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Locked-AzoBenzene: testing the scope of a unique photoswitchable scaffold for cell physiology.

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Abstract

Azobenzenes are the most widely studied photoswitches, and have become popular optical probes for biological systems. The *cis* configuration is normally metastable, meaning the *trans* configuration is always thermodynamically favored. The unique exception to this rule is an azobenzene having a two-carbon bridge between *ortho* positions, substitutions that lock the photoswitch in its *cis* configuration. Only thoroughly chemically characterized relatively recently, we describe the first applications of this locked-azobenzene (or “LAB”) scaffold with two derivatives designed to control ion flow in pyramidal neurons in acutely isolated brain slices. Our LAB derivatives maintain most of the desirable photochemical properties of the parent scaffold, and work as designed in living cells. However, LAB derivitization changes the *trans* photostationary state from >85% of the parent photoswitch to about 50%, suggesting that careful design considerations must be given for future applications of the LAB scaffold in biological areas.

Keywords

photoswitch; azobenzene; glutamate switch; potassium channels; visible light; reversible

Introduction.

Photochemical molecular switches are chromophores that exist in two stable configurations which bestow upon each distinct chemical properties(1–4). In a biological context such molecules were originated by Erlanger and co-workers in 1968(5). They controlled the kinetics of enzyme inactivation by photochemically sculpting of the bulk of an acylating reagent. They found that an azobenzene (AB) derivative of a chymotrypsin-specific drug was five times more reactive in its *cis* configuration than its thermodynamically favored

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Author contributions.

G.C.R.E-D. conceived of the project. ERT synthesized the probes. JZ performed the physiological experiments. ERT and G.C.R.E-D. wrote the paper. All authors edited and approved the final MS.

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Supporting Information

Supporting figures and experimental procedures, and general experimental details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Conflicts of interest.

None.

trans configuration. Ultraviolet light (320 nm) was used to cause *trans* to *cis* isomerization, and violet light (420 nm) photoreversion to the *trans* configuration. Erlanger and co-workers presented their ground breaking a model of naturally occurring “light-sensitive metabolic systems present in living organisms”. Processes such as visual transduction and photosynthesis are now well characterized at the atomic and physiological levels, so perhaps the lasting impact of this seminal contribution is most felt in the new field of what is called “photopharmacology”(6). While chemists have invented many different photochromic systems(2), the AB chromophore used by Erlanger in 1968 remains most popular and useful in this field(1).

A common feature of all AB photoswitches is that the *trans* configuration is the thermodynamically favored form(7). Recently, an exception (**1**, Figure 1) to this rule was well-characterized chemically by the groups of Herges, Renth and Temp(8). Known since 1910(8), the photoswitch scaffold **1**, which we called locked-AB (or “LAB”), exists in the dark in its *cis* configuration *completely*. The two-carbon bridge between the *ortho* positions *locks* the chromophore in what is normally the thermodynamically highly unfavored form. While a few applications of LAB derivatives have appeared(9–11), there has been no report of the use of this unique AB in living cells. Importantly, LAB can be photoswitched in a chromatically independent way with UV and blue/green light(8, 10) to generate spectrally distinct chromophore that compare favorably with chromophores such as tetrafluoro(4F)AB(12) (Figure 1). Thus, we felt that these properties merited exploration of LAB-X photoprobes, where X = glutamate or quaternary ammonium (QA), using well-defined photopharmacological properties from previous studies of other ABs(13–15). We found that conditions could be found when both *cis* LAB-Glu (**2**) and LAB-QA (**3**) did not target their ion channel receptors. Such derivatives disturbed the excellent *trans* photostationary state (PSS) of the parent LAB chromophore (ca. 50% compared to 85%(8, 16)), whilst preserving the 100% *cis* PSS, suggesting that simple photowswitchable drugs might not be able to exploit LAB’s unique properties fully. However, the ring substitution of LAB required to attach the pharmacophore produced the benefit of a bathochromic shift the absorption maximum of *cis*-LAB-X to the violet region, making such probes more compatible with use of visible light in both optical channels.

Results.

Synthesis and photochemical properties of LAB-X probes.

