-to-large diameter recruitment for optical stimulation results in increased threshold irradiance required for action potential generation in larger diameter axons.

Population model

The simulation results displayed in Figs. 4–6 illustrate the general trends of irradiance threshold for myelinated axons stimulated with idealized source-to-axon distances and node

of Ranvier alignment with the light source. However, experimental investigations are faced with the more complicated situation of stimulating an intact nerve. Therefore, we simulated optical stimulation of the mouse sciatic nerve, using theoretical probabilities of axon diameter distributions. Our nerve model was based on histological data, to attempt to reproduce the experimental results of Llewellyn et al. (2010) (Fig. 7). In addition, we used a symmetric axonal branching model (Morgan and Proske, 2001), to estimate a normalized EMG response from our axon activation predictions.

Fig. 7A illustrates the light distribution and axon locations in the population model, which consisted of two axon subpopulations innervating either the Soleus (SOL) or Lateral Gastrocnemius (LG). Previous results from the mouse sciatic nerve show that the LG axons are located closer to the periphery of the nerve where there is higher transmittance of source irradiance (LG: average source-to-axon depth $\approx 38.6 \mu\text{m}$; SOL: average source-to-axon depth $\approx 109 \mu\text{m}$) (Llewellyn et al., 2010) (Fig. 7B). In addition, the LG axons are typically of larger diameter than the SOL axons (LG: $D=6.7\pm0.16 \mu\text{m}$; SOL: $D=4.5\pm0.17 \mu\text{m}$) (Llewellyn et al., 2010) (Fig. 7C). Experimental results also show that the LG muscle requires higher threshold irradiance than the SOL muscle to generate a similar normalized EMG signal (Llewellyn et al., 2010) (Fig. 7D, E).

We attempted to simulate the Llewellyn et al. (2010) findings and compare the ability of the 16 LED cuff light source model to match the experimental results (Fig. 7D–F). In addition, we analyzed optical stimulation with a 4 LED ring. Both light source representations produced a sigmoidal-type recruitment curve where the smaller axon diameter subpopulation innervating the SOL muscle was recruited preferentially. The 16 LED cuff produced recruitment curves that were similar to the experimental results of Llewellyn et al. (2010) at irradiances above 5 mW/mm^2 (Fig. 7D). Not surprisingly, the 4 LED ring at the same pulse duration required much larger irradiances to activate the axons than the 16 LED cuff (Fig. 7E). However, the slopes of the recruitment curves were also different. The more focal light delivery of the 4 LED ring exhibited a much wider dynamic range for activation and accentuated the small-to-large diameter recruitment order at short stimulus pulse durations.

Interestingly, alterations in the pulse duration can have multiple effects on the recruitment curves. For example, the 4 LED ring with 1-ms pulse duration resembles the 16 LED ring with 0.5-ms pulse duration (Fig. 7F). However, in this example the 4 LED ring has a somewhat wider dynamic range and a more accentuated small-to-large diameter recruitment order. Further increasing the pulse duration to 5-ms further reduces the irradiance required to produce maximum EMG response and greatly reduces the effective dynamic range between activation of the small and large diameter axons (Fig. 7F).

DISCUSSION

The goal of this study was to develop a model system to characterize optogenetic stimulation of the peripheral nervous system. We investigated the hypothesis that the internodal spacing relationship plays an important role in the small-to-large diameter recruitment order of PONS. We found that detailed light-axon models were able to simulate

the diameter-dependence of optical stimulation (Figs. 4–6) and were capable of reproducing many features of the available experimental data (Llewellyn et al., 2010) (Fig. 7). Our simulations support the concept that increasing the number of nodes of Ranvier directly illuminated decreases the threshold irradiance and contributes to the small-to-large diameter recruitment order of PONS (Figs. 3 and 7). In addition, when light is applied in a more focal manner to larger diameter axons, with larger internodal spacing, they can be especially hard to activate if one or more of their nodes of Ranvier are not directly aligned with the light source (Fig. 7E).

While we propose that internodal spacing represents the most important parameter in dictating the recruitment order of myelinated axons during optical stimulation, other factors also play a role. For example, morphological parameters, that dictate axon diameter-dependent input impedance and axial resistance, create a general trend for small-to-large recruitment order regardless of internodal spacing (Fig. 6). In addition, the possibility of non-uniform ChR2 expression as a function of axon section and/or axon diameter could also impact the recruitment order.

