Optogenetic approaches for investigating neural pathways implicated in schizophrenia and related disorders

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Optogenetic approaches have been rapidly adopted by neuroscientists in order to control the activity of neurons with high temporal, spatial and genetic specificity. By expressing light-sensitive microbial opsins within a genetically-specified population of neurons, flashes of light can be used to activate these opsins and thereby modulate the targeted cells in a spatially and temporally defined manner. Thus, optogenetics can be used to activate very specific sets of neurons or projections at particular times, either within freely behaving animals, or in reduced preparations such as brain slices. These techniques are ideally suited for dissecting complex interactions within neuronal circuits, and for testing ideas about how changes in these circuits might contribute to abnormal behaviors in the context of neuropsychiatric disorders. Here, we review several studies that have used optogenetics to dissect circuits implicated in schizophrenia, and elucidate the ways in which specific components of these circuits may contribute to normal or abnormal behavior. Specifically, optogenetics can be used to label and excite neurons that express particular genes, in order to study how they interact with other neurons and/or modulate behavior. Optogenetics can also be used to study changes in these interactions or behavioral effects following genetic manipulations. In this way, optogenetics may serve to ‘fill in the gaps’ between genes, circuits and behavior, in a manner that should help to translate the rapidly growing list of genes associated with neuropsychiatric disorders into specific pathophysiological mechanisms.

Optogenetics represents a relatively new technique that has been rapidly adopted throughout neuroscience. As its name implies, optogenetic techniques modulate the activity of cells, typically (though not always) neurons, via a combination of optical and genetic targeting. Specifically, optogenetics uses light-sensitive microbial opsins that have been expressed within a genetically-specified population of neurons. Light is delivered to activate these opsins and thereby modulate the targeted cells in a spatially and temporally defined manner. Pioneering early experiments demonstrated the potential power of this approach to precisely activate specific populations of neurons (1–3). Subsequent studies led to the widespread use of Channelrhodopsin-2 (ChR2), a light-activated cation channel (4,5), due to the fact that it is encoded by a single gene and conducts large photocurrents with relatively rapid kinetics. ChR2 continues to be widely used to activate neurons, and many other channelrhodopsin variants have been developed (6–12). Conversely, halorhodopsin (NpHR) (13–15) and archaerhodopsin (16), light-activated chloride and proton pumps, respectively, are widely used to inhibit neurons.

A full accounting of the history and applications of optogenetics is beyond the scope of this review. Instead, we will attempt to illustrate how optogenetics can complement traditional molecular and genetic approaches to powerfully elucidate neuronal mechanisms involved in both normal brain function and disease. Genomics (17) and translational research (18) have provided major insights into the mechanisms underlying mental illness. However, mounting evidence indicates that psychiatric diseases arise from the complex interplay of a potentially large number of genetic liabilities and adverse environmental events that can disrupt brain developmental pathways, producing myriad changes in neuronal circuits. Optogenetic approaches can be used to measure effects of activating or inactivating neurons that express certain genes, and to study how the particular circuits are altered by genetic manipulations. In this way, optogenetic approaches can be used...
to map out the specific neuronal circuits that are perturbed by various genetic disruptions, and the consequences of these disruptions, in order to understand exactly how genetic disruptions might contribute to neuropsychiatric disorders. As an illustration, this review will describe several studies that have used optogenetics to elucidate various neural pathways believed to contribute to schizophrenia and related disorders.

Schizophrenia is a neuropsychiatric disorder that affects ~1% of the population worldwide, and likely reflects dysfunction in multiple brain systems that arise from a combination of genetic insults (19). The disorder comprises positive symptoms such as hallucinations, delusions and thought disorder, negative symptoms such as affective flattening and anhedonia, and deficits in cognitive domains such as working memory, executive functioning, attention and social cognition. However, currently available antipsychotic medications do not alleviate cognitive deficits which are the primary cause disability associated with schizophrenia, demonstrating the need for better clinical targets (20).

