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Optogenetic investigation of neural circuits underlying brain disease in animal models

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Abstract

Optogenetic tools have provided a new way to establish causal relationships between brain activity and behaviour in health and disease. Although no animal model captures human disease precisely, behaviours that recapitulate disease symptoms may be elicited and modulated by optogenetic methods, including behaviours that are relevant to anxiety, fear, depression, addiction, autism and parkinsonism. The rapid proliferation of optogenetic reagents together with the swift advancement of strategies for implementation has created new opportunities for causal and precise dissection of the circuits underlying brain diseases in animal models.

To improve understanding of psychiatric and neurological disorders, it will be important to identify the underlying neural circuits, to pinpoint the precise nature of the causally important aberrations in these circuits and to modulate circuit and behavioural dysfunction with precise and specific experimental interventions. However, such a deep, circuit-level understanding of neuropsychiatric disorders, or indeed even of normal CNS circuit function, has been challenging to achieve with traditional methods. The complexity of neural circuitry has historically precluded the use of genetically and temporally precise manipulations to probe detailed mechanisms of function and dysfunction.

Optogenetics[1,2] describes the now widespread use of microbial opsins[3], or related tools[4], that can be activated by illumination to manipulate cells with high specificity and temporal precision[5–7] even within intact tissue or behaving animals[8–11]. Here, we briefly review how

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optogenetic approaches have been used to dissect neural circuits in animal models of symptoms that are relevant to fear, anxiety, depression, schizophrenia, addiction, social dysfunction, Parkinson's disease and epilepsy. Successful probing of complex diseases in this way will depend on the validity of animal models used to identify the crucial circuit elements and activity patterns that are involved in each cluster of symptoms, and the precision and efficiency of interventions designed to selectively target these elements or patterns. Therefore, we also discuss new strategies for targeting opsins to specific cells or circuit elements and principles for integrating optogenetics with electrophysiological, pharmacological and behavioural assessments. We also highlight the advantages and practical limitations of these approaches for the study of psychiatric and neurological disease.

Technological advances in optogenetics

The optogenetic toolbox includes a rapidly expanding array of available opsin variants that offer both distinct advantages and individual limitations in controlling cellular activity or signalling[3,12–21]. Other important components of the toolbox are light-delivery methods[6,9,22–28], targeting strategies[16,29–31] and transgenic rodent lines that increase the range of available specific cellular targets[32–34]. For example, the recent development of devices[35,36] and transgenic rat lines[37] that facilitate integration of optogenetic techniques with measures of neural activity have advanced the application of optogenetic tools to investigate the neural bases of complex behaviours that are relevant to neuropsychiatric disease.

Integration of optogenetics with mapping techniques.

The recent integration of fMRI with optogenetic manipulation, now referred to as ofMRI, has not only validated a previously assumed interpretation of the fMRI BOLD signal[38] (that increased neuronal activity in local excitatory neurons can causally trigger, rather than simply correlate with, an increase in the local BOLD signal) but has also shown that it is possible to assay the effects of precise optogenetic manipulations on global brain activity. Given that many neuropsychiatric diseases are likely to involve distributed perturbations, global approaches such as ofMRI may be crucial for identifying and mapping the downstream effects of cell-type or projection-specific manipulations (in an unbiased fashion).

Local, detailed circuit-mapping has also benefited greatly from optogenetics. Continuing a long-standing tradition of mapping neural circuitry in mammals with optical approaches[39], and in certain cases using new classes of light delivery[40], several elegant optogenetic studies have already made substantial advances in detailed circuit mapping[41–43]. These studies have helped to clarify the role of specific cortical layers in the regulation of activity flow, as well as to delineate the detailed pattern of synaptic inputs arising from distinct cortical layers onto distinct subcellular locations in neocortical principal cells. By providing a rich source of information that would have been difficult or impossible to obtain by other means, these studies may lay the groundwork for identifying circuit or connectivity phenotypes that can go awry in disease states.

New opsin variants.

Earlier optogenetic tools, such as channelrhodopsin 2 (ChR2)[5,13] — which enables action potential elicitation to be time-locked to light pulses — or halorhodopsin (NpHR)[16,18,44–46] and proton pumps[16,19,21] — which enable hyperpolarization of membranes to inhibit the production of action potentials — are still useful. However, the expansion of the optogenetic toolbox (FIG. 1) now provides greater flexibility in experimental design and more powerful and refined manipulations. For example, engineered channelrhodopsin variants (including the ChETA family[20,21] and ChIEF[47]) can be used to evoke ultra-fast firing frequencies (up to 200 Hz or more) in fast-spiking neurons.

Although the ability to elicit action potentials that are time-locked to light pulses is powerful, the synchrony and patterning of an experimentally delivered illumination pattern may not represent the physiological neural code. OptoXRs[4] (opsin–receptor chimaeras in which the intracellular loops of rhodopsin are replaced with intracellular loops from other G protein-coupled receptors such as adrenergic receptors) now allow light-activated initiation of specific G protein-coupled signalling cascades in targeted neurons within freely moving mammals. This can lead to altered excitability in a population of cells without dictating precise neural spike times. Alternatively, to increase excitability of neurons without dictating a specific pattern of firing, it is possible to apply a long-lasting and subthreshold membrane depolarization, as seen in cortical UP states. The step-function opsin (SFO)[17] facilitates this kind of intervention by delivering a prolonged, bi-stable, subthreshold depolarization of membranes.

New opsins such as the stabilized step-function opsin (SSFO)[48], which is a double mutant of ChR2 (Asp156Ala and Cys128Ser), are a substantial improvement on the previous[17] single mutant SFO (for example, Cys128Ser) in that the stability of the step function-like depolarization is greater — on the order of 30 min[17,21,48]. As SSFO also has an enhanced sensitivity to light, it enables the non-invasive light-induced activation of SSFO-expressing neurons up to 3 mm below the surface of the brain when an optical fibre is placed just above the brain surface[48]. Thus, the development of the SSFO may facilitate research in large brain regions or in large-brained animals.

Another group of noteworthy opsins is the red-shifted activation wavelength ChR1/VChR1 chimaera (C1V1) family, which includes variants that are significantly more potent[48] than ChR2 and approximately fourfold more potent than the *Volvox* channelrhodopsin 1 (VChR1), a red-shifted opsin developed previously[14]. Members of the C1V1 family and their associated variants have a peak activation wavelength of ~560 nm and can readily be activated by 590 nm light, thus increasing the feasibility of combinatorial excitation or depolarization of different populations of neurons at distinct experimental epochs or patterns[21,48]. These opsins have been used to investigate behaviours that are relevant to autism and schizophrenia[48], and are promising candidates for the study of other diseases of the brain.

Use of transgenic rodents in optogenetics.

Tools for conferring genetic specificity to opsin delivery are also improving. A large number of Cre recombinase-driver mouse lines have already been used in optogenetic research (reviewed in REF. 6). Although transgenic mouse lines have proven to be very useful in the study of cells, circuits and behaviours that are relevant to disease[31,48–55], the more complex behavioural and electrophysiological assays available in rats could provide important additional insight. However, optogenetic research in rats has been hampered by a lack of genetic tools for targeting opsins to specific cell types that are implicated in disease. The recent development of two Cre recombinase-driver rat lines targeting tyrosine hydroxylase and choline acetyltransferase neurons, with cell-type-specific promoter or enhancer regions that were too large to package into most vectors[37], has addressed this need and may facilitate research into animal models of psychiatric and neurological disease states. These, and other new transgenic animals, will set the stage for integrated studies combining *in vivo* electrophysiological and optogenetic studies during sophisticated behavioural assays.

Optogenetics in behavioural studies

Optogenetic approaches have already begun to alter the way in which traditional behavioural assays are used. In addition to cell-type-specific and projection-specific targeting strategies that allow unprecedented precision in manipulation, the temporal properties of optogenetics are reshaping the style of experimental design. For example, the immediate ‘reversibility’ of optogenetic manipulations, compared to traditional pharmacological techniques that require a lengthy wash-out period, allows multi-day tests to be condensed into a single session. The elevated plus maze with optogenetics now allows within-subject comparison of conditions (light on and light off) in a single behavioural session[56]. Similarly, paradigms such as conditioned place preference (CPP) were traditionally performed with a habituation day, a conditioning day and a test day. As the traditional paradigm typically involved the animal being locked into one compartment of the chamber while receiving the treatment (such as a drug infusion) and then restricted in the other compartment during the control treatment (such as saline infusion), the only behavioural ‘readout’ of the effect was on the test day. However, with the temporal agility of optogenetic techniques, the ‘conditioning day’ can now be performed with the animal moving freely and with light delivery only when the animal enters the conditioning chamber[4]. This enables the experimenter to quantify a location preference for the animal during conditioning, thus enriching the data set by providing information about the time course over which a conditioned preference develops. Furthermore, animals can be allowed to freely explore a chamber, and different light stimulation parameters (duty cycle, pulse frequency, pulse duration and intensity) can be triggered depending on the animal’s location. This allows for a much finer measure of optimal stimulation parameters in comparison to different drug doses, which cannot be tested within a single session. This utility of the temporal resolution of optogenetics is particularly transformative in experiments that use temporally specific variables, such as discrete cues or tasks that have behaviours with critical time windows, such as a decision point in a T-maze.