Optogenetic technology has revolutionized neuroscience research (Deisseroth, 2011), but numerous biophysical questions remain on the mechanisms of optical stimulation. One fundamental, but currently unknown question is the density of photon-sensitive ion channels on neural membrane. The channel density directly effects irradiance threshold and the volume of tissue directly stimulated by the light source (Foutz et al., 2012). Our previous ChR2 optogenetic models assumed a constant ChR2 density of 1.3×10^{10} channels/cm². This value represents the only experimental estimate currently available, albeit determined from bacteriorhodopsin expression in an oocyte (Nagel et al., 1995). In this study, we treated ChR2 density as a free parameter and elected to fit it to match experimentally measured threshold irradiance values for axonal activation (Llewellyn et al., 2010) (Fig. 7). The ChR2 density we indirectly estimated (4.41×10^{12} channels/cm²) was more than two orders of magnitude higher than the Nagel et al. (1995) estimate. This discrepancy could be attributed to any number of issues related to the limited scientific characterization of ChR2 basic biophysics and expression patterns in the mammalian nervous system. Therefore, our theoretical results highlight the need for quantitative experimental studies on this topic.

Our analysis supports the hypothesis that the optical activating function is spatially dependent on the internodal spacing relationship in an opposite manner to electrical stimulation. The population-based light-axon model we created was capable of generating sigmoidal recruitment curves that were comparable to experimental results (Fig. 7). Llewellyn et al. (2010) showed that lower threshold irradiance is required to achieve the same normalized EMG response in the SOL as compared to the LG muscle. This was despite the fact that the SOL axonal sub-population was located generally at farther distances from the light source where there is lower transmittance of source irradiance. This interesting result was only reproduced in simulation when there was a very strong axon diameter dependence of the threshold irradiance (Figs. 4–7). We found that the combination of spatially distributed illumination and shorter internodal spacing of small diameter fibers were important parameters in reproducing the experimental results. While our simulations provide a practical understanding of the small-to-large diameter recruitment order for

optogenetic axonal stimulation, additional experimental studies are necessary to explicitly support or refute our hypothesis.

Our light-axon model system was constructed with physiologically relevant parameters based on the available data. However, several assumptions were necessary in the creation of the model that have important implications in the understanding and analysis of the model results. First, the four-state model of ChR2 kinetics is not the most detailed representation available in the biophysical literature. However, the four-state model has been shown to sufficiently model the time-varying dynamics as compared to more complex higher-state models (Nikolic et al., 2009). Second, we assumed that the axon trajectories were purely perpendicular to the light source. While the angle of illumination does have an impact on the threshold irradiance (Foutz et al., 2012), axon trajectories in peripheral nerve should have relatively limited variation over the illuminated area. Third, we relied on the assumption that predictions of activation from an axon subpopulation coupled with a symmetric axonal branching model can provide quantitative normalized EMG comparison to experimental recruitment results. Further, the EMG model implicitly assumes that every terminal muscle fiber has a similar effect on the resultant EMG signal regardless of the parent axon diameter and/or axonal sub-population. Therefore, future PONS experiments preformed with concurrent electrical axonal recordings could provide results that are more directly comparable to our model.

One noticeable discrepancy in our model was its inability to accurately reproduce the EMG findings at low irradiance ($<5 \text{ mW/mm}^2$) (Fig. 7D). We speculate that this discrepancy is due to an inappropriately low weighting of small diameter axons in the production of the simulated EMG signal (Table 3). The low weighting of small diameter axons may be from the exclusion of axon diameters below $3 \mu\text{m}$ in the histological analysis of Llewellyn et al. (2010), presumably due to the image recognition/tracing method(s) used. However, it is possible that the axon sub-population innervating the Soleus muscle may include axons with diameters less than $3 \mu\text{m}$ (Fig. 7B). The addition of such small diameter axons in our model would increase the normalized EMG response at lower irradiances.

We propose that the small-to-large diameter recruitment order for optogenetic axonal stimulation is related to the internodal spacing relationship, which preferentially increases the probability of illumination of higher numbers of nodes of Ranvier in smaller diameter axons. Future PONS strategies may utilize this small-to-large diameter recruitment order to provide therapeutic effect without some of the limitations associated with electrical stimulation therapies (e.g. activation of unwanted muscles and/or accelerated muscle fatigue during stimulation). However, numerous engineering hurdles remain for the clinical application of PONS therapies. We propose that PONS models represent a tool for designing and testing optical nerve cuff concepts and identifying the optical stimulation parameter settings necessary to generate the desired axonal response (Fig. 7).

