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Perspective

# Targeting Neural Circuits

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Optogenetic methodology enables direct targeting of specific neural circuit elements for inhibition or excitation while spanning timescales from the acute (milliseconds) to the chronic (many days or more). Although the impact of this temporal versatility and cellular specificity has been greater for basic science than clinical research, it is natural to ask whether the dynamic patterns of neural circuit activity discovered to be causal in adaptive or maladaptive behaviors could become targets for treatment of neuropsychiatric diseases. Here, we consider the landscape of ideas related to therapeutic targeting of circuit dynamics. Specifically, we highlight optical, ultrasonic, and magnetic concepts for the targeted control of neural activity, preclinical/clinical discovery opportunities, and recently reported optogenetically guided clinical outcomes.

## Introduction

Over the past half-century, electrical, genetic, and pharmacological interventions have been developed and applied to obtain causal insights into the functional significance of nervous system activity. Discoveries ranging from the delineation of critical periods in the developing brain to the characterization of perceptual and memory processes in the adult brain have emerged from studies using these diverse interventions as basic neuroscience tools in laboratory animals. Meanwhile in the clinical realm, pharmacological interventions for neuropsychiatric disease states have greatly increased in number over the same time period (though actual therapeutic impact and conceptual insight have not kept pace with the proliferation in medication options). Recent years have also witnessed the emergence of new electrical interventions in the clinical setting—supplementing the small toolkit that was long largely limited to electroconvulsive therapy (ECT) and used to treat certain psychiatric diseases such as major depression by eliciting brainwide seizure activity. Newer electromagnetic therapies such as transcranial magnetic stimulation (TMS, currently approved for psychiatric clinical use in major depression) and deep brain stimulation (DBS, currently approved for Parkinson's disease and other neurological conditions) are targeted more focally (Figures 1A and 1B) than ECT but thus far tend to be less consistently effective in psychiatric disease.

With these newer electromagnetic stimulation modalities, one brain region is targeted. For example, currently approved use of TMS for depression involves repetitive focal stimulation of left dorsolateral prefrontal cortex with parameters chosen to have the best chance of increasing activity in this directly targeted region, and DBS for Parkinson's disease involves high-frequency current pulses typically delivered to the subthalamic nucleus (STN) with the goal of decreasing activity in the directly targeted region. In contrast, what properties might define a circuit target or, more precisely, a cell-type-resolved circuit-dynamical target? Among the intriguing possibilities, one could imagine temporally precise tuning of the activity of a brain cell population, defined by cell body origin and axonal termination target, to resolve the most debilitating symptom domain of a patient's affective disorder. Another example might involve detecting in real time pathological shifts in activity balance between cell types or among several brain-spanning circuits, followed by appropriate cell-type-specific compensation to terminate the incipient pathological state. In laboratory animal subjects, such cellular-level control over local and global neural circuit activity dynamics is now commonplace; indeed, over the last 10 years, the development of genetically encoded optical tools (Figure 1C) has led to many examples in which such population-level fast-circuit dynamical processes have been identified and shown to underlie physiological and disease-related behavior (Deisseroth, 2015).

While these cell-type- and circuit-element-specific observational and interventional tools have illuminated clinical questions in animal models of disease, direct therapeutic application of these optical methods to the human brain has not yet occurred. Such direct translation would require gene delivery to targeted human brain cells to produce light-responsive proteins, as well as light delivery deep into opaque and photon-scattering human brain tissue. Candidate non-optical targeted modalities (Figure 1D; using magnetic, radio-frequency, or ultrasonic energy) similarly would require gene delivery and might support depth penetration of control for direct clinical applications as with optical methods though typically at the expense of spatial or temporal precision. Shared features unify this broad field; for example, engineering challenges are common to all approaches, since energy delivery of any kind (in electrical, optical, or other forms) will require hardware-based brain interfaces designed

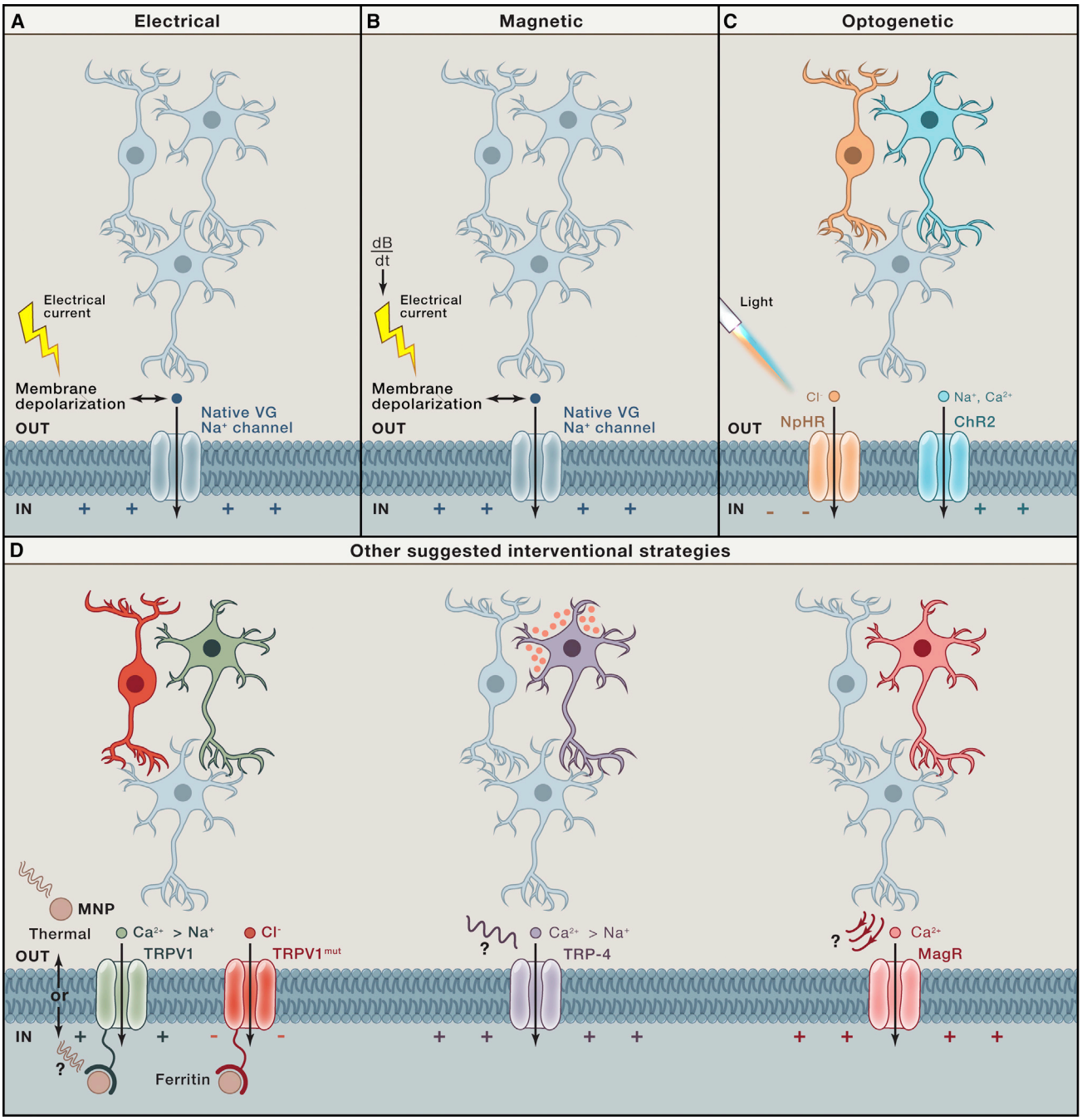


**Figure 1. Established and Proposed Technologies for Targeting Specified Regions and Circuits**

(A) Extracellular stimulation of neurons with electrical current provides regional targeting capability but without neuron-type specificity; capacitance currents are elicited that lead to membrane depolarization, opening of native voltage-gated sodium channels, and further depolarization of the membrane with spike firing.

(B) Stimulation of neurons through application of a transcranial magnetic field provides regional specificity but without precise depth targeting or cell-type specificity. Rapidly changing magnetic fields (dB/dt) induce electrical currents in tissue, causing spike firing as in (A).

(C) Optical stimulation or inhibition of neurons expressing light-sensitive excitatory cation channels (for example, the channelrhodopsin ChR2) or inhibitory pumps (for example, the halorhodopsin NpHR) can be achieved in a cell-type-specific manner (here depicted by NpHR expressed in the orange cell and ChR2 in the blue cell); thin fiber-optic interfaces provide versatile depth focusing for intersectional light-and-gene-targeting strategies (as with optical targeting of opsin-expressing cells by virtue of their axonal projection pattern in projection targeting).

