Potential Utility of Optogenetics in the Study of Depression

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Novel antidepressants are needed to enhance the health and quality of life of the hundreds of millions of depressed individuals worldwide who remain inadequately treated with today’s approaches. In reality, no new class of antidepressant medication has been introduced in over 50 years. This insufficiency of current drug treatments is evident to those eager to pursue invasive experimental options like that of deep brain stimulation. Encouragingly, human brain imaging studies and animal work implicate strong relationships between depressive symptoms and patterns of brain activity, which are now open to more empirical assessments using optogenetics. Recent advances in optogenetics permit control over specific subtypes of neurons or their afferent or efferent projections and can greatly further our understanding of the neural mechanisms involved in depression and the mechanism of action of deep brain stimulation and perhaps chemical antidepressants. Here, we discuss how optogenetic tools are being used to answer a broad range of molecular, cellular, and circuit-level questions pertaining to depression that, up until now, have been resistant to other experimental approaches. The emergence of optogenetic technology, when combined with the best-validated animal models of depression, will dramatically increase knowledge about the basic neurobiology of depression, as well as facilitate the development of more effective antidepressant treatments.

Despite these limitations, knowledge of the brain circuitry involved in depression has led to the experimental use of deep brain stimulation (DBS) in treating severely affected patients. Two brain regions, each implicated in depression, have been shown to be effective: subgenual area 25 (Cg25), a region of the anterior cingulate cortex—part of the prefrontal cortex (PFC), and the anterior limb of the internal capsule, which is thought to involve the nucleus accumbens (NAc), a region of ventral striatum (10–16). Transcranial magnetic stimulation also shows some efficacy for the treatment of depression (17). However, the mechanism by which stimulation of these regions alleviates symptoms of depression is unknown. For example, it is unclear whether the antidepressant effects of DBS are mediated by activation of neurons in the stimulated region, by activation of passing axons, or even by the inactivation of local neurons through depolarization blockade.

The recent development of optogenetic tools has made it possible for the first time to begin to address some of these questions (18). By combining such tools with animal models of depression, work is beginning to causally relate activity in the brain’s limbic circuitry with depression-like and antidepressant-like actions. Here, we review the small handful of studies, to date, including more preliminary reports, employing optogenetic tools in depression models, as well as the tremendous potential of this approach in future years.

How Can Optogenetics Provide Answers?

Most regions of the brain contain several subtypes of excitatory and inhibitory neurons. Subsets are projection neurons, while others are local interneurons. Moreover, the brain contains numerous types of glial cells, which play a critical role in modulating neuronal function. Activation or inhibition of each cell type would be expected to induce a distinct functional, including behavioral, response, although the details in most cases remain poorly understood.

Optogenetics is proving to be uniquely useful in unravelling this information by overexpressing light-sensitive proteins within particular cell types of interest (Figure 1). This is accomplished by the use of viral vectors that infect only certain types of neurons using cell-type-specific promoters such as calcium/calmodulin-depen- dent protein kinase II, which will localize optogenetic proteins to excitatory neurons (19). It is also accomplished by targeted use of viral vectors that express their transgenes in a Cre-dependent manner (20), in combination with mice that express Cre recombinase in...
specific cell types, for example, dopamine (DA) neurons or striatal medium spiny neurons expressing D1 versus D2 receptors (21–24). Additionally, optogenetics can be used to target a particular afferent pathway to a brain region of interest, as just one example, glutamatergic inputs to the NAc coming from the basolateral amygdala as opposed to other afferent regions (25).

The frequency and temporal pattern of cellular activation can also be tightly controlled using optogenetics. Channelrhodopsins (e.g., channelrhodopsin 2) are cation channels expressed in the plasma membrane: when activated by ~470 nm waveforms of light (i.e., blue light), in the case of channelrhodopsin 2, these channels open, followed by a rapid depolarizing current. By utilizing different patterns of light stimulation, it is possible to mimic tonic versus burst patterns of neuronal firing that are seen in vivo under different physiological circumstances (21). Neuronal inhibition (i.e., hyperpolarizing current) can be readily accomplished as well via the expression of halorhodopsin (halorhodopsin from Natronomonas pharaonis), a chloride pump activated by waveforms of light in the ~570 nm range (i.e., yellow light) (26,27). By expressing both proteins within the same neurons, it is possible to study the behavioral consequences of activating or inhibiting the same ensembles of neurons (27).

