# Photochemical control of endogenous ion channels and cellular excitability

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**Light-activated ion channels provide a precise and noninvasive optical means for controlling action potential firing, but the genes encoding these channels must first be delivered and expressed in target cells. Here we describe a method for bestowing light sensitivity onto endogenous ion channels that does not rely on exogenous gene expression. The method uses a synthetic photoisomerizable small molecule, or photoswitchable affinity label (PAL), that specifically targets $K^+$ channels. PALs contain a reactive electrophile, enabling covalent attachment of the photoswitch to naturally occurring nucleophiles in $K^+$ channels. Ion flow through PAL-modified channels is turned on or off by photoisomerizing PAL with different wavelengths of light. We showed that PAL treatment confers light sensitivity onto endogenous $K^+$ channels in isolated rat neurons and in intact neural structures from rat and leech, allowing rapid optical regulation of excitability without genetic modification.**

Eukaryotic cells have ion channels that are directly activated by voltage or ligands, but not by light. Consequently, electrical or chemical stimuli are often used to elicit physiological responses in excitable cells. However, light stimulation has several advantages over electrodes or chemical perfusion devices. Light is noninvasive and can be projected on tissue with great temporal and spatial precision. It can be focused on subcellular structures, single cells or projected diffusely to regulate the activity of many cells simultaneously[1–5]. But how can light be used to manipulate the activity of 'blind' cells that have no natural photoresponsive proteins?

One popular approach to manipulate the activity of excitable cells with light has been to use a 'caged' neurotransmitter (for example, glutamate) that is liberated from a photolabile protecting group (the cage) upon exposure to light[1,5]. Photorelease of caged glutamate accurately mimics the kinetics of synaptic transmission and has been used to map neuronal circuits[6–9]. However, glutamate uncaging is ill-suited for inducing sustained activity because prolonged uncaging can lead to the accumulation of desensitized receptors and to local depletion of the caged neurotransmitter[4]. Photorelease is irreversible, and diffusion of the liberated neurotransmitter can result in unintended activation of receptors on untargeted cells.

To circumvent the limitations associated with using a freely diffusible light-sensitive compound, several types of light-activated proteins have been used to control neuronal activity. A light-activated $K^+$ channel (SPARK), consisting of a photoswitchable ligand attached to a genetically engineered Shaker $K^+$ channel, allows reversible suppression of action potential firing[10]. LiGluR, a light-activated glutamate receptor, containing a different photoswitchable ligand attached to a genetically engineered iGluR6 (ref. 11), reversibly depolarizes cells and promotes neuronal firing[12]. Finally, channelrhodopsin-2 (ChR2) and halorhodopsin (NpHR), which use the natural photoswitch retinal, allow light to trigger or inhibit action potential firing[13–19]. Each of these proteins can impart light sensitivity on neuronal firing, but only if their gene is first introduced into the cell of interest and the protein is expressed in sufficient abundance on the plasma membrane. However, exogenous expression of proteins can be non-uniform and slow, requiring days to weeks, and is not currently practical in some organisms. Genes encoding light-activated proteins can also be introduced transgenically into organisms[12,19–22], but this may perturb the development and function of cells expressing the genes.

Here we describe a new method, based on a photoswitchable affinity label (PAL), to confer light sensitivity to proteins without requiring genetic engineering and exogenous gene expression. Consequently, the PAL approach can be used to photosensitize endogenous proteins and control their activity in freshly obtained, genetically unadulterated cells or tissues. The PAL molecules described here specifically target voltage-gated $K^+$ channels and act as covalently tethered channel blockers. Appropriate design of other PAL molecules, however, should allow the approach to be extended to other ligand-responsive proteins at the cell surface. The tether is photoisomerizable and can be shortened or elongated by exposure to different wavelengths of light, allowing or disallowing the blocking moiety to reach the pore. When applied to neurons, PAL enables

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control of endogenous $K^+$ channels with light, resulting in optical control of electrical excitability without genetic modification.

## RESULTS

### The PAL approach

PALs are derivatives of the photoisomerizable molecule azobenzene (**Fig. 1a**). Connected to one end of azobenzene is a protein-binding ligand, in this case a quaternary ammonium group (QA), which binds to the pore of $K^+$ channels and blocks ion conduction. On the other end is an electrophilic group that enables covalent attachment of the photoswitch to nucleophilic amino acid side chains in the channel. We designed PALs comprising QA, azobenzene and one of three different electrophilic groups: acrylamide (PAL abbreviated AAQ), chloroacetamide (CAQ) or epoxide (EAQ; **Fig. 1b**; see **Supplementary Methods** online for a description of the synthesis). Binding of the QA to the $K^+$ channel pore increases the local concentration of the electrophile at the surface of the channel. This promotes covalent attachment if the channel has a nucleophilic side chain ~20 Å from the QA binding site, matching the length of the PAL molecule. Hence, the covalent attachment of PALs to channels is promoted by ligand binding, as in classical affinity labeling[23]. After the photoswitch is covalently tethered, the QA can reach the pore and block ion conduction only when the azobenzene is in its elongated *trans* form, but not in its bent *cis* form (**Fig. 1c**). Thus, channels can be unblocked by exposure to 360–400-nm light, which photoisomerizes the azobenzene from *trans* to *cis*. The reverse *cis* to *trans* conversion, which restores channel block, occurs slowly in the dark ($\tau$ = ~5 min) and is accelerated by exposure to long-wavelength light (450–560 nm)[10,24].

