Rapid optical control of nociception with an ion-channel photoswitch

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Local anesthetics effectively suppress pain sensation, but most of these compounds act nonselectively, inhibiting activity of all neurons. Moreover, their actions abate slowly, preventing precise spatial and temporal control of nociception. We developed a photoisomerizable molecule, quaternary ammonium–azobenzene–quaternary ammonium (QAQ), that enables rapid and selective optical control of nociception. QAQ is membrane-impermeant and has no effect on most cells, but it infiltrates pain-sensing neurons through endogenous ion channels that are activated by noxious stimuli, primarily TRPV1. After QAQ accumulates intracellularly, it blocks voltage-gated ion channels in the trans form but not the cis form. QAQ enables reversible optical silencing of mouse nociceptive neuron firing without exogenous gene expression and can serve as a light-sensitive analgesic in rats in vivo. Because intracellular QAQ accumulation is a consequence of nociceptive ion-channel activity, QAQ-mediated photosensitization is a platform for understanding signaling mechanisms in acute and chronic pain.

Optogenetic tools enable photoregulation of action potential firing in neurons both in vitro and in vivo1 through the introduction of exogenous genes. In contrast, small-molecule photoswitches enable optical control of neuronal excitability without genetic manipulation2,3. Photoswitch molecules confer light-sensitivity on the intrinsic excitability of neurons within minutes4–6. However, unlike optogenetic tools that can be promoter-targeted for expression in particular types of neurons, photoswitches act nonselectively on all neurons that are exposed to the molecule. Depending on the scientific or biomedical application, it could be a benefit or even a requirement to target photosensitivity to a particular type of neuron.

Here we used a non-genetic strategy to target a photoswitch molecule to pain-sensing (nociceptive) neurons. Nociceptive neurons have been particularly inaccessible to selective electrophysiological manipulation because both their peripheral sensory endings and central synaptic terminals are quite small, either embedded in the skin or interspersed with other neurons in the spinal cord. Nociceptors are unique in possessing a high density of ion channels that respond directly or indirectly to noxious stimuli7. For example, the capsaicin receptor TRPV1, which is sensitive to noxious heat, protons and mediators of inflammation, is expressed in nociceptive neurons, but it is very sparsely expressed elsewhere in the nervous system8. TRPV1 enters into a pore-dilated state upon prolonged agonist activation, allowing permeation of relatively large cations9. This property has been exploited to deliver into nociceptors a membrane-impermeant derivative of the local anesthetic lidocaine, QX-314 (ref. 10). The selective entry and silencing of nociceptors by QX-314 gives this molecule potential as a pain-selective local anesthetic10. However, once QX-314 enters cells, it cannot escape, and silencing persists for many hours11. The irreversibility of QX-314 precludes temporally precise regulation of nociceptor activity.

Here we describe QAQ, a photosomerizable molecule that confers reversible light-sensitivity selectively onto neurons involved in pain signaling, enabling rapid optical control of nociception without genetic manipulation.

RESULTS
QAQ photosensitized voltage-gated ion channels

We developed QAQ, a photoswitch that reversibly suppresses neuronal excitability by optically regulating voltage-gated Na+, Ca2+ and K+ channels. QAQ has a central photoisomerizable azobenzene coupled on both sides to quaternary ammonium groups (Fig. 1a). Upon illumination with 380-nm light, the elongated trans-QAQ converts to the bent cis form (Supplementary Fig. 1a). Cis-QAQ spontaneously reverts to the trans form slowly in the dark (Supplementary Fig. 1b), but this transition occurs quickly (within milliseconds) in 500-nm light.

