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Optical Neural Interfaces

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Abstract

Genetically encoded optical actuators and indicators have changed the landscape of neuroscience, enabling targetable control and readout of specific components of intact neural circuits in behaving animals. Here, we review the development of optical neural interfaces, focusing on hardware designed for optical control of neural activity, integrated optical control and electrical readout, and optical readout of population and single-cell neural activity in freely moving mammals.

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1. INTRODUCTION

The mammalian brain, which is more complex than any electronic circuit yet devised, comprises billions of components with innumerable distinct shapes, sizes, activity patterns, wiring patterns, and molecular phenotypes. Progress toward untangling this complexity had for many years followed two parallel paths, one focusing on function of these circuit elements (electrophysiology) and the other on neurochemical and structural identity (anatomy). Electrophysiological methods can be used to record the activity of neurons in behaving animals, but with limited means to link these neural activity patterns identified in behavior to anatomical identity of the very same neurons. Moreover, the brain can be electrically stimulated to evoke specific memories, behaviors, or affective states (1–3), but the mechanisms by which these manipulations act through specific neural circuit elements and wiring can be difficult to determine.

Recent convergence of the molecular and systems branches of neuroscience, however, has led to unprecedented progress toward a systematic linkage between neuronal structure and function, driven in part by the development of genetically encodable neural actuators and indicators (4–7). With these biological tools, neuroscientists can now control and image the activity of genetically or anatomically defined specific cell types in the brains of behaving mammals and can finally begin to connect this behaviorally relevant function to detailed structure. However, engineering of novel devices is also required, as understanding how circuit elements produce complex behaviors in mammalian systems requires specialized neural interfaces to enable application of optical tools in freely behaving animals. We review this rapidly developing field here.

2. OPTICAL CONTROL OF NEURAL ACTIVITY

2.1. Genetically Encoded Optical Actuators

Recent years have seen the development of a comprehensive toolbox of genetically encoded optical actuators from microbial organisms (8–21). These proteins, which are derived from single-component opsin genes, permit fine control over the activity of genetically or topologically defined neural circuit elements embedded in a matrix of dissimilar cell types. Although electrical stimulation has been used with great efficacy to both control and probe the function of discrete brain regions and to provide therapeutic benefit, it is not capable of targeting genetically specified cell types (**Figure 1*a***), a disadvantage that can be overcome with genetically encoded actuators.

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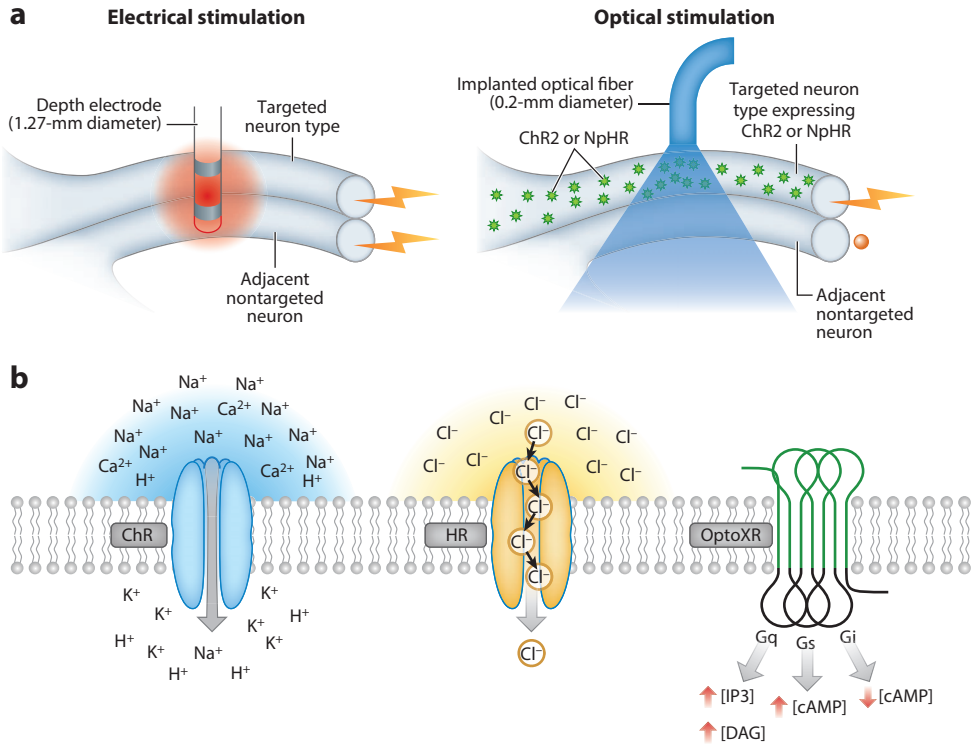

Figure 1

Optogenetics for control of genetically or topologically defined neural subtypes. (*a*) Electrical stimulation can be effective for modulating local neural activity, but the heterogeneity of brain tissue precludes control of single cell types using this method (*left*). Genetically targetable optogenetic constructs enable more precise stimulation of specified neural subtypes intermingled with nontargeted neurons (*right*). (Panel modified with permission from 154.) (*b*) Many different types of optogenetic constructs have been developed for control of neural activity. Currently available tools allow for induction of action potentials (ChR-family light-gated cation channels shown, *left*), silencing of neural activity (HR-family light-gated chloride pumps shown, *middle*; proton pumps may also be used), and modulation of intracellular signaling cascades [OptoXR family (153) shown, *right*; other classes of biochemical tool may also be used], among many other applications. (Panel modified with permission from 4.) Abbreviations: ChR, channelrhodopsin; ChR2, channelrhodopsin-2; HR, halorhodopsin; NpHR, halorhodopsin from *Natronomonas pharaonis*; optoXR, opsin-receptor chimaera.

Optical neural control has been a long-sought goal (22–24), but the microbial single-component tools (8–21) have turned out to be critical for genetically encoded optical actuation, including in mammalian systems, owing to several factors including speed, reliability, and targetability. The first microbial opsin successfully employed to excite neural activity and alter behavior in freely moving mammals (8) was channelrhodopsin-2 (ChR2), a member of the channelrhodopsin (ChR) family (10) (**Figure 1*b***, *left*). These membrane-bound nonselective cation channels could be activated with pulses of blue light to depolarize expressing neurons and induce single action potentials (8). This was quickly followed by the development of the inhibitory halorhodopsin from *Natronomonas pharaonis* (NpHR) (9, 11, 14) (**Figure 1*b***, *middle*), a chloride pump that could hyperpolarize and therefore silence expressing neurons upon the application of yellow light. Further engineering and genomic exploration produced spectrally shifted opsins (12, 20, 25), opsins for biochemical control (153) (**Figure 1*b***, *right*), opsins for proton pumping–based inhibition (14, 15, 21), faster opsins (16, 18), highly expressing opsins (16, 17, 19–21), and bistable step-function opsins (SFOs) (13, 20), creating a wide spectrum of tools for optical neural control (21).

2.2. Single-Site Intracranial Light Delivery

Prior to the development of specialized devices for intracranial light delivery in freely behaving mammals, optogenetic stimulation had been successfully employed to drive neural activity in both cultured neuronal and acute slice preparations (8, 9), as well as to drive simple behaviors in invertebrates (9, 26). In these preparations, light of the appropriate wavelength and power could be delivered from an arc lamp through the imaging objective for full-field optogenetic stimulation, but this approach was not well suited to stimulation deep within the brain, or for flexible behavioral control in freely moving mammals.

The first demonstration of optical control of behavior in freely moving rodents, in 2007 (27), employed an intracranial optical fiber directly coupled to a laser-diode light source; this original optical neural interface device had been developed and described in an optogenetic application to intact anesthetized rodents earlier that same year (27a) (**Figure 2*a***). In this first approach, a metallic/plastic cannula originally designed for intracranial drug delivery (Plastics One) was surgically implanted above the brain region of interest. Immediately following implantation, a viral vector for opsin delivery was injected through the implanted cannula. This design ensured coregistration of the virally transduced neuronal population with the light source. During subsequent behavioral testing, a flexible multimode optical fiber coupled to the laser-diode light source was inserted directly into the cannula (flush with the cannula tip). Using this approach it was possible to induce whisker twitches upon illumination of ChR2-expressing neurons in motor cortex of living rats and mice (27a); this report was followed within a few months by a separate study showing optogenetic control of behavioral state transitions (sleep/wake) in freely moving mice (27).