PAL-modified channels differ in several ways from the previously described SPARK channel[10]. SPARK is based on a Shaker $K^+$ channel engineered to contain an extracellular cysteine that serves as the covalent attachment site for its photoswitch (**Fig. 1d**). The channel also contains additional mutations to shift voltage-dependent activation and minimize inactivation. The SPARK photoswitch is maleimide-azobenzene-QA (MAQ; **Fig. 1b**), which covalently attaches to the channel via a maleimide group often considered selective for cysteines. Thus, to impart light sensitivity using SPARK, two components must be added: the channel gene and the MAQ photoswitch. In contrast, the PAL photoswitch acts on endogenous $K^+$ channels that have no introduced cysteine and no mutations to modify gating. Hence PAL is a one-component system for conferring light sensitivity.

### PAL imparts light sensitivity to $K^+$ channels

We assessed the feasibility of the PAL approach using whole-cell patch clamp recording in cells heterologously expressing Shaker $K^+$ channels. First, we engineered an appropriately positioned nucleophilic site in Shaker (E422C) to maximize the probability of attachment of the PAL photoswitch to the channel. Addition of AAQ to Shaker E422C photosensitized this channel, enabling regulation of ion flow with light (**Fig. 2a**). Notably, AAQ also imparted light sensitivity to a Shaker channel lacking the cysteine substitution (Glu422; **Fig. 2b**) and indeed, conferred a similar degree of light sensitivity on a Shaker channel devoid of extracellular cysteines (data not shown). The fraction of current that could be photoregulated after AAQ treatment (percent photoswitching) was similar for E422C and Glu422 Shaker channels (77 ± 15% and 83 ± 11% respectively, $n = 4$ cells; **Fig. 2c**). CAQ and EAQ also photosensitized Glu422 Shaker (data not shown). Hence, PAL molecules can find a covalent attachment site at an appropriate distance from the channel pore, such that light-elicited changes in photoswitch length allow or disallow block by the QA. PAL-modified channels could be photoswitched repeatedly with little decrement in the fraction of current regulated and no apparent photobleaching of the photoswitch (**Fig. 2a**,**b**). Thus, PAL molecules can be used to endow persistent light sensitivity to a wild-type Shaker channel, allowing rapid and reversible control of channel function.

If PAL molecules can impart light sensitivity to wild-type Shaker, perhaps they can also act on other QA-sensitive $K^+$ channels. We expressed various channels in HEK293 cells and quantified photosensitivity after AAQ treatment (**Fig. 2c**). Several $K^+$ channels became light sensitive, including Kv1.2, Kv1.3, Kv1.4, Kv2.1 and

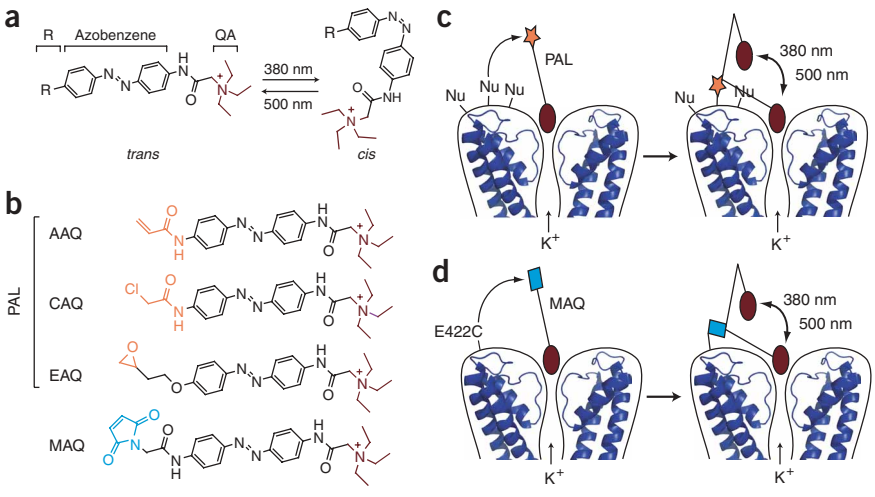


**Figure 1** | The PAL approach for imparting light sensitivity onto native ion channels. (**a**) PAL molecules consist of a photoisomerizable azobenzene group flanked by QA and a covalent attachment group (R). Exposure to 380-nm light isomerizes the azobenzene to its shorter *cis* form, whereas exposure to 500-nm light favors the *trans* configuration. (**b**) Chemical structure of PAL molecules and MAQ. PAL molecules contain a promiscuous reactive group (orange); MAQ contains a maleimide (blue) designed to react with an engineered cysteine. (**c**,**d**) Schematic of the generation of photoswitch-regulated $K^+$ channels. A PAL whose QA (burgundy) is bound to the channel pore covalently attaches via its promiscuous reactive group (orange) to an endogenous nucleophile (Nu) on a $K^+$ channel (**c**). For the SPARK channel, MAQ attaches via its maleimide (blue) to a genetically introduced cysteine in a Shaker (E422C) $K^+$ channel (**d**). In both cases, in 500-nm light the photoswitch is extended, blocking ion conduction, whereas in 380-nm light azobenzene isomerizes to its *cis* form, retracting the QA and allowing ion conduction. Molecular coordinates from KcsA (Protein Data Bank identifier 2A9H) were drawn using MacPyMol (W.L. DeLano, The PyMOL Molecular Graphics System (2002); http://www.pymol.org).

