Fast, repetitive light-activation of CaV3.2 using channelrhodopsin 2

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Introduction

Ion channels are pore-forming transmembrane proteins that regulate rapid movement of ions across the cell membrane along an electrochemical gradient. They undergo conformational changes that lead to opening or closing of the conducting pore. Even minor changes in the structure can drastically alter the conductivity. If such structural changes are genetically-encoded they may cause myotonia, retinitis pigmentosa and hypertension. Since ion channels interconnect the intracellular and extracellular milieu of the cell and are accessible via their extracellular domain, they are interesting drug targets.

The most common approach for ion channel analysis is patch clamp recording that allows tracing of small currents at millisecond time resolution. However, little success has been made during the last decades in the transformation of this electrical method into a high throughput system.

Monitoring ion channel activity with optical ion-sensitive dyes has been proven as a very valuable assay and is now routinely used in many laboratories. Even though most ion channels are sensitive to membrane voltage, such an optical assay lacks the ability to optically control the electrical gradient.

A new and interesting method to achieve optical control of the membrane voltage was recently accomplished by employing channelrhodopsin-2 (ChR2), a light-gated ion channel from the green alga *Chlamydomonas reinhardtii*, in various cells. ChR2 attracted great interest in neuroscience, because it allows to trigger action potentials by application of brief blue light pulses in ChR2 expressing neurons. ChR2 conducts monovalent cations under physiological conditions and to some degrees calcium ions in the absence of monovalent cations. As a ChR2 counterpart, the light-driven chloride pump halorhodopsin of the archa *Natronobacterium pharaonis* (NpHR) enabled hyperpolarisation with yellow light. The membrane potential can then be optically modulated. Blue light depolarizes the cells and promotes action potentials, whereas yellow light hyperpolarizes and suppresses action potentials, respectively. However, since NpHR is an ion pump the desensitizing effect of NpHR is limited by its single-charge per photon coupling. Hence, hyperpolarisation strongly depends on an expression level of NpHR. Since ion channel screening is preferentially done in non-excitable cells most of which exhibit less negative resting potentials than neurons, the resting membrane voltage must be decreased to study the most common voltage-gated ion channels. Consequently,
cells would need to express NpHR in high amount and in addition high intensive yellow background light would be required. However, improvement of NpHR expression was only moderately successful in the past. Therefore, we have chosen a different approach. While using ChR2 for transient depolarization, we employed for persistent hyperpolarization the potassium channel mTrek, which only needs to be expressed at low levels leaving enough space in the membrane for the chosen ion channel drug target. All necessary modules for our approach are shown in Figure 1.

Calcium ions play crucial roles for regulation of a variety of cellular functions as muscle contraction, release of neurotransmitters from nerve terminals, regulate gene expression, and mediate cell death. Accordingly, intracellular calcium is precisely adjusted and acts as a signaling molecule which can be altered by calcium permeable ion channels.

Most ion channels are voltage-gated and of outstanding interest due to their ubiquitous expression in human tissues. Voltage-gated calcium channels can be subgrouped into low voltage activated Ca\(^{2+}\) channels (T-type or LVA) and high voltage activated channels (L-type or HVA). LVA channels may be encoded by the genes: \(\alpha_{1G}, \alpha_{1H}\) and \(\alpha_{1I}\) that all belong to the Ca\(_{3}\)-family.

We have chosen \(\alpha_{1H}\) of the Ca\(_{3}\)-family as a potential drug target due to the clinical relevance in neurological disorders including epilepsy or Parkinson’s disease.