We synthesized LAB-Glu **2** and LAB-QA **3** based on LAB chromophore as outlined in Scheme 1. The stereoselective synthesis of **2** followed(15), and proceeded through palladium catalyzed Stille coupling of known(16) LAB-Br **4** with vinyl stannane **5** to give BOC protected LAB pyroglutamate **6** with 21% yield (Scheme 1). Hydrolysis with KOH and deprotection of BOC group by TFA yielded **2** as a dihydrate, trifluoroacetate salt in an overall 16% yield. LAB-QA was synthesized following(13) by coupling of LAB-amine **7** with known precursor 2-triethyl ammonium acetic acid chloride.

First, we evaluated the photoswitching properties of our LAB derivative **2** and **3**. Both compounds were isolated in 100% *cis* configuration (Supporting Information Figure S1,2). The absorption spectra of these compounds are slightly red-shifted compared to the parent

LAB chromophore(8), with λ_{max} of 402 nm and 400 nm for LAB-Glu (Figure 2a) and LAB-QA (Figure 2b), respectively, for the $n\pi$ transitions. Illumination with a violet light (405-nm-LED) generated the *trans* configurations of **2** and **3** with the expected large bathochromic shift (ca. 70 nm) with λ_{max} at 475nm (green dash for LAB-Glu Figure 2A and LAB-QA Figure 2b). HPLC analysis revealed these PSSs corresponded to 47% and 52% LAB-Glu and LAB-QA, respectively (Supporting Figure S1 and S2). Thus, simple substituents of the LAB chromophore lowered the reported *trans*-PSS (85–92%, (8, 16)). Never the less, the expected quantitative *cis*-PSS was achieved using green light (530-nm-LED) for both **2** and **3** (Supporting Figure S1 and S2). Both probes exhibited slow thermal relaxation of the *trans* configurations to the thermodynamically favored *cis* configurations in the dark, with a half-life times of 17.3 and 0.77 h for *trans*-LAB-Glu and LAB-QA, respectively (Supporting Information Figure S3). Finally, we tested the resistance to photofatigue of our new probes. We were unable to detect photodegradation using UV-vis absorption spectra after multiple switching with violet and green light (Figure 3). Having established that our LAB-X probes preserved many of the desirable properties of the parent LAB scaffold, we tested these photoswitches using living neurons in brains slices acutely isolated from mice.

LAB-Glu – photoswitching of an extracellular ion channel agonist.

First, we tested pharmacology of both configurations of LAB-Glu at their PSSs to check if they could discriminate the binding affinity towards the glutamate receptor in neurons which are endogenously expressing ionotropic glutamate receptors (iGluRs) (Figure 4). Various concentrations of either *cis*-LAB-Glu (ranging from 50 to 250 μM) or *trans*-LAB-Glu (2.35 – 235 μM) were puffed into single hippocampal CA1 pyramidal neurons, and excitatory postsynaptic currents (EPSCs) were recorded in whole-cell voltage clamp mode. We were unable to detect any significant currents by *cis*-LAB-Glu up to a concentration of 0.25 μM (Figure 4a). Thus, *cis*-LAB-Glu functions just like caged glutamate at this concentration, being completely inert towards non-NMDA and NMDA receptors. These data provided a test bed for the assessment of *trans*-LAB-Glu using the violet PSS mixture of *cis* and *trans* configurations. In contrast, significant EPSCs were recorded with *trans*-LAB-Glu (EC_{50} value of $\sim 0.075 \mu\text{M}$, Supporting Information Figure S4a). The amplitude of the trans-mediated EPSCs increased with puffing duration (Figure 4b).

We examined the currents in presence of various antagonists to test the subtype specificity of iGluRs towards LAB-Glu (Figure 4c). First, bath application of CNQX (10 μM), a non-NMDA antagonist (middle panel in Figure 4c), had no effect on EPSCs compared to the control (normal artificial cerebrospinal fluid (ACSF), left panel in Figure 4c). However, in presence of DL-AP5 (40 μM), a selective NMDA antagonist, blocked the evoked EPSC (right panel in Figure 4c). These results demonstrate that *trans*-LAB-Glu activates NMDA receptors selectively. We confirmed the NMDA receptor specificity by monitoring current-voltage relationship with or without Mg^{2+} after puffing *trans*-LAB-Glu (0.047 mM, Supporting Information Figure S4c). We found the J-shaped I-V curve in the presence of external Mg^{2+} and linear I-V curve in the absence of Mg^{2+} , consistent with the NMDA receptors being partially blocked by extracellular Mg^{2+} at resting membrane potentials.