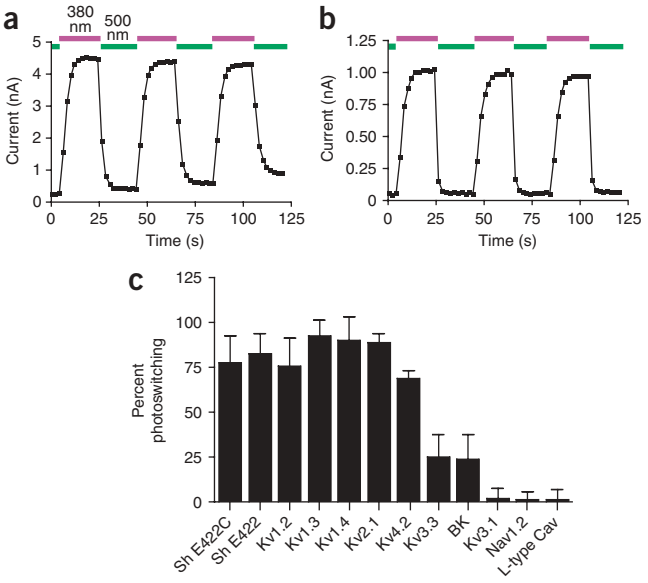


**Figure 2** | Photocontrol of $K^+$ channels expressed in HEK293 cells. (**a**,**b**) Voltage-gated currents from Shaker channels after AAQ treatment. Channels containing either an engineered nucleophilic attachment site (E422C; **a**) or no mutations (**b**) were exposed to 500-nm and 380-nm light as indicated. Voltage-gated $K^+$ currents were elicited by pulsing from −70 to +30 mV for 250 ms. (**c**) Percent photoswitching for the indicated channels treated with AAQ (400 μM, 15 min). We defined percent photoswitching as the difference between the steady-state current in 380 and 500-nm light, divided by the current in 380-nm light. Current was elicited by stepping from −70 to +30 mV ($K^+$ channels), −80 to 0 mV ($Na^+$ channels), and −40 to +20 mV ($Ca^{2+}$ channels). Data are average ± s.d. ($n$ = 4–7 cells for each channel).

Kv4.2 (percent photoswitching for Kv1.2, 76 ± 16; Kv1.3, 93 ± 9; Kv1.4, 90 ± 13; Kv2.1, 89 ± 5; Kv4.2, 69 ± 4; $n$ = 4 cells each; **Fig. 2c**). Light sensitivity was less pronounced for Kv3.3 (25 ± 13% photoswitching; $n$ = 5 cells) and BK (24 ± 13% photoswitching; $n$ = 4 cells). In contrast, Kv3.1 exhibited no detectable photosensitivity after AAQ treatment (2 ± 6% photoswitching, $n$ = 6 cells). Moreover, neither voltage-gated $Na^+$ channels (Nav1.2) nor voltage-gated $Ca^{2+}$ channels (L-type Cav) became light-sensitive after AAQ treatment (1 ± 5% and –2 ± 5% photoswitching, respectively; $n$ = 4–7 cells).

### PAL photosensitizes $K^+$ channels in cultured neurons

We next tested whether PAL molecules could photosensitize endogenous $K^+$ channels in neurons. Endogenous voltage-gated $K^+$ channels set the resting membrane potential and the threshold for triggering action potentials. They also shape the action potential waveform and regulate the propensity to fire repetitively in response to a stimulus. We recorded voltage-gated outward $K^+$ currents from cultured hippocampal neurons before and after AAQ application. Steady-state current-voltage curves indicate that AAQ application and exposure to 500-nm light blocked a large fraction of voltage-gated $K^+$ currents (**Fig. 3a**,**b**; average current blocked 72 ± 9%, $n$ = 5). Photoisomerization of AAQ to its *cis* configuration with 380-nm light completely relieved $K^+$ channel block such that the current-voltage curve overlapped with that before AAQ treatment (**Fig. 3a**,**b**; average current recovered under 380-nm light, 117 ± 29%; $n$ = 5). Voltage-gated $K^+$ currents were photoswitched to a similar extent in neurons treated with CAQ (data not shown). In contrast, MAQ did not impart light sensitivity to endogenous neuronal $K^+$ channels (**Fig. 3c**; 88 ± 5% and 2 ± 13% photoswitching for AAQ and MAQ respectively; $n$ = 6 each).

In darkness, the PAL photoswitch relaxes to its *trans* configuration, blocking PAL-modified $K^+$ channels and potentially causing depolarization of the membrane potential. To prevent tonic blockade of $K^+$ channels, 380-nm light can be used to keep PAL-modified channels unblocked (**Fig. 3a**,**b**). However, because the spontaneous *cis* to *trans* isomerization of the photoswitch occurs over many minutes in the dark[10], flashes of 380-nm light (1 s per minute) were sufficient to maintain 94 ± 4% of the $K^+$ current unblocked in AAQ-treated neurons ($n$ = 10). Thus, the state of PAL-modified $K^+$ channels can be set with the appropriate illumination conditions, preventing tonic $K^+$ channel blockade.

