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Abstract

Optogenetic techniques allow versatile, cell type-specific light-based control of cellular activity in diverse set of cells, circuits and brain structures. Optogenetic actuators are genetically encoded light-sensitive membrane proteins that can be selectively introduced into cellular circuits in the living brain using a variety of genetic approaches. Gene targeting approaches used in optogenetic studies vary greatly in their specificity, their spatial coverage, the level of transgene expression and their potential adverse effects on neuronal cell health. Here, we describe the major gene targeting approaches utilized in optogenetics and provide a simple set of guidelines through which these approaches can be evaluated when designing an *in vitro/vivo* optogenetic study.

Introduction

Optogenetic techniques utilize a wide range of light-sensitive proteins known from a wide variety of organisms. When heterologously expressed in cells of interest, these proteins are capable of producing light-evoked modulations of various physiological functions, ranging from changes in excitability[1-4] to activation or inhibition of distinct biochemical pathways[5-9] , gene expression[10,11] and enzymatic activity[12,13]. Since the discovery of channelrhodopsin[14,15] and the first application of this microbial opsin for activating neurons[1,16], microbial opsins have been extensively used in experimental neuroscience applications including the functional mapping of neural circuit connectivity and dynamics in the brain and the dissection of neural circuits underlying integrated brain functions and behaviours[17,18]. These studies all capitalize on the single-component nature of the optogenetic effectors, which allows the use of gene transfer technology for their introduction into post-mitotic neurons.

In optogenetic experiments, light is used to transiently and reversibly modulate the physiological properties of cellular compartment of circuits *in vitro* and *in vivo* experimental strategies. To assure that the desired physiological effect is achieved in such settings, several key factors should be taken into account, including:

1. **Specificity & selectivity of expression**: optogenetic tool expression should be restricted to the desired neuronal population, with minimal leak to non-targeted cell populations.
2. **Robustness of expression**: The optogenetic actuator should be expressed at sufficient levels to allow modulation with moderate light power, avoiding phototoxicity.
3. **Cytotoxicity and other adverse effects:** The method used to express the selected tool should be well-tolerated and non-toxic for the host cells over the entire duration of the experimental period (and ideally well beyond this time);

The gene targeting methods described below all differ in the degree to which they optimize each of these parameters. Further information about optogenetic technology can be found in many other excellent reviews [18-21]

1. Promoter-based specificity in transgenic expression of optogenetic tools

In optogenetic experiments, a precise assessment of the efficacy of targeting, i.e. the percentage of transduced cells among a genetically-, circuit- or activity-defined population (see below), provides an important estimate before *in vitro* or *in vivo* experimentation. The efficacy of gene expression is dictated by the genetic regulatory elements under which the optogenetic tools are expressed in the targeted cell population. Gene expression is regulated by a large array of non-coding DNA sequences that contain recognition sequences for binding of specific transcription factors, chromatin remodeling proteins and other regulatory elements [22]. Specific genomic promoters allow gene expression selectively within cell types that possess these regulatory proteins. Genomic promoter sequences can span between several hundred bases and several thousands of kilobases (kb). It is therefore often impossible to package complete promoter sequences into a viral vector backbone since viral vectors are limited in their genomic payload size [23,24] Many of the commonly used optogenetic viral vectors utilize minimal promoter sequences (0.2-1.5 kb); these are truncated segments of much longer promoters, or repeated sequences of specific transcription factor recognition sites, and are sufficient for eutopic targeting. Only a few promoters have been identified that can be truncated in this way while retaining sufficient cell-type specificity (see [25-28]).

Lentiviral vectors, due to their larger payload size compared with AAVs, can carry larger minimal promoters and are therefore effective in some cases where a minimal promoter sequence exceeds the 1.5 - 2 kb size, but < 3.5 kb [25,29]. Notably, although minimal promoter sequences can allow specific expression of transgenes, some promoters produce very weak expression of the transgene, thereby limiting their utility in viral vector-based optogenetics since in most cases very strong expression is required to achieve effective light-based control over the targeted neurons. In such cases, alternative methods exist that include classical transgenic mouse engineering (see above). The use of transgenic (multiple random insertion) or knock-in (locus-specific single insertion) recombinant technologies allow one to target the expression of opsins to a specific class of cells from the early embryonic stages of a transgenic animal. For both these approaches, the size of the promoter is less limiting than for the viral targeting. Due to the locus-specific single insertion of the transgene, the knock-in approach is generally preferred for specificity and stability reasons. However, the low single-channel conductance of some optogenetic tools (e.g. channelrhodopsin, halorhodopsin and archerhodopsin), a single copy of the gene encoding them is typically not sufficient for robust optogenetic modulation. Transgenic expression leads to multiple copies of the opsin gene and therefore permits higher photocurrent sizes in targeted neurons and therefore facilitates optogenetic modulation. The first transgenic mouse model with pan-neuronal expression of ChR2 under the Thy1 promoter was reported in 2010 [30]. Since then, several additional mouse lines were generated, expressing ChR2 in GABAergic neurons (VGAT-ChR2), cholinergic neurons (ChAT-ChR2) and others (see www.jax.org for a a list of available and currently generated transgenes). However, there are several limitations inherent to these transgenic approaches. First, despite the use of long promoter sequences, the expression of the transgene can be too low for proper activation/inhibition of cell bodies, but more importantly for stimulation of distant synaptic terminals, which have been shown to require higher light power for efficient optogenetic stimulation[31]. Second, knock-in strategies may results in haplo-insufficiency and preturb the expresison of the endogenous gene, leading to a molecular phenotype that can have significant synaptic behavioural consequences [32]. Third, transgenic strategies may result in the expression of the transgene in multiple neural circuits in the brain, which strongly hamper the selectivity of the optical manipulation. For instance, VGAT-ChR2 animals express ChR2 in all neurons

