Proceedings of the 28th IEEE
EMBS Annual International Conference
New York City, USA, Aug 30-Sept 3, 2006



# Modeling and Engineering aspects of ChannelRhodopsin2 System for Neural Photostimulation

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***Abstract*— It is desirable to be able to stimulate neural cells for many different therapeutic applications. Light stimulation has many advantages over electrical stimulation if it can be achieved. Neural cells are not naturally light sensitive but they can be transformed using different strategies. Here we examine the case of genetically engineered neurons expressing green algae light-gated ion channels, Channelrhodopsin-2. We have developed a mathematical model for the photocycle of this protein, which gives results which are in good agreement with experimental measurements. We have also examined engineering aspects of using this ChR2 system as a phototransduction mechanism. The response characteristics were calculated and potentials of this system-device are discussed.**

## I. INTRODUCTION

Neural stimulation has been the subject of intense research over the last century. Many potential therapeutic applications can be realized with effective stimulation methods. Presently only electrical stimulation has shown the ability to match the signal transduction speeds required. Stimulus is usually applied via biphasic current pulses. These can either be delivered through large area electrodes or through microelectrodes, depending on the required application. Electrical stimulation has however a power overhead which must be considered. Although the potential difference across the neural cell membrane is a few tens of millivolts, it requires volts to provide effective stimulation. In addition the biphasic pulse requires tens of microamps during the stimulation period. This microwatt power consumption during the stimulation pulse is small, but scales with the number of electrodes. The quality of neural implants such as retinal implants scales with the number of electrode stimulation points. However, this scaling can lead to power consumptions which are difficult for implanted devices. In addition these techniques require intimate contact between the stimulation point and the neuron. Placement accuracy can be difficult to achieve. In the long term implanted systems also have long term issues with electrode degradation. Therefore it is highly desirable to achieve cell stimulation in non-invasive way. This is possible by using light, if the neural cells are light sensitized. Apart from the convenience of being non-invasive, optical stimulation offers several other advantages over conventional electrical stimulation methods: better spatial and temporal resolution, flexible spatial control, better cell-electrode contact for multiple electrodes etc. Here we have investigated the Channelrhodopsin-2 (ChR-2) protein as a tool for rendering neural cells photosensitive. We have designed a physical model for the ChR-2 system based on the previous experimental work of Nagel *et.al.* [1], [2], [3] and Ishizuka *et.al.* [4] and then we have examined the engineering consideration aspects of this system as a light transduction mechanism.

The majority of neurons are not intrinsically light sensitive. Signaling is achieved through integration of the post-synaptic potential at synaptic interfaces and expression of action potentials. Specific sensory neurons such as the rods and cones in the eye do express light sensitivity via the G-protein coupled rhodopsin photocascade. However these have analog potential gradients and do not produce action potentials. Several different strategies have been proposed how to create light sensitive neural cells [5]: genetic engineering route [3], [6], photolysis of caged agonists of native cell surface receptors [7] and photoswitching of special ligands attached to ion-channel receptors [8], [9]. In the genetic engineering route cells are induced to stably express a photosensitive protein cascade or ion channel. In the case of opsin-type molecule expression, the molecule is incorporated into the cell plasma membrane and the photoreceptive function usually depends on the presence of cis/trans-isoform of retinaldehyde. The Opsin contains a retinaldehyde molecular core, which undergoes isomer type transformation upon absorption of a photon within the necessary energy range. The isomeric conformation of the retinaldehyde in turn activates the protein. This then can activate a G-protein type cascade (in the case of Rhodopsin or Melanopsin). In the case of photosensitive ion-selective channel expression (e.g. Bacteriorhodopsin, Channelrhodopsin-1 and 2) activation of the opsin directly causes an opening of the ion channel and thus an electrical response.