Despite having a reactive electrophile and causing $K^+$ channel blockade in darkness and 500-nm light, PAL photoswitches did not have obvious deleterious effects on cultured hippocampal neurons. Neurons remained active with a normal resting membrane potential (vehicle, –54 ± 13 mV, $n$ = 10; AAQ, –54 ± 13 mV, $n$ = 23; $P > 0.1$ unpaired $t$-test) and normal membrane resistance (vehicle, 77 ± 20 MΩ, $n$ = 10; AAQ, 113 ± 65 MΩ, $n$ = 6; $P > 0.1$ unpaired $t$-test) for several hours after PAL treatment. Additionally, there was no significant difference in cell death between neurons treated for 15 min with vehicle alone (5 ± 3%), AAQ (9 ± 3%) or CAQ (8 ± 3%; $n$ = 4–8 fields each, $P > 0.05$; one-way ANOVA; **Supplementary Fig. 1** online). EAQ appeared to be more toxic, and we did not use it in subsequent studies (25 ± 11% cell death). We obtained similar results after treatment for 60 min, four times longer than needed to impart light sensitivity (**Supplementary Fig. 1**). We also tested neurons up to 6 d after a 15-min treatment with AAQ and found no significant toxicity (3 ± 1% cell death; $n$ = 4 fields, $P > 0.05$; one-way ANOVA) compared to treatment with vehicle alone (4 ± 1%). Additional experiments showed that AAQ injection into the vitreous humor of the rat eye successfully imparted light sensitivity onto retinal ganglion cells (RGCs) but had no effect on either the histology of the retina or on the rod- and cone-driven light response, as measured in electroretinogram recordings (K.B. and R.H.K.; unpublished observations). Hence, at least for our

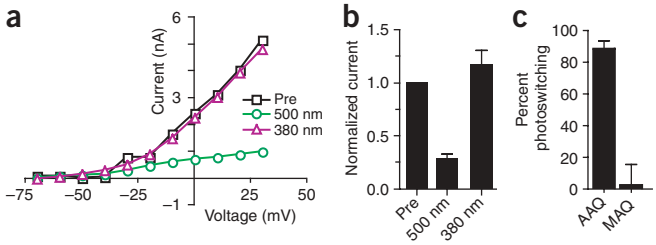


**Figure 3** | Photocontrol of native $K^+$ current in cultured hippocampal neurons. (**a**) Steady-state current-voltage curves from a voltage-clamped hippocampal pyramidal neuron before (Pre) and after a 15-min application of 300 μM AAQ. We measured currents in 380-nm or 500-nm light after AAQ treatment. (**b**) Voltage-gated currents (elicited by stepping from −70 mV to +30 mV) measured after AAQ treatment were normalized to those measured before AAQ application. Data are average ± s.d. ($n$ = 5). AAQ-treated channels are completely unblocked by 380-nm light. (**c**) Percent photoswitching for $K^+$ current in hippocampal neurons treated with AAQ (200 μM) or MAQ (250 μM). Data are average ± s.d. ($n$ = 6 for each).

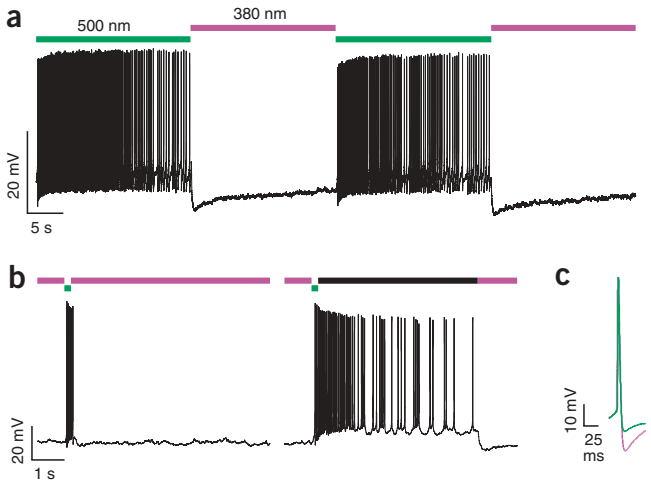


**Figure 4** | Photocontrol of neuronal firing. (**a**,**b**) Current clamp recordings from hippocampal neurons treated with AAQ and exposed to alternating 380-nm and 500-nm light as indicated to control action potential firing (**a**), to a 200-ms flash of 500-nm light followed by either 380-nm light to trigger a transient burst of spikes (**b**; left) or followed by darkness to trigger sustained activity (**b**; right). Depolarizing current was injected throughout. (**c**) Current clamp recordings of single action potentials in an AAQ-treated neuron illuminated with 500-nm light (green) or 380-nm light (violet). Action potentials were induced with 500 ms depolarizing current injection (120 pA in 380-nm light, 100 pA in 500-nm light).

treatment conditions, AAQ does not appear toxic to neurons *in vitro* or *in vivo*.

Like other light-activated ion channels, optical control of PAL-modified $K^+$ channels can alter the frequency of action potential firing in treated neurons. To allow reliable measurement of photoregulation of firing frequency, we induced continuous firing by injecting depolarizing current in AAQ-treated neurons exposed to 500-nm light. Subsequent photoisomerization of AAQ with 380-nm light, which unblocks $K^+$ channels, rapidly suppressed action potential firing (**Fig. 4a**). High-frequency firing resumed upon continuous illumination with 500-nm light. In addition, flashes of 500-nm light followed by exposure to darkness resulted in sustained firing that long outlived the light stimulus (**Fig. 4b**), reflecting the stability of the photoswitch in its *trans* configuration. Hence, light flashes of either wavelength are sufficient to toggle on and off channel blockade and neuronal excitability, eliminating the need for continuous illumination. This feature differentiates PAL-mediated control from regulation by glutamate uncaging and ChR2, which generally affects neurons only during the illumination period. The persistent nature of PAL photoisomers after brief illumination minimizes potential photodamage both to the photoswitch and to target cells by reducing the total amount of light required for optical control. Photocontrol of PAL-modified channels with continuous or intermittent light occurred rapidly, resulting in modulation of neuronal firing within hundreds of milliseconds after the onset of illumination, with the exact delay varying with light intensity. We also found that the after-hyperpolarization at the end of the action potential was more pronounced in 380-nm than in 500-nm light, consistent with $K^+$ channel regulation (**Fig. 4c**).

