Optogenetic Strategies to Dissect the Neural Circuits that Underlie Reward and Addiction

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Optogenetic strategies for perturbation of neural circuit function have begun to revolutionize systems neuroscience. Whereas optogenetics has proven to be a powerful approach for studying neural systems, the tools to conduct these experiments are still continuously evolving. Here we briefly summarize available hardware and reagents that can be used for studying behaviors related to reward and addiction. In addition, we discuss recent studies in which these strategies have been applied to study neural circuit function in brain slices as well as awake and behaving animals. Collectively, this work serves as a brief introduction to optogenetic techniques and highlights how these tools can be applied to elucidate the neural circuits that underlie reward processing and addiction.

Determining causal relationships between neural function and behavior is crucial to understanding the neuropathology underlying addiction. Relationships between function and behavior have traditionally been accomplished by tissue lesioning techniques, electrical stimulation, or pharmacological activation or inactivation. Whereas these methods have uncovered the basic neuroanatomical pathways that mediate reward-related behavior, they often fail to determine how a specific neural pathway or which neuronal cell types mediate a given behavioral response. Site-directed pharmacological manipulations can sometimes be used to address genetically defined pathways (only if a specific receptor), but these manipulations are often over longer timescales, which do not allow for determining how neural activity is required for discrete behavioral events, which can often times last for less than 1 sec. To investigate causal relationships between genetically defined populations of neurons and reward-seeking behavior, techniques allowing for precise control of neural circuitry with millisecond precision are required. Optogenetics allows for pathway-specific manipulation of brain circuitry over a range of timescales, which circumvents many of the technical limitations associated with electrical, lesioning, and pharmacological manipulations. Finally, combining optogenetics with slice electrophysiology and in vivo behavioral...
paradigms can yield an unprecedented insight into how the neural circuitry mediates addiction-related behaviors.

**OPSINS AND HARDWARE TO CONTROL SPECIFIC NEURONAL PATHWAYS WITH LIGHT**

For a full description of the specific opsin proteins that are currently available to study neural circuits, see Yizhar et al. (2011a). The most commonly used opsin to activate neural circuits is channelrhodopsin-2 (ChR2). ChR2 is a light-gated cation channel that was originally isolated from blue-green algae (Nagel et al. 2003). ChR2 is maximally activated by blue, 450–490 nm light. When activated, absorbed photons cause a light-induced isomerization of the all-trans retinal protein, which leads to the opening of the channel allowing sodium and other cations to flow through the cell. When expressed in a neuron, this influx of cations causes depolarization of the cell membrane at resting membrane potentials, which will lead to the opening of endogenously expressed voltage-gated sodium channels to initiate an action potential. More recently, red-shifted channelrhodopsin proteins have been developed, which allow for the possibility of exciting two genetically distinct populations of neurons within the same brain site. Volvox channelrhodopsin (VChR1), the first red-shifted channelrhodopsin characterized (Zhang et al. 2008), has several limitations such as low photocurrents and poor membrane trafficking. However, C1V1, a ChR2-VChR1 hybrid, has been engineered to increase membrane expression and has stronger photocurrents, making it more suitable for excitation of neural circuits (Hegemann and Moglich 2011; Yizhar et al. 2011b).

Optogenetic inactivation of neural circuits is most commonly accomplished using the light-gated chloride pump, halorhodopsin (NpHR), which was first discovered in arachabacteria (Matsumo-Yagi and Mukohata 1977). Introduction of wildtype NpHR into neurons demonstrated that photoinhibition was possible, but initially exogenous NpHR protein was not sufficiently expressed at neuronal membranes for consistent results in vivo (Gradinaru et al. 2010). Further modification of NpHR with an added endoplasmic reticulum (ER) export signal and membrane trafficking peptide sequence, results in robust expression at neuronal membranes, which facilitated its use in vivo for neuronal circuit element inhibition (Gradinaru et al. 2010). NpHR is maximally activated by a yellow/orange, \( \approx 590 \)-nm wavelength of light, but can respond to a broad wavelength range from \( \approx 520 \) to 620 nm. When activated, NpHR pumps chloride from the extracellular space into the cytoplasm of the cell. When expressed in a neuron, this results in hyperpolarization of the cell membrane, and can decrease neuronal firing rates (Fenno et al. 2011). Optical inhibition can also be achieved by the use of outward proton pumps, such as Arch (Chow et al. 2010; Fenno et al. 2011). Arch is maximally activated by a 560-nm wavelength of light, and activation of Arch has been shown to result in robust currents at relatively low light outputs (Chow et al. 2010).