### Results

To verify stable expression of mTrek and \(\alpha_{1H}\) in the \(\alpha_{1H}/\)mTrek cell line, cells were analyzed with standard patch clamp techniques. Representative traces of voltage induced currents by \(\alpha_{1H}\) are shown in Figure 2A. Starting from -90 mV we changed voltage as indicated in the upper voltage protocol in Figure 2A. Depolarization induced transient inward-directed current. Inactivation kinetics is moderately voltage dependent (compare black and blue trace) and \(\tau_{\text{m}}\) is in the range of 10–20 ms in agreement with reported values. The corresponding current-voltage relation is shown as black line in Figure 2B. Maximum peak amplitude was obtained at -25 mV. To assess reactivation of Ca\(_{3.2}\), we used a voltage protocol shown in the lower part of Figure 2A and a current-voltage relation was compiled (grey line Fig. 2B) showing that activity is fully recovered after inactivation below -90 mV.

Next, we confirmed that the mTrek potassium channel, which is in its open state at practically all voltages, provides the option to change membrane potential with extracellular potassium concentration. In a current-clamp experiment the membrane voltage correlates with an increasing extracellular potassium concentration (Fig. 2C). Accordingly, a change of the potassium concentration by 12 mM resulted in a shift in membrane voltage of about 50 mV. In fluorescence experiments with the stable \(\alpha_{1H}/\)mTrek cell line loaded with fura-2, a rapid change from 1 mM to 10 mM potassium concentration triggered a transient increase of fura-2 fluorescence corresponding to an opening of the calcium channel and a Ca\(^{2+}\) influx (Fig. 2D). Small potassium concentration jump from 2 mM to 6 mM results in smaller transient fluorescence change. The T-type calcium channel inhibitor mibefradil completely abolished the fluorescence change. In the next step we tested activation of ChR2 and fura-2 in a dual-beam set-up (Fig. 3A). In a current-clamp experiment under whole cell conditions, current amplitudes for light activation at 340 nm, 380 nm and 470 nm were compared (Fig. 3B). Although absorption of ChR2 is low in the UV-range, the photocurrent at 100% light intensity at 380 nm was similar to that of 470 nm. However, a decrease in fura-2 excitation light to 10% while leaving illumination at 470 nm unchanged, shows only a moderate cross-activation (lower traces Fig. 3B).

Furthermore, we confirmed that all three ion channels operate in concert (Fig. 3C). The calcium channel opened at a voltage jump from -100 mV to -40 mV (shown in the inset). A subsequent illumination at -100 mV caused a ChR2 mediated inward current. Functionality of mTrek was verified by a voltage ramp from -100 mV to 50 mV, showing a small outward rectification at positive voltages. In the presence of 10 \(\mu\)M mibefradil no inhibition was observed for ChR2 or mTrek (Fig. 3C red line).

For ChR2-transfected \(\alpha_{1H}/\)mTrek cells a K+ concentration jump from 1 to 10 mM at 10% fura-2 excitation light caused transient fluorescence increase (Fig. 4A). At 10 mM K+ corresponding to high membrane voltage, a blue light pulse of 250 ms duration did not evoke fluorescence changes. However at 1 mM K+ and a lower membrane voltage blue light pulses evoked fast Ca\(^{2+}\) influx as seen from the transient fura-2 fluorescence increase. Even after 5 min of resting periods, blue light induced Ca\(^{2+}\) influx remained reproducible whereas 10 \(\mu\)M mibefradil completely abolished the response. Ca\(^{2+}\) influx was fully recovered after removal of mibefradil (Fig. 4B).
To counterbalance the low signal-to-noise ratio at low fura-2 excitation we tested different exposure times (Fig. 4C).

Figure 4C shows an enlarged single response upon a blue light pulse, revealing a decay time of 2 s. After testing different mibefradil concentrations, the $K_D$ value for inhibition was determined to be 130 nM with a Hill coefficient of about 5 (Fig. 5A). Signal amplitude was found to depend on the extracellular Ca$^{2+}$ concentration as shown in the inset. Blue light pulses longer than 50 ms resulted in a robust activation of CaV3.2 (Fig. 5B).

