Theoretical principles underlying optical stimulation of a channelrhodopsin-2 positive pyramidal neuron

Thomas J. Foutz¹,², Richard L. Arlow¹,², and Cameron C. McIntyre¹,²

¹Department of Biomedical Engineering, Cleveland Clinic Foundation, Cleveland, OH, USA
²Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH USA

Corresponding Author:
Cameron C. McIntyre, Ph.D.
Cleveland Clinic Foundation
Department of Biomedical Engineering
9500 Euclid Ave ND20
Cleveland, OH 44195
Phone: 216-445-3264
Fax: 216-444-9198
E-mail: mcintyc@ccf.org

Copyright © 2012 by the American Physiological Society.
Abstract

Optogenetics is an emerging field of neuromodulation that permits scaled, millisecond temporal control of the membrane dynamics of genetically targeted cells using light. Optogenetic technology has revolutionized neuroscience research; however, numerous biophysical questions remain on the optical and neuronal factors impacting the modulation of neural activity with photon sensitive ion channels. To begin to address such questions, we developed a computational tool to explore the underlying principles of optogenetic neural stimulation. This “light-neuron” model consists of theoretical representations of the light dynamics generated by a fiber optic in brain tissue, coupled to a multi-compartment cable model of a cortical pyramidal neuron embedded with channelrhodopsin-2 (ChR2) membrane dynamics. Simulations revealed that the large energies required to generate an action potential are primarily due to the limited conductivity of ChR2, and that the major determinants of stimulation threshold are the surface area of illuminated cell membrane and proximity to the light source. Our results predict that the activation threshold is sensitive to many of the properties of ChR2 (density, conductivity and kinetics), tissue medium (scattering and absorbance), and the fiber optic light source (diameter and numerical aperture). We also illustrate the impact of redistributing the ChR2 expression density (uniform versus non-uniform) on the activation threshold. The model system developed in this study represents a scientific instrument to characterize the effects of optogenetic neuromodulation, as well as an
engineering design tool to help guide future development of optogenetic technology.
Introduction

Optical stimulation technology has rapidly advanced since the first characterization of Channelrhodopsin-2 (ChR2) (Nagel et al., 2003). ChR2 is a light activated, nonspecific cation channel (H\(^+\), Na\(^+\), K\(^+\), Ca\(^{2+}\)) (Ehlenbeck et al., 2002; Zhang et al., 2007; Berndt et al., 2010) that is now being expressed in vivo in a range of mammalian species and can be targeted to specific neuron types. ChR2 photoactivation begins with the absorption of blue spectrum light, followed by excitation of the retinal chromophore, leading to a conformational change with opening of the ion channel (Hegemann et al., 2005, Muller et al. 2011). Selective expression of ChR2 channels in neurons and their activation by targeted delivery of light, permits millisecond temporal activation of specific neural populations. This technique has been useful in elucidating numerous sensory pathways, and providing new insights into the function of central nervous system circuits (Deisseroth et al., 2006).

The scientific value of optogenetic technology is unquestioned; however, numerous biophysical questions remain on the mechanism of action potential generation from optical stimulation. Further, definitive relationships that describe light irradiance thresholds as a function of the stimulation parameters are lacking on topics as fundamental as the fiber-optic-to-neuron distance, the ChR2 density/conductivity/distribution, and the optical fiber geometry. In this study, we investigate the complex relationship between an implanted optical fiber and a pyramidal neuron using a “light-neuron” model. Our theoretical analysis attempts to expand current knowledge, parameterizing the model based on the available experimental data,
and provides new quantitative hypotheses on the spatial extent of neural activation induced by optogenetic stimulation.

Characterizing the underlying mechanisms of optical stimulation also has important implications for the creation of new optogenetic technology. Knowledge of neuron orientation and/or photon sensitive ion channel characteristics that best interact with the light source to minimize stimulation power have important implications for the engineering of next generation optical fibers and/or optrodes (e.g. Sparta et al., 2011), as well as new channel constructs (e.g. Berndt et al., 2011). Further down the optogenetic technology development line, proposals of therapeutic application in human conditions such as Parkinson’s disease, spinal cord injury, depression and obsessive-compulsive disorder will need to compete with the current neuromodulation technologies which focus on electrical stimulation (Henderson et al., 2009). Numerous advances in electrical stimulation technology can be attributed to the mechanistic understanding provided by “field-neuron” models (McNeal, 1976). We propose that “light-neuron” models could provide a similar service to the optogenetic community. Preliminary portions of this work have been presented in abstract form (Foutz and McIntyre, 2010; 2011).
Methods

Neuron model

The neuron model used in this study was based on the soma-dendritic cable model of a reconstructed neocortical, layer V pyramidal neuron from cat visual cortex (Mainen et al., 1995; Mainen and Sejnowski, 1996; Shu et al., 2006; Yu et al., 2008; Hu et al., 2009) (Figure 1bc). The ModelDB accession number for the reconstructed pyramidal neuron and endogenous membrane dynamics used in this study is 123897. The neuron has an elliptical cell body (20 µm diameter), with 1 main apical dendrite and tuft (1100µm in length), as well as a basal dendritic tree (Figure 1b). The main axon is composed of an axon hillock (length, 10 µm; diameter, 3.8 to 2.4 µm), axon initial segment (length, 50 µm; diameter, 1.22 µm), unmyelinated axon (length, 400 µm; diameter 1.01µm) and myelinated axon (length, 1300µm; diameter, 1.21 µm; internodal length, 100 µm). The myelinated axon incorporates 14 node compartments (length, 1 µm; diameter 0.91 µm). The membrane electrical properties were uniformly distributed through the cell with the same values as previously published versions of the model (Hu et al., 2009): specific membrane resistivity \( (r_m) \) of 30 kΩ·cm², specific cytoplasmic (axial) resistivity \( (r_i) \) of 150 Ω·cm, and membrane capacitance \( (C_m) \) of either 1 µF·cm⁻² (soma), 0.5 µF·cm⁻² (dendrites, axon hillock, axon initial segment and nodes) or 0.02 µF·cm⁻² (myelinated sections). The input resistance at the soma was 43.6 MΩ. Simulations were run with a nominal temperature of 37 °C.