(D) Diverse additional proposed strategies for specific neural circuit element control. (Left) Magnetic nanoparticles (MNP) can transduce magnetic/radiofrequency fields into thermal energy capable of opening heat-sensitive (TRPV1) depolarizing channels; these can be expressed in a cell-type-specific manner (here depicted as the green neuron; Chen et al., 2015). External magnetic fields have also been proposed to be transduced by paramagnetic ferritin in neuronal control applications if the ferritin is tethered (either directly or indirectly) to TRPV4 (Wheeler et al., 2016) or TRPV1 (Stanley et al., 2016); the TRPV channels have been

*(legend continued on next page)*

to be minimally invasive, biocompatible, stable over chronic clinical timescales, cost effective, and energy efficient. We note that, in contrast, chemogenetic approaches, which may be quite clinically relevant (Urban and Roth, 2015), deliver the actuation signal to targeted cell types through parenteral chemical delivery rather than through pulsed energy delivery; these methods thus obviate the need for an energy-delivery interface, although in doing so sacrifice the temporal and spatial resolution of circuit targeting afforded by (for example) optogenetic fiber-optic neural interfaces. Regardless of modality, therapeutic control of neural activity may be best realized in closed-loop configurations, wherein observations of endogenous activity can be used to inform interventional activity manipulations in real time (Grosenick et al., 2015). Such challenges are currently driving interdisciplinary innovation in both device-hardware engineering and computational approaches.

Framed by these active clinical and preclinical scientific challenges, here we consider the present state and future possibilities of targeting circuits. Setting the stage for such considerations, simply defined locations in the brain currently already serve as clinical targets for electrical intervention, though the precise targeting of cellular-resolution brain circuit dynamics can be routinely only carried out in animals and with optical methods. We will not address here the potential ethical considerations surrounding genetic delivery of precise behavioral modification tools to the human brain, but we do note that optogenetically guided circuit therapies have already made a clinical impact. Indeed, a key emerging theme is that approaches conceptually combining the novel and the traditional (e.g., optogenetically guided interventions that are purely electromagnetic or electromagnetic/pharmacological) may be especially powerful. We highlight ongoing opportunities and challenges relating to the development of such integrated formulations for clinical or basic science application and in central or peripheral nervous systems.

## Optical Control: Opsins, Hardware, and Software for Large Brain Volumes

In the first section of this Perspective, we will review technical advances that build our capacity to manipulate circuits, beginning with an overview of optogenetics. This methodology leverages light-responsive ion-conducting proteins derived from microbes that, when expressed in neurons, can elicit light-dependent activation or inhibition. These optically activated conductance regulators, such as halorhodopsin (HR), channelrhodopsin (ChR), bacteriorhodopsin (BR), and their many variants, have been developed into a diverse palette of tools for single-component and precisely timed control of targeted cell populations in freely moving animals (Deisseroth, 2015; Figure 1C). Recent developments in opsin engineering are particularly relevant to large-brained animal subjects in general and potentially to human and non-human primates. For example, the development of excitatory ChR derivatives that flux cations in response to longer-wavelength red light (redshifted variants include VChR1, C1V1, ReaChR, Chrimson, and bReaChES; Zhang et al., 2008; Yizhar et al., 2011; Lin et al., 2013; Klapoetke et al., 2014; Grosenick et al., 2015; Rajasethupathy et al., 2015) extends optical stimulation to moderately greater depths in large brains compared to the initially identified blue-responsive ChRs, due to reduced scattering of red-shifted light (Yizhar et al., 2011). Far more extensive volume recruitment may be obtained by increasing operational light sensitivity (Mattis et al., 2012) via prolonging the opsin deactivation time (Berndt et al., 2009; Bamann et al., 2010; Yizhar et al., 2011); among other applications, this step-function opsin (SFO) class of excitatory channelrhodopsin confers orders of magnitude greater light sensitivity at the cellular level and has recently been found useful for studying brain-wide circuit-dynamical underpinnings of anhedonia and abnormal social behavior in rodents (Ferenczi et al., 2016; Yizhar et al., 2011). While extended volume recruitment is not as significant for the fast red-activated ChRs, a greater utility of the red opsins may be for enhanced compatibility with optical readouts such as blue-light-activated genetically encoded $Ca^{2+}$ indicators (Akerboom et al., 2013; Chen et al., 2013b; Deisseroth and Schnitzer, 2013; Inoue et al., 2015; Emiliani et al., 2015). For example, certain all-optical combinations (Rajasethupathy et al., 2015; Grosenick et al., 2015; Packer et al., 2015; Rickgauer et al., 2014; Kim et al., 2016) may be useful for closed-loop configurations in basic or clinically inspired applications, wherein observed neural activity is fed back to modify manipulations of neural activity in real time (reviewed in Grosenick et al., 2015).

A major challenge for real-time circuit-dynamical intervention in large-brained organisms has been achieving inhibition over wide brain areas. For many years, the only microbial opsins suitable for inhibition were pumps (HR and BR derivatives) rather than channels, which while broadly useful in laboratory animals tend to be less operationally light sensitive than channels because only one ion is transported per photon by pumping (instead of the hundreds that can be moved across the membrane by channel mechanisms; reviewed in Deisseroth, 2015). Moreover, pumps cannot be converted into the SFO form to achieve the highest levels of operational light sensitivity (Mattis et al., 2012). However, single-component inhibitory channels have at last been engineered and discovered over the past 2 years, beginning with the structure-guided development of light-sensitive chloride channels (Berndt et al., 2014; Wietek

suggested to then open via thermal, mechanical, or other means and may thus implement excitation (if cation selective; Stanley et al., 2016; Wheeler et al., 2016) or inhibition (if chloride selective; Stanley et al., 2016). Plausibility of a magnetic mechanism has recently been challenged (Meister, 2016). (Middle) Ultrasonic activation of neurons through application of low-pressure ultrasound waves that may be transduced by gas-filled microbubbles (blue) into mechanical energy sufficient to open mechanosensitive (TRP-4) channels, which might also be expressed in a cell-type specific manner (here depicted as the purple neuron) (Ibsen et al., 2015). (Right) Application of magnetic fields has been proposed to open magnetically sensitive channels (MagR), allowing the influx of $Ca^{2+}$ with the potential for cell-type-specific activation (here depicted as the pink neuron), as described in the text; plausibility of this magnetic mechanism has also been questioned (Meister, 2016). Generalizable AAV-based cell-type targeting strategies developed for optogenetics might also be used to target these energy-transducing membrane proteins (TRPV1/4, TRP-4, MagR), although it is not clear that these energy modalities themselves can be focused locally deep within the brain to provide the intersectional targeting based on genetic and circuit-anatomy cellular identity crucial in (for example) optogenetic targeting of neurons defined by their output (axonal projections).

et al., 2014), which were made significantly more potent, light sensitive, and functional for behavioral control with further engineering, including introduction of SFO (Berndt et al., 2009) mutations (Berndt et al., 2014, 2015). This initial discovery was followed by identification of naturally occurring chloride channels (Govorunova et al., 2015) that share ion-conduction pathway and selectivity properties with the engineered chloride channels (Berndt and Deisseroth, 2015; Berndt et al., 2015) and, separately, by the engineering of a light-activated potassium channel, also inhibitory in neural systems (Cosentino et al., 2015). In the latter study, the authors fused a small viral potassium channel to the photosensitive LOV2-J$\alpha$ domain of the plant blue-light receptor, with several additional modifications to reduce dark activity; the resulting (though very slow) single-component hyperpolarizing tool (BLINK1) was capable of modulating behavior in transparent zebrafish larvae. Although together these studies show promise for basic science, it remains to be demonstrated that the light sensitivity of these inhibitory channels will functionally suffice for behavioral control in primate brains.

An independent strategy to improve recruitment of cells deep within large volumes (and enable new kinds of cell-type targeting) has been the development of specialized optical hardware to bypass or overcome scattering, building on the basic initial design of the laser diode-coupled fiber-optic for delivering light to deep-brain structures in freely moving mammals (Aravanis et al., 2007; Adamantidis et al., 2007). Many fibers can be employed if needed even in freely moving animals (Kim et al., 2016), and the thin, flexible structure of the now widely used fiber-optic interface (two to three times smaller in diameter than $\sim$1.4 mm clinical DBS electrodes) plays several useful and clinically relevant roles. For instance, these fiber-optic interfaces allow targeting of cells for control of behavior by virtue of projection anatomy alone (Gradinaru et al., 2009; Tye et al., 2011) with no genetic information per se (a crucial capability for genetically less-tractable animals, including human and nonhuman primates); this widely-used "projection targeting" effect is achieved with adeno-associated virus (AAV)-based focal expression of an opsin with good axonal-trafficking properties in one brain region, followed by fiber-optic-based light delivery to another brain region wherein the only light-sensitive elements represent axons of cells residing in the first brain region with projections to the fiber-optic-targeted region; reviewed in Deisseroth (2014). Additionally, these fiber-optic interfaces enable not only delivery, but also collection of light from deep-brain populations and projections during behavior for activity-guided and closed-loop control (Gunaydin et al., 2014; Grosenick et al., 2015; Lerner et al., 2015; Kim et al., 2016; Zalocusky et al., 2016) while also separating from the tissue the major heat-generating elements of the device, thus avoiding local damage as well as direct nonspecific excitation of the neural tissue. These features of fiber-optic light interfaces (especially the precisely localized deep delivery and collection of activity information, not feasible with other energy modalities such as magnetic or ultrasonic) are so critical that investigators in the basic science realm for the most part avoid non-invasive energy delivery even if available; the presence of the fiber-optic interface itself is of fundamental utility.