## II. KINETIC MODEL

The photocycle model for ChR-2 was described in [1], which is based on the recorded photocurrents. Upon the photo absorption, the molecule which is in the closed, sensitized state (C) undergoes very fast transition into excited state ($C^*$). This state leads to the open channel state (O), which then spontaneously turns into closed, but desensitized state (D), when the ion channel is closed but the molecule is not ready to photoswitch again. Molecule then goes into closed but sensitized state (C) after some recovery period. The time constant of the process of turning closed ion channel into open ($C \rightarrow C^* \rightarrow O$) is smaller than 1 ms. The process of closing the ion channels ($O \rightarrow D$) depends strongly on pH of the bath solution (extracellular liquid) and it lasts 10-400 ms.

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Since the opening happens so fast, the model can be reduced to a three-state model (C → O → D → C).

The process of recovery from desensitization (D → C) is not yet fully understood. It takes about 2-10 seconds in the dark, but in the light this process should take only 60 ms in order to explain the experimental photocurrents [1], [2], [3], [4]. This could be explained by assumption that the recovery rate depends on the light flux, or that the recovery process only partially goes via D state, whereas some of molecules go directly from Open to Closed/sensitized state. We have tested both hypothesis and our conclusion was that the first one more closely describes the experimental results. Hence we have adopted a three-state model as show in Fig. 1.

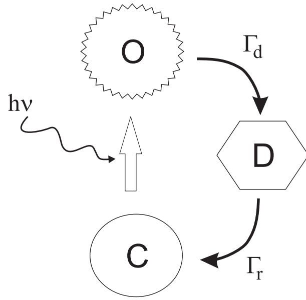


Fig. 1. Three-state model of ChR-2 photocycle.

The model above is mathematically described by:

$$\frac{\mathrm{d}X^*}{\mathrm{d}t} = \varepsilon\phi X - \Gamma_{\mathrm{d}} X^* \quad (1)$$

$$\frac{\mathrm{d}X_{\mathrm{ds}}}{\mathrm{d}t} = \Gamma_{\mathrm{d}} X^* - \Gamma_{\mathrm{r}} X_{\mathrm{ds}} \quad (2)$$

where $X^*$, $X_{\mathrm{ds}}$ and $X$ are the numbers of open/excited (state O), closed/desensitized (state D) and closed/photosensitive (state C) molecules in a cell, $\Gamma_{\mathrm{d,r}}$ are the rates of channel closure and recovery of photosensitivity, $\varepsilon$ is the quantum efficiency of ChR-2 system and $\phi$ is the number of photons hitting the cell per second. If the total number of ChR-2 molecules in a cell is $X_0$ ($X_0 = X^* + X_{\mathrm{ds}} + X$), the system of differential equations (1)-(2) becomes:

$$\frac{\mathrm{d}x^*}{\mathrm{d}t} = p - (p + g_d)x^* - p x_{\mathrm{ds}} \quad (3)$$

$$\frac{\mathrm{d}x_{\mathrm{ds}}}{\mathrm{d}t} = g_d\, x^* - g_r\, x_{\mathrm{ds}} \quad (4)$$

where $x = X/X_0$, $g_{\mathrm{d,r}} = \Gamma_{\mathrm{d,r}}/P_{\max}$, $p = P/P_{\max}$, $P = \varepsilon\phi$, and $P_{\max}$ corresponds to the maximum light intensity in a experiment. The current in the whole cell voltage-clamp measurements in the ChR-2 expressing cells is directly proportional to the number of open channels ($i \propto x^*$). Results of simulations are shown in Fig. 2.

The light illumination almost instantaneously creates current, which was experimentally measured under whole-cell patch clamp by several groups, e.g. [1], [4]. The light source was a blue LED ($\lambda_{\mathrm{peak}} = 470\,\mathrm{nm}$, 2.6eV) in [4], the light intensity varied between 0 and $5\,\mathrm{mW\,mm^{-2}}$. Our simulation results shown in Fig. 2 represent the typical light-evoked response of ChR-2 expressing cells, with a rapid increase to

