











Roadmap for direct and indirect translation of optogenetics into discoveries and therapies for humans

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Optogenetics has transformed basic research on neural circuitry, led to diverse experimental insights into human brain function and dysfunction, and opened pathways for clinical translation based on new understanding of how specific cell types contribute to cognition and behavior. Many of these translational pathways do not rely on the direct application of optogenetics in humans, but rather develop and advance other treatment modalities by leveraging causal knowledge derived from optogenetic circuit neuroscience. However, a recent proof-of-principle study—showing that optogenetics applied directly to the human central nervous system can treat blindness—underscores not only the curative potential but also the need for careful ethical consideration in the extension of direct optogenetic intervention to other disorders. Here, we review relevant considerations—including the selection of clinical indications, identification of molecular and optical strategies for specificity, and navigation of safety and regulatory issues—that together inform the development of optogenetic translation targeting cells and circuits that have been causally implicated through optogenetic discoveries.

Optogenetics¹ enables light-mediated, temporally precise gain- or loss-of-function control of specific cell types or even individual cells, which can be targeted by various features such as location, wiring, gene expression and combinations thereof, within living animals. Modern optogenetic methods also allow for considerable diversity in light-targeting modalities (including implanted fiber optics, three-dimensional holographic illumination of defined neuronal ensembles and noninvasive wearable light-emitting diodes (LEDs)), timescales of intervention (ranging from milliseconds to chronic use) and controllability (with effect sizes tunable by rapid changes in the intensity of light delivered^{2,3}, including in fast closed-loop modes of operation). These features have enabled the general application of optogenetics beyond the nervous system as a fundamental tool for biology (with

>10,000 papers already reporting on discoveries made with optogenetics). Some recent examples in this rapidly expanding field involve the investigation of memory⁴, decision-making⁵ and heart-to-brain communication⁶. Exploring nature in this way has long represented the most important dimension of the optogenetic approach⁷.

In the course of this fundamental research^{3,4}, many neural circuit components that cause or alleviate neuropsychiatric symptoms have been identified, opening the door to empirically grounded pathways to new therapies^{8,9}. One such therapeutic pathway involves drugs or stimulation techniques that target specific cell populations that have been causally implicated in symptoms¹⁰ through optogenetics and its complementary methods. Upon establishing causal links, characterizing the relevant cells by gene expression facilitates the development of

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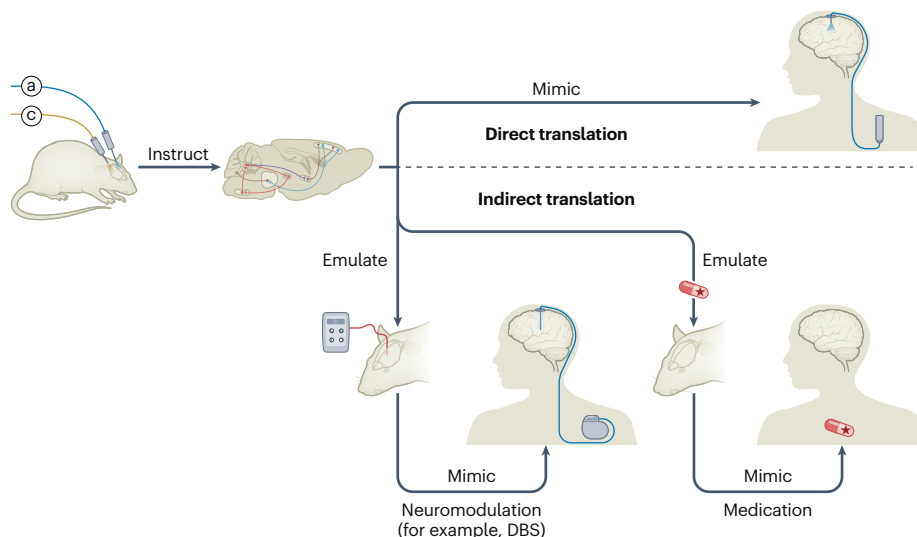


Fig. 1 | Direct and indirect translation of optogenetic circuit discoveries. Basic optogenetic studies guide the development of circuit insights in laboratory animals (for example, rodents) for specific neuropsychiatric symptoms (as an example, circuitry related to essential tremor is depicted as colored arrows superimposed on the mouse brain). When optogenetic methods are directly applied in human treatment (top pathway in the figure), this is known as

‘direct translation’. Conversely, ‘indirect translation’ (also known as immediate or optogenetically inspired translation) occurs when established treatment technologies, such as electrical DBS and/or pharmacological interventions, are developed to target the optogenetically defined cell population, leading to human applications based on these neuromodulatory and/or medication modalities (bottom pathways in the figure).

pharmacological treatments, while defining the causal cells by anatomy further aids in the implementation of neuromodulatory treatments (for example, deep brain stimulation (DBS), transcranial magnetic stimulation, transcranial direct current stimulation and magnetic resonance imaging-guided focal ultrasound¹¹). This immediate pathway to therapies might be termed ‘indirect clinical translation’ (Fig. 1), as it does not require the introduction of genes or light into patients but instead applies the new mechanistic knowledge from optogenetics using medical and neuromodulatory approaches¹² (Box 1). Complementing this immediate approach to therapy development, optogenetic discoveries are also guiding the advancement of basic insights into human brain function^{13,14}, which may yield even greater and longer-term dividends in terms of therapy development.

For direct translation of optogenetics, the delivered gene will typically encode a microbial opsin, such as a channelrhodopsin, from a broad family of seven-transmembrane light-activated ion channels derived from single-celled organisms¹⁵. Microbial and other potential clinical opsins¹⁶ share key clinical safety properties, including (1) sensitive control by visible (non-ultraviolet) light, (2) fast reversibility (in minutes or less) after light is turned off (light-off) and (3) no requirement for the infusion of chemical cofactors. Gene therapy for central nervous system (CNS) applications has advanced broadly^{17–20}. In the first published example of direct optogenetic therapy, the delivery of a channelrhodopsin gene and light to the retina of a blind patient with retinitis pigmentosa (Fig. 2) considerably ameliorated visual perceptual deficits²¹. None of the nine similarly treated patients have thus far experienced safety concerns²², and currently, there are at least four additional clinical trials for optogenetic vision restoration targeting different retinal cell types (NCT04945772, NCT05417126, NCT02556736 and NCT04919473).

Given these advances, along with expanding opportunities from the broad realm of optogenetic basic research (including major advances in the identification of cellular and dynamical principles that are conserved across mouse and human brains^{14,17–19}), we here consider the space of direct and indirect optogenetics for clinical translation. We explore aspects of indication selection, model validity, light delivery, opsin selection, cell type targeting, vector design, immune interactions, regulatory issues and, above all, ethical considerations^{20,23}. While

basic science investigations and immediate clinical pathways for indirect optogenetics will remain of paramount importance, opportunities for direct optogenetics will continue to emerge for patients with neuropsychiatric diseases. Effective communication and cooperation across our clinical and scientific communities are vital, as ideas from circuit analysis continue to provide empirical grounding and inspiration for clinical approaches.

Selecting indications

Common neuropsychiatric disorders, such as depression, anxiety and addiction, contribute enormously to morbidity, mortality and disability worldwide²⁴; however, they need not be the earliest suitable target indications. Factors favoring leading-edge exploration include (1) knowledge of circuit elements to target, (2) accessibility of those circuit elements to genes and light, (3) understanding of the modulation required (excitation, inhibition or plasticity-inducing), and (4) greater benefits and/or reduced risks relative to the standard of care. Targeting retinal degeneration in end-stage retinitis pigmentosa was an outstanding first choice, as the dysfunctional cells are precisely known and the retinal circuitry downstream of these lost photoreceptors remains intact²¹. Here and in other diseases without a current curative therapy, the exploration of new therapies (if not imposing undue risk) is ethically well-grounded²⁵. Neurodegenerative diseases may generally represent interesting indications, as competing curative therapies are lacking, compassionate use principles apply²⁵ and targeting can include healthy circuits that are dysfunctional only because of neuronal loss elsewhere; for example, neuromodulation in the healthy subthalamic nucleus can be effective for parkinsonian symptoms, although the degenerating cells are located elsewhere (in the substantia nigra).

Most common neuropsychiatric disorders present challenges for direct optogenetics consideration without knowledge of intervention targets; however, basic scientific discovery has rapidly identified circuit-based mechanisms for specific symptoms, and the clinical need is both very high and growing. The size of treatable patient populations becomes relevant when considering the reduction of human suffering, as well as when evaluating whether an approach will be practically viable and sustainable. For these

BOX 1

Indirect translation: examples inspired by optogenetic circuit analysis

In one approach to indirect translation, laboratory models of targeted optogenetically induced symptoms provide a precise and reproducible new testing ground for candidate medications, bringing reliability and specificity to a field in which animal models have often been inconsistent. In another approach, laboratory screening of optogenetic treatments for previously validated symptom models provides causal grounding that the targeted cells matter for modifying the symptoms under consideration. With either approach, the discovery of cell types that cause or correct symptoms opens the door to deep molecular and anatomical analyses of known relevant cell types, with the identification of new targets for medications (or neuromodulatory therapies) now resting on a causal foundation.