expressing the VGAT gene (i.e., most of GABAergic cells). Although optical stimulation can be restricted to small brain nuclei, it will activate cell bodies, axons and fiber of passage as well as terminals in the vicinity of the tip of the optical fiber, which often decrease the specificity of the manipulation. To overcome these limitations, recombinase-based methods or multi-virus circuit/connectivity-based targeting can be used (see below).

2 Viral vector-mediated expression of optogenetic tools

Viral vectors are the most popular means of delivering optogenetic tools to the adult brain. Viral vectors are in essence genetically-engineered viruses in which a minimal set of viral genes has been retained to allow host cell entry, transport to the nucleus and expression of the transgene while eliminating virulence functions such as replication and cytotoxicity [33]. Lentiviral (LV) vectors [23] and adeno-associated viral (AAV) vectors [34] have both been widely utilized for introducing optogenetic transgenes into post-mitotic neurons [35]. There are several important differences between these vectors that should be considered when designing an optogenetic study. Recombinant AAV (rAAV) vectors are considered safer than LV since the currently available strains do not broadly integrate into the host genome but rather remain inside the nucleus as episomes [36]. AAV-based expression vectors display lower immunogenicity [37], and in many cases allow larger transduction volumes than LV [38] . Cell type specificity, the topic of the current chapter, can be achieved using both LV and AAV vectors using cell type-specific promoters [25-27], and both vector types support pseudotyping techniques, which in principle enable a wide range of cell-type tropisms and transduction mechanisms [39,40].

2.1 Combined promoter- and recombinase-based specificity

When the required cell-type specific minimal promoter fragment cannot be packaged into the viral genome while retaining cell-type specificity and adequate expression levels, one can utilize transgenic or knock-in mice expressing a recombinase (e.g., Cre, Flp) [41] under the genomic cell-type specific promoter for the same target population. Recombinase-dependent viral vectors allow specific expression only in cells that contain a specific recombinase protein. For example, a viral vector carrying a Cre-dependent expression cassette will be expressed only in inhibitory neurons of a

mouse that expresses Cre under the control of the parvalbumin promoter, which is specific to a population of fast-spiking inhibitory neurons [42,43]. This approach can be extended to mouse lines carrying the Flpo recombinase, which recognizes sequence elements that are incompatible with the Cre recombinase. More complex "boolean logic" gates have been described, utilizing both Cre- and Flpo-dependent expression cassettes that allow Cre-on, Flpo-on, Cre-off, Flpo-off and all combinations of dual-recombinase logic[35]. Apart from the obvious advantage of utilizing a large genomic promoter to generate Cre driver lines, the use of recombinase-dependent expression vectors allows the uncoupling of expression specificity from transgene levels in the targeted cells which result, for instance, in better membrane expression of ChR2 in axons allowing the activation of a subset of targeted cells by restricting the optical simulus to the cell terminals rather than the soma (see below and Fig. 1). The recombinase-dependent approach is quite versatile and economical since a single Cre-driver strain allows: 1) the targeting of different circuits by restricting the virus injection to specific brain nuclei/circuits (e.g., basal forebrain or brainstem cholinergic neurons in ChAT-Cre animal); 2) utilization of many different types of Cre-dependent viral vectors encoding excitatory, inhibitory and other optogenetic tools.