Key advantages of this system include the ability to stimulate either surface brain tissue or subcortical structures, as the cannula can be placed stereotaxically to target any brain region of interest. In addition, simultaneous optogenetic and pharmacological manipulation can be performed through the same cannula, a useful property because pharmacological interventions add an informative mechanistic component to many optogenetic studies. Subsequent work refined this approach to permit secure, long-term fiber placement, as well as free rotation of the animal using a commutator (fiberoptic rotary joint; Doric Lenses), which is crucial for many behavioral experiments in freely moving rodents (5, 20, 27, 28).

A subsequently developed system for optogenetic stimulation in freely behaving rodents used chronic implantation of a short optical fiber stub coupled to a metal ferrule (**Figure 2*b***) (29). During behavioral testing, the metal ferrule could be coupled to an external fiber with a ceramic

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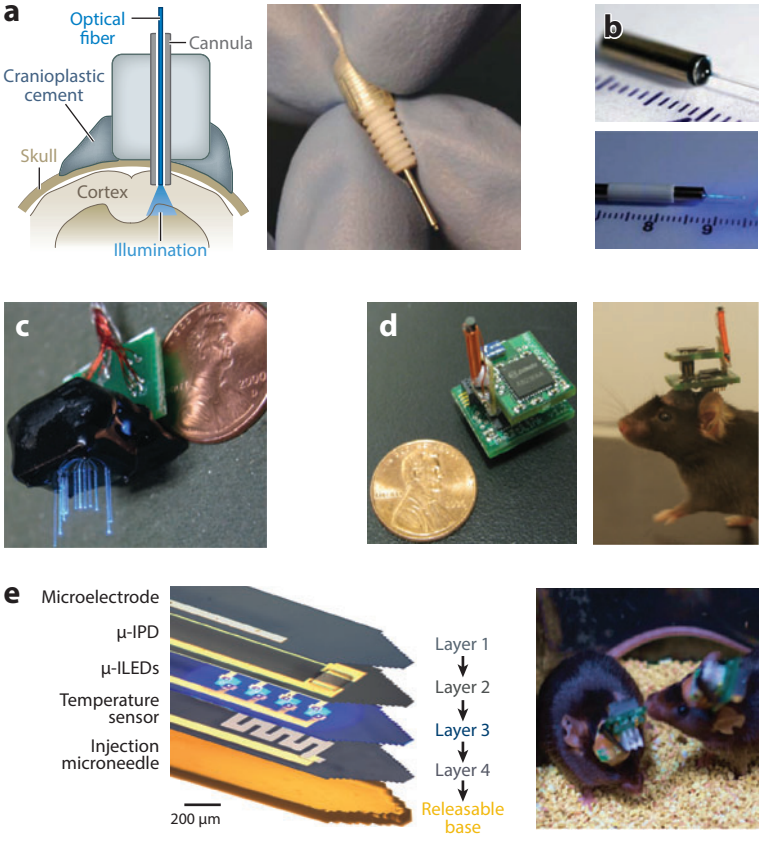

Figure 2

Optical-neural interfaces for light delivery to brain tissue in freely moving mammals. (*a*) The original fiberoptic neural interface (27a), in this case implemented via a cannula implanted over the brain region of interest; the optical fiber coupled to a light source is inserted during behavioral testing. This approach allows for simple integration with pharmacological methods. (Panel modified with permission from 27a.) (*b*) Light delivery through an implanted fiber. An optical fiber stub is implanted over the brain region of interest and is coupled to a fiberoptic tether with a ceramic sheath during behavior. This approach is well suited to high-throughput behavioral testing but cannot be easily combined with pharmacology at the same site. Both cannulas and optical fiber stubs can be used for single- or dual-site illumination. (Panel modified with permission from 29 and 154.) Further advancements include (*c*) multisite light delivery (panel modified with permission from 42) and (*d*) wireless control (panel modified with permission from 37), both appropriately sized for use in freely behaving mice. (*e*) Injectable optoelectronics for wireless multisite multiwavelength optical stimulation/sensing, electrophysiology, and temperature sensing with coregistration and minimal tissue damage. Multiple μ-ILED light sources (a thousandth the size of conventional LEDs) can be independently controlled and can deliver a choice of wavelengths. Abbreviations: μ-IPD, microscale inorganic photodetector; μ-ILED, microscale inorganic light-emitting diode. (Panel modified with permission from 48.)

sheath and commutated for free rotation during behavior as above. Significant advantages of this system over the previously described cannula-based system include (*a*) avoidance of repeated insertion of an external fiber into the brain, thereby reducing damage to neural tissue; (*b*) reduced likelihood of breaking the external fiber upon insertion; and (*c*) reduced handling stress for the rodent. However, the ability to simultaneously perform same-site intracranial

pharmacology is lost. Additionally, viral injections must be performed separately, leading to a possible misregistration of opsin expression and fiber targeting. In practice, this technique is used primarily for high-throughput behavioral screening, whereas the cannula approach is used when integrated optogenetic and pharmacological experiments are performed. Commercially available implantable fibers with ferrules may currently be obtained from several vendors, including Doric Lenses, Thorlabs, and Plexon Inc. Additionally, construction of these devices may be accomplished easily in the laboratory at very low cost (30).

2.3. Light Sources

Light delivery to brain tissue through an intracranial fiber has been accomplished primarily using either laser (diode or diode-pumped solid state, DPSS) or light-emitting diode (LED) light sources. Each has advantages, and the appropriate system for any given experiment will ultimately be driven by the relative importance of variables such as cost, size, light power, spectral tuning, stability, and temporal properties.

Fiber-coupled lasers have been the light source of choice for many recent experiments (20, 27, 27a, 31–36), in large part because the efficient coupling between light source and fiber enables delivery of high-powered illumination directly to neural tissue. Although control of neuronal firing upon photostimulation requires relatively low irradiance values at the microbial opsin-expressing cell (1–5 mW/mm^2) (8, 9), the high degree of light scattering and absorption by neural tissue (5) creates a practical in vivo requirement for orders-of-magnitude-greater light levels at the fiber tip, depending on light sensitivity of the cells expressing the opsin of choice (13, 21). Lasers with the capacity to deliver up to 100-mW (or more) light power are typically used in the laboratory and are commercially available from several sources (OEM Laser Systems, CrystaLaser, Cobolt, LaserGlow, Thorlabs, Omicron, and OptoEngine). A light source of this power enables delivery of light into the brain at the appropriate intensity to induce optogenetic neural activation, even after losses introduced through fiber coupling, fiberoptic rotary joints for commutation, or splitting for bilateral illumination. Additional advantages of laser light sources include very low beam divergence and tight spectral bandwidth—important for multicolor, multiopsin experimental configurations (discussed below).

Despite these advantages, laser systems have several drawbacks. Although some wavelengths are relatively inexpensive (~$1,000 for a 473-nm laser), yellow light sources in particular can be more costly ($5,000–10,000 for a 590-nm laser). Additionally, lasers can be fragile (particularly for yellow wavelengths), require long warm-up times, and are not guaranteed to provide stable illumination power without considerable expense. Laser light sources are also bulky and can require specialized optical components to couple light to the fiber. Although analog modulation can be used with lasers to vary power output, the precision of this manipulation is somewhat lacking. Moreover, although short, millisecond-width pulses can be generated with lasers of some wavelengths, large transients are often seen with pulse shape significantly deviating from a square waveform; indeed currently, yellow lasers cannot be practically modulated on this timescale and are instead routinely used with a shutter for temporal control of illumination, but shutters can, in turn, introduce additional problems. Though effective for the timescale on which this wavelength is typically used for inhibition (seconds to minutes), the mechanical action of shutters produces a sound that may be confounding for behavioral experiments; liquid crystal shutters are significantly quieter but are generally incompatible with the power levels required for in vivo experiments. Finally, laser light sources also require the use of a fiberoptic rotary joint when used with freely behaving mammals, which imposes additional limitations; these joints are expensive, introduce

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variation in light power as the joint is rotated, and must be integrated with electrical commutation systems typically used in neurophysiological experiments.

