Experimental Physiology

Channelrhodopsin as a tool to investigate synaptic transmission and plasticity

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The light-gated cation channel channelrhodopsin-2 (ChR2) has been used in a variety of model systems to investigate the function of complex neuronal networks by stimulation of genetically targeted neurons. In slice physiology, ChR2 opens the door to novel types of experiments and greatly extends the technical possibilities offered by traditional electrophysiology. In this short review, we first consider several technical aspects concerning the use of ChR2 in slice physiology, providing examples from our own work. More specifically, we discuss differences between light-evoked action potentials and spontaneous or electrically induced action potentials. Our work implies that light-evoked action potentials are associated with increased calcium influx and a very high probability of neurotransmitter release. Furthermore, we point out the factors limiting the spatial resolution of ChR2 activation. Secondly, we discuss how synaptic transmission and plasticity can be studied using ChR2. Postsynaptic depolarization induced by ChR2 can be combined with two-photon glutamate uncaging to potentiate visually identified dendritic spines. ChR2-mediated stimulation of presynaptic axons induces neurotransmitter release and reliably activates postsynaptic spines. In conclusion, ChR2 is a powerful tool to investigate activity-dependent changes in structure and function of synapses.

Within only a few years after its first introduction into neurons (Boyden *et al.* 2005), the algal cation channel channelrhodopsin-2 (ChR2) has become a very important tool in neuroscience. Channelrhodopsin-2 can be expressed in virtually any cell type in vertebrates and invertebrates and allows depolarizing and firing of neurons with blue light. The light-gated channel has found prominent application both *in vivo* and *in vitro*, where it enables stimulation of genetically defined cells in complex neuronal networks (Lagali *et al.* 2008; Claridge-Chang *et al.* 2009; Gradinaru *et al.* 2009; Varga *et al.* 2009; Adesnik & Scanziani, 2010; Cruikshank *et al.* 2010; Hagglund *et al.* 2010). A number of studies have provided important insights into the function of neural circuits without the need to control the precise firing patterns in ChR2-expressing neurons because many circuit functions do not depend on the timing of individual action potentials (APs; see e.g. Gradinaru *et al.* 2009; Hagglund *et al.* 2010). In such experiments, the effectiveness of stimulation can be controlled by extracellular recordings to obtain a population response to the light stimulus.

In slice physiology, the experimenter often tries to control firing of individual action potentials with high temporal precision to investigate processes that typically operate on millisecond time scales. Channelrhodopsin-2-based stimulation opens the possibility to stimulate distributed populations of genetically defined neurons using light. However, due to expression level differences, reliability and timing of AP induction varies from cell to cell. Electrophysiological control recordings from individual neurons are possible, but cannot provide information about the entire population of ChR2-positive cells. Furthermore, for certain experiments, such as chronic stimulation over several days, combining photostimulation with electrophysiology may not possible because of deleterious effects on cell viability. In fact, the possibility to stimulate cells without inserting glass or metal electrodes into the tissue is one of the main advantages of optogenetic approaches over traditional electrical stimulation methods. Optimization of ChR2 expression and stimulation as well as careful electrophysiological calibration measurements are

therefore key to ensure reliable firing of ChR2-expressing neurons, in particular in the absence of simultaneous control recordings.

Channelrhodopsin-2 expression and stimulation

If ChR2 is used to elicit APs in neurons, light-induced depolarization has to reach the firing threshold. Owing to the small single-channel conductance of ChR2 (Bamann *et al.* 2008), expression levels required for reliable AP induction are quite high. Moreover, due to variable expression levels in individual transfected cells, there will often be a subpopulation of ChR2-expressing neurons that experience only subthreshold depolarization during blue light stimulation.