However, as well as the new advantages that optogenetic techniques offer, we are presented with several new limitations, caveats and considerations. One very important limitation to consider is the production of heat with illumination. When using high light powers or duty cycles, the light that is emitted from the optical fibre may cause heating. Heating neurons may not only alter their activity in a nonspecific manner but may also be detrimental to cell health. Appropriate controls, as well as assessment of light source stability and performance, must be carefully and frequently examined to ensure precise and reliable light output and interpretation of light effects[6].

Another limitation of optogenetic tools is the potential for toxicity at very high expression levels or long-term expression. As expression of a microbial opsin typically means the insertion of light-activated channels or pumps into the cell membrane, there may be a maximal level of opsin expression that can be tolerated by a given cell. To determine whether opsin expression has altered cell health, it is necessary to perform controls under the same conditions as the experimental parameters. Cell health and all other performance parameters will vary with many parameters, including light intensity, virus titre, injection volume, vector, opsin, cell type, species and incubation time[21]. Finally, transient intracellular or extracellular ion balance changes (over seconds) may occur after modulated (or natural) activity patterns, and optogenetic experiments typically are designed with this in mind. It is important to consider the contribution of many parameters during the growth of this new field, and we recommend that experimenters empirically examine measures of interest in each new preparation.

In vivo optogenetic design

Designing *in vivo* optogenetic experiments for investigation of the neural circuits underlying behaviour in both health and disease requires a unique set of considerations (BOX 1). In selecting an appropriate opsin gene for experiments, it is necessary to recognize the trade-offs that accompany each choice. For example, there is an inverse correlation between light sensitivity and off-kinetics[21]; volume of activation could be sacrificed in favour of greater precision of temporal control or vice versa. Thus, it is important to consider which properties are most important for each experimental aim.

It is also important to determine the best targeting strategy (FIG. 2). One commonly used strategy is viral transduction, an approach that will initially limit opsin expression to the injection site. However, after some incubation time, viral transduction strategies enable ‘anterograde’ control capabilities[6,7], meaning that the protein is expressed in local cell bodies and trafficked to the downstream terminals of those cell bodies; these projections can then be illuminated to control cells by virtue of their outgoing (efferent) connectivity. There are many promoters available for transducing viruses into wild-type animals[6], and the most commonly used viral vectors are lentivirus and adeno-associated viruses. Other viruses that have potential utility include herpes simplex viruses (HSVs) or rabies viruses, which can infect axon terminals and present ‘retrograde’-like targeting opportunities for controlling afferents to a structure; however, there may be more toxicity associated with these viruses.

For promoter sequences that are too large to package into a viral vector, Cre recombinase-driver lines used in conjunction with Cre-dependent opsin-expressing viral vectors offer an attractive alternative, as first demonstrated behaviourally in tyrosine hydroxylase (TH)::Cre mice[31] and parvalbumin (PV)::Cre mice[53,54]. Many such targeting strategies exist[29,33,57], and there are also transgenic mice that constitutively express ChR2 in certain types of cells[32,33,58]. Opsin expression in these mice is not subject to the variability that is associated with viral infection or the burden of a lengthy incubation time. These advantages may be particularly important for some applications; however, they are accompanied by the caveat that the illuminated region may contain not only local photosensitive targeted cell bodies but also photosensitive projections from cell bodies that are located elsewhere in the brain. Another recently developed strategy involves selectively infecting specific neuron types by using a viral vector that recognizes receptors on the exterior of the cell[59,60].

Lastly, it is crucial to determine the most appropriate method for delivery of light into the brain. Optical fibres can be used either acutely (for example, a bare fibre that is itself a patch cable can be inserted using a guide cannula)[9,10,25] or chronically (for example, an implantable optical fibre can be joined to a patch cable outside the brain)[6,28]. Acute optical fibres can deliver pharmacological agents to the same location as light[56,61]; however, because of the delicate nature of optical fibres, the risk of fibres breaking in the guide cannula is a substantial disadvantage. Although chronically implantable fibres do not allow for integration with pharmacology at the same site, they do offer increased durability for multiple-day experiments[6,28]. Laser diodes are a widely used light source and are easy to couple to fibre optics[6]: light-emitting diodes (LEDs) can be used directly at the tissue (although there are associated problems owing to high local heat generation) or coupled to fibres in the same way as laser diodes (but with some light loss)[22,26,62,63]. Finally, once an opsin is expressed and light is delivered to the desired location, the light stimulation parameters should be considered. Possible issues include the effects of heating, light scattering and the physiological capacity of the targeted neurons. With so many variables, it is important to carefully validate — with imaging, physiology, or c-FOS staining — that neurons are being manipulated with the strength and specificity intended before interpreting any experimental results.

Circuitry of fear and anxiety disorders

Anxiety disorders, which include generalized anxiety disorder, panic disorder, post-traumatic stress disorder (PTSD) and phobias, are the most common class of psychiatric diseases, with a lifetime prevalence of ~28% (REFS 64,65). Although anxiety disorders are common, the available treatments are inadequate in terms of efficacy and side effects[66–68].

Anxiety is characterized by a sustained state of apprehension in the absence of an immediate threat[65]. By establishing methods for projection-specific optogenetic targeting in rodent anxiety models, a specific population of amygdala synapses has been identified that can rapidly and reversibly modulate baseline anxiety levels in a freely moving mammal[56]. This study capitalized on the fact that microbial opsins can be expressed throughout the cell, including on the membranes of axons and axon terminals (a property that may be enhanced by the inclusion of specific targeting sequences[16]), allowing axon depolarization to be

generated simply by illumination of the axons themselves. The study also used a bevelled light-delivery cannula to guide light selectively to axons projecting from one brain region to another. It was shown that selective optogenetic stimulation (with a channelrhodopsin) of excitatory basolateral amygdala (BLA) cells with axons that project to the central nucleus of the amygdala (CeA) produced an anxiolytic effect. This phenotype was markedly different from the anxiogenic effect that was observed when BLA excitatory cell bodies were illuminated nonspecifically without regard to projection target[56,69] (FIG. 3). Indeed, BLA projection neurons have many targets, including not only the centrolateral and centromedial nuclei of the amygdala but the nucleus accumbens (NAc), the prefrontal cortex, the bed nucleus of the stria terminalis and many other structures that could have fundamentally different (and even opposite) effects on anxiety. Thus, optogenetic projection targeting enabled resolution of this distinct endogenous pathway for anxiolysis in the amygdala. Moreover, selective illumination of these BLA–CeA axons expressing an enhanced version of the hyperpolarizing halorhodopsin, eNpHR3.0 (REF. 16), induced an anxiogenic effect, establishing that this cellular projection is capable of bidirectional modulation of behaviours that are relevant to anxiety[56].

Panic attacks are briefer and more intense than anxiety episodes and are characterized by intense bursts of terror, apprehension and autonomic arousal, often with chronic consequences such as a debilitating fear of future attacks[64,65,67]. Interestingly, these can be triggered by isolated components (which are not threatening in isolation) of a context that was previously associated with fear. Although it has long been thought that the lateral amygdala is a crucial region for processing fear[70–74], it had not been demonstrated directly that activation of glutamatergic lateral amygdala neurons alone could induce a fear response. Optogenetic tools have now allowed researchers to selectively target the glutamatergic pyramidal neurons of the lateral amygdala, which also contains local GABAergic interneurons. Fear responses in mice were observed in response to light-induced activation of ChR2-expressing lateral amygdala neurons; moreover, pairing the presentation of a neutral stimulus (a tone) with lateral amygdala illumination led to the observation of conditioned fear responses to the tone alone[69].

Fearful responses to specific stimuli or contexts can also include phobias, which can arise from exposure to harm or threat and represent the single largest sub-class of anxiety disorders, affecting up to 12% of the population[67]. Although many lesion and pharmacological studies have implicated the amygdala in the acquisition and expression of conditioned fear[71,73–81], the intricate microcircuits in the amygdala have been difficult to causally dissect with traditional manipulations. Although optogenetic animal studies do not yet directly address panic disorder or phobias *per se*, by using optogenetic techniques to target protein kinase Cδ (PKCδ)-expressing neurons in a subnucleus of the lateral division of the CeA, researchers have recently found an inhibitory microcircuit in the CeA that gates the expression of conditioned fear[82]. Another study defined subpopulations of neurons in the CeA that are involved in the expression and generalization of conditioned fear[83] (FIG. 3). These studies have highlighted the synergistic value of genetic and spatial targeting of optogenetic control by combining the focal injection of opsin-bearing viruses into the amygdala for spatial resolution with genetic targeting strategies (for example, targeting PKCδ cells within the amygdala) for cell type resolution. This approach has enabled

researchers to determine the role of a particular cell type in the control of a symptom related to psychiatric disease in animal models.

