Chapter 8

Manipulating Neural Activity

After reading this chapter, you should be able to:

- Describe the experimental logic behind gain-of-function and loss-of-function experiments
- Compare different approaches for manipulating neural activity in the nervous system
- Discuss approaches to manipulating neural activity in various organisms including humans

Techniques covered:

- **Physical manipulation**: ablation, thermal cooling
- **Electrical manipulation**: microstimulation, electrolytic lesions
- **Pharmacological manipulation**: agonists, antagonists, drug delivery methods
- **Genetic manipulation**: perturbation of endogenous genes, expression of genes to ablate neurons
- **Chemogenetics**: transgenes from other species, DREADDS (hM3Dq, hM4Di)
- **Optogenetics**: Channelrhodopsin-2 (ChR2), Halorhodopsin (NpHR), Archaerhodopsin (Arch), Anion-conducting channelrhodopsins (ACRs), light delivery methods
- **Neuromodulation in humans**: pharmacology, microstimulation, transcranial magnetic stimulation (TMS), ultrasonic neuromodulation (USNM)

One of the ultimate experimental goals in neuroscience is to perturb a neural system to test hypotheses about the role of that system for a behavior or phenotype. For example, if a scientist hypothesizes that a population of neurons in the brain regulates thirst, a key experiment would be to artificially inhibit or stimulate those neurons to determine their necessity or sufficiency for water-seeking behavior. The ability to manipulate neural activity in vivo or in vitro has never been more feasible, and many techniques have been developed and optimized over just the past 10–15 years in a variety of model organisms. Importantly, many of these tools have proven to be relatively cheap and easy to apply in a wide variety of laboratories.

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This chapter surveys approaches for artificially inducing or suppressing electrical activity in neurons (Fig. 8.1). Physical methods ablate neural tissue to determine its role in behavior. Electrical methods apply current to a tissue preparation to stimulate neural activity or cause a lesion. Pharmacological methods employ chemical compounds to alter the function of ion channels and cell receptors. Genetically encoded transgenes ablate neurons or alter baseline physiological firing properties. Combining multiple techniques, such as with chemogenetic tools, allows the targeting of nonendogenous receptors to control neurons with chemical compounds that are normally inert. Finally, optogenetic methods target light-sensitive ion channels and proteins to neurons that allow for neuromodulation with high spatial and temporal precision.