These data are similar to those reported by DiGregorio and Trauner for a related AB-based probe(17) but slightly different from the original reversibly caged glutamate(15).

Having established the basic, stationary-state pharmacology of LAB-Glu, we evaluated the dynamic, bidirectional photochemical modulation of NMDA receptor mediated ion fluxes in CA1 pyramidal neurons (Figure 5). We used local perfusion of *cis*-LAB-Glu (0.1 mM, or ACSF as control) onto CA1 neurons. Irradiation with violet light (405-nm LED) induced significant EPSCs (35 ± 0.9 pA, $n = 4$ cells) from NMDA receptors (Figure 5b bottom) in comparison to control (Figure 5b top, $n = 3$ cells). These currents were quickly silenced by illumination with green light (530-nm LED, Figure 5b bottom). Furthermore, in whole-cell current-clamp mode, continuous local perfusion of *cis*-LAB-Glu (0.1 mM) did not trigger action potentials (Figure 5c), showing again the *cis*-configuration is biologically inert (Note that during this experiment, no current was injected to the neurons to prevent action potential firing). Irradiation with violet light (405-nm LED) triggered action potentials (Figure 5c), which were terminated with green light. In both voltage clamp and current clamp, such bidirectional manipulations of currents/potentials could be performed in succession, numerous times (Figure. 5). Thus, LAB-Glu could be useful to control the neural activity bidirectionally using two colors of visible light with chromatic independence.

LAB-QA - photoswitching of an intracellular ion channel pore blocker.

Having established the photochemical efficacy of LAB-Glu on neurons, we explored the utility of the LAB photoswitch scaffold to modulate ion channels intracellularly using LAB-QA (3). We used the well established paradigm that (photoswitchable) monomeric QA probes block open potassium channels from the inside in a use dependent way(13). We expected the more bulky *cis* configuration to have less access to the inner vestibule of the channel compare to the elongated *trans* form, the latter would therefore block potassium channel currents more effectively (Figure 6a). Thus, we filled single hippocampal CA1 pyramidal neurons in an acutely isolated brain slice with LAB-QA (0.5 mM) via the patch pipette. Voltage dependent currents evoked by step depolarization from -60 to $+40$ mV were monitored (Figure 6b). Irradiation with violet and green light resulted in reversible modulation of potassium current amplitudes (Figure 6b). The result indicated that, like 4FAB-QA(18), *trans*-LAB-QA blocked more effectively (1.3 ± 0.1 nA) (Figure 6b) the open channel ion than the *cis* counterpart (1.6 ± 0.1 nA). All cells were responded to light irradiation. The blocking efficacy was 18.8 ± 0.7 %, calculated as (currents in *cis*-isoform – currents in *trans*-isoform)/current in *cis*-isoform).

Finally, we tested the bidirectional photoswitching of LAB-QA, in CA1 pyramidal neurons during multiple rounds of illumination. LAB-QA (0.5 mM) was loaded into neurons by patch clamp recording pipette, and the currents were recorded with stepwise depolarizations of cells from -60 to $+40$ mV at frequency of 1Hz, illuminating with violet and green light sequentially at an interval of 26 s (Figure 6c). The result clearly demonstrated the reversible modulation of steady state potassium currents using multiple rounds of illumination, showing LAB-QA could be an excellent photoswitch to study Kv channels in neurons with low intensity visible light and high photostability.

Discussion.