The endogenous membrane properties used in this study were identical to the model reported in Hu et al. (2009), with the addition of exogenous ChR2 (Figure 1e; see below) distributed uniformly throughout the neural structure (unless otherwise noted in
the results and figures). The neuron’s resting potential was -70 mV. Stimulation
threshold was defined using the irradiance exiting the tip of the fiber optic (see below).
The minimum irradiance necessary to generate an action potential that propagated to
the penultimate node of the axon was determined by a binary search algorithm.
Simulations were performed with NEURON v7.2 in Python (Carnevale and Hines,
2009).

Channelrhodopsin-2 dynamics
Channelrhodopsin-2 was modeled as a nonspecific ion channel with four states:
two closed states (C1, C2), and two open, conducting states (O1, O2) (Nagel et al.,
2003; Hegemann et al., 2005; Nikolic et al., 2009) (Figure 1d). In this model, ChR2 can
be excited from a closed, ground state (C1) to an open state (O1) secondary to
absorption of a photon of ~470 nm light (Figure 1a). This process occurs with a rate
constant $K_{a1}$. ChR2 in the excited state (O1) can decay back to a closed state (C1, rate
constant $K_{d1}$) or transition to a second excited state (O2, rate constant $e_{12}$). ChR2 in this
second excited state is more stable, but has a lower ion conductance. ChR2 in state O2
can either return to the first open state (O1, rate constant $e_{21}$), or decay to a closed
state (C2, rate constant $K_{d2}$). Finally, channels in state C2 can either be photoexcited
back to O2 (rate constant $K_{a2}$), or be slowly converted thermally to C1 (rate constant $K_r$)
(Grossman et al., 2011). The instantaneous rate of change of these states was defined
by a set of rate equations:

$$\frac{dO1}{dt} = K_{a1} C1 - (K_{d1} + e_{12}) O1 + e_{21} O2$$  \hspace{1cm} (1)
\[
\frac{dO_2}{dt} = K_a O_2 + e_{12} O_1 - (K_{d_2} + e_{21})O_2 
\] (2)

\[
\frac{dC_2}{dt} = K_{d_2} O_2 - (K_{a_2} + K_r)C_2 
\] (3)

\[
1 = O_1 + O_2 + C_1 + C_2 
\] (4)

In these equations, \(O_1\), \(O_2\), \(C_1\) and \(C_2\) represent the fraction of ChR2 molecules in the respective states. The fixed rate constants are summarized in Table 1. The activation rate constants \(K_{a_1}\) and \(K_{a_2}\) are calculated dynamically, since they depend upon the light irradiance.

\[
K_{a_1} = \begin{cases} 
\varepsilon_1 \Phi (1 - e^{-t/\tau}), & \Phi > 0 \\
\varepsilon_1 \Phi_0 (e^{-(t-t_0)/\tau} - e^{-t/\tau}), & \Phi = 0 
\end{cases} 
\] (5)

\[
K_{a_2} = \begin{cases} 
\varepsilon_2 \Phi (1 - e^{-t/\tau}), & \Phi > 0 \\
\varepsilon_2 \Phi_0 (e^{-(t-t_0)/\tau} - e^{-t/\tau}), & \Phi = 0 
\end{cases} 
\] (6)

In these equations, \(\varepsilon_1\) and \(\varepsilon_2\) are the quantum efficiency of photons which attempt to excite channelrhodopsin from a closed state to the corresponding open state; \(\Phi\) is the photon flux per unit area during illumination; \(\Phi_0\) is equal to \(\Phi\) during prior illumination (dark phase); \(t\) is the time since prior illumination began; \(t_0\) is the time since prior illumination ended (dark phase); \(\tau\) is the time constant of channelrhodopsin, shown in Table 1.

Determination of the ChR2 transmembrane channel conductance depends on the transmembrane voltage \(V_m\), the reversal potential \(E_{cat}\) set to 0 mV, and the channel conductance \(g_{ChR2}\). The ChR2 current during illumination \(i_{max}\) is determined by Ohm’s law:

\[
i_{max} = (V_m - E_{cat})g_{ChR2} 
\] (7)
ChR2 channel conductance is dependent on the state of the channel, with zero conductance in states C1 and C2, low conductance \( g_2 \) in state O2, and high conductance \( g_1 \) in state O1 (See Table 1).

After a period of illumination, the ChR2 transmembrane current decays exponentially. This decay has been fit experimentally by separating the current into a fast \( i_{fast} \) and a slow component \( i_{slow} \) (Nikolic et al., 2009; Grossman et al., 2011). The ChR2 transmembrane current, post-illumination, is defined as:

\[
i = i_{slow} e^{-\Lambda_1 (t-t_0)} + i_{fast} e^{-\Lambda_2 (t-t_0)}
\]  

(8)

where \( \Lambda_1 \) and \( \Lambda_2 \) are current decay factors. As time increases, the net transmembrane current decays to zero. The fast and slow components of the current are defined by:

\[
i_{fast} = i_{max} \frac{O_{10}(K_{d1} + (1-\gamma)e_{12,dark} - \Lambda_1) + O_{20}(\gamma(K_{d2} - \Lambda_1) - (1-\gamma)e_{21,dark})}{\Lambda_2 - \Lambda_1}
\]  

(9)

\[
i_{slow} = i_{max} \frac{O_{10}(\Lambda_2 - K_{d1} - (1-\gamma)e_{12,dark}) + O_{20}((1-\gamma)e_{21,dark} + \gamma(\Lambda_2 - K_{d2}))}{\Lambda_2 - \Lambda_1}
\]  

(10)

where \( O_{10} \) and \( O_{20} \) are the fraction of open channels during the prior illumination phase, and \( \gamma \) is the ratio of the conductance of the two states O2 and O1 (\( \gamma = g_2/g_1 \)).

The ChR2 model was typically inserted in all compartments of the neuron model, with a uniform ChR2 channel density of 130 \( \mu \)m\(^{-2} \) (Nagel et al., 1995). To simulate the effect of non-uniform distributions, ChR2 was distributed either in specific compartments (Table 2), or by distributing its density based on distance from the soma (Figure 10b).

Distance-based ChR2 distribution was performed by weighting the channel density by the path distance from the center of the soma to each point on the dendritic arbor, and scaling the density such that the total number of the channels in the soma-dendritic arbor remained constant. For the apical distribution, the most distant compartment had
the maximal density, while the soma had minimal density of ChR2. For the *basal* distribution, the distribution was reversed.