Direct implantation of LEDs for light delivery carries a risk of local heating (except when opsins such as SFOs are used that are bistable or extremely light sensitive). However, new LED engineering may offer other capabilities (Montgomery et al., 2015). For example, ultrathin (50 × 50 × 6 $\mu$m) inorganic gallium nitride LEDs can be directly injected into the brain and controlled remotely by a wireless receiver mounted above the skull (0.7 g; Jeong et al., 2015). This approach provides neural control with minimal hindrance to the subject, as well as the potential for substantial scaling up. In addition to the miniaturized nature of this device, other advantages stem from the fact that the LED strips can be printed in many different configurations, enabling tailoring of dimensions to the target brain area. Though implanted LEDs by themselves (unlike the fiber-optic interface) cannot deliver feedback information regarding local neural activity, other kinds of flexibility may be enabled. For example, LED strips can be bound to microfluidic channels that can simultaneously release pharmacologic agents on demand, or flexible devices that can detect or actuate electrical, mechanical, or thermal information simultaneously might be incorporated (Canales et al., 2015).

## Beyond Optical Control: Diverse Energy Modalities

While existing optical methods are well suited for use in animal subjects, clinical application of optical control and readout methods will be limited by the size and scattering-related opacity of the human brain. Therefore, guided by the core single-component principle of optogenetics (targeting an energy receptor/actuator protein for temporally precise cell-type-specific control), non-optical methods for modulating neural activity are being explored. For instance, cell-type-targeted control of neural activity might be accomplished by transducing a magnetic signal into electrical activity using targeted receptors analogous to microbial opsins. Such an approach would leverage the fact that magnetic fields can penetrate more deeply than light into tissue (though likely without the focal depth delivery desired for spatial or projection targeting). One approach has proposed use of a genetically encoded paramagnetic protein ferritin either fused directly to TRPV4 channels or tethered indirectly to TRPV1 channels (giving rise to a large genetic payload that could be packaged into lentivirus in wild-type $Ca^{2+}$-conducting or mutant $Cl^-$-conducting forms) reported to transduce magnetic energy into thermal and/or mechanical energy sufficient to open the channels. The resulting $Ca^{2+}$ or $Cl^-$ entry (Figure 1D) was reported to suffice for neuronal activation or inhibition, respectively, in vitro and in vivo (Wheeler et al., 2016; Stanley et al., 2015, 2016). This approach (while novel) has been challenged regarding quantitative plausibility of the proposed mechanism (Meister, 2016), and independent of mechanism, it also appears limited by several factors, including potential side effects of confounding signals from the natural biological activity of TRPV channels (since they are known to be activated by endogenous stimuli ranging from temperature and pH to stress and the endocannabinoid system), tissue heating, activation/deactivation kinetics that are many times slower than temporal dynamics of native neural activity signals, and lack of spatial depth targetability of stimulation energy (which is important for projection targeting and other forms of intersectional specificity, as described above).

Another study (Chen et al., 2015) proposed that injection of iron oxide nanoparticles (22 nm in diameter) with efficient heat dissipation could provide depth targeting of thermal energy (Huang et al., 2010) via potent transduction of magnetic energy into thermal energy sufficient to activate TRPV1 channels on neurons and could achieve $Ca^{2+}$ entry on the timescale of seconds (Chen et al., 2015; Figure 1D). Though this approach relinquishes single componency (requiring both gene and nanoparticle introduction), investigators were able to show reliable magnetic-field-induced spiking of neurons (<1 Hz) in primary hippocampal neurons as well as in deep brain tissue. CNS neurons remained responsive for up to a month after iron oxide particle injection, though limited dispersion of the particles from the deep site of injection remained an issue. The investigators also developed a field stimulus protocol that allowed cyclical heating (reaching TRPV1 channel opening thresholds) and cooling (back to 37 degrees) in vivo within a single stimulus pulse (5 s) to avoid prolonged exposure to noxious heat. While this step is noteworthy, 5 s is still extremely slow on the neuronal signaling timescale, and the extent to which even this step would be feasible clinically is unclear.

Challenges for the future thus include increasing temporal precision (into the millisecond domain, perhaps via nanoparticles with greater specific power), reducing temperature requirements by engineering channels with lower activation thresholds while ensuring lack of activity at rest, and crucially increasing the dispersion of nanoparticles within tissue. A related line of research has explored the use of naturally occurring bacterial magnetoreceptors to transduce magnetic signals into local genetically targetable neural modulation (Long et al., 2015; Qin et al., 2016; Figure 1D); these responses have potential utility relevant to the twin challenges of tissue heating and limited nanoparticle dispersion discussed above but nevertheless will need to be reproduced and extended, remain considerably slower than optogenetic responses, and also have been challenged regarding physical plausibility (Meister, 2016). Moreover, a magnetic apparatus remains to be developed that is compatible with free mammalian behavior and depth-targeted energy delivery for projectional/intersectional targeting, as with optogenetics.

Finally, a clinically inspired approach has built upon transcranial pulsed ultrasound to enable non-invasive and potentially localized stimulation of modified neurons (although not yet in a genetically targeted manner), presumably operating through activation of mechanosensitive channels and involving cavitation forces (Figure 1D). Using this modality, a series of studies has demonstrated ultrasound-mediated stimulation of neural activity sufficient to elicit action potentials and synaptic transmission in vitro (Khraiche et al., 2008, Tyler et al., 2008, Menz et al., 2013) and in vivo in mice (motor cortex: Tufail et al., 2010, King et al., 2013; hippocampus: Tufail et al., 2010), without significant elevation in brain temperature (<0.01°C). Average response latencies (~50–150 ms), while better than with the magnetogenetic approaches, remain considerably slower than with typical optogenetic experiments (<5 ms). These approaches also exhibit limited spatial resolution (spanning several cubic millimeters), though this parameter could be improved with higher ultrasound frequencies (at the cost of impaired skull penetration) or through sculpting of the ultrasound beam and use of multiple transducers for further focusing.

Recent attempts to translate these findings to humans have led to demonstrated use of focused ultrasound to modulate the activity of human primary somatosensory cortex, with associated shifts in sensory discrimination (Legon et al., 2014, Lee et al., 2015). A useful feature of ultrasonic modulation is ready compatibility with existing MRI methods, potentially enhancing diagnostic and therapeutic circuit targeting. Indirect means for genetic targeting may be possible in a limited sense—for example, by titrating stimulus properties so that cell populations expressing higher levels of mechanosensitivity preferentially respond. Direct genetic targeting is also theoretically possible via overexpression of mechanosensitive channels as deep-tissue energy receptors, analogous to optogenetic approaches. Indeed, an attempt to transduce sound waves into genetically targeted neuronal activation has recently been reported (Ibsen et al., 2015), with similar caveats to the putative magnetogenetic approaches but representing another energy modality that could find relevance in the research or clinical setting (Figure 1D). While much remains to be understood about the mechanisms and optimal stimulation parameters of ultrasound-mediated brain stimulation, progress in this area (Yoo et al., 2011, King et al., 2013, Moore et al., 2015) suggests that ultrasound (whether targeted or not) may become a useful modality of neural stimulation.

## Neuropsychiatric Diseases with Potential Circuit-Dynamical Targets

When considering interventional therapeutics that extend beyond simply targeting a brain region, a key initial step will be identifying the most causally relevant circuit dynamical property to target for a specific symptom. In general, such information is simply not known for neuropsychiatric disease states, though testable ideas abound. These possibilities include acute alteration of the dynamic traffic along specific long-range projections, modifying the connection strength between specific brain regions, modulation of cellular excitation/inhibition balance or coherence in activity oscillations between brain regions, and recruitment or suppression of brain-wide activity correlations such as specific resting state or task-associated networks. Given the ubiquity of the unknowns, however, the most immediate clinical relevance of circuit-dynamics interrogation technology remains foundational identification of causal principles in animal models. Here, we highlight a few recent specific examples of diseases in which related circuit-targeting progress has been made and in which circuit-dynamical ideas are influencing the design and delivery of effective brain stimulation protocols.