Azobenzene (AB) and AB derivatives are the most widely studied photochromes(19). The effects of *ortho* and/or *para* substituents on the PSS and the rate of relaxation of the metastable *cis* configuration are profound(20, 21). In the current work we were very attracted by the basic photochemical properties of the LAB chromophore(8). Namely, that the PSS for *cis* and *trans* at 100% and 85–92%(8, 16), respectively, and that there is excellent chromatic separation of these two configurations (Figure 1). Absorption spectra of *cis* and *trans* configurations of normal AB have complete overlap in the visible range of 400–500 nm, the region used to drive *cis* to *trans* isomerization(13, 21, 22). In contrast, the spectral separation of the $n\pi^*$ absorption bands of LAB is strikingly large (Figure 1). These seem quite similar, or perhaps even better than the 4FAB chromophore (Figure 1). Furthermore, unlike all other AB photochromes, LAB is uniquely more stable in its *cis* configuration(8). In this report we have explored the effects of ring substitution of the chromophoric properties of LAB, and tested if such probes can exploit the distinctive thermodynamic properties of this AB scaffold. We chose to make two probes with fundamentally different pharmacological targets in living neurons. LAB-Glu (2) was designed to modulate extracellular glutamate receptors(15), whereas LAB-QA (3) has intracellular binding site on voltage-gated potassium channels(13).

We found that when *cis*-LAB-Glu was applied to neurons at quite high concentrations (0.25 mM) no currents were detected, implying that the thermodynamically favored *cis* configuration was biologically inert. This pharmacology reproduces what one normally sees with the vast majority of AB-based bioprobes(1–3). Thus, the dark adapted LAB-Glu which naturally resides 100% is the *cis* form appears “like caged glutamate” for ionotropic glutamate receptors (Figure 3). However, the ring substitution used to create LAB-Glu from LAB subtly changes the absorption spectra, and the *trans* PSS. The former shows a favorable red shift with the absorption maxima of *cis*-LAB-Glu to 402 nm, a value that matches well with the standard violet lasers widely available on confocal microscopes (405 nm). However, the PSS is significantly reduced from the LAB scaffold value of 85–92%(8, 16) to 47%. In spite of this, we were able to use photolysis of *cis*-LAB-Glu to fire action potentials reversibly in CA1 neurons in brains with violet and green light (Figure 4).

Our results with LAB-QA were broadly similar to LAB-Glu. We found the violet PSS of LAB-QA was only 52%. This should be contrasted with those reported by Herges(23) and Woolley(10) for their diamido-LAB derivatives. In the former case, substituents were *para* to the methylene bridge, and gave 54% *trans* PSS, whereas in the latter *para* to the diazo allowed 70% *trans* PSS to be achieved. All of these values are lower than our recently developed 4FAB-QA, which has a violet PSS of 86%(18), a value similar to its parent diamido-4FAB scaffold(24). We suggest the decrease in *trans*-LAB might have important practical consequences for the use of the LAB scaffold under certain circumstances. Typically mono-QA photoprobes are bath applied to cells at low concentrations 25–100 μ M for several mins to allow permeation across the plasma membrane(13). The bath solution is replaced with normal extracellular media before patch clamping with a normal intracellular solution. In the case of LAB-QA we found this strategy did not work (data not shown), and that relatively high concentrations (0.5 mM) were required in the patch pipette for effective

photoswitching (Figure 5). Our data taken together suggest that whilst LAB-Glu and LAB-QA preserve many of the highly attractive photochemical properties of the parent chromophore, the slightly smaller change in molecular geometry for *cis* to *trans* LAB scaffold(8) compare to non-bridged AB molecules(7) probably reduces pharmacological efficacy of the active *trans* configuration of LAB-X tested here.

Finally, it is interesting to note that the thermal stabilities of our *trans*-LAB-X probes are changed compared to the parent *trans*-LAB scaffold(8). The latter is reported to have half-life of about 4.5 h in hexane, whereas LAB-Glu and LAB-QA were 17.3 and 0.77 h in aqueous buffer at RT.

So if one is to select a scaffold beyond “normal AB”(22, 25) for biological application, which derivative is to be preferred? Obviously a comprehensive summary is beyond the scope of this article. But we propose the following brief set of considerations for such planning. Our recent results suggest that if long-term thermal stability of the metastable *trans* configuration is a priority then 4FAB(12) is the scaffold of choice(18). If greater thermal stability of regular AB is desired, then dimethyl scaffolds seem highly promising(26). If one desires the use of longer wavelengths of visible light for photoswitching, then tetramethoxy-ABs work well(27, 28). Finally, some amino-ABs are very thermally unstable in the *cis* configuration, conferring useful photo/thermal control of biological processes(14, 29, 30). Additionally, synthetic flexibility and compatibility with other optical probes that might be used in biological applications are further considerations that go beyond the chemical properties of the azobenzene chromophore itself. In terms of tethered photoswitches(22), we envisage LAB being quite useful when it is required that the *trans* configuration pushes an attached pharmacophore into the binding cleft, since upon thermal or photochemical reversion the 100% *cis* form would then completely turn off the activated receptor.