QAQ resembles lidocaine and its derivative QX-314 (Fig. 1b,c), local anesthetics that block voltage-gated Na+, K+ and Ca2+ channels from the cytoplasmic side12,13. Lidocaine is a tertiary amine that crosses the membrane in an uncharged state and blocks ion channels after becoming protonated in the cytoplasm.
Figure 1 | Intracellular QAQ photosensitized voltage-gated ion channels. (a–c) Chemical structures of cis and trans QAQ (a), lidocaine (b) and QX-314 (c). (d) Na⁺ current in cells with intracellular QAQ (100 μM). Depolarization was from –70 mV to –10 mV. Photoswitching, as defined by (current (I) at 380 nm – I at 500 nm)/I at 380 nm, was 60.5 ± 5.8% (n = 4 cells) (e) Na⁺ current in cells with extracellular QAQ (1 mM). Photoswitching was 1.4 ± 1.3% (n = 7 cells). (f) Current versus membrane voltage (Vm) of peak Na⁺ current. (g) Na⁺ current in cells with intracellular QAQ (100 μM) and repetitive depolarizing pulses (1 Hz). Control with no QAQ is shown. (h) Reversibility of Na⁺ current photoswitching. (i) Ca₂⁺ current utilizing intracellular QAQ (100 μM). Depolarizing pulse was from –60 mV to +10 mV. Photoswitching was 60.5 ± 10.5% (n = 3 cells). (j) Shaker K⁺ channel current utilizing intracellular QAQ (100 μM). Depolarizing pulse was from –70 mV to +40 mV. Photoswitching was 60.3 ± 8.6% (n = 4 cells). (k) Percentage photoswitching of currents through voltage-gated Na⁺ (Nav), voltage-gated Ca²⁺ (Cav), and voltage-gated K⁺ (Kv) channels. ‘Neuronal’, Na⁺ channels from NG108-15 cells; ‘sensory’, Na⁺ channels from rat trigeminal ganglion neurons; TTX sens., tetrodotoxin-sensitive; and TTX res., tetrodotoxin-resistant. L-type, voltage-gated channels from GH3 cells; Ca₂⁺, K₂.1, K₃.1 and K₄.2 were expressed in HEK-293 cells; ‘hippocampal’, K⁺ channels from primary hippocampal cultures (n = 3–13 cells, error bars, s.e.m.). NG108-15 cells were examined in d–h, and HEK-293 cells in i,j.

QAQ contains a permanently charged quaternary ammonium, preventing it from crossing the membrane. However, QAQ is a potent blocker of activity when introduced through a patch pipette into the cytoplasm14.

To test whether QAQ can act like a photoregulated ion-channel blocker, we made whole-cell recordings from NG108-15 cells, a mouse neuroblastoma and rat glioma hybrid cell line that expresses neuronal voltage-gated Na⁺ (Naᵥ) channels15. When we delivered QAQ into the cytosol through the patch pipette, it blocked most of the Na⁺ current in the trans configuration, but blockade was removed in 380-nm light (Fig. 1d). In contrast, bath application of QAQ did not block (Supplementary Fig. 2) or photosensitize the Na⁺ current (Fig. 1e), indicating that QAQ is membrane-impermeant like QAQ-314 (ref. 10). Light-sensitive block of the Na⁺ current occurred at all membrane potentials tested (Fig. 1f). We quantified block of trans-QAQ versus cis-QAQ by examining Na⁺ current during a train of depolarizing stimuli. In the trans form, the amount of QAQ blockade is use-dependent, becoming more complete with increasing duration or frequency of depolarization (56% ± 10% block after 30 s, n = 7 cells, Fig. 1g). In contrast, cis-QAQ decreased the current by 9.6% ± 0.1% (n = 7 cells), indistinguishable from control experiments with no QAQ (8.3% ± 0.1%, n = 5 cells, P = 0.52 Student’s t-test). Photocontrol of Na⁺ current could be elicited repeatedly and rapidly without decrement over many minutes (Fig. 1h and Supplementary Fig. 3).

Local anesthetics are used to silence the activity of sensory neurons, which have a variety of voltage-gated Na⁺ channels, including tetrodotoxin-sensitive and -resistant types7. Whole-cell recordings from rat trigeminal ganglion neurons showed that both channel types could be photoregulated by intracellular QAQ (Supplementary Fig. 4).

QAQ also photosensitized voltage-gated Ca²⁺ channels. We recorded from HEK-293 cells stably expressing voltage-gated Ca²⁺ channel Ca₂⁺, and from GH3 cells, a rat pituitary tumor cell line expressing L-type calcium channels16. In both cell types, internal trans-QAQ blocked the Ca²⁺ current, but blockade was removed in 380-nm light (Fig. 1i and Supplementary Fig. 5a). Photoregulation of both Ca²⁺ channels was rapid, occurred at all voltages tested and exhibited little decrement over time (Supplementary Fig. 5b–f).

Voltage-gated K⁺ channels were also sensitive to QAQ. We recorded from HEK-293 cells expressing the inactivation-removed Shaker K⁺ channel17 and again observed robust photoregulation, with current blocked by trans-QAQ and unblocked by converting the molecule to the cis form (Fig. 1j). QAQ block at 500 nm was voltage-dependent, increasing with depolarization, as observed with other quaternary ammonium molecules5 (Supplementary Fig. 6a). QAQ photosensitized other voltage-gated K⁺ channels exogenously expressed in HEK-293 cells as well as native K⁺ current in hippocampal neurons (Supplementary Fig. 6b–j). Photoregulation of K⁺ channels occurred rapidly and without decrement over time (Supplementary Fig. 6k,l).