We have obtained very good results using gene gun transfection and the human synapsin 1 promoter (Kugler *et al.* 2001) for neuron-specific expression in hippocampal slice culture. High expression levels are reached within about 10 days after transfection. Importantly, we did not observe any signs of cytotoxicity even in the most strongly expressing cells. This may seem surprising given that overexpression of transmembrane proteins can cause endoplasmic reticulum (ER) stress if the ER protein folding machinery is overstrained (Zhang & Kaufman, 2006). The rate of heterologous protein synthesis, rather than absolute protein levels, may therefore determine safe ChR2 levels.

Light-induced action potentials. Large photocurrents lead to rapid cell depolarization, triggering APs with short delay and minimal jitter. Conversely, cells with small photocurrents depolarize more slowly and reach firing threshold with delays of several milliseconds and less precise timing, if the firing threshold is reached at all. Large photocurrents are therefore essential for temporally precise AP induction. However, compared with the very brief AP, ChR2 kinetics are slow (Bamann *et al.* 2008; Ritter *et al.* 2008) and the maximal depolarization is reached several milliseconds after the onset of the light pulse. If the light-evoked AP (AP_L) is fired with a very short delay, the ChR2 photocurrent therefore outlasts the repolarizing K^+ current of the AP_L, resulting in an after-depolarization (Fig. 1*A*).

In two-photon calcium imaging experiments, we have found that the calcium transients in dendrites and spines evoked by AP_L were significantly larger than calcium transients associated with APs triggered by brief somatic current injection (AP_C). Application of TTX completely blocked AP_C and the concomitant calcium influx, whereas light pulses still evoked calcium transients. However, calcium influx was completely abolished when the cell membrane was clamped to −65 mV, indicating that the additional calcium influx during AP_L was mainly due to increased activation of voltage-gated calcium channels

(VGCCs) without any measurable contribution from calcium flux through the ChR2 pore (Zhang & Oertner, 2007).

Light-induced neurotransmitter release. The increased calcium influx during AP_L has important functional consequences. We performed patch-clamp recordings from untransfected hippocampal CA1 cells to compare EPSCs resulting from light activation of ChR2-expressing CA3 cells ($EPSC_L$) with electrically induced EPSCs ($EPSC_C$). The $EPSC_L$ induced by application of blue light in CA1 area had a very short latency compared with light stimulation in CA3 area or electrical stimulation of individual CA3 neurons by somatic current injection, indicating that AP_L were induced locally in the axon (Fig. 1*B*). Furthermore, the amplitudes of $EPSC_L$ evoked by stimulation in CA1 area were very uniform, suggesting a high release probability (p_r). Indeed, by direct two-photon imaging of calcium transients in individual postsynaptic
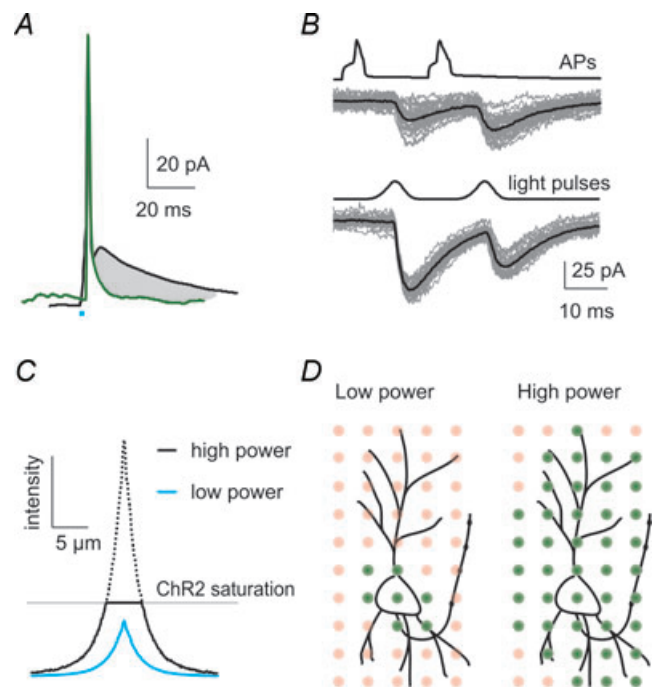