The optogenetic development of new treatments for autism may involve targeting both circuitry involved in social motivation¹⁹¹ and circuitry involved in social cognition¹³⁴. As one example, serotonin 5-HT_{1B} receptor agonists have been shown to promote accumbens–raphe interactions involved in social motivation and reward¹⁹², but they could also favorably shift (by lowering) the excitation–inhibition balance in prefrontal cortex circuitry, an effect thought to enhance social cognition in autism¹³⁹. This approach is rooted in optogenetic discovery work in animal models^{134,139,192,193}, and both effects are predicted to arise from 5-HT_{1B} receptor agonism owing to preferential expression of the inhibitory 5-HT_{1B} receptor in excitatory axons, including those in the prefrontal cortex and accumbens^{194,195}. This principle of cell type-specific modulation of axonal output, while minimizing disruption of information processing in the dendrites and cell body, could have broader implications beyond autism (for example, in depression and anxiety) as well as in subcortical and cortical projection neurons^{196,197}.

Optogenetics may also guide the targeting of causal regions or projections through already approved interventional technologies or further develop established neuromodulation modalities using optogenetically inspired regimens, including new DBS protocols. In mouse models of dopamine-dependent psychiatric disorders, including substance use disorder, evidence implicates drug-evoked synaptic plasticity in supporting addiction-like behavior; for example,

repetitive exposure to cocaine leading to locomotor sensitization is driven by potentiation of cortico–accumbens glutamate synapses in dopamine D1R-expressing spiny projection neurons⁵⁸. While locomotor sensitization could be readily reversed using an optogenetic *in vivo* depotentiation protocol to restore baseline transmission, restoration could not be achieved with electrical stimulation. This effect was discovered to derive from a particular nonspecificity of electrical stimulation (also boosting dopamine release, which, by activation of D1Rs, blocked depotentiation). Only the combination of a chemical D1R antagonist and electrical stimulation yielded the desired effect, successfully emulating the optogenetic protocol⁵⁸.

Finally, in mouse models of Parkinson's disease, the identification of two distinct cell types in the external globus pallidus—parvalbumin and LHX6 neurons—led to the discovery of an optogenetic stimulation protocol with which the selective stimulation of parvalbumin neurons or inhibition of LHX6 neurons reduced symptoms for an extended period⁶¹. The optogenetically established neural circuitry of the external globus pallidus inspired the development of an electrical burst stimulation protocol (100 Hz for 200 ms every 1 s) that achieved cell type-specific neuromodulation and persistent therapeutic benefits in dopamine-depleted mice⁶¹. A pilot study of burst stimulation in patients with Parkinson's disease found this protocol to be safe and effective in an acute setting, warranting future studies to test for persistent therapeutic benefits extending beyond the termination of the acute stimulation¹⁹⁸.

These examples illustrate how optogenetics can be used to understand what should be done to ensure that refined pulse patterns produce the desired effect, medications recruit the relevant cells, or both. Similar workflows are in progress for symptoms related to schizophrenia, Alzheimer's disease-related psychosis and many others. The systematic acquisition of fundamental and causal neural circuit activity information using optogenetics thus informs the design of new medical treatments, involving proper consideration of the global features of brain complexity.

reasons, a transdiagnostic approach aimed at alleviating a specific debilitating symptom across several disorders, rather than addressing many diverse symptoms of a single disorder, may make the most sense¹⁰. The single-symptom approach also aligns well with spatially restricted targeting in direct optogenetics²⁶, which could benefit patients most when addressing a discrete symptom that may be initiated or mediated locally (for example, anhedonia, which is associated with depression, addiction and schizophrenia)⁹. Following the same logic, neurological diseases that are effectively monosymptomatic, such as essential tremor, may be appealing early options. The treatment of essential tremor has the advantage of allowing an immediate, objective readout to monitor efficacy²⁷, and focal epilepsies²⁸ and deafness^{29,30} present interesting possibilities as well. Other opportunities include neuropathic pain³¹, bladder and bowel dysfunction due to spinal cord injury³², and amyotrophic lateral sclerosis³³; these disorders are not well served by current therapies, involve cellular targets that are more accessible to genes and light, and may present a reduced risk owing to the existence of intervention sites residing outside the brain.

Preclinical disease models

The choice of any indication depends on the availability of a valid preclinical model to test interventions for safety and efficacy. For neuropsychiatric disorders, these tests are usually carried out first in small mammals (for example, mice or rats), sometimes followed by studies of large animals (for example, nonhuman primates (NHPs), pigs or sheep)³⁴. Testing in NHPs, which are genetically and anatomically closer to humans, can provide insights into safety³⁴; however, for efficacy, studies remain limited to diseases for which corresponding models exist. Moreover, NHP testing for neuropsychiatric diseases is associated with considerable regulatory and ethical burdens³⁵. Therefore, NHP testing is not an obligatory part of the translational workflow in general for new neuropsychiatric interventions, including optogenetic approaches. Instead, the requirements for model systems in direct optogenetics will vary depending (for a particular disease indication) on similarity to human beings in anatomy, target cell type, side effects, multisystem interactions (for example, immune), and the design of vectors and optics. The animal models used should replicate, as closely as possible, the targeted human symptoms in terms of affected brain

region, cell type (defined by gene expression and anatomy where possible) and/or behavioral deficits. These considerations further favor a symptom-focused approach rather than a disease-focused one for neuropsychiatric diseases, as animal models for individual symptoms, such as anhedonia or compulsive behavior, are more readily available than comprehensive multisymptom recapitulations of human disease^{9,10}.

The wiring of the peripheral nervous system is reasonably conserved between humans and mice, making certain disorders of this system suitable for early selection in direct optogenetics. Examples include regional pain syndromes such as peripheral neuropathy³⁶, reflex sympathetic dystrophy³⁷ and complex regional pain syndrome³⁸, as well as symptoms of impaired bowel and bladder control after spinal cord injury. By contrast, one domain in which NHP testing has been important is retinal optogenetics (outside the brain but within the CNS), as high-resolution vision in humans relies on a specialized central part of the retina called the fovea, which is present only in primate retinas. Furthermore, the cellular architecture of the fovea differs from that of the rest of the retina, and the penetration of gene therapy vectors to the fovea is different compared to other retinal regions^{39,40}.

Considerations of phylogenetic relatedness arise for the brain as well; the human cortex has considerably expanded relative to the cortices of mice or NHPs⁴¹. Yet, many key brain targets share conserved functional anatomy across mice and humans, thanks to ancestral structure–function principles that are common to all mammals⁴². Examples of disease-relevant anatomy conserved from rodent to human brains include the basal ganglia and motor control circuitry relevant to Parkinson's disease, as well as the reward and addiction circuitry (including dopamine afferents to the nucleus accumbens from the ventral tegmental area, as well as regulatory afferents to the nucleus accumbens from the neocortex⁴³). Prefrontal neocortical neurons, subcortical habenula neurons and brainstem dorsal raphe neurons also have distinct, conserved roles in modulating motivation and social interaction that are relevant to depression and autism^{44,45}. Extended amygdala structures, including the basolateral amygdala and the bed nucleus of stria terminalis, share conserved roles and wiring that are relevant to anxiety and post-traumatic stress disorder (PTSD)⁴⁶. Hypothalamic neurons have conserved roles in the implementation and competition of survival drives, including hunger, thirst, temperature regulation and sleep, all of which can become dysfunctional in neuropsychiatric disease states⁴⁷. As a final example, the retrosplenial cortex appears to have a conserved role (from mice to humans) in aligning the sense of self with the sense of the body and in interpreting the spatiotemporal properties of the environment, which are relevant to stress, trauma, epilepsy, intoxication, PTSD and psychotic disorders¹³.

Finally, an area of recent interest is accessing human CNS tissue for preclinical testing. Human CNS slices (for example, from cadaveric retinas or surgical resections in epilepsy or cancer) can be kept alive for hours or days in the laboratory⁴⁸ and used to verify that vectors with particular properties (viral serotype, cell type-specific promoter or opsin payload) express safely, efficiently and specifically in human tissue^{49,50}. Testing in human organoids (millimeter-scale structures derived from stem cells) is also possible if the organoid contains the cell type of interest⁵¹. There are drawbacks; for example, surgical samples provide brain region options limited by clinical resectability. Furthermore, the resected tissue may be atypical if it is diseased. Organoids also often represent early developmental stages and do not always mimic the structure and function of the adult human brain⁵². They are currently limited to subcentimeter size, as they do not develop complete vasculature, and thus cannot easily replicate important spatial aspects of light and vector penetration. However, recent methods for transplanting human organoids into the CNS of living rodents are beginning to address this issue⁵³. Given our limited capabilities for in vivo cellular-level studies of the human brain, these work-arounds enabling human tissue testing represent a valuable opportunity for direct optogenetics.

Choosing the stimulation protocol

Optogenetic interventions can be designed for acute effects as well as long-lasting neural plasticity^{54–59} (Fig. 3). In the former case, the therapeutic effect will be linked to stimulation timing, which is particularly appropriate when disease symptom-related neural activity is transient, as seen with epilepsy or certain pain syndromes. Stimulation may inhibit symptoms in patient-triggered protocols (for example, during pain flare-ups or pre-seizure aura) or closed-loop paradigms (for example, responding to detected pre-seizure electroencephalogram signals or orchestrating the sequences of smooth muscle contraction and relaxation necessary for urinary bladder voiding)⁵⁶. Eliciting activity-dependent neural plasticity (potentiation or depression of synaptic transmission) also aligns well with the temporal and cellular specificity properties of optogenetics^{57,58} and may provide substantial practical advantages. Lasting changes elicited by brief light stimuli minimize power consumption and reduce heating and phototoxicity side effects. Specific optogenetic stimulation protocols can be used to elicit synaptic plasticity (for example, long-term potentiation, long-term depression, depolarization suppression of inhibition and behavioral timescale plasticity-like protocols)^{57–59} in identified cells or projections, to reverse disease-like behaviors relevant to drug addiction, depression, anxiety and PTSD. Ultimately, practical optogenetic therapies may harness the brain's remarkable capability for plasticity in this manner.

