Optical control of neuronal ion channels and receptors

Pierre Paoletti, Graham Ellis-Davies, Alexandre Mourot

Optical control of neuronal ion channels and receptors

Pierre Paoletti[1], Graham Ellis-Davies[2] and Alexandre Mourot[3]

[1] Institut de Biologie de l'Ecole Normale Supérieure (IBENS)
Ecole Normale Supérieure
CNRS, INSERM, Université PSL
Paris, France

[2] Department of Neuroscience
Mount Sinai School of Medicine
New York, NY, USA

[3] Neuroscience Paris Seine - Institut de Biologie Paris Seine (NPS – IBPS)
CNRS, INSERM, Sorbonne Université
Paris, France

Correspondence to Pierre Paoletti (pierre.paoletti@ens.fr), Graham Ellis-Davies (graham.davies@mssm.edu) or Alexandre Mourot (alexandre.mourot@upmc.fr).

Key words: receptors, ion channels, neuron, synapse, optogenetics, optopharmacology, photopharmacology; caged compounds, photoswitch

Abstract

Light-controllable tools provide powerful means to manipulate and interrogate brain function with low invasiveness and high spatiotemporal precision. Although optogenetics permits neuronal excitation or inhibition at the network level, other technologies such as optopharmacology (also known as photopharmacology) have emerged that provide molecular level control by endowing light-sensitivity to endogenous biomolecules. In this Review, we discuss the challenges and opportunities of photocontrolling native neuronal signaling pathways, focusing on ion channels and neurotransmitter receptors. We describe existing strategies for rendering receptors and channels light-sensitive and provide an overview of the neuroscientific insights gained from such approaches. At the cross roads of chemistry, protein engineering and neuroscience, optopharmacology offers great potential in understanding the molecular basis of brain function and behavior, with promises for future therapeutics.

Introduction

Ion channels and neurotransmitter receptors are the linchpin of brain function. They control neuronal membrane potential and excitability as well as synaptic transmission and neuronal communication[1-4]. Neuronal ion channels and neurotransmitter receptors are involved in many neurological and psychiatric disorders, and are thus major drug targets; an analysis in 2011 showed that more than 40% of all then-commercialized drugs targeted an ion channel or a surface receptor[5]. Thus, there is enormous interest in understanding how these proteins work and are regulated both for better comprehension of brain function and for therapeutic purposes.

Neuronal ion channels and receptors show great diversity, each family comprising numerous members (or subtypes) with distinct distribution and properties. This heterogeneity largely derives from the multigenic and multimeric nature of these protein families, resulting in a wide variety of subunit assemblies. As examples, 77 genes encode potassium channel subunits in humans, whereas 19 subunits encode pentameric GABA type A receptors ($GABA_ARs$) which mediate the bulk of synaptic inhibition[6,7]. The physiological relevance of such plurality is largely unknown: whether individual ion channel or receptor subtypes carry out specific tasks or whether redundancy prevails is unknown. Hopefully this diversity can be exploited for new and better medicines. So far, research in electrophysiology, molecular biology, genetics, biochemistry, pharmacology and structural biology have provided much information on the structure and function of ion channels and receptors. However, none of these approaches combines the proper molecular and spatiotemporal precision that is required to probe specific ion channel and receptor subpopulations in live systems. Consequently there is great need for novel approaches to manipulate rapidly and precisely different brain proteins with high molecular, cellular and network control level.

Light permits superior spatiotemporal resolution and combined to genetic and pharmacology allows precise manipulation of protein targets with defined molecular composition[8,9]. Optogenetics has driven huge advances in causal understanding of the neural circuits underlying behaviour, and confers high temporal precision, on-off control of neuronal activity and cell-type specificity[10]. However, classical optogenetics lacks the ability to probe the role of specific receptors or signaling pathways in the control of physiology and behaviors. Optopharmacology (also known as photopharmacology or optochemical genetics or synthetic optogenetics) aims at filling this gap, by endowing light sensitivity to specific protein targets and thus providing a molecular control and understanding of brain function.

Starting with photoreactive ligands in the late 1970s[11-14], the field of optopharmacology boomed in recent years with the development of new methods directly tagging 'blind' proteins (i.e. insensitive to light) with light-sensitive protein modules or synthetic chromophores[15-18]. Such photoactuators allow the manipulation and interrogation of target proteins, and even individual constitutive subunits, with unprecedented precision. Progress has been particularly applicable and striking in neuroscience, where conventional in vivo pharmacology displays many inherent limitations (slow diffusion, off targets, poor spatial confinement, and slow reversibility). In the present review, we present current strategies to render neuronal receptors and ion channels light responsive, and their applications for biophysical and neurophysiological studies. Optopharmacology covers multiple scales from the molecular, synaptic, cellular up to the circuit level. We also discuss practicalities and challenges for in vivo implementation in behaving animals and translational potentialities in the clinic. Optopharmacological approaches are poised to illuminate our understanding of the molecular and functional diversity of key brain biomolecules with important consequences on neuronal signaling in the normal and diseased brain.

Optopharmacological approaches

Three types of approaches have been developed to design of light-controllable receptors and ion channels (**Fig. 1**): chemical approaches, which rely exclusively on exogenous photosensitive chemicals (no modification of the target protein); genetic approaches, which require genetic modification of the protein of interest; and hybrid approaches, which are two-component systems involving both genetic and chemical modifications. As described below (and see **Table 1**), each of these approaches has its own requirements, and concomitant advantages and limitations. Choosing the right tool depends on the biological question and the amenability to genetic modification of the target. Altogether, they provide a large toolset for precise manipulation of membrane proteins and their associated signaling pathways with broad applicability in molecular and neuronal studies.

Chemical approaches

Photochemical approaches target native (that is unmodified, wild-type) proteins and were the first to emerge in the late 1960s. The general idea is to confer light-sensitivity to a freely diffusible ligand, rather than the target protein. Chemists have developed various photochemicals for the control of neurotransmitter receptors and other signaling molecules,

including caged compounds, photoswitches and photolabels (**Fig. 1a**). Such chemical approaches have one crucial advantage: they bestow light sensitivity to endogenous proteins without requiring gene modification, and therefore act on native tissues and signaling molecules. They may lack precise molecular target specificity, however.

Caged compounds. Caged compounds contain a photo-removable protecting group that is cleaved upon light stimulation, resulting in a rapid release of biologically active molecules[15]. The compound is inert (that is caged) in darkness, whereas uncaging following illumination triggers a concentration jump of the molecule. Diffusible caged ligands are arguably the most widely used photochemicals in neuroscience[15]. Several desirable properties of caged compounds explain such wide use[15,19,20]. They are inherently biologically inactive before photolysis while full biological activity is recovered by irradiation. Their rate of uncaging is usually much faster than most biological processes that are being studied and photolysis uses biocompatible wavelengths not absorbed by proteins or DNA. Finally, the chromophoric byproducts are usually non-toxic. Most neurotransmitters have been caged, including glutamate[21-24], GABA[25-29], dopamine[30] and serotonin[31]. Moreover, most of these have been made two-photon sensitive, allowing fine spatial manipulation down to the dendritic spine level[32]. Caged molecules with refined pharmacological spectra have also been developed, such as caged nicotinic[33-36] and glycine[37] receptor agonists, caged NMDA[38,39] and kainate[40], or caged neuropeptide receptor agonists and antagonists[41-43], enabling the control of specific receptor types or subtypes.

Photoswitchable ligands. Photoswitchable ligands (PLs, also known as photochromic ligands (PCLs)) contain a photo-isomerizing group that, unlike caged compounds, have the major advantage of being able to alternate reversibly between an active and an inactive form with two different wavelengths of light[18,44,45]. This results in a reversible on and off control of protein targets. In some rare cases, PLs can covalently attach to an ideally positioned endogenous reactive amino acid on the target wild-type receptor or ion channel[11,14,46]. The use of two different colors of light to drive the active and inactive states of molecular photoswitches means that activation/deactivation (or inhibition/disinhibition) steps can be precisely time-controlled[47]. PLs have been designed for a huge variety of neurotransmitter receptors and ion channels[8,17,18,48,49] (**Table 1**). Examples include blockers of voltage-gated potassium[45,50,51], sodium[52,53] and calcium[54] channels, activators of GIRK channels[55], modulators of delayed rectifiers[56], ATP-sensitive[57] and two-pore domain[56] K^+ channels, activators of TRPV1 channels[58], agonists and antagonists of ionotropic glutamate receptors (iGluRs)[44,59-62], agonists[11,63] and pore blockers[12] of nicotinic acetylcholine receptors (nAChRs), an agonist of adenosine receptors[64], an antagonist

of $GABA_A$ receptors[65], and allosteric modulators of metabotropic glutamate receptors (mGluRs)[66-70] and of $GABA_A$[71,72] receptors.

The most commonly used synthetic photoswitch is azobenzene (**Fig. 2**). Azobenzenes have many advantageous attributes[73,74]: they are small (length <10 Å) and easy to synthetize, display high quantum yield (0.2-0.5) and minimal photobleaching, and can be rapidly and reversibly switched between a bent, *cis* form and an extended, *trans* configuration using two different colors, classically near-ultraviolet (UV) (360–400 nm) and blue-green light (480–-550 nm). Usually, the *trans* form is the most stable isomer (in darkness or visible light), whereas the *cis* form gradually relaxes back to *trans* configuration in darkness with kinetics ranging from milliseconds to days depending on the chemical substituents[75]. 'Bridged' azobenzenes having a two-carbon link between ortho-positions provide an exception whereby the *cis* configuration is thermodynamically favored[76,77]. In the case of slow relaxation lifetimes (in the hour timescale), excellent bi-stability is reached without the need for long-duration light exposure. Rather brief pulses of light are sufficient to rapidly toggle the azobenzene between its two states. To avoid UV irradiation and thus limit phototoxicity and enhance biocompatibility, red-shifted azobenzenes were recently developed[51,78-84]. Red shift usually comes at the expense of faster spontaneous *cis*-to-*trans* photorelaxation kinetics (ms to s; but see ref. [85]). Thus, whereas ideally one photo-isomer of the azobenzene is fully pharmacologically inactive while the other is active, in practice both isomers are active to some extent (for diffusible photoswitches at least), resulting in ten to hundred-fold maximal differences in activity between the two isomers (250-fold in ref. [86]). In addition, since the absorption spectra of the two isomers overlap to some degree, the photostationary state ratio between the two isomers is classically around 95:5 (refs. [87,88]). Remarkably, many pharmacological agents can be converted into photoswitchable drugs by substituting an azobenzene-related structural motif for a genuine azobenzene moiety (termed 'azologization'; ref. [65,66,74,89]). Other photochromic chemicals besides azobenzenes include the spiropyrans[90] and dithienylethenes[91], but their use as photoswitches in biology remains limited.

Photo-affinity labels. Photo-affinity labels (PALs) contain a photoreactive group that is converted to a reactive, alkylating entity upon photostimulation. PALs reversibly bind to receptors in darkness but bind permanently after photo-activation; hence, they can 'trap' receptors or ligand-activated ion channels in a permanently activated (or inhibited) state[92-94].

Chromophore-assisted laser inactivation. Finally, chromophore-assisted laser inactivation (CALI) relies on a synthetic dye (such as malachite green, fluorescein or eosin) that releases short-lived reactive oxygen species upon photostimulation (mostly singlet oxygen), leading to damage and

irreversible inactivation of nearby proteins through amino acid side chain oxidation and protein crosslinking [95,96]. CALI can be targeted to specific endogenous membrane proteins using antibodies[97].

Genetic approaches

Genetic approaches require the incorporation of a protein-based, light-sensitive module in the target protein, resulting in a light-responsive receptor that is fully encoded genetically, without any exogenous co-factors. Genetic and hybrid approaches have one essential superiority over chemical approaches: they allow photosensitization with molecular and cellular specificity, a crucial feature when it comes to dissecting discrete signaling pathways in complex circuits.

Various natural photoreceptors from animals, plants and microorganisms have been fused to mammalian cytoplasmic signaling proteins and enzymes to render them photocontrollable[98], but only in a few cases have they been fused to membrane receptors or ion channels. Natural photoreceptors include opsins, which contain an endogenous retinal photoswitch (**Fig. 1b**), and the light-oxygen-voltage (LOV)-sensing domains of flavoproteins, and cryptochromes (including crytochrome-2 (CRY2)), which are both from plants. Purely genetic methods are easier to use than hybrid approaches, especially *in vivo*, since light-sensitivity is inherent to the protein itself with its natural co-factor (virtually any mammalian cell produces enough quantities of retinal or flavin photosensors).

Opsins. Opsins belong to the G-protein coupled receptor (GPCR) superfamily and can be engineered as chimeric receptors with intracellular loops of (light-insensitive) neuromodulatory GPCRs to produce light-controllable receptors (opto-XRs)[99]. Although restricted to GPCRs, opto-XR engineering has proved highly versatile enabling light control of signaling via adrenergic[100-102], adenosine[103], opioid[104,105], glutamatergic[106], dopaminergic[107], serotoninergic[108-110] and even orphan[111] receptors (**Table 1**).

LOV domains and CRY2. LOV domains contain a flavin mononucleotide chromophore, whereas CRY2 use a flavin adenine dinucleotide chromophore. Blue light triggers large conformational changes in these plant domains **(Fig. 1b)** — protein unfolding in LOV domains and dimerization with CIB1 for CRY2 — that can be exploited to modulate protein-protein interactions and protein localization with subcellular precision[98]. These plant domains are relatively bulky protein modules (~12-19 kDa) with inherently slow on and off kinetics. Consequently, they have been mostly used to control slow cell parameters such as neurite outgrowth, intracellular signaling cascades, gene expression or protein localization, and less often to manipulate rapid signaling

events such as neurotransmission[112]. Ion channels can be indirectly photosensitized by fusing LOV domains to partner proteins such as K^+ channel toxins[113] or the intracellular Ca^{2+} sensor protein STIM1 that interacts with Ca^{2+} release-activated channels[114]. LOV domains can also be fused to GTPases, thereby resulting in photocontrol of voltage-gated calcium channels[115] or dendritic spine size[116-118]. CRY2 has been successfully used as optogenetic devices to inhibit G protein signaling in specific microdomains[119] and to control neurotransmitter release using an engineered botulinum neurotoxin[120] . In only a few examples have LOV or CRY2 domains been directly fused to the membrane protein itself, as demonstrated with potassium channels[121], tyrosine-kinase receptors[122,123] or AMPA receptors (AMPARs) to control their abundance at excitatory synapses[124].

CALI. Genetically-encoded fusion proteins can also be used in the CALI approach (**Fig. 1b**), as tags to anchor the photosensitizer[125] or protein modules directly acting as singlet oxygen generators[126].

Hybrid approaches

Hybrid approaches exploit the chemical and genetic methodologies to precisely manipulate and control protein function with optochemical tools that are genetically anchored to or incorporated in the desired membrane target.

Unnatural amino acids. One hybrid strategy relies on unnatural amino acids (UAAs), which can be inserted at virtually any position into the target protein, including buried sites, using genetic code expansion[127]. This methodology hijacks the translational machinery through the reassignment of a stop codon (usually amber) driven by a suppressor tRNA that is aminoacylated with the UAA of interest[128,129]. Light-responsive UAAs are diverse in photochemical nature and have proved powerful tools to manipulate membrane receptors and ion channels[130-132] (**Fig. 1c**). Amber stop codon suppression was used in particular to design nAChRs harboring caged tyrosine[133,134] and inwardly rectifying potassium channels incorporating caged cysteine[135]. As with diffusible caged compounds, caged amino-acid side-chains enable time-controlled changes, abrupt and irreversible, in protein structure and function. UAAs carrying photocrosslinking or photoswitchable moieties have also been incorporated into various receptors and ion channels [131,132] (see below). An advantage of photosensitive UAAs (and UAAs in general) is that they can be inserted at any desired location in the target protein, including solvent-inaccessible sites, although this UAA incorporation usually results in decreased protein expression[132].