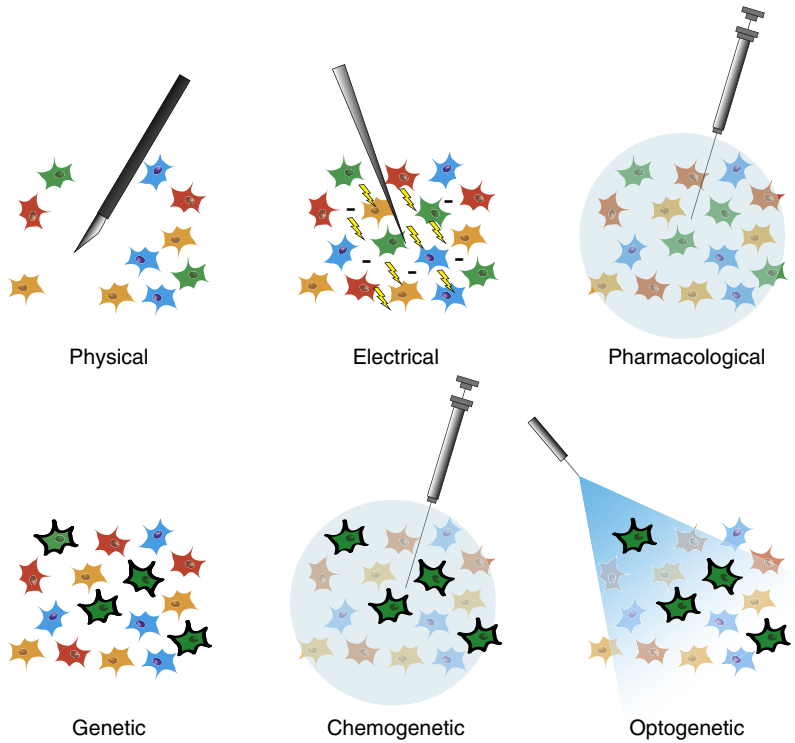

FIGURE 8.1 Methods of manipulating neural activity. Neurons in the brain typically reside within heterogeneous populations composed of many cell types. Physical lesioning techniques ablate cells indiscriminately. Electrical techniques can be used to stimulate cells or cause electrolytic lesions that ablate cells. Pharmacological techniques affect cells via the administration of a psychoactive drug (either centrally or systemically) to affect membrane-bound receptors that regulate neural activity. Genetic methods manipulate gene expression in discrete populations of neurons by taking advantage of cell-type–specific promoters. Altered genes can affect neural activity. Chemogenetic techniques use genetic targeting methods to deliver receptors capable of affecting neural activity in specific cell types. Optogenetic techniques deliver light-activated neuronal actuators to specific cell types.

EXPERIMENTAL LOGIC OF MANIPULATING NEURAL ACTIVITY

Before performing a neuromodulation experiment or choosing any specific technique, it is important to clearly articulate the research goal and comprehend the meaning of experimental outcomes. In general, neural manipulation experiments can be classified in two ways: gain-of-function experiments and loss-of-function experiments. In the context of manipulating neural activity, a **gain-of-function** experiment stimulates activity in neurons and tests their sufficiency in causing a phenotype. In contrast, a **loss-of-function** experiment inhibits activity in neurons and tests their necessity for a phenotype.

For example, consider a population of neurons that are hypothesized to regulate hunger. Perhaps these neurons exhibit increased activity following food deprivation or during conditions of hunger. If artificially stimulating these neurons in freely moving mice causes an increase in food seeking, the scientist can fairly conclude that activity in these neurons is *sufficient* to increase food-seeking behavior. Alternatively, if artificially inhibiting these neurons decreases food seeking, especially when an animal is normally hungry, the scientist can fairly conclude that activity in these neurons is *necessary* for normal food-seeking behavior. Note that the concepts of sufficiency and necessity are completely separable, and it is possible for a neural system to be sufficient but not necessary for a phenotype (or vice versa). For example, perhaps stimulating neurons could increase food seeking behavior while inhibiting neurons would have no significant effect—this result might suggest that there are other neurons in the brain that also regulate food seeking, and no single neural population is necessary for food seeking to occur.

Scientists must be careful not to make inappropriate conclusions from gain-of-function and loss-of-function experiments. For example, if inhibiting a population of neurons decreases food intake, a scientist would be correct that these neurons are necessary for food-seeking behavior, but would be unwise to conclude that these neurons function as "hunger neurons" or "food intake neurons." Perhaps these neurons are also necessary for wakefulness or motor activity, and animals eat less simply because they move less. Thus, concluding that a neural system is necessary for a behavior might not, by itself, be informative. Such a result should impel a scientist to perform other experiments to rule out other potential roles for that neural system in other biological processes.

PHYSICAL MANIPULATION

A physical manipulation is any method that physically disrupts the activity of neurons due to direct, physical contact. Two of the most common methods of physically manipulating neural activity are through ablation experiments and thermal cooling.

Ablation

Ablation, or creating a brain **lesion**, is perhaps the oldest technique used to study the necessity of a particular brain region for a biological function. A scientist can physically lesion the brain by excising or aspirating a piece of brain tissue during a stereotaxic surgery (Chapter 3).

Despite their place in the history of neuroscience research, permanent physical lesions are a relatively crude method for performing loss-of-function experiments in the brain. It is often difficult to control the precision of the lesion or the impact of removing tissue on the remaining brain structures. Because ablating neural tissue damages not only cell bodies but also fibers of passage (the axons traveling through that region of the brain), it is difficult to know if the effects of the lesion are due to the neurons in the ablated area or neurons in a distant area whose fibers have been damaged. Sometimes ablating tissue can also affect the local circulatory system and cause systemic changes in the entire organism. Finally, an obvious limitation to physical ablation is that it is completely irreversible (see Table 8.1 for a comparison of neural lesioning techniques).