PTSD is a class of pervasive anxiety disorder that can occur following a traumatic experience under specific conditions (for example, involving subjective helplessness) and is remarkably debilitating owing to its chronic influence on many aspects of social and occupational functioning and its resistance to treatment or extinction[84–86]. Indeed, an animal model of the processes that may be dysfunctional in PTSD is fear extinction, in which fear responses are gradually eliminated once the aversive stressor is no longer presented with the previously associated contextual stimulus[86,87]. An intriguing idea is that the fear associations in PTSD are pervasive and intractable to treatment because they are stored in several locations and circuits throughout the brain, with many independent and potent memory traces[84,86,87]. Taking this into consideration, it is important to understand where long-term fear memories are stored and how they can be most effectively disrupted or reconfigured.

It has long been suggested that the hippocampus is important for the encoding of contextual fear[88–91] but that long after consolidation (that is, in the ‘remote’ phase many weeks, months or years later) the memory is no longer stored in the hippocampus and is instead maintained in a distributed neocortical network such as in the anterior cingulate cortex[92–96]. However, in a recent study in which eNpHR3.0 was expressed in glutamatergic pyramidal neurons in the CA1 region of the hippocampus, it was shown that the hippocampus is indeed important for the expression of even remote fear memories. Neocortical networks are also important in this remote (long-term) phase and can be recruited to participate more heavily when the hippocampus is dysfunctional[97]. In other words, even for remote fear memories, the memory trace is stored in several locations that may be redundant, that may work especially hard to compensate for each other when needed and that therefore could require a number of simultaneous distinct methods and approaches to resolve pathological forms of recall.

In summary, applying optogenetic tools to the study of fear and anxiety behaviours in rodents has yielded valuable and in many cases surprising results that would have been difficult or impossible to obtain with other methods. Fear conditioning is an excellent target for the early application of optogenetics to brain disease as it involves a simple, robust behavioural paradigm for which the underlying neural circuitry has been extensively characterized and is therefore ideal for validating novel techniques as well as advancing and refining our understanding of an emotional state that is perturbed in many diseases.

Circuitry of addiction

Drug addiction is a chronic, relapsing condition characterized by compulsive drug seeking and substance use despite harmful consequences[98–100]. Addiction has been proposed to ‘hijack’ the brain’s natural reward system[99–102]; therefore, understanding the neural circuitry mediating reward processing may be crucial for understanding the pathophysiology of addiction. Although it has long been known that the mesolimbic dopamine system is involved in reward processing[103–107] and that the NAc is critically involved in both reward

processing and other addiction-related behaviours[100,101,105,108–110], the mechanistic processes and neural codes mediating these behaviours have been incompletely understood.

The motivation to understand these mechanistic processes spurred the development and application of new optogenetic targeting strategies. Promoters that could drive the expression of opsin genes specifically in dopamine neurons are too large to be packaged together with the microbial opsins into conventional viral vectors while maintaining functionality. However, as mentioned above, highly selective Cre-dependent viral vectors have been developed[29] that enable opsins to be expressed exclusively in Cre^+ cells. Notably, this strategy was used to target ChR2 to dopamine neurons in the ventral tegmental area (VTA) to show that phasic, but not tonic, stimulation of VTA dopamine neurons at frequencies that reliably produced NAc dopamine transients was sufficient to support CPP[31], a paradigm that has been used to assay drug reward-related behaviours[111,112]. The use of optogenetics with CPP has enabled researchers to identify the functional contributions of distinct neural substrates in the NAc in reward-related behaviours. Distinct populations of neurons in the NAc that express different dopamine receptor subtypes differentially modulate cocaine place preference; activating D1-expressing neurons enhances cocaine CPP, whereas activating D2-expressing neurons suppresses cocaine CPP[113]. In related work that also combined optogenetics with the use of CPP, cholinergic neurons in the NAc were shown to be crucially involved in modulating cocaine reward-related place preference[50], and acute optogenetic activation of defined G protein-coupled receptor pathways in the NAc supported CPP[4] (FIG. 4).

To better understand the circuits underlying reward-seeking behaviour, a series of studies have examined whether optogenetic activation of specific brain areas and neural pathways can serve as ‘rewards’ to be sought in their own right. These studies draw on a long history of experiments that showed that animals would perform an operant response (usually a lever press) to deliver small amounts of electrical current to specific brain regions[114], a behaviour that is termed intracranial self-stimulation (ICSS). Although such experiments served as a powerful demonstration that reward could be triggered entirely within the CNS, the heterogeneous neural populations activated by this technique precluded definitive knowledge about which cell types and neural projections could sustain ICSS behaviour. Two recent studies used optogenetics to establish causal roles for specific cell types in ICSS. Selectively activating excitatory projections from the BLA to the NAc (defined by a projection-specific targeting strategy) produced robust self-stimulation, whereas stimulation of the same cell bodies did not[61]. Moreover, this self-stimulation of BLA–NAc projections was dependent on D1 dopamine receptor (D1R) signalling[61] (FIG. 4). Optogenetic self-stimulation of dopaminergic neurons in the VTA has also been demonstrated[37,52] (FIG. 4). Together, these findings provide a greater understanding of the precise activity patterns in defined cells or projections that underlie processes that are thought to become pathological in states of substance dependence or abuse.

We anticipate that optogenetic tools will be lever-aged to test ideas that are based on indirect evidence. For example, GABAergic neurons in the VTA are emerging as a new target for addiction research because opioids act to reduce GABAergic suppression of VTA dopamine neurons[115]. With the availability of transgenic mouse lines selectively expressing Cre, direct

manipulation of VTA GABA neurons can be tested in assays of hedonic and reward-seeking behaviours. Relative to fear-conditioning, reward-related behaviours may be more sensitive to motivational state. However, previous *in vivo* electrophysiological recordings performed throughout the corticolimbic system during the acquisition[116–119], maintenance[106,108,110,120–122], extinction[123,124] and reinstatement[123,125] of reward-related behaviours have identified a rich landscape of neural correlates, and have set the stage for optogenetic interrogation in the pursuit of causality.

The strong *in vivo* electrophysiological tradition in this field is also supported by studies that used intracranial pharmacological manipulations to identify circuits relevant to addiction and reward-seeking behaviours. Thus, looking towards the further integration of optogenetics with high-speed readouts for neural activity and neurochemical signals[126,127], the field of reward-related behaviour represents a fertile proving ground for technological synergy set against a back-drop of sophisticated behavioural theory.

Circuitry of depression

Although up to 13% of the population will experience clinically relevant major depression at some point during life[64,128], the pathophysiological underpinnings of depression and other mood disorders are poorly understood. Currently available antidepressant medications are often ineffective and even when effective must generally be taken for 4–6 weeks to show improvements in patients[129–132]. Brain stimulation treatments that are milder and more targeted than electroconvulsive therapy are also being actively explored; for example, high-frequency electrical stimulation of the region near the subgenual cingulate gyrus may improve depressive symptoms in treatment-resistant patients[133,134]. Although this breakthrough finding sparked hope of an improved understanding of the neural substrates of depression, the mechanisms of this phenomenon are still poorly understood; for example, it is not clear whether high-frequency electrical stimulation exerts antidepressant effects by directly influencing axons passing through this area or local cell bodies, or whether the net effect that is causal in symptom remediation is increasing, decreasing or otherwise influencing local cortical neural electrical activity.

In an attempt to better understand the underlying mechanisms of deep brain stimulation (DBS) treatments for depression better, optogenetic techniques have been used to target cell bodies in the prefrontal cortex of mice. Mice with a depression-related phenotype showed an antidepressant-like response to illumination of medial prefrontal cortical neurons expressing ChR2 (REF. 135). Although this study used a more targeted method than electrical stimulation, limiting activation to local cell bodies without activating fibres of passage, new optogenetic tools allow even greater specificity. For example, because the prefrontal cortex projects to many regions, including the dorsal raphe, the NAc, amygdala and other structures proposed to be involved in mood regulation, it will be particularly useful to examine these targets in a pathway-specific manner using projection targeting approaches. In addition, the use of cell-type-specific promoters or Cre-dependent targeting strategies would allow increased cellular, as well as regional, specificity.