**Light Model**

Most of our simulations were performed with the optical fiber oriented perpendicular to the long axis of the neuron (Figure 1b), directed at the soma from a distance of 1 mm, unless otherwise noted. There are four primary factors affecting the distribution of light exiting the fiber optic. These are 1) the source light distribution, 2) the geometric spread of unfocused light, 3) the scattering and 4) the absorbance of light by the tissue (Figure 2). The light at each point in space ($I$) is defined by the source light irradiance ($I_0$, center of fiber optic output) and the transmittance of light between that point and the source ($T$):

$$I(r,z) = T(r,z)I_0$$  \hspace{1cm} (11)

where $r$ is the radial distance and $z$ is the height in a cylindrical coordinate system with the origin defined at the center of the fiber optic output. The transmittance is wavelength dependent, and can be broken down into linear components:

$$T(r,z) = G(r,z)C(z)M(r,z)$$  \hspace{1cm} (12)

where $G$ describes the Gaussian distribution of light emitted by fiber optics, $C$ describes the conical spreading of unfocused light, and $M$ describes the scattering and absorbance of light according to the Kubelka-Munk general theory of light propagation (Kubelka and Munk, 1931; Vo-Dinh, 2003; Aravanis et al., 2007). The light-model parameters are summarized in Table 1.
Light emitted from a fiber optic spreads as a cone of light (Figure 2a) with a divergence half-angle ($\theta_{\text{div}}$) dependent on the tissue index of refraction ($n_{\text{tis}}$) and the numerical aperture of the fiber optic ($NA_f$): 

$$\theta_{\text{div}} = \sin^{-1}\left(\frac{NA_f}{n_{\text{tis}}} \right)$$  \hspace{1cm} (13)

The radius of the light cone ($R$) at height $z$ emitted by a fiber optic with radius $R_0$ spreads according to:

$$R(z) = R_0 + z \tan(\theta_{\text{div}})$$  \hspace{1cm} (14)

As the light diverges, the irradiance decreases according to the law of conservation of energy. Therefore, when considering the effects of geometry independently, the radiant power ($P$) is constant at all distances, and is equal to the irradiance ($I$) times the surface area illuminated:

$$P = I(z) \pi R(z)^2 = I_0 \pi R_0^2$$  \hspace{1cm} (15) 

where $I$ is the irradiance at distance $z$ from the fiber optic. Therefore, the transmittance due to geometrical spreading ($C$) is:

$$C(z) = \left(\frac{R_0}{R(z)}\right)^2$$  \hspace{1cm} (16)

The Gaussian distribution of light ($G$) emitted by a fiber optic (Weik, 1997) can be approximated as a transmittance:

$$G(r,z) = \frac{1}{\sqrt{2\pi}} \exp\left(-2\left(\frac{r}{R(z)}\right)^2\right)$$  \hspace{1cm} (17)
According to this equation, 95.4% (2σ) of light is emitted by the core of the fiber optic, with the remaining 4.6% emitted by the cladding. The Gaussian light distribution with and without geometrical spread is shown in Figure 2a.

The last two factors affecting the light distribution in our model are the scattering and absorptive properties of tissue. To capture these effects, we implemented the Kubelka-Munk general model of light propagation in diffuse scattering media (Vo-Dinh, 2003). The transmittance of light in absorptive, scattering media ($M$) was:

$$M(r,z) = \frac{b}{a \sinh(bS\sqrt{r^2 + z^2}) + b \cosh(bS\sqrt{r^2 + z^2})}$$  \hspace{1cm} (18)$$

where:

$$a = 1 + \frac{K}{S}$$  \hspace{1cm} (19)$$

$$b = \sqrt{a^2 - 1}$$  \hspace{1cm} (20)$$

$S$ is the scatter coefficient per unit thickness (mm$^{-1}$), and $K$ is the absorption coefficient per unit thickness (mm$^{-1}$). This model assumes that the sample is planar, optically homogenous, and illuminated normal to the sample surface with diffuse monochromatic light. Reflection and absorption processes are assumed constant over the illuminated area, and occur at infinitesimal distances. The coefficients for scattering ($S = 7.37$ mm$^{-1}$, 95% CI 6.68–8.06) and for absorbance (0.125 mm$^{-1}$, 95% CI 0.05–0.20) were fit to light transmission data acquired from rodent brain slices (Gradinaru et al., 2009).

Once the irradiance of light has been determined at each point along the neuron, the flux of photons across each retinal molecule must be determined to calculate the rate of ChR2 openings. The flux of photons across a single retinal molecule can be...
determined by the energy content of the irradiated light. The energy of a single photon \( E_{\text{photon}} \) is determined by the Planck relation:

\[
E_{\text{photon}} = \frac{hc}{\lambda}
\]  

(21)

where \( h \) \( (6.63 \times 10^{-34} \text{ m}^2\cdot\text{kg/s}) \) is Planck’s constant, \( c \) is the speed of light \( (2.998 \times 10^8 \text{ m/s}) \), and \( \lambda \) is the wavelength \( (473 \text{ nm}) \). The energy of each photon is therefore \( 4.2 \times 10^{-19} \text{ J} \). The flux of photons \( \Phi \) can be determined by the law of conservation of energy:

\[
\Phi = \frac{I(r,z)\sigma_{\text{rel}}}{E_{\text{photon}}}
\]  

(22)

where \( \sigma_{\text{rel}} \) is the cross-section of a single retinal molecule \( (\sim 1.2 \times 10^{-8} \mu\text{m}^2) \) (Hegemann et al., 2005). The flux of photons across a single retinal molecule \( \Phi \) is used by the rate equation to describe the rates of channel opening (Equation 5, 6).
Results

Action Potential Initiation

As depicted in Figure 1b, our default fiber optic position was oriented such that light projected perpendicular to the long axis of the main apical dendrite, specifically centered on the soma. The transmission of light is described by equation 12, providing an estimate of the light irradiance in the extracellular space at various depths. The light distribution is depicted in Figure 2a. The theoretical irradiance from our default 0.2 mm diameter fiber optic decreased to 3.2 % of $I_0$ at 500 µm, and to 0.56 % of $I_0$ at 1 mm (Figure 2b). This drop is due to the scattering, absorbance and geometric spread of light. The model predicted intensity over this range of distances is consistent with intensity recordings reported for a 0.4 mm diameter fiber optic in rodent brain tissue (Gradinaru et al., 2009).