Hence whereas QAQ was normally membrane-impermeant, it photosensitized current flowing through voltage-gated Na⁺, Ca²⁺ and K⁺ channels when introduced into the cell (Fig. 1k). Intracellular QAQ photosensitized many but not all K⁺ channels; inward-rectifier (Kᵢ) and hyperpolarization-activated cyclic nucleotide–gated (HCN) channels were unaffected by QAQ (Supplementary Fig. 7). Intracellular QAQ did not photoregulate current through N-methyl-d-aspartic acid (NMDA) and non-NMDA receptors (Supplementary Fig. 8).

QAQ enabled photoregulation of neuronal excitability Because it imparted light-sensitive block on voltage-gated Na⁺, K⁺ and Ca²⁺ channels, QAQ should have a strong influence on the electrical excitability of neurons. To examine the net effect of
internal QAQ on action-potential firing we carried out current clamp recordings from dissociated rat hippocampal neurons in culture. Current pulses of increasing amplitude elicited a progressive increase in the number of action potentials when QAQ was in the cis configuration (Fig. 2a). However, when QAQ was converted to the trans form, neurons fired a single spike at the onset of stimulation, but did not fire additional spikes even with the largest current pulse tested, consistent with use-dependent blockade of Na+ channels (Fig. 2a,b). The amplitude of the first spike decreased and its half-width increased when we switched from 380 nm to 500 nm light, in agreement with both Na+ and K+ channels being blocked (Fig. 2c). Higher internal concentration of QAQ (≥200 µM) eliminated all spikes at 500 nm (data not shown).

QAQ affected spiking but had little or no effect on the resting properties of these neurons. Neither the input resistance nor the membrane potential changed over time as QAQ diffused into the neuron (Fig. 2d,e). Moreover, light had no effect on these parameters, in agreement with QAQ not affecting Ki and HCN channels, two channels that have a role in setting the resting membrane potential of neurons. Firing threshold in these neurons was the same with (195 pA ± 31 pA; ± s.e.m.) and without (161 pA ± 12 pA, P = 0.44 Student t-test) QAQ in the pipette and was not affected by changing the wavelength of light (Fig. 2f).

**QAQ entered cells through nociceptive ion channels**

QAQ is normally membrane impermeant, so it does not photosensitize most cells. However, we asked whether QAQ could be delivered into cells without requiring dialysis through a patch electrode. This strategy involves using nociceptive pore channels as a conduit for QAQ entry. We transfected HEK-293 cells with the gene encoding the Shaker K+ channel, which we used as an indicator of intracellular QAQ accumulation. We first used TRPV1, a channel whose pore dilates after exposure to its agonist capsaicin. Control cells treated with capsaicin showed no QAQ-mediated photosensitization (Fig. 3a). However, cells expressing TRPV1 showed photosensitization of Shaker current, but only when we applied QAQ on the cells in conjunction with ATP. We then tested two other TRP channels, TRPA1 and TRPM8, but found no significant loading through either TRPA1 (7.3% ± 4.0%; s.e.m., n = 11 cells, P = 0.39, Student t-test) or TRPM8 (−0.3% ± 1.4%; s.e.m., n = 3 cells, P = 0.81, Student t-test) channels after these channels were activated with allyl isothiocyanate (30 µM) or menthol (30 µM), respectively (data not shown).

Some ionotropic receptors for ATP (P2X receptors) also exhibit pore dilation upon prolonged activation. Therefore we tested whether P2X channels could be used as a conduit for QAQ entry. Control HEK-293 cells treated with ATP showed no QAQ-mediated photosensitization (Fig. 3b). However, cells expressing P2X receptors showed photosensitization of Shaker current, but only when we applied QAQ on the cells in conjunction with ATP. The amount of K+ channel photosensitization was nearly the same 5 and 30 min after ATP application, suggesting that QAQ equilibrated quickly in the cell.

To test whether QAQ can enter into neurons through dilating pore channels, we recorded from cultured rat hippocampal neurons. QAQ alone had no effect on endogenous voltage-gated K+ current (Fig. 3c). However, we could bestow light-sensitivity to neurons that exogenously expressed P2X receptors, by treating them with QAQ and ATP. A cell death assay showed that there was no toxicity resulting from this treatment (Supplementary Fig. 9).
TRPV1 channels are crucial for nociception in peripheral sensory neurons. The presence of endogenous TRPV1 channels suggests that QAQ might enter nociceptive neurons without requiring exogenous gene expression. We examined the effect of QAQ on three different parts of nociceptive neurons: their cell bodies, located in the dorsal root ganglion (DRG), their synaptic terminals, located in the spinal cord, and their sensory nerve endings, located in the periphery.

