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Voltage- and temperature-dependent gating of heterologously expressed channelrhodopsin-2

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Abstract

Channelrhodopsins are light-activated channels originally isolated from algae that are being used increasingly as tools to non-invasively stimulate neurones. Despite their widespread use some aspects of their biophysical properties have not been fully characterised. Here we report detailed investigation of the gating kinetics and voltage-dependence of ChR2 transiently expressed in HEK-293 cells. Currents were elicited using light pulses of defined duration and intensity generated by a blue LED. Datasets were gathered both at room temperature (RT, ~22 °C) and 37 °C. Current responses to light rose rapidly to a peak and then desensitized to a steady state plateau. When illumination was terminated currents rapidly deactivated. Recovery from desensitization at –85 mV was slow with half-times of 1.4 and 3.1 s at 37 °C and ~22 °C, respectively. At both temperatures, the reversal potential of ChR2 responses was a few mV positive to 0 mV. Both the peak and plateau phases of ChR2 responses exhibited strong inward rectification with only small outward currents at positive membrane potentials. The rates of ChR2 activation, deactivation and desensitization were ~2 times faster at 37 °C than at ~22 °C. Both the activation and deactivation kinetics of ChR2 were significantly slowed by depolarization at both temperatures. Additionally, the degree of steady state desensitization was greater at more depolarized potentials. The macroscopic desensitization kinetics were not voltage-dependent, but recovery from desensitization was slowed by depolarization. These gating behaviour data provide an important basis for more detailed analysis of the properties and limitations of ChR2 use in more complex systems.

Keywords

Channelrhodopsin; Electrophysiology; Gating; Kinetics

1. Introduction

Channelrhodopsins are light-activated ion channels that play an important role in the phototactic behaviour of green algae (Harz and Hegemann, 1991; Sineshchekov et al., 2002;

Suzuki et al., 2003). The cloning of the genes that encode these channels (Nagel et al., 2002, 2003; Zhang et al., 2008) and their subsequent heterologous expression in various host cells has provided novel experimental tools to manipulate membrane potential by exposure to light (Nagel et al., 2003, 2005a). This approach has proved particularly useful in neurobiology (Boyden et al., 2005; Nagel et al., 2002, 2005b; Schroll et al., 2006; Li et al., 2005) where channelrhodopsin expression in neurones has allowed action potential firing and down-stream synaptic transmission to be driven by exposure to light pulses (Nagel et al., 2003, 2005a). Indeed, channelrhodopsins have already been used extensively in preparations ranging from dissociated primary neuronal cultures to in vivo studies (Douglass et al., 2008; Adamantidis et al., 2007; Aravanis et al., 2007; Wang et al., 2007; Arenkiel et al., 2007; Schroll et al., 2006; Nagel et al., 2005b; Alilain et al., 2008; Bi et al., 2006). Furthermore, depolarizing channelrhodopsins have been supplemented by halo, a Cl pump from the archaeobacterium *Natronomas pharaonis* which when activated with yellow light can rapidly hyperpolarize cells (Han and Boyden, 2007; Zhang et al., 2007).

Channelrhodopsin-2 (ChR2) is a non-selective cation channel from *Chlamydomonas reinhardtii* that is activated by blue light (Nagel et al., 2003). As a prelude to using this protein in neurones we characterised the macroscopic kinetics and voltage-dependence of ChR2 channels expressed in HEK293 cells under near physiological ionic conditions. Previous biophysical studies have used either *Xenopus* oocytes (Nagel et al., 2003) or normal or electrofused HEK293 cells as an expression host (Nagel et al., 2003; Bamann et al., 2008; Zimmermann et al., 2006). These studies focused primarily on the permeation properties, potential roles of channel protonation/deprotonation in gating behaviour and the nature of the photocycle. In addition, however, it was also reported that recovery from desensitization is quicker at more negative membrane potentials (Nagel et al., 2003). In this study we confirm this observation and have identified additional voltage-dependent modulation of ChR2 gating kinetics that may have implications for the use of these channels in more complex systems where the membrane potential may not be constant.