1.3 Target volume considerations

The size of the target brain structure is a major consideration in the design of an optogenetic study. Experiments targeting small nuclei such as the mouse amygdala [44,45] require different targeting strategies than those involving larger brain regions such as the primate neocortex [46-49]. Therefore, each experimental design necessitates a proper adjustment of the viral delivery methods (e.g. glass pipette, metal needle, *convection-enhanced methods* [50] and viral vector type to allow efficient transduction of the target region. Furthermore, the choice of optogenetic tool and illumination method is critical to assure that the desired effect is achieved in the targeted cells. The spatial distribution of viral vector particles strongly depends on the targeted brain region. Restricted transduction of smaller brain regions can be achieved by choosing the appropriate viral vector and injection volume. For example, LV and AAV2 injection results in expression patterns that are more localized compared with the pseudotyped rAAV2/1, rAAV2/5, rAAV2/8 or rAAV2/9. AAV2 and LV are therefore well-suited for local expression in volumes smaller than 1 mm^3 [38]. Although viral titer

can be reduced in order to decrease the size of the transduced volume, lower-titer injections are also likely to influence the number of genome copies in transduced cells, leading to lower expression levels of the transgene in individual cells within the target region [38]. Compared with AAV2 transduction, LV transduction is more spatially restricted when injected *in vivo* and thus, can be used to target smaller structures [44]. However, LV has been reported to exhibit a bias towards excitatory neurons in cortex [27], an effect which is likely also region-specific since other more specialized cell types have been successfully targeted with lentiviral vectors [25,29,51]. Although such control of viral transduction volume can be achieved with the choice of viral vector, transduction of larger volumes can simply be obtained by performing multiple injections covering a large area. This strategy is commonly used in primate studies, and has also been used successfully in the rodent brain [52].

1.4 Circuit-based expression of optogenetic tools

Neural circuit dissection is one of the most widely-used applications of optogenetic techniques. Optogenetic activation combined with electrophysiological recording allows functional anterograde circuit mapping[53,54]. Introduction of fluorescently tagged channelrhodopsins to the membrane of specific neuronal populations in a defined brain region allows visualization and subsequent photoactivation of long-range axonal connections throughout the brain. Simultaneous electrophysiological recording at the projection site allows the identification of specific post-synaptic components of the circuit both *in vivo* and in the acute brain slice preparation [45,55,56]. While it provides important information regarding the functional properties of specific anterograde projections, it is hardly scalable due to the need to perform electrophysiological recordings at each target site. Circuit-based expression tools utilize neurotropic viruses for tagging neurons based on their connectivity pattern with identified neurons or macroscopic anatomical projection patterns.

Circuit-based expression methods can be divided to two types: those based on the anatomical location of presynaptic terminals (anatomical circuit-based targeting, Fig. 1c) and those that allow targeting of neurons based on specific synaptic connectivity (monosynaptic circuit-based targeting; Fig. 1d). Anatomical circuit-based targeting can be achieved using a variety of viral vectors that are capable of transducing neurons through their axons or presynaptic terminals [57-60]. The herpex simplex virus (HSV) has been utilized in a variety of optogenetic experiments to label neurons

projecting to a specific location in the brain, to attain optogenetic modulation of these cells [61,62] or to "tag" these neurons using combined electrophysiological recording and photostimulation [58]. The type 2 canine adenovirus (CAV2; [59]) has been used for a similar purpose and is perhaps even more efficient than HSV in retrograde targeting. Although these two viruses are both considered retrograde-labeling, some studies have mentioned that these two vectors transduce non-overlapping neuronal populations [63] and that CAV2 might also be capable of transducing axons of passage [64]. It is therefore important to keep these differences in mind when designing and interpreting circuit-based expression experiments, and to conduct proper anatomical controls.

Monosynaptic circuit-based targeting (Fig. 1d) capitalizes on the exquisite capability of the rabies virus to transport its genetic material across synaptic contacts. This approach utilizes a glycoprotein-deleted variant of the rabies SAD B19 strain, SADΔG [60]. The rabies virus glycoprotein (G), which is embedded in the viral membrane, is required for trans-synaptic spread [65]. By introducing the glycoprotein gene in neurons prior to infection of the G-deleted mutant virus, the virus spreads to pre-synaptic neurons and is restricted from further spread due to the lack of this complementary glycoprotein in newly transduced neurons. This enables the dissection of direct connections originating from a population of defined neurons, or even from a single primary infected neuron [66,67].

While this approach provides much more refined selectivity of retrogradely-targeted neurons, it still lacks specificity due to the difficulty in targeting the rabies glycoprotein to the primary neurons. To achieve more refined specificity of the primary viral transduction event, the rabies vector can be directed to genetically-defined post-synaptic neuronal subtypes by using the avian receptor TVA system. In this approach, the SADΔG rabies variant is pseudotyped with an envelope protein from the avian sarcoma and leukosis virus (ASLV). The avian-specific TVA receptor, which is required for infection by the pseudotyped rabies virus, is then expressed in the cells to be targeted for infection by SADΔG along with the rabies glycoprotein. This allows the virus to spread trans-synaptically from only the TVA-expressing cells to their presynaptic partners. The TVA receptor, along with the rabies G protein gene, can be delivered using AAV to specific neurons using the double-floxed Cre-based expression system [55,68]. Under this configuration, only Cre-expressing cells will express the proteins required for both uptake of the pseudotyped rabies virus and

monosynaptic retrograde transport. The advantages of using rabies-based circuit tracing techniques are its efficient uni-directional retrograde transport and its rapid onset of expression. Unfortunately, the time course of survival of SAD B19-transduced neurons is limited to approximately two weeks [60], suggesting that other systems might be required for experiments requiring long-term survival of transduced neurons.