Figure 1. Channelrhodopsin-2-mediated photostimulation
A, peak-aligned spontaneous action potential (AP; green) and light-induced AP (black; 2 ms light pulse indicated by blue square) in a CA1 pyramidal neuron. The grey area indicates the after-depolarization associated with ChR2 activation. *B*, comparison of electrically triggered EPSCs (top) with light-triggered EPSCs in a CA1 neuron (bottom). Neurotransmitter release from ChR2-expressing CA3 neurons was induced by somatic current injection or by photostimulation of axons in CA1 area, respectively (modified from Zhang & Oertner, 2007). *C*, light intensity profile in the focal plane for high and low laser power. High light intensities lead to saturation of ChR2 activation in the laser focus. *D*, schematic illustration of APs (green dots) and subthreshold depolarizations (red dots) induced in a neuron with focal laser stimulation in a grid pattern (*C* and *D* are based on Schoenenberger *et al.* 2008).

spines, we measured release probabilities of approximately 0.9 after light stimulation of presynaptic axons (compared with ∼0.3 at electrically stimulated Schaffer collateral synapses; Oertner *et al.* 2002). Most probably the increase in p_r is caused by the additional calcium influx during AP_L (Zhang & Oertner, 2007).

The recently reported accelerated ChR2 variants exhibit reduced after-depolarizations and might therefore allow firing of neurons without enhancing transmitter release at their output synapses (Gunaydin *et al.* 2010). These novel variants should thus enable the investigator to choose between wild-type ChR2 for experiments profiting from high p_r and the accelerated ChR2 variants for experiments attempting to mimic natural spiking. Since accelerated ChR2 variants typically produce smaller photocurrents, special care has to be taken to achieve high expression levels for reliable AP induction (Gunaydin *et al.* 2010).

Chronic ChR2 stimulation. A general concern associated with the use of light in live tissue is phototoxicity and bleaching of fluorescent reporters or light-controlled effectors. However, $EPSC_L$ recorded in untransfected cells receiving input from ChR2-expressing cells is very stable, and we have not observed any use-dependent run-down of presynaptic release reliability or $EPSC_L$ amplitude in whole-cell recordings of up to 60 min.

To test for potential light-dependent ChR2 degradation or phototoxic effects of chronic light stimulation, we stimulated ChR2-transfected slice cultures in the cell culture incubator for up to 4 days with bright light pulses emitted by high-power light-emitting diodes. We did not observe any signs of cytotoxicity, either in ChR2-expressing cells or in untransfected neighbours. The photocurrents quantified in ChR2-expressing pyramidal cells after 4 days of stimulation were not different from currents in non-stimulated control cells. From these stimulation experiments employing a total of ∼35,000 light pulses, amounting to 17 min of intense blue light illumination, we concluded that ChR2 exhibits no light-dependent degeneration and that repeated blue light exposure is not detrimental to culture viability (Schoenenberger *et al.* 2009).

Channelrhodopsin-2 variants. As already mentioned, several engineered ChR2 variants have been reported. Still, we have performed the large majority of experiments with the original wild-type ChR2 first introduced into neurons by Karl Deisseroth's group (Boyden *et al.* 2005) because it is well tolerated by transfected neurons and under optimal conditions allows induction of single APs at frequencies up to about 40 Hz in pyramidal neurons. If stimulation light intensities or expression levels are low, the H134R mutant may enable more robust firing than wild-type ChR2 because of its enhanced photocurrents (Nagel *et al.* 2005).

Of the engineered ChR2 variants, the so-called bi-stable channelrhodopsins containing amino acid substitutions at the C128 position exhibit most remarkable properties (Berndt *et al.* 2009). In extensive experiments with ChR2(C128A), we have found that this channel can induce long spike trains after a brief stimulation pulse even with minimal light intensities. However, photocurrent amplitudes were dramatically reduced after repeated stimulation, which limited the number of spike trains that could be elicited. Most probably, this was due to accumulation of desensitized channel in a non-conducting state (Schoenenberger *et al.* 2009; Stehfest *et al.* 2010). Despite use-dependent inactivation, the bi-stable ChR2 variants will be valuable tools if a limited number of spike trains or long-lasting subthreshold depolarizations are sufficient for a certain application.