**Discussion**

In this study we demonstrated that ChR2 can be used as a tool for a transient control of the membrane voltage in an ion channel.
Under physiological conditions no Ca\textsuperscript{2+} influx through ChR2 was detected with fura-2 during illuminating with blue light. A Ca\textsuperscript{2+} influx was observed only when monovalent cations were substituted with NMG\textsuperscript{+} and after illumination longer then 10 s (data not shown). This result is in good agreement with reported fluorescence values (data not shown). This result is in good agreement with reported fluorescence values.

In general, all rhodopsins show broad absorption spectra. As hyperpolarisation is necessary for the activation of vast majority of voltage-gated ion channels, the mTrek potassium channel was expressed. Critical depolarisation requires high ChR2 expression levels, high extracellular calcium concentration at neutral pH, and about half saturating light intensities at 470 nm. In contrary, hyperpolarisation with K\textsuperscript{+} only requires low or moderate mTrek expression.\textsuperscript{14} The ion channel under study should be expressed at appropriate levels to get sufficient fluorescence signals at low 340/380 nm light intensities. Under physiological conditions no Ca\textsuperscript{2+} influx through ChR2 was detected with fura-2 at 380 nm and mCherry fluorescence (540 nm). A computer controlled LED light source was used for ChR2 excitation (470 nm). For combining both optical pathways a 70% R/30% T beam splitter (BS) was used. For illumination and detecting the fura-2 and mCherry fluorescence a dual dicroic mirror was used as shown in lower part of (A). In (B) traces at 10% and 100% light intensity for 340/380 nm illumination are shown. At 10% light intensity only small cross-activation of ChR2 at 380 nm is observed. ChR2-illumination at 470 nm was always kept at 100% light intensity, causing a change in membrane voltage of up to 70 mV. In (C) ChR2-mCherry is transiently transfected into the stable α\textsubscript{1H}/mTrek cell line. A typical voltage-clamp trace showing transient calcium influx upon changing membrane potential from -100 mV to -40 mV. A subsequent illumination with 470 nm at -100 mV cause a ChR2 mediated inward current. Outward rectifying current was observed during voltage ramp from -100 mV to 50 mV, indicating functionality of mTrek. Same voltage protocol was applied in presence of 10 μM mibefradil (green line) showing that only Ca\textsubscript{3.2} is inhibited.

Figure 3. Current-clamp experiment of ChR2 transfected α\textsubscript{1H}/mTrek cell line at various light intensities. In (A) the instrumental set-up with dual illumination pathway is shown. Illumination with Polychrom V was used for exciting fura-2 (340 nm and 380 nm) and mCherry fluorescence (540 nm). A computer controlled LED light source was used for ChR2 excitation (470 nm). For combining both optical pathways a 70% R/30% T beam splitter (BS) was used. For illumination and detecting the fura-2 and mCherry fluorescence a dual dicroic mirror was used as shown in lower part of (A). In (B) traces at 10% and 100% light intensity for 340/380 nm illumination are shown. At 10% light intensity only small cross-activation of ChR2 at 380 nm is observed. ChR2-illumination at 470 nm was always kept at 100% light intensity, causing a change in membrane voltage of up to 70 mV. In (C) ChR2-mCherry is transiently transfected into the stable α\textsubscript{1H}/mTrek cell line. A typical voltage-clamp trace showing transient calcium influx upon changing membrane potential from -100 mV to -40 mV. A subsequent illumination with 470 nm at -100 mV cause a ChR2 mediated inward current. Outward rectifying current was observed during voltage ramp from -100 mV to 50 mV, indicating functionality of mTrek. Same voltage protocol was applied in presence of 10 μM mibefradil (green line) showing that only Ca\textsubscript{3.2} is inhibited.