Unlike other light-activated channels, PAL targets native ion channels that control cellular excitability, thereby altering the threshold for action potential firing. Current injections that failed to elicit action potential firing under 380-nm light reliably induced firing when a neuron was illuminated with 500-nm light (**Fig. 5a**). The relative proportion of *cis* and *trans* photoisomers of azobenzene-containing photoswitches is dependent on wavelength[10,24]. This photoequilibrium can be exploited to adjust the extent of $K^+$ channel block in a graded manner to finetune cellular excitability. Thus, the amount of depolarizing current required to induce action potential firing decreased as the number of blocked $K^+$ channels was increased with longer wavelengths (**Fig. 5b**). Photoregulation of voltage-gated $K^+$ channels also allows control of spike frequency adaptation. Neurons responding to depolarizing current injection with a single spike in 380-nm light fired repetitively when illuminated with visible light (400–500 nm;

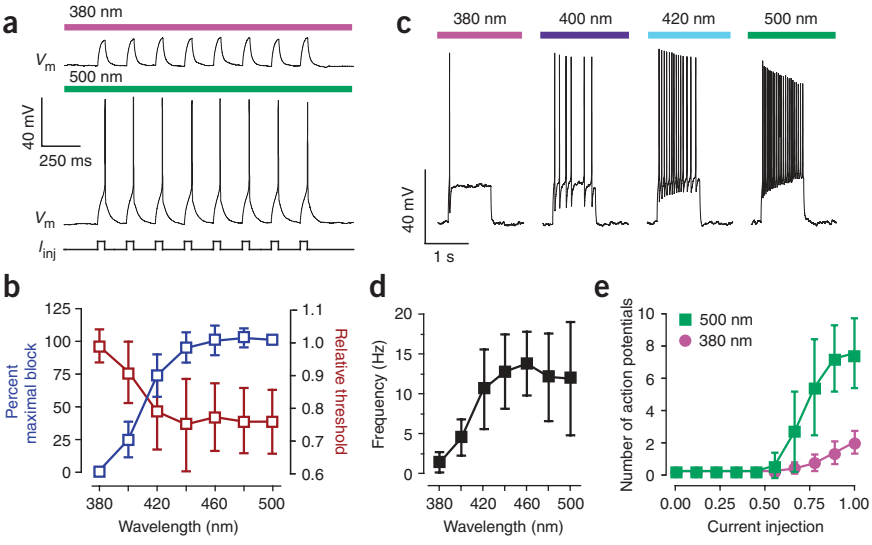


**Figure 5** | Modulation of neuronal excitability with light. (**a**) After PAL treatment identical current pulses ($I_{inj}$ 250 pA, 50 ms, 5 Hz) that did not elicit changes in membrane voltage ($V_m$) sufficient to trigger spikes in 380-nm light (top), reliably elicited spikes in 500-nm light (bottom). (**b**) Effect of different wavelengths of light on voltage-gated $K^+$ channel blockade and action potential threshold in neurons exposed to light of increasing wavelength. For each wavelength, the percent maximal block of voltage-gated $K^+$ current ($n = 5$ cells) was compared to the current injection threshold required to fire a spike ($n = 6$ cells). (**c**) Effect of different wavelengths of light on spike frequency adaptation. Current injection (300 pA, 1 s) in neurons treated with PAL was performed during exposure to 380-, 400-, 420- or 500-nm light, eliciting different firing patterns. (**d**) Relationship between illumination wavelength and average spike frequency during depolarizing pulses ($n = 9$ cells). (**e**) Effect of illumination wavelength on firing threshold and sensitivity to progressively greater current injections in neurons treated with PAL. Currents were normalized to the maximal amount of current injected in each cell. Current injections lasted 500 ms and ranged from 15 to 360 pA ($n = 6$ cells). All data are means ± s.d.

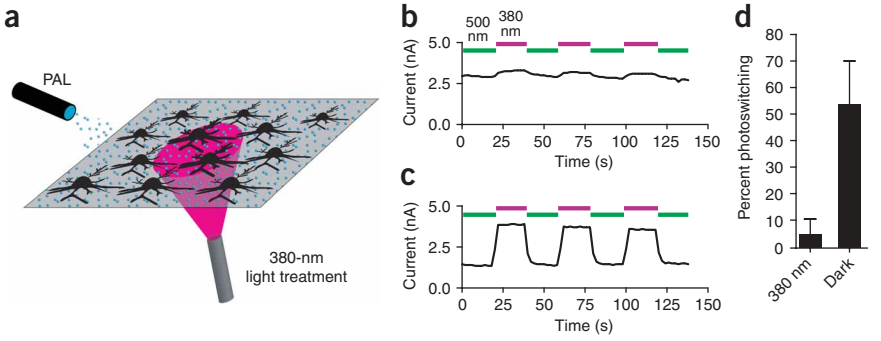


**Figure 6** | Local illumination during PAL treatment imprints photosensitivity onto specific neurons. (**a**) A hippocampal neuronal culture was uniformly exposed to 300 μM AAQ. During treatment, the microscope objective was used to illuminate a subpopulation of neurons with 380-nm light while the remaining neurons were kept in the dark. After treatment, AAQ was replaced with extracellular solution, whole-cell recordings were obtained, and photoresponsiveness was tested by applying alternating flashes of 380-and 500-nm light. (**b,c**) Photoresponsiveness of neurons treated with AAQ either in 380-nm light (**b**) or in darkness (**c**). Currents were elicited by stepping from −70 mV to +30 mV for 250 ms every 2 s while illuminating with 380-nm or 500-nm light. Recordings in **b** and **c** were from neurons on the same coverslip. (**d**) Summary data showing average percent photoswitching of neurons treated with AAQ in 380-nm light or the dark. Data are averages ± s.d. ($n$ = 9–10 neurons).