Treatments for cognitive deficits in schizophrenia remain underdeveloped in large part because the relevant physiological mechanisms remain unclear. Thus, the use of optogenetic techniques both in vitro and in vivo (5,21) may pinpoint cell types, neuromodulatory actions, interactions between brain regions and other pathophysiological mechanisms that play critical roles in aspects of schizophrenia, and may be targets for novel therapeutic interventions. Here we will review several studies that illustrate ways in which optogenetic approaches can be used to probe neural systems, pathways and mechanisms that may contribute to schizophrenia.

INSIGHTS INTO DOPAMINERGIC SYSTEMS

For over 40 years, antipsychotic drugs that antagonize D2-like dopamine receptors (D2Rs) have been used to alleviate positive symptoms (delusions, hallucinations and disorganization) in individuals with schizophrenia. Despite advances in second generation or atypical antipsychotics, to this date, all known clinically-effective antipsychotics block D2Rs. Thus, there is a powerful relationship between D2R blockade and antipsychotic efficacy (22,23). One hypothesis is that excessive dopamine signaling in subcortical limbic regions (e.g. the striatum, amygdala and nucleus accumbens) might account for the positive symptoms of schizophrenia while insufficient dopamine signaling in frontal cortical regions may explain negative symptoms (24). This and many other variants on the original dopamine hypothesis of schizophrenia have focused on the possible role of subcortical structures such as the striatum, which has an extremely high density of dopamine terminals and receptors. The striatum receives most of the cortical input to the basal ganglia, which project to thalamic nuclei, and ultimately to the frontal cortex—thus influencing control over movements, motivation, decision-making and many other behaviors that can be perturbed in schizophrenia (25). One mouse model of schizophrenia has used overexpression of striatal D2Rs to model aspects of schizophrenia (26).

Optogenetic techniques have been used to elucidate cellular effects of dopamine in the striatum and link these to behavior. For example, Higley and Sabatini (27) used optogenetic stimulation of cortical-striatal projections to show that D2Rs can suppress cortical inputs to medium spiny neurons. A more recent study used optogenetics to study cortical inputs to medium spiny neurons (MSNs) in the direct and indirect pathways, which selectively express D1Rs and D2Rs, respectively (28). Contrary to a hypothesis, based on anatomical studies, that direct and indirect pathway MSNs receive distinct streams of cortical input from different populations of layer 5 pyramidal neurons, Kress et al. found that both direct and indirect pathway MSNs receive input from both intratelencephalic and pyramidal tract neurons. These two studies illustrate how optogenetic approaches can be used to define neural pathways and effects of dopaminergic modulation on these pathways. Other studies have taken advantage of the fact that direct and indirect pathway MSNs differentially express dopamine receptors in order to identify roles for these two pathways in behavior. Using D1-Cre or D2-Cre transgenic mice to selectively label direct or indirect pathway MSNs with ChR2, respectively, Kravitz et al. (29,30) found that activating direct pathway MSNs could facilitate movement and reinforce behaviors, whereas activating indirect pathway MSNs led to opposite effects. Another study similarly found that optogenetically activating D1 or D2-expressing MSNs introduces opposing biases during decision-making (31).

Other optogenetic studies have focused on dopamine release itself, rather than its consequences on downstream structures. For example, one early study used optogenetic stimulation to show that phasic firing in dopamine neurons is sufficient to drive behavioral conditioning (32). While this parallels earlier results obtained using electrical stimulation, this study used optogenetics to specifically activate dopaminergic neurons within the ventral tegmental area (VTA). By contrast, electrical stimulation may recruit multiple types of cells and/or axons. A more recent study used optogenetic stimulation to activate dopaminergic neurons at various times relative to reward delivery, and found that dopamine neuron activation at the time of reward delivery could drive long-lasting increases in reward-seeking behavior (33). This demonstrates that dopamine can indeed signal prediction error as has long been hypothesized (34). In this case, both the specificity (for dopaminergic neurons within the VTA), and the temporal precision (to deliver activation concurrent or out of phase with rewards) of optogenetic activation were critical for the study. Another study delivered optogenetic excitation to transgenic mice which selectively express ChR2 within specific classes of neurons within the VTA, e.g. GABAergic or dopaminergic neurons, during in vivo recording (35). In this way, this study was able to ‘tag’, i.e. optogenetically excite, and thereby identify VTA neurons belonging to these two classes in behaving mice. This led to the finding that dopaminergic and GABAergic neurons within the VTA signal reward prediction errors, and expected reward, respectively. Notably, both of these studies using optogenetics in the VTA support the general idea that aberrant dopamine signals may lead to the irregular assignment of salience to environmental signals—this has been proposed as one mechanism that may contribute to the formation of delusions, hallucinations and other aspects of psychosis (36).