2. Methods

The ChR2-eYFP-pcDNA3 construct was a kind gift from Dr Karl Diesseroth (Stanford University).

2.1. Dissociated hippocampal cultures

Briefly, hippocampi were dissected from the brains of E18 Wistar rat embryos. The isolated tissue was then enzymatically dissociated using trypsin treatment (0.5 mg ml^{-1} for 9 min) followed by mechanical dissociation. Cells were then plated on poly-L-lysine-coated glass coverslips at a density of ~ 100 cells per mm^2 and grown under standard cell culture conditions (5% CO_2 , 37°C). The culture medium was composed of Neurobasal medium (Gibco) supplemented with horse serum (9%), B27 (Gibco) and 2 mM glutamine. On the second day, the media was changed for Neurobasal medium supplemented with B27 and 2 mM glutamine and neurons were then fed each week with this serum-free medium until used experimentally after 18–21 days in vitro. To introduce channelrhodopsin into hippocampal cells they were transfected with $3 \mu\text{g}$ DNA per dish using Lipofectamine 2000 (Invitrogen) and used in experiments 2–5 days later.

2.2. HEK293 cultures

HEK293 cells were cultured in Dulbecco's Modified Eagle's Medium (Lonza) containing 9% fetal bovine serum, penicillin/streptomycin and 2 mM glutamine. Cells were passaged 2 days prior to transfection. HEK293 cells were transfected using the Amaxa Nucleofection Technology™ (Amaxa). Cells were resuspended in solution from nucleofector kit V

following the Amaxa guidelines for cell-line transfection. Briefly, 100 μ l of $2\text{--}5 \times 10^6$ cell suspension mixed with 3 μ g DNA was transferred to the provided cuvette and nucleofected with an Amaxa Nucleofector apparatus (Amaxa). Immediately after nucleofection cells were moved to dishes containing poly-lysine-coated coverslips and returned to culture for 2–5 days before experiments were performed.

2.3. Electrophysiological methods

Whole cell patch clamp measurements were made from single cells visualized with an inverted microscope (Nikon TE300). Experiments were performed at room temperature ($\sim 22^\circ\text{C}$) or 37°C . The bath solution was a HEPES-buffered saline consisting of (mM) NaCl, 135; KCl, 5; HEPES, 10; MgCl_2 , 1; CaCl_2 , 2; D-glucose, 30; pH 7.3 with NaOH, ~ 310 mOsm. Patch pipettes were filled with a potassium gluconate-based solution of the following composition (mM) K-Gluconate, 130; KCl, 20; HEPES, 10; EGTA, 0.2; GTP-Na salt, 0.3; ATP Mg salt, 4; pH 7.3 with KOH; ~ 295 mOsm. All voltage-clamp potentials have been corrected for a 15 mV liquid junction potential.

ChR2 was activated using a Cairn OptoLED light-source. This device was connected to the epi-fluorescence adaptor of the microscope and allowed us to use our standard electrophysiological software (Axon Instruments pClamp10) to control the intensity and duration of light pulses delivered from its blue light emitting diode (LED). The light intensity was controlled by varying the current flow through the LED between 0 and 1.1 A. Using a standard optical power meter (Spectra Physics 407A) we confirmed that the LED current (in the range 0–1.1 A) was linearly related to illumination Wattage at the sample plane. Unless otherwise stated experiments employed illumination at our standard LED current of 1 A. In HEK-293 cells current responses to light pulses were collected in voltage clamp using pClamp10 software. Membrane potential responses of neurones were measured in whole cell current clamp (Fig. 1A). In addition, the loose patch configuration in voltage-clamp mode was used to characterise ChR2-mediated action potential responses non-invasively (Fig. 1B). Sample frequencies were 10 or 20 kHz and data were filtered at 3 or 5 kHz using the 4 pole Bessel characteristic filter inherent to the patch clamp amplifier (Axon Instruments, Axopatch 200B).

