
Optical Stimulation of Neural Circuits in Freely Moving Animals

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14.1 INTRODUCTION

Despite all that we have learned about the nervous system, from molecules to networks, we still cannot draw the schematics of, for example, curiosity. Nevertheless, bridging the gap between psychology and biology is a key goal for the neuroscience community (Human Brain Project, n.d.). In order to actually link a behavioral function to its underlying neural mechanisms, animal models must be free from anesthesia and constraints, thus allowing neural circuits to be probed during the behaviors that engage them. Hence, bridging the psychology-biology gap depends upon freely moving animals (Figure 14.1).

Merely observing which aspects of neural activity are correlated with a given behavior may provide invaluable information about the system, but to causally define it, the underlying circuit dynamics must be manipulated. Over the two centuries since Galvani established electricity as the “language” of the nervous system (Galvani, 1791), investigators have continuously perfected our capability to “converse” (i.e. record and manipulate) with neurons (Patil and Thakor, 2016). However, electrical stimulation, a canonical tool for manipulating neuronal activity, exhibits well-documented

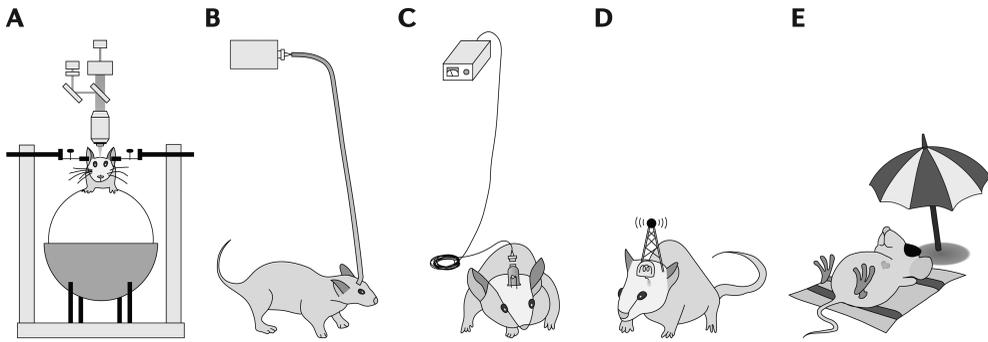


FIGURE 14.1 Concepts of optical stimulation systems. The delivery of light to the brains of behaving rodents can be divided into strategies based on the provided degrees of freedom. (A) Benchtop opto-stimulators (e.g. a two-photon microscope) can be used to record and/or manipulate neuronal activity in head-fixed mammals. (Redrawn with permission from Figure 14.1A in Dombeck et al., 2007.) (B) Animals large enough to receive implants can be tethered to optical fibers (Gradinaru et al., 2007). (C) By placing the light source/s on or in the head of the animal, rigid optical fibers can be replaced with flexible electrical wires, upscaling the number of independently controlled targets and providing many more degrees of freedom (Stark et al., 2012). (D) Head-mounted light sources can be powered without a tether, by a battery or wireless power transfer (Wentz et al., 2011). (E) Closing the loop between neural activity and optical stimulation on the head may enable pacemaker-like user-independent systems.

disadvantages (Tehovnik, 1996), including: (1) limited spatial resolution: we can either stimulate a few isolated neurons or an ill-defined cluster of cells, and (2) low specificity: it is almost impossible to control which cell type is excited. These detriments have been overcome by utilizing light as a stimulating agent (Figure 14.2).

In 2002, Zemelman and colleagues demonstrated that neurons can be genetically manipulated into becoming light-sensitive. Three years later, “optogenetics” took the neuroscience community by storm when the single-component, light-sensitive cation channel Channelrhodopsin-2 (ChR2) was expressed in neurons (Boyden et al., 2005). The ability to selectively activate and/or silence a genetically defined population of neurons with millisecond temporal precision and high throughput, in the absence of direct contact between stimulus and target, marked a new era in neural manipulation.