LED light sources have become increasingly suitable for optogenetic experimentation in freely moving mammals (28, 37, 38) and have been used for motor and behavioral control in several papers (28, 37, 39–41). LED configuration can be quite flexible and modified to align with experimental requirements; these light sources may be used above a thinned skull for a noninvasive experimental manipulation, mounted directly above the cortical surface, inserted into the brain, or remotely coupled to a fiber to target deep brain structures. Less expensive (~$300), smaller, more stable, more reliable, and available in a range of wavelengths, LED light sources have only one serious drawback for single-color experiments: relatively poor light source–fiber coupling and the concomitant difficulty in obtaining light powers high enough to drive behavioral responses upon in vivo stimulation. Efficient coupling to an insertable light guide is important because direct insertion of an LED itself into target tissue typically generates local heating. An additional drawback for multicolor experiments is the relatively broad spectral tuning of LEDs, on the order of 10 to 50 nm at half-max, which may negatively influence both dual-opsin stimulation experiments and simultaneous optical stimulation/readout with fluorescent Ca^{2+} or voltage indicators.

Recent improvements in the efficiency of fiber–LED coupling have enabled significantly higher light powers, particularly for blue wavelengths. LEDs from Plexon Inc. are currently available in nine wavelengths ranging from royal blue to infrared (450–940 nm) and, thus, suitable for many optogenetics experiments. The blue (465-nm) LEDs, in particular, are quite powerful, delivering ~25 mW from the fiber tip if used with a 200-μm fiber. However, some LEDs continue to deliver relatively low powers; for example, yellow LEDs currently deliver little more than 3 mW from the fiber tip. This level of power output may be useful for some experiments, but higher light powers are more commonly desired, particularly if the light must run through multiple coupling stages or be split for bilateral illumination. Available adjacent wavelengths, such as orange (620 nm) or green (525 nm), are accessible with higher-powered LEDs that may potentially be used instead to activate the same opsins. As inexpensive, higher-powered LEDs become commercially available (as from Luxeon), novel configurations of fiber-coupled LED arrays may be assembled for optogenetics applications.

Other advantages of LED-based systems include ease of commutation of the electrical power cable, compared with a diode-coupled optical fiber. Simultaneous commutation with neurophysiological recording equipment is relatively straightforward, as an extra electrical channel can be used for this purpose and need not require a new design. Optical fiber commutation with laser-based systems requires concentric commutation of optical fiber and electrical wires, which may not be easy to integrate with some currently available systems. In addition, when electrical commutation with LEDs, rather than optical commutation, is used, light power fluctuations have been shown to be less pronounced (38). One potential disadvantage of LED-based systems arises from the propensity of head-mounted LEDs to introduce an undesirable electrical artifact when combined with neurophysiological recording, thereby negating one of the key advantages of light-based stimulation over electrical stimulation—namely, minimizing artifactual electrical signals that would make it impossible to record neural activity during ongoing stimulation. Yet fiber-coupled LEDs at a distance have been shown to introduce minimal artifacts when care is taken with experimental setup (38).

2.4. Multisite Optical-Neural Interfaces

Light delivery to multiple intracranial sites is often an experimental requirement. Some optical control experiments, particularly those in which a brain region is inhibited during behavioral testing, demand bilateral modulation in order to affect behavior. Other experiments target large

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brain structures, in which case it may be desirable to stimulate or inhibit a large fraction of the structure with multiple fibers. And for still other applications, precise spatial patterning of an array of stimulation sites would be advantageous, as when optical control of different cortical layers is needed. To address this class of experimental need, device designs for these and other light-delivery configurations have been developed over the past few years to allow for flexible choice in stimulation pattern by the investigator.

Bilateral stimulation can be achieved fairly expeditiously for many brain regions by the implantation of two independent cannulas or ferrule-coupled optical fiber stubs (31–33). This approach has the advantage of not requiring specialized equipment, but it comes at the cost of implanting two separate pieces of hardware, which requires either additional surgical time or the use of a more expensive two-arm stereotaxic apparatus. Additionally, in many cases, the desired stereotaxic targets are too close to permit implantation of both pieces of hardware. For cases such as these, the use of a double cannula (Plastics One) or double optical fiber implant (Doric) may allow bilateral targeting of the structure of interest, as well as a reduction in implantation time. In either configuration, light can be delivered to both stimulation sites by using either a fiber splitter to divide the light from a single light source (Fiber Optic Network Technology Co.) or a 1 × 2 double fiberoptic rotary joint (Doric). And a configurable device for stimulation of multiple brain sites (42) (**Figure 2*c***) would, in principle, allow for flexible targeting of multiple stereotaxically determined target sites, which might also be coupled to a planar array of LEDs or to electrodes for simultaneous neurophysiological recordings. Many innovations in multisite light delivery have been tested only in anesthetized preparations and may require additional engineering for use in freely behaving mammals. Examples include a linear array of independently addressable sites along a single probe axis (43), which has recently been extended to a three-dimensional (3D) matrix of linear probes (44); optical splitter and optical mixer probes (45); and planar arrays of optrodes (46, 47).

2.5. Wireless Optogenetic Control

Recently, several devices have been developed that seek to move toward wireless control of intracranial light delivery (37, 41, 48), which if demonstrated could facilitate chronic stimulation over hours, days, or even weeks (potentially useful for studying long-timescale processes such as learning and development). This approach could also facilitate behavioral experiments wherein a wire tether might be not easily accommodated, such as for control over multiple animals in a single environment without tangling wires. However, these proposed wireless systems (addressed in detail below, but not yet validated in behavioral or physiology settings) are based on LED light sources, with the potential disadvantage of electrical artifacts and heating during stimulation because the LED would be located very close to (or at) the site of stimulation.

One simple battery-powered, head-mountable wireless LED stimulation system developed for use in freely behaving rodents (41) is composed of a wireless receiver coupled to a battery, with a total weight of 3.1 g. The receiver powers a blue LED that is coupled to a 2-mm polymer fiber, which is placed above a thinned skull for transcranial stimulation. The implanted device is controlled by a transmitter, which can send internally or externally triggered transistor–transistor logic (TTL) pulses of arbitrary duration at up to 38 kHz, as well as manually triggered pulses. As power is onboard rather than wirelessly transferred, animals can, in principle, be up to 2 m away from the transmitter without loss of functionality, which is potentially useful for experiments that require a large range of movement, such as mazes or open field tests.

Another type of wireless device is both controlled and powered remotely, in order to reduce weight and increase durability (obviating the local battery and need for continuous power transfer) (37) (**Figure 2*d***). This head-mounted device can contain up to 16 independently controllable

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LEDs, which may either be bare and implanted directly above a surface target or coupled to a fiber for targeting deep structures. Power can be transferred wirelessly from an induction platform under the experimental apparatus or home cage, and a supercapacitor on the head-mounted device can be used to buffer power locally. Steady-state input power of 2 W can be delivered to the LEDs, with the potential for delivering bursts of up to 4.3 W. A wireless base station can be used to transmit updated or altered stimulation protocols to the device; this communication module increases device mass from 2 g to 3 g. This system was designed with freely behaving mice in mind, and it may not work as well in other freely behaving animals, such as rats or songbirds, because peak magnetic field above the induction platform is at a height of 0.5 to 2 cm, with a reduction to 30% at 3 cm; thus, a rearing mouse would likely remain within the maximal power range, but a rearing rat or moving songbird may not.

Most recently, a wireless system utilizing ultrathin microscale inorganic LEDs (μ-ILEDs)—miniature, independently addressable light sources directly injectable into brain tissue—has been developed (48) (**Figure 2*e***). Much smaller (50 × 50 × 6 μm) than the 200-μm fiber typically used for deep light delivery, and a thousandth the size of a conventional bulk LED, a system with such a low footprint has several advantages, including reduced tissue damage and minimized inflammation or glial scarring. The μ-ILEDs used in this system are made of gallium nitride (GaN) and emit blue light (450 nm) when powered. They can be transfer printed onto thin plastic strips in many different configurations, which can then be mounted onto an injection needle and injected deep into neural tissue. After the mounting adhesive is dissolved, the injection needle can be withdrawn, leaving a very small strip within the brain. μ-ILED strips may be bound together with other strips designed for optical, thermal, and electrophysiological sensing and actuating, and they may be simultaneously injected to target the same brain region with perfect coregistration.