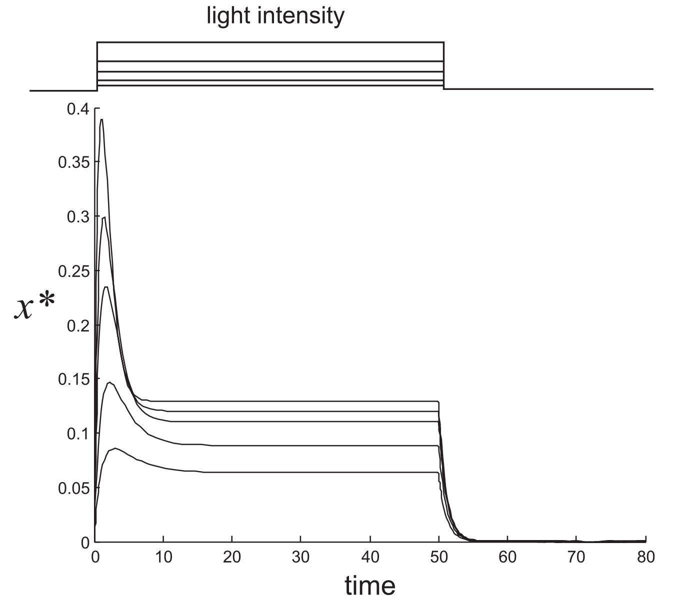


Fig. 2. Cell response to light pulse: time dependent series of the number of open ion channels in the cell (i.e. light-activated currents) for different light intensities. The light pulse duration is 50 time units (i.e. 1 second, if the time unit, which is $1/P_{\max}$, is 20 ms) and the relative light intensities are: 0.1, 0.2, 0.4, 0.6 and 1. $g_{\mathrm{d}} = 1,\ g_{\mathrm{r}} = 0.15$.

the peak current and then a slower decrease to the asymptotic - plateau value. Our model gives the analytic solution for the time-dependent currents, i.e. values for $x^*$:

$$x^*(t) = A^* + A^* e^{-\alpha t}\left[\xi\,\frac{\sinh(\beta t)}{\beta} - \cosh(\beta)\right] , \quad (5)$$

where:

$$A^* = A^*_{\max}\frac{p}{p+K}\ ,\ A^*_{\max} = \frac{g_{\mathrm{r}}}{g_{\mathrm{r}}+g_{\mathrm{d}}}\quad K = \frac{g_{\mathrm{r}}g_{\mathrm{d}}}{g_{\mathrm{r}}+g_{\mathrm{d}}} \quad (6)$$

$$\alpha = (p + g_{\mathrm{d}} + g_{\mathrm{r}})/2 \quad (7)$$

$$\beta = \sqrt{((g_{\mathrm{d}} + g_{\mathrm{r}} - p)/2)^2 - g_{\mathrm{d}}g_{\mathrm{r}}} \quad (8)$$

$$\xi = \frac{p}{A} - \alpha \quad (9)$$

When the light is turned off, the current exponentially turns-off:

$$x^*(t) = A^* e^{-g_{\mathrm{d}} t}\ . \quad (10)$$

By measuring the dark current, the value for $\Gamma_{\mathrm{d}}$ can determined. The peak current as well as the plateau current ($A^*$) are dependent on the light intensity ($p$). The plateau value saturates as the light intensity reaches high illuminations, see (6), whilst the peak values are almost proportional to the light intensity and only slightly saturates for the levels of illumination examined here, see Fig. 3.

The results in Fig. 3 are in good agreement with experimental results from [4]. For example, they have found for the turning-off time constant $\tau_{\mathrm{off}} = 11\,\mathrm{ms}$ (a sample response to a 1 s light pulse at the maximum intensity used in experiment), which gives $\Gamma_{\mathrm{d}} = 1/\tau_{\mathrm{off}} \approx 90\,\mathrm{s^{-1}}$, and then $P_{\max} = 30\,\mathrm{s^{-1}}$ and $\Gamma_{\mathrm{r}} = 6\,\mathrm{s^{-1}}$ (since $g_{\mathrm{d}} = 3$ and $g_{\mathrm{r}} = 0.2$). The peak-to-plateau part of the response (see Fig. 2) can be fitted with a single exponential curve of the