In our experiments, the vigorous firing evoked by ChR2(C128A) induced expression of the immediate early gene c-*fos* in a graded and cell-specific manner (Schoenenberger *et al.* 2009). Potential induction of immediate early genes and other activity-dependent factors may seem an unwanted side-effect of stimulated neuronal activity because it may alter functional and structural properties of cells. However, it opens the door to study activity-related processes at the level of individual neurons (Matsuo *et al.* 2008) and allows identification of stimulated cells without the need to monitor electrical activity during stimulation.

Local ChR2 activation. Most studies employing ChR2 have used wide-field illumination for channel activation. However, spatial restriction of the excitation light can be exploited to probe connectivity between neurons (Petreanu *et al.* 2007, 2009) or to avoid direct activation of presynaptic axons and increased calcium influx at presynaptic terminals. Moreover, local ChR2 activation might allow depolarization of subcellular structures down to the level of single dendritic spines, which is not possible with electrophysiological approaches and therefore constitutes one of the most interesting applications of optogenetics in slice physiology. We therefore investigated the factors determining the spatial resolution of ChR2 activation (Schoenenberger *et al.* 2008).

When we stimulated neurons expressing yellow fluorescent protein (YFP)-tagged ChR2 with a blue laser beam directed at the soma with increasing light intensities, we found that ChR2 activation rapidly saturated in the laser focus. High laser intensities did not only increase the photocurrents, but also led to a loss of spatial selectivity of photocurrent induction. Large currents were induced even when the laser was directed at positions further than 100 μm lateral to the soma. How can the loss of spatial resolution with high light intensity be explained? As a result of focal saturation of ChR2

excitation, the photocurrent amplitude can only be increased by illuminating a larger membrane area for a given pulse duration. Increasing the laser intensity results in stimulation of a larger membrane area by the spatial tails of the laser beam (see Fig. 1*C*) and by scattered light. The additional photons in the laser focus do not enhance photocurrents, but contribute light scattering and thereby increase activation of out-of-focus membrane.

In contrast to photocurrent induction, where resolution can be gradually improved by using lower laser power, APs are only fired if the spike threshold is reached. As a consequence, neurons with high ChR2 expression levels can be fired with low light intensities and therefore high spatial selectivity (Fig. 1*D*). Weakly expressing cells, however, may only fire APs if the entire cell membrane is illuminated.

We have restricted our local stimulation experiments to the soma and main dendrites of neurons, but the principles established here also apply to stimulation of any subcellular structure and should apply to one-photon excitation of any optogenetic agent. Owing to unavoidable stimulation of ChR2 in out-of-focus membrane, one-photon activation is not ideal for local stimulation of small subcellular structures. Two-photon excitation, in contrast, can restrict ChR2 activation to a very small volume and should enable stimulation of subcellular structures with very high spatial selectivity (Rickgauer & Tank, 2009; Zhu *et al.* 2009).

Investigation of synaptic transmission and plasticity with ChR2

Long-term potentiation (LTP), the most extensively studied form of synaptic plasticity, is triggered when presynaptic activity coincides with postsynaptic depolarization. To investigate the sequence of molecular events and morphological changes that occur during potentiation, it would be desirable to visualize the synapses where LTP is induced directly. In classical electrophysiological LTP protocols, however, it is difficult to isolate responses of individual synapses and to identify their location. In addition, wash-out of second messengers and soluble proteins (Tanaka *et al.* 2008) associated with whole-cell patch-clamp recordings limits the time window for effective LTP induction after break-in to the postsynaptic cell. These limitations of purely electrophysiological approaches can be overcome using optogenetics; ChR2-mediated stimulation of fluorescently labelled axons helps to identify active synapses, whereas optogenetic depolarization of the postsynaptic cell eliminates wash-out problems.