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Abbreviations

PONS	Peripheral Optogenetic Neural Stimulation
PNS	Peripheral Nervous System
ChR2	Channelrhodopsin-2
STIN	Stereotyped Internodal Region
FLUT	Fluted Paranodal Main Region
MYSA	Myelin Sheath Attachment Region
SOL	Soleus
LG	Lateral Gastrocnemius
EMG	Electromyogram

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Fig. 1.

Light-axon model. (A–C) Light density distribution models. Three dimensional and cross-sectional planes are presented for (A) single fiber optic, (B) single LED, and (C) 16 LED cuff. The single LED and single fiber optic 3D representations have been sliced in half to visualize the light distribution. (D) Four-state model of ChR2 in which incident light energy ($h\nu$) results in an increase in the K_{a1} and K_{a2} rate constants and subsequent increase in probability of the O1 and O2 channel states, leading to ChR2 current. (E) MRG axon model with ChR2. *Above*: diagram of the various nodal and internodal segments. *Below*: circuit diagram including additions of ChR2 channels in the axon membrane.

Fig. 2.

Action potential initiation. (A) Traces of transmembrane voltage, (B) transmembrane ChR2 current, and (C) ChR2 channel conductance in response to a 0.5-ms blue light pulse (as denoted by the gray bar and vertical dotted gray line) from the single fiber optic light density distribution model. The following parameters were used: axon diameter=5.7 μm , source-to-axon distance=530 μm , and the central axon node was directly under the center of the light source.

Fig. 3.

Activating functions. Activating function values evaluated for individual nodes of Ranvier at the threshold for action potential initiation for a small or a large diameter axon: (A) 16 LED cuff (CUFF), (B) single LED, (C) single fiber optic light density distribution models, and (D) cathodic electrical point source electrode. Source-to-axon distance=530 μm and pulse duration=0.5 ms. The central axon node (i.e. node 10) was directly under the center of the light source for all simulations.

Fig. 4.

Strength–duration relationships. Threshold irradiance as a function of pulse duration for various axon diameters. (A) 16 LED cuff (CUFF), (B) single LED, and (C) single fiber optic light density distribution models. Irradiance is reported as the value immediately exiting the light source. (D) Threshold current for extracellular electrical stimulation of the same axon models. The following parameters were used: source-to-axon distance=530 μm, and the central axon node was directly under the center of the light source.

Fig. 5.

Intensity–distance relationships. Threshold irradiance as a function of source-to-axon distances. (A) 16 LED cuff (CUFF), (B) single LED, and (C) single fiber optic light density distribution models. Irradiance is reported as the value immediately exiting the light source tip. Note different y-axis scale for 16 LED cuff. (D) Threshold current for extracellular electrical stimulation of the same axon models. The following parameters were used: pulse duration= 0.5 ms, and the central axon node was directly under the center of the light source.

Fig. 6.

Relative contribution of internodal spacing. Threshold irradiance as a function of internodal spacing for various axon diameters. (A) 16 LED cuff (CUFF), (B) single LED, and (C) single fiber optic light density distribution models. Irradiance is reported as the value immediately exiting the light source. (D) Threshold current for extracellular electrical stimulation of the same axon models. The dotted line corresponds to the threshold stimulus of the axons with default internodal spacing parameter values. The following parameters were used: source-to-axon distance=530 μm , pulse duration=0.5 ms, and the central axon node was directly under the center of the light source.

Fig. 7. Population model. (A) PONS model visualization. The lateral gastrocnemius axon sub-population (LG) is typically of larger diameter, located closer toward the periphery, and is represented with a thicker line as compared with the soleus axon sub-population (SOL). The probability density function for the SOL (small dotted line) and LG (large dotted line) is shown at varying (B) axon diameters and (C) source-to-axon distances. Position=0 μm corresponds to the center of the sciatic nerve and ±180 μm corresponds to the sciatic nerve boundaries. Probability data are reproduced from Llewellyn et al. (2010). (D, E) Simulated normalized EMG of the SOL (solid thin line) and LG (solid thick line) across varying irradiances. (D) Simulated results from the 16 LED optical cuff model with 0.5-ms pulse duration compared to the experimental results for the normalized EMG of the SOL (dotted smaller line) and LG (dotted larger line) reproduced from Llewellyn et al. (2010). The inset graph displays the percentage of axons activated as a function of the light intensity. (E) Simulated results from the 4 LED ring model with 0.5-ms pulse duration (Red) plotted on the same irradiance scale as D. Inset shows recruitment at higher irradiances. (F) Simulated

results from the 4 LED ring model with 1-ms pulse duration (Blue) and with 5-ms pulse duration (Green) plotted on the same irradiance scale as D. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