We calculated the irradiance required to generate an action potential in the neuron with fiber-optic-to-soma distances ranging from 100 µm to 2.0 mm (Figure 2c). Over these distances, action potential initiation took place in the axon initial segment. The threshold for generating a propagating action potential with a 0.2 mm diameter fiber optic was found to increase from 40.5 mW/mm² at 500 µm, to 190 mW/mm² at 1 mm, consistent with the drop in light irradiance at these distances. Our theoretical results predict activation up to 1.3 mm distant from the tip of a 0.2 mm diameter fiber optic with an irradiance of 380 mW/mm² (5 ms pulse width). Using the same fiber optic and irradiance stimulation parameters, Aravanis et al. (2007) published an estimated stimulation depth in motor cortex of 1.4 mm from a ChR2 rodent model, based on an
estimated firing threshold of 1 mW/mm². Likewise, Han et al. (2009) reported modulation of cortical neuron activity in a ChR2 non-human primate up to 1 mm distant from the tip of a 0.2 mm fiber optic with 80 mW/mm² light (5 ms pulse width). Under those same stimulation conditions, our model predicts direct action potential generation in layer V pyramidal neurons up to 0.7 mm from the fiber optic.

The threshold irradiance for action potential generation is highly dependent on the duration of illumination. Strength-duration curves were calculated at three different fiber-optic-to-soma distances (500 µm, 1000 µm and 1500 µm) (Figure 3). The threshold irradiance followed a pattern seen commonly during electrical stimulation, with short duration pulse widths requiring larger intensities than longer duration pulse widths. The rheobase irradiance levels were 9.0, 42.4 and 148.3 mW/mm² for distances of 500, 1000 and 1500 µm, respectively. The corresponding chronaxia times were 8.32, 8.33 and 8.34 ms.

We stimulated the model neuron for 1 s with trains of 5 ms light pulses, using frequencies ranging from 1–200 Hz, and measured the neural output firing frequency (Figure 4). The model neuron responded reliably to pulse trains up to 90 Hz when using a light irradiance that was greater than the single pulse threshold (120%, 140%). However, the response was not reliable for near threshold light irradiance levels. Furthermore, we tested the impact of varying channel density on the firing frequency rate (Figure 4). We stimulated three neurons with channel densities of 97.5, 130 (estimated default) and 195 ChR2·µm⁻², with an irradiance that was 120% of the single pulse threshold for each density. In the case of increased channel density (150% of estimated) action potentials followed in a 1:1 ratio with the stimulus train successfully up
to 100 Hz. For the low channel density case (75% of estimated), the success rate dropped below 1:1 at 30 Hz, and became unreliable above 80 Hz. These results contrast experimental findings that show action potential following failure typically occurs above ~40 Hz (Gunaydin et al., 2010).

*ChR2 Current*

The light irradiance threshold was highly dependent on which neural compartment types contained ChR2. The threshold irradiance was determined with ChR2 limited to specific compartment types (Table 2). Thresholds were determined with a 1 mm fiber-optic-to-soma distance, a 0.2 mm diameter fiber optic and a 5 ms light pulse. At the end of a threshold stimulus pulse, total current through all ChR2 was calculated ($I_{\text{ChR2}}$), as well as the approximate number of open channels (ChR2$^+$ membrane surface area * channel density). When ChR2 was limited to the soma compartment, the threshold irradiance required for activation was 2 orders of magnitude higher than when limited to the dendrites; however, the number of open channels was similar. Expression in both compartment types further decreased the threshold, but only marginally. Additional expression in the axon had negligible effect on the threshold. The total ChR2 current and approximate number of open ChR2 channels were slightly lower for soma-limited ChR2, and substantially lower for axonal-limited ChR2, but were similar in the scenarios including dendritic ChR2 (Table 2).

We investigated the response of the pyramidal neuron to a constant 1 s pulse of illumination from a 0.2 mm diameter fiber optic with intensities ranging from 10 to 500 mW/mm$^2$ (Figure 5ab). During stimulation, the photocurrent in the model rapidly
increases, peaks, and then decays to a plateau level. After a stimulus pulse, the current rapidly decays to zero. These general characteristics of the ChR2 current match experimental voltage-clamp recordings (Nikolic et al., 2009). For a typical stimulus intensity of 5 mW/mm², the peak and plateau current are 1.6 and 1.2 nA, respectively, with 2.7% (peak) and 2.2% (plateau) of the channels opened. Both the peak and plateau ChR2 currents increased with higher light irradiance. The percentage of channels open follow a similar response (peak/plateau) (Figure 5cd), also demonstrating a proportional response to light irradiance level.

Saturation has been demonstrated with focused laser light (Schoenenberger et al., 2010) when a small subset of compartments (e.g. the soma) was selectively illuminated. When our model neuron was illuminated with a focused laser beam of light, illuminating only the soma, the peak current (~2.3 nA) leveled off at irradiance levels greater than ~1000 mW/mm², while the plateau current (~0.857 nA) leveled off at irradiance levels greater than ~500 mW/mm² (data not shown).

Threshold Profile

The threshold for activation was dependent on the fiber optic-neuron orientation (Figure 6). The illumination threshold was determined for a range of fiber optic locations along the neuron, from a distance of 500, 1000 and 1500 µm, with light directed perpendicular to the long axis of the neuron (Figure 6e). The threshold profile is nonlinear, being influenced by many factors, such as the contribution of depolarization in each given compartment to the generation of an action potential (Figure 6a), the membrane area illuminated (Figure 6b), the number of channels opened due to the flux
of photons across the membrane (Figure 6c), and the distance between the fiber optic and neuron compartments (Figure 6d). The activation threshold was lower near the cell body and the apical dendrites, and higher along the apical shaft and axon. This general relationship was preserved at larger fiber-optic-to-neuron distances. During stimulation of the somatic/basal region, action potentials were initiated in the axon initial segment. As stimulation moved laterally along the apical shaft and into the apical tuft, the site of action potential initiation localized to the apical dendrites.

