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Optogenetics in the behaving rat: integration of diverse new technologies in a vital animal model

Abstract

Rats are the preferred experimental subjects across many fields of neuroscience, for which these large and behaviorally-complex rodents occupy a favorable position that jointly optimizes accessibility for experimental intervention and richness of experimental readout. Yet application of new optogenetic tools in the rat system has been slower than in the mouse system until recently, due in part to technical challenges. These challenges have now largely been overcome, and the neuroscience community is applying optogenetic techniques to the rat system for fast, specific, and potent manipulation of neural circuit elements in the context of diverse readouts ranging from physiology to imaging to behavior. Here we provide an overview of the optogenetic tools and techniques best suited for rat optogenetics, and review current literature employing optogenetics in this way for application to fundamental systems neuroscience, and to models of neurological and psychiatric disease.

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Introduction

Experimental investigations in neuroscience that require multiple electrodes for chronic monitoring or manipulation of neural activity, or bulky hardware for pharmacological interventions and measurements, are often performed most readily in rats. Their large brain size, relative to mice, allows for more precise targeting of pharmacological agents, viral vectors, fMRI regions of interest, or recording electrodes, and their relative strength enables the use of more cumbersome recording, pharmacological, or optogenetic hardware. Moreover, a large array of important behavioral tasks either are best performed in rat models or have been validated and optimized especially well in rat models, including tasks relating to reward [1-3], sensory systems [4], working memory [5-7], and decision making [8,9]. In particular, using standard laboratory strains, the visual acuity of rats has been measured to be approximately twice that of mice [10], and rat performance in memory tests, such as the Morris water maze, also compares favorably [11]. For these and many other reasons, investigations in the rat system promise to continue to contribute in fundamental ways to our understanding of a broad range of complex behaviors (including social [12-14] and cognitive tasks [4,9,15-20]) and clinically-relevant pathologies (including obesity [21], addiction [22,23], and other neuropsychiatric diseases [22-27]).

Optogenetic technologies, which enable fast and precise control over targeted circuit elements within intact tissue and behaving mammals [28-30], in principle provide a new dimension for experimental investigation that would dovetail with the strong foundations and the new opportunities for neuroscience in the rat system. However, application of optogenetics to the rat system has lagged behind applications to the mouse system by several years. Recently, as optogenetic technologies have continued to be refined along with new classes of genetic manipulation [31], the neuroscience community has adapted optogenetics to address a variety of questions in rat models. To effectively utilize microbial opsin-based optogenetic techniques in this way, investigators must consider 1) opsin selection, 2) opsin targeting to the cell population of interest, and 3) integration of optogenetic control with readouts (e.g. behavioral, electrophysiological, and imaging) in the context of the unique constraints and opportunities of the rat model. Here we review these general considerations as well as recent studies that are advancing rat optogenetics in neural systems.

1. Opsin selection and general considerations

A systematic comparative study of currently available opsins for optogenetic application has been published previously [32]. Here we review major opsin categories (Figure 1) and certain general considerations, including those identified and addressed in the comprehensive study, and take special note of parameters with particular relevance to the rat system.

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The channelrhodopsins (ChR, e.g. ChR2; Figure 1) are light-activated cation channels that can be used to depolarize neurons with millisecond precision and thereby drive precisely-timed action potentials [28,33]. Engineered versions include modification for faster deactivation after light-off (to enable the driving of reliable fast spiking up to 200Hz or beyond [34]) or, conversely, modification for slower deactivation after light-off (which in turn allows elicitation of sub-threshold depolarizations-- stable over even tens of minutes-- to be elicited after a single millisecond-scale pulse of light [29,35]; these "step-function opsins" have been used to enhance the excitability of a neural population over prolonged experimental sessions without driving spiking directly [29]). Potentially relevant to rat research, these slower opsins also have the property of rendering expressing cells sensitive to light at much lower levels (by several orders of magnitude) compared with conventional ChRs [29,32,35,36]; this phenomenon carries with it the potential to recruit larger tissue volumes (a highly relevant consideration in larger-brained rodents) at a given experimental light power density. These opsins also help enable more long-term and complex behavioral experiments particularly well-suited to the rat system, for example by allowing rich and interactive social and physical tasks to proceed over many minutes with no optogenetic hardware in place [29].

Opsins also can be used to inhibit or modulate neurons. Halorhodopsins (HR, e.g. NpHR; Figure 1) are light activated chloride pumps [37-39] that, by pumping chloride ions into the cell, can hyperpolarize and therefore inhibit neurons. An amber light-activated form derived from the archaebacterium *Natronomonas pharaonis,* upon engineering for improved expression [40,41], has been shown to be useful for optogenetic inhibition-mediated control of behavior in mammals [26,27,36,42-45] including rats [26]. Another class of hyperpolarizing opsins includes the bacteriorhodopsins [32,39,41,46-49]. Upon illumination, these proteins pump protons out of the cell, thereby hyperpolarizing neurons and inhibiting spiking. Finally, distinct from direct electrical control of neurons, light-activated proteins called optoXRs have been engineered to mimic the activity of endogenous G-protein coupled receptors (GPCRs) [50]. Fusions of the extracellular and transmembrane domains of bovine rhodopsin with intracellular loops of the α_1 (for G_q signaling) or β_2 (for G_s signaling) adrenergic receptors reproduce key specific aspects of the native GPCR second-messenger signaling, in response to light rather than ligand [50]. Recently, similar strategies have led to a light-activated dopamine D1 receptor [51] and light-activated recruitment of G_i signaling [52]. An important caveat relevant to rat work is that, for such biochemical neuromodulation, as well as for electrical inhibition/loss-of-function optogenetics, to ensure successful modification of native signaling it may be important to control relevant structures on both sides of the bilaterally symmetric mammalian brain (in contrast to gain-of-function work with ChRs where delivering the novel neural signal on only one side of the brain may be experimentally suitable to modify behavior). In this regard the larger size of the rat brain compared with the
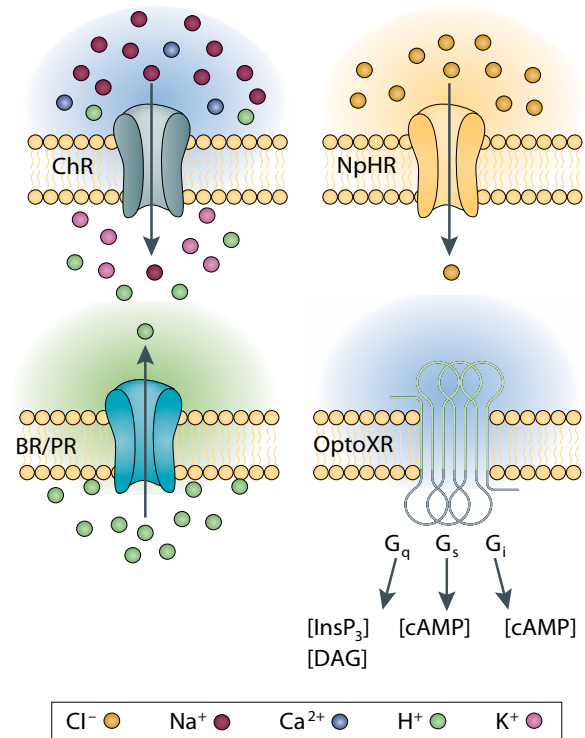

