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Illuminating circuitry relevant to psychiatric disorders with optogenetics

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Abstract

The brain's remarkable capacity to generate cognition and behavior is mediated by an extraordinarily complex set of neural interactions that remain largely mysterious. This complexity poses a significant challenge in developing therapeutic interventions to ameliorate psychiatric disease. Accordingly, few new classes of drugs have been made available for patients with mental illness since the 1950's. Optogenetics offers the ability to selectively manipulate individual neural circuit elements that underlie disease-relevant behaviors and is currently accelerating the pace of preclinical research into neurobiological mechanisms of disease. In this review, we highlight recent findings from studies that employ optogenetic approaches to gain insight into normal and aberrant brain function relevant to mental illness. Emerging data from these efforts offers an exquisitely detailed picture of disease-relevant neural circuits in action, and hints at the potential of optogenetics to open up entirely new avenues in the treatment of psychiatric disorders.

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Conflict of Interest Statement

K.D. and R.C.M. are co-founders of a company, Circuit Therapeutics, Inc., the goals of which are to use optogenetics to improve the drug development process and to develop novel devices to treat disorders of the peripheral and central nervous systems. They are both on the scientific advisory board for Circuit Therapeutics, Inc.

Introduction

Recent decades have seen dramatic improvements in treatments for a number of burdensome human diseases. Once considered to be facing a death sentence, many HIV-infected patients now look forward to life expectancies on par with the general population [1]. Similarly, interventions for cardiovascular disease are more numerous and effective than ever [2]. In stark contrast to these remarkable gains is the paucity of effective treatments for mental illness, with best practices changing little at the fundamental level since the mid-twentieth century [2–4].

An underlying assumption in devising treatments for mental illness has been that effective, orally administered pharmacological interventions can be developed. As a result, considerable effort has been devoted to the identification of novel “druggable” targets capable of rectifying neural dysfunction [2,3,5]. The relative lack of success of this approach [2–4], however, indicates that new approaches are needed. One prominent example of such a new approach is deep brain stimulation (DBS), which rests on the assumption that dysfunctional neural circuits mediating disease symptoms can be identified and subsequently corrected with targeted electrical stimulation of critical circuit nodes. While early efforts have shown promise [6–8], this technique suffers from significant limitations. Electrical stimulation indiscriminately affects both neurons and fibers of passage, so the spatial location of the affected cells is difficult to predict. Targeting specific cell types in heterogeneous brain regions is not possible. Furthermore, the functional consequences of electrical stimulation are often not clear, as the current applied may cause excitation, inhibition, or both, thereby limiting insight into the neurobiological mechanisms that cause or treat disease [9].

Optogenetics circumvents many of these problems by permitting bidirectional manipulation of neural activity with anatomical, genetic and temporal precision [10] and is already revolutionizing the study of disease-relevant circuits in animal models [11]. In this review, we highlight and explore in mechanistic detail recent rodent studies where optogenetic tools have been employed to enhance understanding of the neural basis of psychiatric disorders, with a particular eye towards the specific kinds of clinical breakthroughs these studies may help bring to pass.

Anxiety

Anxiety disorders represent the most common class of psychiatric illness, with a lifetime prevalence of ~30% [12]. Animal models exploit the fact that rodents have an innate aversion to open or brightly lit spaces, where they are more likely to be visible to predators. Thus, the proportion of time spent exploring exposed areas relative to the time spent in safer, covered areas is thought to reflect anxiety. (For discussions of the limitations of animal models of psychiatric disorders, see [4,13]). A number of recent studies have used optogenetics to explore the contribution of the extended amygdala, septum and hippocampus in such tasks. Leveraging the cell-type specificity of this technique, the function of closely apposed or intermingled groups of cells in the dorsal and ventral divisions of the bed nucleus of the stria terminalis (BNST) was assessed [14,15]. Surprisingly, optogenetic manipulation

of neighboring cells produced opposing behavioral effects, either increasing or reducing anxiety. These results highlight what will quickly emerge as a recurring theme in this review: it is difficult to ascribe a single function to a particular brain area, even in relation to a specific behavior, as the net effect of neural activity in any brain region will depend on which cells are active, and at what time.