Photoswitchable tethered ligands. Photoswitchable tethered ligands (PTLs) provide another powerful and popular strategy to confer light responsiveness to receptors and ion channels. The approach relies on the covalent attachment of a photoswitchable ligand either at close proximity to its binding pocket through a single cysteine substitution[136,137], or remotely through bioconjugation to larger protein tags (as in the case of photoswitchable orthogonal remotely tethered ligands (PORTLs))[138-140]. In both cases, different wavelengths of light are used to present or withdraw the pharmacologically active head group to or from its binding pocket (**Fig. 1c**). Bi-anchoring chemical photoswitches (nano-tweezers) are additional optopharmacological tools that can control membrane proteins by evoking light-driven mechanical forces between two cysteine-substituted protein segments[141-143] (**Fig. 1 c**).

PTLs in the form of tethered photoswitchable azobenzene-coupled ligands has proven particularly efficient in photocontrolling neuronal receptors and ion channels[8,17,48,49]. They are tripartite molecules, with a central photoreactive azobenzene chromophore asymmetrically substituted on one ring with a pharmacologically-active head group and on the other ring with a cysteine-reactive moiety, usually a maleimide (for an example see **Fig. 2d**). The conjugation of thiol groups of cysteine residues with maleimides is rapid (minutes time scale), highly selective and irreversible, enabling permanent installation of photoswitchable effectors on the protein target. Engineered light-controllable receptors and channels with PTLs include voltage-gated potassium channels[136,144], two-pore domain potassium channels[145], ionotropic and metabotropic glutamate receptors[137,146,147], nAChRs[14,148], $GABA_AR$s[149,150], purinergic P2X receptors[151] and dopamine receptors[152] (**Table 1**). The kinetics of photocontrol with PTLs are greatly accelerated because they are present in high local concentrations and cannot diffuse away[153]. Another advantage of such tethered ligands, when compared to freely diffusible photoswitches, is that they provide excellent separation in biological activity between the *cis* and *trans* forms. Thus, in the ideal realization, PTLs become truly binary photochemical switches for ion channels and receptors. It also requires minimal modification (single amino acid substitution) of the target protein. Perhaps the most distinctive advantage of PTLs for optical neurophysiology is that they provide control over receptors with subtype specificity. For instance, the entire $GABA_AR$ family (α1-6) was made photocontrollable using the PTL strategy[150]. However, the PTL approach is probably restricted to sites that are accessible to the external solvent for proper protein conjugation, excluding intra-cellular and transmembrane protein domains.

Probing receptors and ion channels

Photo-responsive elements introduced at strategic locations in proteins or as ligands have been used to drive changes in protein structure and function with unsurpassed precision. All core biophysical features of receptors and ion channels —– including ligand binding, activation and gating kinetics, and ionic permeation — can be controlled using optopharmacology. Light actuators can act as all-or-none switches, allowing direct channel opening or receptor activation, or as regulators, modulating the activity of receptors or ion channels activated by their cognate stimuli (**Fig. 2a**). Therefore, light can mimic agonists (full, partial or inverse), antagonists (competitive or non-competitive), channel blockers and openers, or allosteric modulators (opto-NAMs and opto-PAMs). Grading light intensity and/or wavelength confers another level for refined control of the protein target (**Fig. 2b**).

Mapping structural properties. Photoaffinity labeling using photocrosslinking ligands (PALs, **Fig. 1a**) has been extensively used to map ligand binding sites on receptors and channels with atomic details[16,131]. This chemical approach allows direct identification of the amino acid residues that contribute to a ligand binding site without a priori knowledge of the site of interaction. Examples include the agonist-binding sites of nAChRs[16] or the binding sites for general anesthetics within transmembrane cavities of pentameric $GABA_ARs$[154,155]. A photoactivatable cross-linking UAA — classically azido-phenylalanine (AzF) or benzoyl-phenylalanine (BzF) — moiety can also be genetically inserted into a protein. These UAAs crosslink upon UV illumination and have been used to map the ligand-receptor interactions of several neuronal GPCRs, including the neurokinin-1 receptor[156], cortico-releasing factor receptors type 1 (CRFR1)[157] and calcitonin gene-related peptide receptors (CGRPR)[158]. Based on similar covalent attachment to a nearby residue, photocrosslinking UAAs have been employed to trap interacting proteins or to map subunit-subunit interfaces and their dynamics during channel gating or receptor activation[132]. BzF crosslinking revealed a state-dependent interaction between the voltage-gated K^+ channel KCNQ1 and the β-subunit KCNE1[159,160]. BzF and AzF also enabled the capture of distinct functional states of iGluRs, including desensitized states of AMPARs[161], and inhibited[162] or potentiated[163] states of NMDA receptors (NMDARs). Generally, the irreversible nature of photocrosslinking allows the accumulation of trapped proteins in a given conformational state, resulting in strong functional phenotypes.

Controlling channel properties. Azobenzene-based photoswitchable UAAs (PSAAs) provide alternative ways to photocontrol biophysical attributes of target proteins. Introduction of PSAAs within different NMDAR subunits and domains enabled rapid, reversible and allosteric regulation of receptor channel open probability, agonist sensitivity and deactivation kinetics **(Fig. 2c)**, as

well as modulation of ionic conductance and Mg^{2+} pore block[164], without directly interfering with natural gating mechanisms. In that respect, PSAAs differ from most PTLs, which act as surrogate receptor agonists[137] and abolish the need for natural agonist activation. Therefore, in native settings, PSAAs can preserve natural patterns of receptor activation, whereas PTLs can take over immediate control of receptor activation and signaling. As examples, fast photoswitching using PTLs makes it possible to mimic synaptic activation profiles[147], whereas tuning agonist deactivation kinetics using PSAAs[164], could be useful to assess how this gating parameter sculpts the time window for synaptic plasticity and integration.

Pushing the photocontrol even further, photoswitchable nano-tweezers, which covalently attach to two engineered cysteine residues, fully substitute the channel natural gating machinery by triggering forceps-like motions on transmembrane helices upon azobenzene isomerization. This technology permits direct light-dependent opening and closure of ligand-gated ion channels such as P2X receptors[141,142] or acid-sensing ion channels (ASIC)[141]. Opto-tweezers of various sizes also provide useful molecular rulers to probe conformational rearrangements involved in pore dilation and control of ion fluxes[142,143].

Temporal and molecular precision. Generally, optopharmacology offers tools of great precision for the biophysical study of receptors and channels. In the temporal domain, caged agonists permit rapid ligand delivery (<25 µs) useful to probe receptor onset kinetics[22,25,38,165,166]. Similarly, fast light switching using PTLs allows submillisecond control over ligand binding and unbinding[153], surpassing conventional techniques of studying receptors and channels that are based on mechanical fast-perfusion systems and issues related to ligand diffusion. At the molecular level, individual subunit accuracy is achievable. Using PALs as agonist analogs, the contribution of sequential binding events to channel gating in multimeric complexes can be dissected, as performed on tetrameric cyclic-nucleotide-gated channels[92]. Similarly, using PTLs conjugated to specific subunits, the influence of individual subunit occupancy on receptor gating can be resolved, as shown on homomeric and heteromeric iGluRs[153] (**Fig. 2d**) and mGluRs[167] glutamate receptors. Small single crosslinking groups and azobenzene groups seem to be remarkably efficient photo-devices to gain real-time control of large molecular complexes and their functionalities.

Interrogation of neuronal physiology

Optopharmacology opened new windows for the understanding of neuronal physiology by

allowing the control of ion channels and receptors in locations that are poorly accessible to electrophysiological or pharmacological manipulations, such as sub-cellular compartments or pre- vs. post-synaptic sites. Historically, caged neurotransmitters were the first photochemical tools to provide major impact on neurobiology, especially caged glutamate and GABA[19,168]. Neurotransmitter uncaging has two important advantages for neuronal physiology. First, the kinetics of release are very rapid (submillisecond time scale)[22,25,38] and can mimic the time-course of pre-synaptic neurotransmitter release. Second, the measured downstream effect bypasses (and therefore is unambiguously dissociated from) upstream signal production. Hence, laser uncaging enables stereotyped input to be applied at will to any part brain region or part of a neuron in a highly reproducible manner[168,169]. Glutamate uncaging can produce connectivity maps between nearby but also distant neurons[169,170], a task that is nowadays routinely achieved using channelrhodopsin[10].

Receptor and channel trafficking and subcellular compartmentalization. Understanding how ion channels and receptors traffic and compartmentalize in neurons has greatly benefited from optopharmacological techniques. Specific receptors and ion channels can be manipulated or isolated in a compartment-specific fashion using appropriate photoresponsive ligands and illuminations patterns[88]. Two-photon uncaging of glutamate or GABA permits functional mapping of neurotransmitter receptor locations and densities individual-spine resolution[28,171]. In brain slices, the expression of functional AMPARs was found to tightly correlate with spine size and geometry in pyramidal neurons, with mushroom spines containing the largest numbers of receptors[23] (**Fig. 3a**). ANQX, a PAL antagonist that irreversibly silences surface AMPARs, was used to investigate the trafficking of native AMPARs; this approach revealed that AMPAR insertion at synapses occurs primarily through lateral diffusion from extrasynaptic sites[93]. Subcellular mapping of receptors using two-photon uncaging and subunit-specific PTLs also revealed that α5 subunit-containing $GABA_A$Rs are distributed evenly along dendrites of CA1 pyramidal neurons, whereas α1-containing $GABA_A$Rs are concentrated at (inhibitory) synapses[150]. Strikingly, in the same neurons no current was detected following local uncaging of nAChR agonist, whereas large responses were detected on nearby CA1 interneurons, mostly at perisomatic sites[172]. Similar experiments performed in the medial habenula showed that in addition to concentrating close to (or at) the soma[35], nAChRs also populate axons[36], raising questions about the source of acetylcholine and their physiological role. In the axon initial segment (AIS) of gerbil auditory neurons, the contributions of specific ion channel conductances to neuronal firing were dissected using AAQ as a light-sensitive channel blocker. Hyperpolarization and cyclic-nucleotide-gated (HCN) channels located in the AIS, but not in the

soma or dendrites, were found to strongly influence excitability by controlling spike threshold and probability[173] (**Fig. 3b**).

Investigation of spine and dendritic function. Analysis of spine and dendritic function has been extensively studied using two-photon uncaging of glutamate [20]. Although the z resolution of two-photon uncaging (~1.6 μm) is larger than a spine head, laser light power can be tuned such that the currents evoked by photolysis of caged compounds are the same as a single quantum release [23]. Direction of 60 such photochemical quanta at a small spine at 1-2 Hz induced structural LTP lasting over 90 minutes (ref. [24]) (**Fig. 3c**). Coupling two-photon uncaging with genetically encoded fluorescence indicators allows imaging of: the insertion of AMPARs associated with structural LTP[174]; the local life-time of activation of CaMK-II[175]; the spread of RAS induced by LTP[176]; the role of Rho during LTP[177,178]; and long-distance ERK signaling[179] (for review see ref. [180]). Structural LTP was also induced at single spines by directly photo-agonizing synaptic light-sensitive NMDARs (LiGluNs) using PTLs[147] (**Fig. 3c**). Inversely, photo-antagonizing LiGluNs optically prevented the induction of LTP at individual spines following glutamate uncaging, demonstrating that caged compounds and PTLs can potentially be used in concert[147].

Although one photon uncaging has contributed to our understanding on dendritic integration[181], two-photon uncaging at many individual spines along dendritic segments has added a new level of detail by showing that forward-propagating dendritic spikes are initiated by as few as 20 synchronous quanta (release events) on the same dendritic branch[182]. Pairing two-photon calcium imaging with two-photon uncaging at single and multiple spines also allowed detailed study of Ca^{2+} dynamics. The size of the spine neck was found to be crucial for the retention of Ca^{2+} (ref. [183]). Moreover, the sequence at which excitatory synapses are activated turned up to be crucial for controlling the size of the local Ca^{2+} signal and determining how neurons respond[184], confirming an old hypothesis of 'directional selectivity'[185].

Control of synaptic physiology. Synaptic physiology can also be controlled with cell type and micro-domain specificity using genetic and hybrid approaches. For instance, photo-inactivation of SNARE proteins fused with genetically encoded singlet oxygen generators fully disrupted presynaptic vesicular release at targeted synapses in a spatially and temporally precise manner in rat hippocampal slices and in behaving worms[126]. Because it targets synaptic proteins, this approach - known as inhibition of synapses with CALI (InSynC) - has a broad applicability in understanding the contribution of individual synapses to the control of neural circuits and behaviors.

PTLs and opto-XRs have the potential to probe the role of receptors and ion channels in distinct synaptic compartments (including presynaptic, postsynaptic, and extrasynaptic compartments) and in a cell-specific fashion (**Fig. 4**). For instance, a retrograde signal that enhances recovery from presynaptic depression was revealed at the Drosophila neuromuscular junction upon specific photo-activation of post-synaptic light-controllable kainate receptors (LiGluRs)[186]. The probability of neurotransmitter release can also be optically manipulated through presynaptic expression of light-sensitive mGlu2 receptors (LimGluR2) in hippocampal neurons, thereby affording precise control over short-term plasticity[146] (**Fig. 4a**). Synaptic and extra-synaptic GABAergic inhibition was independently controlled using α1- and α5-containing isoforms of light-sensitive $GABA_A$Rs (LiGABARs), respectively, offering ways to evaluate the impact of phasic vs. tonic inhibition in a neural circuit[150] (**Fig. 4b**). Similarly, NMDAR-mediated currents can be precisely controlled at synapses using specific isoforms of LiGluNs. In organotypic hippocampal slices, photo-antagonism of GluN2A-containing receptors during LTP induction is sufficient to reversibly prevent morphological and functional changes, enabling 'time-locked' gating of LTP[147]. Finally, glial cell activity and gliotransmission were also manipulated using PTLs. Exploiting the relatively high conductance and calcium permeability of LiGluRs, light-evoked calcium elevations in cultured cortical astrocytes were shown to trigger non-vesicular glutamate release[187], demonstrating the utility of LiGluR-mediated optopharmacological approaches for studying electrically silent cells (**Fig. 4c**).

Multi-chromophore approaches. Synthetic chromophores offer the possibility of flexible manipulation of wavelength absorption through spectral tuning, a property exploited in the confocal imaging of various colors without any channel cross-talk. Pairing two-photon uncaging of glutamate at 720 nm with blue light activation of ChR2 allows all-optical induction of structural LTP[188] as caged glutamate does not absorb blue while ChR2 is not significantly activated by 720 nm light. Another possibility is to pair a blue-light-sensitive caged GABA with caged glutamate. The poor two-photon cross-section of caged GABA prevents any measurable uncaging from irradiation at 720 nm, thus single spine signaling of glutamate and GABA have been probed using this approach[189,190]. An elegant study using two-color actuation paired circuit-specific expression of ChR2 in dopamine neurons with two-photon uncaging of glutamate on D1 targets to unravel the timing window for the effects of dopamine on single spine plasticity in the nucleus acumbens[191]. Recently, a new caging chromophore was developed that enables the photochemical control of several important signaling molecules such as glutamate[192], GABA[193], and cyclic nucleotides[194,195] with excellent chromatic independence, enabling two-color uncaging and actuation with many wavelengths of light[196]. Moreover, taking advantage of the broad

spectral properties of photoswitches as well as orthogonal strategies for their attachment, manipulation of multiple receptor subpopulations simultaneously and independently is now achievable ('multiplexing', ref. [138,139]). This opens interesting perspectives for studying potential cross-talk between different neurotransmitter systems.

In vivo optopharmacology

One fundamental goal of modern neuropharmacology is to link causally changes in receptor activity in given neuronal pathways with alterations of circuit physiology and ultimately behavior. The utility of classical pharmacology in vivo is limited, because local drug delivery is slow, imprecise, and hardly compatible with electrophysiology. In contrast, optochemical technologies are, in principle, able to mimick the timing, amplitude and spread of naturally occurring modulatory signals. In addition, the advent of *in vivo* optogenetics in the past decade[10] has pushed the advances of complementary technologies including multimodal fibers combining optical, electrical and chemical interrogation. These permit local delivery of light and chemicals in precisely defined brain regions, while simultaneously recording of neuronal activity[197,198]. Controlling the brain's own receptors and channels with light in the behaving animal is thus now in reach.