Conclusions.

This is the first study of the *ortho*-bridged azobenzene (AB) photoswitchable scaffold (which we call locked-AB, or “LAB”) for controlling signaling in living cells. We found that, like the parent LAB chromophore(8), mono-substituted LAB-X probes preserve the 100% thermodynamic stability of *cis* configuration of the core LAB photoswitch. Importantly, ring substitution caused a subtle but useful bathochromic shift in the *cis*-LAB absorption maxima, allowing two colors of visible light (violet and green) to be used for multiple rounds of photoswitching in cuvette and in living cells. Hand in hand with this improvement of LAB with LAB-X, we found significant reduction in the *trans* photostationary state, implying that careful cost benefit analysis should be applied for uses of the unique LAB photoswitch scaffold in future applications.

METHODS

Synthesis and spectroscopic analysis

Details of synthesis are described in the Supporting Information. UV-Vis spectra were recorded using a Cary 50 spectrophotometer (Agilent, Santa Clara, CA, USA).

Photostationary state was determined by continuous illumination with either violet or green LED (M405L3, M530L3, Thorlabs, NJ, USA) until no further change in the absorption spectra, and further *cis-trans* composition was determined by HPLC using the relative integrated areas of the *cis* and *trans* peaks at the isosbestic wavelength, considering the sum of integrated area for two peaks is 100%.

Animal and brain slices preparation

All animal studies were approved by Mount Sinai IACUC review. C57BL/6J mice (8–12 weeks.) were anaesthetized with isoflurane and the brain was quickly removed. Horizontal slice sections (350 μ m) were then made in ice-cold cutting solution containing (in mM): 60 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 7 MgCl₂, 0.5 CaCl₂, 26 NaHCO₃, 10 glucose, 100 sucrose, 3 sodium pyruvate, 1.3 sodium ascorbate equilibrated with 95% O₂ and 5% CO₂ (pH 7.3–7.4). The brain slices were then incubated for 15 min at 33°C in artificial cerebrospinal fluid (ACSF, mM: 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 1 MgCl₂, 2 CaCl₂, 26 NaHCO₃, 10 glucose, 3 sodium pyruvate, 1.3 sodium ascorbate; 95% O₂ and 5% CO₂, pH 7.3–7.4). After incubation, all brain slices were held in ACSF at room temperature > 1 hour before patch clamp recording.

Patch clamp recording and photostimulation

Brain slices were transferred to the submerged chamber of a BX-61 microscope (Olympus, Penn Valley, PA, USA) and perfused with ACSF (in mM: 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 1 MgCl₂, 2 CaCl₂, 26 NaHCO₃, 10 glucose, 95% O₂ and 5% CO₂, pH 7.3–7.4) at room temperature. Hippocampal CA1 pyramidal neurons were visualized with a 60X objective (Olympus) and infrared differential interference contrast optics. Whole-cell recordings were made with an EPC-10 amplifier (HEKA Instruments, Bellmore, NY, USA) in voltage-clamp mode (V_m holding at –60 mV) or current-clamp mode. The electrical signals were recorded at 20 kHz and filtered at 3 kHz with Pulse (HEKA). Recording pipettes (3–5 M Ω) were filled with an internal solution (in mM: 135 potassium gluconate, 4 MgCl₂, 10 HEPES, 4 Na₂-ATP, 0.4 Na₂-GTP, 10 Na₂-phosphocreatine, pH 7.35). Voltage-gated sodium channel blocker TTX (1 μ M) was present in ACSF during voltage clamp recordings in LAB-Glu experiments. Glutamate receptor antagonists CNQX (10 μ M) and DL-AP5 (100 μ M) and the GABA_A receptor antagonist bicuculline (20 μ M) were added to ACSF during recording voltage-dependent currents in LAB-QA experiments.

