Optogenetic Insights into Social Behavior Function

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Cognitive and social deficits lie at the core of many neuropsychiatric diseases and are among the many behavioral symptoms not amenable to pharmacological intervention. Despite significant advances in identifying genes potentially involved in the pathogenesis of complex psychiatric conditions such as autism and schizophrenia, knowledge of the physiological functions that are affected (and are therefore potential targets for clinical intervention) is scarce. In psychiatric disorders with a strong genetic component, animal models have provided important links between disease-related genes and behavioral impairment. Social dysfunction, for instance, is commonly observed in transgenic rodent disease models. However, the causal relationships between the behavioral and physiological abnormalities in these models are not well-understood. Optogenetic techniques have evolved to provide a wide range of experimental paradigms in which neural circuit activity can be perturbed with high spatial and temporal precision, enabling causal investigation of the function of defined physiological events in neuronal subgroups. With optogenetics, researchers have begun to elucidate the basic neural mechanisms of social behaviors and of disease-relevant social and cognitive dysfunction. The synthesis of optogenetic technology with genetic animal models will allow forward- and reverse-engineering approaches to investigating the neural correlates of psychiatric disease. This review outlines the neural systems known to be involved in social behavior, illustrates how optogenetic technology has been applied to analyze this circuitry, and imagines how it might be further developed in future studies to elucidate these complex circuits both from a basic science perspective and in the context of psychiatric disease.

Key Words: Autism, depression, E/I balance, optogenetics, schizophrenia, social behavior

Animal social behavior serves a wide variety of goals. Even in its simplest forms, social behavior is diverse and includes parental care and attachment, pair bonding, mating, aggression, and maintenance of interconnected communities. Although the motivations for social interactions might vary among species, the cognitive challenges they pose are likely similar due to high levels of uncertainty and the requirement for real-time generation, refinement, and selection of models for the ongoing actions of other individuals. One of the key motivations for achieving a mechanistic understanding of social behavior is that social dysfunction is a common symptom in many psychiatric diseases. Although human social behavior is more complex and nuanced than its rodent equivalent, laboratory animals such as the mouse and rat display a wide range of social behaviors that can be quantitatively measured with laboratory techniques (1). Behavioral tests have been developed that allow fine-grained investigation of social interactions in the laboratory setting, including reciprocal social interactions, social approach, social recognition memory, ultrasonic vocalization, and more (1,2). Studying social behaviors in the laboratory setting enables testing of specific hypotheses and elucidating the molecular and physiological principles that underlie specific behavioral phenotypes (3). With these methods, the neural circuits that subserve social behaviors might begin to be systematically delineated.

During ongoing social interactions, a representation of the environment of an animal based on sensory input including olfactory, visual, and tactile modalities must be synthesized and used to direct behavior on a moment-to-moment basis. Research into the neural correlates of social behavior has pointed to several systems that process sensory information relevant to social interactions (4). In the rodent, these systems can be broadly divided into two distinct modules. The first is a subcortical social circuit that carries relevant olfactory information to the amygdala, which modulates hypothalamic activity to regulate the behavioral responses of the animal. A second module consists of cortical regions thought to provide top-down control over the activity of subcortical areas. The cortical involvement is of particular relevance to psychiatric disease, because human and animal studies have indicated that substantial changes in cortical physiology might occur in diseases such as depression, autism, and schizophrenia. Several recent studies, reviewed in the following text, have applied optogenetic techniques to study the role of specific subcortical circuits in aggression and anxiety and to explore the role of the prefrontal cortex (PFC) in social dysfunction. The growing range of optogenetic tools and associated technologies will enable a deeper understanding of the neural systems involved in social behavior and the neural correlates of behavioral dysfunction.