Small organic, diffusible photochemicals have profoundly impacted neurophysiology research in vitro (see above), but come with one major inherent drawback when it comes to in vivo applications: they must be supplied continuously. In frog tadpoles or fish larvae, photoswitch application and photocontrol are relatively easy, because these animals are transparent and the photoswitch can be simply added to the swimming water[66]. Diffusible opto-PAMs and opto-NAMs of mGluRs[66-68], and photoswitchable activators of GIRK channels[55], drove light-dependent motility behavior in zebrafish larvae. A rhodamine-based photocontrollable TRPA1 agonist (optovin) also enables rapid and reversible motor activity in paralyzed zebrafish[199]. Similarly, light-dependent perturbation of behavior in the nematode Caenorhabditis elegans was induced by feeding the animals with photoswitchable ligands of nAChRs[63].

In rodents, reliable and quantitative application of diffusible photochemicals in nervous tissue is more challenging. However, proof-of-concept for in vivo two-photon glutamate uncaging has been demonstrated[200]. Two-photon uncaging of glutamate applied from the pial surface led to single spine stimulation (assessed using calcium imaging and patch-clamp recordings) in superficial cortical layers. The concentration of caged glutamate was estimated to be

approximately homogenous up to 200 µm below the surface, and these in vivo observations of synaptic effects confirmed previous reports from brain slices[23]. Further demonstration of the feasibility of controlling neuromodulatory mechanisms in freely behaving rodents using diffusible photochemicals was achieved in recent studies using photosensitized mGluRs drugs, which were either 'azologized'[68,69] or contained a photo-releasable protecting group[201]..

Unlike other non-tethered small photochemicals, which are associated with diffusion-related issues, quaternary ammonium photoswitchable blockers (including AAQ, QAQ and BENAQ) [45,50,52,202-205] cross membranes and accumulate within cells, where they block voltage-gated ion channels, and photosensitize neural tissue for days after a single infusion[203]. Such long-lasting photosensitization of neurons enables restoration of visual functions in blind mice[206] and control of pain signaling in rats[52]. Another alternative to get around the diffusion issue is to immobilize the photochemical at proximity to its target receptor, for instance using an antibody-based CALI approach. The latter strategy was successfully implemented in mice to inactivate irreversibly synaptic GluA1-containing AMPARs using specific eosin-conjugated antibodies locally delivered either in the hippocampus or the lateral habenula, resulting in light-induced erasing of contextual fear memory[97] or avoidance learning[207], respectively.

The most straightforward means of limiting diffusion is to have the photochemical contained within (or anchored to) the membrane protein itself, as in genetic methods. Ideally, the engineered receptors should be controllable with kinetics that mimic those of neuronal excitability and transmission, while preserving the properties of endogenous receptors (including sensitivity to endogenous stimuli, coupling to signaling pathways, expression patterns and levels, subcellular trafficking and recycling, and so on). In practice, these requirements have proved difficult to combine. For instance, opto-XRs, which contain a naturally occurring retinal group in their agonist-binding pocket, are insensitive to their cognate ligands[101,102,105]. Hence, opto-XRs cannot fully replace endogenous receptors, and require ectopic expression. This results in mixture of receptor populations (exogenous and endogenous) which potentially compete for subcellular targeting[108] or express at non-physiological levels.

Nonetheless, opto-XRs have proved useful for triggering specific intracellular GPCR signaling pathways with high spatial and temporal precision (**Fig. 5a**). Photoactivation of opto-β2ARs, when expressed in principal neurons of the basolateral amygdala, modulates neuronal activity and induces anxiety-like behavioral states[102]. Importantly, these opto-β2ARs showed similar signaling (cAMP production and MAP kinase activation) and dynamics (internalization) to endogenous β2ARs. Likewise, opto-MORs closely match wild-type µ-opioid receptors in terms of

signaling and trafficking, and can promote opposite behavioral responses (reward or aversion) depending on which brain nuclei is targeted[105]. Placing the C-terminal domain of the serotoninergic (5-HT_{1A} or 5-HT_{2c}) receptor onto vertebrate opsins yields chimeric opto-XRs that signal and distribute at the sub-cellular level like their wild-type counterparts[109,110]. Expressing these chimeric receptors in the dorsal raphe nucleus of awake mice reveals how activation of 5-HT_{1A} or 5-HT_{2c} receptors can reduce anxiety, although via different mechanisms. In general, opto-XRs provide valuable optomolecular tools to study signaling cascades in vivo, yet how their activation mimics that of endogenous receptors remains unsettled.

In hybrid approaches, single-cysteine or -UAA substitutions only perturb minimally target proteins. Hence, the modified receptor or ion channel usually behaves like its wild-type counterpart (for example, its sensitivity to endogenous neurotransmitters is unaffected), but is photocontrollable[147,150,208,209]. Nevertheless, implementing hybrid approaches to study brain proteins in animals has been challenging, because of the dual requirement for gene and photochemical delivery (**Fig. 5b**). In vivo use of PTLs (attached to an engineered cysteine) was pioneered in zebrafish larvae[210] using LiGluRs. Since then, several studies have reported the use of this technology in behaving zebrafish[146,147,211,212]. In mice, the PTL approach was originally implemented in the eye — a compartmentalized organ with ideal access to light and drug delivery[213] — with the aim of restoring visual function[214,215]. In most of these studies, however, LiGluRs were utilized as binary on/off switches of action potential firing, as in classical optogenetics, and not to interrogate receptor function. An arsenal of light-activatable and light-inhibitable iGluRs and mGluRs of various subtypes is available[17,49] but remains to be exploited fully in vivo to understand how different GluR subtypes contribute to neural circuit operation and behavior. Results using light-regulated NMDARs on zebrafish and highlighting the role of GluN2A-containinig receptors in the formation of sensory topographic maps have set a precedent for this kind of work[147].

In recent years, cysteine-anchored PTLs were deployed in the mouse brain in vivo, first in superficial cortical layers using LiGABARs[150] and LiGluRs[208] and, more recently, in deep brain areas and behaving mice using LinAChRs[209]. Importantly, even though free cysteines are present on other extracellular sites on cells, this approach to photosensitizing receptors has proved to be remarkably specific both in fish and mice, with wild-type neurons and animals being unaffected by light after PTL treatment[146,150,208,209]. Photoswitches can be applied topically to the cortex after craniotomy and duratomy, but to be targeted deeper in the brain they must be infused locally using cannula. Systemic photoswitch application is likely problematic, owing to

the strong lability and widespread reactivity of the maleimide group, but recent development of self-labeling tags[138,139] may help reduce off-target effects and fast degradation. Although the first generation of PTLs required near-UV light to operate, they could be efficiently photocontrolled up to 350 µm deep below the brain surface[150]. Recently developed red-shifted and two-photon sensitive azobenzenes[84,216-219] should facilitate in vivo photocontrol with deeper penetrating wavelengths of light.

Two options are available to express the mutated subunit: viral expression and transgenic knock-in mice. A molecular replacement strategy using knock-in animals is the optimal option is terms of ensuring unperturbed protein density and localization[150]. Although viral transduction is cheaper and quicker than transgenesis, expression levels may be altered (although not systematically; see ref. [147,209]). Nonetheless, viral transduction has one important added value, which is to afford circuit-specific targeting. For instance, LinAChRs over-expressed in the ventral tegmental area (VTA) enabled acute and reversible inactivation of postsynaptic cholinergic transmission, while leaving pre-synaptic nAChRs outside VTA afferents untouched[209] (**Fig. 5b**). Hence, LinAChRs could reveal, in real time, the cholinergic tone that is broadcasted, and its impact, on VTA dopamine neuron firing in vivo[209]. On the other hand, light-controllable α1-containing $GABA_A$Rs expressed in knock-in mice permitted direct photocontrol of visually-evoked responses and revealed the contribution of such receptors in shaping gamma oscillations in awake animals[150].

Because of constant protein turnover, PTL-labeled receptors are expected to decline in abundance with time. Experimental evidence on LiGluRs indicates that this may not be a major issue for long-lasting behavioral assays. Indeed, light-mediated responses in mice expressing LiGluRs were maintained for one (ref. [208]) up to fourteen (ref. [214]) days after a single bolus supply of photoswitchable ligand demonstrating stable labeling and surface expression of the tagged receptors for prolonged periods of time.

Incorporation of photosensitive UAAs for in vivo studies of neuronal proteins remains in its infancy, although proof-of-concept experiments in various organisms including rodents[135,220-223] indicate that feasibility is in reach. In brain slices, a photocontrollable inwardly rectifying potassium (Kir2.1) channel incorporating a caged cysteine in the channel pore region was successfully expressed in the mouse neocortex [135], allowing acute light-induced suppression of neuronal firing. With its site flexibility and genetic encodability, UAA-based photocontrol combines several advantages; however, the system requires multiple components to be delivered — including the orthogonal tRNA/synthetase pair and the mutated target protein of

interest — and so its in vivo implementation is technically difficult. The bioavailability of the UAA poses another hurdle. Recent successful attempts to address these challenges include the generation of transgenic mice incorporating, in their genome, the necessary tRNA- and synthetase-encoding genes needed for amber stop codon suppression[221,223]. In principle, photoresponsive UAAs provide flexible means not only for optical control of protein activities but also for the identification of interacting partners within signaling complexes (using photo-crosslinking UAAs; ref. [131]).

Concluding remarks and perspectives

With its high flexibility and versatility, optopharmacology yielded a large repertoire of light-tunable ion channels and neurotransmitter receptors allowing fast and remote control over specific neuronal signaling pathways at specific locations within a cell, a network or an organism. By establishing causal relationships between a protein activity and a cellular or behavioral output, optopharmacology appears unrivaled to unlock brain molecular logic. It also holds strong potential for identifying new therapeutic targets in neurological disorders (Box 1). It is thus possible to explore the role and signaling mechanisms of numerous receptor and ion channel subtypes in their natural environment in normal and diseased states. Virtually any protein, membranous or soluble, is amenable to optopharmacological engineering provided that its activity is controllable by interacting ligands or binding partners. The greatest molecular specificity can be obtained from light-activated chemicals that are tethered to specific proteins (such as PTLs). On the other hand, approaches that rely solely on synthetic ligands (such as caged compounds and PLs) have the major advantage not to require genetic modifications of the target protein. Both approaches require synthetic chemistry, however, which can be arduous and costly. Naturally occurring photosensitive proteins, usually from plants of microbes and which bypass the need for chemical synthesis, offer an alternative. They can be easily spliced onto mammalian proteins enabling light-controlled of proteins sitting on the cell surface or intracellularly. Booming genome sequencing from various organisms (including non-animal) augurs bright days for biotechnological applications using native light-sensitive modules. Neuroscience through perfected optogenetics and optopharmacology should greatly benefit from these advances. However, one serious limitation remains to implementing light-based approaches in behaving animals: the necessity to deliver light to the cells of interest often requires invasive surgery. Novel approaches based on transcranial acoustic[224] or magnetic[225] stimuli to remotely manipulate neurons and biochemical pathways may overcome this hurdle, yet

face many technical obstacles. Undoubtedly light-based approaches such as optopharmacology will continue shaping new horizons to tackle fundamental processes in brain physiology and pathology.

References

1. Hille, B. Ionic Channels of Excitable Membranes. Third Edition. *Sinauer Associates, Inc.* (2001).
2. Lemoine, D. et al. Ligand-gated ion channels: new insights into neurological disorders and ligand recognition. *Chem Rev* **112**, 6285-318 (2012).
3. Smart, T.G. & Paoletti, P. Synaptic neurotransmitter-gated receptors. *Cold Spring Harb Perspect Biol* **4**(2012).
4. Foster, D.J. & Conn, P.J. Allosteric Modulation of GPCRs: New Insights and Potential Utility for Treatment of Schizophrenia and Other CNS Disorders. *Neuron* **94**, 431-446 (2017).
5. Rask-Andersen, M., Almen, M.S. & Schioth, H.B. Trends in the exploitation of novel drug targets. *Nat Rev Drug Discov* **10**, 579-90 (2011).
6. Coetzee, W.A. et al. Molecular diversity of K+ channels. *Ann N Y Acad Sci* **868**, 233-85 (1999).
7. Engin, E., Benham, R.S. & Rudolph, U. An Emerging Circuit Pharmacology of GABAA Receptors. *Trends Pharmacol Sci* **39**, 710-732 (2018).
8. Kramer, R.H., Mourot, A. & Adesnik, H. Optogenetic pharmacology for control of native neuronal signaling proteins. *Nat Neurosci* **16**, 816-23 (2013).
9. Scanziani, M. & Hausser, M. Electrophysiology in the age of light. *Nature* **461**, 930-9 (2009).
10. Kim, C.K., Adhikari, A. & Deisseroth, K. Integration of optogenetics with complementary methodologies in systems neuroscience. *Nat Rev Neurosci* **18**, 222-235 (2017).
11. Bartels, E., Wassermann, N.H. & Erlanger, B.F. Photochromic activators of the acetylcholine receptor. *Proc Natl Acad Sci U S A* **68**, 1820-3 (1971).
12. Lester, H.A., Krouse, M.E., Nass, M.M., Wassermann, N.H. & Erlanger, B.F. Light-activated drug confirms a mechanism of ion channel blockade. *Nature* **280**, 509-10 (1979).
13. Kaplan, J.H., Forbush, B., 3rd & Hoffman, J.F. Rapid photolytic release of adenosine 5'-triphosphate from a protected analogue: utilization by the Na:K pump of human red blood cell ghosts. *Biochemistry* **17**, 1929-35 (1978).
14. Lester, H.A., Krouse, M.E., Nass, M.M., Wassermann, N.H. & Erlanger, B.F. A covalently bound photoisomerizable agonist: comparison with reversibly bound agonists at Electrophorus electroplaques. *J Gen Physiol* **75**, 207-32 (1980).
15. Ellis-Davies, G.C. Caged compounds: photorelease technology for control of cellular chemistry and physiology. *Nat Methods* **4**, 619-28 (2007).
16. Hamouda, A.K., Jayakar, S.S., Chiara, D.C. & Cohen, J.B. Photoaffinity labeling of nicotinic receptors: diversity of drug binding sites! *J Mol Neurosci* **53**, 480-6 (2014).
17. Berlin, S. & Isacoff, E.Y. Synapses in the spotlight with synthetic optogenetics. *EMBO Rep* **18**, 677-692 (2017).
18. Hüll, K., Morstein, J. & Trauner, D. In Vivo Photopharmacology. *Chem Rev* **118**, 10710-10747 (2018).

19. Ellis-Davies, G.C. A chemist and biologist talk to each other about caged neurotransmitters. *Beilstein J Org Chem* **9**, 64-73 (2013).

20. Ellis-Davies, G.C.R. Two-Photon Uncaging of Glutamate. *Front Synaptic Neurosci* **10**, 48 (2019).

21. Wilcox, M. et al. Synthesis of photolabile precursors of amino acid neurotransmitters. *J Org Chem* **55**, 1585-1589 (1990).

22. Wieboldt, R. et al. Photolabile precursors of glutamate: synthesis, photochemical properties, and activation of glutamate receptors on a microsecond time scale. *Proc Natl Acad Sci U S A* **91**, 8752-6 (1994).

23. Matsuzaki, M. et al. Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nat Neurosci* **4**, 1086-92 (2001).

24. Matsuzaki, M., Honkura, N., Ellis-Davies, G.C. & Kasai, H. Structural basis of long-term potentiation in single dendritic spines. *Nature* **429**, 761-6 (2004).

25. Gee, K.R., Wieboldt, R. & Hess, G.P. Synthesis and Photochemistry of a New Photolabile Derivative of GABA-Neurotransmitter Release and Receptor Activation in the Microsecond Time Region. *J Am Chem Soc* **116**, 8366-8367 (1994).

26. Rial Verde, E.M., Zayat, L., Etchenique, R. & Yuste, R. Photorelease of GABA with Visible Light Using an Inorganic Caging Group. *Front Neural Circuits* **2**, 2 (2008).

27. Kantevari, S., Matsuzaki, M., Kanemoto, Y., Kasai, H. & Ellis-Davies, G.C. Two-color, two-photon uncaging of glutamate and GABA. *Nat Methods* **7**, 123-5 (2010).