Depression has been linked to many neural pathways, including corticolimbic[134], dorsal raphe[136–138], hippocampal[139–142], amygdalar[143–146,65–68], striatal[147–149] and mesolimbic dopamine[130,150–152] circuits. Furthermore, depression has been linked to distinct neuromodulatory systems and receptors, including serotonin[153,154], noradrenaline[155] and dopamine[130,150–152,155,156]. Most currently available antidepressants modulate monoamines, globally modulating synaptic neurotransmission using serotonin, dopamine and/or noradrenaline. Given the diverse distribution and functionality of these receptors, increasing the specificity of drug targets could dramatically improve drug efficacy or reduce side effects[157]. Future development and implementation of optogenetic tools may inspire drug development with enhanced precision to provide more efficacious anti-depressants with fewer side effects.

An intrinsic obstacle in studying the neural circuits underlying depression is that the constellation of symptoms that define clinical depression is not as readily modelled in animals as other diseases. For example, how can motivation be assessed independently from locomotion, and how can despair and helplessness be reliably assayed in animals? In contrast to addiction research in which the disease is defined by the behaviour, in depression the disease state is mainly defined by the subjective experience of the patient. This is a major challenge with which depression researchers continue to wrestle to advance our understanding of this severely debilitating and common psychiatric disease. It may be effective to focus on the symptoms that have measurable correlates in animals (such as assays for changes in motivated behaviours in the absence of gross locomotor effects, and hedonic behaviour changes). Despite the practical challenges in using optogenetic techniques to dissect the neural underpinnings of depression and other mood disorders, the immense potential benefit to the broader community of this reverse translational approach warrants a proliferation of work in this field.

Circuitry of autism and schizophrenia

Autism and schizophrenia also present major experimental challenges for identifying neural circuits altered in states of disease because of the heterogeneity of symptoms that each nominally unitary condition encapsulates. Patients with autism and schizophrenia display diverse and variable arrays of symptoms that make the search for a single cause a daunting and possibly unrealistic goal[158–161]. At the surface level, these diseases share certain signs and symptoms, such as social dysfunction, although the experienced physician will notice that the social behaviours appear to be quite different, even in dysfunction, in these two diseases.

Schizophrenia symptoms include disorganized speech and behaviour, as well as hallucinations, delusions and negative symptoms such as impaired social function[162,163]. This debilitating disease has been correlated with perturbations in the balance of excitation and inhibition and with altered oscillations of cortical neural networks[164], both of which may in theory lead to a breakdown in the transmission or processing of neural information. Although animal models of schizophrenia are still being optimized, one of the few reliable micro-anatomical abnormalities in psychiatric disease is the reduced number and functionality of neocortical parvalbumin neurons[165,166], and therefore optogenetic

approaches to the circuitry in question have included targeting these neurons in PV::Cre mouse lines[53,54]. These studies have revealed the roles of parvalbumin neurons in modulating gamma rhythms and information processing in neocortex that may help to lay the groundwork for a deeper understanding of information processing deficits in schizophrenia[53,54].

Autism spectrum disease is characterized by impairment in social behaviour and communication as well as stereotyped, repetitive movements[159,160,167]. Autism is co-morbid with neurological disorders such as epilepsy[168,169], and is also linked to anxiety and mood disorders[158,170]. The complexity and variability of symptoms in autism have made it particularly difficult to study, but one theory has been that imbalance in cellular excitation and inhibition (excitation–inhibition imbalance) may be causative[171–173]. Optogenetic tools offered the ability to empirically test this idea by enabling the induction of excitation–inhibition imbalance. The expression of a SSFO, which produces subthreshold membrane potential changes that last many minutes, in glutamatergic prefrontal cortical neurons allowed the level of cortical excitation to be elevated[48]. This resulted in an increase in rhythmic activity in the gamma band (30–80 Hz) — a trait linked to autism[174] — and also virtually abolished unconditioned social behaviour[48]. To determine whether this effect was related to an imbalance in the activity of excitatory and inhibitory cells, a novel red-shifted opsin developed for the purpose of combinatorial excitation was used to partially rescue the phenotype by simultaneously increasing the drive of both excitatory and inhibitory cells[48].

An imbalance in excitation and inhibition may also contribute to other psychiatric disorders, such as anxiety and depression[56], and may give rise to interesting candidate endophenotypes in other circuits, such as rhythmic oscillations (for example, gamma oscillations) and altered activity percolation through the circuit. Indeed, given the highly overlapping circuits that are involved in psychiatric disorders such as anxiety, depression, addiction, schizophrenia and autism, it is possible that excitation and inhibition imbalances throughout the brain could contribute to the high rate of co-morbidity among these disease states[156,158,162,175–178], and optogenetic tools now offer the capability to test these fascinating and potentially unifying theories.

Circuitry of neurological disorders

Optogenetic approaches have been applied to fundamental research questions in a variety of neurological disorders such as Parkinson’s disease[51,55], epilepsy[168,169], blindness due to neuronal loss[179,180], failure of respiration[181,182] and neuropsychiatric sleep disorders[25]. Our basic understanding of motor circuitry has also been enhanced by optogenetic investigations; for example, it had long been suggested that the basal ganglia regulate movement through the balance of two pathways, the ‘direct’ pathway, which promotes locomotion, and the ‘indirect’ pathway, which inhibits locomotion[183–185]. Selective targeting of each of these pathways was made possible by Cre-dependent optogenetic targeting of D1R- or D2R-expressing cells[51], thus providing the first direct empirical confirmation of this long-standing theory.

Parkinson's disease, a neurodegenerative disease, is characterized by rigidity, tremor, postural instability and slow movement[186,187]. Although DBS in the subgenual cingulate cortex has been used in humans to treat depression[133], DBS in the subthalamic nucleus (STN), a part of the basal ganglia circuit, and in other targets has shown remarkable therapeutic effects for treatment of the motor impairments associated with Parkinson's disease[188–190]. However, the cellular mechanisms of this therapy have been unclear and highly controversial. Selective optogenetic control of afferent fibres (but not selective control of local somata) in the STN was shown to have profound therapeutic effects on motor behaviours in a hemi-parkinsonian rat model[103] (FIG. 5). These data suggested a model for DBS treatment in which white matter tracts or axonal pathways are the most effective direct target of control. The reasons for the efficacy of control by this means could include the fact that recruitment of large or asymmetric neural structures with a point source of energy such as an electrode or an optical fibre is most efficient if spatially localized incoming axon tracts are the direct initial target. This concept carries direct implications for electrode contact positioning in DBS for depression as well as Parkinson's disease. Alternatively (or additionally), it may be that the flow of activity between brain regions that can be targeted at the level of axon tracts (rather than neural coding by somata *per se* within a region) is the most functionally relevant endophenotype for neuropsychiatric disease symptomatology.

Pathway-specific optogenetic approaches in combination with whole-cell recordings in a corticothalamic circuit linked to epilepsy revealed a pathological generation of aberrant oscillations relevant to seizure activity[169]. Optogenetic activation of neurons in the retrotrapezoid nucleus–parafacial respiratory group induced active expiration and regulated the rhythm of respiration[181]. It may be worth noting that application of optogenetic approaches can also further the study of stem cell-based interventions for Parkinson's disease[191]; optogenetic targeting of grafted stem-cell-derived dopamine neurons has shown that opsin-expressing grafted cells can be functionally integrated into networks of wild-type mouse striatum[168]. The unconditioned modulation of feeding behaviour was shown to be under the control of two functionally opposing subpopulations of neurons in the hypothalamus[57]. An elegant examination of aggressive behaviour demonstrated that attacks could be evoked with optogenetic activation of a specific subset of ventromedial hypothalamic neurons[192]. Lastly, restoration of vision by optogenetic activation of cones was shown to rescue the blindness associated with retinitis pigmentosa[179,180].

Conclusion

We have provided here a brief summary of some recent investigations relevant to the study of neuropsychiatric disease that used optogenetic tools. In these cases, the precision of optogenetics has provided major experimental leverage[193] and has led to insights into neural circuit function and dysfunction. Although the impact of these recent investigations has already been substantial, there remains much work to do; for example, the application of optogenetics to non-human primates is still in its infancy[24,27,194,195], and many disease states and symptom clusters remain unexplored. We anticipate several major areas of optogenetic tool advancement in the future, from tool development to scientific application, mainly driven by demand in the field.

With respect to developments in opsin engineering, despite the rapidly proliferating library of opsins, there remain areas that require increased attention. For example[18], we anticipate the engineering of blue-shifted hyperpolarizing opsins with narrower activation wavelength spectra to allow for enhanced combinatorial neuronal inhibition experiments[21]. Another much needed development is a hyperpolarizing SFO to allow sustained inhibition of neurons without requiring constant illumination, which can cause heating and may be impractical for chronic inhibition experiments. In addition to light-sensitive pumps and channels, we anticipate the continued expansion of the OptoXR family[4], as light-sensitive domains are being added to an increasing number of receptor and even intracellular signalling proteins so that optogenetics will expand to the study of cell signalling in addition to the study of neural activity.