Since then, continuous efforts have been made to fulfill the promise of optogenetics. From the hardware perspective, the concept is simple: we must shed the right amount of light at the right place at the right time. In reality, the task has proven arduous: the ideal light delivery device should be able to utilize various tunable wavelengths to illuminate, with cellular spatial resolution and sub-millisecond temporal precision, multiple targets positioned deep within the brain while simultaneously recording multi-neuronal activity. And, all this must be done in freely moving animals. Given the technology available today, there are many tradeoffs between these requirements; some of them are even mutually exclusive. Consequently, devices for optical stimulation have typically been designed to answer a pre-defined set of research questions, and no single strategy can be used for all purposes.

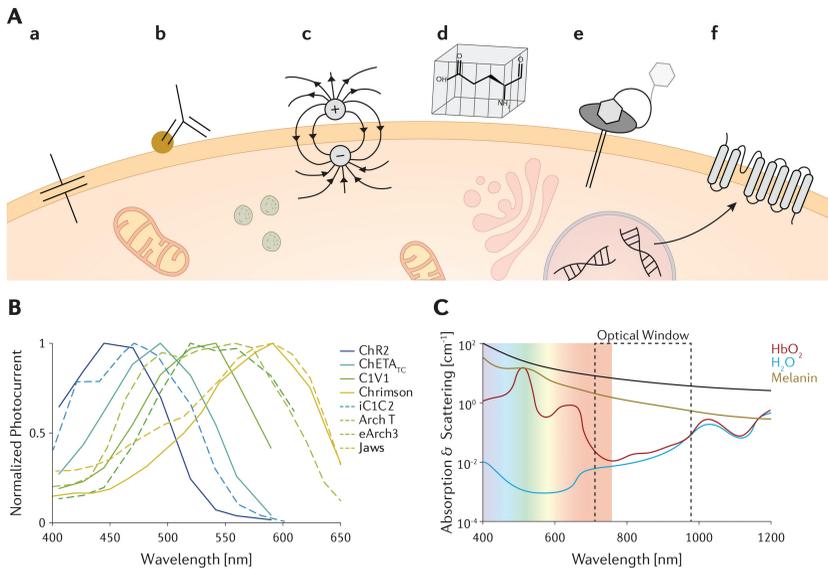


FIGURE 14.2 Optical control of neural activity. **(A)** Optical stimulation methods. **(a)** Infra-red (IR) radiation absorbed by water produces a temperature increase, which transiently alters membrane capacitance and generates depolarizing currents (Shapiro et al., 2012). **(b)** Photothermal effects can be augmented by gold nanoparticles conjugated to antibodies (Carvalho-de-Souza et al., 2015). **(c)** Optoelectric devices (e.g. quantum dots) act as photo-responsive electrodes that generate an electric field when exposed to light (Bareket-Keren and Hanein, 2014). **(d)** Bioactive compounds (e.g. glutamate) can be “caged” within photolabile protecting groups (Callaway and Katz, 1993). **(e)** Photoisomerizable molecules which undergo a reversible conformational change in response to the absorption of two different wavelengths can be tethered to a target protein (e.g. receptor), yielding a photosensitive actuator (Volgraf et al., 2006). **(f)** Microbial light-sensitive channels (opsins) can be encoded within the mammalian DNA (Boyden et al., 2005). **(B)** Action spectra of selected depolarizing (solid lines) and hyperpolarizing (dashed lines) opsins. Note coverage of the visible spectrum. Data adapted from Berndt et al., 2014; Chuong et al., 2014; Klapoetke et al., 2014; Mattis et al., 2012. **(C)** Absorption (colored lines) and scattering (black line) coefficients of biological tissue. Note that the ideal optical window is at near- and infra-red wavelengths (~650–950 nm, dashed box), which is distinct from the action spectra of microbial opsins. (Data adapted from Jacques, 2013.)

The goal of this chapter is to describe current approaches for optical stimulation of neural circuits in freely behaving animals. Specifically, we focus on opsin-based optogenetics and limit the exposition to miniaturized devices compatible with rodents, since these designs can then be adapted to larger animals. The content is organized according to the degrees of freedom for animal movement that the different techniques provide (Figure 14.1).