Optogenetic Tools for Studying Social and Cognitive Behaviors

Optogenetic technology can be readily integrated into social and cognitive behavioral studies, with a growing array of tools that enable genetically defined, light-based control of neural circuit elements (5–8). In optogenetics, genetically encoded light-gated ion channels, pumps, and receptors are expressed in living cells. These are then modulated with controlled illumination of the transduced tissue. Most commonly, this is achieved through stereotactic delivery of genetically modified viruses carrying a microbial opsin gene. Once an opsin gene product is expressed in the target cell population, its activity allows light-based control over cellular physiology (9). Microbial opsins are single-component light-gated ion conductance regulators (10) that, similar to the mammalian eye, use a retinal cofactor that enables light sensation. The most widely used microbial opsins are channelrhodopsin-2 (ChR2), a light-gated depolarizing cation channel (11), and halorhodopsin (12,13), a light-gated hyperpolarizing chloride pump. These two opsins have been adapted to neuroscience in recent years to allow blue light-activated excitation (14) and yellow light-based inhibition (15,16) of neural activity. Due to their high degree of temporal, spatial, and genetic specificity, optogenetic tools can be used to probe neural circuit physiology, for example through mapping of local and long-range projections (17–20), or to examine plasticity in defined pathways (21–24). In behavioral studies, optogenetic tools might be used both to simulate disease-related circuit states and to counterbalance disease-related physiology (25–27).

The optogenetic toolbox has evolved rapidly in recent years and now includes a wide range of tools for controlling neuronal function (reviewed by Mei and Zhang in this issue). In addition to the

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Received Aug 29, 2011; revised Dec 22, 2011; accepted Dec 23, 2011.

0006-3223/$36.00
doi:10.1016/j.biopsych.2011.12.029

channelrhodopsin variants used to depolarize neurons and the chloride and proton pumps used for light-based hyperpolarization, optogenetic tools are available to control cellular biochemical signaling pathways (28–30), extending the range of possible experiments beyond direct modulation of ionic conductance. In experiments that require prolonged modulation over the activity of a particular neuronal population with minimal light delivery, a set of channelrhodopsin variants called step function opsins (SFOs) (31) are used to stably alter neural activity in an on-off fashion with blue light to trigger the opsin on and yellow light to trigger it back to its inactive state. Neurons expressing SFOs respond to a single blue light pulse with sustained depolarization due to the slow decay inherent in SFO photocurrents (31). The stabilized step function opsin (SSFO), generated through combinatorial mutagenesis of the ChR2 sequence, is extremely light-sensitive and can be used to modulate neural activity in behavioral experiments for prolonged periods (>30 min) without the need for attachment of optical hardware during the course of the experiment (25). This property is particularly important when the subject is in direct contact with others, as in some social exploration tests. An additional benefit that stems from the increased light sensitivity of SFOs is that a larger volume of brain tissue can be modulated, despite the substantial decay of light through brain tissue due to scattering and absorption (6,32,33). Channelrhodopsins with red-shifted action spectra provide another solution to the scattering problem, because both scattering and absorption are lower for red-shifted light, therefore allowing for larger excitation volumes (6). C1V1, a red-shifted channelrhodopsin, is triggered on with yellow light and might be used either alone or in combination with the blue light-activated ChR2 by targeting expression of each gene to a different population of neurons (10,25,34). Expression of C1V1 variants can also be combined with a blue light-sensitive SFO, which would allow spectrally and temporally distinct modes of activation (25). With this growing array of optogenetic tools, neuronal function can be reliably controlled in a wide range of experimental paradigms. With these methods, researchers have begun to dissect the various neural circuits involved in social behavior.

Subcortical Systems Involved in Social Behavior

The subcortical social circuit is a major pathway for sensory information specific to social interactions, which directly feeds into motivational and emotional circuits that directly modulate behavioral states and regulate behavioral responses. Sensory neurons in the vomeronasal organ (VNO) respond to nonvolatile chemical cues and thus convey olfactory social information obtained through direct contact between animals (35,36). Olfactory signals transmitted in animal social behavior trigger the release of short nonapeptides of the vasopressin and oxytocin family, through a sensory pathway that transmits information from the VNO to the hypothalamus (37). Chemical cues are processed in the VNO through direct responses of neurons in the sensory epithelium. Sensory information from the VNO is conveyed through direct projections to the accessory olfactory bulb (38), in which cells were shown to respond strongly to physical contact with conspecifics (39). Information from the accessory olfactory bulb is then transmitted to the medial amygdala (36,40,41), which sends efferent projections to widespread brain regions, including the hypothalamus, the nucleus accumbens, and frontal cortical regions (42).