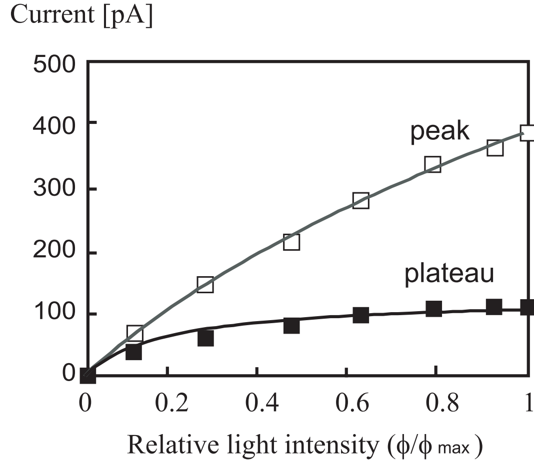


Fig. 3. The peak (open squares) and the plateau currents (closed sqares) in the experiment of Ishizuka *et. al.* [4] and our corresponding theoretical values (lines), $g_{\rm d} = 3,\ g_{\rm r} = 0.2$.

form $\sim \exp(-t/\tau_i)$ where $\tau_i$ is the inactivation time constant [4]. If we approximate the value of $\tau_i$ for the maximum illumination to be $(1/\tau_i) = \alpha - \beta$ then our model (6)-(9) will give $\tau_i \approx 22\,\text{ms}$ and the experimental value is $23 \pm 2\,\text{ms}$.

## III. Engineering Aspects

Our device is a neural cell with ChR2 molecules embedded in the cell membrane immersed into appropriate solution of some salts, sugars etc, with well defined pH value. This device transduces light to effect a change in the membrane conductance. This change leads to depolarization of the cell effecting action potential spiking. The signal transduction process depends on some engineering characteristics of the system which we shall address in the next section.

### A. Small signal response

Let us assume that the system is in the steady state under some constant illumination. The number of open channels ($x^*$), i.e. the current, has its asymptotic value $A^*$ (6). Thus how does the system respond to a small change in the illumination ($\Delta p$)? The change of the current is proportional to $\Delta x^* = x^* - A^*$. The system of equations (3)-(4) gives:

$$\frac{\mathrm{d}(\Delta x^*)}{\mathrm{d}t} = (1 - A^* - A_{\rm ds})\Delta p - (p + g_{\rm d})\Delta x^* - p\Delta x_{\rm ds}$$
$$\frac{\mathrm{d}(\Delta x_{\rm ds})}{\mathrm{d}t} = g_{\rm d}\,\Delta x^* - g_{\rm r}\,\Delta x_{\rm ds}$$

We know that $1 - A^* - A_{\rm ds} = A$ which is the steady state value for the number of closed/ready molecules - this result seems plausible because this is the pool of molecules which is ready to respond to the change of illumination. Otherwise this system of equations is identical to (3)-(4) and could be reduced to a second order differential equation (under assumption that the coefficients $g_{\rm d}$ and $g_{\rm r}$ are not light flux dependent functions):

$$\frac{\mathrm{d}^2(\Delta x^*)}{\mathrm{d}t^2} + 2\alpha\,\frac{\mathrm{d}(\Delta x^*)}{\mathrm{d}t} + \Omega^2(\Delta x^*) = g_{\rm r} A\,\Delta p\ , \qquad (11)$$

where $\Omega^2 = pg_{\rm d} + pg_{\rm r} + g_{\rm d}g_{\rm r}$ and $A = g_{\rm d}g_{\rm r}/\Omega^2$. If the input modulation is a harmonic function of frequency $\omega$ and amplitude $(\Delta p)_0$, the response would be also a harmonic function (a forced oscillator):

$$\Delta x^* = (\Delta p)_0 G(\omega)\,\sin(\omega t - \varphi)$$

where the frequency dependent gain is:

$$G(\omega) = \frac{g_{\rm r}A}{\sqrt{(\Omega^2 - \omega^2)^2 + (2\alpha\omega)^2}} \qquad (12)$$