Physical ablation techniques can also be used in invertebrate model organisms. For example, individual cells in *C. elegans* can be eliminated by lasers under a dissection microscope. These lesions are cleaner and more precise than physical ablations in vertebrates; however, they are also irreversible.

Thermal Cooling

Neurons in the brain function at an optimal temperature, and precise homeostatic mechanisms ensure that the mammalian brain remains at an optimal

TABLE 8.1 Methods of Lesioning the Nervous System.

Category	Tool	Precision
Physical	Surgical blade	Not very precise. Cannot discriminate between cell types or fibers of passage
Electrical	Microelectrode	More spatially precise than surgical (physical) lesions, but cannot discriminate between cell types or fibers of passage
Pharmacological	Toxins (e.g., ibotenic acid)	Spatial precision determined by quality and specificity of injection. Fibers of passage are unaffected.
Genetic	Genetically encoded proteins (e.g., ataxin, diphtheria toxin receptor)	High cell-type specificity

37°C. Cooling neurons a few degrees can temporarily slow down the kinetics of ion channels and cell physiology, effectively decreasing neural activity. Neurons can be easily cooled in in vitro preparations by slightly cooling the temperature of the bath solution. Neurons can be cooled in vivo by placing a piezoelectric implant in the brain. In contrast to physically removing brain tissue, cooling a brain region is reversible assuming that the altered temperatures do not cause long-lasting damage. Therefore, each animal can serve as its own internal control, and behavior can be compared during cooled and noncooled conditions.

Although reversible, cooling the brain is not very spatially precise as it is not possible to discriminate among neurons or fibers of passage within a given area. Cooling is also not temporally precise, as it takes a variable amount of time for the neurons to cool down, and then warm up when the experiment is complete. Finally, thermal manipulation techniques are much more feasible for loss-of-function experiments than gain-of-function experiments because heating the brain often causes damage rather than an increase in neural activity.

ELECTRICAL MANIPULATION

Microelectrodes are used during electrophysiology experiments to record electrical activity from neurons, but they can also be used to deliver electrical current into the brain, to brain slices, or to neurons in culture in a process called **microstimulation**. A microelectrode is inserted into the brain or next to a neuron of interest and current is applied at a fixed frequency, amplitude, and time. The electrode elicits action potentials by changing the extracellular environment such that voltage-gated ion channels open, depolarizing the neuron. If too much current is applied to the electrode, the scientist can kill nearby cells. Often, this is done deliberately to create an **electrolytic lesion** that permanently ablates neurons. Therefore, microelectrodes can be used to either reversibly stimulate neural activity or irreversibly lesion neural tissue. The spatial resolution of electrical manipulation is limited, as injection of current affects all neurons in a local area, regardless of cell type, and it can affect fibers passing through the area of stimulation as well. Microstimulation exhibits very precise temporal resolution because the frequency and timing of current delivery is under the control of the investigator.

PHARMACOLOGICAL MANIPULATION

A scientist can influence neural activity pharmacologically using one of hundreds of compounds that selectively bind to membrane-bound receptors (Fig. 8.2). An **agonist** is a compound that can bind to and cause activation of a receptor, thus mimicking an endogenous ligand. A **partial agonist** is a compound that can produce an agonist-like effect but not to the maximal extent of the full agonist or endogenous ligand. An **antagonist** binds to the receptor but