Photosensitization of neurons in intact DRGs
We developed a system to record and analyze many mouse DRG neurons at once while simultaneously photoregulating their electrical activity (Fig. 4a and Online Methods). We used a three-dimensional multielectrode array containing 60 pin-shaped electrodes. We controlled the isomeric state of QAQ with a light source positioned under the multielectrode array; the array was transparent to 380-nm and 500-nm light. We positioned a microscope objective above the DRG and used an external stimulation unit to elicit action potentials, which could be recorded as extracellular signals by electrodes of the multielectrode array (MEA). (Fig. 4b).

After treatment with QAQ, individual electrodes recorded spikes that could be silenced by switching from 380-nm to 500-nm light, consistent with QAQ photosensitization (Fig. 4c). This suggests that there must be some basal activity of QAQ-permeant channels in DRG neurons. We plotted the activity of 24 neurons on a raster plot (Fig. 4d). Trains of stimuli at 10 Hz elicited trains of action potentials, which could be photoregulated by switching from 380-nm to 500-nm light. At both wavelengths, the number of spikes diminished within a train of stimuli, but the extent of spike-train accommodation was much greater in 500-nm light.

Principal component analysis is a common method in multidimensional data analysis to reduce dimensionality. In this case, the analysis was used to identify patterns in the data that could be attributed to specific neuronal subtypes.

If the TRPV1 channel is the main conduit for QAQ entry, blocking or eliminating TRPV1 channels should reduce QAQ loading and consequently the amount of photosensitization. Consistent with this, we found that N-(4-tertiarybutylphenyl)-4-(3-chlorophyridin-2-yl)-tetrahydro-pyrazine1(2H)-carboxamide (BCTC), a TRPV1 antagonist that inhibits acid- and capsaicin-induced activation, considerably reduced DRG photosensitization (Fig. 4g).
This system can be used as a platform for assessing the activity of TRP channels in the intact DRG in response to various stimuli. We treated the ganglia with capsaicin during QAQ loading, followed by thorough washing with normal saline. Capsaicin is a selective agonist of TRPV1, and as expected, it increased QAQ loading and therefore photosensitization (Fig. 4g). Bradykinin is a neuropeptide that promotes pain hypersensitivity and inflammation. We found that Bradykinin also promoted QAQ-mediated DRG photosensitization, consistent with a signaling cascade that leads to activation of TRP channels.

Direct electrical stimulation of sensory neuron axons in the peripheral nerve also promoted DRG photosensitization (Fig. 4g), indicating enhanced QAQ entry during the stimulation period. Action potential firing may directly promote QAQ entry into TRPV1-containing neurons, but TRPV1 channels are only weakly voltage-sensitive. In addition, action potential firing may promote DRG somata to release neuro-inflammatroy transmitters, and these may indirectly lead to activation of nociceptive channels, a positive feedback mechanism that could contribute to prolonged hypersensitivity and chronic pain.

Photosensitization of neurons in spinal cord slices

TRPV1 is abundantly expressed throughout the entire length of nociceptive neurons, including the central terminals in the spinal cord, but it is thought to be largely absent from non-nociceptive sensory neurons. The central terminals of nociceptive neurons are located in laminae I–II of the dorsal horn of the spinal cord, whereas the terminals of non-nociceptive neurons are located in laminae III–IV (ref. 7). If QAQ loading is selective for nociceptors, it should photosensitize only the subset of sensory neurons that terminate in laminae I–II.

We treated spinal cord slices with QAQ and recorded synaptic responses in dorsal horn neurons triggered by electrical stimulation of the dorsal root (Fig. 5a). In lamina II, the average excitatory postsynaptic current (EPSC) amplitude was reduced by switching from 380-nm to 500-nm light, whereas light had no effect for EPSCs recorded in laminae III–IV (Fig. 5b,c). These results are consistent with preferential photosensitization of nociceptive neurons by QAQ (Fig. 5d).

To distinguish between a pre- versus postsynaptic effect of QAQ, we recorded spontaneous EPSCs in lamina II neurons and analyzed the cumulative distribution of amplitudes and interevent intervals. The amplitude of these EPSCs was unaffected by the wavelength of light but the frequency of these EPSCs was decreased by 500-nm light (Fig. 5e–g) in six of eight cells. A change in EPSC amplitude indicates a postsynaptic alteration in neurotransmitter receptor function, whereas a change in frequency usually indicates a change in presynaptic neurotransmitter release.

QAQ-mediated photosensitization also impacted polysynaptic pathways on the spinal cord. Trains of stimuli generated a strong inward current that persisted for several seconds after the monosynaptic EPSCs should have decayed (Fig. 5h). Switching from 380-nm to 500-nm light caused a dramatic reduction in the amplitude of this current. Switching back to 380-nm light largely restored the initial amplitude of the response (Fig. 5i).