Optogenetics is not limited to the control of neural activity per se, as there are many applications of this technology that are expanding at an exciting rate (28,29). Some examples that appear applicable to the study of depression include optogenetic tools that control glutamate receptor signaling, G protein-coupled receptor signaling, and intracellular trafficking of signaling molecules (Figure 1)(30–35). Many cellular effectors of these various optogenetic tools have the potential to serve as novel targets for the treatment of depression.
treatment of depression (36), which emphasizes the great potential of this technology in drug discovery efforts.

Historical Precursors to Optogenetic Studies in Depression

Before the advent of pharmacotherapies and psychotherapies, psychosurgical methods were used to treat depressive symptoms, as well as other neuropsychiatric syndromes. Archaeological evidence (i.e., human skulls with drilled holes) suggests that intracranial surgeries date back at least 5000 years to Northern Africa and Europe (37–39). Centuries later, the frontal leukotomy (i.e., frontal lobotomy) was introduced. In 1936, Egas Moniz, a Portuguese neurologist, described the PFC as the “psychic center of a person.” He suggested that separating connectivity between cortex and other brain regions would cure psychological pathologies. Moniz, who earned a Nobel Prize in 1949 for introducing leukotomies, initially injected alcohol into the white matter of the anterior frontal lobes to ablate this tissue before he went on to develop more intricate procedures (37,39).

Frontal leukotomies were replaced by more focalized lesions using stereotactic techniques. Areas targeted by these surgical procedures included the cingulate gyrus (i.e., cingulotomy), the subcaudate area to disrupt projections from orbitofrontal cortex to subcortical structures (i.e., subcaudate tractotomy), the anterior limb of the internal capsule, inferior thalamic peduncle, and the dorsomedial nucleus of the thalamus (i.e., anterior capsulotomy), and ablative surgeries aimed at combinations of these brain regions (i.e., lemic leucotomy) (40). Interestingly, such focalized ablative surgeries improved some depressed symptoms despite the invasiveness of these approaches.

High-frequency DBS was originally used to treat severe motor disorders (41–43) and has emerged as an attractive alternative to the ablative surgeries also once used in the treatment of Parkinson's disease, essential tremors, and dystonias (44). Given the successes of DBS in treating motor disorders, combined with imaging studies in depression, DBS procedures were postulated as a new strategy in treating depression (12,13). The first report appeared in 2005 (45): chronic high-frequency (130 Hz) DBS was applied to the white matter adjacent to the Cg25, based on evidence that this region is metabolically overactive in depressed patients (46,47). Results from this initial study were striking: antidepressant effects were seen in roughly half of the treated patients and were accompanied by diminished activity in Cg25 (45,48). Since that time, DBS has been conducted in many other candidate brain areas, including the NAc, anterior limb of the internal capsule, inferior thalamic peduncle, and habenula (10,15,16,49,50). Many of these studies combined the use of DBS with functional imaging to examine its effects on regional patterns of brain activity. Brain areas outside of the stimulation site were also affected by DBS, consistent with the involvement of multiple circuits in the treatment and pathogenesis of depression (10,15,16,49,50).

Similar to primate anterior cingulate cortex, the rodent medial PFC is critically involved in the expression of emotional behaviors (51–53). Deep brain stimulation in the ventromedial PFC of rodents also has prominent antidepressant-like effects (54). In fact, rodent DBS studies laid the groundwork for improving future cortical stimulation approaches by identifying many of the stimulus parameters required for producing antidepressant-like effects. High-frequency stimulation (~100 Hz) at an intensity of ~200 μA into the ventromedial PFC can provide maximal antidepressant-like responses (54). Importantly, the behavioral effect of high-frequency stimulation in the medial PFC is likely due to cortically mediated control over other limbic brain regions, including subcortical monoamineergic nuclei, because lesions that deplete serotonin diminish the antidepressant-like effects of medial PFC stimulation (55). Rodent DBS studies will continue to be useful for detecting the antidepressant potential of controlling the activity of additional brain structures. For instance, high-frequency DBS in the NAc enhances rhythmic and synchronous inhibition within ascending cortical and other subcortical structures (56).

Optogenetics can now be used to extend these stimulation studies in humans and rodents to elucidate the neurobiological underpinnings of depression-like behavior in animal depression models, as well as the antidepressant-like effects of DBS in these models. This approach has already been used to understand DBS action in Parkinson's disease: optogenetic studies of rodent Parkinson's models indicate that the therapeutic effect of DBS on the subthalamic nucleus is achieved through activation of axonal inputs to this target region (57), a finding consistent with indirect interpretations from earlier DBS studies in human patients (58).