1.5 Activity-based tagging and optogenetic control

Cell-type specificity, the topic of this chapter and one of the key advantages of optogenetics over more classical methods of experimental manipulation in neural circuits, relies on the inherent assumption that cells with distinct gene expression properties perform defined function in neural circuits. Yet, from a systems neuroscience perspective, this assumption is inherently flawed since neuronal ensembles can form purely from processes of synaptic activity and in a way that is at least partially independent of genetic "identity". Can we therefore target ensembles of neurons based on their "assembly/ensemble activity" in a particular behavioral paradigm or neural representation of environmental stimuli? For decades, immediate early genes have been used to represent reliable marker of cell activity, though their expression varies between and within cell population. These genes thus allow the identification of neurons that have been active over a short period of time (minutes to hours) and provided functional tagging of such activity-modulated gene promoters for activity-based expression of optogenetic actuators.

The promoter for c-Fos, an activity-dependent immediate early gene, can be used in combination with the rapidly inducible TRE-ttA expression system in order to achieve expression of ChR2 in hippocampal neurons activated during aversive learning[69] and appetitive experience[70]. In this experimental configuration, the tetracycline transactivator (tTA) is expressed under the control of the c-Fos promoter in a transgenic mouse. An adeno-associated viral vector is then introduced into the hippocampus, expressing ChR2 under the control of the tet-response element (TRE), which under this configuration allows expression of ChR2 in the presence of tTA and in the absence of doxycycline[69]. This system allows selective activation of memory engrams in various paradigms [70], but it requires the constant administration of doxycycline except during the experiment in which the cells are to be labeled and is therefore referred to as "tet-OFF". The tet-ON system, while potentially easier to

apply since doxycycline should only be administered during the labeling experiment, is more "leaky" and is therefore potentially less useful for such experiments as they rely on specific optogenetic modulation of cells activated during a strictly defined time window. An alternative approach has been developed which uses the expression of an inducible form of the Cre recombinase (Cre^{ERT2}), expressed transgenically under the control of the promoter for the immediate-early gene Arc [71].

A similar approach, utilizing either the c-Fos or Arc promoters with the Cre^{ERT2} transgene, has been used to generate the Fos-TRAP and Arc-TRAP mice, a general resource for targeting expression of any transgene to recently-activated ("TRAPped") neurons [72]. Finally, work from the Bito lab has led to the development of the E-SARE vectors [73], which utilize tandem repeats of the Arc enhancer sequence to generate a viral vector that expresses an activity-dependent form of the Cre^{ERT2} protein. When injected into the brain this viral vector expresses Cre^{ERT2} in a manner that allows activity-dependent expression of Cre-dependent transgenes. The E-SARE approach has the advantage of allowing activity-dependent modulation in non-transgenic animals, but does not allow the brain-wide screening possible with the Fos/Arc-TRAP mice. Importantly, all of these approaches rely on activity-dependent transcription of immediate-early genes, but they vary with regard to the temporal integration time of activity-dependent expression, in its efficacy and in the level of baseline expression levels. Experiments utilizing these constructs for expression of optogenetic tools should be preceded by detailed characterization of these parameters in the particular cell type, region and behavioral paradigm used.

2 Light delivery in the animal brain

After the proper targeting of optogenetic tools to neural circuit(s) of interest, the next step consists of designing an optical neural interface for *in vitro/vivo* light delivery into (deep) brain structures or brain slices, respectively. *In vitro* whole cell recordings are frequently used to verify the biological functions of the opsins, as well as a first step towards deconstruction of neural circuits. In this case, a light source can be coupled to the objective of the microscope and controlled by integrated TTL generator/electrophysiology stimulator. In the case of a two-photon microscope set up, a laser beam of small diameter (few dozens of microns) can be focused on smaller targets for high-precision synaptic physiology [53,74-76]. Sculpted light holds great promises in shaping 3D light stimulation in in vivo preparation [77-80].

Similar to deep brain electrical stimulation that uses metal electrodes to non-selectively activate cells in brain structure (e.g., self-electrical stimulation paradigms, Parkinson disease, etc.), optogenetic configuration requires optical fibers to deliver sufficient light to shallow or deep brain targets.