In some, but not all lamina II neurons, QAQ photosensitized not only the presynaptic inputs but also intrinsic voltage-gated channels $K^+$ currents (Supplementary Fig. 10a). In contrast, there was little photosensitization of $K^+$ channels in lamina III–IV neurons. Photosensitization of lamina II neurons was eliminated by BCTC (Supplementary Fig. 10b). These results suggest that TRPV1 channels enabling QAQ entry are present and active in lamina II neurons to a much greater extent than in lamina III–IV neurons.

**In vivo photoregulation of peripheral nerve endings**

If QAQ effectively photosensitizes nociceptive neurons, exposure to light should alter pain sensation in vivo. We explored this possibility by testing the pain-avoidance (nocifensive) blinking response that is elicited by mechanical stimulation of the cornea in rats using the von Frey hair test (Online Methods). The cornea is densely innervated with nociceptors that mediate the blink response. Free nerve endings are only a few micrometers below the surface, and the cornea is transparent, facilitating optical control. To enable QAQ entry into nociceptor nerve endings, we topically applied
QAQ with capsaicin in one eye and capsaicin alone in the contralateral eye. We immobilized the rats by mild sedation with xylazine and ketamine at low doses that do not interfere with nociceptive blinking. The von Frey hair test in ambient light showed that the normalized blink threshold was about fivefold higher in the eye treated with QAQ plus capsaicin compared to the eye treated with capsaicin alone (Fig. 6a). Moreover, 380-nm light decreased the normalized blink threshold in the eye treated with QAQ plus capsaicin (Fig. 6b). The decrease in blink sensitivity caused by QAQ was completely removed by exposure to 380-nm light (Fig. 6c). Taken together, these results show that QAQ can serve as a local anesthetic that can be turned off with light.

**DISCUSSION**

Microbial light-sensitive ion transporters, including halorhodopsin and archaerhodopsin-3, have been used as optogenetic inhibitors of neuronal activity. Genes encoding these proteins can be promoter-targeted to subpopulations of neurons. However, for several reasons, nongenetic optical control of nociception with QAQ may be preferable to optogenetic methods.

Unlike optogenetic tools that overpower the natural activity of cells, QAQ acts on endogenous ion channels that underlie initiation and propagation of action potentials. Hence QAQ suppresses electrical excitability at its source. Because the ion-transport rate of transporters is much slower than ion flux through channels, optical silencing with halorhodopsin and archaerhodopsin-3 requires very high expression. Exogenous expression can be achieved by injecting viral vectors into the appropriate part of the nervous system, but expression requires days to weeks and is restricted to neurons that are exposed to an adequate titer of virus. Optogenetic expression can result in permanent genetic alteration of neurons, which may not be necessary or desirable for the acute regulation of pain signaling, either for scientific or biomedical applications. In contrast, QAQ-mediated photosensitization occurs within minutes and persists only until the molecule dissipates, either by being metabolized inside the cell or diffusing away from targeted neurons. Because QAQ is a small molecule, it diffuses readily through tissue and presumably gains access to all neurons that have ion channels that permit its entry into the cytoplasm.

QAQ has potential value as both a scientific and a clinical tool for controlling nociception. Because it selectively accumulates in nociceptors, QAQ could selectively inhibit pain signaling while sparing other sensory modalities and therefore could function as a targeted analgesic. This is similar to the recently proposed therapeutic use of QX-314 (ref. 10). However, QAQ has the added feature of being rapidly controllable with light. In vivo photocontrol would require delivery of sufficient QAQ and projection of sufficient light onto target neuronal tissues. Because it is doubly charged, QAQ is unlikely to cross the blood–brain barrier. But our results show that QAQ penetrates into spinal cord slices and intact DRGs, so if injected, QAQ should have access to other neural structures. Implanted fiber-optic systems such as those developed for deep brain photocontrol of neurons expressing optogenetic tools could be adapted for controlling QAQ administered to internal neural structures (for example, spinal roots or DRGs). QAQ might also be controlled by an external light source after topical administration (for example, for treating corneal pain).

QAQ-mediated photosensitization could facilitate mapping of nociceptive circuit connections mediated by fast conventional synapses or by slow neuromodulatory neurotransmitters that may contribute to central sensitization and pain hypersensitivity. Our synaptic studies in spinal cord were limited to full-field regulation of presynaptic activity, but higher-resolution photocontrol should be possible by projecting through a microscope small spots or patterns of light, for example, to target presynaptic axons or terminals.