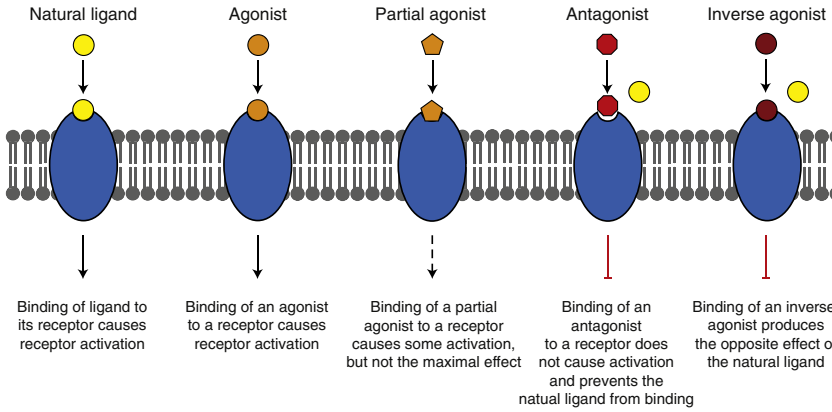

FIGURE 8.2 Different categories of pharmacological compounds cause different effects on membrane receptors.

does not cause activation, preventing the endogenous ligand from binding and thereby blocking its biological activity. An **inverse agonist** binds to the same receptor as the natural ligand, but produces the opposite effect (i.e., inhibition instead of activation of the receptor).

Where do pharmacological compounds come from? Some drugs are engineered in pharmaceutical labs to target specific receptors or other proteins. However, many plants and animals naturally produce pharmacological compounds and neurotoxins for self-defense. Scientists have purified several of these compounds to manipulate neural activity in vitro and in vivo. For example, many fish of the order Tetraodontiformes (the most famous being the pufferfish) produce a compound called **tetrodotoxin (TTX)**. This compound is an antagonist of voltage-gated sodium channels, and therefore can be used to inhibit action potentials.

Many commonly used pharmacological agents target inhibitory GABA receptors. GABA receptor antagonists including **bicuculline** have a net effect of increasing neural excitability, as they prevent the normal inhibitory effects of GABA signaling. GABA receptor agonists decrease neuronal excitability. For example, **muscimol** is a commonly used GABA receptor agonist that is often used during behavioral and/or in vivo electrophysiology experiments to inhibit neural activity.

Some pharmacological agents can be used to irreversibly ablate neurons. For example, **ibotenic acid** causes excitotoxic effects when directly injected into the brain. Pharmacological ablation of neurons is preferable over physical or electrical ablation methods because it spares fiber tracts crossing through the target area. Therefore, if an injection of ibotenic acid is precise, a scientist can make better conclusions about any resulting effects on behavior or physiology.

For in vitro experiments, drugs can be delivered in cell culture media or perfused in the bath solution incubating a brain slice. For in vivo experiments, some drugs can be delivered to the brain via the bloodstream, allowing a scientist to perform an **intraperitoneal (i.p.) injection**. However, many drugs do not cross the blood–brain barrier. In this case, a scientist can perform an **intracerebroventricular (i.c.v.) injection** into the lateral ventricles of the brain. Substances in the cerebrospinal fluid in the ventricles can then diffuse throughout the extracellular milieu of the brain. Alternatively, a scientist can perform a small, local injection into a discrete brain region.

To inject a drug into the brain just before a behavioral experiment, a scientist can first implant a cannula above the brain region of interest during a surgery (Chapter 3), then subsequently inject the drug through the cannula. Injections into the brain can be performed either by pressure injection or **microiontophoresis**, the process of using a small electrical current to drive substances out of a glass pipette. The advantage to microiontophoresis is that it allows for careful delivery of small volumes to discrete, local areas of the brain. For sustained, chronic delivery of a drug over multiple days or weeks, a scientist can use specially implanted **osmotic minipumps** that infuse the drug through an implanted cannula.

Manipulating neural activity using pharmacological agents is usually reversible (except in the case of neurotoxins like ibotenic acid). These agents have varying degrees of spatial precision depending on the specific receptors they target and the receptor expression patterns in the area of injection. They are typically not very temporally precise in vivo because, once injected, they require several minutes or even hours to clear from the system.