14.2 HEAD-FIXED

We begin our discussion on optical stimulation with optical recordings, since the two should be distinguished in the context of freely moving animals. Optical imaging of genetically encoded biosensors is complementary to electrical recordings in a similar way that optical stimulation in the context of optogenetics is complementary to electrical stimulation, i.e. it

may provide better spatial resolution and specificity. However, one technical problem hinders imaging in freely moving animals, namely, the microscope. The detection of intrinsic signals and/or fluorescence requires optics that are difficult to miniaturize, and some optical recording methods (e.g. two-photon microscopy) also require high-energy, active photostimulation (Dombeck et al., 2007). Implantable, head-mounted “miniscopes” are under continuous development (Hamel et al., 2015; “UCLA Miniscope,” n.d.), but such devices have yet to demonstrate optogenetic stimulation capabilities. Consequently, to preserve conventionally sized instrumentation, all-optical neurophysiology, i.e. using light to record and manipulate neural activity, has only been applied to freely moving worms (Leifer et al., 2011) or head-fixed mammals (Packer et al., 2015; Rickgauer et al., 2014; Figure 14.1A).

14.3 FIBER-TETHERED

The first freely moving rodents to be optogenetically manipulated were tethered to an optical fiber (Aravanis et al., 2007; Figure 14.1B, Figure 14.3, Figure 14.7Aa). With one end of the waveguide coupled to an external light source and the other inserted into the brain, this simple interface has become a standard approach for integrating optogenetics with behavioral studies.

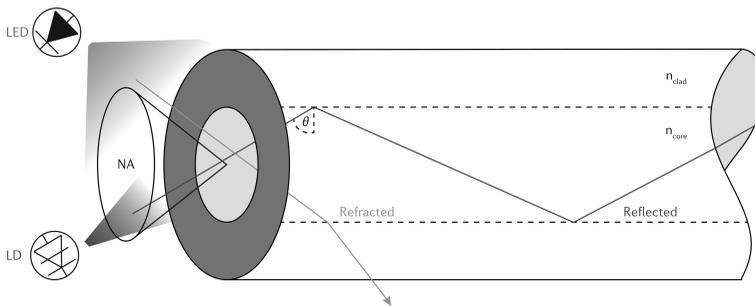


FIGURE 14.3 Optical waveguides. At the border between two media with different refractive indices (propagate light at different speeds), if the angle of the incident light ray is smaller than a particular critical angle (θ), light is transmitted through the second medium with a change in direction (refracted, blue); otherwise, it is reflected (red). The waveguide used most often in neuroscience research is the optical fiber (Keiser et al., 2014), composed of a core and cladding layer with different refractive indices ($n_{\text{core}} > n_{\text{clad}}$). Light transmitted through the core is trapped by reflection at the core-cladding interface, allowing it to propagate along the fiber with minimal losses. Light is coupled into the fiber if it falls within its acceptance cone, described by the numerical aperture:

$NA = \sqrt{n_{\text{core}}^2 - n_{\text{clad}}^2}$. Efficient source-fiber coupling is greater when the source has a small area, when the fiber has a large input diameter and high NA, when the source and fiber are placed in close proximity, and when the source, medium, and fiber have as similar refractive indices as possible (Stark et al., 2012). Both light-emitting diodes (LEDs) and laser diodes (LDs) can be coupled to fibers, but since LDs produce \sim Gaussian (i.e. coherent) beams and LEDs present \sim Lambertian (i.e. wide angle) emission profiles, the coupling efficiency of LDs is at least an order of magnitude higher than of LEDs (Stark et al., 2012). Conversely, LDs may be larger, consume more current, and generate more heat than LEDs. See also Figure 14.6.

Although light can affect the nervous system without even touching the animal (Lima and Miesenböck, 2005), one of the greatest potentials of light as a stimulating agent – non-invasiveness – has limited practical applicability due to the optical properties of biological tissue. Even when the skull is removed and the furthest-reaching wavelengths (i.e. infrared; Figure 14.2B) are utilized, surface illumination cannot penetrate deeper than ~500 μm while maintaining cellular spatial resolution, and is therefore typically accompanied by cortical excavation (Rickgauer et al., 2014). In contrast, waveguides not only release the animal from the bench, but also grant access to the entire depth of the nervous system while displacing no more than their own volume of tissue.