The development of improved targeting strategies on both a cellular and subcellular level is just as important as the development of new opsins and opsin variants. Improved or expanded recombinase strategies are well within reach, and would facilitate circuit-level investigations. We have steadily improved the expression of opsins at the membrane, but further exploration in this area may produce targeting strategies that allow selective opsin expression in subcellular compartments such as dendrites, soma or axon terminals, thereby increasing the level of precision[196]. Currently, we are able to selectively express opsins in cells with a given genetic phenotype or marker, but methods to selectively exclude opsin expression in cells with a given genetic identity will also be useful.

In addition, methods of light delivery will require continual improvement. The optical fibre provides a small zone of illumination and, rather than opsin expression, the illumination pattern may often be limiting. Many experiments may call for broader volumes of illumination (to facilitate achieving behavioural effects from optogenetic manipulations in larger species such as monkeys). We also anticipate continued improvement in the speed and precision of single-cell activation through refinement of the ability to visualize and selectively illuminate individual cells to allow the playback of specific neuronal population codes. The creation of a method to simultaneously perform optogenetic activation and achieve calcium imaging at a single-cell or even dendritic-spine resolution in a freely moving mammal would also be an enormous advance for the field.

It is intriguing to speculate that a very specific (and now testable) neural circuit dysfunction (such as an imbalance in excitation and inhibition) could be causally involved in multiple psychiatric diseases including anxiety, depression, addiction, schizophrenia and autism. Variation in precise symptomatology from disease to disease could be more closely linked to variation in the role of the affected circuit than to fundamentally distinct principles of the pathologically altered neural activity propagation. In particular, given the high rate of co-morbidity among the various mental illnesses and the shared symptomatology between individual diseases, identification of such simplifying themes and unifying theories by optogenetic or other means is one of the most pressing needs and exciting avenues of research into neurological and psychiatric disease.

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Box 1 |

Designing optogenetic experiments to study brain disease

There are at least five major steps in the design of optogenetic experiments to study behaviour in normal function or disease models (see the figure).

Select the opsin best suited to the experimental goals.

There are trade-offs for different aspects of performance, such as peak photocurrent, kinetics, activation wavelength spectrum and light sensitivity.

Select targeting strategy or vector to express opsin in target cells.

Many kinds of viral transduction can restrict cell-body expression of opsin to the injection site, and can be used in wild-type animals or Cre recombinase lines. Some transgenic lines constitutively express opsin.

Select light delivery method.

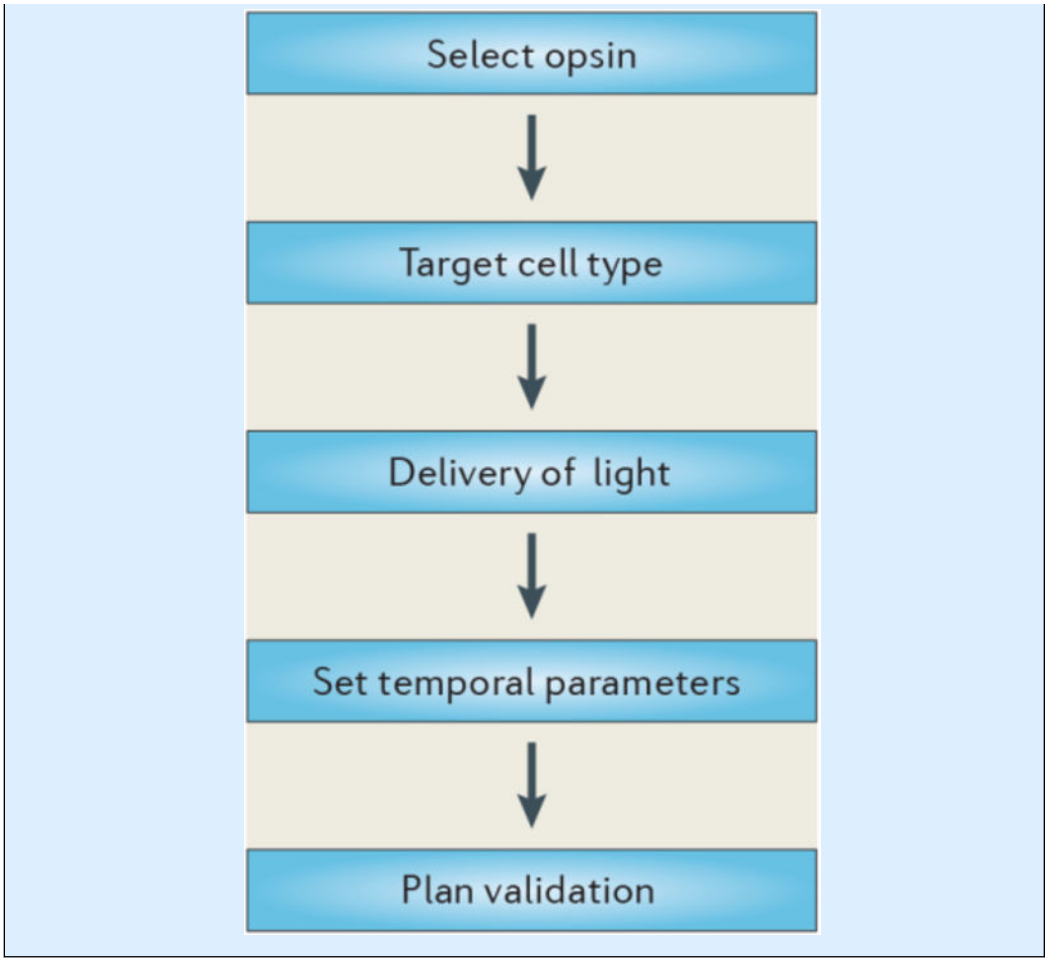
Fibre optics are the most common method of light delivery to deep cell bodies or axon terminals. The numerical aperture, diameter and mode of the fibre will influence the spread of light. There are trade-offs for using acute or chronically implantable fibres, as acute fibres allow for pharmacological manipulations but are delicate and easy to break.

Choose appropriate temporal parameters.

Duty cycle, pulse duration, frequency and epoch pattern are the key light-delivery parameters to select. Depending on the behavioural assay, exploring within-session light manipulations can maximize the utility of optogenetic tools.

Validate the experimental manipulation.

To verify that the opsin, targeting strategy and illumination parameters are manipulating cells in the intended manner, confirmation using electrophysiology, immunohistochemistry or other measures is crucial for data interpretation.



Opsins

Membrane-bound proteins that can incorporate small organic ‘retinal’ molecules to become a light receptor.

fMRI

(Functional magnetic resonance imaging). This method can use detection of blood oxygen levels as a proxy for neural activity, and offers a non-invasive method to globally assay brain activity in humans.

BOLD

(Blood oxygen level dependent). The BOLD signal is one kind of signal that fMRI can use to assess neural activity.

Channelrhodopsin

A light-driven cation channel, found in algae, that can be used to depolarize cell membranes.

Halorhodopsin

A light-driven chloride ion pump found in phylogenetically ancient archaea, known as halobacteria, that can be used to hyperpolarize cell membranes.

UP states

Sub-threshold membrane depolarization states that have been observed to spontaneously occur *in vivo* in some neurons and that may serve to increase the intrinsic excitability of the neuron.

Cre recombinase

DNA recombinase that excises DNA sequences flanked by *loxP* sequences with the same orientation, or inverts sequences flanked by *loxP* sites with opposite orientation. It is effective in mammalian cells *in vitro* and *in vivo*.

Vectors

Vehicles used to transfer genetic material to a target cell.

Conditioned place preference

(CPP). A behavioural test in which an unconditioned stimulus is paired with one distinctive context and a neutral event is paired with a different context. Preference is determined by allowing the animal to move between the two contexts and measuring the amount of time spent in each context.

Duty cycle

The time that a machine, system or light source spends in an active state as a fraction of the total time under consideration.

Conditioned fear responses

A fear-associated stimulus (such as a shock-predictive tone) that may evoke conditioned responses such as freezing, fear-potentiated startle or increases in blood pressure, perspiration or heart rate.

Deep brain stimulation

(DBS). Continuous therapeutic electric stimulation of subcortical areas at high frequencies (~130 Hz) using chronically implanted electrodes.