Postsynaptic activation by ChR2. Channelrhodopsin-2 allowed us to induce brief bursts of action potentials in individual postsynaptic neurons in a non-invasive fashion.

Since the intracellular milieu of the postsynaptic cell was not affected, we had no time limitations to find synapses and follow plasticity-induced changes. One of our goals was to measure volume changes of dendritic spines after potentiation. Frequently used fluorescently tagged ChR2 would have resulted in staining of the cell membrane, which is not optimum for volume measurements. Therefore, we combined unlabelled ChR2 and the soluble red fluorescent protein tdimer2 (Campbell *et al.* 2002) in a single plasmid, which led to reliable co-expression and enabled us to assess ChR2 expression levels in individual neurons. Whole-cell recordings showed that in response to blue light illumination, 19 of 21 cells reliably produced spikes. Knowing that the postsynaptic cells could be activated by light, we stimulated single synapses on identified spines by two-photon uncaging of methoxy-nitroindolinyl (MNI)-glutamate (Fig. 2*A*). Repeated pairing of single uncaging pulses with photostimulation of the postsynaptic cell induced LTP and an increase in volume of the stimulated spines.

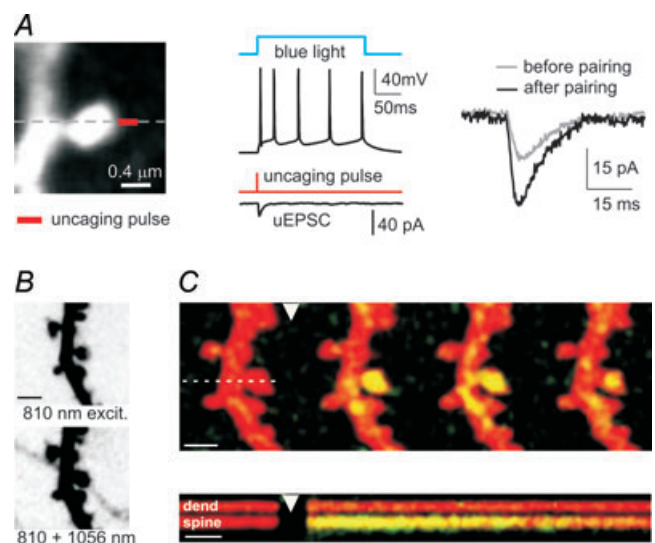We successfully applied this optical pairing protocol to visualize translocation of *alpha calcium/*

Figure 2. Investigation of single synapses using ChR2
A, left panel shows simultaneous two-photon imaging and two-photon glutamate uncaging on a dendritic spine. Middle panel shows optical pairing protocol, in which single uncaging-evoked EPSCs (uEPSCs) were paired with blue light pulses that evoked five or six APs in the postsynaptic neuron. Right panel shows average uEPSCs before and after optical pairing (reproduced from Zhang *et al.* 2008). *B*, visualization of potential synaptic contacts between a ChR2-tdimer2-expressing axon and Alexa 594-filled spines. Scale bar represents 2 μm. *C*, top panel shows an overlay of green (fluo-5F) and red fluorescence (Alexa 594) from the dendrite shown in (*B*). Calcium transient after light stimulation (arrowhead) is restricted to a single spine (yellow). Scale bar represents 2 μm. Dashed line indicates position of line scan. Bottom panel shows line scan across dendrite and spine head. Scale bar represents 50 ms (*B* and *C* are modified from Zhang & Oertner, 2007).

calmodulin-dependent kinase II (αCaMKII), a key component in the LTP signalling cascade, at individual dendritic spines and revealed input-specific accumulation of αCaMKII after potentiation (Zhang *et al.* 2008*b*). An alternative method to induce LTP at individual synapses is glutamate uncaging in the absence of extracellular magnesium. Since removal of magnesium can induce seizure-like activity in brain slices, it is typically combined with TTX to block fast sodium channels and prevent action potential firing (Matsuzaki *et al.* 2004). This protocol strongly enhances calcium influx at stimulated spines, since in the absence of extracellular magnesium the NMDA receptors are fully conductive as long as they are bound to glutamate (>100 ms after the uncaging pulse). We prefer optogenetic induction of APs in the postsynaptic cell because it allows mimicking of spike-timing-dependent plasticity, arguably the most physiological of all plasticity induction protocols.