28. Matsuzaki, M., Hayama, T., Kasai, H. & Ellis-Davies, G.C.R. Two-photon uncaging of gamma-aminobutyric acid in intact brain tissue. *Nat Chem Biol* **6**, 255-257 (2010).

29. Donato, L. et al. Water-soluble, donor-acceptor biphenyl derivatives in the 2-(o-nitrophenyl)propyl series: highly efficient two-photon uncaging of the neurotransmitter gamma-aminobutyric acid at lambda = 800 nm. *Angew Chem Int Ed Engl* **51**, 1840-3 (2012).

30. Araya, R., Andino-Pavlovsky, V., Yuste, R. & Etchenique, R. Two-photon optical interrogation of individual dendritic spines with caged dopamine. *ACS Chem Neurosci* **4**, 1163-7 (2013).

31. Breitinger, H.G., Wieboldt, R., Ramesh, D., Carpenter, B.K. & Hess, G.P. Synthesis and characterization of photolabile derivatives of serotonin for chemical kinetic investigations of the serotonin 5-HT(3) receptor. *Biochemistry* **39**, 5500-8 (2000).

32. Warther, D. et al. Two-photon uncaging: New prospects in neuroscience and cellular biology. *Bioorg Med Chem* **18**, 7753-8 (2010).

33. Walker, J.W., McCray, J.A. & Hess, G.P. Photolabile protecting groups for an acetylcholine receptor ligand. Synthesis and photochemistry of a new class of o-nitrobenzyl derivatives and their effects on receptor function. *Biochemistry* **25**, 1799-805 (1986).

34. Milburn, T. et al. Synthesis, photochemistry, and biological activity of a caged photolabile acetylcholine receptor ligand. *Biochemistry* **28**, 49-55 (1989).

35. Banala, S. et al. Photoactivatable drugs for nicotinic optopharmacology. *Nat Methods* **15**, 347-350 (2018).

36. Passlick, S., Thapaliya, E.R., Chen, Z., Richers, M.T. & Ellis-Davies, G.C.R. Optical probing of acetylcholine receptors on neurons in the medial habenula with a novel caged nicotine drug analogue. *J Physiol* **596**, 5307-5318 (2018).

37. Niu, L., Wieboldt, R., Ramesh, D., Carpenter, B.K. & Hess, G.P. Synthesis and characterization of a caged receptor ligand suitable for chemical kinetic investigations of the glycine receptor in the 3-microseconds time domain. *Biochemistry* **35**, 8136-42 (1996).

38. Gee, K.R., Niu, L., Schaper, K., Jayaraman, V. & Hess, G.P. Synthesis and photochemistry of a photolabile precursor of N-methyl-D-aspartate (NMDA) that is photolyzed in the microsecond time region and is suitable for chemical kinetic investigations of the NMDA receptor. *Biochemistry* **38**, 3140-7 (1999).

39. Palma-Cerda, F. et al. New caged neurotransmitter analogs selective for glutamate receptor sub-types based on methoxynitroindoline and nitrophenylethoxycarbonyl caging groups. *Neuropharmacology* **63**, 624-34 (2012).

40. Niu, L., Gee, K.R., Schaper, K. & Hess, G.P. Synthesis and photochemical properties of a kainate precursor and activation of kainate and AMPA receptor channels on a microsecond time scale. *Biochemistry* **35**, 2030-6 (1996).

41. Banghart, M.R. & Sabatini, B.L. Photoactivatable neuropeptides for spatiotemporally precise delivery of opioids in neural tissue. *Neuron* **73**, 249-59 (2012).

42. Banghart, M.R., Williams, J.T., Shah, R.C., Lavis, L.D. & Sabatini, B.L. Caged naloxone reveals opioid signaling deactivation kinetics. *Mol Pharmacol* **84**, 687-95 (2013).

43. Banghart, M.R., He, X.J. & Sabatini, B.L. A Caged Enkephalin Optimized for Simultaneously Probing Mu and Delta Opioid Receptors. *ACS Chem Neurosci* **9**, 684-690 (2018).

44. Volgraf, M. et al. Reversibly caged glutamate: a photochromic agonist of ionotropic glutamate receptors. *J Am Chem Soc* **129**, 260-1 (2007).

45. Banghart, M.R. et al. Photochromic blockers of voltage-gated potassium channels. *Angew Chem Int Ed Engl* **48**, 9097-101 (2009).

46. Izquierdo-Serra, M. et al. Optical control of endogenous receptors and cellular excitability using targeted covalent photoswitches. *Nat Commun* **7**, 12221 (2016).

47. Szymanski, W., Yilmaz, D., Kocer, A. & Feringa, B.L. Bright ion channels and lipid bilayers. *Acc Chem Res* **46**, 2910-23 (2013).

48. Broichhagen, J. & Trauner, D. The in vivo chemistry of photoswitched tethered ligands. *Curr Opin Chem Biol* **21**, 121-7 (2014).

49. Reiner, A., Levitz, J. & Isacoff, E.Y. Controlling ionotropic and metabotropic glutamate receptors with light: principles and potential. *Curr Opin Pharmacol* **20**, 135-43 (2015).

50. Fortin, D.L. et al. Photochemical control of endogenous ion channels and cellular excitability. *Nat Methods* **5**, 331-8 (2008).

51. Mourot, A. et al. Tuning photochromic ion channel blockers. *ACS Chem Neurosci* **2**, 536-43 (2011).

52. Mourot, A. et al. Rapid optical control of nociception with an ion-channel photoswitch. *Nat Methods* **9**, 396-402 (2012).

53. Schonberger, M., Althaus, M., Fronius, M., Clauss, W. & Trauner, D. Controlling epithelial sodium channels with light using photoswitchable amilorides. *Nat Chem* **6**, 712-9 (2014).

54. Fehrentz, T. et al. Optical control of L-type Ca(2+) channels using a diltiazem photoswitch. *Nat Chem Biol* **14**, 764-767 (2018).

55. Barber, D.M. et al. Optical control of neuronal activity using a light-operated GIRK channel opener (LOGO). *Chem Sci* **7**, 2347-2352 (2016).

56. Leippe, P., Winter, N., Sumser, M.P. & Trauner, D. Optical Control of a Delayed Rectifier and a Two-Pore Potassium Channel with a Photoswitchable Bupivacaine. *ACS Chem Neurosci* (2018).

57. Broichhagen, J. et al. Optical control of insulin release using a photoswitchable sulfonylurea. *Nat Commun* **5**, 5116 (2014).

58. Stein, M., Breit, A., Fehrentz, T., Gudermann, T. & Trauner, D. Optical control of TRPV1 channels. *Angew Chem Int Ed Engl* **52**, 9845-8 (2013).

59. Stawski, P., Sumser, M. & Trauner, D. A photochromic agonist of AMPA receptors. *Angew Chem Int Ed Engl* **51**, 5748-51 (2012).

60. Laprell, L. et al. Optical control of NMDA receptors with a diffusible photoswitch. *Nat Commun* **6**, 8076 (2015).

61. Laprell, L. et al. Restoring Light Sensitivity in Blind Retinae Using a Photochromic AMPA Receptor Agonist. *ACS Chem Neurosci* **7**, 15-20 (2016).

62. Barber, D.M. et al. Optical control of AMPA receptors using a photoswitchable quinoxaline-2,3-dione antagonist. *Chem Sci* **8**, 611-615 (2017).

63. Damijonaitis, A. et al. AzoCholine Enables Optical Control of Alpha 7 Nicotinic Acetylcholine Receptors in Neural Networks. *ACS Chem Neurosci* **6**, 701-7 (2015).

64. Bahamonde, M.I. et al. Photomodulation of G protein-coupled adenosine receptors by a novel light-switchable ligand. *Bioconjug Chem* **25**, 1847-54 (2014).

65. Huckvale, R., Mortensen, M., Pryde, D., Smart, T.G. & Baker, J.R. Azogabazine; a photochromic antagonist of the GABAA receptor. *Org Biomol Chem* **14**, 6676-8 (2016).

66. Pittolo, S. et al. An allosteric modulator to control endogenous G protein-coupled receptors with light. *Nat Chem Biol* **10**, 813-5 (2014).

67. Rovira, X. et al. OptoGluNAM4.1, a Photoswitchable Allosteric Antagonist for Real-Time Control of mGlu4 Receptor Activity. *Cell Chem Biol* **23**, 929-34 (2016).

68. Gomez-Santacana, X. et al. Illuminating Phenylazopyridines To Photoswitch Metabotropic Glutamate Receptors: From the Flask to the Animals. *ACS Cent Sci* **3**, 81-91 (2017).

69. Zussy, C. et al. Dynamic modulation of inflammatory pain-related affective and sensory symptoms by optical control of amygdala metabotropic glutamate receptor 4. *Mol Psychiatry* **23**, 509-520 (2018).

70. Bossi, S. et al. A Light-Controlled Allosteric Modulator Unveils a Role for mGlu4 Receptors During Early Stages of Ischemia in the Rodent Cerebellar Cortex. *Front Cell Neurosci* **12**, 449 (2018).

71. Stein, M. et al. Azo-propofols: photochromic potentiators of GABA(A) receptors. *Angew Chem Int Ed Engl* **51**, 10500-4 (2012).

72. Yue, L. et al. Robust photoregulation of GABA(A) receptors by allosteric modulation with a propofol analogue. *Nat Commun* **3**, 1095 (2012).

73. Beharry, A.A. & Woolley, G.A. Azobenzene photoswitches for biomolecules. *Chem Soc Rev* **40**, 4422-37 (2011).

74. Broichhagen, J., Frank, J.A. & Trauner, D. A roadmap to success in photopharmacology. *Acc Chem Res* **48**, 1947-60 (2015).

75. Lin, W.C., Tsai, M.C., Rajappa, R. & Kramer, R.H. Design of a Highly Bistable Photoswitchable Tethered Ligand for Rapid and Sustained Manipulation of Neurotransmission. *J Am Chem Soc* **140**, 7445-7448 (2018).

76. Samanta, S., Qin, C., Lough, A.J. & Woolley, G.A. Bidirectional photocontrol of peptide conformation with a bridged azobenzene derivative. *Angew Chem Int Ed Engl* **51**, 6452-5 (2012).

77. Thapaliya, E.R., Zhao, J. & Ellis-Davies, G.C.R. Locked-Azobenzene: Testing the Scope of a Unique Photoswitchable Scaffold for Cell Physiology. *ACS Chem Neurosci* (2019).

78. Chi, L., Sadovski, O. & Woolley, G.A. A blue-green absorbing cross-linker for rapid photoswitching of peptide helix content. *Bioconjug Chem* **17**, 670-6 (2006).

79. Samanta, S. et al. Photoswitching azo compounds in vivo with red light. *J Am Chem Soc* **135**, 9777-84 (2013).

80. Kienzler, M.A. et al. A red-shifted, fast-relaxing azobenzene photoswitch for visible light control of an ionotropic glutamate receptor. *J Am Chem Soc* **135**, 17683-6 (2013).

81. Rullo, A. et al. Long wavelength optical control of glutamate receptor ion channels using a tetra-ortho-substituted azobenzene derivative. *Chem Commun (Camb)* **50**, 14613-5 (2014).

82. Hoppmann, C., Maslennikov, I., Choe, S. & Wang, L. In Situ Formation of an Azo Bridge on Proteins Controllable by Visible Light. *J Am Chem Soc* **137**, 11218-21 (2015).

83. Dong, M., Babalhavaeji, A., Samanta, S., Beharry, A.A. & Woolley, G.A. Red-Shifting Azobenzene Photoswitches for in Vivo Use. *Acc Chem Res* **48**, 2662-70 (2015).

84. Dong, M. et al. Near-Infrared Photoswitching of Azobenzenes under Physiological Conditions. *J Am Chem Soc* **139**, 13483-13486 (2017).

85. Passlick, S., Richers, M.T. & Ellis-Davies, G.C.R. Thermodynamically Stable, Photoreversible Pharmacology in Neurons with One- and Two-Photon Excitation. *Angew Chem Int Ed Engl* **57**, 12554-12557 (2018).

86. Borowiak, M. et al. Photoswitchable Inhibitors of Microtubule Dynamics Optically Control Mitosis and Cell Death. *Cell* **162**, 403-411 (2015).

87. Gorostiza, P. et al. Mechanisms of photoswitch conjugation and light activation of an ionotropic glutamate receptor. *Proc Natl Acad Sci U S A* **104**, 10865-70 (2007).

88. Mourot, A., Tochitsky, I. & Kramer, R.H. Light at the end of the channel: optical manipulation of intrinsic neuronal excitability with chemical photoswitches. *Front Mol Neurosci* **6**, 5 (2013).

89. Schoenberger, M., Damijonaitis, A., Zhang, Z., Nagel, D. & Trauner, D. Development of a new photochromic ion channel blocker via azologization of fomocaine. *ACS Chem Neurosci* **5**, 514-8 (2014).

90. Kocer, A., Walko, M., Meijberg, W. & Feringa, B.L. A light-actuated nanovalve derived from a channel protein. *Science* **309**, 755-8 (2005).

91. Lachmann, D. et al. Photochromic Dopamine Receptor Ligands Based on Dithienylethenes and Fulgides. *Chemistry* **23**, 13423-13434 (2017).

92. Ruiz, M.L. & Karpen, J.W. Single cyclic nucleotide-gated channels locked in different ligand-bound states. *Nature* **389**, 389-92 (1997).

93. Adesnik, H., Nicoll, R.A. & England, P.M. Photoinactivation of native AMPA receptors reveals their real-time trafficking. *Neuron* **48**, 977-85 (2005).

94. Mortensen, M. et al. Photo-antagonism of the GABAA receptor. *Nat Commun* **5**, 4454 (2014).

95. Jay, D.G. Selective destruction of protein function by chromophore-assisted laser inactivation. *Proc Natl Acad Sci U S A* **85**, 5454-8 (1988).

96. Sano, Y., Watanabe, W. & Matsunaga, S. Chromophore-assisted laser inactivation--towards a spatiotemporal-functional analysis of proteins, and the ablation of chromatin, organelle and cell function. *J Cell Sci* **127**, 1621-9 (2014).

97. Takemoto, K. et al. Optical inactivation of synaptic AMPA receptors erases fear memory. *Nat Biotechnol* **35**, 38-47 (2017).

98. Goglia, A.G. & Toettcher, J.E. A bright future: optogenetics to dissect the spatiotemporal control of cell behavior. *Curr Opin Chem Biol* **48**, 106-113 (2018).

99. Spangler, S.M. & Bruchas, M.R. Optogenetic approaches for dissecting neuromodulation and GPCR signaling in neural circuits. *Curr Opin Pharmacol* **32**, 56-70 (2017).

100. Kim, J.M. et al. Light-driven activation of beta 2-adrenergic receptor signaling by a chimeric rhodopsin containing the beta 2-adrenergic receptor cytoplasmic loops. *Biochemistry* **44**, 2284-92 (2005).

101. Airan, R.D., Thompson, K.R., Fenno, L.E., Bernstein, H. & Deisseroth, K. Temporally precise in vivo control of intracellular signalling. *Nature* **458**, 1025-9 (2009).

102. Siuda, E.R. et al. Optodynamic simulation of beta-adrenergic receptor signalling. *Nat Commun* **6**, 8480 (2015).

103. Li, P. et al. Optogenetic activation of intracellular adenosine A2A receptor signaling in the hippocampus is sufficient to trigger CREB phosphorylation and impair memory. *Mol Psychiatry* **20**, 1339-49 (2015).

104. Barish, P.A. et al. Design and functional evaluation of an optically active mu-opioid receptor. *Eur J Pharmacol* **705**, 42-8 (2013).

105. Siuda, E.R. et al. Spatiotemporal control of opioid signaling and behavior. *Neuron* **86**, 923-935 (2015).

106. van Wyk, M., Pielecka-Fortuna, J., Lowel, S. & Kleinlogel, S. Restoring the ON Switch in Blind Retinas: Opto-mGluR6, a Next-Generation, Cell-Tailored Optogenetic Tool. *PLoS Biol* **13**, e1002143 (2015).