GENETIC MANIPULATION

Unlike physical, thermal, electrical, and pharmacological techniques, genetic methods of modulating neural activity can target specific cell types in the brain by taking advantage of endogenous promoters and other genetic regulatory elements that limit expression of genes to specific populations of neurons (Chapter 12). Therefore, if a cell type of interest exists in a heterogeneous population of neurons with one or more other cell types, it is possible to selectively target these neurons and not surrounding cell types using modern transgenic and gene targeting technologies.

To permanently ablate a specific cell type, a scientist can express a transgene that causes the death of the cell. For example, the **ataxin** transgene efficiently causes apoptosis when expressed in neurons. However, one potential limitation to using the ataxin transgene is that the neurons die immediately upon expression. To gain more temporal control over expression of the ataxin gene, it is possible to use transgenic technologies that allow for controlled expression at specific timepoints (Chapter 12). Alternatively, it is possible to express the **diphtheria toxin receptor** to ablate neurons at a

timepoint of the investigator's choosing. This receptor can cause the death of neurons, but only in the presence of the diphtheria toxin. Therefore, a scientist can target the diphtheria toxin receptor to a specific cell type and ablate those neurons at any timepoint during an animal's lifespan by injecting the toxin.

Instead of ablating neural activity, it is possible to express transgenes that, when expressed, allow a neuron to fire action potentials normally but not release neurotransmitter. Therefore, these transgenes effectively silence neural activity. The neurotoxins **tetanus toxin (TeNT)** and **botulinum toxin (BoNT)** inhibit neurotransmitter release by blocking the exocytosis of synaptic vesicles in mammals. These transgenes are irreversible, therefore, once expressed, the neurons will be silent throughout the life of the animal. In *Drosophila*, the temperature-sensitive **shabire** (dynamin) transgene can reversibly silence neurons at specific temperatures.

Another method of modulating neural activity using genetics is to overexpress or knockout a naturally occurring ion channel or membrane-bound receptor that regulates cellular excitability. For example, knocking out an endogenous GABA receptor subunit might increase neuronal excitability. However, neurons have a remarkable ability to compensate for alterations in cell physiology, and other proteins affecting cell physiology may be upregulated or downregulated to return the intrinsic excitability to normal. Furthermore, even if no compensatory mechanisms occur, genetically manipulating neurons does not offer temporal control in manipulating neural activity.

To overcome these limitations, scientists have developed ingenious methods of targeting genetically encoded actuators to neurons that allow for reversible stimulation or inhibition of neural activity with exceptional temporal precision. Two major categories of genetically encoded neuromodulation tools include chemogenetics and optogenetics.

CHEMOGENETIC MANIPULATION

Chemogenetics refers to a set of membrane-bound receptors that are activated by chemical compounds ("chemo-") and are genetically encoded ("-genetics"). The overall strategy is to genetically target neurons with nonendogenous receptors that are capable of being activated by a normally inert ligand. Because other cell types in the brain do not naturally respond to the ligand, only the cells that express the receptor are affected.

Some chemogenetic strategies utilize receptor–ligand systems from other species. For example, the mammalian **capsaicin receptor (TrpV1)** is a ligand-gated channel that depolarizes a neuron upon exposure to capsaicin. Flies and other invertebrates do not normally express this channel, so capsaicin exposure will depolarize only those neurons that express the receptor. A similar strategy can be used to inhibit activity and hyperpolarize neurons in the presence of a ligand. For example, the insect **allatostatin receptor (Alstr)** is not expressed

in mammals. In the presence of the insect protein allatostatin, this receptor inhibits neural activity. Thus, if Alstr is expressed in the mammalian brain, addition of allatostatin will reversibly inactivate genetically targeted populations of neurons. These strategies have proven useful in many studies, but there are substantial disadvantages to using these systems in rodents. The capsaicin receptor is endogenous in rodents and causes a painful sensation when activated, so its use has been limited to TrpV1 knockout mice. The allatostatin receptor is not endogenous in rodents; however, allatostatin does not cross the blood–brain barrier, so it must be injected centrally through a surgically implanted cannula.