Presynaptic activation by ChR2. Although two-photon glutamate uncaging allows for excellent spatial resolution, this technique completely bypasses the presynaptic terminal and is therefore restricted to investigation of postsynaptic mechanisms. An interesting application of ChR2 is the selective activation of visually identified axons in intact tissue. To stimulate and image axons in brain slices, we tagged ChR2 with tdimer2 (Campbell *et al.* 2002) based on the following considerations. Firstly, the optical properties of tdimer2 make it ideal for simultaneous imaging with synthetic dyes such as Alexa 594 and fluo-5F in the postsynaptic spines. The emission spectrum of Alexa 594 overlaps with that of tdimer2. However, these two fluorophores can be easily separated by two-photon excitation at different wavelengths (Fig. 2*B*). Secondly, inspection of red labelling with green excitation light allowed us to select cultures with a favourable expression pattern without fully activating ChR2-positive cells. Finally, tdimer2 does not form aggregates even at high expression levels.

Using two-photon microscopy, we identified potential points of contact between the dendrite of a dye-filled CA1 pyramidal cell and ChR2-positive axons. In response to blue light, ChR2-expressing axonal terminals provided a reliable and precisely timed source of glutamate, which was clearly revealed by two-photon calcium imaging at postsynaptic spines (Fig. 2*C*). As a result, pairing of light-evoked synaptic release with postsynaptic depolarization led to lasting potentiation of synaptic connections (Zhang & Oertner, 2007). In these experiments, postsynaptic depolarization was still provided by a somatic patch electrode, but presynaptic terminals were not perturbed. This approach is therefore suitable for optical analysis of proteins involved in vesicular release and presynaptic plasticity.

Future directions

To tap the full potential of optogenetics in slice physiology – remote control of neuronal activity without the need for electrical access to cells or tissue – ChR2-based stimulation has to be combined with optical measurements of the structure or function under investigation, as we have demonstrated for αCaMKII translocation. Rapidly improving fluorescent reporters of electrical activity, ion influx and enzyme activity (Dimitrov *et al.* 2007; Lee *et al.* 2009; Tian *et al.* 2009) extend the potential of such all-optical experiments. Channelrhodopsin-2-based stimulation will also benefit from the combination with genetically encoded calcium indicators to provide a direct and rapid stimulation feedback (Mank *et al.* 2008; Tian *et al.* 2009). Since currently available genetically encoded calcium indicators are based on green fluorescent protein (GFP) or cyan fluorescent protein (CFP)–YFP FRET (fluorescence resonance energy transfer) pairs and thus have excitation spectra overlapping with the ChR2 action spectrum, optical read-out is always associated with some degree of ChR2 activation. Genetically encoded calcium sensors based on red fluorescent protein (RFP) would therefore be advantageous for all-optical experiments.

A second important focus of current efforts is the engineering or identification of spectrally shifted channelrhodopsins (Zhang *et al.* 2008*a*). Dual or even multicolour control of different optogenetic agents will allow probing of the contributions of distinct neuronal populations to phenomena ranging from dendritic integration to behaviour. The challenge for protein engineers will be to alter the action spectra without compromising channel conductance, kinetics or expression levels. A third largely unexplored territory is targeting of ChR2 to specific regions of the cell (Lewis *et al.* 2009) or to intracellular organelles. Given the range of possibilities, the optogenetic revolution might have only just begun.