For both acute and plasticity-inducing protocols, stimulation may interact with the natural dynamics of targeted neural circuitry. Representing a potential convergence of direct and indirect optogenetics for clinical translation, computational models along with modern cellular-resolution optogenetics have begun to provide insights into how externally delivered activity interacts with endogenous activity, as well as how focal changes in synaptic strength or neural excitability can alter the dynamics of networks that shape behavior^{54,60–62}. The exact form of stimulation delivered will also depend on the coding strategies of the targeted cells, which may differ between neuromodulatory systems (for example, ascending dopamine projections activating G-protein-coupled receptors (GPCRs)) and faster neurotransmission systems (for example, glutamatergic or GABAergic cortical, thalamic or striatal projections). Stimulation protocols for the former may involve coordinated or even synchronous recruitment of the targeted cells, while the latter may include strategies for biasing the excitability of targeted cells without directly driving population spiking, allowing for greater asynchrony.

Accessing specific cell types in human beings

Cell types, defined by gene expression, anatomy and function, are the elemental building blocks of all biological tissues, including nervous systems¹⁸. Exploring and leveraging this complexity by identifying and accessing the cell types that control symptom-relevant neural circuit activity helps guide both basic discovery and the translation of optogenetics.

Relevant for translational animal models, many cell types in the brain appear to be conserved across mammalian species (for example, from rodents to primates). Large-scale collaboration with single-cell transcriptomics has recently led to comprehensive cellular atlases spanning mouse, NHP and human brains, revealing both extraordinary diversity and substantial conservation across thousands of molecularly distinguishable cell types^{17–19,63}. By defining cell types using quantitative metrics for gene expression clustering, these atlases provide a foundation for the systematic investigation of neural circuit operation in health and disease. Optogenetic approaches are well suited for integrating these molecular typology datasets. Indeed, many years before the new atlases became available, a critical step in the development of optogenetics was creating generalizable and versatile opsin-targeting strategies based on cellular anatomy and wiring, cell type-specific gene expression, or both.

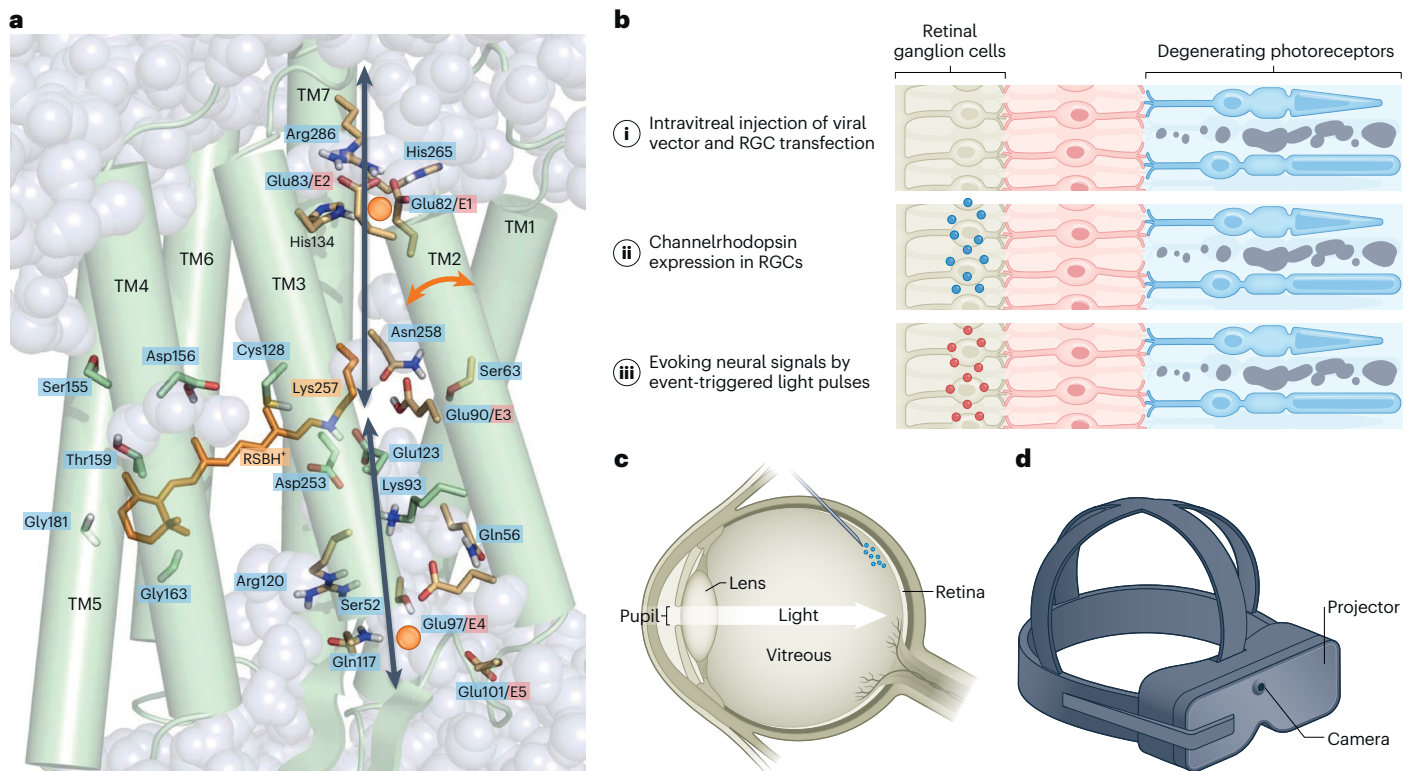


Fig. 2 Elements of direct optogenetic therapy. **a**, Light-activated mechanism for transmembrane ion conduction. Shown are the inner workings of a cation-conducting (excitatory) channelrhodopsin. The orange structure is the all-*trans* retinal chromophore that receives the photon and isomerizes to drive channel opening; the gray spheres represent water molecules; the green cylinders depict the transmembrane α -helices; the orange circles represent sodium ions; and the vertical arrows indicate the cation conduction pathway. **b**, Established strategy for direct optogenetic therapy: optogenetic vision restoration in retinitis pigmentosa. (i) AAV delivering the channelrhodopsin gene is injected into the patient's retina, leading to (ii) expression (represented by blue dots on the cell

surface) in retinal ganglion cells (RGCs). (Note that this disease involves the loss of photoreceptors, not ganglion cells.) (iii) Light delivery activates expressing cells (represented by red dots on the cell surface). **c**, Diagram illustrating the delivery route for the gene (indicated by the gray needle delivering viral particles) and light (indicated by the arrow following the physiological route through the pupil and lens to the retina at the back of the eye). **d**, Light is emitted at sufficient intensity by special goggles, which capture the environment through a camera and stimulate the retina through a projector to generate an image. Panel **a** adapted from ref. 15, AAAS.

For the former, the development of projection targeting allowed the optogenetic recruitment or inhibition of cells defined by anatomy alone^{64,65}. For example, to target cells with axonal projections from region A to region B, cell bodies in region A are transduced by injecting an opsin-expressing virus, while it is the axonal projections of those same cells in region B that are accessed by local light delivery (for example, with fiber optics)^{66,67}. This light- and gene-targeting method does not require transgenic animals and may thus be readily translated into direct optogenetic therapeutics. For example, axonal light delivery in humans could, in many cases (for example, regional pain syndromes), be provided to peripheral projection fields by wearable noninvasive LEDs, especially with the more recently discovered highly light-sensitive microbial opsins for excitation^{62,68,69} and inhibition^{70–72}. Opsin GPCRs may enable efficient inhibitory modulation when activated directly in the presynaptic compartment^{16,73}, as with many inhibitory microbial opsins (both allowing for projection-specific modulation; reviewed in refs. 74,75).

Recombinase-dependent opsin-expressing adeno-associated viruses (AAVs) were created to use with recombinase-expressing transgenic lines with recombinase expression (Cre or Flp) genetically restricted to particular cell types using large transcriptional regulatory regions that exceed the capacity of a virus. This approach later allowed for expanded dimensions of discovery through opsin expression conditioned on Boolean logic operations (AND, OR and NOT) with the presence or absence of multiple specifically expressed recombinases in behaving mammals^{66,77}. Although recombinase-expressing

transgenic lines are not relevant to human therapeutics, it may be possible to target human cells anatomically using these viral tools by delivering the recombinase-dependent viruses to one region and a recombinase-producing virus to another region⁷⁸ (especially if using one of the several AAV serotypes known to exhibit robust transduction of axons⁷⁹). More generally, translational targeting strategies benefit from the increasingly powerful use of cell type-specific promoter and enhancer elements that fit within viral vector genomes and confer cell type specificity; a recent example relevant to pain and substance use disorders is the creation of μ -opioid promoter (OPRM1) fragments with high cell type specificity in primates⁸⁰. This approach may facilitate translation; indeed, recent studies have made it clear that modern chromatin structural methods and computational tools can resolve small enhancer elements conferring cell type specificity entirely within single viral vectors^{81,82} (Supplementary Table 2). One important example is the identification of enhancers that work across species, from rodents to primates, for targeting subtypes of GABAergic inhibitory cells^{81,83,84}, with relevance to epilepsy, autism and schizophrenia⁸⁵.