3. Results

3.1. ChR2-mediated changes in spike latency in hippocampal neurones

A major application for ChR2 is to depolarize neurones to generate action potential firing (Boyden et al., 2005; Aravanis et al., 2007; Wang et al., 2007). In initial experiments we investigated ChR2-mediated excitation of primary cultured hippocampal neurones prepared from E18 rat embryos. Fig. 1A illustrates 3 sweeps from a typical whole cell current clamp recording of action potential firing in a ChR2 transfected neurone. A train of five 2 ms blue light flashes was applied at 10 Hz to stimulate ChR2 gating and each stimulus was sufficient to generate firing. Fig. 1B shows an example of ChR2-mediated action potential generation measured using the non-invasive loose patch method. The latency from applying the flash to the generation of a spike by the neurone becomes successively longer as the train of 20 stimuli progresses. On average the spike latency for first stimulus was 3.3 ± 0.2 ms from the onset of the 5 ms light pulse, whereas the spike latency for 20th spike was 5.3 ± 0.5 ms. In these same recordings, when a single flash was applied 10 s after the last stimulus in the train it triggered a spike with response latency (3.4 ± 0.3 ms), not significantly different from that generated by the first flash in the train (3.3 ± 0.2 ms). Thus, using ChR2 activation to repeatedly stimulate hippocampal neurones at 10 Hz causes a short-term change in the latency to generate an action potential.

Alterations to the intrinsic excitability of hippocampal neurones triggered by their recent activity are one possible explanation for the changes to action potential generation shown in Fig. 1B and C, in essence a form of short-term intrinsic plasticity. Alternatively, the observed changes may be attributable to alterations in the ChR2-mediated stimulus during the train. For example, if the amplitude of light-activated ChR2 currents declined during the train then smaller and slower depolarizing voltage responses would occur in the host neurone, a change likely to result in a longer latency to firing. To determine between these possibilities we carried out voltage-clamp analysis of the gating behaviour of ChR2 expressed in a mammalian cell-line.

3.2. Stimulus duration versus response relationships

We characterised the responses to light pulses of various durations using a 1 A LED current. Traces from an example recording are shown in Fig. 2A. At $\sim 22^\circ\text{C}$ ($\sim 22^\circ\text{C}$) a flash duration of >3 ms was required to elicit peak current and the activation 10–90% time of ChR2-mediated currents at $\sim 22^\circ\text{C}$ was never <5 ms. After 300 ms the extent of macroscopic desensitization approached a steady state level of $75 \pm 1\%$ of peak current. The degree of desensitization generated was not dependent on the peak amplitude of the current prior to desensitization (Fig. 2B), indicating that ions passing through the pore were not responsible for generating desensitization.

For some ligand-gated channels, such as the GABA_A receptor, the rate of deactivation is dependent on the level of desensitization established prior to agonist removal (Jones and Westbrook, 1995; Mellor and Randall, 1998). A similar coupling can be seen between inactivation and deactivation of certain voltage-gated channels, for example CaV₃ Ca²⁺ channels (Warre and Randall, 2000). We found that desensitizing most of the population of ChR2 caused only a very small ($<10\%$) change in deactivation rate. Thus, deactivation after a single 1 ms light flash, which produces $<5\%$ desensitization (see Fig. 2A) had a mean time constant of 10.7 ± 0.4 ms at $\sim 22^\circ\text{C}$ whereas a 300 ms light stimulus, which desensitized 75% of channels, deactivation occurred with a time constant of 11.5 ± 0.5 ms ($p < 0.05$, paired *t*-test).