A violet and green LED (405 and 530 nm, respectively; Thorlabs, NJ, USA) were mounted on the epifluorescence port of the microscope and light beams were combined and directed to the objective by standard long-pass dichroic mirrors. Light power was controlled via the LED driver (LEDD1B, Thorlabs) by external voltage modulation. Timing and duration of LED lights were controlled by TTL signals and synchronized with EPC-10 system.

Data analysis and statistics

The amplitudes of LAB-Glu evoked currents were calculated as the difference of the peak and baseline. The amplitudes of Kv currents were calculated as the difference of the steady state and baseline. In the figure of long term multiple photoswitches of LAB-QA, each point

represents as the mean amplitudes of 12 Kv current steps. All data were expressed as the mean $\pm$ SEM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References.

1. Beharry AA, and Woolley GA (2011) Azobenzene photoswitches for biomolecules, *Chem Soc Rev* 40, 4422–4437. [PubMed: 21483974]
2. Szymanski W, Beierle JM, Kistemaker HA, Velema WA, and Feringa BL (2013) Reversible photocontrol of biological systems by the incorporation of molecular photoswitches, *Chem Rev* 113, 6114–6178. [PubMed: 23614556]
3. Fehrentz T, Schönberger M, and Trauner D (2011) Optochemical genetics, *Angew Chem Int Ed* 50, 12156–12182.
4. Gostl R, Senf A, and Hecht S (2014) Remote-controlling chemical reactions by light: Towards chemistry with high spatio-temporal resolution, *Chemical Society Reviews* 43, 1982–1996. [PubMed: 24413363]
5. Kaufman H, Vratsanos SM, and Erlanger BF (1968) Photoregulation of an enzymic process by means of a light-sensitive ligand, *Science* 162, 1487–1489. [PubMed: 5700068]
6. Velema WA, Szymanski W, and Feringa BL (2012) Photopharmacology: beyond proof of principle, *J Am Chem Soc* 136, 2178–2191.
7. Bandara HM, and Burdette SC (2012) Photoisomerization in different classes of azobenzene, *Chem Soc Rev* 41, 1809–1825. [PubMed: 22008710]
8. Siewertsen R, Neumann H, Buchheim-Stehn B, Herges R, Nather C, Renth F, and Temps F (2009) Highly efficient reversible Z-E photoisomerization of a bridged azobenzene with visible light through resolved S(1)(n pi*) absorption bands, *J Am Chem Soc* 131, 15594–15595. [PubMed: 19827776]
9. Eljabu F, Dhruval J, and Yan H (2015) Incorporation of cyclic azobenzene into oligodeoxynucleotides for the photo-regulation of DNA hybridization, *Bioorg Med Chem Lett* 25, 5594–5596. [PubMed: 26592170]
10. Samanta S, Qin C, Lough AJ, and Woolley GA (2012) Bidirectional photocontrol of peptide conformation with a bridged azobenzene derivative, *Angew Chem* 51, 6452–6455. [PubMed: 22644657]
11. Li S, Han G, and Zhang W (2018) Concise Synthesis of Photoresponsive Polyureas Containing Bridged Azobenzenes as Visible-Light-Driven Actuators and Reversible Photopatterning, *Macromolecules* 51, 4290–4297.
12. Bleger D, Schwarz J, Brouwer AM, and Hecht S (2012) o-Fluoroazobenzenes as readily synthesized photoswitches offering nearly quantitative two-way isomerization with visible light, *J Am Chem Soc* 134, 20597–20600. [PubMed: 23236950]
13. Banghart MR, Mouro A, Fortin DL, Yao JZ, Kramer RH, and Trauner D (2009) Photochromic blockers of voltage-gated potassium channels, *Angew Chem* 48, 9097–9101. [PubMed: 19882609]
14. Mouro A, Kienzler MA, Banghart MR, Fehrentz T, Huber FM, Stein M, Kramer RH, and Trauner D (2011) Tuning photochromic ion channel blockers, *ACS Chem Neurosci* 2, 536–543. [PubMed: 22860175]
15. Volgraf M, Gorostiza P, Szobota S, Helix MR, Isacoff EY, and Trauner D (2007) Reversibly caged glutamate: a photochromic agonist of ionotropic glutamate receptors, *J Am Chem Soc* 129, 260–261. [PubMed: 17212390]