Finally, QAQ provides insight into the activity status of ion channels implicated in pain and inflammation. Previously, the activity of nociceptive ion channels has been studied almost exclusively in isolated neurons that had been enzymatically and mechanically dissociated from DRGs. This disruptive procedure could alter the activity and expression of these channels. QAQ enabled investigation of nociceptive ion-channel activity in undisrupted neural structures. Moreover, QAQ photosensitized regions of a nociceptor that are largely inaccessible to electrodes. Hyperalgesia in both inflammatory and neuropathic pain is associated with upregulation of TRP channel gene expression in peripheral nociceptors, but the activation status of these channels in chronic pain is unknown. Because QAQ-mediated photosensitivity is a consequence of the cumulative activity of nociceptive channels, it serves as an ultrasensitive reporter that provides new insights about when and where these channels are active, in both physiological and pathological conditions.

**METHODS**

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturemethods/.

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

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AUTHOR CONTRIBUTIONS
A.M. and R.H.K. wrote the paper. A.M., T.F., Y.L.F., C.M.S., F.N., D.T. and R.H.K. designed experiments. A.M., T.F., Y.L.F., C.M.S. and C.H. performed electrophysiological experiments and analyzed data. A.M. and D.D. performed in vivo experiments.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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ONLINE METHODS

**General.** All animals were housed in the centralized animal facilities as assigned by the University of California Berkeley and were provided food and water ad libitum. Animal care and experimental protocols were approved by the University of California Berkeley Animal Care and Use Committee. All chemicals were purchased from Sigma-Aldrich, except BCTC (N-(4-tertiarybutylphenyl)-4-(3-cholorphyridin-2-yl)-tetrahydro-pyrazine1(2H)-carboxamide) and AITC (allyl isothiocyanate) that were purchased from Tocris.

**QAQ synthesis and spectroscopic characterization.** QAQ was synthetized as previously described. UV-vis spectra of QAQ were measured using a smartSpec Plus spectrophotometer (Bio-Rad) in combination with illumination using the Polychrome V (Till Photonics), through an optic fiber positioned perpendicular to the detection beam of the spectrophotometer.

**Cell culture.** HEK-293 cells were cultured under standard conditions (Dulbecco’s modified Eagle medium (DMEM) containing 10% FBS). We grew GH3 cells in F-12K medium containing 15% horse serum and 2.5% FBS. NG108-15 cell medium contained 95% DMEM mixed with HAT (0.1 mM hypoxanthine, 400 nM aminopterin and 0.016 mM thymidine) and 5% FBS. Cells were plated on poly(ℓ-lysine) (0.1 mg/ml) treated coverslips in a density of 12,000 cells per cm² for electrophysiological measurements. Dissociated hippocampal neuronal preparations were performed from neonatal Sprague-Dawley rats according to standard procedures. Hippocampi were dissected, dissociated and cells were plated on poly(ℓ-lysine)-coated coverslips at a density of 100,000/cm². We grew hippocampal neurons in minimum essential medium containing 5% FBS, 20 mM glucose, B27 (Invitrogen), glutamine and Mito+ Serum Extender (BD Biosciences). Trigeminal ganglion (TG) neurons from neonatal rats were prepared as previously described. TGs were dissected and neurons were dissociated (with collagenase and trypsin) and plated on poly(ℓ-lysine)-coated coverslips. We grew TG neurons in minimum essential medium containing 5% horse serum, MEM vitamins (Invitrogen), glutamine and penicillin-streptomycin. HEK-293 cells were transfected using calcium phosphate precipitation and measured after 24–48 h (ref. 4). GH3 and NG108-15 cells were recorded 24 h after plating. Hippocampal neurons were transfected 7 d after plating and measured 10–14 d after plating. TG cells were measured 10–14 d after plating. TG cells were measured 12–48 h after plating.

**Dorsal root ganglia (DRG) preparation.** Mice, C57/BL6 wild type or Trpv1−/− aged 1–6 months of either sex, were deeply anesthetized with isoflurane and quickly beheaded. The spinal column and surrounding muscles were removed and dissected in ice-cold oxygenated low calcium, low magnesium ACSF (101 mM NaCl, 3.8 mM KCl, 18.7 mM MgCl₂, 1.3 mM MgSO₄, 1.2 mM KH₂PO₄, 10 mM HEPES, 1 mM CaCl₂ and 1 mM glucose). After laminectomy, the spinal roots were cut, the spinal cord was gently removed, and its lumbar part was placed into a small agarose block. We prepared 300-µm-thick slices using a Leica VTS 1000 vibratome. The slices were then transferred in warm (31 °C) ACSF equilibrated with 95% O₂ and 5% CO₂ for at least 1 h before starting patch-clamp recordings.