In general, all rhodopsins show broad absorption spectra. This ChR2 stimulation and parallel measurements of fura-2 fluorescence is challenging.\textsuperscript{18} Since ChR2 does not absorb light above 550 nm, ion-sensitive dyes with longer wavelength absorption are eligible such as Crimson\textsuperscript{TM} or calcium Orange\textsuperscript{TM} (Invitrogen, Carlsbad, CA). However, the fluorescence responses were too small for our systems, most likely due to a low sensitivity or low dye loading (data not shown). Calcium dyes like Fluo4 are more sensitive than fura-2 but the absorption overlaps with the ChR2 spectrum. Consequently, fura-2 was chosen as intracellular calcium probe. Although fura-2 excitation light (380 nm) at high intensity caused full activation of ChR2, cross-activation could be minimized by reducing the light intensity to 10% (Fig. 3B). In addition, mTrek hyperpolarized the membrane voltage to the appropriated level. Under these optimized conditions Ca\textsuperscript{2+} influx through the α\textsubscript{1H} channel can be triggered with bright blue light and monitored with weak UV-light.

The red-shifted channelrhodopsin-1 of Volvox carteri (VChR1) shows a lower absorption in the UV range, which would allow stronger fura-2 excitation and might enable the use of genetically-coded calcium sensors without cross-activation of VChR1.\textsuperscript{19} But, due to insufficient VChR1-expression in HEK293T-cells a reliable depolarization with green light was not achieved with VChR1 yet (data not shown).

Since lower excitation light reduces cross-activation of ChR2 on the cost of lower fluorescence signal-to-noise ratio, a semi-optical approach was used to depolarize the cells optically with 470 nm light and to monitor intracellular calcium changes with fura-2 fluorescence. As hyperpolarisation is necessary for the activation of vast majority of voltage-gated ion channels, the mTrek potassium channel was expressed. Critical depolarisation requires high ChR2 expression levels, high extracellular calcium concentration at neutral pH, and about half saturating light intensities at 470 nm. In contrary, hyperpolarisation with K\textsuperscript{+} only requires low or moderate mTrek expression.\textsuperscript{14} The ion channel under study should be expressed at appropriate levels to get sufficient fluorescence signals at low 340/380 nm light intensities. Under physiological conditions no Ca\textsuperscript{2+} influx through ChR2 was detected with fura-2 during illuminating with blue light. A Ca\textsuperscript{2+} influx was observed only when monovalent cations were substituted with NMG\textsuperscript{+} and after illumination longer then 10 s (data not shown). This result is in good agreement with reported values.\textsuperscript{8,9}

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Sampling rate is limited in our system due to incapability to activate ChR2 and measure fura-2 emission simultaneously. Thus, the sampling rate was limited to 8 Hz corresponding to a minimum exposure time of 125 ms with minimum blue light activation of 50 ms for ChR2 (Figs. 4C and 5B).

Whereas mibefradil did not inhibit ChR2 and mTrek, a relatively high hill coefficient was obtained (Fig. 5A). This is most likely due to long exposure time of 500 ms which averages Ca\textsuperscript{2+} influx to zero.

ChR2 was transiently expressed to establish the ion-channel assay. Thus the degree of depolarization was dependent on ChR2 expression levels. As characterized previously, channelrhodopsins show a relatively linear light-intensity/current profile.\textsuperscript{20,21} A stable expression of ChR2 in a host-cell in combination with mTrek will offer more precise and defined voltage control.
**Materials and Methods**

**Cell culture.** HEK293 cells stably expressing α_{1H} subunit of Ca_{3.2} and mTrek1 were kindly provided by Paula Q. Barrett. Generation and properties of this cell line are described previously.17

Cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine and 1% (w/w) penicillin and streptomycin (Biochrom, Berlin, Germany) at a constant level of 5% CO₂ and 37°C. To maintain the stable expression of both genes, a final concentration of 400 μg/ml G418 and 0.4 μg/ml Puromycin (Sigma-Aldrich, St. Louis, MO) were added to the medium. For measurements 2.5 x 10⁵ cells were seeded on a glass cover slip. After 24 h cells were transfected with ChR2-mCherry plasmid using TransPass (NEB, Beverly, MA) and the cultivation was continued for another 24 h. Prior fluorescence or patch-clamp measurements, cells were treated with DME + 10% FBS with 2 μM all-trans retinal.