The behavioral endpoint of BNST activity depends on the efferent target of activated neurons. Separable behavioral phenotypes observed in low-anxiety states (i.e. increased risk tolerance, lower respiration rate and positive valence attributed to places associated with anxiolysis) were individually controlled by projections from the anterodorsal BNST to the lateral hypothalamus, parabrachial nucleus and ventral tegmental area (VTA), respectively [15]. Such anatomical segregation of function could theoretically be exploited to develop individualized treatments for patients with differing symptoms or minimize undesired side-effects. Similarly, distinct behavioral effects were observed when either basolateral amygdala (BLA) neurons or their projections to the central amygdala (CeA) were individually modulated with optogenetics. Activation of BLA projections toward the lateral aspect of the CeA was found to be anxiolytic, while activation of BLA neuronal somata caused an increase in anxiety, suggesting that projections from the BLA to other brain areas increase anxiety [16].

In the lateral septum, selective activation of neurons that express the type 2 corticotropin-releasing factor receptor (CRFR2) was found to promote anxiety via projections to the anterior hypothalamus. Surprisingly, activation of these neurons *prior to* as well as during anxiety assays was anxiogenic, indicating that CRFR2 neurons in the septum can trigger a persistent state of anxiety [17]. In another study, activation of the ventral hippocampus (vHC) was found to be anxiolytic [18]. Collectively, these studies suggest that complex microcircuits located in multiple brain regions modulate anxiety-like behaviors in the mouse. Whether similar circuits are altered in human anxiety disorders remains to be determined.

Conditioned Fear

The ability to react appropriately to stimuli that signal impending danger is an adaptive and highly conserved trait. Decades of research have uncovered the BLA as a critical mediator of this learning process [19]. These studies primarily utilize auditory or contextual fear conditioning tasks, in which neutral stimuli are paired with an aversive event, such as a mild footshock. After pairing, presentations of the stimulus (or “cue”) alone come to elicit the unconditioned behaviors previously only elicited by the aversive event itself. As this robust form of learning is observed in humans [20], it is hoped that the insights garnered from studies of fear conditioning may eventually have implications for the treatment of post-traumatic stress disorder (PTSD) [21].

Given the robustness of fear conditioning in rodents, it is not surprising that the application of optogenetics has greatly expanded our understanding of the neural circuits that mediate fear learning. A series of elegant studies has provided compelling evidence that inhibitory neurons in the amygdala and cortex play a pivotal role in fear learning [22–26]. In the BLA,

parvalbumin-positive (PV+) interneurons are excited by cues and inhibit somatostatin-positive (SOM+) interneurons [22]. Since this latter class of interneurons targets excitatory principal neurons, shock-predictive cues cause enhanced BLA output via disynaptic disinhibition of the principal neurons [22]. In the lateral nucleus of the CeA, a subpopulation of protein kinase C-δ-expressing inhibitory neurons inhibit medial CeA neurons that project to brainstem regions important for generating freezing [25]. Some of these neurons are inhibited by fear cues, consistent with their anatomical position [25]. In contrast to their role in the BLA, PV+ interneurons in the dorsomedial prefrontal cortex (dmPFC), a region that is reciprocally connected with the BLA, were inhibited by shock-predictive cues [23]. Time-locked inhibition of these interneurons caused a phase resetting of cognitively-important theta oscillations, which allowed BLA-projecting excitatory neurons in the dmPFC to fire synchronously [23]. PV+ interneurons in the auditory cortex were also inhibited during tone-shock pairing, and optogenetic disruption of this inhibition disrupted fear learning [24].

The role of specific excitatory neuron populations has also been studied with optogenetics. Using a combinatorial viral strategy that permitted the identification of BLA neurons that projected to either the infralimbic (IL) or prelimbic (PL) sub-regions of the medial PFC (mPFC), it was found that IL-projecting BLA neurons were selectively activated by an extinguished cue, while PL-projecting BLA neurons were activated by non-extinguished shock-associated cues [27]. Accordingly, optogenetic inhibition of IL-projecting neurons impaired extinction learning, while inhibition of PL-projecting neurons resulted in enhanced extinction learning [27]. Projection-specific optogenetic modulation has also revealed roles for the BLA to entorhinal cortex pathway in contextual fear conditioning [28] and pointed to the importance of hypothalamic orexin neurons projecting to the noradrenergic locus coeruleus in cued fear conditioning [29]. Finally, brain slice recordings following ChR2 expression in defined neuronal populations *in vivo* revealed that extinction of a tone-shock association was associated with a reduction in excitatory transmission between mPFC and BLA principal neurons, likely due to a change in the probability of neurotransmitter release [30].