Although the highest powers have been obtained with blue light (40 mW/mm^2 with an array of four blue LEDs receiving wired power), other colors are available, including red (675 nm and made from gallium arsenide, GaA) and green (530-nm, fluorescein phosphor-coated GaN μ-ILEDs). A potential advantage of this device is small size, which may facilitate precise targeting of smaller neural circuits. In addition, the direction of light may be tailored by engineering the plastic strip with reflective or absorbent surfaces, which could enable horizontal stimulation (useful for some target structures). A current disadvantage is low power output, particularly under wireless control (with this radio frequency–scavenging operational mode, the maximal output light power is 7 mW/mm^2). Another disadvantage is heating of neural tissue; with continuous 23.5-mW/mm^2 light, a rise in brain temperature of 10°C at an implantation depth of 0.3 mm was detected, which would be more than enough to influence local circuit dynamics. Pulsed 20-Hz stimulation, by contrast, led to a minimal rise of ~0.5°C, and deeper implantations were also associated with more subtle increases in temperature.

Wireless control of intracranial light delivery may be a useful direction for future technological innovation, particularly for animals that are very sensitive to handling (e.g., young animals and songbirds) and for behavioral tests that may not easily accommodate wire couplings to the experimental animal. Currently, a primary limitation is restricted output light power (particularly in the yellow wavelengths), which falls below optimal strength for all available designs; this factor may be prohibitive for operation in real-world conditions, and more work is needed to map out this appealing space of possibilities.

3. INTEGRATED OPTICAL CONTROL AND ELECTRICAL READOUT

It is potentially of great utility to integrate optical control of neural activity with neurophysiological readout of circuit activity in freely behaving mammals. This direction of technology development

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has been pursued since the advent of behavioral optogenetics (27, 28) and is primarily motivated by two core goals: (*a*) characterization of changes in circuit dynamics upon silencing or activation of specific cell types and (*b*) optical identification, or tagging, of electrically recorded neurons. Both of these pursuits are likely to prove informative in determining the roles of different neuronal cell classes in normal and pathological circuit function.

The quest to find correspondence between electrically recorded in vivo neurophysiological profiles of single neurons and descriptive anatomical markers has been a long one, and many approaches have been proposed over the years. One method has involved the use of neuronal spike properties to identify genetically defined cell types. For example, dopamine (DA) neurons in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) are typically thought to possess a broad, triphasic waveform and slow, irregular firing rates when compared with other neurons in this region (such as inhibitory GABA neurons) (49, 50), and cortical fast-spiking inhibitory neurons have a distinctive narrow waveform and faster firing rates when compared with nearby cortical excitatory neurons (51–53). However, these identification methods are imperfect, and recent experiments have shown that misidentification of neurons is an issue, although this may be less of an issue for cortex than for subcortical regions (54). Optogenetic tagging (described below) provides a potentially improved means for linking neural activity and genetic or anatomical characteristics and is a prime motivator driving the development of devices for integrated optical control and neurophysiology.

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3.1. Silicon Probes with Integrated Light Guides

The first attempts at pairing optical control of neural activity with neurophysiology in the intact mammalian brain were in anesthetized rats and mice. These studies typically either used a device called an optrode, which in its early forms consisted of an optical fiber glued to a sharp metal extracellular recording electrode (28, 55, 56), or separately positioned and advanced electrodes and optical fibers (57). Rapid progress was soon made in combining electrical readout with optical control in freely moving mammalian systems, beginning with electroencephalography (EEG) (27) and soon leading to integration of an optical fiber into a preexisting system for neurophysiological recordings (20, 36, 58–65).

One group (58) combined multisite silicon recording probes with micrometer-scale light guides to build the first integrated recording system suitable for recording from units in freely behaving rodents (**Figure 3*a***). Silicon probes with eight recording sites each (66, 67) were epoxied to single-mode fibers etched to an external diameter of 5 to 20 μm and coupled to a laser light source. Each probe could accommodate 1–2 fibers, and the probes were combined in groups of 4 to 8 for a total capacity of 32 to 64 recording sites and 8 to 16 light sources per device. These assemblies were attached to an implantable micromanipulator and could be advanced after implantation to target multiple depths. An advantage of this type of device design is the close coupling between light output and recording probe (within 100 to 300 μm), enabling the use of very low light powers to stimulate nearby neurons with great spatial precision. This is advantageous in avoiding simultaneous activation of many neurons in parallel, which would otherwise lead to superimposed waveforms and unsortable population spikes. Another advantage of lower light powers is reducing local field potential (LFP) artifacts and heating. This device was tested and performed well in freely moving rats, although it may be too large for routine use in freely moving mice. This design was subsequently modified and extended to incorporate device-mounted LED light sources (62), which simplifies commutation in freely moving animals as discussed above and is particularly useful for the simultaneous use of multiple light sources of different wavelengths.

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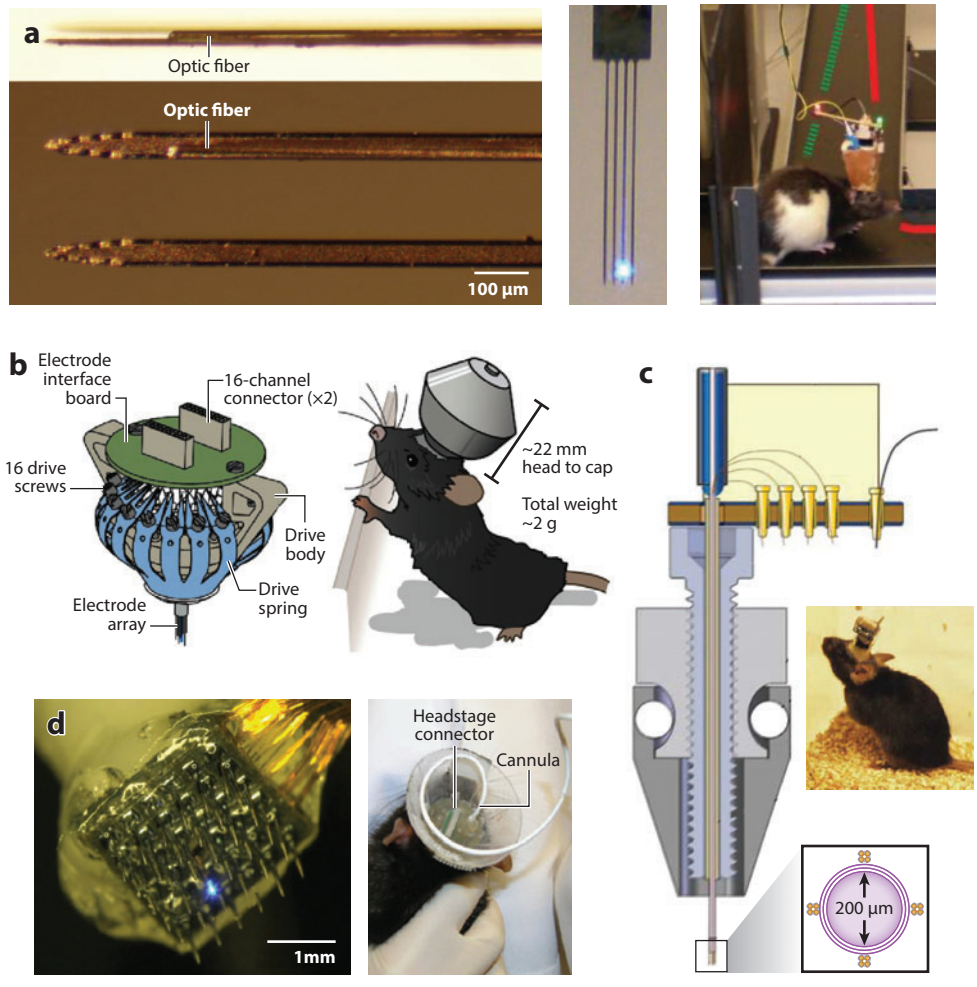

Figure 3

Integrated neurophysiology and optical control. (*a*) Optical fibers can be incorporated into multisite silicon probe designs suitable for use in freely behaving rats. (Panel modified with permission from 58.) (*b*) A tetrode microdrive with independently drivable tetrodes can be modified to incorporate either a fixed or freely movable optical fiber for use in large, superficial brain regions such as cortex or the hippocampus. (Panel modified with permission from 63.) (*c*) Simultaneous optical stimulation and electrical recording from small, deep structures, such as the brainstem neuromodulatory nuclei, requires a more compact design. A drivable device achieves this with tetrodes attached to a central fiber core. (Panel modified with permission from 61.) (*d*) An optical fiber can replace an electrode on a multielectrode array to enable simultaneous optical stimulation and high-density cortical recording. (Panel modified with permission from 65.)