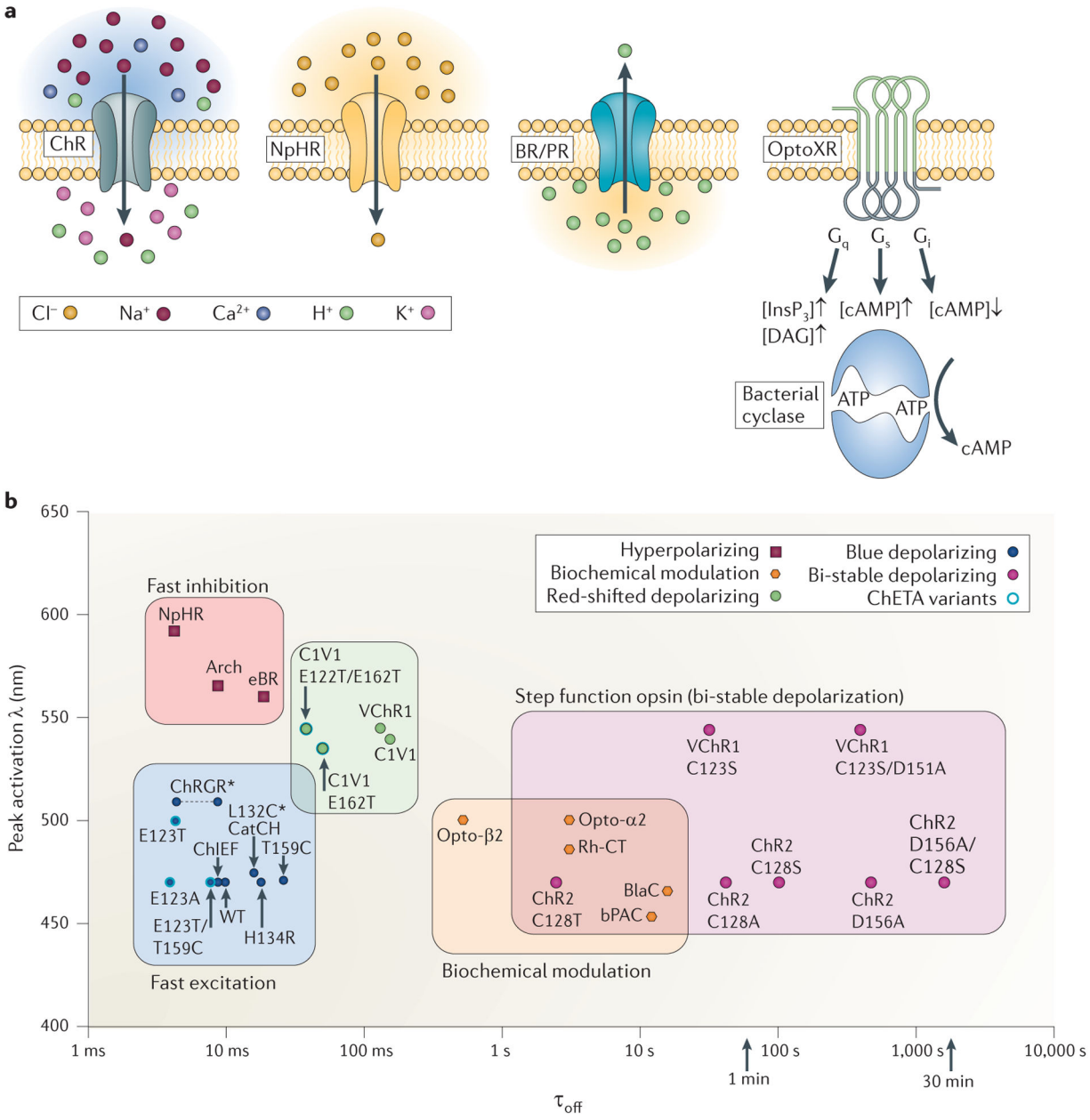


Figure 1 |. Optogenetic tools.

a | Major classes of single-component optogenetic tools include cation-permeable channels for membrane depolarization (such as channelrhodopsins (ChRs)), chloride pumps (for example, halorhodopsin (NpHR)) and proton pumps (such as bacteriorhodopsin or proteorhodopsin (BR/PR)) for membrane hyperpolarization, and light-activated membrane-bound G protein-coupled (OptoXR) or soluble (bacterial cyclase) receptors that mimic various signalling cascades. **b** | Tools that have been characterized in terms of wavelength activation spectra and decay kinetics. The chart shows peak activation wavelength plotted against decay kinetics and illustrates groupings of tools over the range of spectral and temporal characteristics. This also demonstrates why it is feasible to use tools that are well

separated in spectral and/or temporal domains to achieve dual-channel control. It should be noted that the kinetics for OptoXR were characterized *in vivo* using an assay that measured a downstream readout (spiking) and probably represent an upper bound for these properties. Decay kinetics are temperature-dependent; all reported values except for channelrhodopsin-green receiver (ChRGR) were recorded at room temperature (an ~50% decrease in decay kinetics is expected if the temperature is increased to 37°C). ChRGR has only been studied at 34°C. We have therefore extrapolated the likely range for this protein at room temperature. The decay kinetics for the L132C mutation (calcium translocating channelrhodopsin (CatCH)) were not measured in neurons, and these properties may depend on factors including the presence of other channels in the host cell and the host cell tolerance of, and response to, elevated intracellular Ca^{2+} levels[197]. The recently determined[198] crystal structure of ChR2 may allow the design of additional classes of optogenetic tools. The '/' indicates the combination of two mutations. Figure is modified, with permission, from REF. 6 © (2011) Elsevier. Arch, archaerhodopsin; bPAC, bacterial photoactivated adenylyl cyclase; C1V1, ChR1/VChR1 chimaera; cAMP, cyclic AMP; DAG, diacylglycerol; eBR, enhanced bacteriorhodopsin; $InsP_3$, inositol trisphosphate; VChR1, *Volvox* channelrhodopsin 1.

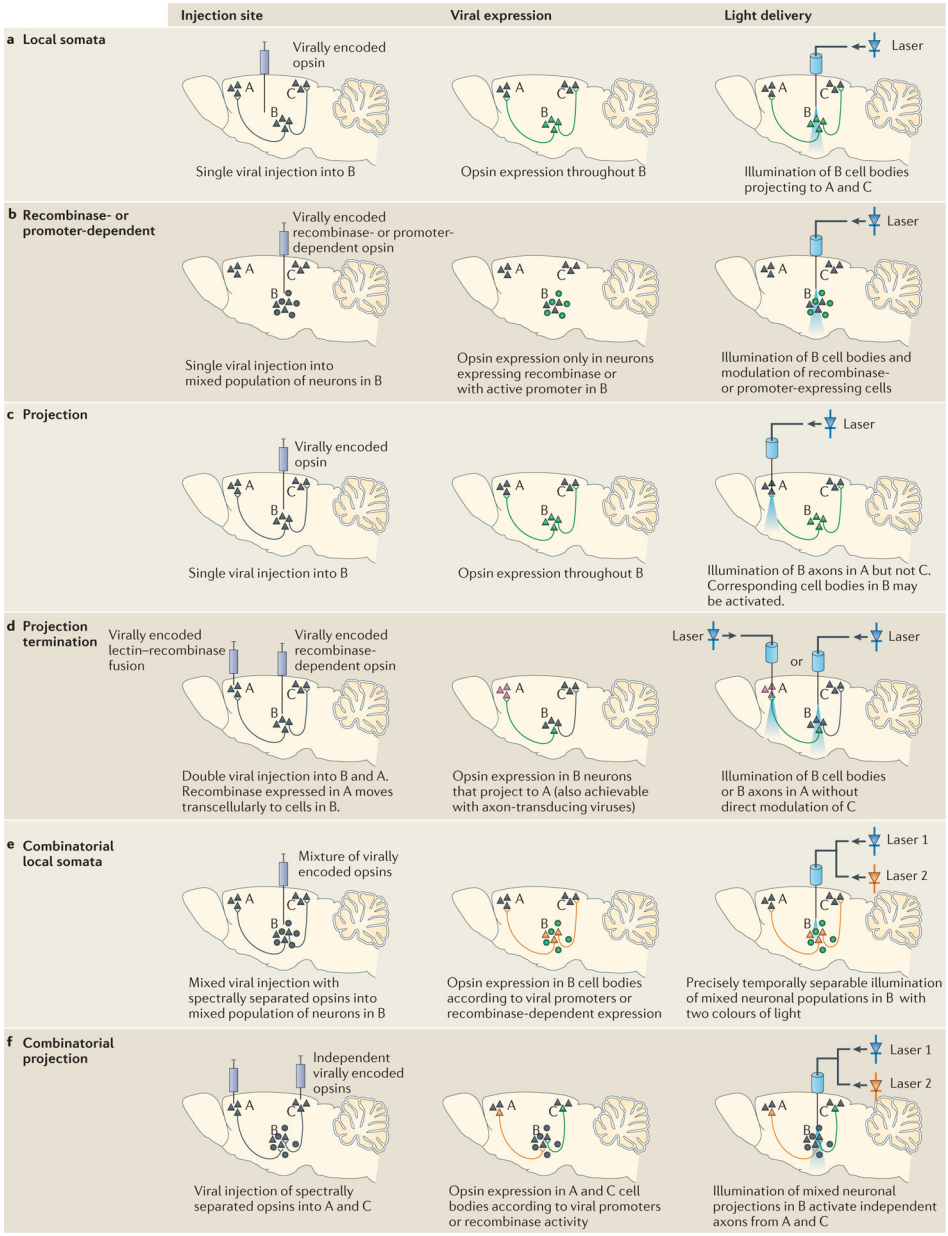


Figure 2 |. Targeting strategies with optogenetic tools *in vivo*.