Hypothalamic neurons were shown to be strongly activated during both mating and aggressive behavior (43,44). However, it has not been clear whether these behaviors are mediated by distinct neuronal populations within the hypothalamus. A recent study addressed this question elegantly by targeting neurons in the ventrolateral aspect of the ventromedial hypothalamus, a region shown to receive direct projections from the posteriodorsal medial amygdala (42). Lin *et al.* (45) used single unit recordings in behaving mice to show that neurons in ventromedial hypothalamus respond to both male and female intruders before physical contact and are strongly modulated during aggressive encounters. With virally delivered ChR2, the authors show that activation of neurons in this hypothalamic subregion can directly induce attack behaviors, whereas electrical stimulation of the same region is ineffective in producing this behavioral effect. This is an important distinction, because it emphasizes the fundamental differences between electrical and optogenetic stimulation in the context of mapping the behavioral significance of defined neuronal populations. Optogenetic stimulation of virally transduced cell populations mostly modulates neuronal cell bodies expressing the optogenetic proteins and spares nonexpressing fibers-of-passage (26). By contrast, such axonal fibers are preferentially activated with electrical microstimulation, triggering back-propagation of evoked action potentials to distant somata projecting to the stimulated area (46). Because multiple neuronal populations with diverse roles might be recruited in this way, the anatomical specificity conferred by the combination of viral spread and restricted illumination in optogenetic excitation enables the specific dissection of anatomically defined neuronal populations, as exemplified in this study.

Excitation of channelrhodopsin in axonal terminals, through recruitment of endogenous sodium and calcium channels, leads to neurotransmitter release in the modulated axonal terminals (47). Stimulation of ChR2-expressing axons therefore allows for activation of specific projection pathways in neural circuits. This approach was applied in the dissection of amygdalar circuitry underlying unconditioned anxiety (48), where specific projections from the basolateral amygdala (BLA) to the central amygdala were stimulated with ChR2. In this study, the authors identified a feed-forward inhibitory neural circuit within the central amygdala that regulates anxiety behavior. Activation of excitatory projections from the BLA into the centrolateral amygdala evoked feed-forward inhibitory responses in the centromedial nucleus, leading to an anxiolytic effect, whereas direct stimulation of BLA excitatory neuron somata did not affect anxiety. Because amygdala nuclei are highly interconnected (49), and given the direct projections from the medial amygdala to the hypothalamus (42), it is likely that the activity of these individual subcortical circuits—controlling anxiety, aggression, and mating—integrates to control the engagement in social behavior and the progression of social encounters. Application of optogenetic technology to additional subsystems within the subcortical social circuit is expected to yield valuable information with regard to the role of these systems in mediating social behavior.

Neocortical Control of Social Behavior and Its Dysfunction in Psychiatric Disorders

In the setting of active social interactions, both automatic and volitional processes converge in guiding behavior on a moment-to-moment basis. Top-down control through cortical modulation of behavioral responses has been proposed as a link between higher cognitive processes and subcortical structures such as the amygdala and hypothalamus (4). Despite the differences in the sensory cues used and the complexity of human social behavior, primate (50) and human studies (51–53) have consistently shown the amygdala to be a key player in social processing, providing a possible link between a more primitive, olfactory-based social cognition in rodents and its human homologue. Amygdala activity is

strongly modulated through top-down control by the PFC (50), which directly projects to the amygdala in both rodents and primates (54–57). It is therefore likely that, through modulation of amygdala activity, PFC neurons can exert a strong modulatory effect over social behaviors, integrating higher cognitive processing with innate behavioral responses.

The involvement of the PFC in regulation of amygdala activity might also provide an important link to disease states. Imaging studies have reported significant structural and functional changes to the PFC in patients with major depression (58). In human patients with treatment-resistant depression, electrical stimulation to the subcallosal cingulate gyrus—a prefrontal hub containing many fiber tracts to and from the PFC (59)—was shown to be effective in alleviating depressive symptoms (60,61). Consistently, postmortem studies in depression patients have shown reduced expression of immediate early genes in the PFC, indicating reduced neuronal activity in this region that might contribute to disease symptoms (27). Covington *et al.* have now shown that in mice that are susceptible to social defeat stress, similar reductions in the expression of immediate early genes occur within the medial prefrontal cortex (mPFC). In these experiments, mice are repeatedly exposed over a 10-day period to an aggressor mouse both in direct physical encounters and indirectly through a perforated partition that allows for sensory contact. After this period, some mice develop signs of social defeat-related stress that manifests as anhedonia (measured as a reduced preference for sucrose over water) and a low interaction time in a social approach test. To test the hypothesis that increased activity in the mPFC could rescue this pathological state, optogenetic stimulation was delivered to the mPFC in susceptible mice and control subjects (27). Increased mPFC activity, achieved through activation of ChR2 expressed in socially defeated mice, restored normal social interaction and sucrose preference. The authors mimicked a stimulation pattern associated with the antidepressant effects of deep brain stimulation (60) by delivering short bursts of high-frequency light pulses to drive ChR2-expresing neurons. By contrast, this same stimulation pattern did not lead to increased social approach in control (nondefeated) mice, indicating that the effects of burst stimulation in PFC are specific to the depressive-like state induced by the social defeat paradigm. These results confirm the clinical data implicating the importance of the mPFC in regulation of depression-related social symptoms and provide a starting point for more detailed optogenetic characterization, possibly through projection-based targeting techniques (62), of the diverse subcortical projections of the PFC in modulation of social behavior deficits. Such studies will provide important links between the cortical and subcortical circuits involved in social behavior.