3.2. Stereotrode and Tetrode Microdrives

Other groups have combined fiberoptic light delivery with stereotrode (68) or tetrode (69–71) devices and drives of various designs. One stereotrode device developed for use in freely behaving mice (59) was composed of a fixed optical fiber glued to an electrode interface board (EIB) and surrounded by eight fixed stereotrodes. This device was later improved by incorporating independent drives for each tetrode or stereotrode (60), enabling fine control over the position of each

and the ability to gather higher-quality data with more isolatable single units. Although this device used a fixed rather than drivable fiber, this configuration may enable more stable recordings. It is worth noting that the kind of optical fiber used in these devices has an outer diameter 10 times as large as that of an individual stereotrode or electrode and would likely be capable of some amount of local tissue damage through either blunt trauma or rupture of blood vessels, in addition to the tissue deformation that would impair isolation on nearby channels. Most recently, some reengineering led to a device with reduced weight (2 g) and enhanced channel count (16 tetrodes) that can accommodate either a fixed or a movable fiber (63) (**Figure 3*b***).

Another group (61) took an entirely distinct approach, choosing instead to situate at the core of the device a movable optical fiber, which was then surrounded by attached tetrodes (**Figure 3*c***). As a consequence, the whole optetrode assembly could move up and down in brain tissue as a bundle, which is advantageous for maintaining close positioning of the tip of the fiber and the recording sites—particularly useful for recording in small, deep nuclei. In a typical tetrode hyperdrive (69), tetrodes are free to emerge from different sites at the base of the device and have a tendency to diverge as they are driven into the brain; this adjustable hyperdrive approach is useful for sampling recording sites in large brain areas, such as the dorsal hippocampus, but falls short when recording from smaller, deeper structures such as the VTA, where light-mediated identification of neural type would be especially useful (54). The optetrode device is particularly lightweight at 2 g and was shown to be suitable for routine use in freely behaving mice (61).

3.3. Fixed-Wire Arrays

Other approaches have made use of fixed-wire implanted arrays. The chronic multisite optrode (CMO) consists of four tungsten wires glued to an implantable fiberoptic light guide (Doric) and cut at 500-μm intervals (20). This device is most suited for LFP and multiunit recordings and is relatively inexpensive and simple to make within the lab. Also, its small size enables use of more than one device per mouse. However, because of the potential for an optoelectronic artifact introduced by pulsed light (72, 73), this device is best used with opsins that do not require continuous light input, such as the SFOs (13, 20).

3.4. Multielectrode Arrays

Finally, an integrated fiber–multielectrode array (MEA) device, the optrode-MEA, has been developed for chronic recording and stimulation in freely moving rodents (64) (**Figure 3*d***). In this setup, 10×10 intracortical microelectrode arrays (Blackrock Microsystems) were repurposed to include a single tapered optical fiber in place of one of the electrodes. This optical fiber was coated with gold and insulated with epoxy, with a small exposed optical aperture at the end, in order to permit simultaneous recording from this site. Achieved impedance measurements ranged from 200 kΩ to 1 MΩ, within the range typically used for extracellular recordings. The optrode was strong enough to penetrate rat dura without deformation, enabling high-quality recordings from cortical areas. Improvements in this device have been shown to permit chronic recordings of up to 8 months in rat cortex (65) (**Figure 3*d***), which would be useful for long-term experiments investigating neural plasticity or changes in neural dynamics on this timescale.

Ultimately the choice of device will depend on the application. Optical control in combination with arrays of silicon probes or individually controllable tetrode or stereotrode microdrives is ideally suited for recording in large regions with dense cell packing, such as the CA1 or CA3 pyramidal cell layers in the hippocampus (58, 60, 62, 63). If the desired application is simultaneous recording of single isolated neurons and stimulation or inhibition in deeper, smaller brain regions, such as the VTA, a unified device with a fiber core is likely more appropriate (61). If cortical

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recordings of well-isolated single units over long periods of time are desired, optrode-MEAs would be the best solution, whereas if an inexpensive and simple method for recording cortical LFPs is needed, a chronic multisite optrode would be ideal.

A remaining challenge for optimal functionality of these devices is the optoelectronic artifact almost always seen in LFP recordings, in which illumination of the metal recording electrode can produce a significant slow-voltage change (72, 73). In some cases, optical fibers and electrical recording devices can be separated to avoid this artifact, but this configuration is not practical for many applications. This problem may be partially resolved by appropriate configuration of light delivery and recording electrode, by choice of electrode coating, or by choice of electrode material (72).

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4. OPTICAL READOUT OF NEURAL ACTIVITY

The past few years have seen an explosion in development of both genetically encoded fluorescence-based indicators of neural activity (74–90) and devices designed to read out these fluorescence signals (91–103). Complementing the optical neural activity actuators described above, these optical neural activity reporters enable fast-timescale readout of neural activity, currently approaching the measurement of single neural action potentials (89). Though still under development, these activity reporters in many respects have the potential to stand alongside electrophysiology as standard tools for measuring neural circuit activity in freely behaving mammals. In vivo fluorescence-based neural activity imaging promises to expand the number of simultaneously recorded neurons by orders of magnitude, to enable the unambiguous mapping of recorded neural activity onto underlying genetic or anatomical identity regardless of local circuit architecture, and to provide long-term recording of identified single neurons over days to weeks, allowing investigators to probe circuit dynamics during extended processes such as learning or development (104).

4.1. Genetically Encoded Activity Indicators

Genetically encoded activity indicators can report as fluorescence changes certain neuronal properties that are influenced by neural activity, such as intracellular Ca^{2+} or transmembrane voltage. These indicators have some significant advantages over chemicals such as bulk-loaded Ca^{2+} or voltage-sensitive fluorescent dyes, which can be quite fast and sensitive but do not typically allow for cell-type-specific imaging [although retrogradely and anterogradely transported indicators can certainly be used for neural projection-specific labeling (105, 106), and indicators can be used in combination with cell-type-specific fluorescent labels (107)]. In addition, these dyes must be loaded at the beginning of each imaging session, making chronic neural activity recordings by this means problematic. By contrast, genetically encoded indicators can be cell-type specific and exhibit persistent expression, enabling monitoring of the same neural population over weeks. Like microbial opsins, these genetically encoded sensors can be introduced into neurons using a variety of methods, including viral vectors (84, 108) and mouse transgenesis (109, 110).

Genetically encoded Ca^{2+} indicators (GECIs) have made great strides in recent years through many iterations of optimization (74–89). These engineered proteins, which typically include both green fluorescent protein (GFP)-based components and Ca^{2+}-binding domains (83), report changes in local Ca^{2+} concentration as changes in fluorescence. Action potentials and synaptic input trigger Ca^{2+} influx into neurons over well-studied spatial distributions (111, 112), and GECIs can be used to report changes in these processes. The first GECI constructs had a signal-to-noise ratio that could be used to monitor bursts of action potentials, but not single spikes, whereas modern variants (optimized to be faster and more stable with improved signal-to-noise ratio) are fast

closing in on the goal of discriminating individual action potentials in vivo (89). One caveat is that Ca^{2+} signals may vary significantly across cell types owing to differences in ion channel expression, Ca^{2+}-binding protein expression, and other regulatory biochemical pathways, but many currently available Ca^{2+} indicators have been fully characterized only in excitatory pyramidal neurons.