To better confine the distribution of exiting light and alleviate some of the pressure on neural tissue, waveguide dimensions can be greatly reduced by *de novo* fabrication (Wu et al., 2013), chemical etching in acidic solution (Royer et al., 2010), machining (Ozden et al., 2013), or heating and pulling (“tapering”: Godwin et al., 1997, Figure 14.4A). Narrowing a single end of a fiber is advantageous also in terms of coupling efficiency: the larger input aperture can accept more light, while the other, smaller, output aperture can produce higher irradiance.

Fiber bundles, comprised of 1–100,000 fiber cores sharing a common cladding, have been used as brain-insertable microendoscopes, where each fiber core transmits one “pixel” of the final image (Flusberg et al., 2005). However, fiber bundles can also relay light in the other direction (Figure 14.5). For instance, multiple targets can be sequentially

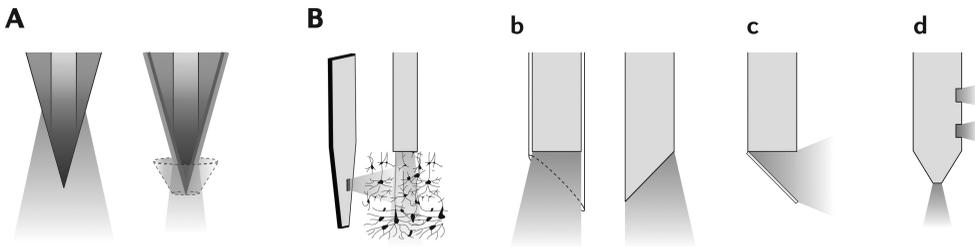


FIGURE 14.4 Waveguide manipulation. **(A)** During tapering of an optical fiber (left), core and cladding might be melted and mixed together, resulting in a homogeneous medium that can no longer serve as a waveguide. Consequently, a sizeable fraction of the light originally confined in the core is lost through the sidewalls of the taper (Godwin et al., 1997). This byproduct can be bypassed by coating the tapered fiber with reflective material (right), which can in turn serve as a recording electrode (Zhang et al., 2009). A problem with such coaxial optrodes is that the electronically monitored (within dashed line) and illuminated (blue shade) regions do not fully overlap. **(B)** Solutions to direct emitted light. **a.** μLEDs can provide lateral (laminar-specific) illumination (left), whereas waveguides preferentially illuminate a cone of tissue below the waveguide (right). **b.** Side-biased illumination can be produced by encasing a fiber in a beveled metal cannula (left; Tye et al., 2011) or by machining the fiber tip (right; Pashaie and Falk, 2013). **c.** Side-limited illumination can be achieved by placing a miniature mirror at the waveguide end (Zorzos et al., 2010). **d.** Multi-site illumination using a single waveguide is produced by etching multiple apertures and manipulating the coupling strategy (e.g. the power and angle of input light; Pisanello et al., 2014) or by wavelength division multiplexing (Segev et al., 2015).

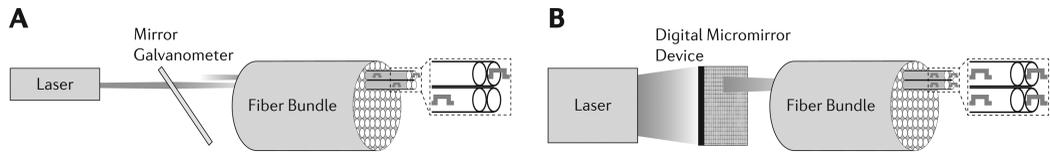


FIGURE 14.5 Patterned illumination using fiber bundles. (A) A mirror galvanometer, resonant scanner, or acousto-optic device can rapidly scan a single focused laser beam across multiple positions. (B) Parallel illumination techniques allow all targets to be illuminated simultaneously via spatial light modulators (e.g. a digital micromirror device). The temporal resolution of the latter approach is inherently higher, but available light power is divided between multiple targets, limiting the number of targets that can be illuminated at a given input power. All strategies based on fiber bundles require surface mounting, cortical excavation, and/or considerable volume of compressed/displaced neuronal tissue.

activated by rapidly positioning a galvanic mirror across different cores of the fiber bundle (Hayashi et al., 2012), or alternatively, they can be simultaneously activated by passing the laser beam through a digital micromirror device (DMD: Szabo et al., 2014; Zorzos et al., 2012). These two approaches for controlling multiple targets represent some of the possibilities for patterned illumination (Emiliani et al., 2015).