We analyzed the spatial sensitivity of fiber optic stimulation by evaluating the threshold as we moved the fiber optic in a plane 500 µm above the long axis of the neuron (Figure 7a). High spatial resolution has been achieved using focused light, with the minimum threshold observed in the somatic region (Schoenenberger et al., 2008; Rickgauer and Tank, 2009). Our simulations suggest that there is an additional region of minimal irradiance threshold in the apical tuft region. At close distances (100 µm), there is some degree of additional spatial dependence, particularly among the apical dendrites (Figure 7b-c), though to a lesser extent than would be possible with focused light.

Optical stimulation of the cortex commonly involves illumination perpendicular to the surface of the brain, as depicted in Figure 8. A population of 101 neurons was composed with 50 µm lateral spacing, spanning 5 mm. This population was stimulated with a 0.2 mm diameter fiber optic. The distance between the tip of the fiber optic to the closest neuron’s soma was 1.5 mm, and approximately 0.5 mm to the closest neuron’s apical dendritic tuft. The irradiance threshold of the closest neuron was 0.1 mW/mm², which increased substantially within a few hundred µm. Neurons 250 µm lateral to the
center neuron had thresholds of 190 mW/mm² (left) and 202 mW/mm² (right) (Figure 8c).

Parameter Sensitivity

We performed a sensitivity analysis to determine the robustness of the single spike threshold estimates to changes in model parameters, as well as to give insight into the design of future ChR2 variants and optical fibers (Figure 9). The results of this analysis reveal that optogenetic neural stimulation is highly sensitive to changes in many parameters. For example, a 50% increase in the ChR2 channel density was found to result in a decreased irradiance threshold from 190 mW/mm² to 126 mW/mm² (Figure 9a) for our default fiber-optic-neuron orientation (Figure 1b). Positive correlation was seen in the absorption/scattering coefficients of brain tissue, fiber-optic-to-neuron distance, numerical aperture of the fiber optic, and the rate of channel desensitization ($K_{d1}$). A decrease in any of these parameters resulted in a lower stimulus threshold. Negative correlation was seen in the tissue index of refraction, ChR2 channel density, fiber optic radius, stimulus duration, single channel conductance and quantum efficiency. An increase in any of these parameters would result in a lower stimulus threshold. Other parameters that had no effect on stimulus thresholds were not shown, which include $K_{d2}$ and $K_r$, rather than influencing the stimulus threshold, these two parameters affect plateau currents, as well as channel conductance post-illumination. Therefore, it should be noted that these parameters could play a more important role in repetitive stimulation analyses.
Results from the fiber-optic radius parameter manipulation may be potentially counterintuitive. Increasing fiber-optic radius from 0.2 mm to 0.3 mm decreases the irradiance threshold from 190 mW/mm² to 104 mW/mm²; however, it also increases the surface area of the fiber optic, with a net increase in the radiant power (see Equation 15) from 5.97 mW to 7.34 mW. Instead, decreasing the fiber-optic radius to 0.1 mm is predicted to decrease the radiant power requirement to 4.74 mW. For all other parameters in Figure 9, radiant power is proportional to irradiance.

Non-Uniform ChR2 Expression

The default neuron model used in our analysis assumed a uniform ChR2 distribution. However, the ChR2 distribution and overall channel density has a large impact on the stimulus threshold (Figure 9), with higher channel densities demonstrating a lower stimulus threshold (Figure 10a). We investigated the impact of non-uniform ChR2 distributions by constructing neuron models in which the same numbers of soma-dendritic channels (10 million) were distributed primarily in 1) the somatic/basal region, or 2) the apical region (Figure 10b). Higher densities of channels in the apical region led to lower thresholds in the apical region, at the cost of higher thresholds in the somatic/basal region. Higher somatic/basal distributions resulted in the opposite relationship. In the case of apical stimulation parallel to the long axis of the neuron (e.g. cortical stimulation perpendicular to the surface of the cortex) (Figure 10c), there are substantial decreases in the irradiance threshold with an apical distribution. For example, the 1 mm distance threshold of 299 mW/mm² with a uniform distribution dropped to 99 mW/mm² with an apical distribution.
Taken together, these results of this study demonstrate that the activation threshold is dependent on the biophysical parameters of ChR2, its distribution, the tissue properties, the characteristics of the fiber optic and its orientation with respect to the neuron.

Discussion

The goal of this study was to generate a theoretical understanding of optogenetic action potential generation in neocortical layer V pyramidal neurons expressing ChR2. The results demonstrate that optical stimulation power with a fiber-optic is minimized when illuminating a large cross sectional area of ChR2+ neural membrane from a short distance. We found that the threshold for activation is highly dependent on 1) the fiber optic design, 2) the irradiance of light, and 3) the ChR2 characteristics, including distribution, conductivity, and temporal kinetics. While limited experimental data currently exists to directly support or refute many of our quantitative predictions, the model does provide several new hypotheses that could be tested in vitro with patch-clamp recordings of individual neurons and fine control of the fiber optic position relative to the neuron.

Our results indicate that the threshold irradiance for generating an action potential is highly dependent on the number of ChR2 channels illuminated. As such, quantitative definition of the ChR2 expression density (and its neuron-to-neuron variability) represents one of the most important, but currently unknown, values necessary for the characterization of optogenetic stimulation spread (or activation volumes). When assuming a uniform ChR2 channel density, the surface area is a direct determinant of the number of channels available for illumination. Figure 6 demonstrates
that the stimulation irradiance is minimized in regions of large surface area illumination (e.g. dense apical dendritic arbors) and highest along the axon. This is in contrast to the threshold profile of neurons with electrical stimulation (McIntyre and Grill, 1999), where the threshold minima occur along the axon.

Since the discovery of ChR2, numerous genetic variants have been developed to deal with the limitations of ChR2 (Lin, 2010). For example, ChETA is a ChR2 genetic variant that has faster opening and closing rates than ChR2, with a reduced photocurrent amplitude (Gunaydin et al., 2010), whereas ChEF demonstrates slower temporal kinetics, but increased light sensitivity (Lin et al., 2009). ChR2 is also being targeted to specific regions of the neuron, such as the dendrites (Lewis et al., 2009), the axon (Lewis et al., 2011). When ChR2 was limited to the axon initial segment (Grubb and Burrone, 2010) action potentials could not be generated, presumably due to the small number of channels available for recruitment. This is consistent with Table 2, demonstrating the inverse relationship between available surface area (i.e. number of channels illuminated) and threshold irradiance.