Light distribution model parameters

Light distribution model parameters			
Parameter	Description	Value	References
I_o	Irradiance at the light source tip	1–700 mW/mm ²	
D_o	LED diameter	600 μ m	LED datasheet of Llewellyn et al. (2010)
	Fiber optic diameter	200 μ m	Aravanis et al. (2007)
$NA_{\text{Fiber Optic}}$	LED numerical aperture	0.9323	LED datasheet of Llewellyn et al. (2010)
	Fiber optic numerical aperture	0.37	Aravanis et al. (2007)
η_{Tissue}	Tissue index of refraction	1.36	Vo-Dinh (2003). Foutz et al. (2012)
h	Planck's constant	6.627×10^{-34} m ² kg/s	
c	Speed of light	2.99×10^8 m/s	
λ	Wavelength of light	465 nm	Llewellyn et al. (2010)
σ_{Ret}	Cross-sectional area of retinal	1.2×10^{-20} m ²	Hegemann et al. (2005)
K	Absorbance coefficient	7.37 mm ⁻¹	Gradinaru et al. (2009) Foutz et al. (2012)
S	Scattering coefficient	0.125 mm ⁻¹	Gradinaru et al. (2009) Foutz et al. (2012)

Table 2

ChR2 model parameters

ChR2 model parameters			
Parameter	Description	Value	References
g_{01}	Conductance of the O1 channel state	$50 \times 10^{-15} \text{ S}$	Grossman et al. (2011)
g_{02}	Conductance of the O2 channel state	$250 \times 10^{-17} \text{ S}$	Nikolic et al. (2009)
γ	g_{02}/g_{01}	0.05	Nikolic et al. (2009)
U_0	Empirical constant	40 mV	Grossman et al. (2011)
U_1	Empirical constant	15 mV	Grossman et al. (2011)
E_{cat}	ChR2 equilibrium (Nernst, reversal) potential	0 mV	Nagel et al. (2003)
$e_{12 \text{ LIGHT}}$	Rate constant	0.053 ms^{-1}	Nikolic et al. (2009)
$e_{12 \text{ DARK}}$	Rate constant	0.022 ms^{-1}	Nikolic et al. (2009)
$e_{21 \text{ LIGHT}}$	Rate constant	0.023 ms^{-1}	Nikolic et al. (2009)
$e_{21 \text{ DARK}}$	Rate constant	0.011 ms^{-1}	Nikolic et al. (2009)
A_1	Empirical constant for slow component of current in dark	0.03 ms	Nikolic et al. (2009)
A_2	Empirical constant for fast component of current in dark	0.15 ms	Nikolic et al. (2009)
K_{d1}	Rate constant	0.13 ms^{-1}	Nikolic et al. (2009)
K_{d2}	Rate constant	0.025 ms^{-1}	Nikolic et al. (2009)
K_r	Rate constant	0.0004 ms^{-1}	Nikolic et al. (2009)
ϵ_1	Quantum efficiency of O1 state	0.5	Nikolic et al. (2009)
ϵ_2	Quantum efficiency of O2 state	0.1	Nikolic et al. (2009)
τ_{ChR2}	Time constant	1.3 ms	Nikolic et al. (2009)

Table 3

Axon models

Axon models					
Axon diameter (μm)	Internodal spacing (μm)	Fiber number (FN)	Probability		
3	281	1.002	SOL	0.004	
			LG	0.031	
3.5	322	1.726	SOL	0.192	
			LG	0.02	
4	362	2.764	SOL	0.214	
			LG	0.026	
4.5	403	4.188	SOL	0.192	
			LG	0.049	
5	443	6.073	SOL	0.191	
			LG	0.123	
5.7	500	9.641	SOL	0.07	
			LG	0.126	
6.1	563	12.246	SOL	0.02	
			LG	0.096	
6.5	625	15.321	SOL	0.017	
			LG	0.093	
6.9	688	18.913	SOL	0.015	
			LG	0.087	
7.3	750	23.072	SOL	0.018	
			LG	0.104	
8	875	31.868	SOL	0.021	
			LG	0.081	
8.7	1000	42.839	SOL	0.016	
			LG	0.108	
10	1150	70.011	SOL	0.003	
			LG	0.05	
11.5	1250	114.617	SOL	0	
			LG	0.027	
12.8	1350	167.226	SOL	0	
			LG	0.005	