14.4 WIRE-TETHERED

Although optical fibers are commercially available and relatively straightforward to use, they are usually made of glass (silica) and thus stiff and intolerable to sharp bends. Consequentially, optical fibers tethered to a behaving animal may suffer from decreased transmission efficiency, irreversible physical damage (breakage), restriction of animal movement, or all three. While mechanical compliance can be improved using a rotary joint (optical “commutator”; Gradinaru et al., 2007), or fabricating flexible polymer fibers (Canales et al., 2015), such solutions are not scalable with respect to the number of independently illuminated targets.

Compared to optical waveguides, electrical wires provide many more degrees of freedom. Substituting the fiber with a wire enables using miniaturized light sources (light emitting diodes, LEDs, or laser diodes, LDs; Figure 14.6), moved from the bench onto the animal’s head. Light can then reach the neuronal target through a cranial window (Huber et al., 2008) or, if deeper regions and/or higher resolutions are of interest, via optical fibers (Stark et al., 2012), or fabricated waveguides (Kampasi et al., 2016; Schwaerzle et al., 2013) confined to the implant. In this manner, instead of delivering light from a remote light source via rigid fibers, electricity can be delivered from a remote power source via highly flexible lightweight wires (e.g. multi-stranded Litz wires: Stark et al., 2012).

Moving the light source from the bench to the head is one conceptual leap; the natural next step is to insert the light source into the brain of the animal. In 2013, advances in micromachining techniques enabled the production of miniature light sources, i.e. μ LEDs (Cao et al., 2013; Kim et al., 2013; McAlinden et al., 2013). When implanted within the brain, μ LEDs present three unique properties. First, they bypass the delicate process of

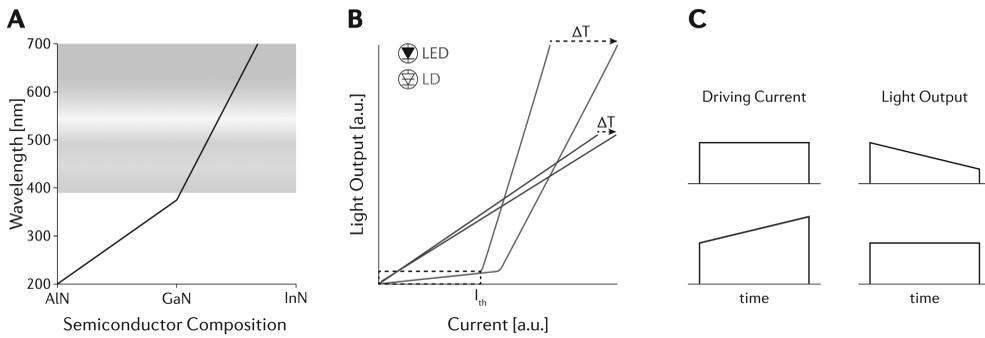


FIGURE 14.6 LDs and LEDs. **(A)** In LEDs, the emitted light wavelength depends on the energy gap between the p- and n-type semiconductors. Thus, a wide spectrum of colors from red (644 nm) down to UV (197 nm) can be achieved by varying the materials forming the diode. Semiconductors typically used include aluminum nitride (AlN), gallium nitride (GaN), indium nitride (InN) and their alloys. Values adapted from Schubert, 2006. **(B)** Current-to-light conversion (I-P transfer function) for LEDs (red) and LDs (blue) at two different temperatures. Although both LEDs and LDs exhibit a non-linear voltage-to-current relationship (V-I transfer function, not shown), these two diode types differ in the way they convert electric power to light. The I-P relationship of LEDs (red) is nearly linear (until the maximal light output), simplifying its control. In contrast, LDs (blue) initially behave as typical LEDs but then, upon crossing a threshold (I_{th}) for stimulated emission, rapidly increase their light output. Even though the light output at this “sub-threshold” domain (dashed box) is minuscule, it may affect low-light applications (e.g. optogenetics) and hence cannot be neglected. **(C)** I-P transfer functions, especially of LDs, are strongly dependent on device temperature, resulting in light power variance due to a poorly-controlled variable in a positive feedback loop: as temperature rises, more current must be applied to maintain light output constant, which further increases the heat. Temperature can be easily controlled on the bench, but is more challenging to stabilize in miniature implanted devices.