3.3. Recovery from desensitization

Desensitization is key feature of ChR2 gating and is a key determinant of how well this optogenetic tool can drive various patterns of neuronal activity. As well as defining the kinetics of desensitization it is also important to understand ChR2 recovery from desensitization (Nagel et al., 2003). We characterised the recovery from desensitization with a standard variable interval paired stimulus protocol, as illustrated in Fig. 3A. This consisted of an initial 200 ms stimulus, used to produce near steady state desensitization followed at various latencies by a second shorter flash to establish the degree of recovery that had occurred in the intervening dark phase. A plot of fractional recovery versus inter-stimulus interval for experiments at $\sim 22^\circ\text{C}$ and a membrane potential of -85 mV is shown in Fig. 3B. Under these conditions >10 s were required to fully recover function of a strongly pre-desensitized ChR2 population. Recovery from inactivation at 37°C was faster, although prolonged recovery periods were still required to generate full dedesensitization (Fig. 3B). The interpolated half-times for dedesensitization were 1.4 and 3.1 s at 37°C and $\sim 22^\circ\text{C}$, respectively.

3.4. Responses to trains of light stimuli

Given this slow recovery from desensitization we decided to question how well ChR2 responses could follow 500 ms trains of brief light stimuli applied across a range of frequencies. At 22°C flashes of 2 ms duration produced currents that rose rapidly~(mean time to peak 2.9 ± 0.1 ms) and decayed monoexponentially (time constant 7.2 ± 0.7 ms, $n =$

10), kinetics that are quite similar to AMPA receptor-mediated EPSCs recorded at neuronal cell bodies. The rapid deactivation of ChR2 meant application of flashes at 10 Hz produced responses that were well isolated from each other. A significant decline in response amplitude, however, was observed from stimulus to stimulus such that the 5th response was $18 \pm 5\%$ smaller than the 1st (Fig. 4A, $n = 10$). At 20 Hz the responses remained well separated and the incremental decline in response continued throughout the train (Fig. 4B). At 40 Hz the rise of each subsequent response clearly overlapped with the latter part of the deactivation phase of its predecessor. Desensitization continued to increase throughout the train (Fig. 4C). At 80 Hz stimulation, the greater summation of responses meant the largest current deflection from baseline occurred following the second flash. After this initial summation-induced potentiation of response amplitude, however, subsequent current amplitudes declined by some 50% as the 40 light pulses were applied (Fig. 4D). When 2 ms flashes were applied at still higher frequencies (150–400 Hz) the responses largely merged into continuous currents, although an underlying oscillation at the applied stimulus frequency was detectable both visually and by calculating power spectra (Fig. 4E). Trains applied at 37 °C displayed similar declines in response amplitude as the train progressed. Due to faster deactivation kinetics, however, responses exhibited less summation for example at 80 Hz (Fig. 4F).

3.5. Voltage-dependent gating of ChR2

The permeation properties and gating kinetics of many channels outside the voltage-gated channel family also display voltage-dependence. For example, deactivation and desensitization of GABA_A receptors are voltage-dependent and the TRP channel, TRPV1, exhibits time and voltage-dependent rectification (Gunthorpe et al., 2000). We initially characterised the voltage-dependence of the steady state response of ChR2 (i.e. the response remaining after steady state desensitization was established). To do this we used a voltage-clamp protocol consisting symmetrical paired ramps from -85 to $+55$ mV initiated 1 s into a light response, a time at which ChR2 desensitization had reached steady state (Fig. 5A). A similar ramp was applied with the LED turned off and then the current–voltage relationship of the light-activated current was determined by subtracting the ramp response in the dark from that recorded during illumination (Fig. 5B). Consistent with previous reports (Nagel et al., 2005a; Bamann et al., 2008), this protocol revealed inward rectification in the steady state current with little or no outward current generated at positive potentials (Fig. 5C). Similar results were also obtained at 37 °C (data not shown).