For $\omega = 0$, $G(0) = g_{\rm r}A/\Omega^2$. If the "operating point" was $p = 0$ (dark) then $\Omega^2 = g_{\rm d}g_{\rm r}$ and $A = 1$ so $G(0) = 1/g_{\rm d}$. For small input modulation $(\Delta p)_0 \ll 1$, the output is $(\Delta p)_0/g_{\rm d} \approx A^*$, which is expected. However, this system does not go into resonance, because the resonant frequency is $\omega_{\rm rez} = \sqrt{\Omega^2 - 2\alpha^2}$, and for our system: $\Omega^2 - 2\alpha^2 = -(p^2 + g_{\rm d}^2 + g_{\rm r}^2)/2 < 0$. The amplitude characteristics are shown in Fig. 4.

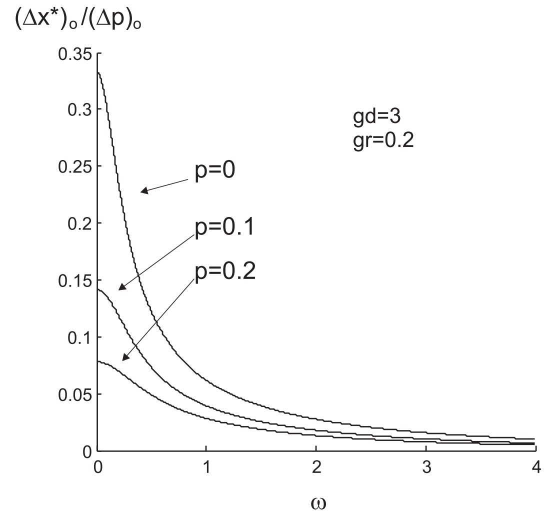


Fig. 4. The amplitude characteristic of the neuron-ChR2 device. For adopted values for $g_{\rm d}$ and $g_{\rm r}$, value for $\omega = 1$ is 30 Hz.

The results suggest that this device is most sensitive when it is initially in the dark and the ChR-2 channels are in the closed state.

### B. Rapid kinetics of ChR-2

System response to a series of light pulses is of special interest for us. The ChR-2 system shows the ability of spontaneous recovery in dark, because retinal can re-isomerize to the all-trans ground state in dark, without need for special enzymes. However this recovery is relatively slow $\tau_{\rm r,dark} \sim 2\!-\!10\,\text{s}$. This fact can be included in our model, but $\tau_r$ has to be dependent on the light flux, or alternatively we can adopt two different values for $\tau_r$: one for light ($\tau_{\rm r,light}$) and one for dark conditions ($\tau_{\rm r,dark}$). Now the recovery of the ChR-2 molecules in the dark can be examined by applying two light pulses, but at different intervals, results are shown in Fig. 5. The recovery time of the peak current is very slow, but the plateau currents are not affected.

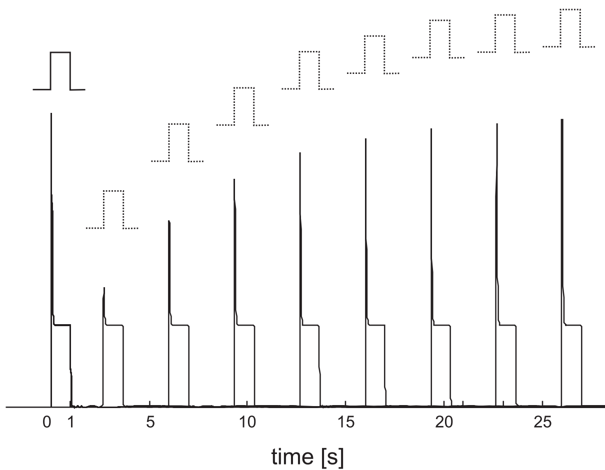


Fig. 5. The recovery rate for the peak current investigated by applying two light pulses (first pulse shown with full line, the second pulse is one of the broken lines) at various intervals. The time scale is for the values: $\tau_{\rm d} = 11\,{\rm ms}$, and $\tau_{\rm r,light} = 165\,{\rm ms}$ and $\tau_{\rm r,dark} = 6.5\,{\rm s}$.