Progress has also been made in modifying these proteins to fluoresce at different wavelengths, including in the red and blue spectral bands (80, 82, 113–115), which may eventually facilitate both dual-wavelength fluorescent imaging (for simultaneously imaging two different neuronal cell types) and combined imaging/optogenetic control, discussed below. Other constructs have been developed for optically reporting the release of specific neurotransmitters (90, 116), which may eventually complement technologies such as microdialysis (117, 118) or fast-scan cyclic voltammetry (119, 120). There has also been progress on the development of genetically encoded voltage indicators (GEVIs), which may ultimately enable monitoring of single spikes and subthreshold membrane potential in vivo (121–129), although improved signal strength and specificity are needed.

4.2. Population Monitoring with Single Fibers and Fiber Bundles

The development of hardware designed to image neural activity in freely moving mammals has proceeded in parallel with the development of genetically encoded activity indicators. These devices tend to be designed for certain broad categories of experiment: monitoring of bulk population neural activity (without cellular resolution), two-photon cellular imaging, and one-photon cellular imaging. These designs have most often been validated with chemical activity indicators, but recent work has seen a greater emphasis on integration with the genetically encoded activity indicators described above.

One recent focus has been on detection of bulk population neural activity using single fibers or fiber bundles (91–96). These relatively low-cost techniques have been used with success to study cortical Ca^{2+} waves, investigate the neural dynamics of whisker touch, and probe the circuit mechanisms underlying action initiation in the striatum (91–96). Rather than detecting the activity of single neurons, as with one-photon and two-photon head-mounted microscopy (discussed below), these lower-resolution detection and imaging approaches instead monitor total fluorescence changes driven by the activity of populations of neurons (130, 131) through a single fiber or bundle of fibers implanted over the brain structure of interest, often without the use of onboard microscopy. Correlated activity among relatively large groups of neurons produces a signal that can be measured using these methods, but these approaches are less useful for monitoring decorrelated population activity or the activity of small groups of rare neurons (91). Fiber-based methods are particularly straightforward for freely behaving rodent models because the flexibility and low weight of the implanted fiber or fiber bundle do not greatly interfere with natural behavior.

Early development of these in vivo population–recording fiber methods preceded the widespread use of GECIs and relied instead on bulk-loaded Ca^{2+}- or voltage-sensing dyes (132). First used in anesthetized preparations (133, 134), this method was quickly extended to freely moving mammalian systems. One group measured cortical Ca^{2+} waves in newborn mice using a single implanted multimode fiber for both excitation and detection (91). A Ca^{2+} dye was injected into cortex, and a 200-μm, 0.48-NA multimode fiber implanted over the same region. Photon collection was performed using a single photomultiplier tube (PMT), which enabled detection of bulk fluorescence intensity over time from a single cortical site, as well as observation of spontaneous cortical Ca^{2+} transients in the behaving mouse. Experiments in vitro with simultaneous bulk fiber imaging and two-photon imaging showed that signals detectable using this method

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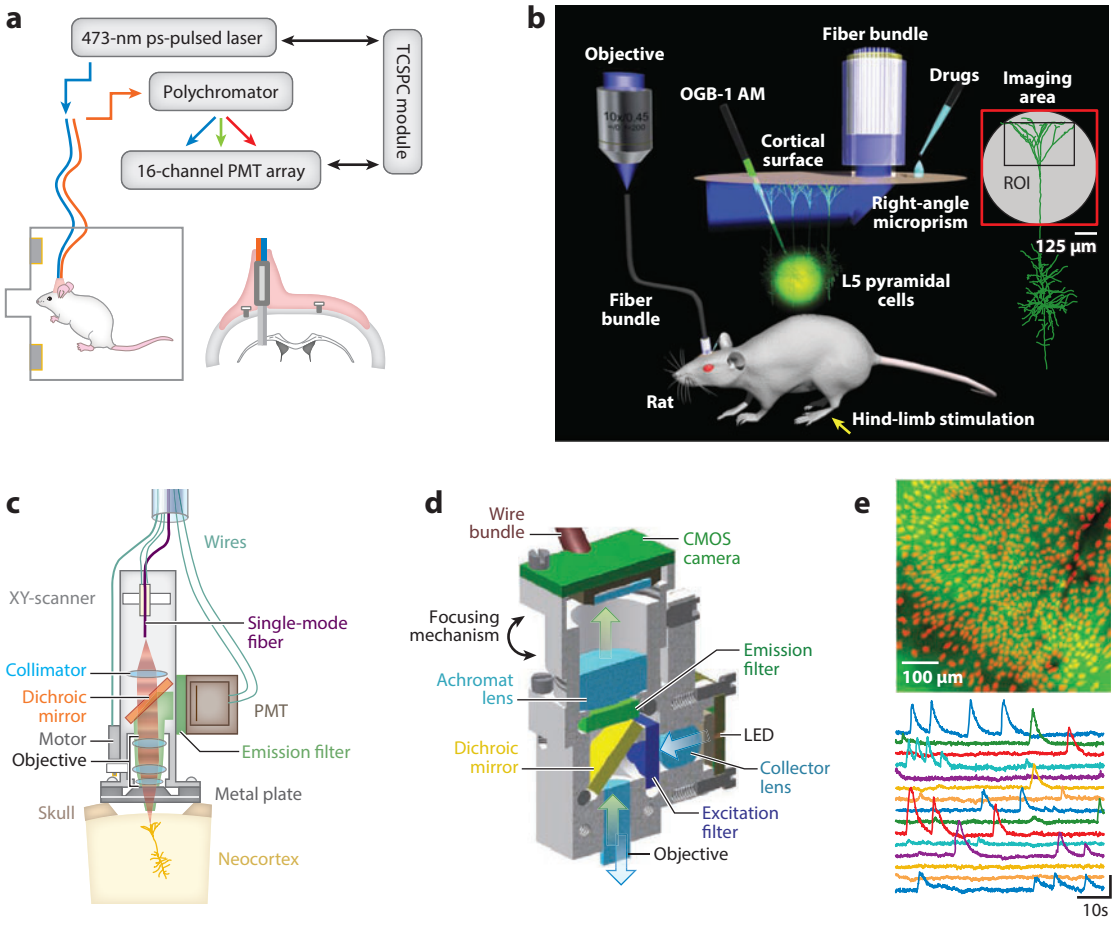

Figure 4

Optical-neural interfaces for readout of neural signals. Several approaches have been developed for optically monitoring neural signals in freely behaving mammals. (*a*) Fiber-based bulk tissue optical recording. A single multimode optical fiber is implanted over a brain area of interest in order to record fluorescence signals from neurons expressing genetically encoded Ca^{2+} or voltage indicators. This system is particularly suitable for use in freely behaving mice owing to the small form factor and minimal weight of the implanted fiber. (Panel modified with permission from 96.) (*b*) One-photon imaging through a fiber bundle permits the construction of population-scale two-dimensional images, which can be used to monitor layer-specific neural activity. (Panel modified with permission from 93a.) (*c*) Head-mounted two-photon microscopy suitable for imaging cellular-resolution cortical fluorescence signals in behaving rats (97). (*d*) A lightweight one-photon endoscopic microscopy system that can be used to image cellular-resolution neural activity in deep structures in behaving mice. (Panel modified with permission from 101.) (*e*) Ca^{2+}-imaging data collected with the system depicted in panel *d*. (*top*) CA1 principal neurons identified by Ca^{2+} imaging. (*bottom*) Ca^{2+} signals recorded from this neural population in a freely behaving mouse. (Panel modified with permission from 146.) Abbreviations: CMOS, complementary metal-oxide semiconductor; F, fluorescence; LED, light-emitting diode; OGB-1 AM, Oregon Green 488 BAPTA-1; PMT, photomultiplier tube; ROI, region of interest; TCSPC, time-correlated single-photon counting.

represented activity of thousands of cortical neurons, whereas asynchronous Ca^{2+} transients were not detectable by fiber (91). Another group used a similar single-fiber bulk detection method to study striatal pathways in action initiation (96) (**Figure 4*a***). This study targeted GCaMP3 (84), a GFP- and calmodulin-based GECI, to neurons belonging to the direct or indirect pathways of the striatum, using a single-mode fiber to deliver excitation pulses and a multimode fiber for detection.