Lesion or other targeting of the STN can offer temporary therapeutic benefit for a subset of Parkinsonian symptoms in some patients. Point sources for intervention, such as electrodes for DBS (Whitmer et al., 2012) or fiber-optics for optogenetic control (Gradinaru et al., 2009), appear to be most effective if their initial direct target is afferent white matter tracts—an especially potent focal way to modulate a large downstream structure as well as to exert retrograde effects back at the cell bodies of tract origin. Consistent with this afferent-tract targeting principle, recent DBS protocols in humans have revealed potential therapeutic

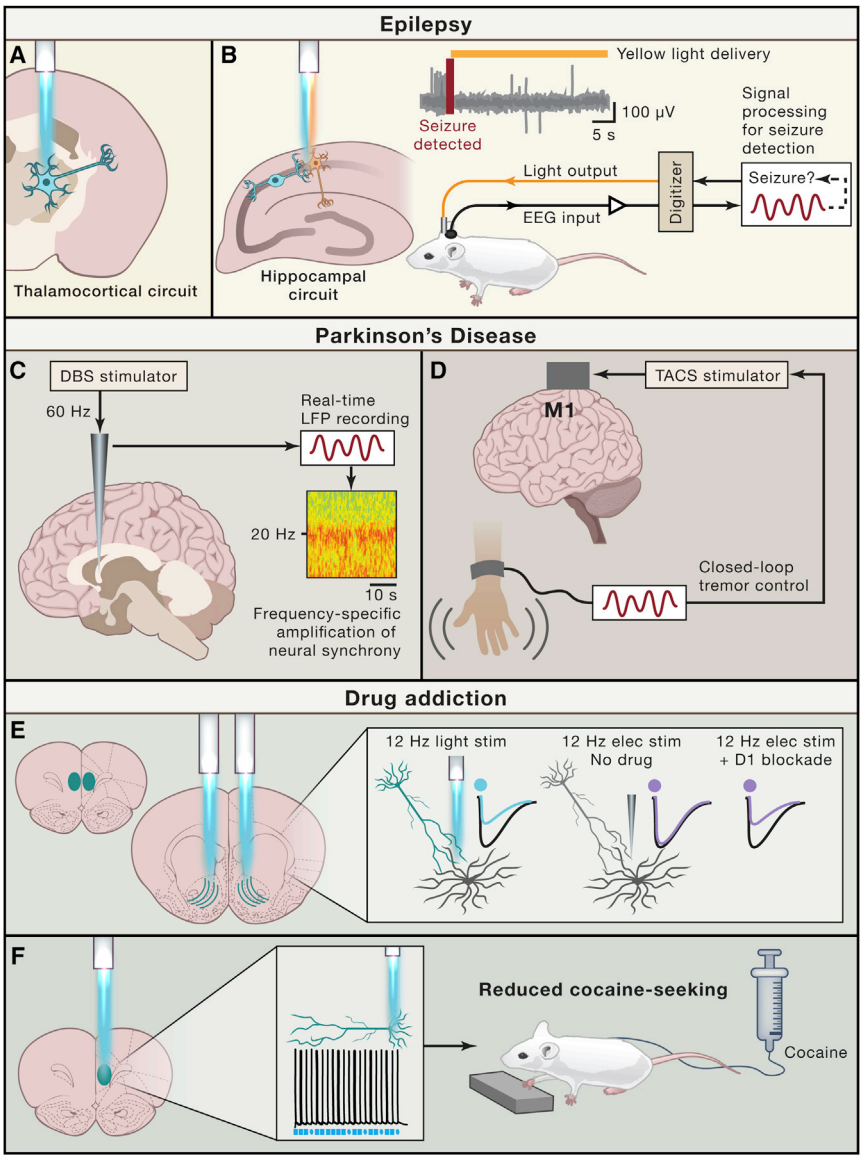


**Figure 2. Disease-Related Circuit-Targeting Demonstrations**

(A and B) (A) Targeting thalamocortical neurons and (B) hippocampal neurons with closed-loop strategies to cause real-time interruption of EEG- and behaviorally defined seizures (Paz et al., 2013; Krook-Magnuson et al., 2013). (B, right) Depiction of closed-loop setup wherein EEG inputs are used to detect seizure onset, after which real-time inhibition can be administered through optogenetic manipulation.

(C) Low-frequency DBS stimulation (60 Hz), in contrast to traditional high-frequency stimulation (130 Hz), results in significant amplification of subthalamic neural synchrony and alpha/beta band power thought to underlie improved gait symptoms in Parkinson's (Blumenfeld et al., 2015).

(D) Use of an alternative non-invasive approach termed transcranial alternating current stimulation (TACS) is depicted for stimulation of motor cortex; guided by real-time readout of cortical oscillations linked to tremor, TACS was found to result in significant cancellation of the resting-state tremor (Brittain et al., 2013).

(E) 12 Hz optical stimulation was reported to produce robust LTD, whereas 12 Hz electrical stimulation did not. The discrepancy in this study was attributed to off-target recruitment of D1 receptors by electrical stimulation, which impeded LTD; a combination of low-frequency DBS together with D1 antagonists provided reliable LTD and restoration of behavior in cocaine-seeking animals (Creed et al., 2015).

(F) Optogenetic prelimbic cortical excitation significantly prevented cocaine seeking in rats (by compensating for hypoactivity observed in cocaine-seeking rats), pointing to the prefrontal cortex as a promising therapeutic target for compulsive drug use (Chen et al., 2013a; Terraneo et al., 2016).

value for supporting memory function by targeting structures afferent to the hippocampus, such as the fornix (Fontaine et al., 2013) or the entorhinal cortex (Suthana et al., 2012), but not by attempting to target the entire elongate hippocampus directly itself. In fact, at this time, a large-scale randomized clinical trial (NCT01608061) of DBS in the fornix is underway to assess safety and efficacy at 1 year for memory improvement in Alzheimer's patients.

DBS for psychiatric disorders, including for treatment-resistant depression and obsessive compulsive disorder, has recently witnessed a rapidly broadening potential application domain, in which targeting of afferent white matter tracts again may be most important (Mayberg et al., 2005; Ressler and Mayberg, 2007; reviewed in Deisseroth, 2014). Corresponding larger-scale clinical trials are ongoing, but in all of these studies, challenges are expected, as many questions remain unanswered regarding: (1) the most suitable site for clinical efficacy; (2) spurious consequences of electrical stimulation, which as described below (in contrast to optogenetic or other genetically targeted control methods; Figure 2), will directly recruit off-target cells or passing fibers unrelated to the target population or region (e.g., Creed et al., 2015); and (3) the likely need for individualized targeting aligned to patient-specific wiring (for example, as assessed by MRI tractography) and to the specific dynamical context of the patient (for example, as assessed by open-loop or closed-loop detection of native activity patterns that might span spatially segregated circuits and extended timescales).

Circuit targeting in the widespread and debilitating disease of drug abuse, using not optogenetics itself but, rather, optogenetically guided treatment, has recently reached the clinic. DBS had earlier been considered for the treatment of certain forms of drug addiction, with much attention focused on the nucleus accumbens (NAc; a brain structure closely associated with reward and abused drug action) and on the medial prefrontal cortex (mPFC)-NAc projection. While high-frequency DBS delivered to NAc has in some cases been found to ameliorate drug-abuse-related symptoms acutely, the mechanism remained largely

unknown and symptoms typically reappeared once stimulation was stopped. Triggering long-term depression (LTD)-type synaptic plasticity in this projection (and hence lasting behavioral benefit) was robust with optogenetic low-frequency stimulation (LFS) in mice but more challenging with electrical LFS, thus frustrating efforts to define a simple clinically relevant electrical stimulation therapy. But a recent study reported that this discrepancy between optogenetic and electrical effects might be attributable to the additional recruitment (only with electrical stimulation) of an off-target pathway recruiting dopamine type 1 (D1) receptors, which was inhibiting LTD; consistent with this hypothesis, electrical low-frequency DBS together with the application of D1 antagonists to cocaine-adapted mice was able to provide long-term restoration of normal behavior (Figure 2E; Creed et al., 2015).

An earlier paper addressing a similar question (Chen et al., 2013a) came to a different optogenetically inspired treatment concept, which has now led to therapeutic clinical benefit, as reported in a published clinical trial outcome. In a rat model of compulsive drug seeking, prolonged cocaine self-administration decreased intrinsic excitability of deep-layer mPFC pyramidal neurons, which was especially pronounced in compulsive drug-seeking animals. Compensating for the hypoactivity of these projection neurons with in vivo optogenetic mPFC stimulation prevented compulsive cocaine seeking, and the authors suggested that this could represent "a promising therapy for treating compulsive drug use" (Figure 2F; Chen et al., 2013a). Recent clinical studies using TMS as the clinical interventional tool now indeed suggest that stimulation of the dorsolateral prefrontal cortex reduces cocaine use in human cocaine abusers (Terraneo et al., 2016; Ferenczi and Deisseroth, 2016) and curbs cue-induced craving in human heroin addicts (Shen et al., 2016). Potential causal brain-wide mechanisms for this effect have been described (Ferenczi et al., 2016) and are discussed in greater detail below.

Some of the most interesting optogenetic preclinical explorations relevant to neuropsychiatric disease have been in the field of epilepsy. Closed-loop studies in mouse models of temporal lobe epilepsy have shown that seizure onset can be detected in real time and abolished by closed-loop optogenetic inhibition of thalamocortical neurons (Figure 2A) or granule cells in dentate gyrus (Figure 2B; Paz et al., 2013, Krook-Magnuson et al., 2013) or by closed-loop optogenetic excitation of midline cerebellar parvalbumin neurons (Krook-Magnuson et al., 2014). Closed-loop modulation of brain circuits (regardless of intervention modality) may be especially well suited for paroxysmal conditions like epilepsy, although even chronic conditions such as depression could, in principle, benefit from closed-loop control as the field continues to develop deeper knowledge of the underlying causal dynamics and plasticity of affective brain states.