**Patch-clamp measurements.** All voltage clamp measurements were performed on an Axiovert 35 (Carl Zeiss Jena, Jena, Germany) with a 40x N.A 1.3 objective. For electrical measurements an Axopatch200B with peltier-cooled headstage (Molecular Devices, Foster City, CA) or EPC-7 (List Electronic, Darmstadt) were used. Analogue data were digitized with Digidata1440 (Molecular Devices, Foster City, CA). For applying protocols for data recording, pClamp software version 10.1 (Molecular Devices, Foster City, CA) was used. Patch pipettes were pulled with micropipette puller model P-97 (Sutter Instrument Co., Novato, CA) from micro-haematocrit-tubes (Hecht-Assistant, Sondheim, Germany). Pipette solution contained [in mM]: 110 NaCl, 10 EGTA, 2 MgCl₂, 2 CaCl₂, 2 KCl and 10 Hepes. pH was adjusted with NMG/HCl to 7.2 and 280 mOsm. All external solution were adjusted to 320 mOsm with glucose and

![Figure 4.](image)

_A ChR2-mCherry expressing HEK-α_{1H}/mTrek cells were loaded with fura-2 and subjected to pulses of blue light in different extracellular buffer conditions. Only when cells are hyperpolarized by a low concentration of K⁺, light pulses evoked transient fluorescence increase due to opening of voltage sensitive calcium channel Ca_{3.2}. Those responses can be evoked repetitively even after a longer pause in same size and kinetic. In (B) a blue light pulse evoke calcium influx. Adding 10 μM mibefradil suppresses influx. After a wash-out for more than 3 min, blue light again can evoke calcium influx showing the reversibility of activation. In (C) fluorescence responds from a single cell upon blue light stimulation with different exposure times. Higher sampling rate corresponding to smaller exposure times leads to a smaller signal-to-noise ratio. (D) Shows enlarged, single responds revealing a decay time ~2 s for the fluorescence increase._

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**Note:** The image contains a figure with graphs and annotations that are not fully described in the text. The description of the figure is provided below:

*Figure 4.* A ChR2-mCherry expressing HEK-α_{1H}/mTrek cells were loaded with fura-2 and subjected to pulses of blue light in different extracellular buffer conditions. Only when cells are hyperpolarized by a low concentration of K⁺, light pulses evoked transient fluorescence increase due to opening of voltage sensitive calcium channel Ca_{3.2}. Those responses can be evoked repetitively even after a longer pause in same size and kinetic. In (B) a blue light pulse evoke calcium influx. Adding 10 μM mibefradil suppresses influx. After a wash-out for more than 3 min, blue light again can evoke calcium influx showing the reversibility of activation. In (C) fluorescence responds from a single cell upon blue light stimulation with different exposure times. Higher sampling rate corresponding to smaller exposure times leads to a smaller signal-to-noise ratio. (D) Shows enlarged, single responds revealing a decay time ~2 s for the fluorescence increase.
Cells were loaded with fura-2-AM solved in DMSO and diluted in DMEM + 10% FBS to a final concentration of 4 µM. Cells were then incubated for 20 min at 37°C with 5% CO₂. Afterwards cells were washed 3 times with [in mM] 140 NaCl, 2 KCl, 2 MgCl₂ and 2 CaCl₂ and incubated for another 10 minutes. After mounting the cover slip into the measuring chamber and connecting the chamber to the perfusion system, cells were then checked for mCherry and fura-2 fluorescence. 

**Data analysis.** Data were exported from Tillvision 4.3 Software (TILL Photonics, Planegg, Germany) and analyzed using a self-written MatLab 7.0.1.24704 (R14) program that allows background fluorescence subtraction and analysis of single cells. For fura-2 experiments at high excitation intensities, fura-2 fluorescence ratios at 340 nm/380 nm are graphically presented. For ChR2 experiments with low excitation light intensity, only 380 nm emission was recorded and displayed. Graphs were designed with SigmaPlot (SPSS Science, Chicago, IL).