The ability to apply this technology to depression is derived from at least six major interdisciplinary achievements. First, decades of human brain imaging and postmortem results have identified pertinent brain areas in depression (e.g., subregions of cerebral cortex, NAc, and habenula). Second, the advent of in vivo methods for controlling protein expression in brain, including the use of viral-mediated gene transfer and inducible transgenic animals, has allowed for examining a loss or gain of function for virtually any gene within a defined brain region. Third, ethologically valid animal models of depression, particularly those involving chronic social stress, have high levels of construct, face, and predictive validity, which allows for the examination of neural mechanisms involved in depression as well as its treatment. Fourth, improvements in severe cases of depression after DBS confirm a functional role for certain key brain regions in antidepressant responses and define frequency and patterns of stimulation necessary for these actions. Fifth, techniques to record the functional activity of specific brain areas (or multiple areas simultaneously) in vivo have been incorporated in animal models of psychiatric disease (59). Finally, advancements in optogenetics, as stated earlier, make it possible to manipulate specific cell types, individual afferent and efferent pathways, and patterns of activity generated within discrete brain regions.

Use of Optogenetics in Animal Models of Depression

The ability to use optogenetics to understand depression and its treatment depends on the availability of useful animal models. In the absence of bona fide genes that cause depression in humans with high penetrance and specificity, animal models of depression have focused on exposing normal rodents to different forms of chronic stress. While the validity of all such models must be evaluated with caution and skepticism (60), there are several rodent models that demonstrate some etiologic and face validity, as well as neurobiological abnormalities that are validated in postmortem tissue from depressed humans (61,62). The temporal nature of neural plasticity leading to depressive-like behaviors can be studied in these animal models using in vivo imaging and electrophysiologic recording techniques (63–65). The time is ripe, therefore, to use optogenetics to directly control patterns of neural activity to understand circuit-level events related to depression-related behavioral abnormalities and their reversal by antidepressant treatments.

We recently employed the chronic social defeat stress model to explore antidepressant-like actions of optogenetic stimulation of the PFC (66). In this model, 10 days of continuous social stress...
induces a behavioral syndrome characterized by social avoidance; anhedonia-like symptoms, including reduced preference for sucrose, high-fat diet, and sexual behavior; anxiety-like symptoms; disrupted circadian rhythms; a hyperactive hypothalamic-pituitary-adrenal axis; and a metabolic syndrome characterized by increased eating and weight and insulin and leptin resistance (67–70). Many of these symptoms are long-lived and can be reversed by chronic, not acute, administration of antidepressants, such as imipramine or fluoxetine; anxiolytics are ineffective (67,71). About a third of mice subjected to chronic social defeat stress avoid most of these symptoms; we refer to these mice as resilient compared with the major-susceptible mice (68,71). Many of the neurobiological changes that occur in limbic regions of the brain in susceptible mice have been validated in the brains of depressed humans (67,68,72–75).

In our optogenetic study with this model, we focused on medial PFC, based on human data for the importance of this region in depression and the antidepressant effects of DBS (see above) and based on prior work that has shown that stress can modify neurons in this brain region in rodent models (76–79). Long-lasting deficits in the functional activity of the ventral portion of the medial PFC have been inferred by the reduced expression of immediate early genes after social defeat stress in rats (79). In line with these results, we found that within postmortem tissue collected from the human anterior cingulate cortex zif268 and arc messenger RNA expression were significantly downregulated in clinically depressed cases (66). In parallel, we found that chronic social defeat stress in mice causes decreased levels of zif268 and arc messenger RNA in the ventral medial PFC, one of several regions of the PFC that may have some functional homology with the human anterior cingulate cortex (80). These effects predominate in mice that are susceptible to defeat stress. Our hypothesis is that deficits in immediate early gene expression reflect reduced cortical activity induced by stress; specifically, reduced medial PFC burst firing could result from social stress in susceptible mice (81,82). We hypothesize further that these abnormalities may contribute to the emergence of emotional disturbances. Consistent with this, we found that driving a 100-Hz pulse with an interpulse interval of a few seconds, essentially a burst-like pattern of cortical activity, using in vivo optogenetic stimulation of the medial PFC, induced rapid antidepressant-like responses in susceptible mice (Figure 1) (66). These antidepressant-like effects brought on by optogenetic stimulation did not disrupt other ongoing behaviors, such as locomotor activity, generalized anxiety-like responses, or social memories. These data closely follow the antidepressant effects of direct DBS in medial PFC of rats, mentioned earlier (54). Further work is now needed to confirm that the rapid antidepressant action of optogenetic stimulation of medial PFC is mediated via activation of local pyramidal neurons in the stimulated region, as well as to characterize the circuit-level changes in several other brain regions that result from this action.