Figure 1. Single-component optogenetic tool categories. Four major classes of opsin commonly used in optogenetics experiments, each encompassing light sensation and effector function within a single gene, include: 1) channelrhodopsins (ChR), which are light-activated cation channels that give rise to inward (excitatory) currents under physiological conditions; 2) halorhodopsins (NpHR shown), which are inhibitory (outward-current) chloride pumps; 3) bacteriorhodopsins and proteorhodopsins (BR/PR), proton pumps that tend to be inhibitory and include archaerhodopsins; and 4) optoXRs, which modulate secondary messenger signaling pathways. Adapted with permission from ref. 220.

mouse brain may provide both challenges and opportunities—facilitating in some cases the implantation of multiple fiberoptic interfaces, while at the same time increasing the net size of brain area that must be controlled (already large in the rat system).

Several classes of control experiment are important in optogenetics to ensure that observed effects are specifically due to optical recruitment of opsins in targeted cells. First, we have cautioned that powerful and prolonged light delivery can cause heating effects that could, in principle, alter neural activity even in non-expressing cells, and we have provided quantitative estimates of the magnitude of this effect [28]. This potential caveat can be addressed by maintaining moderate-intensity [28] or pulsed-light protocols and by including experimental cohorts in which no opsin is expressed but all other manipulations are performed, including (if relevant) surgery, viral transduction, hardware implantation, and light delivery [28]. Similar controls are useful for identifying and/or correcting for confounds linked to any sensory perception of the light (for example, response to laser light as it scatters through the brain and impinges on the back of the retina) [53].

Second, overexpression of any foreign protein could cause altered structure, function, or survival of host cells, and opsins provide no exception to this rule. However, optogenetic methods do intrinsically provide a powerful means to control for such effects by allowing light-on and light-off assessment of physiology or behavior in each experimental subject to ensure normal baseline behavior in the same animal at virtually the same time. Moreover, overexpression of control proteins in parallel experimental animals allows the experimenter to ensure that light effects are not observed only because the animal or tissue is in an unusual state imposed by opsin expression. Fluorescent proteins (XFPs) are most often employed as this control protein, since opsins are often expressed as XFP fusions; ongoing work is focused on developing photocurrent-null opsins for improved experimental control purposes [54].

Third and finally, any form of strong neural activity (ranging from physiological, to electrically-driven, to optogenetically induced) can cause transient (seconds-scale) shifts in the extracellular or intracellular ionic milieu; we have cautioned [55-58] that experimental assessments and behavioral exposures therefore in most cases should be carried out during, rather than immediately after, optogenetic or electrical stimulation to ensure that assessments are operative during the known optogenetic control period rather than during a period of less well-defined recovery. Here again optogenetic tools provide a uniquely powerful way to track any such effects, enabling (for example) electrophysiological interrogation at the same time as optogenetic stimulation to track evolution of ion balance shifts (such electrical recording would be interfered with by artifacts arising from electrical stimulation, discussed in more detail below). This class of caveat, as with the previous two classes of control experiment, applies to the rat as with any experimental system.

2. Opsin targeting

Although rats have long served as a crucial experimental system for neuroscience, genetic and molecular tools suitable for targeting defined cell populations in rats have lagged behind those in the mouse system. However, there now are many methods to target specific neural populations in rats, even without transgenesis (Figure 2a-c) [28]. Either adeno-associated viruses (AAV) or lentiviruses (LV) can be engineered to carry an opsin (for example, under the control of a general neuron-specific promoter such as the human synapsin promoter hSyn, or an astroglial-specific promoter such as GFAP [28]), and can then be injected into a target brain region to transduce local cell bodies. Even this simple approach provides an immense spatial-specificity advantage beyond electrical stimulation, since AAV and especially LV do not efficiently transduce axons, and therefore will sensitize for stimulation local cells and not fibers of passage. The larger rat brain may provide even more opportunities for such specificity than the mouse brain, allowing for improved region-specific targeting given virus diffusion (typically on the millimeter scale) after injection. Yet the flip side

of this advantage for the rat system is that, for controlling large regions of the brain, researchers may have to consider multiple injections or larger volumes of virus to ensure adequate spatial extent of transduction.

Further specificity can be obtained in certain cases by using cell-subtype specific promoters. Although most such genetic control regions are too large to package into AAV or LV, cell-subtype specific opsin expression has been successfully demonstrated in (for example) rat serotonin [59], somatostatin [60], CaMKIIα [61], and GFAP [25]-expressing cells using this method [28]. An important cautionary note is that a promoter fragment showing specificity in one species or in one virus type may not show the same specificity in another preparation. The specificity and penetrance of cell-type specific promoter strategies varies across species, across brain regions within a species, and even across viral titers when all other conditions are matched [62]. As a result, each promoter strategy must be validated as it will be used experimentally and in the brain region and species of interest.