The voltage-dependence of ChR2-mediated currents and their kinetics were also investigated by applying 250 ms flashes at 22 °C or 37 °C at a range of different holding potentials (Fig. 6A). Peak current versus voltage plots yielded I – V relationships with strong inward rectification. At both temperatures the reversal potential was slightly positive to 0 mV (Fig. 6B and C). For these same responses we characterised the voltage-dependence of the rates of activation, deactivation and desensitization, as well as the steady state extent of desensitization. All rates were faster at 37 °C than ~ 22 °C. In addition, both the activation and deactivation processes were faster at more negative potentials (Fig. 7Aii and iv). The rate of desensitization was not significantly voltage-dependent (Fig. 7Aiii) but the extent of desensitization measured relative to peak current became greater as the cells were depolarized (Fig. 7Ai). This is additionally illustrated in Fig. 7B which plots peak-normalised average responses from cells clamped at -85 and -25 mV, respectively ($n = 8$ at room temperature and $n = 6$ at 37 °C).

4. Discussion

Channelrhodopsins and other light-activated ion channels and transporters provide powerful new tools with which to control neuronal activity (Boyden et al., 2005). They allow “non-

invasive” activation or inhibition of neurones, or other cell types, with light pulses. Their expression in defined neuronal cell types via use of appropriate gene delivery methods and/or promoter sequences (Bi et al., 2006; Mahoney et al., 2008) can be used to alter the activity of single cells, discrete neuronal assemblies or substantial brain areas. In addition multiphoton excitation allows subcellular-resolution activation in vivo (Mohanty et al., 2008). These possibilities have led to an explosion in their use both in vertebrate and invertebrate systems. We set out to use ChR2 to study mechanisms of intrinsic and synaptic plasticity in hippocampal neurones. Fundamental aspects of ChR2 biophysics have been reported (Nagel et al., 2002; Bamann et al., 2008; Zimmermann et al., 2006), but our initial findings led us to consider if, and how, the gating of ChR2 is effected by changes to membrane potential, light intensity and temperature.

In a system where ChR2 is used to depolarize cells to generate action potentials, decreased illumination intensity at the cell could substantially slow membrane charging thereby causing changes in action potential latency or even failure to generate spikes. Thus care must be taken to ensure that, for example, drugs applied to a preparation during an experiment, do not significantly absorb the blue light applied to trigger ChR2 activation. In longer-term experiments, for example those performed in vivo (Aravanis et al., 2007), changes to the optical properties of the tissue around implanted optical fibres may also lead to changes to responsiveness over time. Such changes could result from time-dependent gliosis triggered by the inevitable tissue damage associated with implantation. Indeed, in such experiments in vivo, cells near the implanted optical source may act fast and effectively whereas those further away, and consequently experiencing lower intensity stimulation, will activate more slowly and perhaps less reliably. The experimental approach seemingly least likely to lead to issues arising from changes to intensity of illumination seems to be to apply short, supramaximal intensity flashes. With such stimuli a loss of some illuminating power would do little to alter either current amplitude or waveform.

The recovery from desensitization of ChR2 is a slow process, which is further slowed by depolarization (Nagel et al., 2003; Fig. 3). At $\sim 22^{\circ}\text{C}$ and a membrane potential of -85 mV , complete recovery takes $>20\text{ s}$. This facet of ChR2 gating is a potential disadvantage when aiming to use this optical tool to repeatedly activate cells at high frequencies. Indeed, our experiments at $\sim 22^{\circ}\text{C}$ using trains of 2 ms flashes applied at $10\text{--}80\text{ Hz}$ indicate that even a single 2 ms flash triggers $\sim 5\%$ desensitization (Fig. 5A and B). Such desensitization is not seen as macroscopic desensitization during the current response, but is made apparent by the depressed amplitude of the next response in the train. This slow rate of recovery from desensitization means that little or no recovery can occur during the time between pulses when stimuli are applied at 10 Hz or more.

ChR2 recovery from inactivation remains slow at 37°C with similar decreases in trains of flash responses (Fig. 4D). However at 37°C there was little summation of responses at frequencies as high as 80 Hz due to faster deactivation (Fig. 7). Some potential limitations imposed by ChR2 desensitization can be avoided by higher levels of ChR2 expression but since ChR2 has significant Ca^{2+} permeability this strategy can produce undesired elevations in intracellular Ca^{2+} (see for example Caldwell et al., 2008).