**Fig. 5c**), and the frequency of firing was graded depending on the wavelength of illumination (**Fig. 5d**). Photoregulation of AAQ-modified channels thus modulates firing threshold (that is, rheobase) as well as the number of action potentials fired in response to a given current injection (**Fig. 5e**).

### Targeted photosensitivity with optical imprinting

Channel modification by AAQ is dependent on the presence of a nucleophile at the correct distance from the QA binding site. We showed that covalent attachment occurs when cells are treated in the dark with AAQ in its extended *trans* form. However, the reactive moiety of PAL will encounter different amino acids when the photoswitch is in its shorter *cis* configuration. To test whether AAQ can attach to $K^+$ channels in its *cis* form, we added AAQ while illuminating a subpopulation of neurons with 380-nm light (**Fig. 6a**). The remaining neurons were kept in darkness and thus were exposed to AAQ in its *trans* form. Neurons illuminated with 380-nm light during PAL treatment showed little photosensitivity, whereas those kept in darkness exhibited light-regulated $K^+$ currents (**Fig. 6b–d**; treatment in 380-nm light, 5 ± 5% photoswitching, $n$ = 10; treatment in darkness, 54 ± 16% photoswitching, $n$ = 9). The magnitude of the unblocked $K^+$ current in the two groups of cells was not significantly different (3,515 ± 1,022 pA, $n$ = 9 and 4,355 ± 1,466 pA, respectively, $n$ = 10; $P > 0.1$, Student's unpaired $t$-test). Hence, photosensitization of $K^+$ channels by PAL is itself sensitive to light. This feature can be exploited to pre-program specific cells to become light-sensitive by using selective illumination during PAL treatment.

### PAL-mediated optical control of activity in neural tissue

So far we showed that PAL molecules enable optical control of cells in culture. For the approach to be effective in tissue, the only additional requirement is that the photoswitch and light reach the cells of interest. We evaluated PAL-mediated optical control of neuronal firing in freshly obtained rat cerebellar slices. After pretreatment with AAQ, we recorded from cerebellar basket cells using the loose-patch configuration. We recorded in the presence of AMPA, NMDA and $GABA_A$ receptor antagonists, leaving the basket cells synaptically isolated from the rest of the circuit. Blocking AAQ-modified $K^+$ channels with 500-nm light promoted the firing of basket cells whereas unblocking channels with 360-nm light decreased their firing (**Fig. 7a**; we observed photoregulation in 6/6 basket cells in AAQ-treated slices and in 0/8 basket cells in untreated slices). These results demonstrate that the penetration of AAQ and the delivery of light are not substantially impeded in brain tissue.

We next tested AAQ on the medicinal leech *Hirudo medicinalis*, a system in which the introduction of foreign genes required for other types of light-activated proteins is not widely used. Specifically, we obtained extracellular recordings from the heart central pattern generator interneurons (HN cells). HN cells control the contraction of the heart by bursting in alternation and imposing this rhythm on heart motor neurons. The neuropeptide FMRFamide decreases the burst period of HN cells, possibly by modulating voltage-gated $K^+$ currents[25]. By allowing specific and reversible photoregulation of $K^+$ channels, the PAL approach provides a means to assess the contribution of $K^+$ channels in the bursting pattern of HN cells. We found that unblocking AAQ-modified

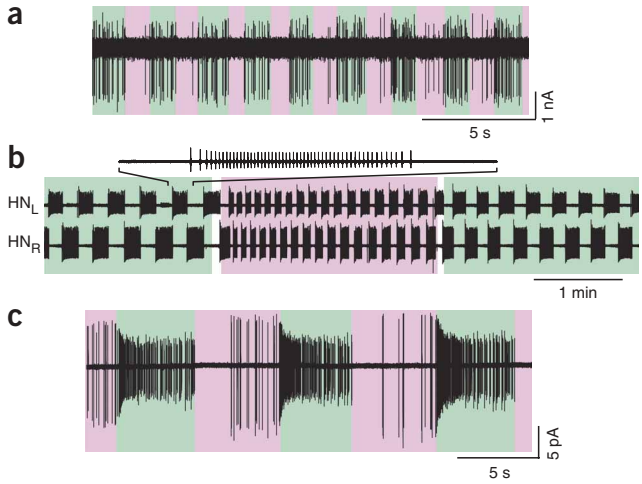


**Figure 7** | Photocontrol of action potential firing in intact neural circuits. (**a**) Rat cerebellar slices were treated with PAL, and a loose-patch recording of spontaneous spiking was obtained from a cerebellar basket cell during alternating 360-nm and 500-nm light. Basket cells were synaptically isolated from other neurons by using GABA and glutamate receptor antagonists. (**b**) Simultaneous loose-patch recordings of bursting activity from the left and right heart interneurons ($HN_L$ and $HN_R$) in the medicinal leech heartbeat central pattern generator after PAL treatment and subsequent illumination with 380-nm and 500-nm light. (**c**) Loose-patch recording of PAL-mediated photoresponses in a rat RGC from a flat-mounted retina under alternating illumination as in **b**.