A more generalizable approach, which has seen widespread use in behaving mice over the past few years [27-29,42,43,63-80], involves the introduction of viruses (engineered so that opsin expression is Cre recombinase-dependent) into mouse driver line subjects (in which Cre is present only in the cell type of interest). Though this method is successful in mice (and growing in use and potential as recombinase-driver mouse lines are rapidly generated around the world), there had been until recently little opportunity to apply this approach in rats since there were no specific Cre-driver rat lines. However, optogenetic specificity in rats now can be achieved in versatile fashion this way, as shown with the first specific Cre-driver rat lines [31]. One of these lines expresses Cre under the tyrosine-hydroxylase (TH) promoter region, allowing for dopamine neuron-specific targeting in the ventral tegmental area (VTA) and substantia nigra (SN), as well as norepinephrine neuron-specific targeting in the locus coeruleus (LC) [31]. Another of these lines expresses Cre under a choline acetyltransferase (ChAT) promoter region, allowing for targeting of acetylcholine-expressing neurons in the medial septum, nucleus accumbens, and nucleus basalis [31]. As this technique appears to be generalizable, there will likely be a proliferation of such genetic tools for rats in the near future. Moreover, other recombinases (e.g. Flp) and versatile genomic targeting tools (TALENs, Zn-finger-related tools, and other strategies [81-87]) can now be brought to bear in rats.

Anatomical (non-genetic) targeting strategies are also versatile and effective, and suitable for the rat system. For example, individual layers of cortex have distinct connectivity and functional properties [88-90] that may help give rise to the hypothesized characteristics of the canonical cortical microcircuit [91,92], but without optogenetics it is difficult to causally manipulate individual layers [79,80]. The facility with which one can stimulate and record in multiple regions in rats would render these animals valuable for future investigation of this fascinating topic, but transgenic strategies for cortical layer targeting do not yet exist in rats. However, in-utero
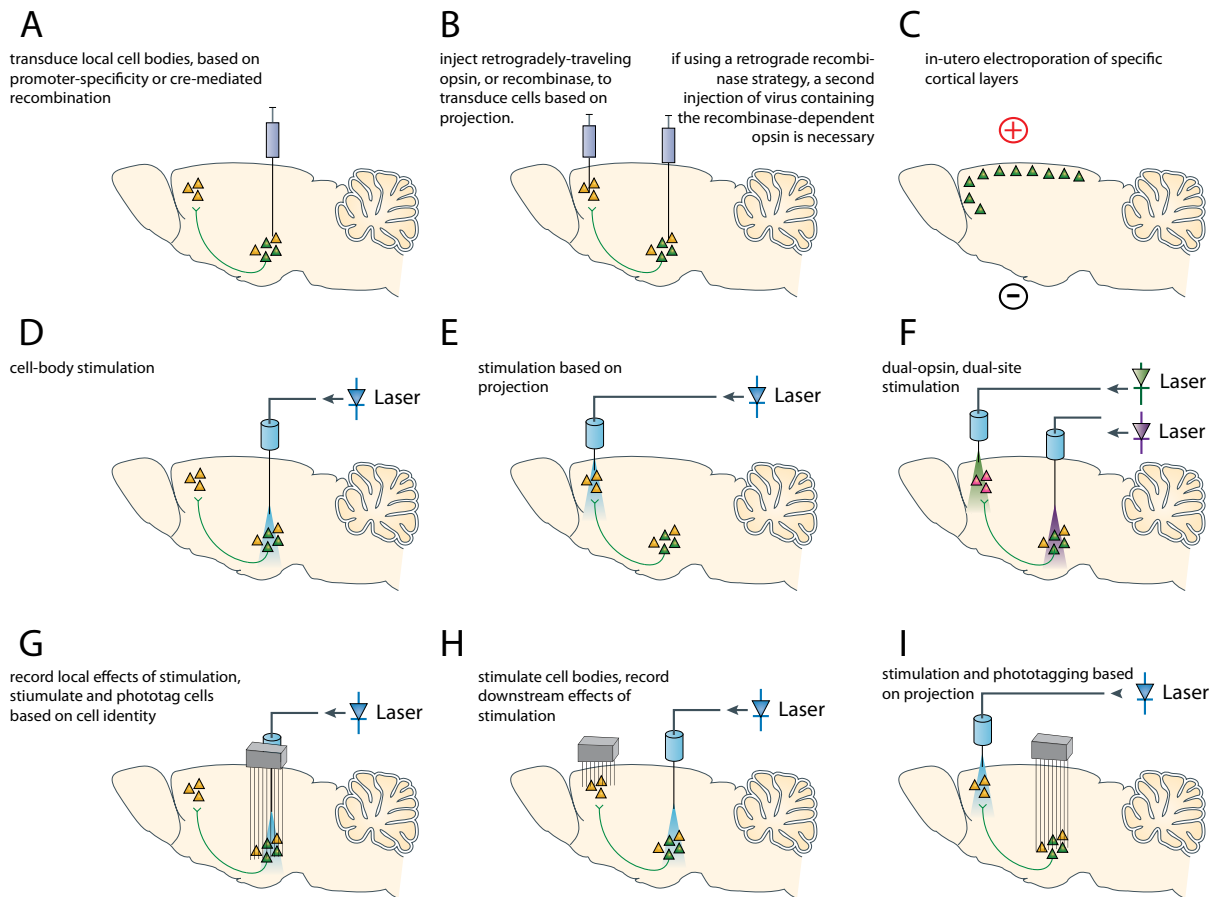
Figure 2. Optogenetic targeting and experimental design suitable for the rat system. Panels A-C illustrate rat-compatible strategies for transducing the cell population of interest with an opsin. These include A) transduction of cell bodies via viral injection, B) single or dual-virus retrograde strategies for projection-specific opsin expression, and C) in-utero electroporation for cortical layer specific expression. Panels D-F illustrate possible configurations for optical stimulation, including D) illumination at the site of transduced cell bodies, E) illumination of downstream projections, F) illuminating multiple distinct populations of cells at the same or different locations, which can express opsins sensitive to different wavelengths of light. Panels G-I illustrate combinations of electrical recording with optical stimulation. Possible configurations include: G) recording at the site of optical stimulation, H) recording downstream of optical stimulation, I) recording at transduced cell bodies, while stimulating downstream projections. Adapted with permission from ref. *28, 220.*

electroporation (Figure 2c), through which a transgene-encoding construct is introduced into the developing cortex [93], enables anatomical layer-specific opsin expression with no requirement for genetic information at all. But perhaps the most versatile approach to anatomical targeting specificity (and one particularly well-suited to the rat system) is "projection targeting" [25,28]. Because microbial opsins are trafficked efficiently along neural processes (especially in the molecularly engineered trafficking-enhanced forms [41]), even distant axons and axon terminals of opsin-expressing neurons will become photosensitive. This property enables the projection-targeting approach, which requires knowledge of neuroanatomy but little or no genetic information or genetic tools (perfectly suited to the rat model), in which neurons are transduced with opsin at the site of the cell bodies but illuminated at the site of downstream projection targets— thereby defining the population to be controlled by virtue of 1) cell body location, 2) promoter expression properties, and 3) axonal projection pathway (Figure 2e,i). This approach

has been widely used in mouse [44,45,76] and also in rat [25,26,31,61,94,95] behavior.