Indeed, many types of clinical circuit targeting might also leverage detection of local circuit activity patterns. For example, while many patients with Parkinson's disease have benefited from high-frequency DBS stimulation, low-frequency 60 Hz stimulation (as compared with the more classical >100 Hz stimulation) may be well suited for amplifying resting state neural synchrony in the STN with concomitant improvements in gait and speech, but not tremor (Figure 2C; Blumenfeld et al., 2015). On the other hand, high-frequency stimulation is classically associated with alleviation of symptoms of tremor, but not necessarily of gait or speech. This dichotomy therefore opens the door to the intriguing possibility of circuit-dynamical "knock in" and "knock out" of patient-specific resting-state synchrony bands for highly precise symptom alleviation (Blumenfeld et al., 2015). Other opportunities in Parkinson's include minimally invasive and closed-loop strategies using transcranial ultrasound (TUS) and transcranial alternating current stimulation (TACS) for the treatment of tremor. Recent pilot work used a closed-loop strategy to deliver TACS over motor cortex during tremor to induce phase cancellation of tremor rhythm, with promising results (Figure 2D; Brittain et al., 2013).

## Outside the Brain

In addition to the domain of neuropsychiatric disease, there is emerging interest in circuit targeting in oncology, in part for deeper understanding of the cancer micro-environment. For example, neocortical neuronal activity has recently been found to promote the growth of malignant gliomas in a circuit-specific manner through activity-regulated secretion of specific growth factors (Venkatesh et al., 2015). Beyond the central nervous system, peripheral nerve circuit activity has been shown to contribute to the progression of prostate (Magnon et al., 2013), gastric (Zhao et al., 2014), pancreatic (Stopczynski et al., 2014), and skin (Peterson et al., 2015) cancers.

With the advent of genetically resolved, bidirectional optogenetic modulation of peripheral nerves (e.g., Towne et al., 2013, Iyer et al., 2014), opportunities exist for enhanced understanding and treatment of other diseases originating outside of the brain, including chronic pain, autoimmunity, and blindness (http://clinicaltrials.gov/ct2/show/NCT02556736?term=optogenetics). Yet as in the CNS, a primary goal may simply be fundamental research rather than direct clinical application; any resulting optogenetically guided clinical intervention need not be optogenetic itself, and basic-science optogenetic investigations in animals are already illuminating circuit-targeting therapies employing diverse electrical, optical, magnetic, and pharmacological modalities in the peripheral and autonomic as well as central nervous systems.

## Outlook for the Next Decade

Some of the most important growth into the future for fast cellular-resolution, circuit-dynamical intervention will actually be in the readout domain. Readout quality is crucial for circuit targeting, in particular for (1) obtaining a particular known magnitude of desired response in the intended circuit elements, (2) aligning the timing of a desired response in the intended circuit elements to a naturally occurring activity pattern or to other events, (3) closed-loop control to account in real time for shifting brain state/response properties and to test models of system function, and (4) tracking brain-wide relationships and activity patterns, since the desired circuit response may involve both local and global components.

In addressing the first two issues, matching desired magnitude (Kim et al., 2016) and timing (Zalocusky et al., 2016) of activity in deep-brain-targeted circuit elements corresponding to patterns

naturally observed in behavior has recently been achieved—a long-sought goal in optogenetics. Kim et al. (2016) developed frame-projected independent-fiber photometry (FIP) to precisely deliver and match the magnitude of optogenetically evoked activity to that of naturally occurring activity in genetically targeted circuitry deep in the brain of the same freely behaving mouse; Zalocusky et al. (2016) likewise integrated fiber photometry and optogenetics to precisely deliver and match the timing of a naturally occurring activity signal within D2-receptor-expressing nucleus accumbens neurons of freely moving rats. Regarding issue (3) (closed-loop) noted above, closed-loop optogenetic control has taken major steps forward since the initial work of Sohal et al. (2009), with the advent of increasingly precise fluorescent, electrical, and behavioral system readouts, as described above and recently reviewed (Grosenick et al., 2015; Emiliani et al., 2015). Closed-loop systems involving one or more of these readouts could ultimately become part of advanced clinical interventions relevant to neuropsychiatric disease states.

Fourth, real-time brain-wide activity readout in the setting of optogenetic control has been possible since 2010 (Lee et al., 2010; Desai et al., 2011; Gerits et al., 2012), and recently Ferenczi et al. (2016) used this clinically relevant global readout (optogenetic fMRI, or ofMRI) in awake rats to identify and quantify brain-wide indirect consequences of accurate direct targeting of a small and discrete cortex region, with resulting insights into the adaptive and maladaptive regulation of hedonic behaviors in the very same experimental subjects. Focal and subtle modulations were found to change the state of the entire brain, including the manner in which diverse distant brain regions interacted with each other (Ferenczi et al., 2016), revealing potent mechanisms by which natural processes such as neuromodulation or attention might be able to give rise to global brain-state changes with behavioral significance. Importantly, the temporal flexibility of optogenetic methods allows these effects to be elicited and studied on any acute or chronic timescale from milliseconds to many days or more (Berndt et al., 2009; Goshen et al., 2011; Yizhar et al., 2011; Ahmari et al., 2013; Ferenczi et al., 2016) while maintaining precision of direct control over the same correctly targeted circuit elements. The broad temporal versatility unique to optogenetic control is also particularly useful when combined with brain activity measurements that span both acute and chronic timescales. This enables, for instance, the study of adaptive circuit dynamics across the intact brain and tuning interventions to match activity patterns naturally observed during behavior in the same individual; this principle might be extended beyond fMRI to freely moving brain-spanning modalities such as multisite fiber photometry (FIP; Kim et al., 2016) or miniaturized positron emission tomography (PET; Schulz et al., 2011).

Ongoing success with optogenetically guided therapeutic interventions (e.g., Terraneo et al., 2016; Shen et al., 2016; Ferenczi and Deisseroth, 2016) will encourage further innovative uses of circuit knowledge. However, as with any other therapy, a single circuit-targeting treatment may not be applicable to every patient suffering from a particular symptom domain or disorder. Personalized circuit diagnostics, through the use of biomarkers in the form of structural or functional readouts, will be valuable for optimizing individual safety and efficacy and for guiding treatment decisions. Benefits of circuit targeting may be further complemented by molecular diagnostics and therapeutics as well; indeed, circuit-guided identification and intervention targeted to molecular, transcriptional, or genomic/epigenomic features (Koester and Insel, 2016) that are causal to observed circuit dynamics and behavior will develop alongside advances in protein-based biologics and nucleic acid (e.g., siRNAs, CRISPR-Cas9) interventions. Such molecular circuit-targeting strategies may further leverage advanced cellular-resolution intact-tissue analyses in the laboratory. For example, once a projection or population activity pattern is identified as causally important for a specific symptom or behavior, high-content structural information (Chung et al., 2013; Dodt et al., 2007; Ertürk et al., 2012; Hama et al., 2015; Kuwajima et al., 2013; Tomer et al., 2014, 2015), protein-expression data (Chung et al., 2013, Murray et al., 2015; Renier et al., 2014; Susaki et al., 2014; Yang et al., 2014), and transcriptional readouts (Sylwestrak et al., 2016) can be collected to provide molecular leverage on causal circuit elements, and in laboratory animals, such data streams may even be registered at cellular resolution to the same intact volume on which activity dynamics were recorded and controlled.

Important molecular information relevant for circuit targeting will also emerge from clinical genetics, just as clinical genetics may influence pharmacological intervention (e.g., Le Clerc et al., 2015; Hodgson et al., 2016; Trivedi et al., 2016), and the coming decade is certain to witness ongoing identification of genes linked to neuropsychiatric disease (e.g., McCarroll et al., 2014; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). This accelerating genetic data stream will be powerfully enhanced by understanding of the circuits involved in causal processes leading from genes to behavior. Bidirectional information flow (both using circuit-dynamical knowledge for mechanistic insight into influences of genes and molecules on behavior and using genetics knowledge and tools to guide interventions and diagnostics relevant to causal circuit-activity deficits) may further advance the broad goal of linking patient genotype to disease phenotype via circuit dynamics. On long timescales, as engineered interfaces and multimodal energy-delivery technologies improve in targetability, efficacy, and safety, concomitant improvements in the design of clinical trials will increasingly need to include tailored recruitment of patients with shared circuit etiology (e.g., through the use of biomarker sets that jointly represent circuit genetics and physiology). It will also be important to improve the design and alignment of treatment and placebo arms, as well as clinical endpoint nature and timing, in order to ensure that the intended circuit targets are, in fact, engaged with maximal potency and specificity.

Diverse circuit-targeting interventional strategies (using tissue-penetrating energy delivery and cellular-resolution receptor/transducer targeting, as with optogenetics; Figure 1) will also continue to evolve, with refinements likely to include improvements in temporal resolution, spatial resolution, sensitivity, local side effect profile, and other issues. In their eventual mature forms, diverse energy-delivery modalities may have the potential to complement optogenetics in providing a broad palette of flexible and multiplexed neural circuit control tools to help guide and implement clinical interventions, following and building upon the

optogenetic principle of targeting a single-component receptor/actuator to specific cells in order to confer real-time responsivity to exogenous pulses of information. Immediate or near-future opportunities for targeting clinically relevant circuits (based on principled predictions from animal studies) now already include: (1) developing concomitant pharmacological (Figure 2E) or behavioral-stimulus interventions to be used synergistically with currently available brain interventions; (2) developing and leveraging potential long-term plasticity effects of available stimulation modalities for more lasting benefit; (3) improving patient-specific localization of interventions with individualized anatomy and open-loop activity mapping; and (4) implementing online data acquisition and analysis pipelines to enable closed-loop interventions. And as with so many fields of medicine, for neuropsychiatric diseases, the most crucial process (now and likely for many decades to come) will be the ongoing basic science discovery of key principles in the laboratory.