optical coupling by eliminating the need for a waveguide. Second, due to their size and lack of coupling losses, μ LEDs have very low power requirements compared to LEDs or lasers, and efficient optical stimulation can be achieved with nano-watt scale light (Wu et al., 2015). Third, the illumination path emitted from on-probe μ LEDs is perpendicular to the diode surface, providing a natural way to stimulate cells in a laminar-specific manner (Figure 14.4B). Two major concerns with implanted diodes are (1) tissue integrity, which strongly affects the performance of all implantable devices (Figure 14.7); and (2) thermal management, since all of the electrical energy that is not converted to light is released to the tissue as heat (typically over 95%; Wu et al., 2015). Nevertheless, experiments with mice implanted chronically with multi-site diode-probe arrays (Stark et al., 2015) and with μ LED-probe arrays (Wu et al., 2015) have shown that the generated heat by itself is insufficient to modify neuronal activity.

14.5 WIRELESS

Flexible as they may be, electrical wires constrain animal movement, are at constant risk of damage, and limit the range of possible behaviors. The only way to circumvent these shortcomings is to remove the tether and provide power to head-mounted light sources by

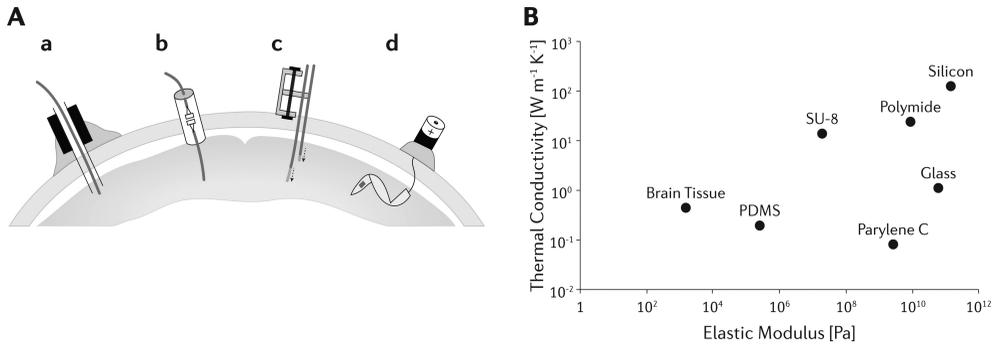


FIGURE 14.7 Implant-brain mechanical interaction. (A) Implantation strategies. (a) Removable fiber. A metal tube (“cannula”) can be fixed to the skull, through which an optical fiber is inserted at the beginning of every experiment (Aravanis et al., 2007). (b) Permanently-implanted fiber. A mating sleeve (“ferrule”) can be used to connect two fibers, one fixed inside the brain, the other coupled to an on-bench light source, thus reducing the tissue damage caused by repeated insertions (Sparta et al., 2012). (c) Movable fiber/s. A microdrive capable of independently maneuvering multiple devices (optical fibers, diode-tetrode, etc.) can be used to modify the depth of each device over extended durations (Stark et al., 2012). (d) Fiber-less devices. Instead of a stationary implant secured to the skull, an entire device can be implanted within the parenchyma, permitting movement with the brain rather than with the skull (Jeong et al., 2015). (B) Thermal conductivity and rigidity (elastic modulus) of the materials used for neural implants are major concerns when optimizing tissue integrity. (Data adapted from Hassler et al., 2011; Kaster et al., 2011; Scholten and Meng, 2015; Wu et al., 2015.)

a head-mounted battery (Iwai et al., 2011; Jeong et al., 2015) or via wireless power transfer (WPT; Figure 14.1D). WPT technologies can be divided into three strategies, according to the properties of the applied electromagnetic energy (Table 14.1). (1) At a certain distance from the emitting source ($r > 2\lambda$), the “far-field”, electromagnetic energy radiates as planar electric and magnetic waves perpendicular to each other and to the direction of propagation, and can be harvested using radio-frequency scavenging (Kim et al., 2013; Park et al., 2015). (2) At distances close to the emitting source ($r \ll \lambda$), the “near-field”, emitted energy is stored as either electric or magnetic fields, and can be harnessed via inductive coupling (Wentz et al., 2011). (3) At the transition zone between the near- and far-fields ($r \sim \lambda$), the “midfield”, electromagnetic irradiance can be tailored to the dielectric properties of the animal, and thus provide power via inductive coupling to micro-implants (Ho et al., 2014; Montgomery et al., 2015).