One cautionary note is that, with this technique, all opsin-expressing axons passing through the illuminated region will be activated, such that fibers of passage continuing on to multiple distinct target regions may be optically modulated. To address this issue and provide a further level of specificity (beyond projection-pathway specificity to projection-target specificity), retrogradely-propagating viruses that transduce presynaptic terminals can be introduced into the desired postsynaptic target region. Examples of viruses that can transduce axon terminals and give rise to expression in the corresponding cell bodies include herpes simplex virus 1 (HSV1), pseudorabies (PRV), rabies virus, and vesicular stomatitis virus (VSV) [96,97]. Promising additional developments in anatomy-based viral targeting include a lymphocytic choriomeningitis virus glycoprotein (LCMV-G) for trans-synaptic anterograde transduction [97] and a glycoprotein deleted rabies virus (ΔG-rabies) for trans-synaptic transduction

of cells exactly one synapse retrograde [98]. However, it must be cautioned that these viruses tend to exhibit unstable expression or toxicity over weeks or even days, requiring careful timing and interpretation of experiments. Emerging methods that avoid toxicity and instability of virus-mediated gene expression include dual-virus strategies in which an AAV carrying a recombinase-dependent opsin construct is injected in the upstream cell body region (Figure 2b) and another virus carrying the recombinase is injected into the target population terminal field. This second virus can be 1) another AAV via which the encoded recombinase is delivered from postsynaptic to presynaptic cell as a protein fused to a transcellularly transported lectin such as WGA [41], or 2) a canine adenovirus (CAV2) which moves retrogradely with efficiency comparable to HSV1 [99] but with stable expression (60-70% of baseline six months post-injection) [99]. Through methods such as these, specific neural cells and projections can be targeted for optogenetic control with versatility, stability, and tolerability in the rat system.

3. Readouts

The capacity of optogenetics for spatially-, genetically-, and temporally-precise circuit manipulation can be integrated particularly well in rat with multiple layers of readout, including electrophysiology, complex behavior, fMRI, and cellular imaging. In this section, we discuss practical considerations and experimental possibilities for these various readout modalities incorporating optogenetics in rats.

3.1 Readouts: integrating electrophysiology with optogenetics in rats

Unlike electrical stimulation, which suffers from stimulation-artifact constraints, optogenetic stimulation in principle allows simultaneous *in vivo* neural manipulation and recording in real time [55]; the word "optrode" was introduced in this context to describe an integrated device for optogenetic stimulation and recording [40]. Rat proportions facilitate the implantation of multiple individual light guides or multi-fiber arrays along with high-throughput neural recording devices [26,27,100,101], real-time measurement of neuromodulator concentrations, and/or cannula-based pharmacological manipulations [26]—all in freely-moving animals. The advent of chronic, implantable electrodes for fast-scan cyclic voltammetry [102] opens another potential avenue for circuit dissection, allowing the measurement of catecholamines—including dopamine, serotonin, or norepinephrine [103]—with sub-second precision in freely-behaving animals. While there can be electrical recording artifacts arising from direct illumination of metallic recording wires [53,104,105] that in some cases can complicate local field potential recordings [104], these effects are unlikely to adversely influence extracellular spike recordings, and strategies for mitigation of these photovoltaic effects have been identified [104]. Many groups have now designed and tested optrode devices and configurations that appear suitable for the rat preparation (Figure 2g-i, Figure 3) [26,29,101,106-109].

In one simple configuration, a light guide directly above the somata of opsin-expressing cells (Figure 2d) can be combined with electrical recording devices (Figure 2g,h; Figure 3) [25,29,74,101,107-109]. Interestingly, this method can also allow for confirmation of recorded-cell opsin-expression status (i.e., determining if the cell generating a particular set of light pulse-elicited spikes is a ChR-expressing cell or a downstream cell) through optical tagging [110]. A refinement of this configuration involves implanting the light guide in a downstream terminal region of the opsin-expressing cells (Figure 2e). This configuration allows, not only separable control of neurons within a given region that may have similar genetic signatures but divergent projection patterns and behavioral functions (as discussed above) [26,111-114], but also— when combined with neural recordings (Figure 2i)— opens the possibility for identifying recorded cells based on their projection patterns. If the recorded cells express an opsin, then stimulation of the cells' terminal fields can generate back-propagating action potentials, allowing for optical tagging by projection pattern, though care must be taken in considering variance in latency of antidromic spike propagation in the context of possible orthodromic and recurrent mechanisms. And in yet another configuration, neural recordings (e.g. electrical or voltammetric) can be made postsynaptically to optical cell-body stimulation (Figure 2h) [27]. This arrangement combines causal cell-type specific manipulations at one node of a neural circuit with simultaneous recording of potential effects at a downstream node or layer, providing a novel window into the workings of complex neural circuitry. Beginning in 2009 [25], all three of these multi-probe configurations now have been shown to be well-suited to the dimensions of the rat system, and are currently widely applied [26,27,102,115].