Much of our work on the social defeat model has implicated the brain’s reward circuitry, dopaminergic projections from the ventral tegmental area (VTA) to the NAC, as also being crucial in the development of susceptibility versus resilience (67,68). We previously demonstrated that susceptibility to social defeat stress is associated with increased firing of VTA DA neurons in brain slices and in vivo (68,83). Using optogenetics, we recently demonstrated that activation of these neurons in awake, behaving mice promotes susceptibility (84,85). We are now using optogenetics to selectively activate subpopulations of VTA dopamine neurons that project to distinct limbic brain regions to identify the specific dopaminergic pathway that mediates this effect. It will be interesting to test whether optogenetic suppression of VTA dopamine neuron activity reverses susceptibility and induces a more resilient state. However, recent observations reveal that stimulation of DA neurons in the VTA can also promote antidepressant-like effects in mice in the chronic mild stress paradigm (86). These data suggest plausible differences in the neurobiological mechanisms underlying responses to different forms of stress (e.g., chronic social defeat stress vs. chronic mild stress) and one cannot rule out that different populations of DA neurons projecting to different brain regions (e.g., NAC vs. PFC) may play selective roles in different types of stress. These distinct DA projection pathways can now be examined with optogenetics.

Optogenetic studies of the NAC are also in order, given the promise of DBS to this region in depressed patients. One rationale for pursuing such studies is that DBS and optogenetic activation of the NAC similarly modify the rewarding effect of drugs of abuse, like cocaine (23,87). High rates of comorbidity between addiction and depression, along with the fact that these two syndromes have significantly overlapping mechanisms in the brain’s reward circuitry, further substantiate the need for optogenetic exploration of this region in the context of depression models. Recent optogenetic studies of the NAC in addiction models, for example, have defined very different roles played by the activation of the two major subpopulations of medium spiny neurons—those expressing D1 versus D2 dopamine receptors and cholinergic interneurons—in mediating the addicting properties of cocaine (23,88). Examining the influence of these various cell types in depression models is now a high priority.

The hippocampus has long been considered to be an important structure in depression (89). Here, treatment with conventional antidepressants such as fluoxetine or imipramine significantly alters cellular activity and the survival of newborn neurons (90–93), and these events are linked to the behavioral effects produced by these drug treatments (90). The use of advanced high-speed imaging techniques in hippocampal brain slices further highlights the importance of hippocampal subdivisions during both the expression of a stress-induced depressive-like behavior and subsequent treatment with either fluoxetine or imipramine (63). Specifically, following exposure to chronic mild stress, activity of the dentate gyrus decreases relative to increases in cornu ammonis 1 activity. A reversal of both stress-induced effects in the hippocampus can be achieved with chronic antidepressant treatment (63). This decreased dentate gyrus and increased cornu ammonis 1 activity extends earlier observations whereby depression has been associated with elevated hippocampal output and decreases in hippocampal input (89,90). Deep brain stimulation reduces activity within the stimulated region of anterior cingulate cortex, which receives major excitatory input from the hippocampus (46). Therefore, this pathway is likely critical for the expression of some depressive symptoms, and optogenetics will be useful in directly testing this possibility.

While most of the work using optogenetics in affective-like behavior has pertained to mice, this technique is also applicable to rats. The reason for the current bias toward studies in mice is simple. While the field has moved rapidly toward understanding the roles for specific cell types involved in behavior, the tools for enabling such cell type specificity has been sparse for rats. The lack of tools may change as transgenic rat lines further develop, particularly those involving Cre drivers in specific cell types or those labeling activated neurons (94,95). Advantages of employing optogenetic approaches in rats include the more sophisticated behavioral assays available in this species and the larger size of the rat brain, which makes parsing neural circuitry more feasible.

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The Future of Optogenetics in Depression

Neuroimaging studies are useful for identifying brain areas that potentially contribute to symptoms of depression. It is now essential that we better assess such aberrant patterns of activity in specific cell types and across neural circuits, as this is feasible in validated animal models of depression. Current high-speed imaging and in vivo imaging technologies like optical microendoscopy or bioluminescence imaging of activity-related genes or calcium probes (63,64) can be combined with cell-type-specific and circuit-specific genetics (20). These examples will be useful for defining changes in neuronal or glial activity that directly control depressive-like behavior, as well as mechanisms of antidepressant action.