**Spinal cord slice preparation.** C57/BL6 mice were deeply anesthetized with isoflurane and quickly beheaded. The spinal column and surrounding muscles were removed and dissected in ice-cold oxygenated low calcium, low magnesium ACSF (101 mM NaCl, 3.8 mM KCl, 18.7 mM MgCl₂, 1.3 mM MgSO₄, 1.2 mM KH₂PO₄, 10 mM HEPES, 1 mM CaCl₂ and 1 mM glucose). After laminectomy, the spinal roots were cut, the spinal cord was gently removed, and its lumbar part was placed into a small agarose block. We prepared 300-µm-thick slices using a Leica VTS 1000 vibratome. The slices were then transferred in warm (31 °C) ACSF equilibrated with 95% O₂ and 5% CO₂ for at least 1 h before starting patch-clamp recordings.

**Whole-cell electrophysiology.** Patch clamp recordings of mammalian cells were performed at room temperature. Bath solution for K⁺ current contained 138 mM NaCl, 1.5 mM KCl, 1.2 mM MgCl₂, 2.5 mM CaCl₂, 1 µM tetrodotoxin (for hippocampal cells only), 5 mM HEPES and 10 mM glucose. Bath solution for Na⁺ current contained NaCl 145 mM, CdCl₂ 0.5 mM, CaCl₂ 2 mM, HEPES 5 mM and glucose 5 mM. Bath solution for Ca²⁺ current contained NaCl 138 mM, KCl 5.4 mM, MgCl₂ 0.8 mM, BaCl₂ 20 mM, tetrodotoxin 1 µM (for GH3 cells only), HEPES 10 mM and glucose 5 mM. Bath solution for K⁺ current contained NaCl 138 mM, KCl 5.4 mM, MgCl₂ 1.2 mM, CaCl₂ 2.5 mM, HEPES 5 mM and glucose 10 mM. Pipette solution for K⁺ current contained NaCl 138 mM, K⁺ gluconate 135 mM, HEPES 10 mM, MgCl₂ 2 mM, MgATP 2 mM, EGTA 1 mM. Pipette solution for Na⁺ current contained NaCl 30 mM, CsCl 100 mM, HEPES 10 mM, MgCl₂ 2 mM, CaCl₂ 1 mM, MgATP 2 mM, NaGTP 0.05 mM, EGTA 10 mM and glucose 5 mM. Pipette solution for Ca²⁺ current contained CsCl 120 mM, HEPES 20 mM, CaCl₂ 1 mM, MgATP 2 mM, NaGTP 0.35 mM and EGTA 0.35 mM. All solutions were adjusted to pH 7.4. Electrophysiological measurements were performed with an Axopatch 200A (Molecular Devices) or a Patch-Clamp PC505B (Warner) amplifier. Patch pipettes resistances were 2–4 MΩ. Sodium channel currents in NG108-15 cells and calcium channel currents in GH3 cells were corrected by P/N leak subtraction. pClampex 8.2 software (Molecular Devices) in combination with a Digidata 1200 interface (Molecular Devices) were used to create and apply pulse protocols. Voltage clamp recordings were low-pass–filtered at 2 kHz and current clamp measurements were low-pass filtered at 5 kHz. Illumination of cells was based on a xenon lamp either in combination with narrow band-pass filters or with a monochromator Polychrome V, as described previously. For direct internal application through the patch pipette, QAQ was dissolved to a final concentration of 100 µM. Measurements were started after 5–10 min of equilibration time for HEK-293, NG108-15, GH3 cells and TG neurons, and after 15–20 min for hippocampal neurons. For bath incubation, cells were incubated with QAQ (classically 1 mM) in the presence or absence of agonist (1–2.5 mM ATP or 1 µM capsaicin) at 37 °C in the dark. Loading solution is similar to K⁺ current recording solution but with no calcium.
Treated coverslips were rinsed with regular calcium-containing recording solution before measurement.

For spinal slice electrophysiology, slices were placed in a recoding chamber bathed with warmed (31 °C) ACSF (NaCl 130.5 mM; KCl 2.4 mM, CaCl2 2.4 mM, NaHCO3 19.5 mM, MgSO4 1.3 mM, KH2PO4 1.2 mM, HEPES 1.25 mM and glucose 10 mM, pH 7.4) equilibrated with 95% O2 and 5% CO2. Electrophysiological measurements were performed under the control of an Olympus BX51 microscope using an Axoclamp 2B (Molecular devices). Patch pipettes (7–11 MΩ) were filled with appropriate pipette solution (potassium gluconate 120 mM, KCl 20 mM, CaCl2 0.1 mM, MgCl2 1.3 mM, EGTA 1 mM, HEPES 10 mM, GTP 0.1 mM, cAMP 0.2 mM, leupeptin 0.1 mM, Na2ATP 3 mM and d-mannitol 77 mM, pH 7.3). Illumination of preparations was performed using two different wavelength diodes (380 nm and 500 nm) controlled by transistor-transistor logic (TTL) pulses. A glass suction electrode connected to Master-8 (A.M.P.I.) stimulator was used to stimulate dorsal roots. Non-nociceptive primary afferent fibers were specifically recruited using low-threshold stimulations (50 μs, less than 100 μA), whereas nociceptive fibers were recruited using high-intensity stimulations (500 μs, more than 250 μA).