3.2 Readouts: integrating freely-moving behavior with optogenetics in rats

Although the size and strength of rats can facilitate complex optogenetic experimentation, these same characteristics imply that special care is required when designing the relevant equipment. For example, although mice can carry relatively little weight, mice are also unlikely to damage or destroy fiber-optic patch cords, whereas rats can and will pull apart and chew through even heavily jacketed cords. Diverse behavioral rigs can, however, be outfitted for optogenetic experimentation in rats [25-27] (Figure 4). In a typical arrangement, a computer or stand-alone function generator is used to control the light source (laser or LED-based), which is connected to a fiber-optic patchcord (200-300 mm in diameter). This in turn connects to an optical commutator, allowing the rat to rotate in the behavioral apparatus without tangling the patchcord. A second patchcord connects the optical commutator to the fiber cannula that is implanted on the rat, and can be protected from mechanical damage with the same type of lightweight metal spring often used to protect in-vivo microdialysis equipment (Plastics One, part 6Y000123101F). This patchcord assembly can be kept out of reach of the rat by support with a counter-weighted
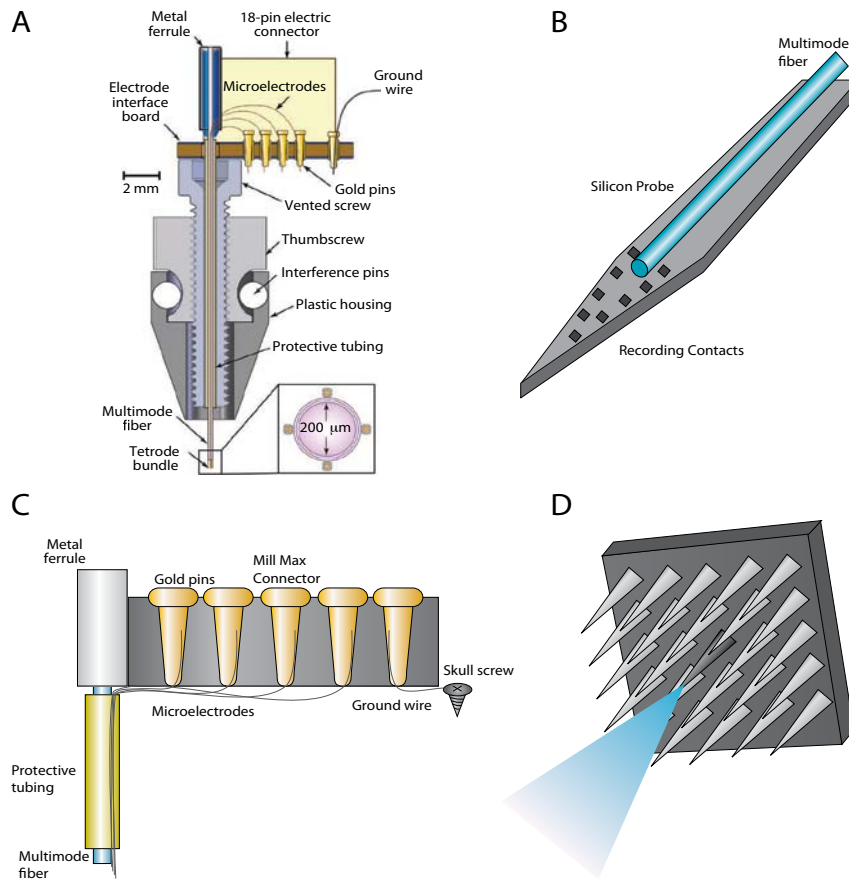
Figure 3. *Integrating optogenetics with electrophysiology in rats. Several groups have developed optrode devices for simultaneous optical manipulation and neural recording; four classes of optrode device include: A) the optetrode, which combines an optical fiber with four bundles of drivable tetrodes (adapted from ref. 74; multiple additional optetrode devices have been designed and fabricated [29,101,106-109]); B) the multisite silicon probe optrode [106], which allows for illumination patterns across many recording sites (adapted with permission from reference 106); C) the chronic multisite optrode [29,101], a set of fixed microwires bundled with an optical fiber, enabling local multiunit and LFP recordings; and D) the optrode multielectrode array [109], for recording and stimulation across many cortical units (adapted with permission from reference 109).*
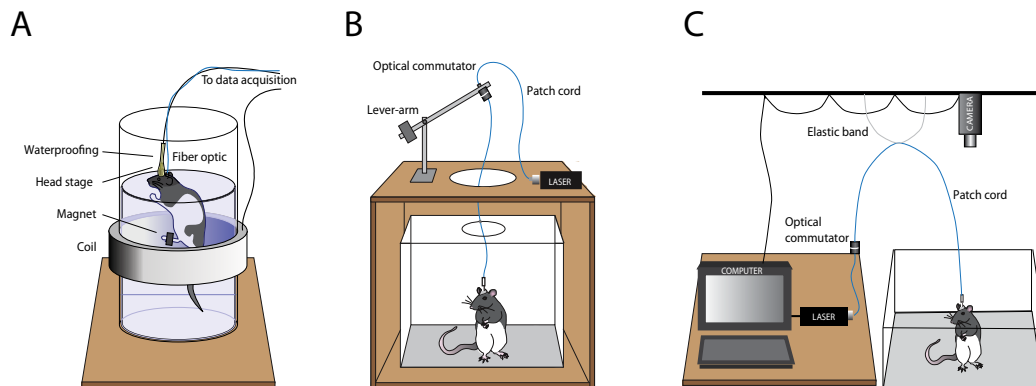

Figure 4. *Integrating optogenetics with behavior in rats. Diverse behavioral rigs can be outfitted for rat optogenetic experimentation. A) The forced swim test has been automated with magnetic induction-based detection of kicks combined with optogenetic stimulation and electrical recording (adapted with permission from ref. 26). B) Operant behavior in rats can also be combined with optogenetics [16,31]. The chamber itself is modified to accommodate the entry of fiber optics and recording wires, and the stimulation/recording assembly is kept out of the reach of the rat with a counter-weighted lever arm (adapted with permission from ref. 31). C) Optogenetic manipulations can also be combined with behavior in open fields or large mazes [115,116]. In these experiments, an elastic band, rather than a lever arm, is used to support stimulation/recording equipment. Video recording combined with custom or commercially available software can be used to synchronize optical stimulation with behavior.*

arm (Figure 4b) or with an elastic band suspended above the behavioral apparatus (Figure 4c).