Using optogenetics to determine molecular correlates of depression is particularly promising. This can be achieved by changing neuronal activity in specific cell types with optogenetics to modify depression-like behaviors followed by profiling gene expression changes in the manipulated regions or in regions to which they send inputs. For example, we show that loss of brain-derived neurotrophic factor (BDNF) signaling in NAc is correlated with enhanced activity in both the D1 and D2 neuronal subtypes in this region by use of optogenetics (23). We further found that optogenetic stimulation of D1 type neurons decreases levels of the activated form of extracellular signal-regulated kinase 1/2, a downstream target of BDNF signaling. More recently, we were able to reverse a blunted behavioral response to morphine, seen after increasing BDNF signaling in the VTA, by optogenetic control of VTA projections to the NAc (Ja Wook Koo, Ph.D.; unpublished data; November 2011). These types of studies in the drug abuse reward circuitry can now be translated to depression-like behaviors in rodents.

Given our increasing knowledge of the complex molecular events that mediate depression-like behavior in animal models (8,36,62,71,73–75), the ability to activate or inhibit a range of signaling proteins with the precise temporal and spatial specificity afforded by optogenetic approaches will be a major boon to the study of the molecular basis of depression. Thus, it is likely that, over the coming years, we will see increased optogenetic dissection not only of neuronal activity but also of a host of signaling proteins involved in the pathophysiology of depression and the mechanisms of antidepressant action (Figure 1). The OptoRXr, G protein-coupled receptor (GPCR)-opsin chimera, in which the intracellular loops of rhodopsin are replaced with those of specific GPCRs, represents a viable approach since they have led to insights in GPCR signaling in the NAc in reward behaviors (31). More recently, another group has produced a chimera of rhodopsin and the serotonergic GPCR, G12α-coupled serotonin 1A (5-HT1A), by tagging rhodopsin with the 5-HT1A C-terminus (96); this light-responsive 5-HT1A receptor will be extremely useful given the role of serotonergic signaling in depression-related phenomena. Researchers are also making use of phototactivatable nucleotidyl cyclases from bacteria to manipulate cyclic adenosine monophosphate and cyclic guanosine monophosphate levels using light in living cells (97,98).

Additionally, the light-oxygen-voltage (LOV) protein domain from Drosophila phototropin, which can allosterically regulate proteins fused to this domain upon exposure to blue light, is a very promising optogenetic tool for controlling molecules. Light-oxygen-voltage sterically inhibits proteins to which it is fused in the dark and, upon exposure to blue light, a helix linking LOV to the protein becomes unwound, relinquishing the protein so that it is then able to interact with its binding proteins (33,34). This group fused LOV to Rac1, a small guanine triphosphatase that plays an important role in regulating act cytoskeletal dynamics, and demonstrated that photoactivatable-Rac1 (PA-Rac1) in the presence of blue light can produce cell protrusions and ruffling, alter cell motility, and control the direction of cell movement in cell cultures and in cells in vivo Drosophila (33,34). Our group activated PA-Rac1 in vivo in mouse brain to investigate its role during critical time periods of cocaine exposure and found robust regulation of cocaine-induced behaviors and cytoskeleton regulation when PA-Rac1 was activated in NAc (David Dietz, Ph.D.; unpublished data; November 2011); such studies can now be translated to depression-like behaviors. Another group used the LOV domain to target transcriptional regulation by fusing LOV to the Escherichia coli trp repressor, which they term LovTAP, and this fusion protein selectively binds DNA when illuminated with blue light (99). The ability to translate this to mammals and create photoswitchable molecules to temporally regulate transcriptional activity is a very powerful prospect, considering the profound role of transcriptional and epigenetic regulation in depression and other neuropsychiatric disorders (8,36,62,71,73–75).

Finally, the promising therapeutic effects of DBS in depression and other neuropsychiatric disorders and of optogenetics in animal models of these illnesses, raises the question of whether optogenetics is itself a viable treatment in humans. Optogenetics, combined with viral vectors that target particular cell types, has the potential of exerting more selective stimulatory or inhibitory effects compared with DBS. Technical challenges remain with this approach, which indicates the need for much further research to evaluate the therapeutic potential of optogenetics. Nonetheless, researchers have effectively translated these optogenetic tools to nonhuman primates (100), setting the stage for potential future use in human patients. We will likely see many novel studies using diverse types of optogenetic tools to provide new insights into the neurobiology of depression, which can potentially lead to novel therapeutics that offer more effective treatment in humans.

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