However, this is a recovery of the photo-current under voltage-clamping, but we are also interested to the kinetics under current-clamp conditions, when action potential spikes are generated. It has been experimentally shown that neuron-ChR2 can reliably generate trains of spikes as a response to a series of light pulses of 10 ms duration and down to 100 ms interpulse interval [3]. In order to simulate these experimental results we need to examine the response of the system to a train of light pulses which are fast switching on and off, see Fig. 6.

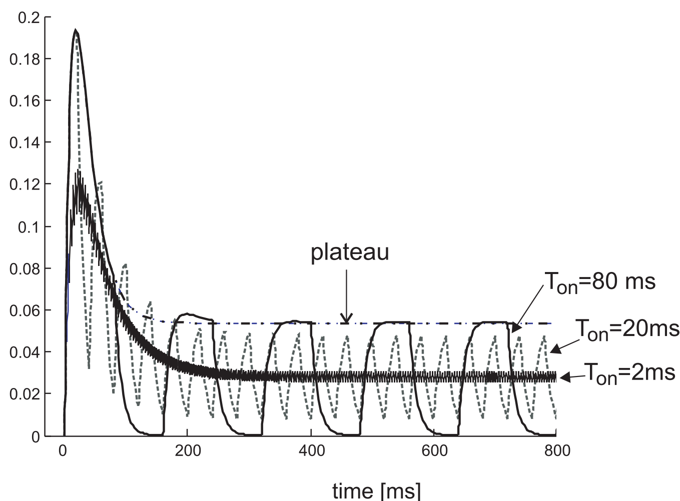


Fig. 6. Current response depends on the frequency and duration of light pulses. The light-on time $T_{\rm on}$ and and the light-off time $T_{\rm off}$ are equal. Maximum light intensity. Time scale same as in previous figure.

From results in Fig. 6 is clear why action potentials can be controllably generated up to the frequency of about 15 Hz (for the parameter choice taken here, but which is in accordance to the experiments of Ishizuka *et.al.* [4]). Since the change of the neuron membrane potential is proportional to the integral of the current during certain period, the current due to ChR2 ion channels has to be turned on and off (sufficiently fast) so that depolarization and subsequent repolarization of the membrane is possible during one cycle. Hence for the case $T_{\rm on} = T_{\rm off} = 2\,{\rm ms}$ the current through ChR2 channels is almost constant and light has almost no effect on neuron spiking. Only for $T_{\rm off} \sim 100\,{\rm ms}$ there appears to be enough time during which the ion channels are closed and the internal pumps can repolarize the membrane and make it ready for new action potential. The new action potential will be created during the light-on period which could be as short as ten to twenty milliseconds.

## IV. CONCLUSIONS

ChR2 system provides reasonably fast kinetics and high conductance which could be used in light stimulation of neurons. It offers good temporal resolution which is the main requirement for external stimulation of neurons and control of neuron firing. However, the ChR2 system has no built in amplification mechanism, such as G-protein cascade, hence relatively high light intensities combined with high expression levels of ChR2 are required for driving this system. The photocycle of CrR2 was successfully modeled to reproduce experimental results. This modeling gives us valuable insight into the working mechanism of the device. The maximum frequency of the light pulses which could be reliably reproduced into neural spike trains depends on the deactivation rate of the ChR2 molecules and it is not affected by slow recovery rate in dark. The engineering aspects of the neuron-ChR2 device established here could be used for future circuit models of some neural systems [10].

## V. ACKNOWLEDGMENTS

The authors gratefully acknowledge the funding contribution of Advanced Nanotech.

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