Instead of using naturally occurring transgenes from other species, a relatively newer set of engineered chemogenetic tools has been specifically designed for use in a wide range of model organisms. Referred to as **Designer Receptors Exclusively Activated by Designer Drugs (DREADDs)**, these tools consist of artificially engineered G-protein–coupled receptors that activate intracellular signaling pathways to affect neuronal activity. The **hM3Dq** receptor (human M3 muscarinic DREADD coupled to Gq) takes advantage of the endogenous Gq signaling pathway to increase neuronal excitability and induce firing, while the **hM4Di** receptor (human M4 muscarinic DREADD coupled to Gi) takes advantage of the endogenous Gi signaling pathway to decrease neuronal excitability and silence neural activity. These receptors are sensitive to the normally inert ligand **clozapine-N-oxide (CNO)**. Importantly, CNO is capable of crossing the blood–brain barrier, so animals can be injected intraperitoneally during in vivo experiments. The effects of CNO typically last 6–10 h, the time it takes CNO to be metabolized and neuronal signaling cascades to return to baseline. In in vitro culture experiments using cells or tissue expressing DREADDS, CNO can be directly added to the bath solution.

Though CNO is biologically inert, recent work has suggested that, in vivo, CNO can be converted to clozapine, a biologically active compound that can bind to multiple endogenous receptors. Thus, when using CNO for chemogenetic experiments, it is vitally important to use proper controls to rule out any off-target effects. Alternatively, one may choose to use a different DREADD agonist, such as compound-21.

Chemogenetic techniques are spatially precise, as neuromodulation is restricted to the genetically targeted population of cells. However, these techniques do not offer temporal precision greater than traditional pharmacological approaches. For experiments in which greater temporal precision is necessary, researchers may consider optogenetic approaches.

OPTOGENETIC MANIPULATION

Optogenetics refers to a set of neuromodulation tools that are activated by light ("opto-") and are genetically encoded ("-genetics"). Like other

transgenes, optogenetic tools can be delivered with high spatial precision to specific cell types in the nervous system. However, because scientists can deliver light to neurons at any duration or frequency, optogenetics allows for unparalleled spatial *and* temporal precision. In the years since its development, optogenetics has become a staple of modern neuroscience. Hundreds of labs, working in a variety of model organisms, now routinely use optogenetics to make major advances to our understanding of neural systems and circuits.

Optogenetic Transgenes

The most commonly used optogenetic actuators are engineered versions of microbial opsins, proteins that increase ion permeability across membranes in response to activation by specific wavelengths of light (Fig. 8.3).

Optogenetic Stimulation

Channelrhodopsin-2 (ChR2) is a nonspecific cation channel derived from the green algae *Chlamydomonas reinhardtii*. ChR2 absorbs blue light, causing a conformational change that allows H^+, Na^+, K^+, and Ca^+ ions to passively diffuse down their concentration gradients. When ChR2 is expressed in neurons, its opening causes a rapid depolarization of the plasma membrane that can cause action potentials. Importantly, the channel also closes very rapidly when the blue light is switched off. Therefore, single action potentials can be generated with a brief pulse of blue light, with no long-lasting residual effects of stimulation. ChR2 is capable of driving neuronal activity at frequencies from 1 to 40 Hz.

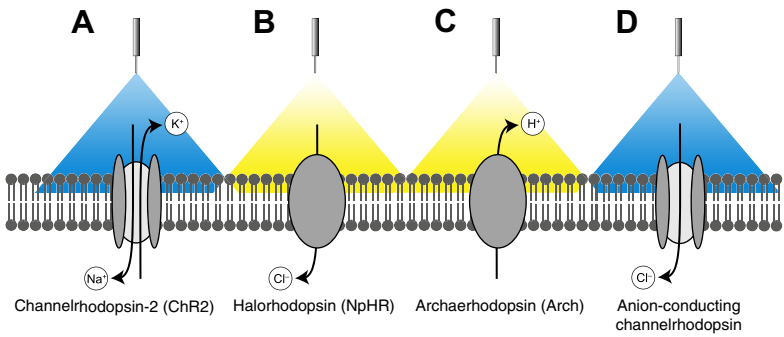

FIGURE 8.3 Genetically encoded actuators used in optogenetics. (A) Channelrhodopsin-2 (ChR2) is a nonspecific cation channel that opens in response to blue light. (B) Halorhodopsin (NpHR) is a chloride pump that actively translocates chloride ions from outside to inside a cell in response to yellow light. (C) Archaerhodopsin (Arch) is a hydrogen (proton) pump that actively translocates hydrogen ions from inside to outside a cell in response to yellow light. (D) Anion-conducting channelrhodopsins (ACRs) are chloride channels that open in response to blue light.