Optogenetics has been used to replace the exogenous stimuli normally used in fear conditioning protocols and thereby reveal minimal neural elements that are sufficient to drive learned fear. Conditioned freezing could be elicited by simply pairing a tone with optogenetic activation of glutamatergic neurons in the lateral amygdala (LA) in the absence of a naturally aversive stimulus [31]. Conversely, pairing optical stimulation of auditory inputs to the LA (i.e., acting as an artificial “tone”) with shock produced conditioned fear behavior that was indistinguishable from that of rats receiving actual tone-shock pairings [32]. This memory could be erased and reinstated with optical LTD and LTP protocols, providing strong evidence that LTP and LTD at these synapses are cellular mechanisms of memory generation and elimination [32].

In contrast to cued fear conditioning, contextual fear conditioning requires the hippocampus. A sophisticated genetic strategy was used to demonstrate that optical reactivation of hippocampal neurons that were previously active during context-shock pairing was sufficient to subsequently induce freezing in a new context that had never been paired with shock [33]. Similarly, optogenetic reactivation of hippocampal neurons that were previously

active in a novel, neutral context could be used to create a false contextual fear memory [34].

Addiction

The brain's reward circuitry, in particular dopamine (DA) release in the nucleus accumbens (NAc), plays an important role in the actions of addictive drugs and the development of addiction [35]. Because of its critical role in addiction, depression (see below) and all forms of adaptive reinforcement-dependent behaviors, the neural and behavioral effects of optogenetically manipulating the brain's reward circuitry, especially DA neurons, have been the focus of a substantial number of studies that have been recently reviewed [36–38]. Here we will confine our discussion to recent studies that utilize optogenetics to elucidate the circuit changes that mediate behavioral actions of addictive drugs.

Studies that have focused on the synaptic changes induced by drugs of abuse, in particular psychostimulants, commonly use either experimenter-administered drug injections or self-administration protocols that more closely resemble human addiction. Both modes of drug administration trigger long-lasting, complicated, changes in the properties of excitatory synapses on VTA DA neurons and NAc medium spiny neurons (MSNs) [35]. These cell types receive inputs from a large number of brain areas and using traditional electrical stimulation techniques it was essentially impossible to examine the input specificity of drug-induced synaptic changes and their behavioral importance. Optogenetics approaches have overcome this limitation, and not surprisingly, a complex picture of the role of different inputs is emerging. Inputs from the mPFC [39] and vHC [39,40] were found to be potentiated after cocaine exposure, a change that appears to be specific to D1 MSNs. Optogenetic depotentiation of mPFC synapses on NAc MSNs reversed cocaine-induced locomotor sensitization following passive cocaine administration [41] and reversed cue-elicited cocaine seeking during extinction from self-administration [39]. Consistent with these findings, direct optogenetic inhibition of mPFC inputs to NAc blocked cue-elicited reinstatement of cocaine-seeking [42]. Optogenetic excitation or inhibition of vHC inputs to the NAc also bidirectionally modulate cocaine-induced locomotor sensitization [40] whereas depotentiating these synapses following self-administration reduced the vigor of responses during drug seeking [39].

Consistent with the importance of BLA inputs to NAc, using ChR2 to perform a minimal stimulation assay selectively in this projection, "silent synapses" were detected shortly after cocaine self-administration but were "unsilenced" after a prolonged withdrawal period that facilitates the development of cocaine craving [43]. Optogenetic induction of LTD at these synapses during withdrawal reduced cocaine-seeking behavior [43] while optogenetic silencing of this pathway blocked cue-induced reinstatement of cocaine seeking [44]. Together these studies demonstrate how optogenetics has allowed the study and manipulation of specific NAc inputs in animal models of addiction. However, the results to date do not allow specific and distinct behavioral functions to be attributed to these inputs.

Nonetheless, the notion that changes in excitatory drive to NAc neurons have behavioral relevance is further supported by studies that have directly manipulated NAc neuron

activity. Optical activation of D1 MSNs was found to potentiate cocaine conditioned place preference (CPP), while activation of D2 MSNs suppressed the same behavior [45] and reduced cocaine self-administration [46]. Optical inhibition of cholinergic interneurons, which potently inhibit MSNs despite constituting only a small fraction of NAc neurons, also reduced cocaine CPP [47]. Although the specific NAc projection targets that influence cocaine related behaviors are largely unknown, the NAc projection to dorsolateral ventral pallidum is likely to be important, as optical inhibition of this pathway blocked cocaine/cue-primed reinstatement [48].