a | Neuronal cell bodies can be directly stimulated by injecting a viral vector into the target region and implanting a local light-delivery device in the same region. **b** | Specific expression of the transgene in defined cell populations can be achieved by including cell-type-specific promoters within the viral vector or by injecting a recombinase-dependent virus into an animal that is engineered to express a recombinase (such as Cre) in particular cell types. **c** | The optogenetic tool can be targeted to axonal projections by injecting the virus at the location of neuronal cell bodies and delivering light to the target region harbouring opsin-expressing processes. **d** | In projection termination labelling, cells are targeted by virtue of their synaptic connectivity to the target region, probably excluding axons that are simply passing through the area. In the example shown, transcellular labelling

is achieved using a recombinase-dependent system. The synaptic target site is injected with a virus expressing Cre that is fused to a transneuronal tracer (such as a lectin), and the cell body region is injected with a Cre-dependent virus. This results in cells that project to the Cre-injected area becoming light sensitive. Similar effects can be obtained using retrograde viruses (those that transduce the axon terminal), such as rabies or herpes simplex viruses (HSVs), although these approaches do not enable control over the postsynaptic cell type. **e**,**f** | Combinatorial manipulations at either neuronal somata (**e**) or projections (**f**) can be achieved with two different optogenetic tools that have well-separated activation spectra (responding to different wavelengths of light) and by using a light-delivery tool to merge multiple wavelengths of light. Figure is modified, with permission, from REF. 6 © (2011) Elsevier.

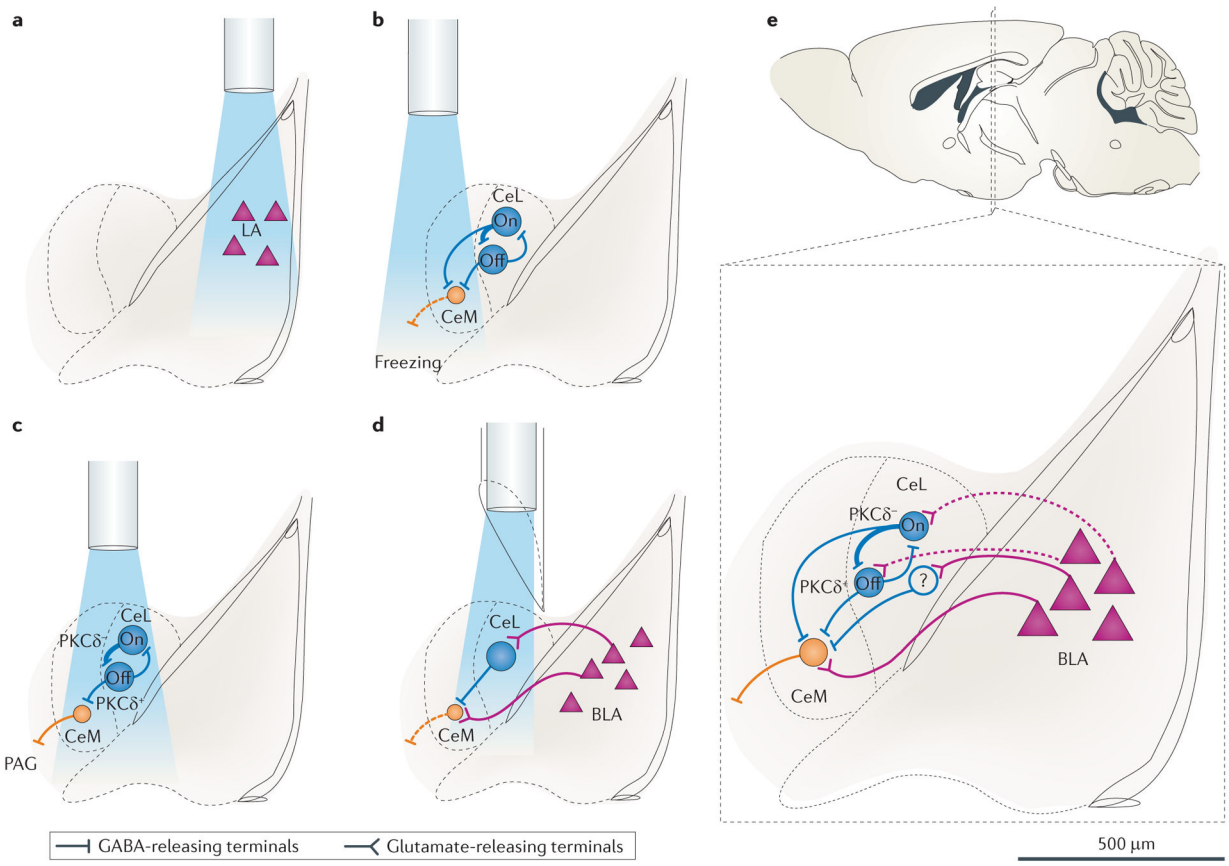


Figure 3 |. Functional dissection of amygdala microcircuitry using an integrated approach involving optogenetic tools.

a–d | Four recent papers[69,56,82,83] have used optogenetic tools to dissect subpopulations of amygdala neurons or projections in fear and anxiety studies. The diagrams show connectivity but are not intended to depict ultrastructural anatomy. **a** | In one study[69], adeno-associated virus (AAV) 2/1 serotype was used to express channelrhodopsin 2 (ChR2) in glutamate neurons in the lateral amygdala (LA), and LA somata illumination (in lieu of shock) was paired with a conditioned stimulus to produce fear responses. This showed that activation of LA neurons produced unconditioned fear responses (freezing), which when paired with a stimulus was able to support stimulus-evoked freezing. **b**,**c** | Recent studies[82,83] identified two interesting subpopulations of neurons in the centrolateral nucleus of the amygdala (CeL). In the experiment shown in **b**, a recombinant AAV (rAAV) was used to express ChR2 in the CeL and illumination of the centromedial nucleus of the amygdala (CeM) was used to produce fear responses[83] (**b**). When the conditioned stimulus was presented, two populations of CeL neurons were identified: CeL On cells showed excitation, whereas CeL Off cells were inhibited. In the experiment shown in **c**, AAV2/5 expressing ChR2 was injected into the CeL of protein kinase Cδ (PKCδ)::GluClα-ires–Cre mice[82] to show that PKCδ-expressing neurons inhibited periaqueductal grey (PAG)-projecting CeM neurons (**c**). PKCδ^{+} cells corresponded to CeL Off cells. **d** | Another study[56] examined amygdala function in the context of unconditioned anxiety rather than conditioned fear. In this study, basolateral amygdala (BLA) neurons were transduced with an AAV expressing

ChR2, and it was shown that activating BLA–CeL projections reduced anxiety-related behaviours, whereas activating the BLA cell bodies without specificity for projection target increased anxiety-related behaviours. This study also demonstrated the opposite effects of BLA–CeL projections using optogenetic inhibition. However, this study did not determine whether BLA neurons provided monosynaptic excitatory input to a particular subpopulation of CeL neurons. **e** | Information from these four studies, synthesized to display certain current optogenetically obtained knowledge about amygdala microcircuitry that is causally involved in behaviour. Although many questions remain, these studies show the advantages of integrating optogenetic tools with molecular, electrophysiological, pharmacological and imaging techniques in the context of behaviour relevant to psychiatric disease.