A 473-nm picosecond-pulsed laser was used for excitation, a 16-channel PMT array was used for detection, and a time-correlated single-photon counting (TCSPC) module was used for photon counting, together enabling a quantitative description of neural activity in these two cell populations aligned with behavioral events in behaving mice. This method has recently been extended to permit monitoring of projection-specific neuronal firing (94). These single-fiber methods, by utilizing bulk detection of emitted light rather than cellular-resolution imaging, provide a low-cost complement to optogenetic control in freely behaving mammals and are made particularly powerful by the concomitant use of genetically encoded fluorescent activity indicators.

Fiber bundles have also been used to measure evoked fluorescence changes. One approach used a 300 × 300 bundle of small (8-μm) single-mode fibers with a voltage-sensitive dye in mice (92). This design, unlike the single-fiber approaches described above, allowed for the reconstruction of two-dimensional (2D) fluorescent signal maps from a patch of cortical tissue over time, though not detection of signals from individual identified neurons. Excitation and imaging were performed through the same bundle of fibers. Although the fiber bundle was relatively large (with a diameter of several millimeters), free movement and natural behavior in mice were possible because the component fibers were left unglued and free to flex in the middle of the bundle. This technique would be most appropriate for surface cortical recordings because the bundle size would likely cause too much tissue damage in accessing deep brain structures, although as with hippocampal imaging, overlying cortex can be aspirated to image underlying structures in vivo.

Advancements in fiber bundle imaging enabled imaging of dendritic Ca^{2+} signals in rat cortex (93, 93a) (**Figure 4*b***). This design also utilized a bundle of small single-mode fibers, but in this case the bundle was attached to a gradient refractive index (GRIN) lens with a right-angle microprism at the end. This cortical periscope was implanted in the upper cortical layers, enabling transverse imaging of the superficial dendrites of deep cortical neurons following layer 5 (L5) loading of Ca^{2+} indicator. This positioning allowed the investigators to measure population Ca^{2+} signals specifically from the distal dendritic tufts of L5 neurons without signal contamination from deeper cortical layers. One group (95) recently reported, for use in mice, an adaptation of this device in which GECIs were used instead of bulk-loaded dyes, opening the door to observing the activity of specific populations of genetically defined neurons.

Future experiments using this technology are likely to focus primarily on the use of genetically encoded fluorescent activity indicators, rather than bulk-loaded dyes. Dyes are typically useful in experiments for which neural activity is imaged for a few minutes to hours following dye injection and device implantation, whereas genetically encoded indicators enable repeated imaging of the same brain structure after implantation over days to weeks. Choice of a single fiber or fiber bundle depends on application, but single-fiber implementations are particularly attractive for off-the-shelf setup, low cost, and minimal effects on movement in the freely behaving rodent.

4.3. Cellular Imaging with Head-Mounted Two-Photon Microscopy

A prime goal of systems neuroscience is to read out neural activity—ideally at high enough temporal and spatial resolution to detect single action potentials in individual neurons, but with sufficient scope to encompass hundreds to thousands of genetically identified neurons spanning the relevant neural circuits in freely behaving animals. Cellular-resolution one-photon and two-photon head-mounted intracranial microscopies bring the field closer to achieving this goal (102, 103).

Imaging neural activity in the brain is complicated by strong light scattering and absorption in neural tissue (135, 136). One-photon microscopy generally cannot be used to image the activity of single neurons deeper than 50–100 μm (99, 137) because of these factors, but two-photon imaging (135, 136, 138) makes use of near-infrared excitation wavelengths that can penetrate farther into

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neural tissue and excite only a small volume at the focal plane, enabling imaging at depths of up to 500 μm (139, 140) with superior resolution, reduced background fluorescence, and greater robustness to scattering. This is a significant improvement, but it comes at a cost of scanning time (in part addressed by recent advances in resonance scanning) and a smaller field of view, as discussed below. Other challenges associated with the application of these technologies to freely behaving mammals include miniaturization of microscopy hardware and minimization of motion artifacts (which can arise from movements of imaging hardware relative to the skull and/or brain pulsation, presenting considerable difficulties in the behaving animal). Some of these problems may be avoided by imaging in head-fixed animals, but this approach currently precludes the use of many standard laboratory behavioral tests.

A miniaturized head-mountable scanning two-photon microscope for use in freely behaving rats (97) (**Figure 4*c***) weighed in at 25 g and spanned 7.5 cm, which is large but not exceeding standard dimensions of the tetrode hyperdrive in common use for high-density single unit neurophysiological recordings in behaving rats. As in standard two-photon microscopy, near-infrared excitation light was provided by a Ti:Sapphire laser, but in this design the light was coupled to a 2-m flexible single-mode optical fiber tether for transport to the head-mounted hardware; the tether also contained electrical lines for power and data transmission. The fiber was mounted on a miniature piezoelectric plate that could be driven at the resonant frequency of the fiber (typically 300–800 Hz), causing vibration of the fiber tip and enabling scanning of the focal spot across neural tissue. A stiffening rod was added along one side of the fiber, creating two orthogonal resonant frequencies. The piezoelectric plate could be driven with a superposition of these two frequencies, enabling 2D scanning in a Lissajous pattern across a patch of neural tissue; a single resonant frequency could be used for higher-resolution line scanning. The actual movement of the fiber tip was phase-shifted from the driver, requiring calibration before implantation and experimentation. Excitation and barrier filters, a dichroic mirror, imaging lenses, and a PMT were additionally miniaturized and contained within the housing of the head-mounted hardware, which was mounted above a cranial window. As only a small volume of neural tissue was excited at any given time, all of the emitted light from this focal spot could be collected by the PMT and assigned to the correct pixel to produce an image. With this design, it was possible to image 200–250 μm below the cortical surface in L2/3, to resolve dendritic features, and to measure dendritic Ca^{2+} transients in the awake behaving rat. Although imaging quality was quite good with this system, significant motion artifacts were encountered; imaging during slow movement and rest was possible, but quick head movements led to permanent shifts in imaging frame, limiting utility for some behavioral paradigms.

Motion artifacts are a crucial issue in behaving two-photon microscopy (both freely moving and head-fixed) and are exacerbated by the relatively small field of view and long scan times of this imaging modality (97). These artifacts may arise from device movement relative to the skull, heartbeat, breathing, licking, and animal motion, all of which interfere with image acquisition (141–144). There has been recent progress toward minimization of these artifacts using heartbeat-triggered scanning (143), licking correction with a tunable lens (144), and an adaptive imaging-based movement compensation system (142). Although much of this movement compensation has been developed in head-fixed or anesthetized preparations, application of these technological innovations to head-mounted imaging should enable higher-quality data acquisition.

The development of endoscopic two-photon imaging in freely moving mice (98) represented a key advance, enabling deep-brain two-photon imaging—importantly, in a genetically tractable animal. Fluorescence microendoscopy can be used with either one- or two-photon excitation for imaging of deep tissues, typically with compound GRIN lens–based microendoscopic probes. This approach was initially tested in head-fixed animals (102, 145) and then incorporated into

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elegant devices suitable for freely moving mice (98). The latter application was made possible by a significant reduction in device mass and size but used the same basic strategy for optical scanning—fiber vibrated in a Lissajous pattern, enabling scan fields spanning 145 to 215 μm. Reduction in size was due largely to replacing internal optics with small lightweight GRIN lenses, and spatial resolution was increased by using a flexible photonic bandgap fiber with an air core, rather than a single-mode fiber, for carrying the excitation light (this modification enables transmission of ultrashort pulses free of distortion). The lack of an onboard PMT also aided in weight reduction; emitted photons were collected instead by a multimode fiber for detection on the bench top. The device had a lateral resolution of 1.21 μm and an axial resolution of 9.8 μm, which represent a significant advance enabling definition of single neuronal cell bodies in vivo.

4.4. Cellular Imaging with Head-Mounted One-Photon Microscopy

Rapid progress has been made in miniaturization of one-photon epifluorescence imaging hardware for freely behaving mammals and has already led to key scientific results that would not have been possible with standard neurophysiological techniques (101, 146). Penetration depths are not as good as with two-photon microscopy (discussed above), but the faster frame rates characteristic of one-photon imaging reduce motion artifacts and greatly facilitate cellular imaging during natural behavior.