References

Adesnik H & Scanziani M (2010). Lateral competition for cortical space by layer-specific horizontal circuits. *Nature* **464**, 1155–1160.

Bamann C, Kirsch T, Nagel G & Bamberg E (2008). Spectral characteristics of the photocycle of channelrhodopsin-2 and its implication for channel function. *J Mol Biol* **375**, 686–694.

Berndt A, Yizhar O, Gunaydin LA, Hegemann P & Deisseroth K (2009). Bi-stable neural state switches. *Nat Neurosci* **12**, 229–234.

Boyden ES, Zhang F, Bamberg E, Nagel G & Deisseroth K (2005). Millisecond-timescale, genetically targeted optical control of neural activity. *Nat Neurosci* **8**, 1263–1268.

Campbell RE, Tour O, Palmer AE, Steinbach PA, Baird GS, Zacharias DA & Tsien RY (2002). A monomeric red fluorescent protein. *Proc Natl Acad Sci U S A* **99**, 7877–7882.

Claridge-Chang A, Roorda RD, Vrontou E, Sjulson L, Li H, Hirsh J & Miesenbock G (2009). Writing memories with light-addressable reinforcement circuitry. *Cell* **139**, 405–415.

Cruikshank SJ, Urabe H, Nurmikko AV & Connors BW (2010). Pathway-specific feedforward circuits between thalamus and neocortex revealed by selective optical stimulation of axons. *Neuron* **65**, 230–245.

Dimitrov D, He Y, Mutoh H, Baker BJ, Cohen L, Akemann W & Knopfel T (2007). Engineering and characterization of an enhanced fluorescent protein voltage sensor. *PLoS ONE* **2**, e440.

Gradinaru V, Mogri M, Thompson KR, Henderson JM & Deisseroth K (2009). Optical deconstruction of parkinsonian neural circuitry. *Science* **324**, 354–359.

Gunaydin LA, Yizhar O, Berndt A, Sohal VS, Deisseroth K & Hegemann P (2010). Ultrafast optogenetic control. *Nat Neurosci* **13**, 387–392.

Hagglund M, Borgius L, Dougherty KJ & Kiehn O (2010). Activation of groups of excitatory neurons in the mammalian spinal cord or hindbrain evokes locomotion. *Nat Neurosci* **13**, 246–252.

Kugler S, Meyn L, Holzmuller H, Gerhardt E, Isenmann S, Schulz JB & Bahr M (2001). Neuron-specific expression of therapeutic proteins: evaluation of different cellular promoters in recombinant adenoviral vectors. *Mol Cell Neurosci* **17**, 78–96.

Lagali PS, Balya D, Awatramani GB, Munch TA, Kim DS, Busskamp V, Cepko CL & Roska B (2008). Light-activated channels targeted to ON bipolar cells restore visual function in retinal degeneration. *Nat Neurosci* **11**, 667–675.

Lee SJ, Escobedo-Lozoya Y, Szatmari EM & Yasuda R (2009). Activation of CaMKII in single dendritic spines during long-term potentiation. *Nature* **458**, 299–304.

Lewis TL Jr, Mao T, Svoboda K & Arnold DB (2009). Myosin-dependent targeting of transmembrane proteins to neuronal dendrites. *Nat Neurosci* **12**, 568–576.

Mank M, Santos AF, Direnberger S, Mrsic-Flogel TD, Hofer SB, Stein V, Hendel T, Reiff DF, Levelt C, Borst A, Bonhoeffer T, Hubener M & Griesbeck O (2008). A genetically encoded calcium indicator for chronic *in vivo* two-photon imaging. *Nat Methods* **5**, 805–811.

Matsuo N, Reijmers L & Mayford M (2008). Spine-type-specific recruitment of newly synthesized AMPA receptors with learning. *Science* **319**, 1104–1107.

Matsuzaki M, Honkura N, Ellis-Davies GC & Kasai H (2004). Structural basis of long-term potentiation in single dendritic spines. *Nature* **429**, 761–766.