TABLE 14.1 Wireless Power Transfer Techniques

	Near-field	Midfield	Far-field
EM source	Coils (LC circuits)	Resonant cavity (metal plates)	Antennas
Transmission frequency	<20 MHz	1.5 GHz	910 MHz
Dimensions	2 g, 1 cm ³	20 mg, 10 mm ³	~1 g, ~20 mm ³
Freedom of movement	20-cm diameter arena	21-cm diameter arena	~1 m
Reference	Wentz et al. (2011)	Montgomery et al. (2015)	Kim et al. (2013)

Despite these advances, currently available tether-free designs fail to provide the desired experimental freedom; WPT technologies suffer from low efficiencies and require confined environments (e.g. 21-cm diameter: Montgomery et al., 2015), while batteries add considerable weight to the animal's head and limit the duration of experiments. Moreover, when combining electrical readout with optical stimulation, removing the tether necessitates the recorded data to be stored on the animal's head and/or transferred telemetrically. A potentially promising third direction for wireless devices is based on biological energy harvesting (e.g. muscle movements; Dagdeviren et al., 2014).

14.6 ELECTRICAL READOUT

Manipulating neurons with light can make a fly jump (Lima and Miesenböck, 2005), a worm withdraw (Nagel et al., 2005), a mouse turn (Huber et al., 2008), and a monkey gaze sideways (Cavanaugh et al., 2012). Yet, understanding how this happens requires more than simply measuring the end result; neuronal activity must be registered. Electrical recordings enjoy a ground-truth status since they are inherent to neural communication, and indeed, many of the classical electrical recording techniques have been used alongside a separate optical stimulation device (e.g. tungsten electrode and optical fiber, Figure 14.9A; Han et al., 2009). “Optrodes”, electrodes supplemented with optical stimulation capabilities, can be produced by gluing two instruments together (e.g. Gradinaru et al., 2007; Figure 14.8A) or by combining them in a single multifunctional apparatus (e.g. Wu et al., 2015; Figure 14.8B,C).

Combining electrical recordings with optical stimulation in an implantable device requires more than a compact mechanical design and an efficient optical interface. The proximity of the light sources and the recording electrodes gives rise to two distinct types of artifacts which resemble spontaneous neural activity (local field potentials and spikes) and hinders the interpretation of the recorded data. First, when light directly hits a metal electrode, a “light artifact” generated by the photovoltaic (Bequerel) effect may arise (Han et al., 2009; Figure 14.9A). Second, when current passes through a light source close to an un-buffered neuronal signal, electromagnetic interference (EMI) caused by capacitive coupling may produce artifacts (Stark et al., 2012; Figure 14.9B). Light artifacts are mitigated using low-power light (Stark et al., 2012), deflecting light away from the metal electrodes (Cardin et al., 2010), and/or using non-metal/specialized electrodes (Zorzos et al., 2011). EMI artifacts can be removed by offline adaptive filtering (Wu et al., 2015), or avoided completely by shielding each light source within a grounded Faraday cage (Stark et al., 2012), or by buffering the neuronal signal before the EMI source.

14.7 OUTLOOK

Once electrical recordings are combined with optical stimulation in a compact, noise-free device, the output of (or the activity in) a neuronal circuit can be harnessed to control the input it receives in a closed-loop manner (Grosenick et al., 2015; Stark et al., 2012). Such a closed-loop, “user-independent” system is especially appealing when applied to an un-tethered subject, enabling complete freedom from the experimental rig (Figure 14.1E). Since the late 1950s pacemakers have been implanted in the heart of patients (Kusumoto