The mPFC has long been the focus of attention in investigations of addiction [49] and recent optogenetic studies point to a complex role for this structure. Optical inhibition of PL decreased cocaine-primed reinstatement during extinction from self-administration, but paradoxically increased cocaine seeking during a reinforced session [50]. It is also puzzling that both activation and inhibition of ventromedial PFC disrupts memory for cocaine CPP, although the effect depends on whether the memory being expressed was recent or remote [51].

One of the defining features of addiction is the persistence of drug-seeking despite severe adverse consequences. Thus, investigation into the neural adaptations that accompany aversion-resistant drug-seeking in animal models has the potential to be particularly informative in understanding the etiology of human addiction. In rats that continued to seek cocaine despite receipt of footshock (shock-resistant rats), PL neurons exhibited reduced excitability relative to shock-sensitive rats [52]. Correcting this hypofunction with optical excitation reduced cocaine seeking in shock-resistant rats specifically during sessions when cocaine was paired with shock, and had no effect on cocaine-seeking during regular cocaine self-administration sessions [52]. Similarly, optical inhibition of projections from either the mPFC or the insula to NAc reduced alcohol self-administration when it was accompanied by a negative outcome but failed to reduce alcohol seeking when not accompanied by an aversive event [53]. Taken together, these studies suggest that it may be possible to selectively modulate neural pathways that mediate problematic drug-seeking in human addicts without impairing normal behavior.

Depression and social dysfunction

Major depressive disorder (MDD) affects ~5–10% of the U.S. population in a given year with ~30% of those afflicted categorized as severe [12]. In rodents, chronic stress protocols are commonly used to induce depression-like states, the major symptoms of which are often assessed using the sucrose preference test to measure anhedonia, the forced swim (FST) or tail suspension tests (TST) as proxies for “behavioral despair”, and social interaction tests to measure social avoidance [13,54]. Although the usefulness of some of these assays has appropriately been questioned [13], they remain established surrogates for human symptoms.

Optogenetic manipulations have provided provocative and complex clues to the neural circuit dysfunctions mediating depression symptoms. Given that anhedonia is one of the most devastating symptoms of depression, manipulations of reward circuitry are of clear

relevance [55]. Using a subthreshold social defeat protocol, the chronic version of which generates a depression-like phenotype in the majority (~65%) of "susceptible" mice but not in a subset (~35%) of "resilient" mice, it was found that phasic activation of VTA DA neurons was sufficient to trigger a susceptible phenotype as evidenced by increased social avoidance and decreased sucrose preference [56]. Consistent with the hypothesis that the functional role of VTA DA neurons depend on their projection targets [36] optical activation of NAc-projecting or inhibition of PFC-projecting VTA DA neurons induced susceptibility to social-defeat stress [56]. Molecular mediators of the susceptible state include stress-elicited enhancement of BDNF release in the NAc due to the action of corticotrophin-releasing factor [57].

In contrast to these results, following a chronic mild stress protocol, which is different from the acute social defeat stress noted above, optogenetic activation of VTA DA neurons alleviated deficits in the TST and sucrose preference assays while inhibition of these neurons in control mice induced a depression-like phenotype [58]. Pharmacological manipulations and electrophysiological assays suggest that the consequences of these optogenetic manipulations are due in large part to modulation of the NAc-projecting VTA DA neurons [58]. One possible explanation for the dramatic differences in the consequences of optogenetic activation of VTA DA neurons in the two different depression models is that susceptibility in the chronic social defeat stress model results from a failure to completely engage the protective mechanisms found in resilient mice. In susceptible mice, social defeat stress leads to hyperexcitability of VTA DA neurons. Surprisingly, chronic optogenetic activation of VTA DA neurons caused long-lasting adaptations that actually *decreased* the firing rate in these neurons; this normalization promoted a resilient phenotype *in vivo* [59]. This study highlights a new approach to developing therapeutics where resilience-generating mechanisms are promoted instead of attempting to revert the brain to a stress-naïve state.