Nagel G, Brauner M, Liewald JF, Adeishvili N, Bamberg E & Gottschalk A (2005). Light activation of channelrhodopsin-2 in excitable cells of *Caenorhabditis elegans* triggers rapid behavioral responses. *Curr Biol* **15**, 2279–2284.

Oertner TG, Sabatini BL, Nimchinsky EA & Svoboda K (2002). Facilitation at single synapses probed with optical quantal analysis. *Nat Neurosci* **5**, 657–664.

Petreanu L, Huber D, Sobczyk A & Svoboda K (2007). Channelrhodopsin-2-assisted circuit mapping of long-range callosal projections. *Nat Neurosci* **10**, 663–668.

Petreanu L, Mao T, Sternson SM & Svoboda K (2009). The subcellular organization of neocortical excitatory connections. *Nature* **457**, 1142–1145.

Rickgauer JP & Tank DW (2009). Two-photon excitation of channelrhodopsin-2 at saturation. *Proc Natl Acad Sci U S A* **106**, 15025–15030.

Ritter E, Stehfest K, Berndt A, Hegemann P & Bartl FJ (2008). Monitoring light-induced structural changes of Channelrhodopsin-2 by UV-visible and Fourier transform infrared spectroscopy. *J Biol Chem* **283**, 35033–35041.

Schoenenberger P, Gerosa D & Oertner TG (2009). Temporal control of immediate early gene induction by light. *PLoS ONE* **4**, e8185.

Schoenenberger P, Grunditz A, Rose T & Oertner TG (2008). Optimizing the spatial resolution of Channelrhodopsin-2 activation. *Brain Cell Biol* **36**, 119–127.

Stehfest K, Ritter E, Berndt A, Bartl F & Hegemann P (2010). The branched photocycle of the slow-cycling channelrhodopsin-2 mutant C128T. *J Mol Biol* **398**, 690–702.

Tanaka J, Horiike Y, Matsuzaki M, Miyazaki T, Ellis-Davies GC & Kasai H (2008). Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. *Science* **319**, 1683–1687.

Tian L, Hires SA, Mao T, Huber D, Chiappe ME, Chalasani SH, Petreanu L, Akerboom J, McKinney SA, Schreiter ER, Bargmann CI, Jayaraman V, Svoboda K & Looger LL (2009). Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. *Nat Methods* **6**, 875–881.

Varga V, Losonczy A, Zemelman BV, Borhegyi Z, Nyiri G, Domonkos A, Hangya B, Holderith N, Magee JC & Freund TF (2009). Fast synaptic subcortical control of hippocampal circuits. *Science* **326**, 449–453.

Zhang F, Prigge M, Beyriere F, Tsunoda SP, Mattis J, Yizhar O, Hegemann P & Deisseroth K (2008*a*). Red-shifted optogenetic excitation: a tool for fast neural control derived from *Volvox carteri*. *Nat Neurosci* **11**, 631–633.

Zhang K & Kaufman RJ (2006). Protein folding in the endoplasmic reticulum and the unfolded protein response. *Handb Exp Pharmacol* (172), 69–91.

Zhang YP, Holbro N & Oertner TG (2008*b*). Optical induction of plasticity at single synapses reveals input-specific accumulation of αCaMKII. *Proc Natl Acad Sci U S A* **105**, 12039–12044.

Zhang YP & Oertner TG (2007). Optical induction of synaptic plasticity using a light-sensitive channel. *Nat Methods* **4**, 139–141.

Zhu P, Narita Y, Bundschuh ST, Fajardo O, Scharer YP, Chattopadhyaya B, Bouldoires EA, Stepien AE, Deisseroth K, Arber S, Sprengel R, Rijli FM & Friedrich RW (2009). Optogenetic dissection of neuronal circuits in zebrafish using viral gene transfer and the Tet system. *Front Neural Circuits* **3**, 21.