$K^+$ channels with 380-nm light decreased burst period of HN cells whereas exposure to 500-nm light extended the period (**Fig. 7b**, $n = 4$ pairs). This is consistent with modeling studies that predict changes in HN cell burst pattern upon modulation of $K^+$ channels[26]. The ability to photoregulate neurons in the leech heartbeat central pattern generator demonstrates that the PAL approach is a powerful method to control $K^+$ channels and electrical activity in an intact neural circuit without genetic modification.

The installation of light sensitivity on neurons may enable the artificial input of information downstream from sites of damage or degeneration. For example, the loss of visual function caused by degeneration of rods and cones could be alleviated by treatments that impart light sensitivity on downstream neurons that are normally light insensitive. We tested whether PAL treatment could impart light sensitivity on RGCs, which relay information from the retina to the brain. Loose-patch recordings obtained from RGCs after AAQ treatment showed that their firing increased in 500-nm light and decreased in 380-nm light (**Fig. 7c**), owing to the block and unblock of AAQ-modified $K^+$ channels. We observed robust photocontrol of firing in 15/23 RGCs in AAQ-treated retina and 0/10 RGCs in non–AAQ-treated retina. The retina contains a small fraction of RGCs ($\sim$2.5%) that express melanopsin and are intrinsically photosensitive[27], but it is unlikely that these cells account for the AAQ results reported here.

## DISCUSSION

Traditional affinity labels consist of a ligand that covalently attaches to its target protein via a reactive group. Affinity labels have been used to identify amino acids involved in ligand binding as well as to map distances between selected residues and a ligand binding site in a variety of proteins. Affinity labels can cause permanent activation or inhibition of protein function because covalent attachment results in persistent occupancy of the ligand binding site[23]. We expanded the affinity labeling concept by including a photoisomerizable azobenzene linker between the ligand and the reactive group, enabling photoregulation of ligand occupancy and protein function. By using the pore-blocking QA as the ligand, we designed PALs selective for $K^+$ channels. The inclusion of a promiscuous reactive group resulted in covalent attachment and photoregulation of several types of $K^+$ channels, but not $Na^+$ and $Ca^{2+}$ channels, presumably because labeling is selective for channels that have a QA binding site. The modular nature of PALs enables easy modification of each functional group in the molecule, yielding a combinatorial toolkit for optical regulation of endogenous proteins. For instance, the reactive group could be altered to enable selective attachment to particular amino acids or protein sequences, and the photoisomerizable moiety could be modified to alter the spectral sensitivity or photoswitching kinetics. Finally, inclusion of the appropriate ligand should target PALs to particular types of ion channels and receptors, allowing optical control of a variety of physiological functions.

Genetically encoded light-activated proteins, such as ChR2, LiGluR, SPARK and NpHR, have emerged as potent tools for mapping microcircuitry because their expression can be restricted to subpopulations of neurons. In contrast, PAL molecules target intrinsic cellular proteins and thus impart photosensitivity to all treated cells, as long as they express the photoswitch target. Widespread photosensitivity may facilitate functional analysis of processes that involve the coordinated firing of multiple cells. However, if regulation of particular cells is desired, there are three ways to restrict PAL-mediated photocontrol. First, PAL can be applied locally so that only a restricted cell or group of cells becomes photosensitized. Second, local illumination can be used during treatment to optically imprint PALs and thus photosensitize particular cells. Third, after PAL treatment, light of the appropriate wavelength can be projected locally to regulate excitability in individual cells or groups of cells. The key asset of the PAL approach is that long-term light sensitivity can be installed in freshly obtained tissue, unadulterated by exogenous gene expression and possible developmental consequences of ectopic protein expression.

ChR2 and LiGluR are effective tools for driving precise spike trains in neurons[12–14,16,17] because they encode nonspecific cation channels that directly lead to membrane depolarization. In contrast, the molecular targets of PAL are voltage-gated $K^+$ channels, which act as a 'brake' rather than a direct trigger of activity. Thus, although light flashes can induce action potential firing in PAL-treated neurons, the temporal fidelity is lower than with other light-activated ion channels that directly depolarize the membrane. Instead, the strength of PAL is in its persistent and effective control over excitability, which enables light to modulate neuronal firing reversibly. Moreover, because photoisomerization can be controlled bi-directionally, the level of cellular excitability can be titrated by controlling the equilibrium between *cis* and *trans* photoisomers using different wavelengths of light. Although the isomerization of azobenzene-containing photoswitches occurs over a broad range of wavelengths (*cis*, 360–400 nm; *trans*, 450–560 nm), wavelengths longer than 560 nm do not affect the photoswitch[10,24]. Thus, it may be possible to combine PAL-mediated optical control with imaging of activity-dependent fluorescent dyes to generate an all-optical system for controlling and recording neuronal excitability.

The distinct mechanism of action of PAL explains why it is particularly effective in controlling the activity of neurons that are spontaneously active; their voltage-gated $K^+$ channels are continually opening and therefore continuously subject to optical regulation with PAL. But in theory, PAL should allow modulation of excitability even in quiescent neurons when they are receiving depolarizing input. More complex features of excitability, such as action potential firing threshold and spike frequency adaptation, can also be rendered light-sensitive using PAL. In addition, AAQ treatment allows optical neuromodulation of pacemaker activity in the leech heartbeat central pattern generator, mimicking the actions of a neuropeptide. Voltage-gated $K^+$ channels participate in the control of excitability in many cell types and thus play a crucial role in the regulation of neuronal communication, endocrine and exocrine secretion, cardiac signaling and vascular contraction. By enabling photocontrol of endogenous $K^+$ channels, the PAL approach provides a powerful means to control these physiological functions with light.