This system can also be used to support recording equipment such as headstage wires or microdialysis tubing, and has been shown to work even in challenging environments, such as the forced swim test (with appropriate waterproofing) [26], enclosed operant chambers [16,31], and large mazes [116] (Figure 4). Closed-loop systems are also emerging, combining electrophysiological recording with optogenetics to enable the triggering of optical stimulation or inhibition based not only on behavioral events [16,31], but also on neural events [101]. Simultaneous video recording, combined with commercial or custom software, allows synchronization of neural recordings with behavior as well as the triggering of laser stimulation based on behavioral events (Figure 4c). Additionally, magnetic induction-based methods [26,27] can be used to pick up millisecond-resolution behavioral events such as paw movements in the setting of simultaneous optogenetic control and electrical recording in the rat.

3.3 Readouts: integrating imaging with optogenetics in rats

Functional magnetic resonance imaging (fMRI) has become a useful (and widely used) read-out of human and experimental-animal brain activity. Because the fMRI-measured blood oxygenation level dependent (BOLD) signal is an indirect measure of activity relying on a temporally complex hemodynamic response to local brain metabolism, the degree to which the complex BOLD signal could be causally elicited by activity patterns in defined local cell types had been unclear. In 2010 it was demonstrated that optogenetic excitation of spiking in principal cell bodies sufficed to initiate a robust local positive BOLD signal with classical temporal dynamics [61]; this approach was named ofMRI [61]. Most usefully, it was found [61] that upon optically stimulating principal cells in rat primary motor cortex, positive BOLD signals could be measured downstream in thalamus, indicating ofMRI utility for functional circuit mapping since activation of other networks and circuit elements occurs as dictated by activity of the optogenetically targeted components [61,118]. Many groups have now measured ofMRI signals, including in the mouse, which will facilitate application to transgenic animals [119,120]. Rats may provide a unique advantage for ofMRI since the much-larger spatial dimensions of the brain will improve the resolution by ofMRI of smaller structures and signals at a given field strength and scanner configuration.

As methods for ofMRI improve alongside genetic and optogenetic targeting in rats [120], ofMRI will continue to develop as a tool for the characterization of causal and specific functional connectivity in awake and even behaving animals, both in normal functioning and in disease models. But other imaging modalities also can be integrated with optogenetics in rat or mouse, operating at smaller spatial and faster temporal scales. For example, organic dye-based imaging has been combined with optogenetic control [121-123], and improved genetically encoded sensors for neural activity [124-

126] enable new possibilities for cell-type-specific readout information. Spectral and kinetic properties of the newer channelrhodopsins, such as the C1V1 family [28,29,127,128] in which peak excitation is redshifted away from both the fura-2 and GCaMP spectra especially in the infrared two-photon band, enable additional possibilities for all-optical circuit interrogation.

4. Applications

Here we move beyond experimental and technical considerations to review the successful applications and scientific findings obtained with rat optogenetics.

4.1 Applications: behavioral conditioning and reward in the rat

We begin with a series of papers in which optogenetic tools were used to dissect behavioral conditioning and reward circuitry in the rat.

Self-Stimulation Reward

Early research into the neural substrates of reward included studies of intracranial self-stimulation (ICSS) in rats [1]. This research demonstrated that rats will work for direct electrical stimulation of certain brain regions, and provided the first clues regarding structures mediating behavioral reinforcement. Sites effective for ICSS strongly overlap with major projections of the dopamine (DA) system [2], and lesions of DA neurons or drugs that block DA neurotransmission diminish ICSS behavior [129,130], together suggesting that DA mediates sustained ICSS. Electrical stimulation recruits a spatially complex population of neurons [131,132], however, and not all sites that support ICSS receive dense DA innervation [133]. With the advent of TH::Cre transgenic rats discussed above [31], it became possible to directly test the causal impact of a temporally-precise DA neuron signal in models of conditioning/reinforcement learning and ICSS. Replacing classical electrical stimulation of the VTA with optogenetic stimulation specific to local TH+ (DA) neurons, it was found that phasic optical stimulation of VTA-DA neurons was sufficient for the acquisition and maintenance of robust ICSS behavior, and that stimulation of the dopaminergic VTA projection to the nucleus accumbens (NAc) was itself sufficient to support ICSS.

Over the last two decades, it has been widely hypothesized that DA signals could be used for learning and for reward prediction [134-140], and it has been postulated that the firing of DA neurons represents the reward prediction error of classical temporal difference learning [141]. Until recently, however, researchers have been unable to manipulate DA neurons with the temporal specificity needed to address the role of DA in these learning models. Current research utilizing the TH::Cre transgenic rats in combination with temporally-precise optogenetic excitation or inhibition in these classical tasks [142] is beginning to cast light on long-held predictions surrounding dopamine signaling, learning, and reward.

Addiction

It has been postulated that addiction causes pathological plasticity in the mesolimbic DA system [143]; moreover the NAc, a major projection target for VTA DA neurons, has received attention as a potential locus for neural processes underlying addiction [23,42,144-147]. Evidence from human neuroimaging and rodent pharmacology has also implicated the prefrontal cortex (PFC) in addiction and other impulse-control disorders [148-151]. Until recently, however, the role of the direct projection from PFC to the NAc in drug-seeking behavior remained unknown. In a recent study [95], rats were trained over many days to self-administer cocaine. After a ten day extinction period, rats were then tested either for cocaine-induced or cocaine+cue-induced reinstatement of drug-seeking behavior. The authors showed that optogenetic inhibition of either the PFC or the NAc was sufficient to inhibit reinstatement of cocaine-seeking, and that strikingly, inhibition of the projection from PFC to NAc was itself sufficient to block the drug-seeking behavior in rats. This study opens the door to further elucidation of the circuitry necessary to maintain addictive behaviors, and may suggest insights into human disease and treatment.