107. Gunaydin, L.A. et al. Natural neural projection dynamics underlying social behavior. *Cell* **157**, 1535-51 (2014).

108. Oh, E., Maejima, T., Liu, C., Deneris, E. & Herlitze, S. Substitution of 5-HT1A receptor signaling by a light-activated G protein-coupled receptor. *J Biol Chem* **285**, 30825-36 (2010).

109. Spoida, K., Masseck, O.A., Deneris, E.S. & Herlitze, S. Gq/5-HT2c receptor signals activate a local GABAergic inhibitory feedback circuit to modulate serotonergic firing and anxiety in mice. *Proc Natl Acad Sci U S A* **111**, 6479-84 (2014).

110. Masseck, O.A. et al. Vertebrate cone opsins enable sustained and highly sensitive rapid control of Gi/o signaling in anxiety circuitry. *Neuron* **81**, 1263-1273 (2014).

111. Morri, M. et al. Optical functionalization of human Class A orphan G-protein-coupled receptors. *Nat Commun* **9**, 1950 (2018).

112. Rost, B.R., Schneider-Warme, F., Schmitz, D. & Hegemann, P. Optogenetic Tools for Subcellular Applications in Neuroscience. *Neuron* **96**, 572-603 (2017).

113. Schmidt, D., Tillberg, P.W., Chen, F. & Boyden, E.S. A fully genetically encoded protein architecture for optical control of peptide ligand concentration. *Nat Commun* **5**, 3019 (2014).

114. He, L. et al. Near-infrared photoactivatable control of Ca(2+) signaling and optogenetic immunomodulation. *Elife* **4**(2015).

115. Ma, G. et al. Optogenetic Control of Voltage-Gated Calcium Channels. *Angew Chem Int Ed Engl* **57**, 7019-7022 (2018).

116. Wu, Y.I. et al. A genetically encoded photoactivatable Rac controls the motility of living cells. *Nature* **461**, 104-8 (2009).

117. Dietz, D.M. et al. Rac1 is essential in cocaine-induced structural plasticity of nucleus accumbens neurons. *Nat Neurosci* **15**, 891-6 (2012).

118. Hayashi-Takagi, A. et al. Labelling and optical erasure of synaptic memory traces in the motor cortex. *Nature* **525**, 333-8 (2015).

119. O'Neill, P.R. & Gautam, N. Subcellular optogenetic inhibition of G proteins generates signaling gradients and cell migration. *Mol Biol Cell* **25**, 2305-14 (2014).

120. Liu, Q. et al. A Photoactivatable Botulinum Neurotoxin for Inducible Control of Neurotransmission. *Neuron* (2019).

121. Cosentino, C. et al. Optogenetics. Engineering of a light-gated potassium channel. *Science* **348**, 707-10 (2015).

122. Grusch, M. et al. Spatio-temporally precise activation of engineered receptor tyrosine kinases by light. *EMBO J* **33**, 1713-26 (2014).

123. Chang, K.Y. et al. Light-inducible receptor tyrosine kinases that regulate neurotrophin signalling. *Nat Commun* **5**, 4057 (2014).

124. Sinnen, B.L. et al. Optogenetic Control of Synaptic Composition and Function. *Neuron* **93**, 646-660 e5 (2017).

125. Tour, O., Meijer, R.M., Zacharias, D.A., Adams, S.R. & Tsien, R.Y. Genetically targeted chromophore-assisted light inactivation. *Nat Biotechnol* **21**, 1505-8 (2003).

126. Lin, J.Y. et al. Optogenetic inhibition of synaptic release with chromophore-assisted light inactivation (CALI). *Neuron* **79**, 241-53 (2013).

127. Noren, C.J., Anthony-Cahill, S.J., Griffith, M.C. & Schultz, P.G. A general method for site-specific incorporation of unnatural amino acids into proteins. *Science* **244**, 182-8 (1989).

128. Liu, C.C. & Schultz, P.G. Adding new chemistries to the genetic code. *Annu Rev Biochem* **79**, 413-44 (2010).

129. Chin, J.W. Expanding and reprogramming the genetic code. *Nature* **550**, 53-60 (2017).

130. Beene, D.L., Dougherty, D.A. & Lester, H.A. Unnatural amino acid mutagenesis in mapping ion channel function. *Curr Opin Neurobiol* **13**, 264-70 (2003).

131. Huber, T. & Sakmar, T.P. Chemical biology methods for investigating G protein-coupled receptor signaling. *Chem Biol* **21**, 1224-37 (2014).

132. Klippenstein, V., Mony, L. & Paoletti, P. Probing Ion Channel Structure and Function Using Light-Sensitive Amino Acids. *Trends Biochem Sci* **43**, 436-451 (2018).

133. Miller, J.C., Silverman, S.K., England, P.M., Dougherty, D.A. & Lester, H.A. Flash decaging of tyrosine sidechains in an ion channel. *Neuron* **20**, 619-24 (1998).

134. Philipson, K.D., Gallivan, J.P., Brandt, G.S., Dougherty, D.A. & Lester, H.A. Incorporation of caged cysteine and caged tyrosine into a transmembrane segment of the nicotinic ACh receptor. *Am J Physiol Cell Physiol* **281**, C195-206 (2001).

135. Kang, J.Y. et al. In vivo expression of a light-activatable potassium channel using unnatural amino acids. *Neuron* **80**, 358-70 (2013).

136. Banghart, M., Borges, K., Isacoff, E., Trauner, D. & Kramer, R.H. Light-activated ion channels for remote control of neuronal firing. *Nat Neurosci* **7**, 1381-6 (2004).

137. Volgraf, M. et al. Allosteric control of an ionotropic glutamate receptor with an optical switch. *Nat Chem Biol* **2**, 47-52 (2006).

138. Broichhagen, J. et al. Orthogonal Optical Control of a G Protein-Coupled Receptor with a SNAP-Tethered Photochromic Ligand. *ACS Cent Sci* **1**, 383-93 (2015).

139. Levitz, J. et al. Dual optical control and mechanistic insights into photoswitchable group II and III metabotropic glutamate receptors. *Proc Natl Acad Sci U S A* **114**, E3546-E3554 (2017).

140. Farrants, H. et al. SNAP-Tagged Nanobodies Enable Reversible Optical Control of a G Protein-Coupled Receptor via a Remotely Tethered Photoswitchable Ligand. *ACS Chem Biol* **13**, 2682-2688 (2018).

141. Browne, L.E. et al. Optical control of trimeric P2X receptors and acid-sensing ion channels. *Proc Natl Acad Sci U S A* **111**, 521-6 (2014).

142. Habermacher, C. et al. Photo-switchable tweezers illuminate pore-opening motions of an ATP-gated P2X ion channel. *Elife* **5**, e11050 (2016).

143. Harkat, M. et al. On the permeation of large organic cations through the pore of ATP-gated P2X receptors. *Proc Natl Acad Sci U S A* **114**, E3786-E3795 (2017).

144. Fortin, D.L. et al. Optogenetic photochemical control of designer K+ channels in mammalian neurons. *J Neurophysiol* **106**, 488-96 (2011).

145. Sandoz, G., Levitz, J., Kramer, R.H. & Isacoff, E.Y. Optical control of endogenous proteins with a photoswitchable conditional subunit reveals a role for TREK1 in GABA(B) signaling. *Neuron* **74**, 1005-14 (2012).

146. Levitz, J. et al. Optical control of metabotropic glutamate receptors. *Nat Neurosci* **16**, 507-16 (2013).

147. Berlin, S. et al. A family of photoswitchable NMDA receptors. *Elife* **5**(2016).

148. Tochitsky, I. et al. Optochemical control of genetically engineered neuronal nicotinic acetylcholine receptors. *Nat Chem* **4**, 105-11 (2012).

149. Lin, W.C. et al. Engineering a light-regulated GABAA receptor for optical control of neural inhibition. *ACS Chem Biol* **9**, 1414-9 (2014).

150. Lin, W.C. et al. A Comprehensive Optogenetic Pharmacology Toolkit for In Vivo Control of GABA(A) Receptors and Synaptic Inhibition. *Neuron* **88**, 879-891 (2015).

151. Lemoine, D. et al. Optical control of an ion channel gate. *Proc Natl Acad Sci U S A* **110**, 20813-8 (2013).

152. Donthamsetti, P.C. et al. Optical Control of Dopamine Receptors Using a Photoswitchable Tethered Inverse Agonist. *J Am Chem Soc* **139**, 18522-18535 (2017).

153. Reiner, A. & Isacoff, E.Y. Tethered ligands reveal glutamate receptor desensitization depends on subunit occupancy. *Nat Chem Biol* **10**, 273-80 (2014).

154. Li, G.D. et al. Identification of a GABAA receptor anesthetic binding site at subunit interfaces by photolabeling with an etomidate analog. *J Neurosci* **26**, 11599-605 (2006).

155. Yip, G.M. et al. A propofol binding site on mammalian GABAA receptors identified by photolabeling. *Nat Chem Biol* **9**, 715-20 (2013).

156. Valentin-Hansen, L. et al. Mapping substance P binding sites on the neurokinin-1 receptor using genetic incorporation of a photoreactive amino acid. *J Biol Chem* **289**, 18045-54 (2014).

157. Coin, I. et al. Genetically encoded chemical probes in cells reveal the binding path of urocortin-I to CRF class B GPCR. *Cell* **155**, 1258-69 (2013).

158. Simms, J. et al. Photoaffinity Cross-Linking and Unnatural Amino Acid Mutagenesis Reveal Insights into Calcitonin Gene-Related Peptide Binding to the Calcitonin Receptor-like Receptor/Receptor Activity-Modifying Protein 1 (CLR/RAMP1) Complex. *Biochemistry* **57**, 4915-4922 (2018).

159. Murray, C.I. et al. Unnatural amino acid photo-crosslinking of the IKs channel complex demonstrates a KCNE1:KCNQ1 stoichiometry of up to 4:4. *Elife* **5**(2016).

160. Westhoff, M., Murray, C.I., Eldstrom, J. & Fedida, D. Photo-Cross-Linking of IKs Demonstrates State-Dependent Interactions between KCNE1 and KCNQ1. *Biophys J* **113**, 415-425 (2017).

161. Klippenstein, V., Ghisi, V., Wietstruk, M. & Plested, A.J. Photoinactivation of glutamate receptors by genetically encoded unnatural amino acids. *J Neurosci* **34**, 980-91 (2014).

162. Zhu, S. et al. Genetically encoding a light switch in an ionotropic glutamate receptor reveals subunit-specific interfaces. *Proc Natl Acad Sci U S A* **111**, 6081-6 (2014).

163. Tian, M. & Ye, S. Allosteric regulation in NMDA receptors revealed by the genetically encoded photo-cross-linkers. *Sci Rep* **6**, 34751 (2016).

164. Klippenstein, V., Hoppmann, C., Ye, S., Wang, L. & Paoletti, P. Optocontrol of glutamate receptor activity by single side-chain photoisomerization. *Elife* **6**(2017).

165. Matsubara, N., Billington, A.P. & Hess, G.P. How fast does an acetylcholine receptor channel open? Laser-pulse photolysis of an inactive precursor of carbamoylcholine in the microsecond time region with BC3H1 cells. *Biochemistry* **31**, 5507-14 (1992).

166. DiGregorio, D.A., Rothman, J.S., Nielsen, T.A. & Silver, R.A. Desensitization properties of AMPA receptors at the cerebellar mossy fiber granule cell synapse. *J Neurosci* **27**, 8344-57 (2007).

167. Levitz, J. et al. Mechanism of Assembly and Cooperativity of Homomeric and Heteromeric Metabotropic Glutamate Receptors. *Neuron* **92**, 143-159 (2016).

168. Eder, M., Zieglgansberger, W. & Dodt, H.U. Shining light on neurons--elucidation of neuronal functions by photostimulation. *Rev Neurosci* **15**, 167-83 (2004).

169. Shepherd, G.M. Circuit mapping by ultraviolet uncaging of glutamate. *Cold Spring Harb Protoc* **2012**, 998-1004 (2012).

170. Callaway, E.M. & Katz, L.C. Photostimulation using caged glutamate reveals functional circuitry in living brain slices. *Proc Natl Acad Sci U S A* **90**, 7661-5 (1993).

171. Denk, W. Two-photon scanning photochemical microscopy: mapping ligand-gated ion channel distributions. *Proc Natl Acad Sci U S A* **91**, 6629-33 (1994).

172. Khiroug, L., Giniatullin, R., Klein, R.C., Fayuk, D. & Yakel, J.L. Functional mapping and Ca2+ regulation of nicotinic acetylcholine receptor channels in rat hippocampal CA1 neurons. *J Neurosci* **23**, 9024-31 (2003).

173. Ko, K.W., Rasband, M.N., Meseguer, V., Kramer, R.H. & Golding, N.L. Serotonin modulates spike probability in the axon initial segment through HCN channels. *Nat Neurosci* **19**, 826-34 (2016).

174. Patterson, M.A., Szatmari, E.M. & Yasuda, R. AMPA receptors are exocytosed in stimulated spines and adjacent dendrites in a Ras-ERK-dependent manner during long-term potentiation. *Proc Natl Acad Sci U S A* **107**, 15951-6 (2010).

175. Lee, S.J., Escobedo-Lozoya, Y., Szatmari, E.M. & Yasuda, R. Activation of CaMKII in single dendritic spines during long-term potentiation. *Nature* **458**, 299-304 (2009).

176. Harvey, C.D., Yasuda, R., Zhong, H. & Svoboda, K. The spread of Ras activity triggered by activation of a single dendritic spine. *Science* **321**, 136-40 (2008).

177. Murakoshi, H., Wang, H. & Yasuda, R. Local, persistent activation of Rho GTPases during plasticity of single dendritic spines. *Nature* **472**, 100-4 (2011).

178. Hedrick, N.G. et al. Rho GTPase complementation underlies BDNF-dependent homo- and heterosynaptic plasticity. *Nature* **538**, 104-108 (2016).

179. Zhai, S., Ark, E.D., Parra-Bueno, P. & Yasuda, R. Long-distance integration of nuclear ERK signaling triggered by activation of a few dendritic spines. *Science* **342**, 1107-11 (2013).

180. Yasuda, R. Biophysics of Biochemical Signaling in Dendritic Spines: Implications in Synaptic Plasticity. *Biophys J* **113**, 2152-2159 (2017).

181. Schiller, J., Major, G., Koester, H.J. & Schiller, Y. NMDA spikes in basal dendrites of cortical pyramidal neurons. *Nature* **404**, 285-9 (2000).

182. Losonczy, A. & Magee, J.C. Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. *Neuron* **50**, 291-307 (2006).

183. Noguchi, J., Matsuzaki, M., Ellis-Davies, G.C. & Kasai, H. Spine-neck geometry determines NMDA receptor-dependent Ca2+ signaling in dendrites. *Neuron* **46**, 609-22 (2005).

184. Branco, T., Clark, B.A. & Hausser, M. Dendritic discrimination of temporal input sequences in cortical neurons. *Science* **329**, 1671-5 (2010).

185. Rall, W., Burke, R.E., Smith, T.G., Nelson, P.G. & Frank, K. Dendritic location of synapses and possible mechanisms for the monosynaptic EPSP in motoneurons. *J Neurophysiol* **30**, 1169-93 (1967).

186. Kauwe, G. & Isacoff, E.Y. Rapid feedback regulation of synaptic efficacy during high-frequency activity at the Drosophila larval neuromuscular junction. *Proc Natl Acad Sci U S A* **110**, 9142-7 (2013).

187. Li, D., Herault, K., Isacoff, E.Y., Oheim, M. & Ropert, N. Optogenetic activation of LiGluR-expressing astrocytes evokes anion channel-mediated glutamate release. *J Physiol* **590**, 855-73 (2012).

188. Zhang, Y.P., Holbro, N. & Oertner, T.G. Optical induction of plasticity at single synapses reveals input-specific accumulation of alphaCaMKII. *Proc Natl Acad Sci U S A* **105**, 12039-44 (2008).

189. Chiu, C.Q. et al. Compartmentalization of GABAergic inhibition by dendritic spines. *Science* **340**, 759-62 (2013).