Gene delivery

Viral vectors delivered through stereotaxic injections (Fig. 4) result in spatially confined transduction zones. This is highly desirable when targeting local circuits, but it can be a limitation when targeting large brain regions. As the volume of the human brain is approximately 3,000 times larger than that of a mouse brain, it can be challenging to test dosing in rodents to guide therapeutics for humans. Animal

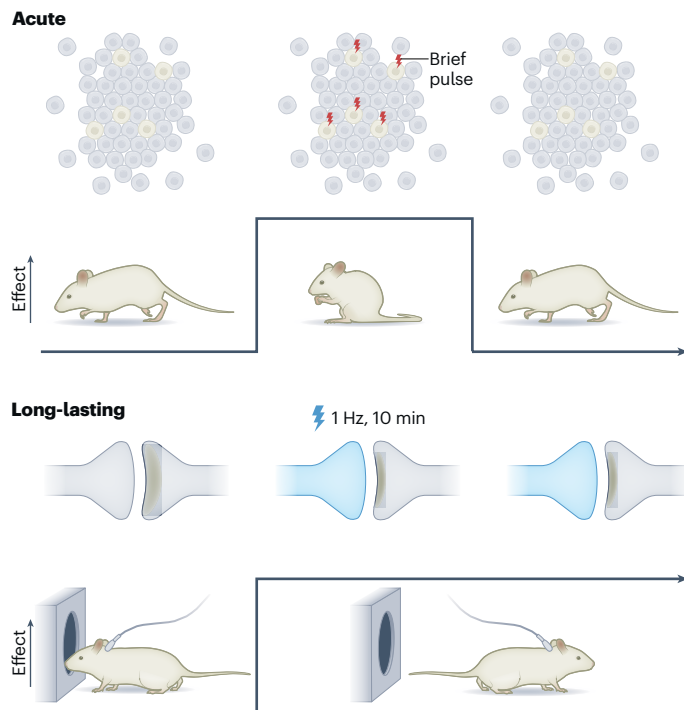


Fig. 3 | Acute versus long-lasting effects of optogenetic manipulations. Top: after the transduction of the neural population expressing an excitatory or inhibitory opsin (amber-colored cells), light is briefly applied to yield an acute effect (indicated by the arrow). Once the intervention is stopped, the effect quickly wanes, governed by opsin deactivation kinetics. One example of this type of intervention is the acute inhibition of D1 receptor (D1R)-expressing medium spiny neurons in the nucleus accumbens, which immediately and reversibly increases food intake in mice; this behavioral effect of the brief light delivery is illustrated¹⁸⁰. Bottom: optogenetic modulation of afferent projections for applying plasticity-inducing stimulation protocols. The precise protocol for light delivery depends on the type of plasticity intended (for example, theta bursts to potentiate synaptic strength or low-frequency stimulation to depress synaptic strength). The resulting plasticity can be cell type- and pathway-specific. The figure shows a synaptic depressing effect, as indicated by the reduced postsynaptic density (brown crescents). Such effects may outlast the actual stimulation for days or weeks. One example of this type of intervention is the targeting of excitatory afferents onto striatal medium spiny neurons to depotentiate synapses and reduce compulsive cocaine self-administration; the lasting behavioral effect of the 1-Hz/10-min light delivery is illustrated¹⁸¹.

models with larger brains than those of mice, such as pigs and sheep, may thus be useful for validating the spatial extent of virus spread. Viral capsid proteins can influence not only the spatial extent of transduction but also cell specificity, by favoring the infection of certain cell types or regions, depending on the molecular interactions of capsid proteins with extracellular biomolecules displayed on or near target cells. Directed evolution-based engineering of the capsid may further enhance cell type specificity or improve the transduction of axon terminals, facilitating the retrograde targeting of neurons through their projection targets⁷⁹.

More recently, noninvasive delivery mechanisms (for example, intravascular infusion, which does not require injection into the brain) have become available by engineering viral capsids to pass the blood–brain barrier (BBB)⁸⁶. First-generation BBB-permeant AAVs efficiently transduced various regions in the rodent brain, largely sparing peripheral organs; however, they largely failed to cross the BBB in primates^{87,88}. This limitation was addressed using directed evolution to develop capsids for whole-brain genetic access^{89,90}. One such AAV now allows for the targeting of neurons in the adult marmoset brain with only limited viral protein, DNA or RNA expression in the liver⁸⁹.

In Old World monkeys (young macaques and African green monkeys), a promising family of BBB-crossing AAVs has been identified and tested⁵⁰ for intravascular delivery, resulting in broad (albeit sparse) neuronal transduction throughout the brain. These AAVs have enabled anatomical and functional studies in macaques, including the delivery of multicolor cell-filling fluorophores and genetically encoded Ca^{2+} indicators (GCaMPs) for imaging in acute brain slices⁵⁰. A panel of similarly potent AAVs from distinct sequence families is now available for efficient crossing of the BBB in macaques and marmosets^{49,89,91,92}. There are currently at least two active US Food and Drug Administration (FDA)-approved programs⁹³ with BBB-crossing AAVs, establishing noninvasive gene delivery to the human nervous system (and for direct optogenetics, dovetailing with increasingly less invasive opsin and light delivery).

Any tissue tropism (for example, glia versus neurons and detargeting of the liver for the clinical safety of systemic gene delivery⁸⁹) should be tested in suitable animal models, with the caveat that some tropisms and enhancers are species-specific. The central question arises: is expression sufficiently specific to the target cells in humans to yield acceptable benefit–side effect ratios? Small changes in transduction selectivity must be considered in any therapy that depends on cell type targeting, as modulating off-target cell types may undermine the therapeutic effect. For instance, if an optogenetic therapy aims to activate excitatory projection neurons in a brain region, the unintentional excitation of a small population of local GABAergic neurons could diminish its efficacy. Such mutually counteracting cell type configurations are present throughout the brain—for example, in the striatum (direct or indirect pathway neurons). To maximize specificity and efficacy, spatially resolved transcriptomic mapping of the brain region targeted for transduction with a particular vector is helpful^{94,95}, ideally comparing human cases to animal models. For increased safety, detargeting can be addressed through both capsid and payload engineering⁹⁶; for example, while some engineered capsids redirect liver transduction from hepatocytes (which express the transgene) toward resident macrophage Kupffer cells (which destroy the vector without expressing the transgene)⁹⁷, miRNA target sites could also be incorporated into the payload to decrease toxicity associated with liver or dorsal root ganglion expression^{96,98,99}.

In summary, BBB-permeant AAVs may synergize with direct optogenetics by enabling brain-wide genetic screens to (1) identify regulatory elements for cell type targeting¹⁰⁰ and (2) induce or relieve disease symptoms to test circuit candidates for optogenetic targeting. AAVs engineered to reach the brain from the blood and transduce specific cell types may enable further insights, including the discovery of BBB transcytosis receptors¹⁰¹ and other transduction-relevant receptors, which may lead to next-generation viral or nonviral noninvasive delivery vectors. Nonviral delivery methods may, in the long run, prove beneficial by reducing immunogenicity or allowing for a larger payload; the latter parameter currently constrains AAV-based therapies^{83,102}, which nevertheless work well for small payloads such as single-component opsins in direct optogenetics.

Expression stability and immune system interactions

With any new therapeutic, potential toxicity needs to be carefully considered. Mortality events have been reported in recent years during non-optogenetic high dose AAV-based gene therapies for serious medical illness¹⁰³; improvements in AAV technology, as well as exploration of alternative gene delivery modalities, will be ongoing to establish and maintain adequate risk/benefit profiles. In particular, viral vectors or gene therapy payloads can trigger local immune responses, which in the brain can manifest in part through the activation of microglia. Human-derived organoids that incorporate microglia⁵² may thus be suitable for screening human-specific local tissue responses. Viral vectors and gene therapy payloads could, in principle, also trigger global