A recent study involving computation analysis of ChR2 genetic variants demonstrated the irradiance threshold dependence on the parameters of each variant (Grossman et al., 2011). This analysis also found that the light irradiance threshold was dependent on ChR2 channel density and conductivity. However, the emphasis of that study was on homogenous illumination, and did not include the light dynamics of fiber optic stimulation.

Our results show that the location of the fiber optic relative to the neuron has a large impact on the source irradiance required to generate an action potential. We found
two local minimal irradiance regions: the soma, and the apical tuft (Figures 6e, 7ab, 10a). In the case of illumination near the soma, the low threshold is achieved in part by the substantially larger membrane area illuminated, and the relatively short distance from the fiber optic to the basilar dendrites that project out towards the fiber optic. On the other hand, for apical illumination, a smaller total surface area is illuminated, but the endogenous channel properties of that section of the neuron make it more excitable (Figure 6a). The apical shaft is difficult to stimulate due to the greater distance from the fiber optic to each compartment. Similarly, when channels were expressed only in the soma, the threshold irradiance was 2 orders of magnitude higher than when expressed globally (Table 2). In general, the number of channels required to be open to induce action potential initiation is similar under many different stimulation conditions. However, opening a sufficient number of channels in a small membrane patch (e.g. apical shaft, soma) with an unfocused beam of light requires a very large irradiance.

The results from our parameter sensitivity analysis demonstrate that the activation threshold is dependent on 1) the design of the fiber optic 2) the tissue parameters and 3) the biophysical parameters of ChR2 (Figure 9). Fiber optic design can take advantage of these findings to decrease irradiance thresholds by decreasing the fiber optic diameter (to decrease radiant power), and increasing the stimulus duration (to increase the depth of activation). Tissue parameters such as absorbance, scattering and the index of refraction are frequency dependent. Therefore, more light would reach the neural membrane by stimulating with a longer wavelength, and this concept is under active exploration with the creation of red-shifted channelrhodopsin variants (Zhang et al., 2008). Our results indicate that higher channel densities, a larger
conductance, and a slower desensitization rate ($K_{d1}$) would result in more responsive ChR2 variants.

The distribution of ChR2 also has a high impact on the threshold irradiance level. Our model predicts that redistributing ChR2 to the apical dendrites for apical directed stimulation may decrease irradiance levels by up to 3-fold. Recent advances in the development of not only neuron specific, but also neuron section specific, promoters for ChR2 (Lewis et al., 2009) suggest that such channel distributions are possible. This concept could be especially advantageous for large volume optical stimulation with LEDs that rest on the cortical surface, while still targeting selective activation of layer V pyramidal neurons.

This study presents a novel model of optogenetic neural stimulation and attempts to incorporate the most relevant components of the overall system (i.e. light source, tissue, neuron, and light sensitive channel). Nonetheless, important simplifications have been made in the creation of the model. First, the model for the absorption and scattering of light assumes that the sample is optically homogenous and illuminated normal to the sample surface, while biological tissue is optically inhomogeneous on the microscopic and macroscopic scale. However, our simplistic model was able to recreate experimental slice transmittance data with good agreement (Gradinaru et al., 2009) (Figure 2b). Second, the pyramidal neuron morphology originates from cat visual cortex and its membrane dynamics represent a simplification of the actual currents responsible for action potential initiation. As such its application to other neural populations may be limited. However, as our model development process evolved over the years we transitioned from the original Mainen et al. (1995) membrane dynamics to the more
recent Hu et al. (2009) model. Reassuringly, even with these substantial differences in
model description, our basic predictions and conclusions remained unchanged. One
caveat of even the recent Hu et al. (2009) model is the lack of hyperpolarization-
activated cation current ($I_h$) in the apical dendrites (Stuart and Spruston, 1998), which
could impact our threshold estimates in that region. Third, the trajectory of illumination is
likely not going to be purely perpendicular (Figure 1b) or parallel (Figure 10c) to the
neuron, but rather somewhere in between. However, we found that the angle of
illumination has a relatively small impact on the threshold intensity outside of those two
extremes (data not shown). Fourth, we implemented a four-state model for ChR2. The
photocycle of ChR2 has been proposed to involve either 3 states (Nagel et al., 2003), 4-
5 states (Hegemann et al., 2005), or 6 states (Ernst et al., 2008; Ritter et al., 2008),
each capturing progressively more detailed representations of ChR2 dynamics at the
single channel level (Nikolic et al., 2009). We elected to use the 4-state model because
of its ability to capture the salient features of ChR2 voltage-clamp data. However, the
ability of the model to respond with action potentials in a 1:1 fashion to high frequency
stimulation suggests limitations in the kinetics of the 4-state model when pushed to an
extreme. Fifth, we only considered the original ChR2 channel, as it remains the most
widely used optogenetic tool and our goal was to demonstrate general principles.
However, there are many new variants of channelrhodopsin, each with different
characteristics that would change the specific results (Grossman et al., 2011). In spite of
these limitations, our results provide some of the first quantitative descriptions of the
complex interactions between the fiber optic, tissue, neuron and ChR2.
Due to the complexity of directly measuring individual neuron irradiance thresholds for action potential initiation (in vivo or in vitro) we are unaware of published experimental results from which we can directly compare our theoretical threshold estimates. Comparison of our model results with the experimental estimates of Aravanis et al. (2007) and Han et al. (2009), suggest that our model underestimates the stimulus spread. However, the discrepancies can be accounted for by the uncertainty regarding some of the model parameters, in particular the ChR2 density. For example, our model would match their experimental estimates well if we assumed a higher ChR2 density as in Figure 10a. Nonetheless, we elected to present the majority of our results using the only available quantitative measurement of photosensitive channel density (Nagel et al, 1995), albeit from bacteriorhodopsin in Xenopus oocytes.