2.1 Optical fiber-based light delivery system to deep brain structures

Typical light sources include high-power diode pumped solid-state lasers (DPSSLs) or light-emitted diodes (LEDs) that are controlled by a waveform generator and commercially-available optical shutters. Glass or plastic optical fibers are used for connecting light sources to *in vivo* preparation (see below). Non-invasive optical fibers or light-emitting diodes can be used with fiber implants or cranial windows for optical stimulation of neuronal networks located in the superficial layers of the cortex or in deep targets, respectively [81]. Deeper targets may require the use of optical fiber implants [82] that are chronically implanted and connected to an optical tether for longitudinal experimental strategies. In vivo optogenetic studies have used a variety of multi-mode fibers that have larger core-size than single-mode fibers and thus, higher numerical apertures and increased "light-gathering" capacity. Light pulses propagate down the fiber-optic by total internal reflection, reaching the fiber tip with minimal power loss. However, it is important to note that the desired experimental application will determine the number of fibers, their shape, length and diameter. Additionally, it is necessary to render the optical fibers opaque (using dark coating or black furcation tubing), since even a small amount of light diffraction through the optical tether can cause sensory stimuli during behavioral testing, particularly in dark environments.

Factors to consider when delivering light to brain structures include 1) the use of appropriate wavelength to activate opsin; 2) the necessity of using one or several optical fiber implants to optimize light delivery to the entire target area (e.g., unilateral *vs* bilateral, multisite fibers, etc.); 3) sufficient light power through the use of either high power lasers or LEDs; 4) the use of optical swivel to allow free movement of the tethered animals.

2.2 Emerging techniques: non-invasive optical stimulation (red shifted opsins, step-function opsins, nanoparticles)

Aternative to the invasive use of optical fiber implant include red-shifted optins, step-function opsins and nanoparticules. The blue light wavelength used for activation of channelrhodopsin is both absorbed and scattered when it penetrates throught he brain tissue, leading to an exponential decay in light power density with distance from the tip of the optical fiber or light source [18,83]. This is minimized by the use of red and far red wavelength and red-shifted channelrhodospin. Although these wavelengths are more prone to generate heat (that must be assesed with proper control conditions), they allow optical control of cortical circuits through thin/polished skull preparation [84,85].

Furthermore, the use of channelrhodopsin requires constant optical stimulation at different frequencies to elicit action potentials. An extraordinary alternative consists in using step-function opsins (SFO) - turn ON and OFF with single pulse of blue and yellow light, respectively. Not only do SFOs avoid imposing hypersynchrony and non-natural firing of action potentials, they have been successfully used to turn ON and OFF cortical circuits remotely, through thinned skull, instead of invasive optical fiber implants [18,82]. A potential limitation, however, is the need to carefully titrate the amount of light delivered to activate these opsins, since excessive depolarization by these opsins could potentially lead to depolarization block and effective silencing of the targeted neurons.

3 Recording light evoked neuronal activity.

A major strength of the optogenetics technology is its compatibility with fast *in vitro*/*vivo* electrophysiological/optical/chemical readout methods[86-88]. Indeed, if an electrophysiological/optical/chemical probe (tetrodes, glass pipette, dialysis probe, fiber photometry, etc.) is implanted in the vicinity of the targeted neurons, it offers a direct confirmation of optical modulation First, it allows one to verify that optogenetic that the optogenetic manipulations work as intended. Although the causality of light-evoked neuronal activity is somewhat debatable, it remains a relatively important verifying step in an experimental procedure. Second, it can be used to characterize the spontaneous activity of these cells during specific behaviour. Defining the precise pattern of firing of opto-tagged cells is particularly important when conducting an optogenetic experiment since it allows one to define optical

stimulation parameters that remain within a physiological range for the particular neuronal population targeted. Third, if the probe is implanted in the vicinity of the terminals, rather than the soma, of targeted neurons, functional circuit mapping experiments can be conducted *in vivo* to reveal a direct and temporally-precise readout of circuit modulation before, during and after optogenetic manipulations. On a deeper mechanistic prospectives, *in vitro* assays of opsin function have been used to study neurotransmitter release from a variety of cell types, including dopaminergic , cholinergic , noradrenergic , hypocretins/orexins neurons , and MCH . [references for these?]

This rapid feedback from *in vivo* electrophysiology or electrochemical detection is valuable for measuring the light-evoked response of neuronal activation/silencing in an *in vivo* preparation, besides the use of classical immunohistochemistry and *in vitro* electrophysiological procedures. Importantly, it allows the fine-tuning of optical stimulation parameters for efficient control of circuit activity in a physiologically-relevant range.