Anti-reward

In addition to circuits mediating reinforcement or reward, rapid advances are also occurring in the study of aversion, disappointment or "anti-reward". Studies in both primates and rodents suggest that neurons in the lateral habenula (LHb) can encode this "anti-reward" signal [152,153]. This finding is of both scientific and clinical interest, as inputs to the LHb are potentiated in animal models of learned helplessness [154], the LHb is a potential target for deep brain stimulation (DBS) in refractory depression [155], and the LHb can inhibit VTA-DA neurons (which have been linked to depression [26,27,78]) disynaptically via the rostromedial tegmental nucleus (or "tail of the VTA") [156,157]. The LHb could in turn receive anti-reward signals from neurons in the globus pallidus interna (GPi, corresponding to the entopeduncular nucleus/EPN in rodents) [158], and a recent study used rat optogenetics to investigate this possibility [94]. The authors expressed ChR2 in EPN and optically stimulated EPN axon terminals in LHb during patch-clamp recordings of LHb cells, observing that the projection from EPN to LHb is chiefly excitatory. The authors also found that stimulation of this projection was itself aversive in a conditioned place preference test in rats, providing additional evidence that the EPN-LHb projection carries an "anti-reward" signal. Finally, it was observed that serotonin (but not dopamine) decreased the amplitude of light-evoked excitatory currents in LHb, providing insight into opposing interactions between serotonin and dopamine in reward [159].

4.2 Applications: cognition in the rat

Memory-guided orienting

Homology between the primate frontal eye fields (FEF, which play an important role in gaze control, orientation, and attention [160-168]) and the rat frontal orienting fields (FOF) has been

suggested since the late 1960s [19,169]. Over the last few decades, increasing anatomical [170-172], lesion [173-175], and microstimulation [176] evidence has continued to support assignment of this homology, and recent electrophysiological evidence [19] has lent further credence to this hypothesis. To directly test the role of the rodent FOF in behavior, a recent rat optogenetic study [177] utilized a memory-guided orienting task, in which rats were trained to distinguish between a "long" and a "short" series of clicks. The authors employed NpHR-mediated inhibition of the FOF to bias behavior, comparing a memory-dependent with a non-memory dependent version of the task, and also tested for an effect of inhibition of the superior colliculus, which receives a direct projection from FOF [172]. Surprisingly, although bias was only present in the memory dependent version of the task, the effect of inhibition was more pronounced if delivered during the cue period than during the memory period, illustrating the power of precisely-timed optogenetic inhibition for the probing of complex brain circuitry and behavior in the rat.

Exploration and encoding

The canonical trisynaptic hippocampal circuit has been suggested to operate in two distinct states: one for encoding while the animal is active and one for consolidation while the animal is resting [178,179]. To test this two-state model, Kemere et al. expressed ChR2 in excitatory cells of the rat dentate gyrus (DG) [115]. The authors then optically stimulated DG cells and recorded from the other (downstream) hippocampal subfields of the trisynaptic circuit while the rat explored a novel environment. Interestingly, the strength of optically-evoked excitation was not simply binary or based on whether the rat was running or resting. Instead, the strength of evoked excitation varied smoothly with the rat's speed. Furthermore, this modulation seems to be limited to a distal step in the circuit, the Schaffer collateral pathway, and was enhanced in a novel environment relative to a familiar environment. This study illustrated the use of rat optogenetics for a novel kind of functional, targeted, real-time circuit mapping in which the strength of defined projections is tracked during complex behavior integrating aspects of memory, novelty detection, exploration, locomotion, and context.

Decision making

Although sensory decision-making is perhaps most extensively studied in the context of primate vision [180,181], multiple researchers have successfully utilized olfactory or auditory stimuli to study sensory decision–making in rat models [8,9,182]. One recent study [182] developed an auditory "tone cloud" task, in which rats are presented with a mixture of high and low tones. The rats indicate whether the cloud is "high" or "low," and behavior smoothly varies with the composition of the tone cloud. In this study, the authors used a retrograde viral strategy to express microbial opsins in neurons projecting from auditory cortex (A1) to the striatum. Stimulation of this corticostriatal projection was found to bias behavioral choice in a tonotopic fashion, while inhibition of the projection biased choice in the opposite direction. These results show that the projection from

A1 to striatum helps mediate decision making in this task, and illustrate the use of optogenetic projection targeting for complex behavioral tasks in the rat.

4.3 Applications: Neurological disease-related pathology in the rat

The potential utility of electrical stimulation in the study of neural function was demonstrated as early as 1870, when Fritsch and Hitzig documented muscle contractions in response to electrical stimulation of motor cortex in the dog [183]. Cortical electrical stimulation was translated to humans just four years later, when Bartholow successfully used the method to generate muscle contractions in patient Mary Rafferty [184]. The therapeutic use of electrical stimulation did not develop until much later, however; for example, the use of chronic stimulation for alleviation of motor disorders was first reported in 1972 [reviewed in ref. 185]. Since then, therapeutic use of electrical stimulation has expanded to include treatment for many movement disorders [186-192] as well as for chronic pain [193], epilepsy [194] and psychiatric diseases [195-200]. Despite extensive use, the effects of electrical stimulation remain poorly understood [131,132]. Since rat models for many of the relevant diseases exist, optogenetic studies in these rat models may improve our understanding of the etiology of these diverse disorders and may also provide insight into treatment mechanisms.

Parkinson's Disease

Parkinson's Disease (PD) is a debilitating movement disorder, marked by bradykinesia, tremor, and rigidity. The vast majority of patients also exhibit comorbid symptoms and signs, including depression, dementia, anxiety, and sleep disturbances [201]. Although dopaminergic medications are highly effective for many PD patients, these medications become less efficacious as the disease progresses, and randomized controlled studies have shown favorable outcomes for deep brain stimulation (DBS) [191,192]. Both globus pallidus stimulation and (more commonly) subthalamic nucleus (STN) stimulation are used [190], but in no case has it been clear how the DBS is working or which circuit element is the direct target of DBS in giving rise to the therapeutic effect. Interestingly, using a rodent model of STN DBS treatment of parkinsonism, it was found that neither direct optogenetic excitation or inhibition of local cell bodies was sufficient to restore normal behavior [25]. However, stimulation of primary motor cortex (M1) projections to STN, or direct stimulation of M1 cell bodies was sufficient to improve behavior, suggesting that the initial direct target of DBS in the STN is afferent axons, including those from motor cortex. These findings were later supported by other rat PD work [202] and together these findings from the rat literature may point to deeper understanding and refined application of DBS as a treatment modality for CNS disease.