A goal of this study was to identify factors that impact the energy efficiency of optical stimulation. The energy efficiency of bench-top lasers is not of much concern to researchers working with slice preparations from rodent brains. However, optogenetics also represents a technology that could someday find application in clinical neuromodulation (Henderson et al., 2009). Numerous barriers exist for the clinical adoption of optogenetic technology, but encouraging preclinical studies suggest it may be a worthwhile goal to pursue (Tønnesen et al., 2009; Gradinaru et al., 2009; Llewellyn et al., 2010). However, the large energy costs of optical stimulation will need to be addressed to enable realistic implantable pulse generators. These challenges may prove to be surmountable, but will require the application of substantial genetic and biomedical engineering advances. As such, we propose that light-neuron models could represent an important design tool for the evaluation of future optogenetic technology.
Acknowledgements

The authors thank Murtaza Mogri for providing light transmittance data. This project was supported by the National Institutes of Health R01 NS047388.

References


Gradinaru V, Mogri M, Thompson KR, Henderson JM, Deisseroth K. Optical


Figure 1. Light-neuron model. (a) Blue light is absorbed by ChR2, followed by a conformational change, opening the channel for the passage of cations. ChR2 structure image adapted from Müller et al. (2011) (b) Representation of a fiber optic illuminating a layer V pyramidal neuron. (c) Neuron represented by a multi-compartment cable model. ChR2 is only activated in compartments directly illuminated by the light source. (d) ChR2 is modeled in four states, with two open and two closed states. Channels can transition between states with rate constants as described by Nikolic et al. (2009) (e) Electrical circuit representation of the soma-dendritic compartments. ChR2:

Channelrhodopsin-2. \( Na^+ \), \( Na^{+1.2} \), \( Na^{+1.6} \): sodium channels. \( K^+ \): potassium channel. \( K^+_{\text{slow}} \): slow, non-inactivating potassium channel. \( K^+_{\text{Ca}} \): calcium-dependent potassium channel. \( Ca^{2+} \): high-voltage-activated calcium channel, with decay of internal calcium concentration. \textit{Leak}: passive leakage channel. \( C_m \): membrane capacitance.

Figure 2. Fiber optic light model. (a) Three-dimensional representation of fiber optic light output. \textit{Lossless} demonstrates Gaussian-distributed light in a vacuum, with no geometric spread. \textit{Geometric} spread and \textit{scattering} are shown independently, and then combined. (b-c) Light transmission and irradiance as a function of the fiber-optic-to-soma distance. The fiber optic was directed at the soma from distances ranging 0.1 to 2.0 mm (50 \( \mu \text{m} \) resolution). Optical stimuli were applied with 5 ms duration. Three different fiber optic diameters (0.1, 0.2 and 0.4 mm) were simulated. (b) Transmission of light through diffuse, scattering tissue. Transmission is a measure of the drop in irradiance, due to conical geometry, absorbance and scattering of emitted light. Comparison to results reported in rodent brain tissue with 0.4 mm diameter fiber optic
(Gradinaru et al., 2009). (c) Threshold source light irradiance required to generate a propagating action potential in a layer V pyramidal neuron with a fiber optic-neuron orientation depicted in Figure 1b.

Figure 3. Strength-duration relationships. The fiber optic-neuron orientation was as shown in Figure 1b. Irradiance required to generate an action potential determined for 10 µs-10ms pulse widths. Fiber optic diameter 0.2 mm. Fiber-optic-to-soma distances were 0.5, 1 and 1.5 mm.

Figure 4. Repetitive stimulation. Neuron firing frequency rates in response to optical stimulation with pulse frequencies ranging from 1 to 200 Hz. (upper) Response at different stimulation irradiance levels: 100%, 120% and 140% of single pulse action potential threshold. (lower) Response with different ChR2 channel densities: 75%, 100% and 150% of our default density. Irradiance level is 120% of single spike threshold.

Figure 5. Response of ChR2 channels. Membrane voltage clamped to -70 mV. Fiber optic-neuron orientation as shown in Figure 1b. (a) Total ChR2 current induced in the neuron with irradiance ranging from 10–500 mW/mm² for one second. (b) Peak and Plateau ChR2 currents, both of which increase for larger irradiance. (c) Percentage of all ChR2 channels opened by given irradiance levels. (d) Peak and plateau percentage of channels opened, both of which increase with larger irradiance levels.
Figure 6. Optical stimulation profile. (a) Current injection threshold at each individual model compartment required to generate an action potential in a non-illuminated neuron. 500 µm scale bar is valid for all x-axes. (b-d) Fiber optic is oriented perpendicular to the long axis of the neuron (1 mm fiber-optic-to-neuron distance), illuminating the neuron at 40 µm intervals along the longitudinal position of the neuron. Stimulations were performed with a 0.2 mm diameter fiber optic, and 5 ms stimulus duration. The threshold irradiance was calculated for each longitudinal position. (b) Area of membrane illuminated (transmittance > 0.001). (c) Percent of total ChR2 channels in the open state at the end of a threshold stimulation pulse. (d) Distance from the output end of the fiber optic to either the closest illuminated neuron compartment or the average position of all illuminated compartments (transmittance > 0.001). (e) Threshold irradiance level (± 0.1%) required to generate a propagating action potential from different fiber-optic-to-neuron distances.

Figure 7. Threshold irradiance contours. Color represents light irradiance threshold (mW/mm²) required to generate a propagating action potential (± 0.1%). Simulations were performed with 0.2 mm diameter fiber optic and stimulus duration of 5 ms. (a) Threshold for action potential generation at over 8000 different fiber-optic-to-neuron positions with the fiber optic incrementally moved in 10 µm steps in a plane 500 µm above the long axis of the neuron. (b-c) Detail views of stimulation from a plane 100 µm above the neuron. Threshold irradiance color bar in (c) also applies to (b).

Figure 8. Cortical stimulation. (a-b) Artificial rendering of light-neuron model integration with a Thy1-ChR2 transgenic mouse line 18 (Wang et al., 2007) (coronal histological
(b) Close-up of cortical layers, with model neurons overlaid in the layer V region and fiber optic oriented perpendicular to the surface of the cortex. (c) Irradiance threshold to generate a propagating action in a population of neurons. The fiber optic tip was 0.5 mm from the apical tuft of the closest neuron (1.5 mm from the soma of the closest neuron). Neuron color corresponds to the irradiance threshold.