Although proton pumps such as Arch show robust inhibition of neuronal membranes, it remains undetermined the deleterious effects these proteins have in neuronal tissues and if they show any noncell-type-specific effects (Fenno et al. 2011).

Expressing opsin proteins under the control of cell-type-specific promoters is one method of targeted manipulations of genetically defined neuronal subtypes. Using this technique, optogenetic manipulation of glutamatergic basolateral amygdala (BLA) neurons to the nucleus accumbens (NAc) have been investigated (Stuber et al. 2011). Calcium-calmodulin-dependent protein kinase IIa (CamKIIa) is preferentially expressed in glutamatergic projection neurons in the BLA (McDonald 1992). ChR2 or NpHR3.0 was introduced into these glutamatergic neurons using an adeno-associated virus (AAV) vector with the opsins under the control of a fragment of the CamKIIa promoter. Stereotoxic injection of viral constructs encoding these proteins into the BLA results in opsin positive neurons constrained to glutamatergic projection neurons within the BLA. As discussed in detail below, implantation of an optical fiber
into the NAc allows for precise control over excitatory BLA inputs into the NAc. Other studies using the CamKIIα promoter have investigated BLA efferents to other regions of interest such as the central amygdala (Tye et al. 2011) and to study cortical pyramidal neurons (Aravanis et al. 2007; Sohal et al. 2009; Yizhar et al. 2011b).

A transgenic approach is also a common method to achieve targeted manipulation of genetically defined cells. There now exist a number of transgenic mouse lines that selectively express ChR2 or NpHR in specific subtypes of neurons (Arenkiel et al. 2007; Zhao et al. 2011). Whereas this method ensures that virtually all neurons of a specific genetically defined population will express opsin proteins, it oftentimes does not provide anatomical specificity of expression to a discrete brain region of interest. Thus, to reliable target neuronal populations within specific brain nuclei, cre recombinase-inducible expression systems have been used in conjunction with transgenic animals expressing cre in specific population of neurons. Using this method, cre-inducible opsins are stereotaxically injected into transgenic rodents expressing cre recombinase in genetically identified neuronal populations (Atasoy et al. 2008; Cardin et al. 2009; Sohal et al. 2009; Tsai et al. 2009; Witten et al. 2011). Cre-inducible AAV vectors contain DNA cassettes with two pairs of incompatible lox sites (LoxP and lox2722), with an opsin inserted between the two lox sites in the reverse orientation. Cre recombinase catalyzes recombination between the two lox sites, resulting in the opsin reversing its orientation, allowing mRNA of the opsin to be transcribed. Thus, delivery of these cre-inducible opsins into a specific brain region results in opsin expression in only the genetically identified cell type in the brain region of interest. Cholinergic interneurons in the NAc have been targeted using this method (Witten et al. 2010). Here, BAC transgenic choline acetyltransferase (ChAT)::Cre mice were injected with a cre-inducible double-floxed recombinant AAV vector coding for ChR2 or NpHR3.0 into the NAc. Dopaminergic (DAergic) neurons in the ventral tegmental area (VTA) have also been targeted using a transgenic approach in which tyrosine hydroxylase (TH)::Cre (Tsai et al. 2009) in mice or rats (Witten et al. 2011) or dopamine transporter (DAT)-cre mice (Stuber et al. 2010; Cohen et al. 2012) are injected with a double-floxed cre-inducible opsin vector. The use of cre-mice paired with double-floxed opsins, or the use of cell-type promoters, allows for precise control over genetically defined populations of neurons.