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**References**

1. Hille B. Classical Description of Channels. In: Sinauer Associates, Inc., Ionic Channels of Excitable Membranes 2nd 1992; 22-58.
2. Ashcroft F. From gene to protein. In: Ion Channels and Disease. NY: Academic Press, 2000; 3-19.
3. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 1981; 391:85-100.
4. Veliçelebi G, Stauderman KA, Varney MA, Akong M, Hess SD, Johnson EC. Fluorescence techniques for measuring ion channel activity. Methods Enzymol 1999; 294:20-47.
5. Nagel G, Staflas T, Huhn W, Kateriya S, Adeeishvili N, Berthold P, et al. Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. Proc Natl Acad Sci 2003; 100:13940-5.
6. Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K. Millisecond-timescale, genetically targeted optical control of neural activity. Nat Neurosci 2005; 9:1263-8.
7. Zhang F, Wang LP, Boyden ES, Deisseroth K. Channelrhodopsin-2 and optical control of excitable cells. Nat Methods 2006; 10:785-92.
8. Lin JY, Lin ZM, Steindach P, Tien R. Characterization of engineered channel rhodopsin variants with improved properties and kinetics. Biophysical J 2009; 96:1803-14.
9. Satoshi Aito Tsunoda, Peter Hegemann. Glu87 of Channelrhodopsin-1 causes pH-dependent color tuning and fast photocurrent inactivation. Photochem Photobiol 2009; 85:564-9.
10. Han X, Boyden ES. Multiple-color optical activation, silencing and desynchronization of neural activity with single-spike temporal resolution. PLoS ONE 2007; 2:299.10.1371/journal.pone.0000299.
11. Sohal VS, Zhang F, Yizhar O, Deisseroth K. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. Nature 2009; 459:698-702.
12. Gradinaru V, Thompson KR, Deisseroth K. eNpHR: a Natronomonas halorhodopsin enhanced for optogenetic applications. Brain Cell Biol 2008; 36:129-39.
13. Zhao S, Cunha C, Zhang F, Liu Q, Gloss B, Deisseroth K, et al. Improved expression of halorhodopsin for light-induced silencing of neuronal activity. Brain Cell Biol 2008; 36:141-54.
14. Perez-Reyes E. Three for T: molecular analysis of the low voltage-activated calcium channel family. Cell Mol Life Sci 1999; 56:666-9.
15. Jeanmonod D, Magnin M, Morel A, Siegemund M, Cacero A, Lanz M, et al. Thalamocortical dysrhythmia II. Clinical and surgical aspects. Thalamus Related Syst 1 2001; 245-54.
16. Mishra SK, Hrnsmeyer K. Selective inhibition of T-type Ca" channels by Ro 40-5967. Circ Res 1994; 75:144-8.
17. Yao J, Davies LA, Howard JD, Adney SK, Welshy PJ, Howell N, et al. Molecular basis for the modulation of native T-type Ca" channels in vivo by Ca"/calmodulin-dependent protein kinase II. J Clin Invest 2006; 116:2403-12.
18. Konstantin N, Grossman P, Grubb MS, Burrone J, Toutziaou C, Patrick Degenaar P. Photocycles of chanelrhodopsin-2. Photochem Photobiol 2009; 85:400-11.
19. Zhang F, Prigge M, Beyriere F, Tsunoda SP, Mattis J, Yizhar O, et al. Red-shifted optogenetic excitation: a tool for fast neural control derived from Volvox carteri. Nat Neurosci 2008; 11:631-3.
20. Ehlenbeck S, Gradmann D, Braun FJ, Hegemann P. Evidence for a Light-Induced H" Conductance in the eye of the green alga Chlamydomonas reinhardtii. Biophysical J 2002; 82:740-51.
21. Bammann C, Kirsch T, Nagel G, Bamberg E. Spectral characteristics of the photocycle of channelrhodopsin-2 and its implication for channel function. J Mol Biol 2008; 375:686-94.