An early version of this kind of microscope (99), weighing only 1.1 g and therefore suitable for use in behaving mice, allowed imaging of single neurons during natural behavior at frame rates up to 100 Hz. This major weight reduction was made possible by moving most of the hardware, including illumination source and detector, filters, and dichroic mirror, off the animal and onto the tabletop; only three GRIN lenses and a focusing motor were mounted directly on the mouse. Light was transported to and from the animal by a high-density fiber bundle, which was coupled to an optical commutator for freedom of movement. The device was designed to make use of either 1.4-mm or 6.2-mm endoscopic GRIN lens objectives, which could be used for surface or deep imaging, respectively. It was possible to obtain a 240–370-μm field of view, with lateral resolution of ~2.8 μm and 10-μm depth of field, which were stable through normal movement. With this device, Ca^{2+} transients could be imaged in the dendritic arbors of individual cerebellar Purkinje neurons.

A significant advance toward commercialization and widespread use of this technology was achieved a few years later (101) (**Figure 4*d***). Although the concept of this device was similar to the one-photon epifluorescence microscope described above, almost everything in this instantiation was moved to the head-mounted hardware, without a prohibitive increase in weight (1.9 g). Using an integrated light source (a blue LED), excitation filter, dichroic mirror, objective, barrier filter, focusing mechanism, and complementary metal-oxide semiconductor (CMOS) camera enabled the use of only-electrical leads to the animal for power and data transmission in the tether—a significant advance for greatest flexibility. In addition, the device was made largely from mass-producible parts, including the semiconductor light source and camera, which may enable widespread, low-cost use in the field. The microscope can be used to image individual neurons from freely behaving mice in a field of view of 600 to 800 μm with lateral resolution of 2.5 μm, and it can be run at 36 or 100 frames per second, allowing the acquisition of high-quality neural population data.

An initial application of this microscope was to image Ca^{2+} transients in over 200 simultaneously recorded Purkinje neurons, in the process identifying previously unsuspected high-order correlations in up to 30 neurons, organized by cerebellar microzone, during motor behavior (101). Subsequent experiments yielded equally surprising results in the CA1 subfield of the dorsal

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hippocampus (146) (**Figure 4*e***). In this series of experiments, the authors tracked thousands of place cells over several weeks in freely behaving mice, with yield an order of magnitude higher than typical neurophysiological recordings with tetrode hyperdrives. With this new technology, long-term stochasticity in place field expression was observed that would not be detected with standard electrophysiological techniques, as it is generally not possible to record the activity of a single identified neuron over the course of several days. With cellular-resolution imaging, however, frames could be spatially registered from day to day with certainty of neural identification, enabling true long-term monitoring of neuronal activity. Although place fields of individual monitored neurons did not change with time, cells fluctuated between active participation in place coding and quiescence on a daily basis. Two-photon and one-photon imaging in freely moving mammalian systems each possess key advantages, but one-photon microscopy is currently more robust to imaging disruptions caused by animal movement. Although it is not possible to image more than 100 μm deep into neural tissue with this technology, this limitation has not proved prohibitive for the demonstration of new and important scientific results.

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5. FUTURE DIRECTIONS

Promising domains of exploration in the coming years will involve development of advanced optical neural interfaces for increasingly sophisticated research applications. And beyond basic science applications, clinically inspired devices such as peripheral nerve cuff optogenetic devices designed to wrap around nerves (much like the vagus nerve stimulation cuffs used for epilepsy and depression, but with LED outputs instead of electrode contact outputs) are being tested in animal models (147–149) and may in the long run even lead to clinically applicable optical neural interfaces.

In the basic science realm, important long-term directions for optical control devices include fast 3D cellular-resolution, spatially addressable light patterning, minimization of optoelectronic artifacts, advanced development of LEDs and other light-generation technologies, and higher-powered wireless control. Long-term goals for optical readout devices include faster and deeper imaging with higher resolution, improved image stability during free behavior, and reliable single-spike detection in vivo during behavior. And integrative optical neural interfaces for combined imaging and control will be a major area of further development, as all-optical interrogation of neural activity is likely to powerfully advance the implementation of sophisticated closed-loop experimental designs for flexible and precise read-write circuit-level control in freely behaving mammals.

In the simplest forms of such an integrative all-optical system, fiber-based population measurements may be combined with optical control using the same implanted probe for imaging and light delivery; the input and output optical channels must be separable, but progress along this front has already taken steps through development of spectrally shifted optical indicators and actuators (12, 20, 25, 80, 82, 113). However, the excitation spectrum of GCaMP6 probes (89) (perhaps the best currently available family of GECIs) substantially overlaps the activation spectra of ChR2 (8, 10), C1V1 (ChR1/VChR1 chimaera) (20, 21), NpHR (9, 11), and Arch (archaerhodopsin-3 from *Halorubrum sodomense*) (15, 21), the most effective optogenetic control tools. Compounding this challenge in combining imaging and optogenetics is overload of imaging PMTs when opsins are stimulated (as they typically would be) at much higher power than is required for imaging. One approach to this problem with combining readout and control would be to use the SFO ChR derivatives, which confer orders-of-magnitude-increased light sensitivity to expressing cells (19–21). Although the most sensitive SFO tools have not yet been applied in this context in freely behaving mammals, a T159C ChR mutant that confers higher light sensitivity on expressing cells

while retaining good temporal precision (19, 21) has been applied in combination with RCaMP, a newly developed red GCaMP derivative with the eGFP domain replaced by circularly permuted mRuby (82). These two constructs work well together with minimal cross talk as long as relatively low light powers are used (e.g., 56 mW/cm^2). However, other red-shifted indicators (mApple-derived R-GECO and RCaMP1.07) artifactually photoswitch under intense blue light by altering red fluorescence even in the absence of Ca^{2+} changes, and therefore should be combined with optical actuators only in the setting of appropriate controls (113, 114). Many other highly light-sensitive engineered opsins, and other input/output combinations such as blue/green spectrum GCaMP indicators combined with red-spectrum C1V1-family opsin actuators, should be explored with this motivation (21). These issues underscore that it will be essential for the development of optical neural interfaces to proceed tightly intertwined with the development of the biological wetware and in the setting of the relevant in vivo biological applications.

A final area of growth will be the development of optical neural interfaces that allow not only imaging at single-cell resolution but also optical stimulation with single-cell resolution, in freely behaving mammals. Single-cell optogenetic stimulation has already been achieved in cortex of living mice using the C1V1 channelrhodopsin and raster-scanning two-photon illumination (150); this kind of setup would already be compatible with behaviors measured in head-fixed mice using existing technologies. Next-generation optical interfaces for single-cell-resolution stimulation in behaving mammals may enable independent control over multiple isolated cells with user-defined temporal relationships and may be based on digital micromirrors, light field illumination, and/or holographic spatial light modulators (150–152). Miniaturization and adaptation to mammalian behavioral setups will be crucial fronts in this exciting and expanding field.

In summary, recent years have witnessed the rapid development of paradigm-shifting technologies for both optical control and readout of neural activity in freely moving mammalian systems. Genetically encoded optical actuators together with devices for intracranial light delivery have made it possible to selectively modulate neural activity, and genetically encoded fluorescent indicators and devices for head-mounted imaging have allowed the detection of activity in defined populations and individual cells. As the molecular tools and dedicated hardware continue to advance in close association, the transformative opportunities for basic science and preclinical investigation will continue to expand rapidly. These technologies are already enabling groundbreaking discoveries in neuroscience, and defining a new era in the study of neural circuits and behavior.

DISCLOSURE STATEMENT

M.R.W. and K.D. have disclosed aspects of the work described here to the Stanford Office of Technology Licensing, which has filed a patent application for the possible use of the findings and methods. K.D. is a cofounder of Circuit Therapeutics Inc., which has licensed some of these technologies to develop treatments for peripheral nervous system interventions to treat disease.

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available for academic and nonprofit research in perpetuity through the Deisseroth Lab optogenetics website (**www.optogenetics.org**).

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