Multielectrode array recordings. A DRG was placed onto a three-dimensional multielectrode array (MEA) chip (MEA60 200 3D GND, Ayanda Biosystems) and secured in place with a ‘harp’ made from dialysis membrane stretched over thick platinum wire and bonded with super glue; the wire was U-shaped to allow the nerve to exit without being crushed. The MEA chip was mounted on an MEA1060-Up amplifier (Multi Channel Systems) and placed on the stage of an IX71 inverted microscope (Olympus). The nerve was led into a manipulator-mounted glass suction electrode of appropriate size driven by a DS2 stimulus isolator (Digitimer) triggered by pClamp v10.0 software through a Digidata 1440A data acquisition system (Molecular Devices).

Except during drug incubations, the MEA chamber was continuously perfused with oxygenated ACSF at ~2 ml/min. Recordings were done at a stimulation rate of 10 Hz while illuminating the DRG with 380-nm or 500-nm light. Each experiment consisted of 5 cycles of 30 s under 380-nm light followed by 30 s under 500-nm light. The DRG was stimulated with 1-ms pulses at 10 Hz for the last 5 s under each wavelength of light, allowing 25 s to recover from adaptation in between stimulation episodes.

Illumination was provided by a U-LH100HGAPO mercury lamp (Olympus) through a 4× objective, resulting in intensities of 17–28 mW/mm². Filters for 380 nm and 500 nm were switched by a Lambda 10-3 system (Sutter Instrument Company) under the control of Metamorph v7.5.3.0 software (Molecular Devices). Evoked responses were recorded at 20 kHz with MC_Rack v4.0 software (Multi Channel Systems). Pictures were taken using Metamorph with a CoolSNAP HQ2 camera (Photometric) connected to the microscope.

Multielectrode array data analysis. Data were recorded in 40-ms-long sweeps synced to stimulation pulses, so that the stimulation produced an artifact at the beginning of the sweeps. Evoked spikes were detected by a negative threshold manually set beyond the noise level. For each detected spike, the first millisecond before the peak and the two milliseconds after were extracted into text files by MC_DataTool software (Multi Channel Systems), for processing with a custom Matlab (MathWorks) program.

Our custom Matlab program calculated the area under each spike to the threshold level. A region of interest (ROI) was also set manually for each recording to exclude the stimulus artifact. The total integrated area of all spikes was calculated for each sweep, and averaged over the five cycles in each wavelength (Fig. 4e,f). This averaged area per sweep was summed over the 5 s of stimulation to quantify the total evoked response in each wavelength of light. For each active channel, the normalized photosensitization was calculated as (area380nm − area500nm) / (area380nm + area500nm).

Channels with excessively small and/or irregular signals were conservatively culled. The per-channel photosensitization values, generated from at least three separate DRGs per drug condition, were pooled by condition and compared for significance using a Mann-Whitney U test (5% significance level).

Cornea-evoked reflex blinks. Sprague-Dawley rats (3–6 weeks old of either sex) were sedated using intraperitoneal injection of xylazine (9 mg/kg) and ketamine (60 mg/kg). We placed rats on a warming pad, and we initiated behavioral testing when rat spontaneous movements ceased but while pinching the rat's paw with a pair of forceps elicited a brisk withdrawal reflex. We used a series of von Frey hairs, nylon fibers of increasing diameter, which we pressed against the cornea to impart increasing force with high accuracy. We held von Frey hairs perpendicular to the cornea for ~2 s, or until a blink initiated, using progressive increase in force from 8 mg to a maximal value of 1 g. Stimuli were presented three times for each stiffness, at intervals of several seconds. Both eyes were tested, and a positive response was noted if the rat blinked two or three times for a given force. Capsaicin (10 μM) was then topically applied on one cornea using a pipette (10 μl volume), and the contralateral cornea was treated with a mixture of capsaicin (10 μM) and QAQ (20 mM). Von Frey testing was done again 10–15 min after drug application. Immediately after von Frey testing, light was applied using an LED (Prizmatix, λ max = 385 nm, 30 mW/cm²) for 1 min and von Frey testing was done again.

Statistical analysis. Unless otherwise noted, all data are presented as ± s.e.m. and statistics were analyzed using a Student t-test.

30. Mckemy, D.D., Neuhausser, W.M. & Julius, D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature 416, 52–58 (2002).