Perspectives

In this chapter, we focused on the use of cell-type specific targeting strategies for optogenetic modulation of neural activity. Over the last decade, the advent of multiple genetically-encoded tools for imaging and manipulation of neuronal activity have expanded the repertoire of techniques for studying the cellular substrates of brain functions and the mechanisms underlying innate, acquired and pathological behaviors. The field of optogenetics had developed rapidly since its first *in vivo* application [25,89]. Current progress in protein engineering is expected to lead to the discovery of novel light sensitive membrane, cytoplasmic or nuclear proteins for remote control of selective ionic flow, cellular signalling, and gene regulation, while the concurrent developement of genetic targeting strategies will allow refined selection of circuit elements for manipulation. The growing application of optogenetic techniques hold great promises on improving our understanding of mamalian brain functions, and identification of novel therapeutical strategies.

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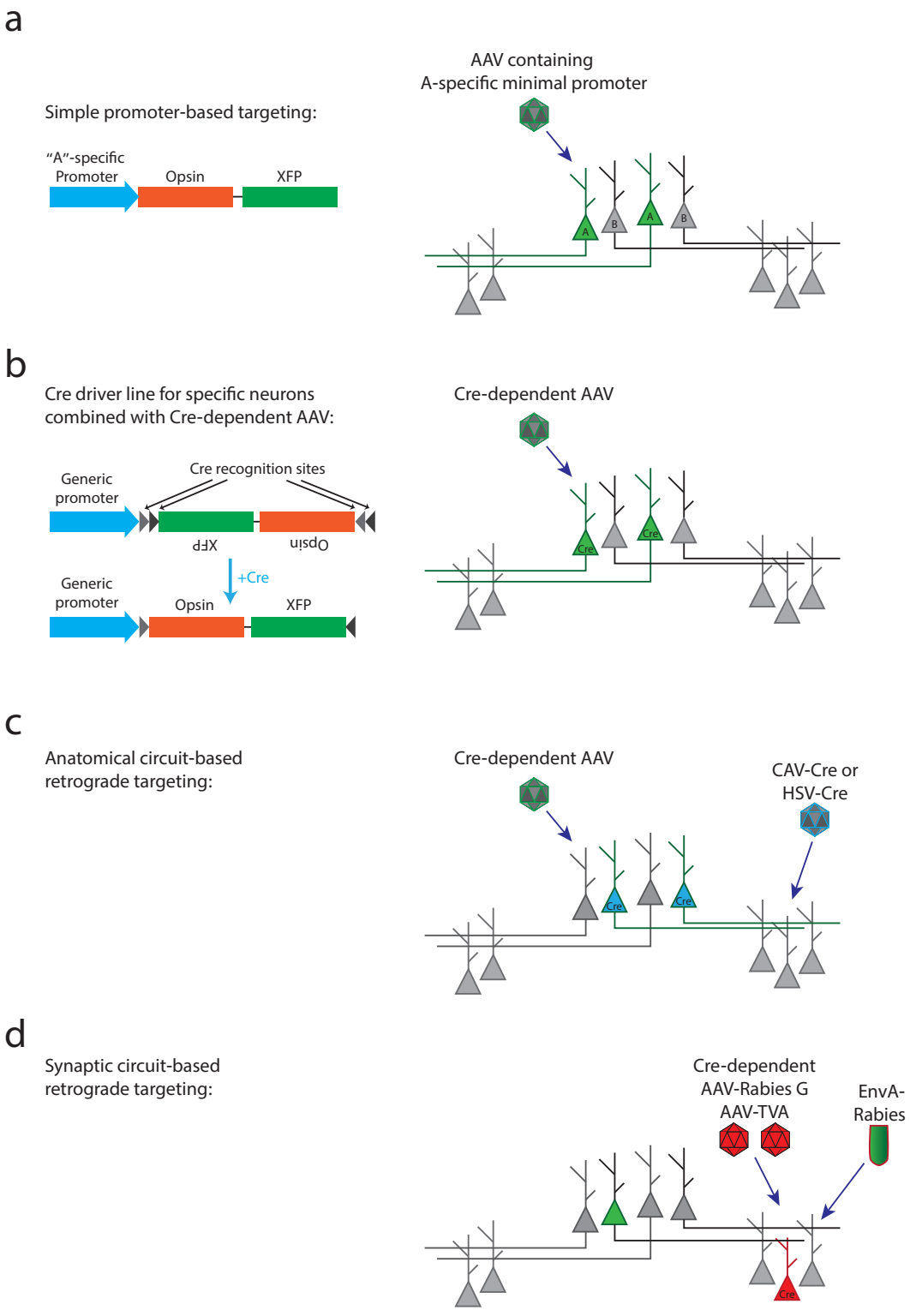

Figure 1: Schematic of genetic targeting of neural circuits.