More recently, optogenetic approaches have also been used to probe potential functions of dopamine in cortical, rather than striatal targets. An imbalance of D1R and D2R activation in the prefrontal cortex has been proposed as a possible pathophysiological
mechanism underlying cognitive deficits in schizophrenia (37,38). A recent study from our laboratory used optogenetics to activate synaptic inputs to prefrontal neurons (39). Under these conditions, we found that D2Rs can increase the excitability of a specific subpopulation of layer 5 pyramidal neurons in the prefrontal cortex which project to subcortical targets.

Finally, many studies have used optogenetics to study connectivity within dopaminergic pathways, and its relevance to various behavioral effects. For example, one study combined the use of rabies viruses (to label neurons which project to specific targets) with optogenetics (to activate specific sets of projections) to show that distinct sets of inputs to the VTA (from the laterodorsal tegmental nucleus or lateral habenula) mediate reward or aversion, respectively, by engaging distinct sets of neurons within the VTA, that project to the nucleus accumbens or medial prefrontal cortex (mPFC), respectively (40). Another study used the approach described previously, to optogenetically tag glutamatergic or GABAergic neurons within the bed nucleus of the stria terminalis during recordings in vivo, and found that these neurons, which excite or inhibit non-dopaminergic VTA neurons, respectively, are activated or suppressed, respectively, by aversive stimuli (41).

**INSIGHTS INTO INTERNEURONS AND OSCILLATIONS**

Synchronized neural oscillations in the gamma frequency range (~30–100 Hz) are associated with higher cognitive functions such as attention (42), working memory (43) and cognitive flexibility (44). A specific class of GABAergic interneurons that express the calcium-binding protein parvalbumin (PV) and have fast-spiking (FS) electrophysiological properties are widely believed to generate cortical gamma oscillations (45). Notably, markers for PV interneurons in the prefrontal cortex are consistently abnormal in postmortem brain tissue from patients with schizophrenia (46). Gamma oscillations are also abnormal in individuals with schizophrenia (47), and together, these observations have led to the hypothesis that deficits in PV interneurons disrupt gamma oscillations in ways that contribute to cognitive symptoms of schizophrenia.

Many observations have long suggested a key role for PV interneurons in gamma oscillations, and two recent studies used optogenetics to directly test this role. Cardin et al. (48) demonstrated that exciting PV interneurons at 40–50 Hz is sufficient to induce gamma frequency local field potential (LFP) oscillations in the barrel cortex of PV-Cre mice. Using a range of light frequencies (8–200 Hz) for light stimulation, they observed the greatest increases in LFP power using stimulation in the gamma-frequency range. Furthermore, they showed that rhythmically-stimulating pyramidal neurons did not elicit LFP gamma oscillations to a similar extent. Finally, this study showed that gamma-frequency LFP oscillations generated by PV interneurons can gate sensory inputs, demonstrating a potential role for interneuron-driven oscillations in cortical processing. A second study used the optogenetic silencing tool halorhodopsin (eNpHR) to selectively inhibit PV interneurons, and found that this selectively-suppressed LFP oscillations in the gamma frequency range (49). This study also used ChR2 to excite PV interneurons in a simplified circuit, in order to show that the presence of feedback inhibition from PV interneurons is sufficient to generate rhythmic activity at gamma-frequencies.