Epilepsy

Another disorder that has proven amenable to study and treatment through neural stimulation (including in rodent models) is epilepsy. The first application of optogenetics to epilepsy came in 2009, when cell-type targeted control of NpHR in mouse hippocampus was demonstrated to inhibit epileptiform activity [56]. Later work brought optogenetics to rat models in which epilepsy is initiated by a photothrombotic model of cortical stroke [101]. Stroke is among the more common etiologies of epilepsy [203], with 11.5% of stroke patients experiencing seizure onset within five years [204]; moreover, cortical stroke leads to epileptiform events in thalamus, increased excitability in thalamocortical cells, and EEG signatures similar to those of corticothalamic absence seizures. Although a role for thalamocortical projections had previously been suggested in human cases of focal epilepsy [205], and a causal role for the thalamus had been suggested by lesion studies [206], the role of thalamocortical projections in focal epilepsy was poorly understood. In this recent rat optogenetics study [101], seizure activity was detected in real time from electrophysiological recordings, and seizures could be terminated in real time through optogenetic inhibition of thalamocortical neurons. Optogenetic approaches have also extended to a model of focal cortical epilepsy induced by local injection of tetanus toxin [207]. As in human cases of epilepsia partialis continua [208], following this treatment, rats exhibited almost continuous, drug-resistant seizure activity. The tetanus toxin injection also enhanced the intrinsic excitability of layer V pyramidal neurons and increased the incidence of EEG-monitored epileptiform events; moreover, local, acute optogenetic inhibition at the seizure focus was sufficient to reduce high-frequency EEG power. Together, these findings from epilepsy models both improve our understanding of the causal impact of defined circuit elements in epileptiform activity and suggest possible new therapeutic avenues for the treatment of seizures.

4.4 Applications: Psychiatric disease-related pathology in the rat

Habit circuitry relevant to compulsive behaviors

Habits are a fascinating class of almost-automatic behaviors that can be surprisingly and frustratingly resistant to change, and when maladaptive in their influence on behavior are linked to major human health issues, including obsessive-compulsive disorder, addiction, and obesity. Evidence from rodent studies has implicated the dorsolateral striatum [209-211] and the infralimbic region [212,213] of medial prefrontal cortex (IL) in habit expression. To examine the degree to which deeply-ingrained behaviors are subject to cortical control, Smith et al. overtrained rats in a t-maze alternation task until behavior had become habitual [116] and maladaptive in the sense of no longer being reward-related. The authors then expressed NpHR in principal cells of IL cortex and demonstrated that online inhibition of IL cells disrupted this established habitual behavior, typically within just three maze-runs. This intriguing finding from rat optogenetics suggested that habitual behavior can be deeply ingrained, insensitive to reward devaluation, almost automatic—and still under cortical control, with profound implications for fundamental understanding of corticostriatal interactions, and with substantial implications for the understanding and treatment of human disease.

Motivation circuitry relevant to depressive behaviors

Major depressive disorder (MDD) is highly prevalent, affecting 12% of men and 20% of women at some point in their lifetimes [214]. MDD is also highly comorbid with other classes of disorders, including anxiety and substance abuse [215]. Current front-line treatments for depression have a slow (weeks-long) therapeutic onset time course and are ineffective in many patients; in fact, less than half of sufferers who seek care report adequate treatment [214]. Furthermore, researchers remain divided on the primary etiology for depression, with little deep understanding and many fundamentally different models of disease causality [216].

Regardless of depression etiology, monoamine modulators can play a role in treatment [216], and many clinically-effective antidepressants block the reuptake of serotonin at the synapse (e.g. SSRIs) or otherwise increase the availability of monoamines (e.g. MAOIs) [216]. Interestingly, stimulation of the subgenual/subcallosal regions of the prefrontal cortex has also been shown to improve symptoms in clinically depressed patient populations [198,199] (a seemingly unrelated treatment from the perspective of monoaminergic medications, although the prefrontal cortex is known to send and receive projections to and from brainstem monoaminergic regions [217,218]). A recent rat optogenetics paper [26] indeed provided a causal link between prefrontal circuitry and serotonergic nuclei in depression-related behaviors, by synchronizing neural recording and optogenetics with the magnetic induction-based millisecond-scale behavioral readouts noted above, in the forced swim test (FST, a classical and well-validated model of behavioral despair). The authors found that optogenetically stimulating excitatory cells in the rat PFC did not change behavior in the FST, but that optogenetic stimulation of the projection from PFC to the serotonergic dorsal raphe nucleus (DRN) increased (and optogenetic inhibition decreased) antidepressed-like escape behaviors in the FST [26]. Conversely, stimulating the projection from PFC to LHb—a nucleus known to have inhibitory effects on downstream dopaminergic and serotonergic neurons as discussed above [157,159,219]—increased expression of the depressive phenotype (i.e. reduced escape-related behavior in the FST) [26].

Another recent paper integrated many of the technologies described above, including the rat optogenetic FST task [26], the TH::Cre rats [31], and multiunit-recording readouts suitable for integration with optogenetic control, to examine the role of VTA-DA neurons in depressive behaviors [27]

after induction of a depressed-like state with chronic mild stress [24]. Optogenetic stimulation of VTA-DA neurons was found to increase escape (antidepressed-like) behavior in the FST; moreover, the increase in escape-related behavior was correlated with the number of NAc neurons modulated by optical stimulation of the VTA [27]. Additionally, NAc neurons were found to encode escape-related behaviors, and optogenetic drive of VTA-DA neurons modulated the neural encoding of this depression-related action in NAc [27]. Together with evidence that the NAc and closely-connected regions may be suitable targets for deep brain stimulation in clinical treatment of depression [200], these findings in rat optogenetic models have helped illuminate the underlying neural circuitry that may cause, correct, and encode complex depression-related behaviors in the mammalian brain.

5. Summary

Here we have reviewed general considerations as well as specific published studies focused on rat optogenetics in neural systems. For reasons discussed (including the natural advantages of the rat model, the long history of pioneering development and validation of rat behavioral tasks and readout methods, and ongoing rapid advances not only in optogenetics but also in rat genetic tools), we expect this field of neuroscience to continue to grow rapidly. Together with associated enabling technologies, rat optogenetics will likely play a crucial role in contributing to our deepening understanding of how diverse classes of neural circuit components interact to give rise to complex behaviors, pathological conditions, and therapeutic responses.

Acknowledgments

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