Two other key nodes of depression circuitry that have been targeted using optogenetics are the PFC and dorsal raphe (DR). In an initial study high frequency optogenetic stimulation of mPFC was found to have a potent anti-depressant effect in susceptible mice following chronic social defeat stress [60]. Not surprisingly, further work revealed that the behavioral consequences of activation of mPFC projections appears to depend on their specific target. Activation of mPFC terminals in the NAc elicited anti-depressant like effects following social defeat stress whereas mPFC terminal activation in the BLA had anxiolytic effects [61]. In contrast, activation of mPFC terminals in the DR increased motor activity during the FST, but not more general locomotor activity in the open field test, suggesting that this projection is particularly important for responding to behavioral challenges [62]. On the other hand, optogenetic activation of ventromedial PFC inputs to DR appears to drive GABAergic cell activity that in turn inhibits 5-HT neuron activity [63]. The net result of this was to promote social avoidance whereas inhibiting this input or inhibiting DR GABAergic neurons directly prevented the acquisition of social avoidance following social defeat stress [63,64].

Social approach/avoidance could also be manipulated by selectively changing the cellular balance of excitation and inhibition in the PFC during social approach [65]. Increased excitation of excitatory pyramidal neurons impaired social approach behavior while

concurrent activation of inhibitory PV+ interneurons was sufficient to restore excitation/inhibition balance and partially ameliorate this deficit. Persistently heightened excitation in the PFC also caused an increase in 30–80 Hz rhythmicity, a phenomenon observed in schizophrenia and autism [65]. A BLA-vHC circuit also can regulate social approach behaviors [66].

OCD

Over time actions that are regularly performed, such as navigating a familiar route, can become engrained habits. In patients with obsessive compulsive disorder (OCD), this normal propensity for habit-formation is exaggerated and takes the form of ritualistic, repetitive behaviors and thought patterns [67]. Corticostriatal circuits have long been considered an important substrate for OCD but only recently have optogenetic approaches been used to probe their function in animal models. Perturbation of activity in IL and lateral orbitofrontal cortex (lOFC) was found to promote goal-directed actions at the expense of habitual behavior [68,69]. Specifically, when rats were over-trained to induce habit formation on a maze navigation task, brief inhibition of IL effectively reverted their behavior back to a goal-directed state [68,69]. Similarly, when mice were able to alternate between performing habitual and goal-directed actions, acute optical activation of lOFC promoted goal-directed responding [70].

Other studies have focused on identifying neural circuitry that mediates repetitive grooming, a murine behavior that models some aspects of OCD. Optogenetic activation of lOFC itself or lOFC terminals in the centromedial striatum (CMS) reversed abnormal excessive grooming behavior in *Sapap3* knockout mice by increasing feed-forward inhibition onto MSNs within the CMS [71]. While activation of lOFC *suppressed* repetitive grooming and promoted goal-directed responding [70,71], repeated optogenetic activation of medial OFC inputs to the ventromedial striatum (VMS) triggered a progressive *increase* in grooming behavior in parallel with increases in VMS activity [72]. These latter optically-induced neural and behavioral changes were reversed by long-term fluoxetine administration, a common treatment for OCD patients [72]. Thus, while multiple lines of evidence point to the involvement of OFC-striatal interactions in rodent behaviors relevant to OCD, the relationship between neural activity in these brain regions and behavior appears to depend on the precise sub-region under investigation.

Conclusions

The impact that optogenetics has had on preclinical brain research is difficult to overstate. Its obvious advantages have led to its rapid adoption as a core technique in neuroscience. By helping to parse the function of highly complex neural circuits that mediate disease-relevant behaviors, optogenetics has great potential to inform the rational development of therapeutic interventions for psychiatric disorders. With time, these new therapies may eventually take the form of long-sought pills that non-invasively cure or ease the symptoms of mental illness with minimal undesirable side effects. However, the heavy burden of psychiatric disease demands that treatment options beyond the pharmacological, including electrical, magnetic,

or optical tuning of the human brain, all guided by optogenetic insights, should be considered in the long run.

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Highlights

- Optogenetics has facilitated elucidation of circuits mediating psychiatric symptoms
- Projections from bed nucleus of the stria terminalis are important for anxiety
- The detailed amygdala circuitry involved in conditioned fear has been dissected
- Depression symptoms involve complex regulation of VTA dopamine neurons
- Cue elicited cocaine seeking can be reversed with optogenetic manipulations