These two studies clearly demonstrated the long-hypothesized ability of PV interneurons to generate cortical gamma oscillations. Subsequent studies have begun using optogenetics to relate changes in PV interneuron function to aspects of schizophrenia. For example, N-methyl-d-aspartate antagonists, phencyclidine (PCP) and ketamine, are known to induce positive and cognitive symptoms resembling those associated with schizophrenia (50). Therefore, another hypothesis about schizophrenia is that hypofunction of glutamatergic (specifically NMDA receptor-mediated) synapses in the prefrontal cortex contributes to aspects of schizophrenia, possibly by reducing activation of PV interneurons, leading to disinhibition of prefrontal circuits. Carlen et al. (51) studied mice which lack NMDA receptors (NMDARs) in PV interneurons and found that baseline gamma power was increased in the somatosensory cortex of these mice, whereas driving ChR2-expressing PV cells at gamma frequency range recruited markedly less gamma power in mutant mice compared with controls. This may model the complex pattern of gamma oscillation abnormalities observed in patients with schizophrenia, in which baseline gamma power may be increased, while gamma oscillations evoked by tasks or sensory stimulation may be deficient (45,47).

Other studies have used the optogenetic ‘tagging’ procedure outlined above to label genetically-defined subtypes of GABAergic interneurons with ChR2, then identify them via their responses to light within in vivo recordings (52). This approach has shown that PV interneurons in frontal cortex have relatively synchronized and similar patterns of firing during behavior, and can potently inhibit target neurons. Finally, other studies have used optogenetics to define the targets of genetically-defined classes of interneurons. For example, a recent study from our laboratory showed that within deep layers of the prefrontal cortex, PV interneurons preferentially inhibit subcortically, rather than callosally, projecting pyramidal neurons (53). Notably, these subcortically-projecting neurons also express dopamine D2 receptors (39), making them a point of convergence for multiple mechanisms related to schizophrenia.

**STUDIES OF EXCITATORY/INHIBITORY BALANCE**

Many symptoms, such as social deficits, can be common to schizophrenia and autism. A disruption in the homeostatic balance between cortical excitation and inhibition (E/I balance), possibly reflecting mechanisms such as impairments in PV interneurons, may give rise to some social and cognitive deficits in autism and possibly in other neuropsychiatric disorders such as schizophrenia (54). As a simple and direct test of this hypothesis, Yizhar et al. (9) used multiple optogenetic tools to acutely perturb the E/I balance in various directions. Consistent with the hypothesis outlined above, this study found that increasing activity in excitatory neurons within the mPFC disrupted the social behavior of mice, whereas optogenetically elevating activity within prefrontal interneurons did not. This study highlights the use of optogenetics as a means to directly test how the interplay of activity within specific populations of neurons may shape behaviors.

**CONCLUSIONS**

Optogenetics has also made advances in our understanding of other psychiatric disorders such as autism spectrum disorders.
(55), Parkinson’s disease (56), obsessive-compulsive disorder (57,58), depression (59–63), anxiety (64–67) as well as addiction (68). The optogenetic toolbox is ever growing, and new techniques may enable optical control over presynaptic neurotransmitter release (69), bistable photo-control of metabotropic glutamate receptors (70) or light-induced activation of intracellular signaling pathways (71). LITEs, light-inducible transcriptional effectors can be used in combination with TALEs, transcription activator-like effectors, to study genes of interest by controlling transcription via light (72).

Obviously, if numerous technical hurdles can be overcome, optogenetics may eventually be useful for exerting temporally precise and cell-type specific control over cells in the human body, bypassing many of the limitations of current strategies, e.g. deep brain stimulation. But in the meantime, these and other new tools will undoubtedly continue to further accelerate investigations into the mechanistic processes that execute and regulate behaviors in both health and disease. As described in this review, optogenetic techniques are particularly useful for mapping out the details of neuronal circuits, identifying ways in which neuromodulators and other manipulations alter the responsiveness of individual neurons, tagging specific classes of neurons within in vivo recordings, and linking changes in the activity of particular neurons to behavioral consequences. In this way, optogenetics can ‘fill in the gaps’ between genes, circuits and behavior, in a manner that should help to translate the rapidly growing list of genes associated with neuropsychiatric disorders into specific pathophysiological mechanisms.

REFERENCES