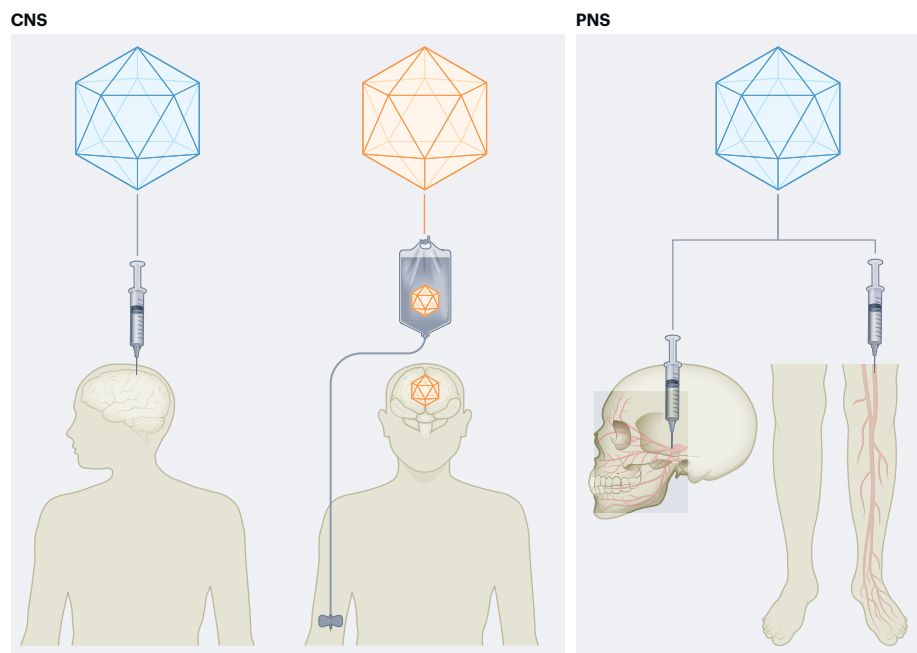


Fig. 4 | Direct delivery methods for optogenetic actuators in clinical patients. Gene delivery for direct optogenetics may take several forms. Injection of specific brain regions (leftmost image) will be relevant to many neuropsychiatric symptom targets, where regional specificity derived from the injection site may intersect with cell type specificity (as enabled by the promoter and enhancer strategies discussed in the main text and Supplementary Table 2). Gene vector delivery for peripheral optogenetics may be targeted to the cell bodies (for example, at the trigeminal ganglia to treat neuralgia; middle-right image) or to

projection targets for additional specificity (thereby targeting cells that connect to the affected region, such as with an AAV that is taken up by nerve terminals locally). When injected intravenously, systemic delivery may be possible using AAVs with modified capsids for peripheral nervous system (PNS) targeting (rightmost image) or those that cross the BBB for CNS targeting (middle-left image). In all of these cases, further cell type specificity can be achieved through focal light delivery (not shown) to target cell bodies or projections as needed for the clinical indication.

innate or adaptive immune responses that are toxic to the brain or other cells in the body. While animal models may be helpful in assessing these immunotoxicity possibilities¹⁰⁴, linking of these models to humans is not fully established and may depend on diverse factors, including the brain region and cell type targeted, vector dose, and specific genes expressed.

A key difference between peripheral organs and the CNS is the innate immune response to viral vectors¹⁰⁵. In peripheral organs, dendritic cells (DCs) take up and process protein antigens, migrate to draining lymph nodes and present these antigens in the context of MHC molecules to naive or memory T cells. Although DCs are found in the meninges and choroid plexus, healthy (noninflamed) brain parenchyma lacks cells with DC markers and analogous functions (antigen uptake, migration to draining lymph nodes and education of T cells). Thus, atraumatic injection of viral vectors is expected to elicit little innate immune response and a muted adaptive response; indeed, the injection of AAV vectors directly into the CNS is generally well-tolerated (Fig. 5). Nonetheless, detailed studies of human immune responses to intraparenchymal CNS injection of AAVs are scarce in the literature, with reported results often being contradictory between studies owing to differences in characterizing innate responses¹⁰⁶.

Beyond toxicity, specific adaptive immune responses can also reduce efficacy (particularly when the vector is delivered systemically; Fig. 5), affecting the durability of payload expression through adaptive immune responses to the viral vector, transgene product or both¹⁰⁷. The ability of animal models to predict the durability of expression has been variable owing to factors including the relatively short lifespan of mice, the lower threshold for the activation of immune cells in humans compared to other species¹⁰⁸ and the presence in humans of memory immune cells to some parental viral strains from which vectors are engineered^{109,110}. As with organ transplants, immunomonitoring may be necessary, possibly alongside concomitant immunomodulatory

therapies such as oral glucocorticoids or cyclosporine, which, while effective, carry risks of infection and other side effects. Experimental approaches (yet to be tested in the clinic) exist to turn off or stabilize transgene expression to chosen levels¹¹¹.

Despite these challenges, published data from humans (providing the most reliable information on durability) have been encouraging for AAV-mediated gene delivery targeting the CNS, with diverse examples of durable expression of human transgenes (Supplementary Note) along with useful vector dose parameters related to immune responses¹¹². For example, subretinal injection of AAV vectors at 1×10^{12} viral genomes (vg) per eye revealed clinical evidence of inflammation in multiple trial participants¹¹³, while 1.5×10^{11} vg per eye did not produce evidence of cellular or humoral immunity¹¹⁴. The route of administration is also important; the threshold for ocular immune responses is lower with intravitreal administration of AAV vectors¹¹⁵ compared to the subretinal route. Other factors that may reduce human immune responses include the use of tissue-specific promoters¹¹⁶, a low CpG content in the vector¹¹⁷, a low number of empty capsids in AAV vector preparations and low impurity levels in vector preparations¹¹⁸.

For CNS targeting, AAV vectors expressing human transgenes have been administered by direct intraparenchymal injection (doses up to 4×10^{12} vg)¹¹⁹, into the cerebrospinal fluid (doses up to 3.5×10^{14} vg)¹²⁰ or systemically¹²¹. For systemic injection, developing vectors that more efficiently cross the BBB may reduce dose-dependent adverse events such as hepatotoxicity and thrombotic microangiopathy^{122,123}. Conversely, for intracranial delivery, precise targeting with refined delivery vectors and tropism for specific cell types may reduce dosage requirements and allow more product to transduce target cells rather than toxicity sources (for example, liver or antigen-presenting cells). Delivery into the cerebrospinal fluid reduces systemic exposure compared to intravascular delivery; however, based on preclinical studies, it cannot completely prevent vector entry into the systemic circulation

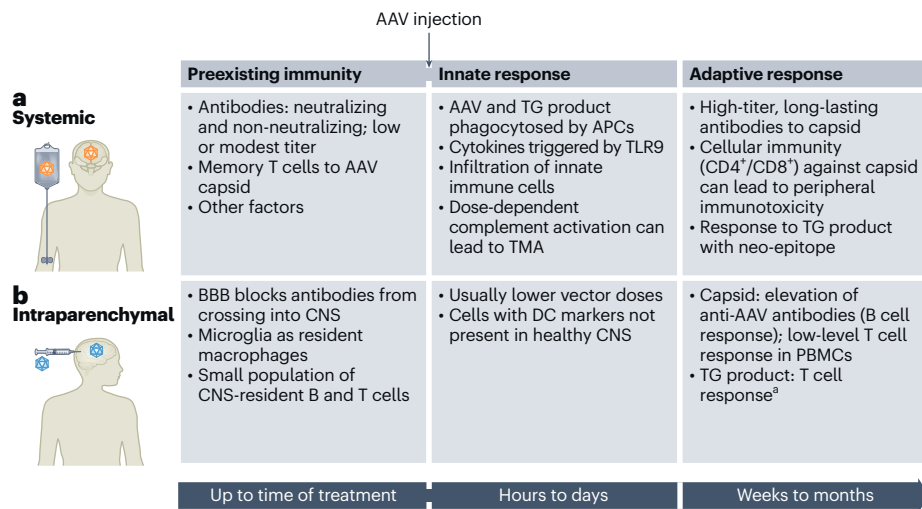


Fig. 5 | Human immune responses to AAV delivery. **a**, Human immune responses to intravenously infused AAV targeting the CNS. Left: patients may have preexisting immunity to certain AAVs based on prior exposure to wild-type viruses or, in the case of infants, due to maternal antibodies. Neutralizing and non-neutralizing antibodies can be screened before treatment, although seronegative patients may have memory B cells that drive a rapid increase in antibody titers. Cellular immune responses can be tracked using immunoassays such as enzyme-linked immunosorbent spot (ELISpot) assays. Middle: immediately following intravenous infusion, the AAV can be taken up by antigen-presenting cells. Activation of Toll-like receptors within or on these cells leads to the downstream production of cytokines, including interferon type I (IFN-I), tumor necrosis factor (TNF) and interleukin-6 (IL-6). High doses of AAV can trigger complement activation, leading to thrombotic microangiopathy. Right: following intravenous infusion of AAV, high-titer, long-lasting antibodies to AAV develop, making repeat infusions difficult. Cellular immune responses to both the capsid and the transgene products may also develop. Although these processes may lead to toxicities in peripheral tissues (for example, hepatotoxicity), clinical experience to date has not revealed destruction of

transduced target cells behind the BBB. **b**, Human immune responses to AAV vectors following direct intraparenchymal injection. Left, the BBB blocks antibodies from the CNS, ensuring that preexisting antibodies do not interfere with transduction. Middle, direct intraparenchymal administration typically allows for lower vector doses; moreover, canonical DCs are absent in the healthy, noninflamed CNS. Thus, atraumatic injection does not necessarily trigger typical innate immune responses. Right, studies have documented AAV DNA in the systemic circulation after direct intraparenchymal injection; many studies have also found increased AAV antibody titers following injection¹²⁵, and some have reported low-level T cell responses to the capsid as well¹⁸². T cell responses to the transgene product have been documented by ELISpot assays (IFN γ , TNF, IL-2) following intraparenchymal delivery in children genetically deficient in the transgene product, but these do not appear to interfere with the production of the transgene by cells in the CNS in children maintained on immunosuppression¹⁰⁶. ^aOne study¹⁰⁵. APCs, antigen-presenting cells; TLR9, Toll-like receptor 9; TMA, thrombotic microangiopathy; TG, transgene; PBMCs, peripheral blood mononuclear cells.