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## REFERENCES

Adamantidis, A.R., Zhang, F., Aravanis, A.M., Deisseroth, K., and de Lecea, L. (2007). Neural substrates of awakening probed with optogenetic control of hypocretin neurons. Nature *450*, 420–424.

Ahmari, S.E., Spellman, T., Douglass, N.L., Kheirbek, M.A., Simpson, H.B., Deisseroth, K., Gordon, J.A., and Hen, R. (2013). Repeated cortico-striatal stimulation generates persistent OCD-like behavior. Science *340*, 1234–1239.

Akerboom, J., Carreras Calderón, N., Tian, L., Wabnig, S., Prigge, M., Tolö, J., Gordus, A., Orger, M.B., Severi, K.E., Macklin, J.J., et al. (2013). Genetically encoded calcium indicators for multi-color neural activity imaging and combination with optogenetics. Front. Mol. Neurosci. *6*, 2.

Aravanis, A.M., Wang, L.P., Zhang, F., Meltzer, L.A., Mogri, M.Z., Schneider, M.B., and Deisseroth, K. (2007). An optical neural interface: in vivo control of rodent motor cortex with integrated fiberoptic and optogenetic technology. J. Neural Eng. *4*, S143–S156.

Bamann, C., Gueta, R., Kleinlogel, S., Nagel, G., and Bamberg, E. (2010). Structural guidance of the photocycle of channelrhodopsin-2 by an interhelical hydrogen bond. Biochemistry *49*, 267–278.

Berndt, A., and Deisseroth, K. (2015). Expanding the optogenetics toolkit. Science *349*, 590–591.

Berndt, A., Yizhar, O., Gunaydin, L.A., Hegemann, P., and Deisseroth, K. (2009). Bi-stable neural state switches. Nat. Neurosci. *12*, 229–234.

Berndt, A., Lee, S.Y., Ramakrishnan, C., and Deisseroth, K. (2014). Structure-guided transformation of channelrhodopsin into a light-activated chloride channel. Science *344*, 420–424.

Berndt, A., Lee, S.Y., Wietek, J., Ramakrishnan, C., Steinberg, E.E., Rashid, A.J., Kim, H., Park, S., Santoro, A., Frankland, P.W., et al. (2015). Structural foundations of optogenetics: determinants of channelrhodopsin ion selectivity. Proc. Natl. Acad. Sci. USA *113*, 822–829.

Blumenfeld, Z., Velisar, A., Miller Koop, M., Hill, B.C., Shreve, L.A., Quinn, E.J., Kilbane, C., Yu, H., Henderson, J.M., and Brontë-Stewart, H. (2015). Sixty hertz neurostimulation amplifies subthalamic neural synchrony in Parkinson's disease. PLoS ONE *10*, e0121067.

Brittain, J.S., Probert-Smith, P., Aziz, T.Z., and Brown, P. (2013). Tremor suppression by rhythmic transcranial current stimulation. Curr. Biol. *23*, 436–440.

Canales, A., Jia, X., Froriep, U.P., Koppes, R.A., Tringides, C.M., Selvidge, J., Lu, C., Hou, C., Wei, L., Fink, Y., and Anikeeva, P. (2015). Multifunctional fibers for simultaneous optical, electrical and chemical interrogation of neural circuits in vivo. Nat. Biotechnol. *33*, 277–284.

Chen, B.T., Yau, H.J., Hatch, C., Kusumoto-Yoshida, I., Cho, S.L., Hopf, F.W., and Bonci, A. (2013a). Rescuing cocaine-induced prefrontal cortex hypoactivity prevents compulsive cocaine seeking. Nature *496*, 359–362.

Chen, T.W., Wardill, T.J., Sun, Y., Pulver, S.R., Renninger, S.L., Baohan, A., Schreiter, E.R., Kerr, R.A., Orger, M.B., Jayaraman, V., et al. (2013b). Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature *499*, 295–300.

Chen, R., Romero, G., Christiansen, M.G., Mohr, A., and Anikeeva, P. (2015). Wireless magnetothermal deep brain stimulation. Science *347*, 1477–1480.

Chung, K., Wallace, J., Kim, S.Y., Kalyanasundaram, S., Andalman, A.S., Davidson, T.J., Mirzabekov, J.J., Zalocusky, K.A., Mattis, J., Denisin, A.K., et al. (2013). Structural and molecular interrogation of intact biological systems. Nature *497*, 332–337.

Cosentino, C., Alberio, L., Gazzarrini, S., Aquila, M., Romano, E., Cermenati, S., Zuccolini, P., Petersen, J., Beltrame, M., Van Etten, J.L., et al. (2015). Optogenetics. Engineering of a light-gated potassium channel. Science *348*, 707–710.

Creed, M., Pascoli, V.J., and Luscher, C. (2015). Addiction therapy. Refining deep brain stimulation to emulate optogenetic treatment of synaptic pathology. Science *347*, 659–664.

Deisseroth, K. (2014). Circuit dynamics of adaptive and maladaptive behaviour. Nature *505*, 309–317.

Deisseroth, K. (2015). Optogenetics: 10 years of microbial opsins in neuroscience. Nat. Neurosci. *18*, 1213–1225.

Deisseroth, K., and Schnitzer, M.J. (2013). Engineering approaches to illuminating brain structure and dynamics. Neuron *80*, 568–577.

Desai, M., Kahn, I., Knoblich, U., Bernstein, J., Atallah, H., Yang, A., Kopell, N., Buckner, R.L., Graybiel, A.M., Moore, C.I., and Boyden, E.S. (2011). Mapping brain networks in awake mice using combined optical neural control and fMRI. J. Neurophysiol. *105*, 1393–1405.

Dodt, H.-U., Leischner, U., Schierloh, A., Jährling, N., Mauch, C.P., Deininger, K., Deussing, J.M., Eder, M., Zieglgänsberger, W., and Becker, K. (2007). Ultramicroscopy: three-dimensional visualization of neuronal networks in the whole mouse brain. Nat. Methods *4*, 331–336.

Emiliani, V., Cohen, A.E., Deisseroth, K., and Häusser, M. (2015). All-optical interrogation of neural circuits. J. Neurosci. *35*, 13917–13926.

Ertürk, A., Becker, K., Jährling, N., Mauch, C.P., Hojer, C.D., Egen, J.G., Hellal, F., Bradke, F., Sheng, M., and Dodt, H.-U. (2012). Three-dimensional imaging of solvent-cleared organs using 3DISCO. Nat. Protoc. *7*, 1983–1995.

Ferenczi, E., and Deisseroth, K. (2016). Illuminating next-generation brain therapies. Nat. Neurosci. *19*, 414–416.

Ferenczi, E.A., Zalocusky, K.A., Liston, C., Grosenick, L., Warden, M.R., Amatya, D., Katovich, K., Mehta, H., Patenaude, B., Ramakrishnan, C., et al. (2016). Prefrontal cortical regulation of brainwide circuit dynamics and reward-related behavior. Science *351*, aac9698.

Fontaine, D., Deudon, A., Lemaire, J.J., Razzouk, M., Viau, P., Darcourt, J., and Robert, P. (2013). Symptomatic treatment of memory decline in Alzheimer's disease by deep brain stimulation: a feasibility study. J. Alzheimers Dis. *34*, 315–323.

Gerits, A., Farivar, R., Rosen, B.R., Wald, L.L., Boyden, E.S., and Vanduffel, W. (2012). Optogenetically induced behavioral and functional network changes in primates. Curr. Biol. *22*, 1722–1726.

Goshen, I., Brodsky, M., Prakash, R., Wallace, J., Gradinaru, V., Ramakrishnan, C., and Deisseroth, K. (2011). Dynamics of retrieval strategies for remote memories. Cell *147*, 678–689.

Govorunova, E.G., Sineshchekov, O.A., Janz, R., Liu, X., and Spudich, J.L. (2015). Natural light-gated anion channels: A family of microbial rhodopsins for advanced optogenetics. Science *349*, 647–650.

Gradinaru, V., Mogri, M., Thompson, K.R., Henderson, J.M., and Deisseroth, K. (2009). Optical deconstruction of parkinsonian neural circuitry. Science *324*, 354–359.

Grosenick, L., Marshel, J.H., and Deisseroth, K. (2015). Closed-loop and activity-guided optogenetic control. Neuron *86*, 106–139.

Gunaydin, L.A., Grosenick, L., Finkelstein, J.C., Kauvar, I.V., Fenno, L.E., Adhikari, A., Lammel, S., Mirzabekov, J.J., Airan, R.D., Zalocusky, K.A., et al. (2014). Natural neural projection dynamics underlying social behavior. Cell *157*, 1535–1551.

Hama, H., Hioki, H., Namiki, K., Hoshida, T., Kurokawa, H., Ishidate, F., Kaneko, T., Akagi, T., Saito, T., Saido, T., and Miyawaki, A. (2015). ScaleS: an optical clearing palette for biological imaging. Nat. Neurosci. *18*, 1518–1529.