Different hardware setups have been used to deliver light in vitro and in vivo. The most common in vitro light delivery systems include filtered light from mercury arc lamps (Boyden et al. 2005; Gunaydin et al. 2010), lasers (Stuber et al. 2010), and LEDs (Wang et al. 2009; Adesnik and Scanziani 2010). In vivo, lasers coupled to optical fibers are most commonly used to deliver light into the brain (Cardin et al. 2010; Stuber et al. 2011; Tye et al. 2011). For a more in-depth description of in vivo light delivery to the brain, see the section entitled In Vivo Optogenetic Strategies.

Finally, interfacing lasers with behavioral equipment allows for optogenetics to be employed in a wide range of reward-related behavioral paradigms including conditioned place preference (Tsai et al. 2009; Lobo et al. 2010), operant conditioning (Adamantidis et al. 2011; Stuber et al. 2011), and Pavlovian conditioning (Stuber et al. 2011). Combining these paradigms with optogenetics allows for subsecond precision control of neural circuitry time locked to discrete behavioral events. Behavioral paradigms associated with other neuropsychiatric diseases, such as open field test and elevated plus maze, have also been interfaced with in vivo optogenetics (Tye et al. 2011). This can be achieved using real-time video tracking hardware and software to restrict optical stimulation when the animal enters a specific area of a behavioral arena.

**SLICE ELECTROPHYSIOLOGY PAIRED WITH OPTOGENETICS TO PARCEL OUT LOCAL CIRCUITS**

Anatomical tracing studies and electrophysiological techniques using electrical stimulation are often used to study the synaptic connectivity within neural circuits. However, there
are significant limitations associated with both of these techniques. Anatomical tracing studies often fail to address the strength and functionality of the synaptic connections. Electrophysiological studies using electrical stimulation can address functionality, but they often fail to determine cell-type-specific projections because most neural tissues are heterogeneous. For example, electrical stimulation of neurons in the VTA will nonspecifically activate both DA and GABA neurons, in addition to any afferents or fibers of passage through the area. Patch clamp electrophysiology paired with optogenetics circumvents the limitations associated with both of these methods because it allows for cell-type-specific activation and assessment of the strength and functionality of these connections. Using this method, it is possible to record from cell-type identified postsynaptic neurons (using mice expressing fluorescent proteins in specific neurons or by post hoc immunohistochemistry), while optically stimulating site-specific or genetically defined afferents that are expressing ChR2. These techniques have been successful in parsing out neural circuits involved in addiction. In one example of this application, Chuhma et al. (2011) used optogenetics in NAc brain slices to define the functional connectivity of medium spiny neurons. By conditionally expressing ChR2 in medium spiny neurons, these authors were able to investigate connections within the striatum and projections to the globus pallidus and substantia nigra (Chuhma et al. 2011), as well as examine how striatal cholinergic interneurons can regulate function of other populations of striatal neurons (English et al. 2012).

Optogenetics paired with slice electrophysiology has also been used to examine the possibility of neurotransmitter co-release. DA and glutamate coincident signaling is crucial for a variety of motivated behaviors including responding to motivationally significant stimuli. A subset of tyrosine hydroxylase positive DA neurons in the VTA also express vesicular glutamate transporter-2 (VGluT2), indicating that these DA neurons are capable of packaging glutamate into synaptic vesicles (Hnasko et al. 2010). Furthermore, pharmacological and electrophysiological studies have suggested that DA neurons co-release glutamate (Sulzer et al. 1998; Bourque and Trudeau 2000; Chuhma et al. 2009); however, these studies only provided indirect evidence as a result of the technical limitations. Selective optogenetic stimulation of ChR2-positive DAergic terminals in the NAc shell results in excitatory postsynaptic currents (Stuber et al. 2010; Tecuapetla et al. 2010), confirming that midbrain DA neurons are capable of coreleasing glutamate in the NAc. Similar studies have now confirmed that other neurons that release neuromodulators, such as acetylcholine, are also capable of glutamate co-release, such as projection neurons in the medial habenula (Ren et al. 2011). Utilizing optogenetic approaches to study neurotransmitter release will likely yield a plethora of novel information on the intraneuronal signaling dynamics of defined neural circuits.