Scientists have made slight genetic modifications to ChR2 to create other channels with desirable neuromodulatory properties. For example, a mutation in the coding sequence of ChR2 causing a single amino acid substitution allows for stimulation of frequencies up to 200 Hz (scientists have named this tool **ChETA**). To cause activation of neurons over longer time periods without the need to continuously deliver light, scientists have created amino acid substitutions in ChR2 that cause the channel to remain open for several minutes or even hours in response to a single pulse of blue light. Called **step function opsins**, these ChR2-derived tools can be closed by a single pulse of yellow light. Scientists have also engineered mutations that alter the wavelength of light used to activate ChR2. The ability to express different versions of ChR2 that react to different colors of light allows for combinatorial experiments in which neuronal activity in multiple cell types can be distinctly manipulated. These variants also allow for optogenetic activation and fluorescent imaging of neural activity to occur simultaneously in the same brain region.

Optogenetic Inhibition

There are three major transgenes used for optogenetic inhibition. **Halorhodopsin (NpHR)** is a chloride pump derived from the halobacterium *Natronomonas pharaonis*. NpHR actively pumps Cl^- ions into cells in response to yellow light. **Archaerhodopsin-3 (Arch)** is derived from *Halorubrum sodomense* and actively pumps hydrogen ions outside of the cell in response to yellow light. The translocation of Cl^- ions into the cell or H^+ neurons out of the cell effectively hyperpolarizes neurons. Alternatively, **anion-conducting channelrhodopsins (ACRs)** are light-gated anion channels. For example, **GtACRs** are blue light—activated ACR variants derived from *Guillardia theta,* a cryptophyte algae. In contrast to NpHR and Arch, which are ion pumps, GtACRs conduct Cl^- ions down their concentration gradient. This Cl^- conductance clamps a neuron close to its resting potential, which inhibits the generation of action potentials.

One limitation of using either NpHR or Arch for optogenetic inhibition is the need for continuous yellow light illumination to sustain hyperpolarization. However, for experiments in which a scientist wants to inhibit neural activity for minutes or even hours, it is not practical to continuously deliver yellow light since this can heat up the tissue and cause neuronal damage. GtACRs solve this issue, as they are more light-sensitive and require lower intensity light to sustain inhibition of neurons. GtACRs also have higher temporal precision than either NpHR or Arch. One limitation of using GtACRs, however, is that under some physiological conditions, activation of these channels results in paradoxical excitation of a neuron. Given the above caveats, a scientist must carefully consider whether optogenetic inhibition is the best tool for a given experiment. For experiments in which neural inhibition is necessary over multiple hours, perhaps a chemogenetic strategy may be the best approach.

Methods of Delivering Light to the Nervous System

Optogenetic methods require the delivery of light to targeted neurons of interest. For cell culture preparations, such as recording neurons in acute brain slice preparations (Chapter 13), scientists can position a light source directly next to the bath or culture plate. Alternatively, light can be directly coupled to the light path of a microscope, and specialized shutters can be installed to deliver light at specific frequencies.

For experiments in invertebrate model organisms, light is typically delivered to the entire housing chamber and passes into the brain directly. In vivo light delivery to rodents is more challenging because of the need to implant a light delivery device capable of penetrating the skull. Light must be delivered very close to the target neurons because brain tissue scatters light exponentially, with only about 10% light intensity remaining at a distance 500 μm from the light source. Furthermore, light delivery systems must not weigh more than a freely moving animal can carry.