(with some serotypes more likely to leak than others¹²⁴). AAV DNA could also be detected in the systemic circulation after direct intraparenchymal injection into the CNS¹²⁵. Because the BBB blocks antibodies from crossing into the CNS, intraparenchymal injection of AAV, in principle, can obviate problems caused by preexisting antibodies to the capsid. Nonetheless, in the only gene therapy product currently approved for intraparenchymal delivery into the CNS—an AAV2 expressing aromatic L-amino acid decarboxylase administered to children with a genetic deficiency of this enzyme—the titer of serum neutralizing antibodies to the AAV increased beginning at week 3 and persisted through week 48 (with the highest titers ranging from 1:80 to 1:10,240) in all patients in the single clinical study supporting registration¹²⁶. Thus, in the setting of intraparenchymal injections, even at low doses (1.8×10^{11} vg total), acquired antibody-mediated (B cell) responses to the capsid still occur.

Even intraparenchymal administration of an AAV vector expressing a human protein can lead to T cell responses to the transgene product¹⁰⁶. A key feature of optogenetics, as with CRISPR–Cas genome editing strategies, is the use of a nonhuman protein, which could heighten immune responses to the transgene product following systemic delivery of the vector (less so when delivered into the CNS¹²⁷). More data are beginning to emerge on this question, with the first clinical trials of optogenetics in advanced retinal degeneration²²; a patient who received an intravitreal injection of 5×10^{10} vg of channelrhodopsin-encoding AAVs exhibited no clinical or imaging evidence of intraocular inflammation over 84 weeks following the injection²¹. Ongoing studies are valuable in determining whether the vector or the expressed transgene may be more immunogenic through a particular delivery route, in exploring whether the immune response

can be modulated pharmacologically and in testing the safe limits of vector dosing.

Choice of the optimal optogenetic effectors and light source

Suitable optogenetic effectors must generate robust neuronal responses to light delivered at safe intensities. Here, we first consider the fundamental parameters that characterize the delivered light and then address the appropriately informed selection of optical hardware and opsins.

In contrast to the typically brief optogenetic interventions in basic science, therapeutic applications may aim to deliver light over months to years in a safe and well-tolerated manner. For light delivery in basic science, the fiber-optic neural interface has been the workhorse of optogenetics since 2007 (refs. 128,129), with broad utility arising from turning an apparent limitation (restricted depth penetration of light in scattering brain tissue) into a versatile advantage. Circuit specificity can be achieved, as restricted illumination zones at the tip of (or along) stereotaxically implanted fiber optics enable region- and projection-specific targeting^{10,74,130}. In addition, modern (micro-)LED devices are highly portable and can be coupled as needed to devices for light guidance, wireless power, electrical recording, and other features suitable for both peripheral and central targets.

Brain-targeting light sources that have demonstrated robust and diverse applicability in laboratory animals will recruit a relatively smaller fraction of the relevant neural circuitry in the much larger human brain (Fig. 6). As a result, opsin considerations loom large, as the spatial extent of directly affected cells at a given safe light level

will be determined by the light sensitivity of these opsin-expressing cells. Even the initial approach to optogenetic vision restoration, while leveraging the natural light receptivity of ocular anatomy with its minimal light scattering, could not rely on environmental light levels to activate opsin-expressing cells. Instead, engineered goggles with an array of digital micromirrors were required to project features of the visual scene at sufficient light intensity, using a 595-nm LED (Fig. 2)²¹.

The effective light sensitivity of opsin-expressing neurons is governed by several parameters, including intrinsic quantum efficiency, opsin expression at the membrane and effector type; ion channels conduct more ions per photon than ion pumps, while G-protein-coupled opsins exhibit signaling that is amplified by downstream pathways (see Supplementary Table 1 for examples of candidate opsin types and their properties). Kinetics are also important; opsins with slower deactivation after light-off have higher effective light sensitivity¹³¹, as prolonging the photocurrent impulse response to a brief pulse of light turns the expressing cell into a photon integrator, with increasing currents for longer pulses of even very weak light¹³². Conversely, desensitization during light stimulation can reduce the effective light sensitivity of an opsin, with faster opsins typically showing stronger desensitization than slower ones¹³². Several strategies for improving light sensitivity have emerged. First, slowing channelrhodopsin deactivation after light-off increases the sensitivity of expressing cells. The first of these reported 'step-function' opsins¹³¹ slowed channel deactivation by many log units (from -10 ms to -100 s)¹³¹, resulting in orders-of-magnitude greater light sensitivity¹³². These step-function opsins work in NHP brains¹³³ and were further developed^{134,135} into stabilized step-function opsins with even slower deactivation, allowing the elicitation of persistent behavioral changes in mice long after the discontinuation of light delivery¹³⁴. These stabilized variants were recently combined with a mutation for increased conductance that had been previously identified¹³⁶, which is suitable for transcranial illumination in mice¹³⁷. G-protein-coupled opsins that are naturally bistable exhibit similarly high light sensitivity and allow highly efficient, albeit long-lasting, optogenetic silencing of cells and axon terminals^{16,73,138}.

While highly effective at enhancing light sensitivity, this slowed deactivation strategy limits temporal resolution, as the greatest sensitivity advantage is achieved with long light pulses; photocurrents can still be terminated with temporal precision using different colors of light than those used for activation^{131,134}. Nevertheless, slowed deactivation, while limiting temporal resolution, is well suited for many research and clinical applications, including those that involve steady or tonic biasing of the excitability of key cellular populations (for example, shifting the excitation–inhibition balance in targeted circuitry)^{134,139}. For other therapeutic applications, it may be helpful to combine high temporal resolution (such as for playing in particular frequencies of activity or a precisely timed action potential) with exquisite sensitivity. This creates a protein design challenge, as, in general, higher sensitivity correlates with slower deactivation and thus with reduced temporal resolution¹³².

Higher photocurrents for a channelrhodopsin-expressing cell at a given light level could also be achieved in principle by (1) increasing quantum efficiency (the likelihood of channel opening in response to a successfully delivered photon) or (2) increasing the membrane expression level of the channel, leading to a higher number of successfully photon-targeted channelrhodopsins at a given light level. Wild-type channelrhodopsin variants already exhibit quantum efficiencies of ~50%, leaving little room for substantial improvement in that regard. However, safely increasing channel expression at the membrane has been a powerful approach¹³². Providing mammalian protein tags (for example, for endoplasmic reticulum export or membrane insertion) to guide membrane trafficking^{140–142} has been discovered to be critically and generally important for improving microbial opsin expression levels, membrane localization and overall tolerability in mammalian cells. Therefore, this approach is now standard practice in the field.

It has also been observed that chimeric opsins often express at the membrane more robustly than either parent construct alone^{134,143,144}.

Indeed, the discovery, structural resolution and further engineering of diverse naturally occurring channelrhodopsins have advanced the tuning of their optical properties^{62,131,135,144–147}. Improvements in the precision and fidelity of waveforms have resulted from the ongoing engineering of ever-faster deactivating channelrhodopsins^{145,146,148}. However, typically, the acceleration of deactivation results in more modest photocurrents (at least for the best-known class of channelrhodopsins, which are dimers of two seven-transmembrane monomers, each with its own light-regulated ion-conducting pore). A more recent finding has shifted the landscape of sensitivity, with the observation of greatly increased neuronal photocurrents for given optical irradiance levels⁶² in a distinct family of channelrhodopsins discovered to be trimeric⁶⁸, not dimeric (interestingly, sharing this principle of structural organization with nonchannel ion pumps^{149,150}). High-resolution structural work^{151,152} on trimeric cation-conducting channelrhodopsins suggests that high ion-flux rates are achieved in part by pore neutrality (with the electrostatic properties crucial for cation-versus-anion selectivity^{152–155} less within the pore, as in dimeric channelrhodopsins, than at the extracellular and intracellular vestibules⁶⁸). These channelrhodopsins (pump-like among the channelrhodopsins in both trimeric structure and primary sequence homology) exhibit not only enormous photocurrents for given light levels but also other unusual pore properties, implementing selectivity for monovalent cations⁶⁸ (in contrast to the canonical dimeric channelrhodopsins, which flux divalent cations such as Ca²⁺ and Mg²⁺, in addition to the monovalent cations Na⁺, K⁺ and H⁺).

Initial testing of these trimeric channelrhodopsins for neuroscience revealed both monovalent cation selectivity and high light sensitivity of expressing neurons⁶⁸, including for members of the trimeric family that show even further refinement of selectivity to favor K⁺ over Na⁺ ions^{70–72}. This K⁺ selectivity could be improved through high-resolution structure-guided design⁷⁰, giving rise to variants that enable highly light-sensitive inhibitory optogenetics while maintaining the photocurrent size and sensitivity characteristics of the trimeric subfamily⁷⁰. In fact, both excitatory and inhibitory variants in this family have been shown to enable fully noninvasive optogenetics (with fast optical control even in deep bodily structures exerted through the skin and/or skull without the need for penetrating optical hardware)^{6,81}. While these studies have thus far been carried out in mice, the demonstrated centimeter-depth access for fast noninvasive optogenetics now opens the door to certain classes of noninvasive human translational applications, such as control over peripheral nerves through wearable LEDs.

Optical hardware design involves a complementary series of considerations and opportunities in the context of these channelrhodopsin structure–function discoveries. For chronic therapeutic applications, light sources should be compact and wearable by freely moving humans—a consideration that rules out essentially all advanced multiphoton methods, which facilitate fundamental discoveries on single-cell mechanisms *in vivo* but require bulky lasers^{2,156}. Instead, practical one-photon methods are likely to involve the use of implantable or even wearable LED-based devices. Implantable LEDs^{157,158} can be coupled to light guides such as optical fibers or micro-endoscopes if safe invasive light delivery is needed for spatial targeting^{129,159–161}. Fast noninvasive pacing of the heart in mice was achieved with excitatory trimeric channelrhodopsins, wearable surface LEDs and a 25% illumination duty cycle that was shown not to cause tissue heating⁶; indeed, for safety, light in clinical settings will typically be provided in pulses to mitigate heating effects^{162,163}. Adapting preclinical findings to humans while avoiding thermal damage or photodamage will necessitate the use of temperature diffusion models with parameters that closely describe the characteristics of human anatomy and/or the use of microscale thermal sensors for precision thermometry^{162,163}.