**IN VIVO OPTOGENETIC STRATEGIES**

In vivo optogenetic approaches can be used for a variety of different experiments, from targeted manipulations of genetically defined cells to manipulation of specific neural pathways on a physiologically relevant timescale. Furthermore, utilizing these in vivo optogenetic approaches in awake and behaving animals allows for precise control over neural circuitry time locked to discrete events, necessary for determining a causal relationship between neural function and reward-related behaviors.

Delivering light into the brain is most often accomplished by implanting an acute or chronic optical fiber into the region of interest (Zhang et al. 2010; Sparta et al. 2012). Using the acute optical fiber method, a guide cannula is chronically implanted in either the virus-targeted region or the projection region of interest. Then, the optical fiber is acutely implanted immediately prior to the experiment. One benefit of employing an acute fiber is the ability to combine local pharmacology through the cannula before implantation of the fiber. However, a major caveat to this method is the risk of tissue damage and fiber breakage as a result of repeated insertion and removal of the fiber. This is
especially of concern when working with behavioral paradigms that involve weeks of training and testing. Chronic fibers, on the other hand, are cemented into the skull during stereotaxic surgery and allow for multiple testing sessions over an extended time period with minimal light loss (Sparta et al. 2012).

Chronic or acute optical fibers can be placed in the same brain area as the virus injection to examine the effects of optical stimulation or inhibition on genetically targeted cell bodies of interest. For example, optical activation of D2 positive neurons in the NAc expressing ChR2 suppresses cocaine reward, whereas activation of D1 positive neurons increases cocaine reward (Lobo et al. 2010). Optical fibers can also be placed in projection targets to investigate the effects of altering pathway-specific circuits on behavior. Opsins are trafficked across neuronal membranes and can be visualized in axons and terminals (Yizhar et al. 2011a). This technique has been employed to look at BLA efferents to different brain regions (see below) (Stuber et al. 2011; Tye et al. 2011), but can be used to look at other neural circuits important in addiction such as DAergic afferents in the NAc and prefrontal cortex (PFC). In addiction, this strategy can identify neural circuit elements or genetically defined populations of neurons that are necessary or sufficient for a discrete behavior such as conditioned approach behavior to a reward-predictive cue. For example, BLA glutamatergic afferents to the NAc have been hypothesized to be important in cue-triggered motivated behavior, but because of the inability to specifically modulate this pathway during time-locked cues, the causal functional role of this pathway in cue-reward behavior was previously not well defined. Using optogenetics, activation and inactivation of BLA terminals in the NAc demonstrated that this circuitry is both necessary and sufficient for cue-driven motivated behavior (Stuber et al. 2011).

One caveat to the optogenetic projection targeting technique is that oftentimes afferent fibers are bundled together, and stimulating terminals in one region may also stimulate fibers of passage that are en route to a more distal target region. For example, DA afferents from the VTA projecting to the PFC pass through the NAc (Beckstead et al. 1979; Herbert et al. 1997), and stimulation of DA terminals in the NAc will likely also stimulate PFC-projecting fibers. Another limitation to this method is the possibility of back-propagating action potentials. Optical stimulation of terminals in one region may lead to back-propagating action potentials that activate the cell bodies, and can then activate afferents projecting to other regions. However, this limitation can be dealt with by injecting lidocaine to prevent back propagating action potentials at the level of the cell bodies of the population of neurons that was transduced (Stuber et al. 2011). In addition, future techniques that allow for retrograde delivery of viruses encoding opsins to specific presynaptic inputs may circumvent some of these limitations.

**CONCLUSIONS AND FUTURE DIRECTIONS**

Optogenetic manipulations of the neural circuitry involved in reward and addiction have aided in supporting and refuting many hypotheses that were previously untestable as a result of technical limitations associated with traditional techniques. Many of the optogenetic studies to date investigating these circuits have used optogenetic stimulation of neurons, but optogenetic inhibition is likely to prove to be an even more powerful tool to determine both necessity and sufficiency of neural circuits for mediating reward-related behaviors. In addition, combining optogenetics with in vivo monitoring techniques such as in vivo electrophysiology, and neurochemical techniques such as microdialysis and voltammetry, allows for actuation of neural circuits, while simultaneously measuring the neurophysiological output. The ever-increasing methods for targeted genetic manipulations of neurons as well as the continued development and refinement of optogenetic methods are unprecedented.

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