16. Jun M, Joshi DK, Yalagala RS, Vanloon J, Simionescu R, Lough AJ, Gordon HL, and Yan H (2018) Confirmation of the Structure of Trans-Cyclic Azobenzene by X-Ray Crystallography and Spectroscopic Characterization of Cyclic Azobenzene Analogs, *Chemistryselect* 3, 2697–2701.
17. Laprell L, Repak E, Franckevicius V, Hartrampf F, Terhag J, Hollmann M, Sumser M, Rebola N, DiGregorio DA, and Trauner D (2015) Optical control of NMDA receptors with a diffusible photoswitch, *Nat Commun* 6, 8076. [PubMed: 26311290]
18. Passlick S, Richers MT, and Ellis-Davies GCR (2018) Thermodynamically Stable, Photoreversible Pharmacology in Neurons with One- and Two-Photon Excitation, *Angew Chem Int Ed Engl* 57, 12554–12557. [PubMed: 30075062]
19. Baroncini M, Ragazzon G, Silvi S, Venturi M, and Credi A (2015) The eternal youth of azobenzene: new photoactive molecular and supramolecular devices, *Pure Appl. Chem.* 87, 537–545.
20. Pozhidaeva N, Cormier M, Chaudhari A, and Woolley G (2004) Reversible photocontrol of peptide helix content: Adjusting thermal stability of the cis state, *Bioconjugate Chem* 15, 1297–1303.
21. Tochitsky I, Banghart MR, Mourot A, Yao JZ, Gaub B, Kramer RH, and Trauner D (2012) Optochemical control of genetically engineered neuronal nicotinic acetylcholine receptors, *Nat Chem* 4, 105–111. [PubMed: 22270644]
22. Banghart M, Borges K, Isacoff E, Trauner D, and Kramer RH (2004) Light-activated ion channels for remote control of neuronal firing, *Nat Neurosci* 7, 1381–1386. [PubMed: 15558062]
23. Sell H, Nather C, and Herges R (2013) Amino-substituted diazocines as pincer-type photochromic switches, *Beilstein J Org Chem* 9, 1–7. [PubMed: 23399830]
24. Knie C, Utecht M, Zhao F, Kulla H, Kovalenko S, Brouwer AM, Saalfrank P, Hecht S, and Blegler D (2014) ortho-Fluoroazobenzenes: visible light switches with very long-Lived Z isomers, *Chemistry, Eur. J* 20, 16492–16501.
25. Kumita J, Smart O, and Woolley G (2000) Photo-control of helix content in a short peptide, *P Natl Acad Sci Usa* 97, 3803–3808.
26. Lin WC, Tsai MC, Rajappa R, and Kramer RH (2018) Design of a Highly Bistable Photoswitchable Tethered Ligand for Rapid and Sustained Manipulation of Neurotransmission, *J Am Chem Soc* 140, 7445–7448. [PubMed: 29874068]
27. Dong M, Babalhavaeji A, Samanta S, Beharry AA, and Woolley GA (2015) Red-Shifting Azobenzene Photoswitches for in Vivo Use, *Acc Chem Res* 48, 2662–2670. [PubMed: 26415024]
28. Beharry AA, Sadvski O, and Woolley GA (2011) Azobenzene photoswitching without ultraviolet light, *J Am Chem Soc* 133, 19684–19687. [PubMed: 22082305]
29. Kienzler MA, Reiner A, Trautman E, Yoo S, Trauner D, and Isacoff EY (2013) A red-shifted, fast-relaxing azobenzene photoswitch for visible light control of an ionotropic glutamate receptor, *J Am Chem Soc* 135, 17683–17686. [PubMed: 24171511]
30. Izquierdo-Serra M, Gascon-Moya M, Hirtz JJ, Pittolo S, Poskanzer KE, Ferrer E, Alibes R, Busque F, Yuste R, Hernando J, and Gorostiza P (2014) Two-photon neuronal and astrocytic stimulation with azobenzene-based photoswitches, *J Am Chem Soc* 136, 8693–8701. [PubMed: 24857186]

Figure1.