190. Hayama, T. et al. GABA promotes the competitive selection of dendritic spines by controlling local Ca2+ signaling. *Nat Neurosci* **16**, 1409-16 (2013).

191. Yagishita, S. et al. A critical time window for dopamine actions on the structural plasticity of dendritic spines. *Science* **345**, 1616-20 (2014).

192. Olson, J.P. et al. Optically selective two-photon uncaging of glutamate at 900 nm. *J Am Chem Soc* **135**, 5954-7 (2013).

193. Amatrudo, J.M. et al. Wavelength-selective one- and two-photon uncaging of GABA. *ACS Chem Neurosci* **5**, 64-70 (2014).

194. Olson, J.P., Banghart, M.R., Sabatini, B.L. & Ellis-Davies, G.C. Spectral evolution of a photochemical protecting group for orthogonal two-color uncaging with visible light. *J Am Chem Soc* **135**, 15948-54 (2013).

195. Agarwal, H.K., Zhai, S., Surmeier, D.J. & Ellis-Davies, G.C.R. Intracellular Uncaging of cGMP with Blue Light. *ACS Chem Neurosci* **8**, 2139-2144 (2017).

196. Amatrudo, J.M., Olson, J.P., Agarwal, H.K. & Ellis-Davies, G.C. Caged compounds for multichromic optical interrogation of neural systems. *Eur J Neurosci* **41**, 5-16 (2015).

197. Herrera, C.G. & Adamantidis, A.R. An integrated microprobe for the brain. *Nat Biotechnol* **33**, 259-60 (2015).

198. Canales, A., Park, S., Kilias, A. & Anikeeva, P. Multifunctional Fibers as Tools for Neuroscience and Neuroengineering. *Acc Chem Res* **51**, 829-838 (2018).

199. Kokel, D. et al. Photochemical activation of TRPA1 channels in neurons and animals. *Nat Chem Biol* **9**, 257-63 (2013).

200. Noguchi, J. et al. In vivo two-photon uncaging of glutamate revealing the structure-function relationships of dendritic spines in the neocortex of adult mice. *J Physiol* **589**, 2447-57 (2011).

201. Font, J. et al. Optical control of pain in vivo with a photoactive mGlu5 receptor negative allosteric modulator. *Elife* **6**(2017).

202. Polosukhina, A. et al. Photochemical restoration of visual responses in blind mice. *Neuron* **75**, 271-82 (2012).

203. Tochitsky, I. et al. Restoring visual function to blind mice with a photoswitch that exploits electrophysiological remodeling of retinal ganglion cells. *Neuron* **81**, 800-13 (2014).

204. Tochitsky, I. et al. How Azobenzene Photoswitches Restore Visual Responses to the Blind Retina. *Neuron* **92**, 100-113 (2016).

205. Tochitsky, I., Trautman, J., Gallerani, N., Malis, J.G. & Kramer, R.H. Restoring visual function to the blind retina with a potent, safe and long-lasting photoswitch. *Sci Rep* **7**, 45487 (2017).

206. Tochitsky, I., Kienzler, M.A., Isacoff, E. & Kramer, R.H. Restoring Vision to the Blind with Chemical Photoswitches. *Chem Rev* **118**, 10748-10773 (2018).

207. Trusel, M. et al. Punishment-predictive cues guide avoidance through potentiation of hypothalamus-to-habenula synapses. *Neuron* (2019).

208. Levitz, J., Popescu, A.T., Reiner, A. & Isacoff, E.Y. A Toolkit for Orthogonal and in vivo Optical Manipulation of Ionotropic Glutamate Receptors. *Front Mol Neurosci* **9**, 2 (2016).

209. Durand-de Cuttoli, R. et al. Manipulating midbrain dopamine neurons and reward-related behaviors with light-controllable nicotinic acetylcholine receptors. *Elife* **7**(2018).

210. Szobota, S. et al. Remote control of neuronal activity with a light-gated glutamate receptor. *Neuron* **54**, 535-45 (2007).

211. Wyart, C. et al. Optogenetic dissection of a behavioural module in the vertebrate spinal cord. *Nature* **461**, 407-10 (2009).

212. Janovjak, H., Szobota, S., Wyart, C., Trauner, D. & Isacoff, E.Y. A light-gated, potassium-selective glutamate receptor for the optical inhibition of neuronal firing. *Nat Neurosci* **13**, 1027-32 (2010).

213. Caporale, N. et al. LiGluR restores visual responses in rodent models of inherited blindness. *Mol Ther* **19**, 1212-9 (2011).

214. Gaub, B.M. et al. Restoration of visual function by expression of a light-gated mammalian ion channel in retinal ganglion cells or ON-bipolar cells. *Proc Natl Acad Sci U S A* **111**, E5574-83 (2014).

215. Berry, M.H. et al. Restoration of patterned vision with an engineered photoactivatable G protein-coupled receptor. *Nat Commun* **8**, 1862 (2017).

216. Izquierdo-Serra, M. et al. Two-photon neuronal and astrocytic stimulation with azobenzene-based photoswitches. *J Am Chem Soc* **136**, 8693-701 (2014).

217. Gascon-Moya, M. et al. An Optimized Glutamate Receptor Photoswitch with Sensitized Azobenzene Isomerization. *J Org Chem* **80**, 9915-25 (2015).

218. Carroll, E.C. et al. Two-photon brightness of azobenzene photoswitches designed for glutamate receptor optogenetics. *Proc Natl Acad Sci U S A* **112**, E776-85 (2015).

219. Cabre, G. et al. Rationally designed azobenzene photoswitches for efficient two-photon neuronal excitation. *Nat Commun* **10**, 907 (2019).

220. Ernst, R.J. et al. Genetic code expansion in the mouse brain. *Nat Chem Biol* **12**, 776-778 (2016).

221. Han, S. et al. Expanding the genetic code of Mus musculus. *Nat Commun* **8**, 14568 (2017).

222. Zheng, Y., Lewis, T.L., Jr., Igo, P., Polleux, F. & Chatterjee, A. Virus-Enabled Optimization and Delivery of the Genetic Machinery for Efficient Unnatural Amino Acid Mutagenesis in Mammalian Cells and Tissues. *ACS Synth Biol* **6**, 13-18 (2017).

223. Chen, Y. et al. Heritable expansion of the genetic code in mouse and zebrafish. *Cell Res* **27**, 294-297 (2017).

224. Tufail, Y. et al. Transcranial pulsed ultrasound stimulates intact brain circuits. *Neuron* **66**, 681-94 (2010).

225. Nimpf, S. & Keays, D.A. Is magnetogenetics the new optogenetics? *EMBO J* **36**, 1643-1646 (2017).

226. Lerch, M.M., Hansen, M.J., van Dam, G.M., Szymanski, W. & Feringa, B.L. Emerging Targets in Photopharmacology. *Angew Chem Int Ed Engl* **55**, 10978-99 (2016).

227. Laprell, L. et al. Photopharmacological control of bipolar cells restores visual function in blind mice. *J Clin Invest* **127**, 2598-2611 (2017).

228. Roska, B. & Sahel, J.A. Restoring vision. *Nature* **557**, 359-367 (2018).

229. Basbaum, A.I., Bautista, D.M., Scherrer, G. & Julius, D. Cellular and molecular mechanisms of pain. *Cell* **139**, 267-84 (2009).

230. Frank, J.A. et al. Photoswitchable fatty acids enable optical control of TRPV1. *Nat Commun* **6**, 7118 (2015).

231. Schonberger, M. & Trauner, D. A photochromic agonist for mu-opioid receptors. *Angew Chem Int Ed Engl* **53**, 3264-7 (2014).

232. Mourot, A., Herold, C., Kienzler, M.A. & Kramer, R.H. Understanding and improving photo-control of ion channels in nociceptors with azobenzene photo-switches. *Br J Pharmacol* **175**, 2296-2311 (2018).

233. Kim, T.I. et al. Injectable, cellular-scale optoelectronics with applications for wireless optogenetics. *Science* **340**, 211-6 (2013).

234. Montgomery, K.L. et al. Wirelessly powered, fully internal optogenetics for brain, spinal and peripheral circuits in mice. *Nat Methods* **12**, 969-74 (2015).

235. Park, S.I. et al. Soft, stretchable, fully implantable miniaturized optoelectronic systems for wireless optogenetics. *Nat Biotechnol* **33**, 1280-1286 (2015).

236. Park, S.I. et al. Stretchable multichannel antennas in soft wireless optoelectronic implants for optogenetics. *Proc Natl Acad Sci U S A* **113**, E8169-E8177 (2016).

237. Shin, G. et al. Flexible Near-Field Wireless Optoelectronics as Subdermal Implants for Broad Applications in Optogenetics. *Neuron* **93**, 509-521 e3 (2017).

238. Jeong, J.W. et al. Wireless Optofluidic Systems for Programmable In Vivo Pharmacology and Optogenetics. *Cell* **162**, 662-74 (2015).

Box 1 | Applications in biomedicine

Photocontrollable drugs and receptors may open up exciting new opportunities for light-guided therapeutic interventions[206,226]. Light delivered at the appropriate wavelength and intensity could potentially trigger signals at specific time and places and with accurate dosing pattern, thereby reducing off-target effects. This concept has shown promise in animal models for two neurological applications: vision restoration and pain management.

Vision restoration

Degenerative retinal diseases are characterized by progressive loss of photoreceptors, ultimately leading to blindness. One therapeutic strategy for advanced retinal degeneration, after photoreceptors have been lost completely, is to photosensitize remaining retinal neurons. Photoswitchable blockers of voltage-gated ion channels[45,50-52] have been tested for their ability to photosensitize the blind retina in animal models of retinal degeneration[202-205,227]. The first generation elicited robust light responses in blind retinas ex vivo, and restored the pupillary light reflex and light-avoidance behavior in blind mice[202]. Newly developed, improved photoswitches that fully operate in the visible spectrum and that quickly relax back to *trans* in darkness were later designed, eliminating the need for two different wavelengths of light (including potentially damaging UV light) for on and off control. These molecules photosensitized blind retinas from mice and rats[203-205], and restored visual fear conditioning behaviors in blind mice[203]. One photoswitch, named BENAQ, photosensitized blind retinas for almost a month following a single intraocular injection of low micromolar photoswitch solution[205]. Interestingly, certain photoswitches do not photosensitize all retinal neurons indiscriminately[204], and therefore permit restoration of complex visual processing in the blind retina, such as ON and OFF light responses[227].

Completely restoring visual functions may still require cell-specific targeting of retinal neurons, for instance using optogenetic gene therapy. A phase II/II clinical trial with channelrhodopsin-2 for advanced retinitis pigmentosa is currently underway (clinical trial #NCT02556736)[228]. Meanwhile, other optogenetic technologies based on engineered mammalian receptors, which may be less immunogenic, are being preclinically evaluated. Expression of LiGluRs (included red-shifted versions) in retinal ganglion cells or ON-bipolar cells restored light sensitivity to blind retinas from mice and dogs[213,214]. A combination of two orthogonal systems, LiGluR and SNAG-mGluR2 (a light-controllable mGluR with a remotely-tethered photoswitch) improved ON/OFF responses and enhanced visual acuity[215]. Finally, the mGluR-melanopsin chimera opto-mGluR6, which, unlike LiGluRs does not require photoswitch

delivery, is another interesting alternative to restore ON responses in blind mouse retinas with moderate light levels[106].

Pain management

Many ion channels and receptors expressed at the periphery and/or centrally are involved in nociception[229], and represent obvious targets for focused and photo-titrated analgesia. Photo-controllable drugs potentially useful for controlling pain signaling include TRPV1 agonists and antagonists[58,230], TRPA1 agonists [199], μ opioid receptor agonists [231], $GABA_AR$ potentiators[71], blockers of voltage-gated sodium channels[52,232] and mGluR modulators[66-69,201]. Photochemical[41,231] or optogenetic[105] regulators of opioid signaling could potentially provide analgesia at peripheral targets, while avoiding brain pathways linked to addiction. Photoswitchable allosteric modulators of mGluRs - one an opto-PAM of mGlu4[69], and the other an opto-NAM of mGlu5[68] - function as photoreversible analgesics in behaving mice, at peripheral nerve endings and in the amygdala[68,69]. Photoreversible local anesthetics (QAQ and QENAQ) also demonstrated spatio-temporal and phototitrable control of pain signaling in behaving rodents[52,232]. In addition, QAQ and QENAQ only enter and silence TRPV1-expressing (that is, pain-sensing) neurons, minimizing undesirable off-target effects on other sensory neurons.

Outlook

A major hurdle for implementing optopharmacology in clinical settings is light delivery to precise locations in the body, the eye being an obvious exception. Recent advances in preclinical developments have generated light-delivery devices that are miniaturized, injectable, programmable and wirelessly controlled, affording efficient remote photocontrol and minimal damage to neural tissue[233-237]. Concomitant local drug delivery is also possible with wireless opto-fluidic probes that combine a micro-LED with soft microfluidic delivery systems[238]. In parallel, photochemicals that respond to deeper-penetrating red or infra-red light[84] have been developed, possibly eliminating the need for LED implantation. Despite rapid technological advances, optopharmacological approaches are still far from ready to use in the clinic. Tolerability and reduced invasiveness to liberate the subject's mobility remain significant challenges for clinical success.

Figure Legends

Fig. 1 l: Main strategies to endow light-sensitivity to neuronal receptors and ion channels.

a l Chemical approaches are based exclusively on modified ligands that are rendered responsive to light (hv). No modification of the protein target is required. With caged compounds, light triggers the release of biologically-active ligands. Photoswitchable ligands reversibly alternate between active and inactive forms using two different wavelengths of light. Photolabels bind a target receptor and upon light irradiation from a covalent link with the protein binding site. Contrasting with photoswitchable ligands, caged compounds and photolabels act irreversibly.

b l Genetic approaches are based on the insertion of genetically encoded light-sensitive protein modules in the target protein of interest. Color coding: light and dark grey represent two different states (e.g. inactive and active) of the protein. Light-sensitivity is endowed by photosensitive co-factors (such as retinal or flavin) that are endogenously present in mammalian cells. Opsin chimeras consist in the fusion between a light-sensitive opsin (grey) and a light-insensitive G-protein coupled neurotransmitter receptor (black). Illumination triggers the conversion from 11-*cis* to 11-*trans* retinal, which causes conformational change in the fusion protein and its activation. In LOV-domain chimeras, light triggers unfolding of the LOV domain, which directly or indirectly modulates protein function. In CALI, light activates the flavin chromophore and generates reactive oxygen species, which results in irreversible inactivation of nearby proteins (shown in white with dashed borders).

c l Hybrid or chemogenetic approaches are based on a photosensitive synthetic chemical and its genetic attachment to or incorporation within the target protein. Different types of light-sensitive unnatural amino-acids (UAAs) can be incorporated into proteins: caged, photocrosslinking (i.e. photolabels), or photoswitchable (i.e. alternating between two configurations upon illumination with different wavelenghts) as depicted. Photoswitchable tethered ligands can be covalently attached to receptors in two ways: either to a cysteine-substituted site usually through maleimide-sulfhydryl chemistry (as depicted), or to a self-labeling protein tag (not depicted), resulting in both cases in reversible control of protein activity. In nanotweezers, a bis-maleimide photoswitch bridges two cysteine mutants. Light-induced conformational changes of the photosensitive moiety exert mechanical forces on the protein, potentially triggering its activation in the absence of ligand. Note that caged compounds, photolabels and CALI are unidirectional while photoswitches (both synthetic and natural) allow for bidirectionality.