Cortical deficits have also been suggested to play important roles in the pathophysiology of autism and schizophrenia, which are both associated with severe social impairment and are among the most debilitating psychiatric diseases (63,64). These disorders share many behavioral and biological commonalities, including the apparent involvement of dysregulation of cortical excitation and inhibition (65–67). Changes in PFC function have been documented in human imaging and postmortem studies and are considered key evidence to the etiology of autism and schizophrenia (68,69). These changes include altered expression of the neurotransmitter γ-aminobutyric acid (70) and other inhibitory neuron markers (71–74), the most remarkable of which is parvalbumin, a calcium-binding protein expressed in fast-spiking inhibitory interneurons that are involved in the generation of γ-band oscillations (69,75–80). These observations have led to and supported a hypothesis stating that changes in the balance between excitation and inhibition (E/I balance) plays a central role in the pathogenesis of autism and schizophrenia (65–67,81–85).

One widely used approach to studying candidate genes with regard to disease etiology has been to introduce analogous mutations to the mouse genome by transgenic manipulation (1,3,86–88). Indeed, many animal models of autism and schizophrenia display deficits in social interaction (89–104) and have enabled the examination of the neural systems involved. The E/I balance abnormalities have been reported in several mouse models of autism (99,105–107) and schizophrenia (79), supporting the link between disease-related genes, E/I balance regulation, and behavioral impairment (67,108,109). Interestingly, although the molecular and physiological abnormalities induced by genetic or developmental changes in these models are quite distinct, many animal models of autism and schizophrenia show similar impairments in social behavior. Whether these social deficits arise from similar neural circuit impairments has not been established. Direct support for this hypothesis comes from our recent optogenetic study in which the E/I balance in PFC was directly modulated by expression of SSFO, in excitatory pyramidal neurons or in fast-spiking inhibitory interneurons. This allowed light-controlled increases in excitatory or inhibitory activity, respectively, within the transduced region. These results have indicated that increased excitation within the PFC leads to behavioral dysfunction, whereas increased inhibition in the same region does not impair behavioral performance (25). As demonstrated in this study, the use of step-function opsins in behavioral studies allows greater flexibility in the design of behavioral experiments. The kinetic stability of SSFO allows persistent modulation of neural activity throughout the course of many common behavioral paradigms, and the superior light sensitivity of these opsins enables minimally invasive light delivery methods in these experiments.

Summary

Optogenetic technology allows precise manipulation of intact neural circuits in vitro and in vivo. Researchers can choose from a wide range of optogenetic tools along with associated enabling technologies for targeting these proteins to particular cell types. Combined with transgenic models of psychiatric disease, optogenetics will serve a unique role in functional dissection of the neural circuits subserving social and cognitive behaviors and aid in the formulation of refined hypotheses to guide future therapies. Study of social behaviors under more naturalistic settings will necessitate the development of novel methods for delivering light to the brain with minimal constraints on animal handling and movement. Techniques for wireless light delivery and telemetric electrophysiological recordings will greatly enhance the scope of possible experiments and allow the examination of sensitive social interactions with minimal intervention. One of the current limitations in applying optogenetic methods to the study of neural circuits is the specificity with which behaviorally relevant groups of neurons can be chosen for modulation. Refined genetic methods for activity-based and connectivity-based expression of the optogenetic tools will greatly increase the precision with which individual circuit elements can be explored. Combined with an expanding range of animal models of psychiatric disease, optogenetics can be used to dissect disease-related circuits and possibly inform novel treatment strategies.

Dr. Yizhar is supported by the Israeli Center of Research Excellence in the Cognitive Sciences. Dr. Yizhar reported no biomedical financial interests or potential conflicts of interest.

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