ChR2 has been used in both homeo- and poikiothermic species. In homeotherms experiments have been performed at both $\sim 22^{\circ}\text{C}$ and at physiological temperatures, including work performed in vivo (Aravanis et al., 2007). We therefore compared ChR2 responses at $\sim 22^{\circ}\text{C}$ and 37°C . As expected, ChR2 activation, deactivation, desensitization and recovery from desensitization are all faster at 37°C than $\sim 22^{\circ}\text{C}$, with an increase in rate at 37°C of around 2-fold at all membrane potentials examined (Fig. 7A).

The membrane potential of neurones is constantly changing as a result of both synaptic input and cycles of intrinsic membrane activity. Furthermore, there is considerable variability in the resting potential of neurones across cellular populations generally regarded as functionally homogeneous. For example, hippocampal CA3 pyramidal cells have resting potentials that are normally distributed over a range spanning at least 30 mV (Brown and Randall, 2009). In addition, certain forms of intrinsic neuronal plasticity involve long-lasting changes to neuronal resting potentials and experimentally induced pathological states can be associated with substantial changes to the average membrane potential. It is therefore important to determine how membrane voltage effects the activation of ChR2. Simple current versus voltage relationships reveal that ChR2 exhibits strong inward rectification as described by others previously (Nagel et al., 2005a; Bamann et al., 2008; Zimmermann et al., 2006). This was observed when either voltage ramps (Fig. 5) or different steady state voltage-clamp potentials (Fig. 6) were used to characterise voltage-dependence.

Our data show that at ~22 °C and 37 °C both activation and deactivation were significantly slowed by depolarization (Fig. 7A) and there is a voltage-dependence in the extent of desensitization generated at steady state (Fig. 7A and B). These voltage-dependent changes may have functional consequences in experimental scenarios. For example the voltage-dependence of activation means that in a neurone at –55 mV ChR2 will take 30% longer to activate than in a neurone at –85 mV (Fig. 7A). Similarly, due to the voltage-dependence of deactivation responses to brief light flashes (e.g. 1–5 ms) will produce responses that are 30% longer in cells at –55 mV than cells at –85 mV. Given that the membrane potential of cells activated non-invasively by ChR2 is not known, but will certainly vary from cell to cell within a population, the timecourse of the ChR2-mediated conductance changes within a neuronal population will exhibit a degree of kinetic variability. This variability in response waveform is over and above that produced by the cell-to-cell variance in peak conductance available which arises from the differences in expression levels between different cells.

In summary, ChR2 and related proteins provide powerful and convenient tools for investigating neuronal function and dysfunction. However, here we describe various aspects of the gating of ChR2 expressed in a mammalian cell-line which indicate that a number of factors determine the effectiveness and timecourse of activation. Our findings have implications for the pattern of ChR2 invoked depolarization in particular cells under particular circumstances. Factors that will influence the cellular response to ChR2 activation include the intensity of illumination, when ChR2 was last activated and for how long, the membrane potential of the cell, and the extracellular pH. Furthermore, the interplay between these different factors to the ChR2 response will vary with temperature.

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Fig. 1.

Changes to ChR2-mediated action potential latency during trains of light flashes. (A) Three consecutive whole-cell current clamp recordings from a cultured hippocampal neurone transfected with ChR2. The rapid upward deflections are action potentials elicited by trains of brief light flashes (2 ms, 1 A LED current) delivered at 10 Hz. (B) Loose patch recording of action potentials elicited by 20 repeated flashes delivered at 10 Hz. Note the progressive increase in latency from the onset of the light flash. (C) Pooled data from 6 recordings like that in (B) showing the increase in spike latency during a 10 Hz train of light flashes. Also shown is the mean spike latency elicited by a single flash applied following a 10 s recovery period after the 20th stimulus of the train of light flashes (open symbol).

Fig. 2.