Comparison of LAB and 4FAB absorption spectra. Absorption spectra of *cis*-LAB (cyan) and *trans*-LAB (green) in hexane. The former is the pure *cis* configuration isolated from synthesis, and corresponds to the thermodynamically stable and green light photostationary state (PSS). The latter is abstracted from an HPLC chromatogram. Note that *cis*-LAB is transparent above 500 nm, a region where *trans*-LAB absorbs strongly. For comparison, the absorption spectra of 4FAB, also at its PSSs(18), are plotted, showing a similar but smaller separation of the $n\pi^*$ absorption bands in the visible region.

Figure 2.
Spectra of LAB-X.

Spectra of pure *cis* configurations for LAB-Glu (a) or LAB-QA (b) isolated from synthesis (violet lines) in Hepes (pH 7.4). These spectra are identical to green PSS arising irradiation of *trans*-LAB-X. Absorption spectra of the PSS from 405 nm LED-irradiation are shown (green dashes). Calculated all *trans* spectra are shown for both probes (green lines). Calculated all *trans* spectra were obtained by subtracting spectra at the violet PSS from the pure *cis* spectra, then normalising these data from the PSSs determined by HPLC.

Figure 3.

Photoswitching of LAB-X without photofatigue.

Solutions of LAB-Glu (a) and LAB-QA (b) in Hepes (pH 7.4) could be irradiated with violet and green light through multiple cycles without any detectable photofatigue as monitored by absorption spectroscopy. Green and violet colors represent the *trans* and *cis* configurations, respectively. Photon fluxes: violet light, 107 mWcm^{-2} from a 405-nm LED; green light, 5.5 mWcm^{-2} from a 530-nm LED.

Figure 4.
Pharmacology of *cis* and *trans*-LAB-Glu.

a) Puffing *cis*-LAB-Glu to a hippocampal CA1 pyramidal neuron at different concentrations (0.05, 0.1, 0.25 μ M) for 100 ms. b) Left: Puffing *trans*-LAB-Glu (0.047 mM, i.e. 0.1 mM LAB-Glu at the violet PSS) onto a CA1 pyramidal neuron for different durations (5, 10, 25, 50 ms). Right: Quantification of the relationship between *trans*-LAB-Glu evoked currents and puffing durations. c) The currents evoked by 1 sec puffing of *trans*-LAB-Glu (control) were blocked by DL-AP5 (40 μ M), but not CNQX (10 μ M), implying LAB-Glu activates NMDA receptors. All experiments were repeated in 3 cells.

Figure 5.

Photoswitching of LAB-Glu on CA1 neurons.

a) Cartoon illustrating how LAB-Glu binds to glutamate receptors and activates current flow.

b) Currents evoked by bidirectional photoswitching of LAB-Glu. Control: puffing normal ACSF continuously, LAB-Glu: puffing of 0.1 mM *cis*-LAB-Glu continuously (n = 3 or 4 cells, control and LAB-Glu, respectively).

c) Bidirectional photoswitch of action potentials by LAB-Glu (n = 3 cells).

Figure 6.

Photoswitching of LAB-QA inside CA1 neurons.

a) Cartoon illustrating how LAB-QA binds to the inner vestibule of potassium channels in the *trans* configuration. b) Currents from a CA1 pyramidal neuron responding to voltage steps (−60 to +40 mV) with LAB-QA (0.5 mM) in patch recording pipette with violet and green light (left). Bidirectional modulation of voltage-gated ion channels with LAB-QA. The electrophysiological protocol consisted of depolarizing voltage steps from −60 mV to +40 mV for 200 ms at a frequency of 1 Hz. This depolarization activated voltage-gated potassium channels were reversibly blocked by violet and green light (right). c) Multiple rounds photoswitching of LAB-QA inside a CA1 neuron. This is representative of traces obtained from 3 cells. Voltage steps as in b).

Scheme 1.

Synthesis of LAB-X.

Reagents and conditions: a) $\text{Pd}(\text{PPh}_3)_4$, CuI , CSF , 21%; b) LiOH , H_2O , THF , 80%; c) 3M HCl , EtOAc , RT, 1h, 16%; d) Benzophenone imine, $\text{Pd}(\text{OAc})_2$, BINAP , CsCO_3 , THF , 30%; e) DIPEA/DMF , 40% (counter ion not shown for clarity).