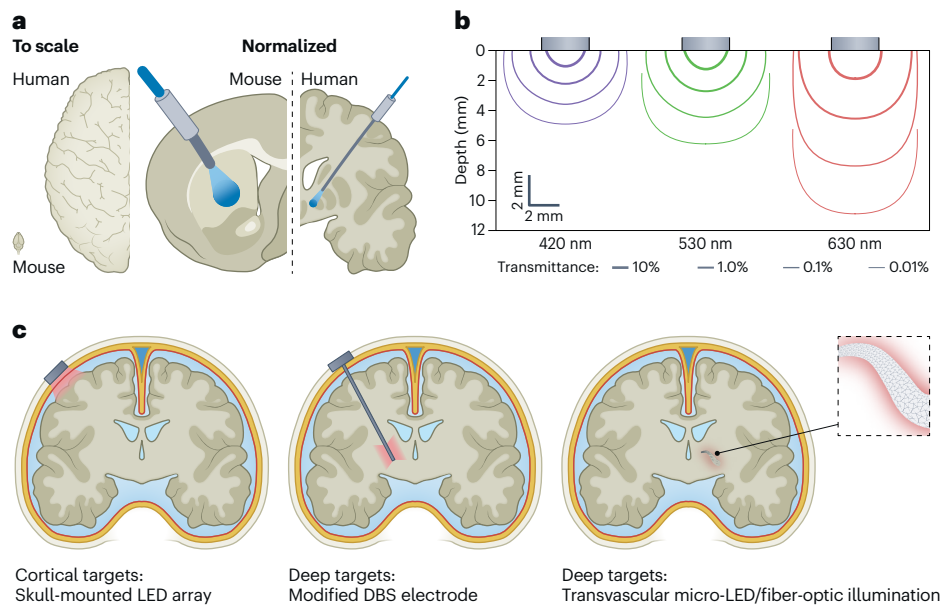


Fig. 6 | Light delivery considerations. **a**, A key parameter in scaling optogenetic techniques from mouse models to human patients is the larger size of the human brain (as illustrated on the left). This distinction affects the tissue volume required to control comparable fractions of relevant circuitry and achieve desired behavioral effects—a consideration pertinent to both gene and light delivery. For example, targeting the dorsal striatum with an optical fiber in mice will affect a much larger proportion of the striatum than it does in the human brain (as illustrated on the right). The effectively more restricted action zone in the human brain can further be turned into an advantage, enabling refined selection of regions and projections, when informed by prior basic science investigations of causal cells and circuits in animal models. If needed, highly

light-sensitive opsins and light delivery protocols can be tuned to achieve greater volumes of affected tissue, as described in the text. **b**, Modeling of light transmittance from the tip of a light source with a diameter of 2 mm, implanted in brain tissue (parameters for 420-, 530- or 630-nm light, based on measurements in human brain tissue^{163,183}). **c**, Potential configurations for light delivery devices for human brain applications: cortical targets (left) may be illuminated with a skull-mounted LED array; deep targets (for example, basal ganglia or thalamic structures) may be illuminated with a modified DBS electrode that includes LED arrays (middle) or an optical fiber linked to an extracranial illumination device¹⁶⁷; transvascular illumination may be envisioned using technology inspired by FDA-approved stent electrodes (right)¹⁸⁴.

Finally, red-shifted opsins allow the use of longer-wavelength light, which propagates deeper into brain tissue (Fig. 6) and induces less photodamage. FDA requirements reflect this distinction, allowing higher doses for light at longer wavelengths. Going forward, it will be important to preserve the key clinical safety principles described above: (1) sensitive control by visible (non-ultraviolet and preferably longer-wavelength) light, (2) reasonably fast reversibility (within minutes or less) after light-off and (3) no requirement for the infusion of chemical cofactors. These design constraints can be jointly met with certain engineered or naturally occurring inhibitory opsins that exhibit moderately slow deactivation kinetics after light-off^{16,153,164–167}, allowing pulsed light delivery to be used for sustained silencing¹⁶. For excitatory interventions, engineered or naturally occurring opsins that adhere to the clinical safety principles noted here have been developed over many years to cover a broad range of profiles that can be explored for different indications^{131,132,134,135,137,144,164,168}. Large photocurrents elicited by brief light pulses^{62,81} can ameliorate heating effects by allowing thermal dissipation between pulses^{6,169}; if deactivation is preserved to some extent, the use of brief light pulses will also enable temporal coding precision (for example, playing in more naturalistic patterns of neural activity, including those representing dynamic natural scenes for retinal therapy in optogenetics). Moreover, the design of adaptive light delivery devices with sophisticated patterning approaches may be helpful across various indications, such as wearable LEDs on wristbands or gloves for conditions such as diabetic neuropathy.

Regulatory process

Regulatory pathways aim to ensure that a new therapeutic intervention is safe and effective for patients, carefully considering the benefit–risk ratio throughout the process. Direct optogenetic therapy requires a regulatory assessment of the safety of administering genes and photons

to targeted circuits in human beings, while taking into account any potential procedure-related side effects, inflammatory or immune responses, and adverse consequences of modulating the targeted cells.

Optogenetic proteins are foreign to the human immune system and thus fall into a broader category of therapies that must be evaluated for immunogenicity, along with the effects of associated protocols, to limit potentially damaging immune responses, such as with corticosteroid administration. In general, for any gene therapy, tolerance for effective gene expression levels must be determined, usually during the initial phases of clinical trials (phase 1b–2a; Fig. 7) through dose-escalation protocols primarily conducted on patients at advanced stages of the disease. At this stage, safety must be demonstrated, but benefits can also be monitored¹⁷⁰. Including children is more challenging, because any investigational therapy with more than minimal risk must offer a prospect of direct benefit, preventing pediatric participants from receiving low or subtherapeutic doses of one-time agents such as gene therapy. This challenge has been dealt with in a number of ways, including (1) conducting initial studies in adults and including pediatric participants only after a safe and potentially effective dose has been established (for example, voretigene neparvovec (Luxturna) for a rare form of inherited blindness)¹⁷¹ and, for diseases that only involve the pediatric population, (2) estimating a safe and potentially therapeutic dose based on animal studies and beginning with that dose (for example, onasemnogene abeparvovec (Zolgensma) for spinal muscular atrophy)¹⁷².

Another consideration is potential illumination phototoxicity, a risk shared with many approved medical interventions that involve light, heat or other forms of energy deposition in tissue. Currently available optogenetic tools for highly sensitive excitation⁶⁸ and inhibition⁷⁰, enabling moderate illumination intensities and duty cycles, may mitigate this aspect. The light delivery devices themselves, if needed, also