(**a**) Simple promoter-based targeting relies on a single viral vector (AAV or lentiviral) carrying the opsin gene under the control of a cell-type specific minimal promoter fragment. Expression of the opsin in the soma and axonal efferents is guided by the activity of the promoter. (**b**) Cre-dependent expression using viral vectors. In a mouse

that expresses Cre under a cell-type specific promoter, a Cre-dependent AAV is injected to the target circuit. The opsin and fluorescent protein ("XFP") genes are positioned in an inverted orientation with regard to the promoter. Cre-mediated recombination "flips" the opsin and fluorophore genes into the forward orientation, permitting the expression of the opsin-reporter fusion protein only in cells expressing Cre. (**c**) Anatomical circuit-based targeting is carried out by injecting a recombinase-expressing viral vector with the capacity to undergo retrograde transport through transduction of presynaptic nerve terminals. A Cre-dependent opsin-expressing viral vector (as in **b)** is injected at the site of the presynaptic cell bodies, where Cre is expressed only in neurons projecting to the site injected with the Cre-carrying vector. (**d**) Synaptic circuit-based targeting. In this approach, a pseudotyped rabies virus is injected following expression of two Cre-dependent expression vectors encoding the rabies glycoprotein (Rabies G) and the avian receptor TVA. The Env-A pseudotyped rabies virus can only transduce TVA-expressing cells, and requires the Rabies G protein to exit the cell and perform retrograde trans-synaptic transport to its pre-synaptic partners. Expression of the opsin from the rabies genome (green) occurs only in cells that provide monosynaptic input to the first-order neurons (red).

Figure 2: Representative timeline for opto-tetrodes recording experiment.
a, Mice are stereotactically injected with ChETA-eYFP, ArchT-eYFP or control AAV at 6-weeks old before chronic implantation of optical fiber implants or opto-tetrodes, EEG, LFPs and EMG (shown in b). Schematic of the Cre-inducible AAV vector backbone is shown. 15 day after injection, mice will be habituated to recording conditions before baseline recording and actual stimulation/recording experiments at 12-weeks old. Novel Obejct Recognition Task and Fear conditioning will be conducted 2 and 4 weeks later, respectively. b, Illustration of recording sheme for opto-tetrode (up) and -probe (bottom) recording in freely-moving rodents. d, Example traces of cortical EEG (top), multi-units (middle) and EMG (bottom) recordings in the LH area in freely-moving mouse.

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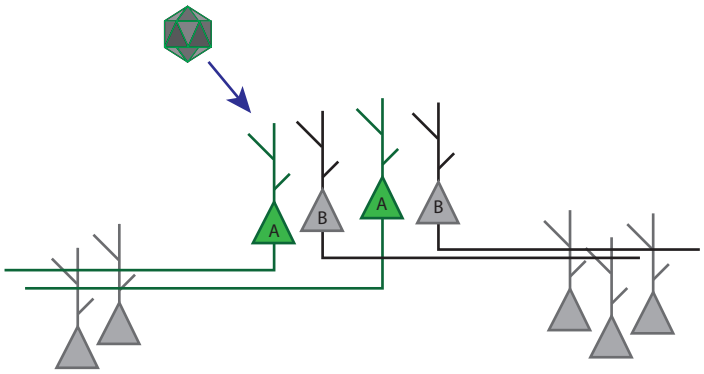
a

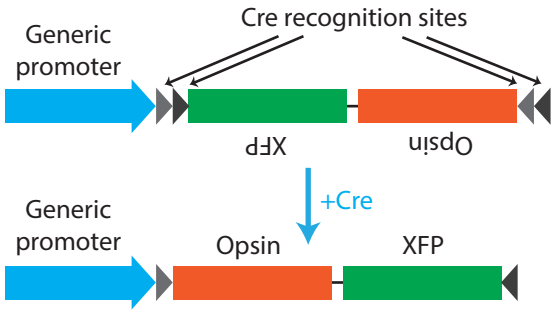
Simple promoter-based targeting:

AAV containing
A-specific minimal promoter

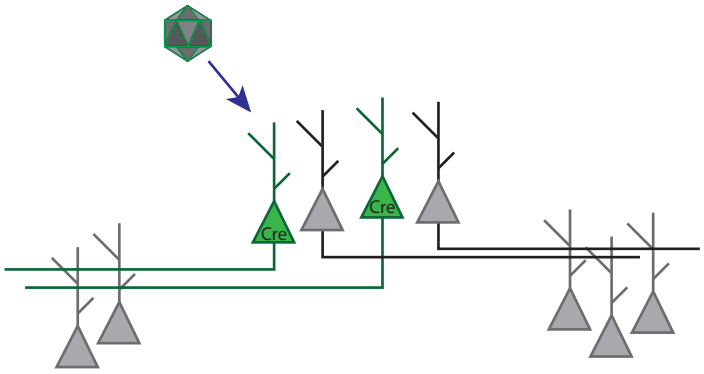


b

Cre driver line for specific neurons
combined with Cre-dependent AAV:



Cre-dependent AAV

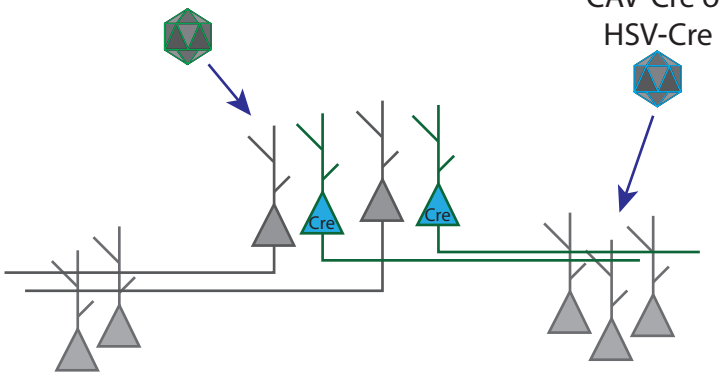


c

Anatomical circuit-based
retrograde targeting:

Cre-dependent AAV

CAV-Cre or
HSV-Cre

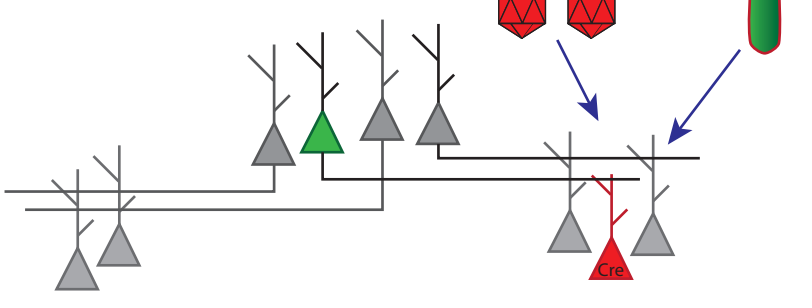


d

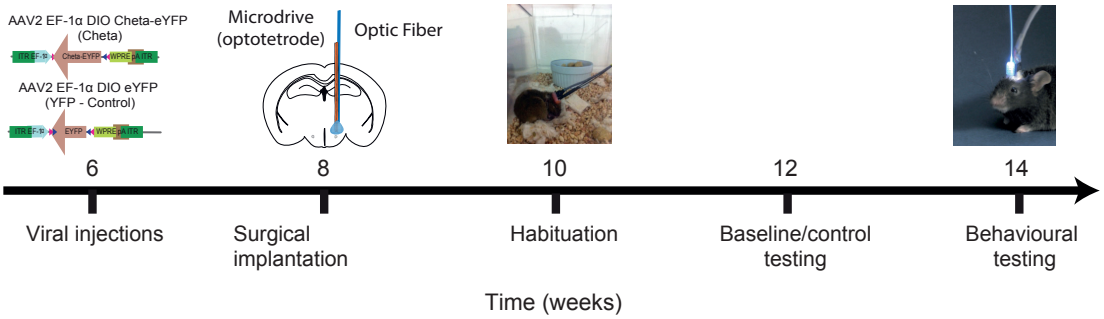
Synaptic circuit-based
retrograde targeting:

Cre-dependent
AAV-Rabies G
AAV-TVA

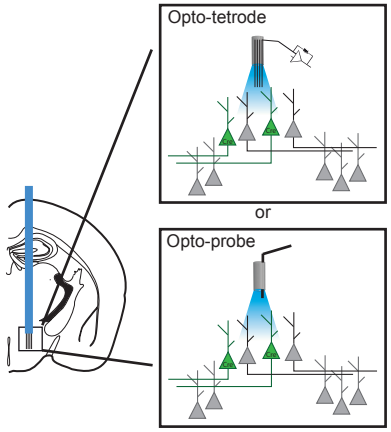
EnvA-
Rabies



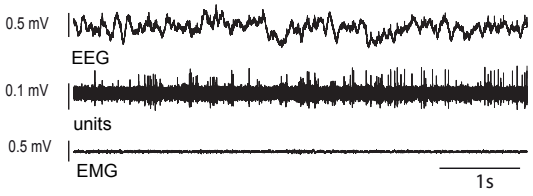
a


AAV2 EF-1α DIO Cheta-eYFP
(Cheta)
AAV2 EF-1α DIO eYFP
(YFP - Control)
Microdrive
(optotetrode)
Optic Fiber
6
8
10
12
14
Viral injections
Surgical
implantation
Habituation
Baseline/control
testing
Behavioural
testing
Time (weeks)

c


Opto-tetrode
or
Opto-probe

d


0.5 mV
EEG
0.1 mV
units
0.5 mV
EMG
1s