Fig. 2 | Photocontrol of ion channel and receptor biophysics and pharmacology.

a | With appropriate optopharmacological tools, light can be used to directly activate receptors or ion channels (agonist), to inhibit them (antagonist or negative allosteric modulator (NAM)), or to positively modulate their function (positive allosteric modulator (PAM)).

b | Activity of the target receptor or ion channel can be adjusted in a graded manner using different light intensity and/or wavelength. The graph illustrates various levels of photo-antagonism associated with changes in light intensity or wavelength.

c | Photomodulation of NMDA receptors (NMDARs) using photoswitchable amino acids (PSAAs). Left: Chemical structures of *trans* and *cis* PSAA in the context of a protein. The azobenzene moiety is highlighted in shaded grey. Right, upper part: Schematic representation of the mechanism of NMDAR photomodulation. The PSAA is incorporated in the GluN1 subunit (dark grey), close to the GluN2 subunit (light grey). GluN1 binds glycine (orange), whereas GluN2 binds glutamate (red). Right, lower part: Illumination with 365 nm light isomerizes PSAA to *cis*, a conformational change sufficient to destabilize glycine binding (that is, leading to a decrease in glycine affinity). This change results in a reduction of current amplitude during agonist applications (Glu, glutamate; Gly, glycine) (left trace) and in an acceleration in deactivation kinetics upon glycine washout (right trace; current normalized). I, current amplitude; $I_{norm.}$, normalized current amplitude; t, time.

d | Photocontrol of kainate receptors using photoswitchable tethered ligands (PTLs). Left: Chemical structures of the PTL maleimide azobenzene glutamate (MAG) in the *trans* and *cis* configurations. The azobenzene moiety is highlighted in shaded grey. Right, upper part: Schematic representation of heteromeric GluK2/K5 kainate receptors. MAG is covalently attached to an engineered cysteine residue on the GluK2 subunit (light grey). Under 380 nm light, MAG adopts its *cis* configuration allowing the glutamate moiety to dock in the agonist binding pocket. Right, lower part: Heteromeric kainate receptors conjugated with two PTLs can be directly activated with 380 nm light which isomerizes the photoswitch to *cis*, and deactivated with 500 nm light which reverts the photoswitch to *trans* (left trace). In such conditions, no or little desensitization is observed. In contrast, when two agonists (pale red) are pre-bound selectively to GluK5 subunits, photoswitching leads to full receptor occupancy and almost complete receptor desensitization (right trace). Part **c** is adapted from ref. [164]. Part **d** is adapted from ref. [153].

Figure 3: Optopharmacology for subcellular neuronal studies.

a. Functional receptor mapping. Left: Chemical structure of MNI glutamate and photorelease of glutamate following either one-photon (1P) or two-photon (2P) illumination. Right: Two-photon uncaging of glutamate at different spots along the dendrite for functional glutamate receptor mapping. Receptor activity is revealed using whole-cell patch-clamp recordings in voltage-clamp mode. I, current amplitude; t, time.

b. Compartment-specific blockade of HCN channels using the photoswitch AAQ. Left: Chemical structures of *trans* and *cis* AAQ. Middle: Schematic representation of photoreversible HCN blockade using AAQ. Right: Probability of spiking is increased when HCN channels are blocked in the axon initial segment (AIS), but probability of spiking is decreased when these channels are blocked in the soma. Neuronal excitability is measured using whole-cell patch-clamp recordings in current-clamp mode. Vm, membrane potential; t, time.

c. Single-spine structural LTP induced using two-photon (2P) uncaging of glutamate or one-photon (1P) activation of light-controllable NMDA receptors (LiGluN) expressed in transfected neurons (green). The left and middle cartoons show how illumination leads to LiGluN activation, while the right cartoon depicts the resulting long-lasting changes in dendritic spine morphology. The graph on the right illustrates the evolution of the spine volume as a function of time before and after LTP induction (red arrow). Part **a** is adapted from ref. [23]. Part **b** is adapted from ref. [173]. Part **c** is adapted from ref. [24] and ref. [147].

Figure 4: Optopharmacology for cell- and receptor-specific interrogation of synaptic physiology. In all panels, the cell selectively expressing the photosensitized receptors is shown in green. **a I** Optical control of neurotransmitter release using light-controllable metabotropic glutamate receptors (LimGluRs) expressed in axon terminals. Photo-antagonizing LimGluR with 380 nm light decreases neurotransmitter release and post-synaptic currents (bottom traces). I, current amplitude; t, time.

b I Optical control of post- and extra-synaptic currents. Phasic (synaptic) and tonic (extra-synaptic) $GABA_A$ receptor-mediated inhibitory currents (bottom traces) can be photo-antagonized under 380 nm light using α1- and α5-containing light-inhibitable $GABA_A$ receptors (LiGABARs), respectively. I, current amplitude; t, time.

c I Optical control of gliotransmission using light-activatable glutamate receptors (LiGluRs) expressed in astrocytes. When LiGluRs are activated with pulses of 380 nm light, intracellular

calcium concentration increases (bottom trace) triggering non-vesicular release of glutamate. ΔF/F, changes in calcium-dependent fluorescence; t, time. Part **a** is adapted from ref. [146]. Part **b** is adapted from ref. [150]. Part **c** is adapted from ref. [187].

Fig. 5 | Optopharmacology for behavioral studies

a | In vivo optical manipulation of GPCR signaling with opto-XRs. A guide cannula for concomitant photocontrol and electrical recording is depicted. Inset: Light-stimulation of opto-β2AR activates downstream Gαs pathway in transduced neurons, leading to an increase in intracellular cAMP and Ca^{2+} concentrations, to the phosphorylation of ERK, and eventually to an increase in cellular excitability.

b | In vivo optical control of neurotransmitter receptors and associated behaviors with PTLs. The cannula guide allows for local delivery of the photoswitch and light as well as for electrical recordings. Inset: Schematic showing conditional expression of LinAChRs in dopamine (DA) neurons of the ventral tegmental area (VTA) using a Cre-dependent expression system (AM, unpublished data). In this scenario, LinAChRs are absent in other neurons of the VTA or in cholinergic afferents from extra VTA regions, allowing acute disruption of nicotinic transmission at the post-synaptic level. The cell selectively expressing LinAChRs is shown in green. In the original study[209], LinAChRs were non-selectively expressed in both DA and non-DA cells of the VTA. Top right: Spontaneous activity of VTA DA neurons is reduced under 380 nm light, when LinAChRs are photo-antagonized. Bottom right: Behavioral experiment using the nicotine-induced conditional place preference test. Preference to nicotine is reversibly disrupted under 380 nm light. Part **a** is adapted from ref. [102]. Part **b** is adapted from ref. [209].

Glossary

Cis-to-*trans* isomerization

Photostationary state

Metabotropic and ionotropic receptors

Singlet oxygen generators

Genetic code expansion

Allosteric modulation

Acronyms (in addition to acronyms of optopharmacological tools listed in Table 1)

CALI: Chromophore-Assisted Laser Inactivation

CRY2: Cryptochrome 2

FAD: Flavin Adenine Dinucleotide

FMN: Flavin Mononucleotide

LOV: Light-Oxygen-Voltage

MAG: Maleimide Azobenzene Glutamate

Opto-NAM: photoswitchable negative allosteric modulator

Opto-PAM: photoswitchable positive allosteric modulator

Opto-XR: light-controllable G-protein coupled receptor

PAL: PhotoAffinity Label

PL (or PCL): Photoswitchable Ligand (or PhotoChromic Ligand)

PORTL: Photoswitchable Orthogonal Remotely Tethered Ligands

PSAA: photoswitchable unnatural amino acid

PTL: Photoswitchable Tethered Ligand

UAA: Unnatural Amino Acid

Table 1 | Main optopharmacological tools used in neurophysiology.

All methods, either purely chemical, purely genetic or hybrid, have strengths and weaknesses. Key advantages of each approach are amply discussed in the Main Text, whereas main drawbacks are listed in the far right column. Choosing one approach or the other depends on the biological problem at hand, and on the genetic tractability of the target protein.

Tool	Name (full definition)	Action	Refs	Limitations
Chemical				
Caged compounds	MNI-Glu (4-methoxy-7-nitroindolinyl-caged-L-glutamate)	Activates glutamate receptors in UV light	[23]	- Requires chromophore delivery - Irreversibility - High concentrations required (potential off target effects, in vivo use limited) - Should be inert and stable in darkness
Diffusible Photoswitches	AAQ (acrylamide azobenzene quaternary ammonium)	Blocks voltage-gated K^+ and HCN channels in dark or green light	[45,50,173,203]	- Requires chromophore delivery - Activity difference between the two isomers can be limited (no all-or-none effects) - Require UV illumination (although red-shifted versions
	DENAQ (diethyl aminoazobenzene quaternary ammonium)	Blocks voltage-gated K^+ and HCN channels in dark	[51,203,204]	
	BENAQ (benzyl ethyl aminoazobenzene quaternary	Blocks voltage-gated K^+ and HCN channels in dark	[51,203,204]	

	ammonium)			available)
	QAQ (quaternary ammonium azobenzene quaternary ammonium)	Blocks voltage-gated K^+, Na^+ and Ca^{2+} channels of TRPV1-expressing neurons in dark or green light	[45,52,232]	
	QENAQ (quaternary ammonium aminoazobenzene quaternary ammonium)	Voltage-gated K^+, Na^+ and Ca^{2+} channels in dark	[232]	
	LOGO (light-operated GIRK-channel opener)	Activates GIRK channels in dark	[55]	
Photo-affinity labels	ANQX (6-azido-7-nitro-1,4-dihydroquinoxaline-2,3- dione)	Inactivates AMPA receptors in UV light	[93]	- Requires chromophore delivery - Requires UV light
Genetic				
Opto-XRs	Opto-α1AR Opto-β2AR	Activates adrenergic receptors (α1 and β2) in blue light	[100-102]	- Requires gene delivery - Fusion proteins do not bind cognate neurotransmitters - Applicability restricted to GPCRs
	Opto-A2A	Activates adenosine receptors (A2A) in blue light	[103]	
	Opto-MOR	Activates opioid receptors (μ) in blue light	[104,105]	

	Opto-mGluR6	Activates metabotropic glutamate receptors (mGlu6) in blue light	[106]	
	Opto-D1R	Activates dopamine receptors (D1) in blue light	[107]	
	CT-5HT	Activates serotoninergic receptors (5HT2c and 5HT1a) in blue light	[108-110]	
	Opto-GPR1–183	Activates orphan receptors in blue light	[111]	
LOV or CRY2 domains	Lumitoxin	Blocks voltage-gated K^+ channels (Shaker, Kv1.1, Kv1.2) in dark	[113]	- Requires gene delivery - Slow on and off kinetics (min - hours) - Relatively large protein domain (>12 kD)
	BLINK1 (blue-light–induced K^+ channel 1)	Activates small viral K^+ channels Kcv in blue light	[121]	
CALI	InSynC (inhibition of synapses with CALI)	Inactivate SNARE proteins (VAMP2 and Synaptophysin) with blue light	[126]	- Requires gene delivery - Irreversibility - Inactivation only (no activation)
Hybrid				
Tethered photoswitches	SPARK (synthetic photoisomerizable azobenzene-regulated K^+	Blocks voltage-gated (Shaker, Kv1.3, Kv3.1, Kv3.4, Kv7.2) and	[136,144]	- Requires gene + photoswitch delivery

	channel)	calcium-activated (SK2) K^+ channels in dark		- Stability of the *cis* configuration - Restricted to solvent-accessible sites (usually extracellular) - Potential for unspecific cysteine conjugation (yet no adverse effect observed so far, and see PORTL for alternative, orthogonal bioconjugation) - Requires UV illumination (although red-shifted versions available)
	TREKLight (light-gated TREK channel)	Blocks two-pore domain (K2P) K^+ channels (TREK1) in dark	[145]	
	LiGLuR (light-gated ionotropic glutamate receptor)	Activates kainate receptors (GluK2) in UV light (alternative version using blue, yellow or red light exist)	[80,81,137]	
	LiGluN (light-gated NMDA receptor)	Activates or inhibit NMDA receptors (GluN1, GluN2A, GluN2B) in UV light	[147]	
	LimGluR (light-gated metabotropic glutamate receptor)	Activates of inhibit metabotropic glutamate receptors (mGlu2, mGlu3 and mGlu6) in UV or blue light	[146,218]	
	SNAG-mGluR (SNAP-tagged-azobenzene-glutamate receptor)	Activates metabotropic glutamate receptors (mGlu2, mGlu7, mGlu8) in UV or blue light	[138,139]	
	LinAChR (light-controlled nicotinic acetylcholine receptor	Activates of inhibit nicotinic acetylcholine receptors (β2 and β4) in UV light	[148]	

	LiGABAR (light-controlled GABA type A receptor)	Antagonize $GABA_A$ receptors (α1-6) I, UV or dark	[149,150]	
	LiDAR (light-controlled dopamine receptor)	Inhibit dopamine receptors (D1 and D2) in UV light	[152]	
	Light-activated P2X receptors	Activates P2X receptors in UV or green light	[151]	
Nanotweezers	Light-controlled P2X receptors and Acid-sensing ion channels (ASICs)	Activates P2X receptors or ASICs in UV or green light	[141-143]	- Requires gene + photoswitch delivery - Specificity and toxicity not determined in vivo - Applicability to other receptor types unknown - May hinder ion flow and kinetics
UAAs	PSAA (azobenzene-based photoswitchable amino acid)	Any (photoswitch)	[164]	- Requires gene + UAA delivery - Only UV-sensitive probes available - Expression level of target protein potentially affected
	AzF (azido-phenylalanine)	Any (photocrosslinker)	[131,162]	
	BzF (benzoyl-phenylalanine)	Any (photocrosslinker)	[131,161]	