**Figure 9.** Parameter sensitivity analysis. Each model parameter was varied independently (±50%), and the threshold irradiance level necessary to generate an action potential was determined (±0.1%). Default values are listed in Table 1.

**Figure 10.** Variable ChR2 expression. (a-b) The fiber optic was placed perpendicular to long axis of the neuron (1 mm distance) and the threshold irradiance required to generate an action potential was calculated (±0.1%). (a) Stimulation profile of a neuron with 5, 10 or 50 million channels distributed uniformly in the soma-dendritic compartments of the model. ChR2 expression in the axon was uniform and constant at the default model value. (b) Stimulation profile with 3 different distributions of 10 million ChR2 channels: *basal/soma* with higher expression of ChR2 in the basal tuft and somatic compartments, *uniform* with equal channel density across all compartments, and *apical* with higher expression of ChR2 in the apical tuft. (c) The fiber optic was placed along the longitudinal axis of the neuron, directed at the apical tuft. The threshold irradiance was calculated as a function of the fiber optic distance from the apical tuft for the 3 different ChR2 distributions used in (b).
# Table 1. Standard model parameters.

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Channelrhodopsin-2 properties</strong></td>
<td></td>
</tr>
<tr>
<td>$K_{d1}$ Decay rate</td>
<td>130 s$^{-1}$</td>
</tr>
<tr>
<td>$K_{d2}$ Decay rate</td>
<td>25 s$^{-1}$</td>
</tr>
<tr>
<td>$\Lambda_1$ Decay Factor</td>
<td>30 µs</td>
</tr>
<tr>
<td>$\Lambda_2$ Decay Factor</td>
<td>150 µs</td>
</tr>
<tr>
<td>$e_{12}$ Transition rate: Light</td>
<td>53 s$^{-1}$</td>
</tr>
<tr>
<td>$e_{21}$ Transition rate: Dark</td>
<td>22 s$^{-1}$</td>
</tr>
<tr>
<td>$\rho_{\text{ChR2}}$ ChR2 density</td>
<td>130 µm$^{-2}$</td>
</tr>
<tr>
<td>$g_1$ O1 state conductivity</td>
<td>50 fS</td>
</tr>
<tr>
<td>$g_2$ O2 state conductivity</td>
<td>2.5 fS</td>
</tr>
<tr>
<td>$\sigma_{\text{ret}}$ Retinal cross section</td>
<td>$1.2 \times 10^{-8}$ µm$^2$</td>
</tr>
<tr>
<td>$\varepsilon$ Quantum efficiency</td>
<td>0.5</td>
</tr>
<tr>
<td>$\tau$ ChR2 time constant</td>
<td>1.3 ms</td>
</tr>
<tr>
<td>$K_r$ Recovery rate</td>
<td>0.4 s$^{-1}$</td>
</tr>
<tr>
<td>$\rho_{\text{ChR2}}$ ChR2 density</td>
<td>130 µm$^{-2}$</td>
</tr>
<tr>
<td><strong>Fiber optic properties</strong></td>
<td></td>
</tr>
<tr>
<td>$R_0$ Optical fiber radius</td>
<td>0.1 mm</td>
</tr>
<tr>
<td>$Z$ Distance</td>
<td>1 mm</td>
</tr>
<tr>
<td>$NA_{\text{fib}}$ Optical fiber numerical aperture</td>
<td>0.37</td>
</tr>
<tr>
<td>$pw$ Illumination duration</td>
<td>5 ms</td>
</tr>
<tr>
<td><strong>Tissue properties</strong></td>
<td></td>
</tr>
<tr>
<td>$K^{**}$ Absorbance coefficient</td>
<td>7.37 mm$^{-1}$</td>
</tr>
<tr>
<td>$S^{**}$ Scattering coefficient</td>
<td>0.125 mm$^{-1}$</td>
</tr>
<tr>
<td>$n_{\text{tis}}$ Tissue index of refraction</td>
<td>1.36</td>
</tr>
<tr>
<td><strong>Cell properties</strong></td>
<td></td>
</tr>
<tr>
<td>$r_m$ Membrane resistivity</td>
<td>30 kΩ·cm$^2$</td>
</tr>
<tr>
<td>$c_m$ Membrane capacitance</td>
<td>0.75 µF·cm$^{-2}$</td>
</tr>
<tr>
<td>$r_i$ Axial resistivity</td>
<td>150 Ω·cm</td>
</tr>
<tr>
<td>$V_m$ Rest membrane potential</td>
<td>-70 mV</td>
</tr>
</tbody>
</table>

1Nikolic et al., 2009, Fig 8  6Aravanis et al., 2007
2Nikolic et al., 2009  7Gradinaru et al., 2009
3Nagel et al., 2003  8Vo-Dinh et al., 2004
4Hegemann et al., 2005  9Hu et al., 2009
5Nagel et al., 1995

*ChR2 density assumed to be similar to that of bacteriorhodopsin measured in Xenopus oocytes.
**Fit to data using least squares method.
Table 2. ChR2 compartment localization.

<table>
<thead>
<tr>
<th>ChR2+ Compartment(s)</th>
<th>Threshold (mW/mm²)</th>
<th>$I_{ChR2}$ (nA)</th>
<th># Open ChR2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soma</td>
<td>14,511</td>
<td>1.11</td>
<td>176,945</td>
</tr>
<tr>
<td>Dendrite</td>
<td>205</td>
<td>1.72</td>
<td>247,975</td>
</tr>
<tr>
<td>Soma + Dendrite</td>
<td>197</td>
<td>1.72</td>
<td>247,259</td>
</tr>
<tr>
<td>Soma + Dendrite + Axon</td>
<td>190</td>
<td>1.71</td>
<td>243,283</td>
</tr>
<tr>
<td>Axon</td>
<td>2,104</td>
<td>0.33</td>
<td>42,386</td>
</tr>
</tbody>
</table>
Figure 1.

(a) Extracellular Cations

(b) Intracellular Cations

(c) 470 nm

(d) Cations

(e) Ca²⁺, K⁺, Na⁺, Na⁺, Leaky, Slow CmChR2, Leak, Cm

Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.
Figure 6.
Figure 7.
Figure 8.
Figure 9.
Figure 10.