Hodgson, K., Tansey, K.E., Powell, T.R., Coppola, G., Uher, R., Zvezdana-Dernovsek, M., Mors, O., Hauser, J., Souery, D., Maier, W., et al. (2016). Transcriptomics and the mechanisms of antidepressant efficacy. Eur. Neuropsychopharmacol *26*, 105–112.

Huang, H., Delikanli, S., Zeng, H., Ferkey, D.M., and Pralle, A. (2010). Remote control of ion channels and neurons through magnetic-field heating of nanoparticles. Nat. Nanotechnol. *5*, 602–606.

Ibsen, S., Tong, A., Schutt, C., Esener, S., and Chalasani, S.H. (2015). Sonogenetics is a non-invasive approach to activating neurons in Caenorhabditis elegans. Nat. Commun. *6*, 8264.

Inoue, M., Takeuchi, A., Horigane, S., Ohkura, M., Gengyo-Ando, K., Fujii, H., Kamijo, S., Takemoto-Kimura, S., Kano, M., Nakai, J., et al. (2015). Rational design of a high-affinity, fast, red calcium indicator R-CaMP2. Nat. Methods *12*, 64–70.

Iyer, S.M., Montgomery, K.L., Towne, C., Lee, S.Y., Ramakrishnan, C., Deisseroth, K., and Delp, S.L. (2014). Virally mediated optogenetic excitation and inhibition of pain in freely moving nontransgenic mice. Nat. Biotechnol. *32*, 274–278.

Jeong, J.W., McCall, J.G., Shin, G., Zhang, Y., Al-Hasani, R., Kim, M., Li, S., Sim, J.Y., Jang, K.I., Shi, Y., et al. (2015). Wireless optofluidic systems for programmable in vivo pharmacology and optogenetics. Cell *162*, 662–674.

Khraiche, M.L., Phillips, W.B., Jackson, N., and Muthuswamy, J. (2008). Ultrasound induced increase in excitability of single neurons. Conf. Proc. IEEE Eng. Med. Biol. Soc. *2008*, 4246–4249.

Kim, C.K., Yang, S.J., Pichamoorthy, N., Young, N.P., Kauvar, I., Jennings, J.H., Lerner, T.N., Berndt, A., Lee, S.Y., Ramakrishnan, C., et al. (2016). Simultaneous fast measurement of circuit dynamics at multiple sites across the mammalian brain. Nat. Methods *13*, 325–328.

King, R.L., Brown, J.R., Newsome, W.T., and Pauly, K.B. (2013). Effective parameters for ultrasound-induced in vivo neurostimulation. Ultrasound Med. Biol. *39*, 312–331.

Klapoetke, N.C., Murata, Y., Kim, S.S., Pulver, S.R., Birdsey-Benson, A., Cho, Y.K., Morimoto, T.K., Chuong, A.S., Carpenter, E.J., Tian, Z., et al. (2014). Independent optical excitation of distinct neural populations. Nat. Methods *11*, 338–346.

Koester, S.E., and Insel, T.R. (2016). Understanding how non-coding genomic polymorphisms affect gene expression. Mol. Psychiatry *21*, 448–449.

Krook-Magnuson, E., Armstrong, C., Oijala, M., and Soltesz, I. (2013). On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy. Nat. Commun. *4*, 1376. http://dx.doi.org/10.1038/ncomms2376.

Krook-Magnuson, E., Szabo, G.G., Armstrong, C., Oijala, M., and Soltesz, I. (2014). Cerebellar directed optogenetic intervention inhibits spontaneous hippocampal seizures in a mouse model of temporal lobe epilepsy. eNeuro *1*, 3.2014.

Kuwajima, T., Sitko, A.A., Bhansali, P., Jurgens, C., Guido, W., and Mason, C. (2013). ClearT: a detergent- and solvent-free clearing method for neuronal and non-neuronal tissue. Development *140*, 1364–1368.

Le Clerc, S., Taing, L., Fond, G., Meary, A., Llorca, P.M., Blanc, O., Beaune, P., Rajagopal, K., Jamain, S., Tamouza, R., et al. (2015). A double amino-acid change in the HLA-A peptide-binding groove is associated with response to psychotropic treatment in patients with schizophrenia. Transl. Psychiatry *5*, e608.

Lee, J.H., Durand, R., Gradinaru, V., Zhang, F., Goshen, I., Kim, D.S., Fenno, L.E., Ramakrishnan, C., and Deisseroth, K. (2010). Global and local fMRI signals driven by neurons defined optogenetically by type and wiring. Nature *465*, 788–792.

Lee, W., Kim, H., Jung, Y., Song, I.U., Chung, Y.A., and Yoo, S.S. (2015). Image-guided transcranial focused ultrasound stimulates human primary somatosensory cortex. Sci. Rep. *5*, 8743.

Legon, W., Sato, T.F., Opitz, A., Mueller, J., Barbour, A., Williams, A., and Tyler, W.J. (2014). Transcranial focused ultrasound modulates the activity of primary somatosensory cortex in humans. Nat. Neurosci. *17*, 322–329.

Lerner, T.N., Shilyansky, C., Davidson, T.J., Evans, K.E., Beier, K.T., Zalocusky, K.A., Crow, A.K., Malenka, R.C., Luo, L., Tomer, R., and Deisseroth, K. (2015). Intact-brain analyses reveal distinct information carried by SNc dopamine subcircuits. Cell *162*, 635–647.

Lin, J.Y., Knutsen, P.M., Muller, A., Kleinfeld, D., and Tsien, R.Y. (2013). ReaChR: a red-shifted variant of channelrhodopsin enables deep transcranial optogenetic excitation. Nat. Neurosci. *16*, 1499–1508.

Long, X., Ye, J., Zhao, D., and Zhang, S.-J. (2015). Magnetogenetics: remote non-invasive magnetic activation of neuronal activity with a magnetoreceptor. Sci. Bull. *60*, 2107–2119.

Magnon, C., Hall, S.J., Lin, J., Xue, X., Gerber, L., Freedland, S.J., and Frenette, P.S. (2013). Autonomic nerve development contributes to prostate cancer progression. Science *341*, 1236361.

Mattis, J., Tye, K.M., Ferenczi, E.A., Ramakrishnan, C., O'Shea, D.J., Prakash, R., Gunaydin, L.A., Hyun, M., Fenno, L.E., Gradinaru, V., et al. (2012). Principles for applying optogenetic tools derived from direct comparative analysis of microbial opsins. Nat. Methods *9*, 159–172.

Mayberg, H.S., Lozano, A.M., Voon, V., McNeely, H.E., Seminowicz, D., Hamani, C., Schwalb, J.M., and Kennedy, S.H. (2005). Deep brain stimulation for treatment-resistant depression. Neuron *45*, 651–660.

McCarroll, S.A., Feng, G., and Hyman, S.E. (2014). Genome-scale neurogenetics: methodology and meaning. Nat. Neurosci. *17*, 756–763.

Meister, M. (2016). Magnetic magic in nature. arXiv, arXiv: 1604.01359v1, http://arxiv.org/abs/1604.01359#.

Menz, M.D., Oralkan, O., Khuri-Yakub, P.T., and Baccus, S.A. (2013). Precise neural stimulation in the retina using focused ultrasound. J. Neurosci. *33*, 4550–4560.

Montgomery, K.L., Yeh, A.J., Ho, J.S., Grosenick, L., Tsao, V., Ferenczi, E.A., Iyer, S.M., Tanabe, Y., Deisseroth, K., Delp, S.L., and Poon, A.S. (2015). Wirelessly powered, fully internal optogenetics for brain, spinal and peripheral circuits in mice. Nat. Methods *12*, 969–974.

Moore, M.E., Loft, J.M., Clegern, W.C., and Wisor, J.P. (2015). Manipulating neuronal activity in the mouse brain with ultrasound: A comparison with optogenetic activation of the cerebral cortex. Neurosci. Lett. *604*, 183–187.

Murray, E., Cho, J.H., Goodwin, D., Ku, T., Swaney, J., Kim, S.Y., Choi, H., Park, Y.G., Park, J.Y., Hubbert, A., et al. (2015). Simple, scalable proteomic imaging for high-dimensional profiling of intact systems. Cell *163*, 1500–1514.

Packer, A.M., Russell, L.E., Dalgleish, H.W., and Häusser, M. (2015). Simultaneous all-optical manipulation and recording of neural circuit activity with cellular resolution in vivo. Nat. Methods *12*, 140–146.

Paz, J.T., Davidson, T.J., Frechette, E.S., Delord, B., Parada, I., Peng, K., Deisseroth, K., and Huguenard, J.R. (2013). Closed-loop optogenetic control of

thalamus as a tool for interrupting seizures after cortical injury. Nat. Neurosci. *16*, 64–70.

Peterson, S.C., Eberl, M., Vagnozzi, A.N., Belkadi, A., Veniaminova, N.A., Verhaegen, M.E., Bichakjian, C.K., Ward, N.L., Dlugosz, A.A., and Wong, S.Y. (2015). Basal cell carcinoma preferentially arises from stem cells within hair follicle and mechanosensory niches. Cell Stem Cell *16*, 400–412.

Qin, S., Yin, H., Yang, C., Dou, Y., Liu, Z., Zhang, P., Yu, H., Huang, Y., Feng, J., Hao, J., et al. (2016). A magnetic protein biocompass. Nat. Mater. *15*, 217–226.