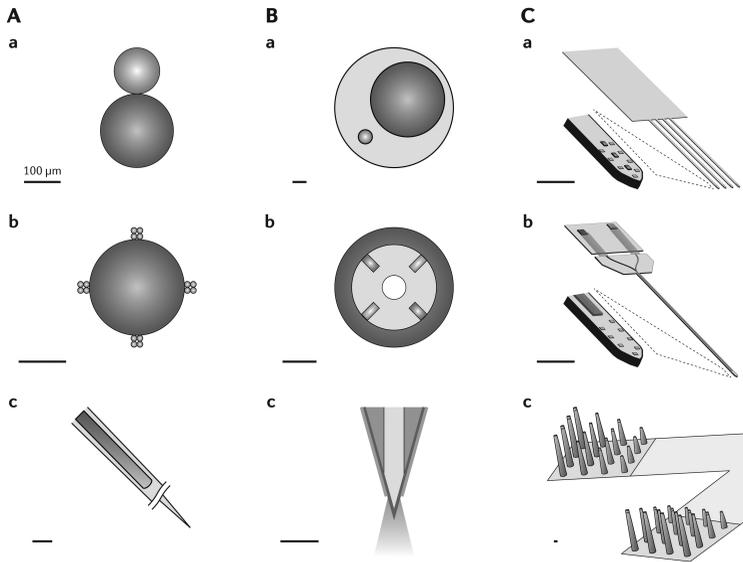


FIGURE 14.8 Optrode designs. Optical stimulation devices combined with electrical recording capabilities. Scale bars, 100 μm . **(A)** Commercially available optical fibers. **a.** Optrode constructed by gluing a 200 μm diameter optical fiber to a 125 μm diameter tungsten electrode (Gradinaru et al., 2007). **b.** “Optetrode”, constructed by fixing four tetrode bundles to a single optical fiber (Anikeeva et al., 2012; English et al., 2012). **c.** “Optopatcher”, constructed by inserting an optical fiber through a glass pipette (Katz et al., 2013). **(B)** Specialized optical fibers. **a.** Dual-core fiber, with a hollow core for extracellular recordings produced near a light-propagating optical core (LeChasseur et al., 2011). **b.** Multi-core, all-polymer flexible fiber, produced with integrating electrode material (Canales et al., 2015). **c.** Concentric electrode and fiber, produced by coating the tapered tip of an optical fiber with gold film (Zhang et al., 2009). **(C)** Fiber-less designs. **a.** Multi-shank (Michigan-like) array. Each monolithic shank is embedded with three neuron-sized μLEDs and eight Ti/Ir recording sites (Wu et al., 2015). **b.** Multi-color monolithic array, produced by coupling two LDs to a common waveguide via GRIN lens. This design is of special interest as it allows the same spot to be illuminated by different wavelengths, enabling, for instance, activating and silencing the same cell (Kampasi et al., 2016). **c.** Multi-electrode (Utah-like) array, containing multiple coaxial optrodes (Kwon et al., 2015).

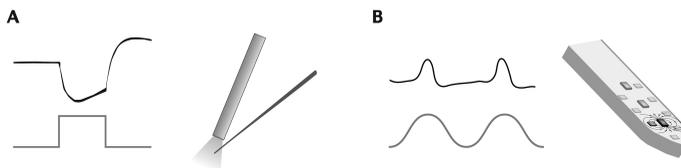


FIGURE 14.9 Opto-electrical artifacts. Combining optical manipulations with electrophysiological recordings can give rise to two distinct types of artifacts. **(A)** Photovoltaic artifacts. Direct illumination (blue line, applied voltage) of metal electrodes can induce large potentials on the recording electrodes (black line, recorded signal; Han et al., 2009). **(B)** Electromagnetic artifacts. When a diode (or any other load), is located close to an un-buffered neural potential recorded by an electrode, artifacts can be caused by electromagnetic interference (EMI). Any dielectric (including air) causes capacitive coupling of the light source and the electrode, and thus the resulting artifacts resemble the waveform of the current ($i = C \cdot dV / dt$) passing through the diode.

and Goldschlager, 1996). In essence, pacemakers are wireless stimulators that can operate in a metronomical fashion or in response to an event sensed by the device itself (e.g. conduction failure). An equivalent opto-electrical implant, capable of sensing and blocking epileptic seizures (Krook-Magnuson et al., 2013), or supplementing/substituting a brain region permanently damaged following a stroke, may provide invaluable scientific insights and, in the long term, beneficial clinical applications.

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