Because it targets endogenous proteins, the PAL approach is applicable to systems in which introduction of foreign genes is impractical or difficult. Indeed, it is conceivable that modified PAL molecules might ultimately be useful for imparting optical control of neurons in humans, without requiring gene therapy. A particularly relevant tissue for PAL-mediated optical regulation is the retina, the sole part of the nervous system that is exposed to light *in vivo*. Our data show that AAQ confers light-sensitivity to RGCs that are not intrinsically light-sensitive, raising the possibility that PAL treatment, along with an appropriate optical system, may be

used as an alternative to multielectrode-based retinal prosthetic devices[28] to restore visual function in retinas with damaged or degenerated rod and cone photoreceptors.

## METHODS

**Cell culture, plasmids and transfection.** We grew HEK293 cells in DMEM containing 5% FBS. For HEK293T cells, we also included 500 μg/ml G-418. For electrophysiology studies, we plated cells at $12 \times 10^3$ cells/cm$^2$ on poly(L-lysine)–coated coverslips and transfected the cells using the calcium phosphate method[29]. The Shaker H4 used here contained a deletion of amino acids 6–46 to minimize fast inactivation[30]. We transfected plasmids encoding Kv4.2 and KChIP3 at a 4:1 ratio[31]. We recorded $K^+$ channel currents 24–48 h after transfection of HEK293 cells and Nav1.2 currents 96 h after transfection of HEK293T cells. We recorded currents for L-type $Ca^{2+}$ channels from GH3 cells grown in F-12K containing 15% horse serum and 2.5% FBS, 48 h after plating. We prepared hippocampal neurons from neonatal rats according to standard procedures[32], plated them at $50 \times 10^3$ cells/cm$^2$ on poly(L-lysine)–coated coverslips and grew them in minimum essential medium containing 5% FBS, 20 mM glucose, B27 (Invitrogen), glutamine and Mito+ Serum Extender (BD Biosciences). We recorded currents 14–25 d after plating. Animal care and experimental protocols were approved by the University of California Berkeley Animal Care and Use Committee.

**PAL treatment.** We incubated cells at 37 °C in the dark for 15 min with 200–300 μM (for hippocampal neurons) or 400 μM (for HEK293, HEK293T and GH3 cells) AAQ diluted in bath solution. We treated the cerebellar slices at room temperature in the dark with 200 μM AAQ for 8 min, and we treated retinas and leech ganglia with 150 μM and 400 μM AAQ, respectively.

For illumination for photoswitching, we used a xenon lamp (175 W) with narrow band-pass filters (380BP10 and 500BP5). We measured light output using a handheld Newport meter (840-C model). At the back of the objective, light output was 0.3 mW/cm$^2$ for 380-nm light and 2.5 mW/cm$^2$ for 500-nm light. When measured through a 40× objective and normalized to the focal area at the specimen plane, light output was 0.5 mW/mm$^2$ and 3.5 mW/mm$^2$ for 380-nm and 500-nm light, respectively. For some experiments, we used a monochromator (Polychrome V; TILL Photonics) for illumination.

All data reported are averages ± s.d.

**Electrophysiological recordings from cultured cells.** We recorded currents in the whole-cell patch clamp configuration using pipettes with 3–5 MΩ resistance. To elicit voltage-gated $K^+$ currents from neurons and HEK293 cells, we set holding potential to –70 mV and stepped to +30 mV for 250 ms. To elicit voltage-gated $Na^+$ currents, we held HEK293T cells expressing Nav1.2 at –80 mV and stepped to 0 mV for 250 ms. We recorded L-type $Ca^{2+}$ currents in GH3 cells and elicited the currents by stepping to +20 mV for 200 ms from a holding potential of –40 mV. Bath and intracellular solutions compositions are available in **Supplementary Methods**.

**Tissue preparation and recordings.** We prepared parasagittal cerebellar slices (300 μm) from postnatal day 14–20 rats using standard techniques approved by the University of California Los Angeles Animal Care Committee. After sectioning, we incubated slices for 30 min at 35 °C in aCSF (composition in **Supplementary Methods**) then brought them to room temperature for PAL treatment and recording. We made loose-patch recordings in the presence of 6,7-dinitroquinoxaline-2,3-[1*H*,4*H*]-dione, gabazine and [*RS*]-3-[2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid using pipettes with 3–5 MΩ resistance. The depth of recorded basket cells was approximately 25–30 μm. We dissected retinas from postnatal day 50–60 rats under continuous flow of oxygenated saline solution (composition in **Supplementary Methods**). We treated dissected retinas at 37 °C with 20 U/ml cysteine-activated papain for 8–20 min, then washed them with saline containing 10 mg/ml BSA and 10 mg/ml trypsin inhibitor followed by PAL treatment and recording using the loose-patch configuration. Dissections and recordings from leech ganglia were conducted as described previously[33]. We obtained extracellular signals from HN cells using the loose-patch configuration.

**Additional methods.** Detailed descriptions of the chemical syntheses and the electrophysiology solutions are available in **Supplementary Methods**. All data reported are averages ± s.d.

*Note: Supplementary information is available on the Nature Methods website.*

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