Rajasethupathy, P., Sankaran, S., Marshel, J.H., Kim, C.K., Ferenczi, E., Lee, S.Y., Berndt, A., Ramakrishnan, C., Jaffe, A., Lo, M., et al. (2015). Projections from neocortex mediate top-down control of memory retrieval. Nature *526*, 653–659.

Renier, N., Wu, Z., Simon, D.J., Yang, J., Ariel, P., and Tessier-Lavigne, M. (2014). iDISCO: a simple, rapid method to immunolabel large tissue samples for volume imaging. Cell *159*, 896–910.

Ressler, K.J., and Mayberg, H.S. (2007). Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. Nat. Neurosci. *10*, 1116–1124.

Rickgauer, J.P., Deisseroth, K., and Tank, D.W. (2014). Simultaneous cellular-resolution optical perturbation and imaging of place cell firing fields. Nat. Neurosci. *17*, 1816–1824.

Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014). Biological insights from 108 schizophrenia-associated genetic loci. Nature *511*, 421–427.

Schulz, D., Southekal, S., Junnarkar, S.S., Pratte, J.F., Purschke, M.L., Stoll, S.P., Ravindranath, B., Maramraju, S.H., Krishnamoorthy, S., Henn, F.A., et al. (2011). Simultaneous assessment of rodent behavior and neurochemistry using a miniature positron emission tomograph. Nat. Methods *8*, 347–352.

Shen, Y., Cao, X., Tan, T., Shan, C., Wang, Y., Pan, J., He, H., and Yuan, T.-F. (2016). 10 Hz repetitive transcranial magnetic stimulation of the left dorsolateral prefrontal cortex reduces heroin cue craving in long-term addicts. Biol. Psychiatry. Published online February 12, 2016. http://dx.doi.org/10.1016/j.biopsych.2016.02.006.

Sohal, V.S., Zhang, F., Yizhar, O., and Deisseroth, K. (2009). Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. Nature *459*, 698–702.

Stanley, S.A., Sauer, J., Kane, R.S., Dordick, J.S., and Friedman, J.M. (2015). Remote regulation of glucose homeostasis in mice using genetically encoded nanoparticles. Nat. Med. *21*, 92–98.

Stanley, S.A., Kelly, L., Latcha, K.N., Schmidt, S.F., Yu, X., Nectow, A.R., Sauer, J., Dyke, J.P., Dordick, J.S., and Friedman, J.M. (2016). Bidirectional electromagnetic control of the hypothalamus regulates feeding and metabolism. Nature *531*, 647–650.

Stopczynski, R.E., Normolle, D.P., Hartman, D.J., Ying, H., DeBerry, J.J., Bielefeldt, K., Rhim, A.D., DePinho, R.A., Albers, K.M., and Davis, B.M. (2014). Neuroplastic changes occur early in the development of pancreatic ductal adenocarcinoma. Cancer Res. *74*, 1718–1727.

Susaki, E.A., Tainaka, K., Perrin, D., Kishino, F., Tawara, T., Watanabe, T.M., Yokoyama, C., Onoe, H., Eguchi, M., Yamaguchi, S., et al. (2014). Whole-brain imaging with single-cell resolution using chemical cocktails and computational analysis. Cell *157*, 726–739.

Suthana, N., Haneef, Z., Stern, J., Mukamel, R., Behnke, E., Knowlton, B., and Fried, I. (2012). Memory enhancement and deep-brain stimulation of the entorhinal area. N. Engl. J. Med. *366*, 502–510.

Sylwestrak, E.L., Rajasethupathy, P., Wright, M.A., Jaffe, A., and Deisseroth, K. (2016). Multiplexed intact-tissue transcriptional analysis at cellular resolution. Cell *164*, 792–804.

Terraneo, A., Leggio, L., Saladini, M., Ermani, M., Bonci, A., and Gallimberti, L. (2016). Transcranial magnetic stimulation of dorsolateral prefrontal cortex reduces cocaine use: A pilot study. Eur. Neuropsychopharmacol. *26*, 37–44.

Tomer, R., Ye, L., Hsueh, B., and Deisseroth, K. (2014). Advanced CLARITY for rapid and high-resolution imaging of intact tissues. Nat. Protoc. *9*, 1682–1697.

Tomer, R., Lovett-Barron, M., Kauvar, I., Andalman, A., Burns, V.M., Sankaran, S., Grosenick, L., Broxton, M., Yang, S., and Deisseroth, K. (2015). SPED light sheet microscopy: Fast mapping of biological system structure and function. Cell *163*, 1796–1806.

Towne, C., Montgomery, K.L., Iyer, S.M., Deisseroth, K., and Delp, S.L. (2013). Optogenetic control of targeted peripheral axons in freely moving animals. PLoS ONE *8*, e72691.

Trivedi, M.H., McGrath, P.J., Fava, M., Parsey, R.V., Kurian, B.T., Phillips, M.L., Oquendo, M.A., Bruder, G., Pizzagalli, D., Toups, M., et al. (2016). Establishing moderators and biosignatures of antidepressant response in clinical care (EMBARC): Rationale and design. J. Psychiatr. Res. *78*, 11–23.

Tufail, Y., Matyushov, A., Baldwin, N., Tauchmann, M.L., Georges, J., Yoshihiro, A., Tillery, S.I., and Tyler, W.J. (2010). Transcranial pulsed ultrasound stimulates intact brain circuits. Neuron *66*, 681–694.

Tye, K.M., Prakash, R., Kim, S.Y., Fenno, L.E., Grosenick, L., Zarabi, H., Thompson, K.R., Gradinaru, V., Ramakrishnan, C., and Deisseroth, K. (2011). Amygdala circuitry mediating reversible and bidirectional control of anxiety. Nature *471*, 358–362.

Tyler, W.J., Tufail, Y., Finsterwald, M., Tauchmann, M.L., Olson, E.J., and Majestic, C. (2008). Remote excitation of neuronal circuits using low-intensity, low-frequency ultrasound. PLoS ONE *3*, e3511.

Urban, D.J., and Roth, B.L. (2015). DREADDs (designer receptors exclusively activated by designer drugs): chemogenetic tools with therapeutic utility. Annu. Rev. Pharmacol. Toxicol. *55*, 399–417.

Venkatesh, H.S., Johung, T.B., Caretti, V., Noll, A., Tang, Y., Nagaraja, S., Gibson, E.M., Mount, C.W., Polepalli, J., Mitra, S.S., et al. (2015). Neuronal activity promotes glioma growth through neuroligin-3 secretion. Cell *161*, 803–816.

Wheeler, M.A., Smith, C.J., Ottolini, M., Barker, B.S., Purohit, A.M., Grippo, R.M., Gaykema, R.P., Spano, A.J., Beenhakker, M.P., Kucenas, S., et al. (2016). Genetically targeted magnetic control of the nervous system. Nat. Neurosci. http://dx.doi.org/10.1038/nn.4265.

Whitmer, D., de Solages, C., Hill, B., Yu, H., Henderson, J.M., and Bronte-Stewart, H. (2012). High frequency deep brain stimulation attenuates subthalamic and cortical rhythms in Parkinson's disease. Front. Hum. Neurosci. *6*, 155.

Wietek, J., Wiegert, J.S., Adeishvili, N., Schneider, F., Watanabe, H., Tsunoda, S.P., Vogt, A., Elstner, M., Oertner, T.G., and Hegemann, P. (2014). Conversion of channelrhodopsin into a light-gated chloride channel. Science *344*, 409–412.

Yang, B., Treweek, J.B., Kulkarni, R.P., Deverman, B.E., Chen, C.-K., Lubeck, E., Shah, S., Cai, L., and Gradinaru, V. (2014). Single-cell phenotyping within transparent intact tissue through whole-body clearing. Cell *158*, 945–958.

Yizhar, O., Fenno, L.E., Prigge, M., Schneider, F., Davidson, T.J., O'Shea, D.J., Sohal, V.S., Goshen, I., Finkelstein, J., Paz, J.T., et al. (2011). Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature *477*, 171–178.

Yoo, S.S., Bystritsky, A., Lee, J.H., Zhang, Y., Fischer, K., Min, B.K., McDannold, N.J., Pascual-Leone, A., and Jolesz, F.A. (2011). Focused ultrasound modulates region-specific brain activity. Neuroimage *56*, 1267–1275.

Zalocusky, K.A., Ramakrishnan, C., Lerner, T., Davidson, T.J., Knutson, B., and Deisseroth, K. (2016). Nucleus accumbens D2R neurons signal past unfavorable outcomes during decision-making and control risky choice. Nature *531*, 642–646.

Zhang, F., Prigge, M., Beyrière, F., Tsunoda, S.P., Mattis, J., Yizhar, O., Hegemann, P., and Deisseroth, K. (2008). Red-shifted optogenetic excitation: a tool for fast neural control derived from Volvox carteri. Nat. Neurosci. *11*, 631–633.

Zhao, C.M., Hayakawa, Y., Kodama, Y., Muthupalani, S., Westphalen, C.B., Andersen, G.T., Flatberg, A., Johannessen, H., Friedman, R.A., Renz, B.W., et al. (2014). Denervation suppresses gastric tumorigenesis. Sci. Transl. Med. *6*, 250ra115.