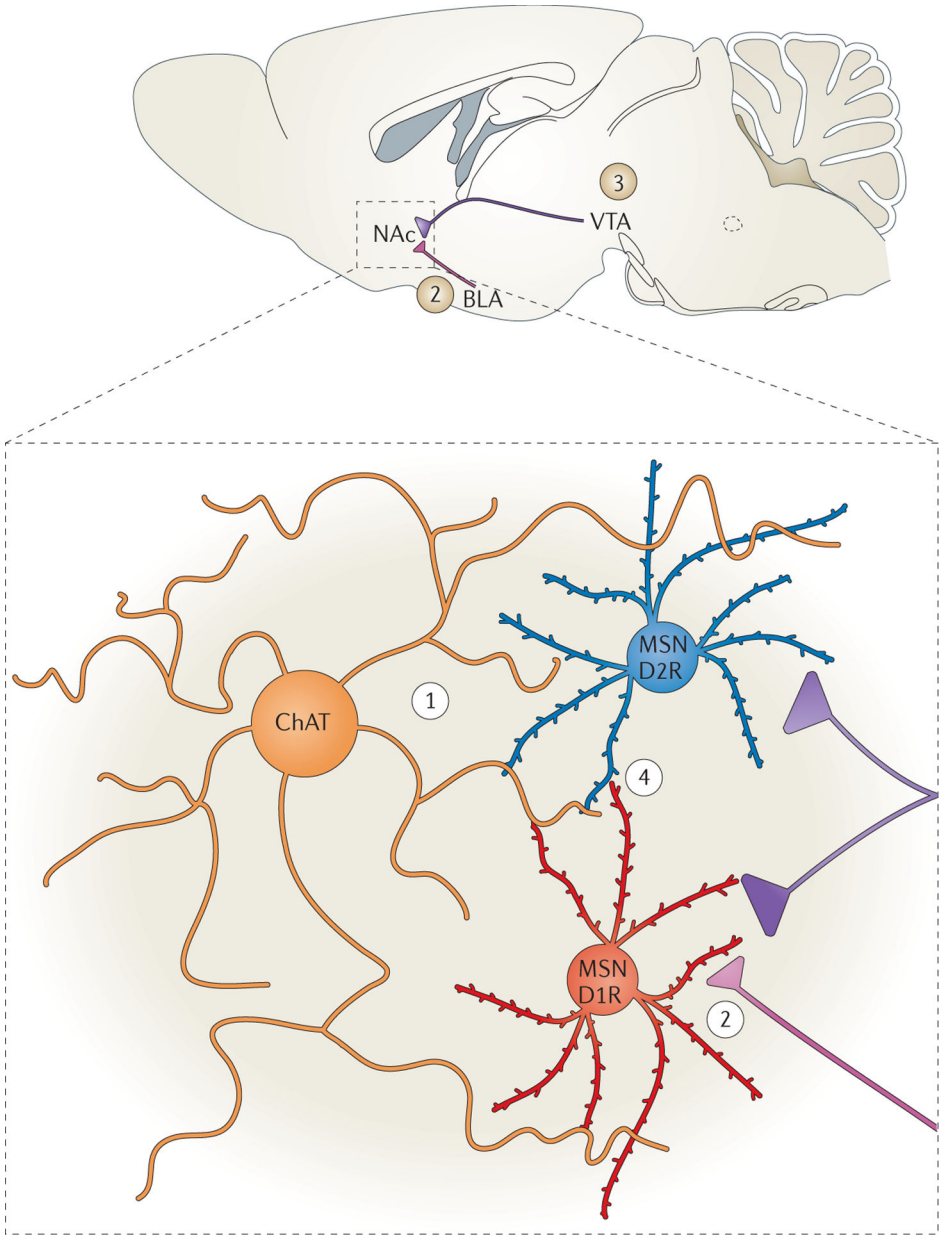


Figure 4 |. Optogenetic dissection of limbic circuits in the context of reward-seeking behaviour. A schematic diagram (top panel), showing key neural projections involved in reward, and an expanded view of intra-accumbens microcircuitry (bottom panel; numbers indicate independent findings). The diagram shows connectivity but is not intended to depict ultrastructural anatomy. Cholinergic interneurons expressing choline acetyltransferase (ChAT) in the nucleus accumbens (NAc) modulate the activity of medium spiny neurons (MSNs) and modulate the ability of the animal to develop cocaine-conditioned place preference (1)[50]. Mice will readily work (nose poke) to receive illumination of channelrhodopsin 2 (ChR2)-expressing basolateral amygdala (BLA) axon terminals in the NAc (2). This self-stimulation behaviour is D1 dopamine receptor (D1R)-dependent, and does not occur when ChR2-expressing prefrontal cortex (PFC) axon terminals in the NAc

are illuminated[61]. It has been shown that activation of dopaminergic neurons in the ventral tegmental area (VTA) can support operant responding in both rats and mice[52], and in rats, illumination of tyrosine hydroxylase and ChR2-expressing axon terminals in the NAc also supports operant responding (3)[37]. The activation of D1R- or D2R-expressing NAc neurons shows differential effects on cocaine-conditioned place preference (4)[113].

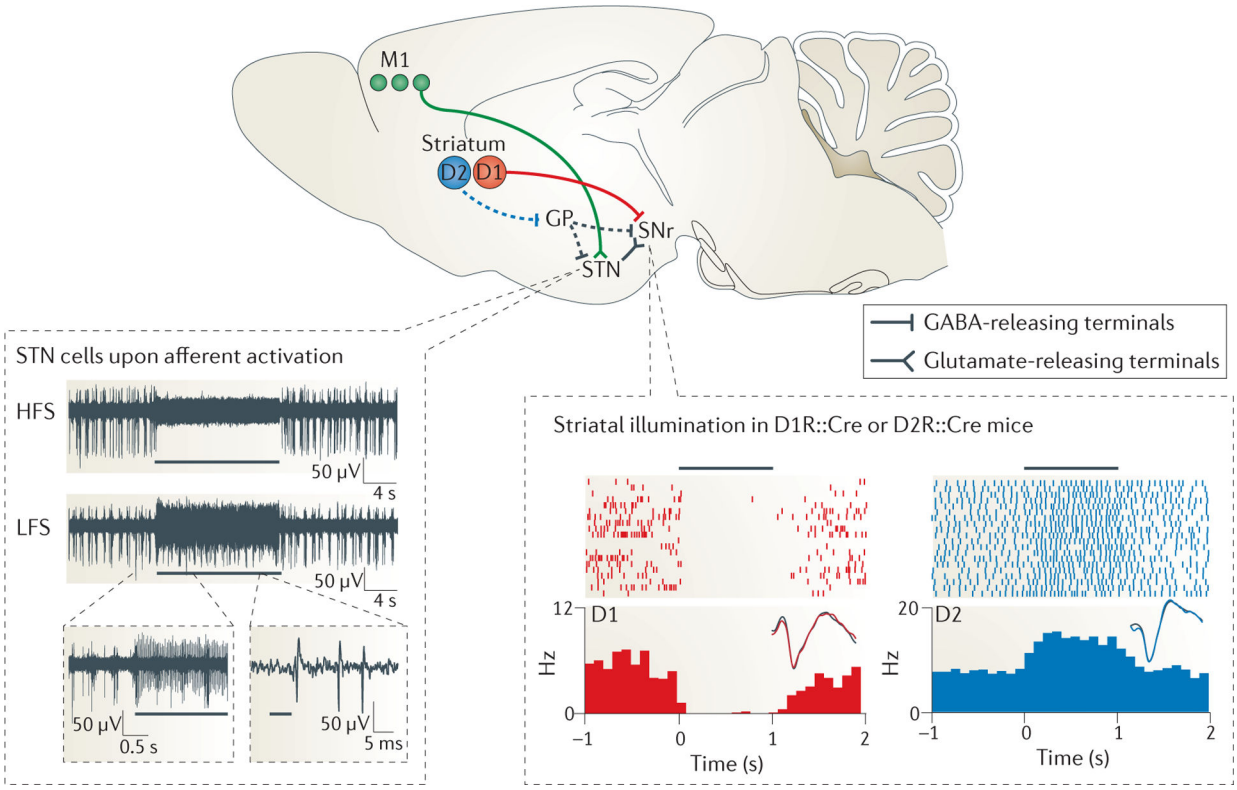


Figure 5 |. Functional mapping of basal ganglia circuitry using optogenetics in the context of Parkinson's disease.

A schematic diagram (top panel) shows key neural projections that are involved in parkinsonian behaviour and treatment. Data in the bottom left panel are from a study that used a constitutively expressing channelrhodopsin 2 (ChR2) mouse line (Thy1::ChR2) to identify a mechanistic explanation for the therapeutic effects of deep brain stimulation (DBS)[55]. By illuminating and recording in the subthalamic nucleus (STN), this paper showed that afferent fibres entering the STN, rather than local cell bodies themselves, are likely to be the direct target of DBS in the correction of parkinsonian motor activity. High-frequency stimulation (HFS) of the afferent fibres into STN potently silenced the structure as shown and reversibly abolished the parkinsonian symptoms. By contrast, low-frequency stimulation (LFS) of the afferents simply added spikes on top of endogenous spikes and worsened parkinsonian symptoms. Data in the bottom right panel are from a study that used a Cre-dependent adeno-associated virus (AAV) to selectively express ChR2 in either D1 dopamine receptor (D1R)::Cre or D2R::Cre mice to examine the differential contributions of the direct and indirect pathways with respect to motor output. Activation of D1R-expressing neurons silenced local basal ganglia activity and increased ambulation, whereas activation of D2R-expressing neurons increased this activity and enhanced immobile or bradykinetic (slow) behaviour[51]. Black bars indicate the duration of illumination. The bottom left panel is reproduced, with permission, from REF. 55 © (2009) American Association for the Advancement of Science. The bottom right panel is reproduced, with permission, from REF. 51 © (2010) Macmillan Publishers Ltd. All rights reserved. GP, globus pallidus; M1, primary motor cortex; SNr, substantia nigra pars reticulata.