Stimulus duration versus response in ChR2-transfected HEK293 cells. (A) An example recording in which a ChR2 expressing cell was exposed to blue light flashes (1 A LED current) of 1, 10, 30 and 300 ms duration. (B) Graph plotting the peak current amplitude (filled symbols, left axis) and charge transfer (open symbols, right axis) in response to light flashes of increasing duration. (C) A scatter plot showing the lack of correlation between the peak current amplitude and the extent of desensitization generated during a 300 ms light stimulus.

Fig. 3.

Recovery from light-induced desensitization in ChR2-transfected HEK293 cells. (A) An example of a typical dual flash experiment used to characterise the kinetics of recovery from desensitization of ChR2. Flash one (F1) induced desensitization and was followed at a variable latency by a second briefer flash (F2) to assess the degree of recovery. (B) Right pooled data from recordings like those in (A) made at either room temperature (~22 °C; filled symbols, $n = 7$) or 37 °C (open symbols, $n = 5$). The dotted lines indicate the time required to generate 50% recovery from desensitization.

Fig. 4.

Responses to trains of brief light flashes in ChR2-transfected HEK293 cells. (A) The traces are current responses to 500 ms duration trains of 2 ms flashes applied at 10, 20, 40 and 80 Hz. In all cases the LED current was 1 A. Note the accumulation of desensitization at all stimulus frequencies. (B) Pooled data plotting the amplitude (filled symbols) of each response in a stimulus train normalised to the amplitude of the first response in a train. Data are pooled from 10 cells and each of the four frequencies illustrated in (A). Also shown is the mean latency to peak for each response (open symbols). (C) At frequencies above 100 Hz, responses to individual light flashes were less easily resolved as a result of increased current summation (top). Spectral analysis of these traces, however, revealed membrane current oscillations at the applied train frequency (bottom). (D) Responses to trains of 2 ms light flashes delivered at 10 and 80 Hz at 37 °C. Note that the level of summation is reduced, but substantial desensitization still occurs.

Fig. 5. Voltage-dependent properties of light-induced responses in ChR2-transfected HEK293 cells. (A) Current responses to symmetrical voltage ramps (-85 mV to $+55$ mV to -85 mV) delivered either during a light pulse (intensity, 1 A; black trace) or in the absence of light (grey trace). (B) Subtractively determined light-activated current responses from 6 recordings like that shown in (A). (C) Averaged normalised current-voltage relationship of the light-induced response in ChR2-transfected HEK293 cells ($n = 6$). Prior to averaging, each I - V plot was normalised to the current at -85 mV.

Fig. 6.

Voltage-dependent properties of light-induced responses in ChR2-transfected HEK293 cells at room temperature and 37 °C. (A) Current responses to 250 ms light pulses (intensity, 1 A) in a cell voltage-clamped at membrane potentials between -85 mV to +25 mV, in 10 mV intervals. The largest currents were evoked at the most negative potentials. (B) Pooled $I-V$ relationships at room temperature (filled symbols; $n = 8$) and 37 °C (open symbols; $n = 6$). (C) The same data in (B), but in this plot, each $I-V$ curve was normalised to the response at -85 mV prior to averaging.

Fig. 7.

Voltage-dependent kinetics of light-induced responses in ChR2-transfected HEK293 cells at room temperature and 37 °C. (A) Pooled data showing (clockwise from top left) the ratio of the sustained current to the peak current ($I_{\text{sus}}/I_{\text{peak}}$), the 10–90% rise time, the time constant of deactivation (τ_{deact}) and the time constant of inactivation (τ_{inact}) at a range of membrane potentials. Data from cells recorded at room temperature (~22 °C) are represented by filled symbols ($n = 8$). Data from cells recorded at 37 °C are represented by open symbols ($n = 6$). (B) Left: peak-normalised average current responses compiled from 8 cells recorded at ~22 °C and stimulated with 250 ms light pulses (intensity, 1 A). Traces recorded at voltage-clamp potentials of –85 and –25 mV are shown. Right: identical pooled data sets for recordings from 6 different cells made at 37 °C.