Fig 1

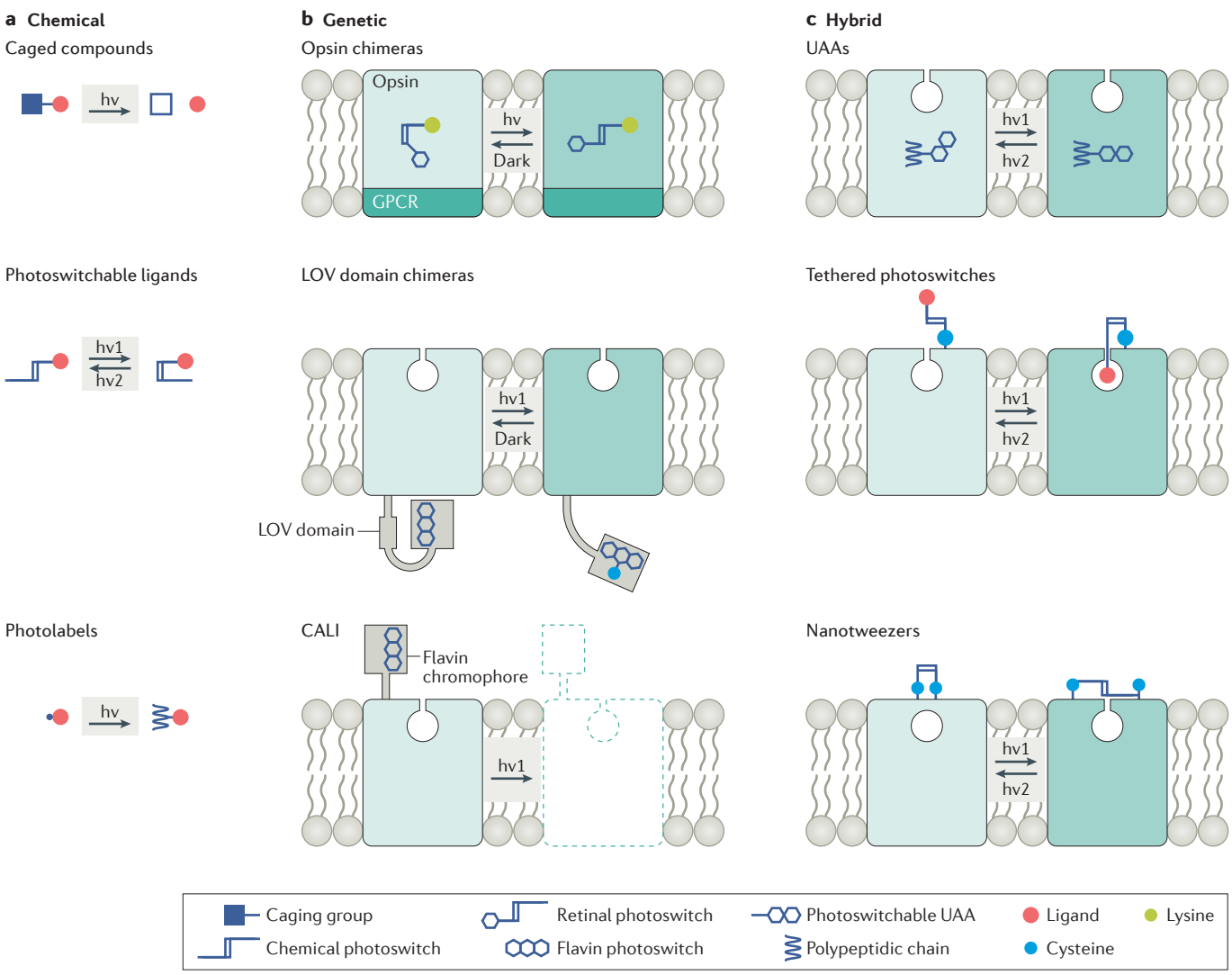
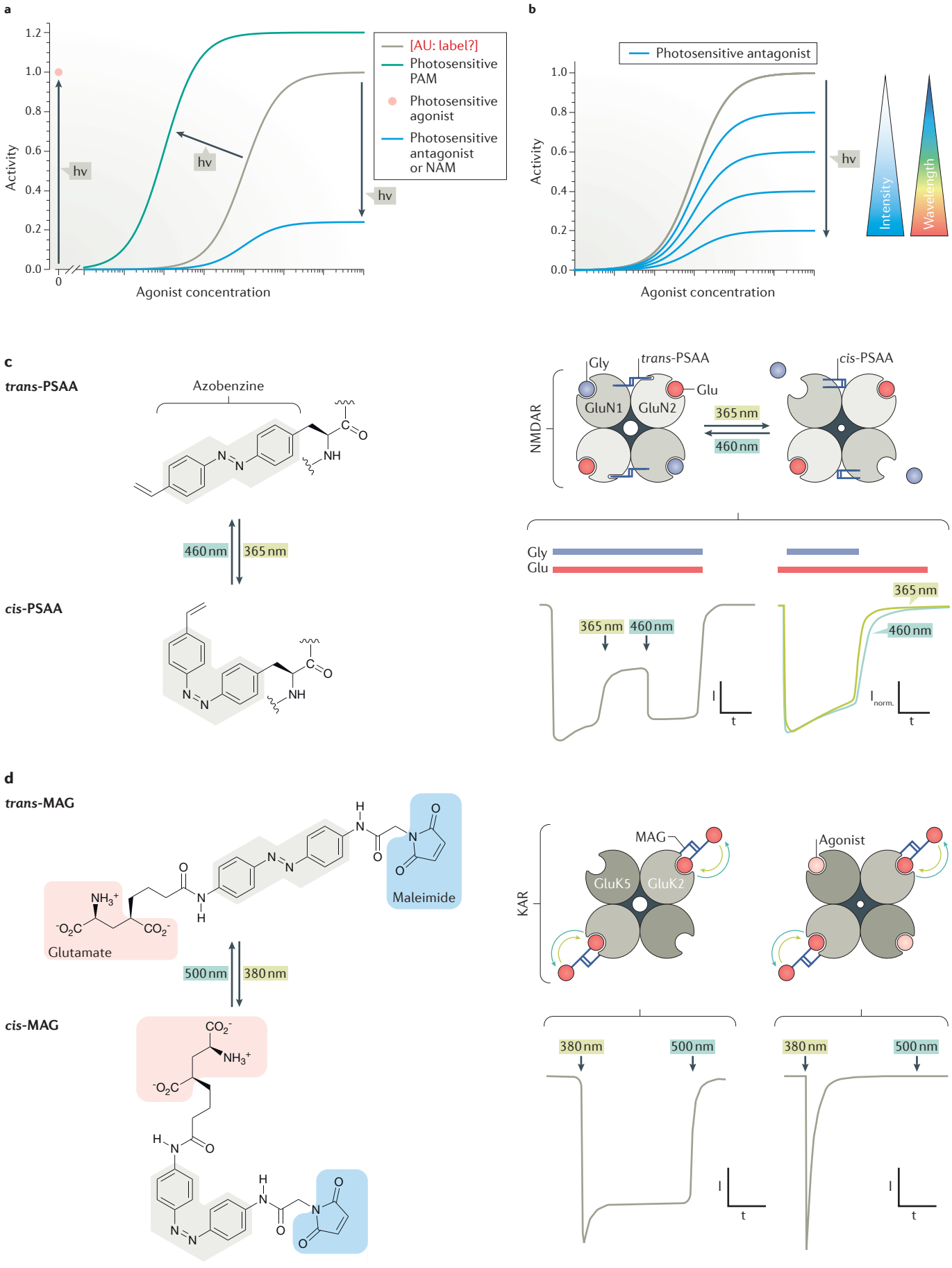

a Chemical
Caged compounds
hv
Photoswitchable ligands
hv1
hv2
Photolabels
hv
b Genetic
Opsin chimeras
Opsin
GPCR
hv
Dark
LOV domain chimeras
hv1
Dark
LOV domain
CALI
Flavin chromophore
hv1
c Hybrid
UAAs
hv1
hv2
Tethered photoswitches
hv1
hv2
Nanotweezers
hv1
hv2
Caging group
Chemical photoswitch
Retinal photoswitch
Flavin photoswitch
Photoswitchable UAA
Polypeptidic chain
Ligand
Cysteine
Lysine

Fig 2


a
Activity
Agonist concentration
hv
[AU: label?]
Photosensitive PAM
Photosensitive agonist
Photosensitive antagonist or NAM
b
Photosensitive antagonist
Intensity
Wavelength
c
trans-PSAA
Azobenzine
460 nm
365 nm
cis-PSAA
Gly
trans-PSAA
Glu
cis-PSAA
NMDAR
GluN1
GluN2
d
trans-MAG
Maleimide
Glutamate
cis-MAG
MAG
Agonist
KAR
GluK5
GluK2
380 nm
500 nm

NRN-18-103 Paoletti 23|05|19

Fig 3

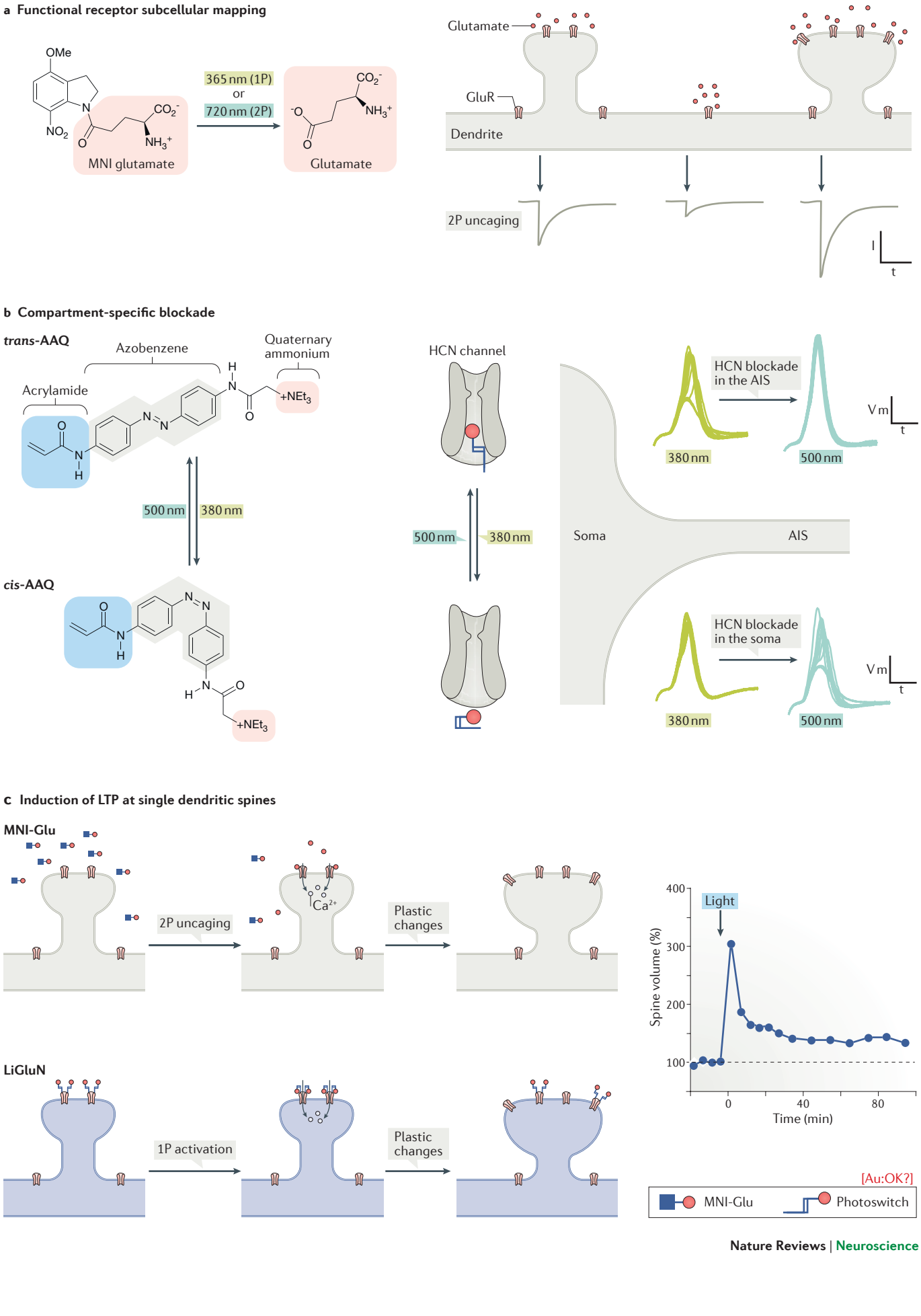
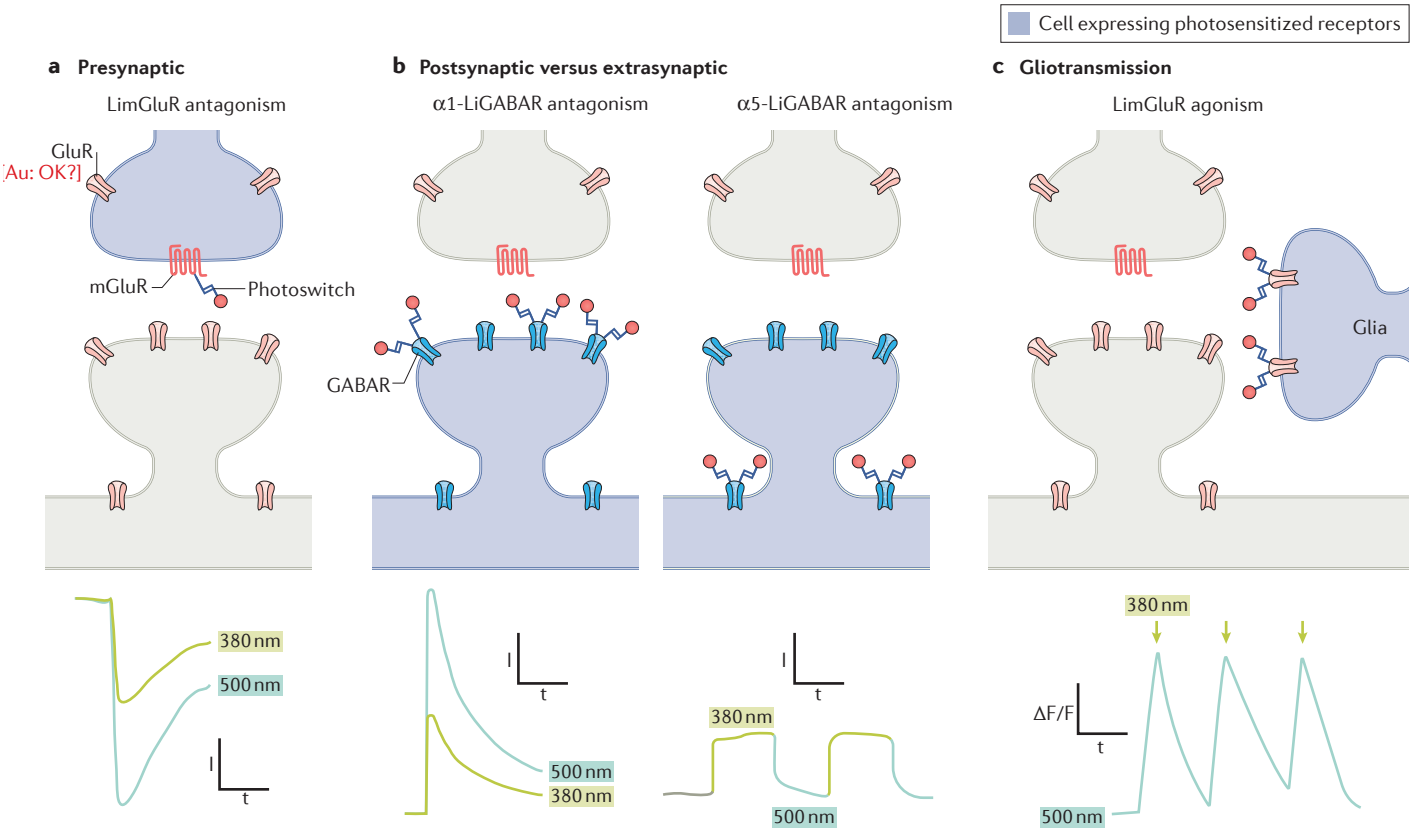
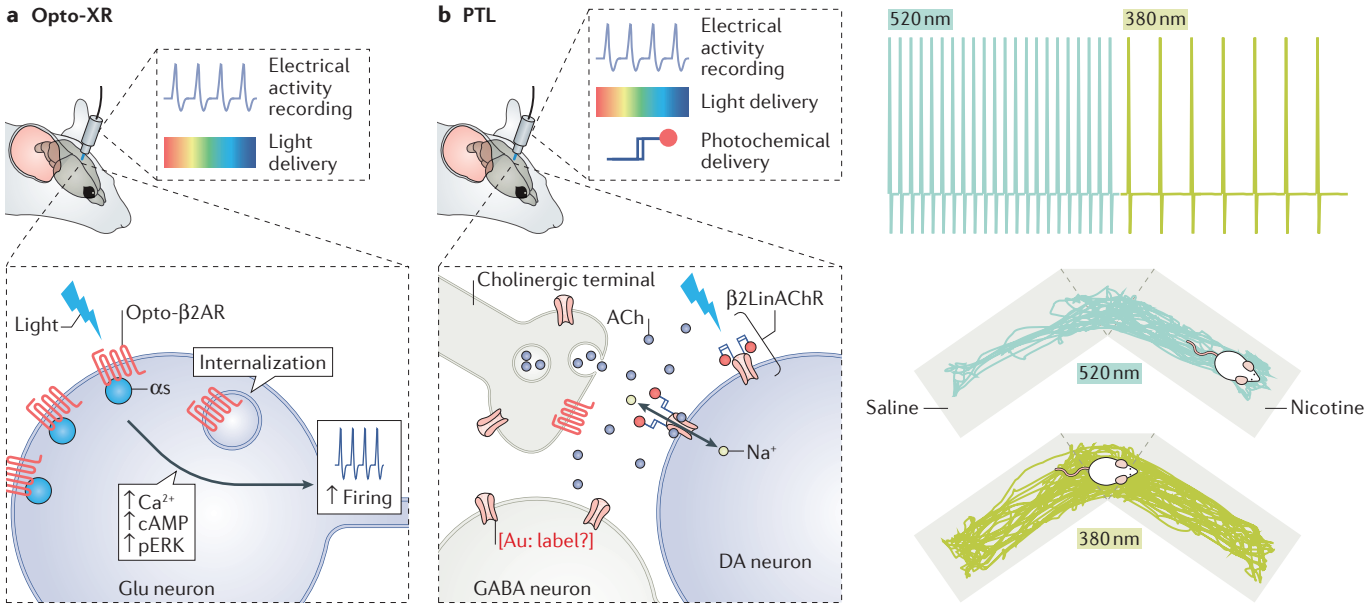


Fig 4



Nature Reviews | Neuroscience

Fig 5



Nature Reviews | Neuroscience