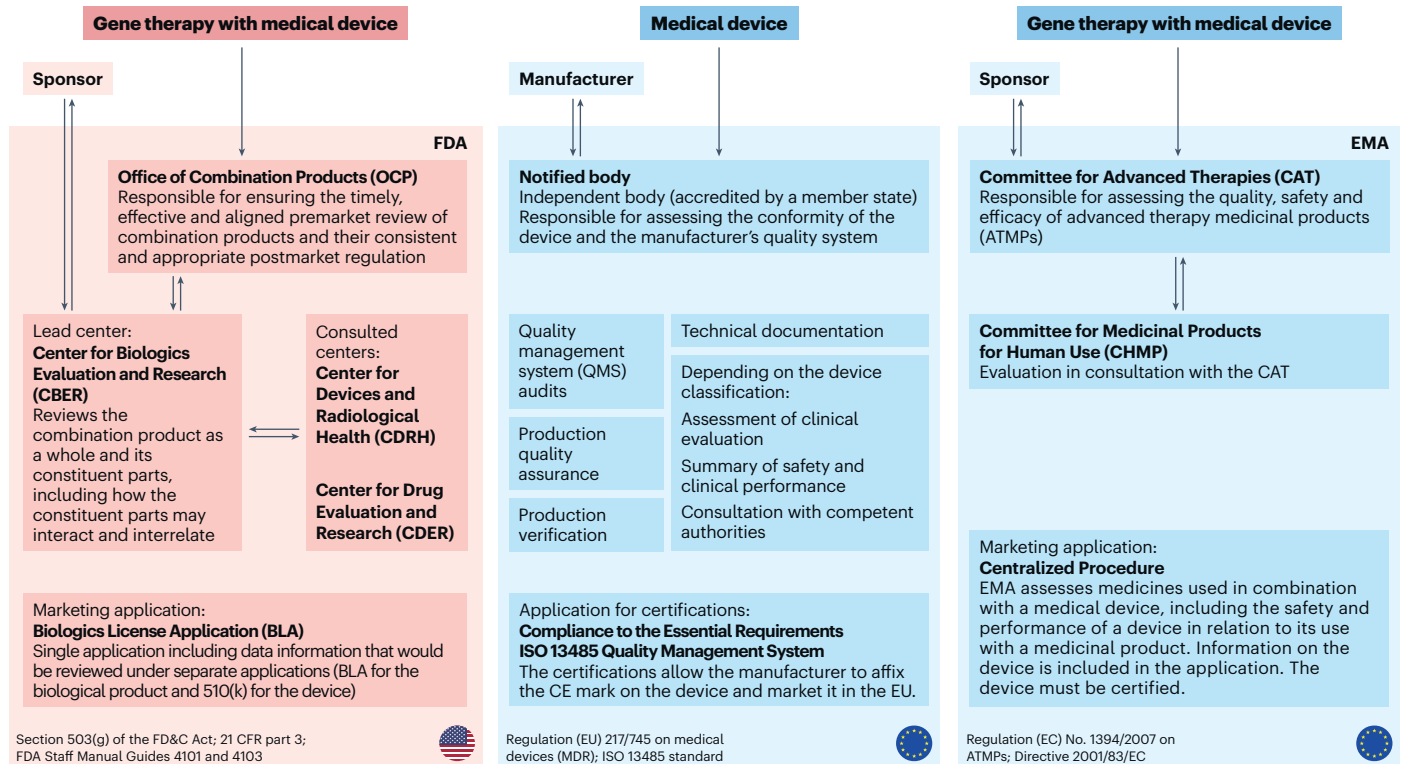


Fig. 7 | The translational pathway for combining gene therapy with a medical device depends on regionally varying governmental regulations. In the US, the FDA approaches the topic with a dedicated combined therapy route (Office of Combination Products)^{185,186}. In Europe, the European Medicines Agency (EMA) considers two parallel avenues with a notified body (medical device)

and the Committee for Advanced Therapies (gene therapy)^{187–190}. As combined therapies may be developed in the future, these current regulatory pathways will undoubtedly evolve in parallel, and regulatory convergence should be promoted to accelerate the international development of modern complex therapies.

require evaluation for robustness and safety, and such device approval was essential for the first application of optogenetics for blindness in human beings²¹. The utility of light delivery devices will extend to non-retinal applications; for example, LEDs may be coupled to elongated optical delivery devices for light delivery to deep tissues, or to the skin surface through wearable devices.

For direct optogenetics, regulatory approval comes from both the governing bodies responsible for biological therapies and the relevant device regulatory bodies; a joint assessment is required. Before entering clinical trials, safety can be established in models such as rodents and, in some cases, NHPs¹⁷³. However, previous studies using the same vectors for other indications can carry weight with regulatory agencies, so only certain safety studies might require retesting. No animal model can fully address safety and efficacy in isolation; thus, human tests are needed. The pivotal clinical protocol will define the comparison arm and doses studied (often two doses), as well as the outcome measures. An ultimate demonstration of meaningful clinical benefit will rely on functional measurements, such as disease symptom scores, biomarkers such as electroencephalogram, assessment of daily life activities using rigorous validated rating scales, patient-reported outcomes and other performance-based human behavioral testing.

Ethical considerations

Optogenetics is a rapidly advancing field. Less than 15 years elapsed between the initial functional introduction of microbial opsins into neurons (Stanford, July 1, 2004)¹⁷⁴ for the initial paper and the first introductions of microbial opsins into human beings showing safety and efficacy (London, October 26, 2018; Paris, March 12, 2019)¹⁷⁵ for the first clinical paper demonstrating treatment of human disease²¹. Fast-moving and powerful technologies require careful ethical consideration, and the optogenetic approach is no exception to this rule.

Discovery science using optogenetics in laboratory animals, along with the enablement of immediate indirect clinical applications, continues to grow swiftly; however, direct clinical applications bring to the forefront ethical considerations that must be addressed rigorously.

These ethical aspects extend beyond simple risk–benefit considerations common to any clinical trial, and even beyond the challenges faced by the pioneering retinal translation of optogenetics, as direct optogenetics in the brain can alter cognitive processes such as mood, motivation and memory, as well as fundamental survival drives common to all mammals—including anxiety, fear, hunger, thirst, reward-seeking, aggression, mating, nurturing and parenting. Together, these capabilities underscore the profound ethical implications of optogenetics for altering human behavior, desires and decisions. The stakes are high for ensuring both the realization of healing potential and the mitigation of risk. Accordingly, we here outline the neuroethics approach to this class of considerations, which arise in analogous forms across science and technology more broadly.

The ethical principle of prioritizing cognitive liberty—the right to mental self-determination—supports the use of optogenetics in human beings, especially for initial clinical applications in severe cases where traditional treatments have fallen short. Nevertheless, this approach should be fortified with regulatory oversight, embracing both complete transparency about the technique’s intentions and impacts and a solid framework to evaluate effects on participants’ mental states and beliefs, in order to harness the transformative power of optogenetics while safeguarding participants’ well-being. Drawing parallels with other interventional technologies (for example, CRISPR–Cas9), which also exhibit both direct and indirect clinical impacts, public comprehension is crucial for responsible scientific advancement. Addressing potential misconceptions and fostering a collaborative ethical discourse—encompassing scientists, ethicists, legal experts,

patients, families and other public stakeholders—will be critical for informing the applications and understanding of optogenetics.

Clinical trials must champion the principles of voluntariness, complete transparency and the unequivocal right to withdraw. Regarding the latter principle, the temporal precision of optogenetics presents a clear distinction compared to other technologies^{176–179} in the neuroethics space, as the simple withholding of light can immediately terminate effects on neural circuitry and behavior. Additionally, a commitment to long-term monitoring after the trial is imperative to detect any unforeseen consequences, particularly concerning human agency. There should also be a clear commitment to neural data privacy, technological integrity and cybersecurity to safeguard individuals' mental privacy and freedom of thought.

Finally, cost and accessibility are important elements to consider from an ethical perspective for direct optogenetics, as with any new kind of therapy, while immediate and indirect clinical applications of optogenetics can already leverage existing clinical workflows. Medical technologies (including CRISPR-based methods and new device-based interventions) have always faced challenging economics at the outset. Thus, initial cost alone should not be viewed as a contraindication, as the industry will adapt in seeking scaling strategies. Indeed, for direct AAV administration, new Investigational New Drug applications are rapidly proliferating (including those for systemic delivery), and AAV production at a human scale is already occurring in the industry. As optogenetics continually evolves, the ethical discourse should remain agile, adapting responsively to emerging challenges and insights. A commitment to prudent vigilance will ensure a balanced approach, weighing immediate risks and benefits while fostering innovation and addressing concerns as they arise.

Conclusions

Although the era of direct clinical optogenetics is already upon us, it is essential to keep in mind that optogenetics is fundamentally a basic science technology, designed for discoveries through which most of its effects on human health will be realized. Indeed, direct optogenetics is not even necessary for clinical translation, as advancing human therapies need not involve the delivery of opsin genes and light to patients (Box 1).

When developing targeted treatments, it is essential to consider the complexity and brain-wide interconnectedness of the mammalian neural circuitry, as conventional hypotheses for treatment targets may be inadequately informed. Therefore, an optimal workflow for optogenetic basic discovery may require brain-wide access, wherever possible, carrying out global and unbiased 'activity screens' analogous to genome-wide genetic screens. The resulting enormous physiological and behavioral datasets may benefit from advanced computational and statistical methods, which, when coupled with mechanistic circuit-based optogenetic modulation, will help reveal additional targets and advance our understanding of brain-wide dynamical principles.

The basic science of human and animal cognition will be advanced by the clinical search for treatment targets—a process that illuminates patterns conserved across mammalian phylogeny, from global dynamical principles to elemental single-cell typology. As is often the case in the history of science, exploring therapeutic indications is a rising tide that lifts all boats, including those of basic discovery. Indeed, fundamental and clinical sciences are already interacting bidirectionally, with basic science researchers both providing impetus for and benefiting from the development of new clinical pipelines for molecular tissue anatomy and human brain activity recordings.

Bridging scales from cells to nervous systems (powered by the fundamental connection between optogenetics and cell type targeting), as well as bridging species through the design of translatable assays and interventions, will validate optogenetic targets and advance our understanding of nervous system evolution. The most vital contributions of rodent and NHP research to translational optogenetics may indeed be through simply identifying the cells and dynamics that

matter. At any scale, a pattern that has been conserved for millions of years of evolution is likely to be important.

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Competing interests

J.-A.S. is a cofounder of SparringVision and Gensight Therapeutics, which supported the clinical trial on optogenetics in retinal degeneration²¹. K.A.H. serves as an officer and holds equity in

RhyGaze, an early-stage privately held biotechnology company investigating the use of optogenetics in therapeutics. She also serves on the Board of Directors of CRISPR Therapeutics AG, Incyte Corporation and Tr1x. V.G. is a cofounder of Capsida Biotherapeutics, which is involved in AAV engineering for gene therapy. H.Z. is on the scientific advisory board of Maplight Therapeutics, which is translating optogenetic findings into medicinal therapies (including those for autism, schizophrenia, Parkinson's disease and Alzheimer's disease). O.Y. is a co-founder and O.Y. and K.D. serve as consultants for Modulight Biotherapeutics, which is involved in GPCR opsin development for human therapeutics. K.D. is a cofounder and scientific advisor of Maplight Therapeutics, as mentioned above, and Stellaromics, which is involved in the translation of discoveries from hydrogel tissue chemistry to human therapies. B.R. is a co-founder of Gensight Therapeutics and RhyGaze, both developing optogenetic therapies for restoring vision, B.R. is also a consultant for RhyGaze. The other authors declare no competing interests.

Additional information

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