The most common method for delivering light to freely moving rodents is implanting an optical fiber directly above a brain region of interest (Fig. 8.4A). This strategy allows for targeting of relatively deep brain structures. During an experiment, the exposed shaft of the optical fiber is coupled to a fiber optic cable (usually about 2 m in length) connected on the other end to a light delivery source, often a laser. Newer, lightweight light emitting diodes (LEDs) can be implanted onto a rodent's head for wireless light delivery. These LEDs can either deliver light to internal optical fibers to penetrate deeper brain structures (Fig. 8.4B) or deliver light via cranial windows to illuminate superficial cortical neurons (Fig. 8.4C).

Strategies for Studying Neural Circuits Using Optogenetics

In a typical optogenetic experiment (although typical does not imply easy), a scientist transduces a population of neurons with an optogenetic transgene and

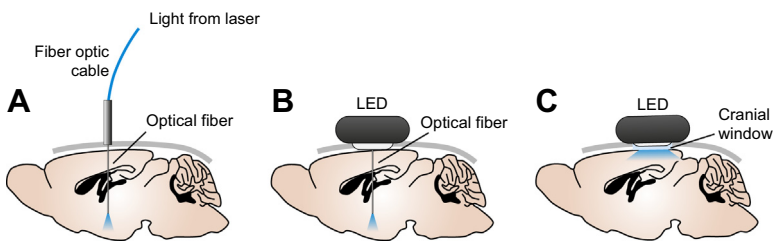

FIGURE 8.4 Methods of delivering light into the brain. (A) The most common form of light delivery in rodent optogenetic experiments is to implant an optical fiber above a cell population of interest and attach the fiber to a longer fiber optic cable connected to a laser. Alternatively, an LED can be directly implanted onto the skull to deliver light (B) into an optical fiber to illuminate deep brain structures or (C) through a cranial window to illuminate superficial cortical neurons.

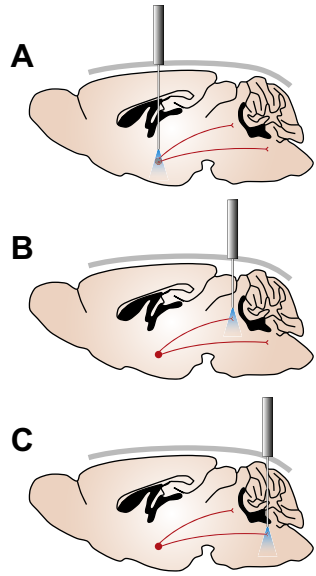

FIGURE 8.5 Modulation of neural projections using optogenetics. (A) After optogenetic transgenes are targeted to a cell population of interest, light can be delivered to the cell bodies themselves to study the effects of neuronal stimulation or inhibition. (B and C) Alternatively, illumination of specific downstream projections can test the effects of stimulation or inhibition on each discrete projection.

then investigates the effects of directly stimulating or inhibiting those neurons with light. In addition to stimulating the cell bodies of transduced neurons, it is possible to stimulate fiber projections to distinct downstream target regions (Fig. 8.5). The ability to selectively stimulate or inhibit individual downstream projections from a neuronal population provides scientists with an unparalleled neural dissection tool. For example, if stimulating a population of neurons is sufficient to cause a phenotype, a scientist can test the hypothesis that this effect is mediated by a specific projection to a downstream brain region. The only caveat to these experiments is that stimulating fiber projections may cause antidromic effects that propagate action potentials up axon tracts back to neuronal cell bodies. Therefore, scientists should perform careful controls to ensure that their stimulation effects are truly specific to the targeted projections of interest.

NEUROMODULATION TECHNIQUES USED IN HUMANS

The rapid development of cutting-edge neuromodulation tools begs the question about whether these tools can be applied in humans. The tools described in this chapter have obvious therapeutic potential in neurodegenerative diseases and neuropsychiatric disorders. Interestingly, they also have

potential for "neuroenhancement"—improving human attention, memory, and cognitive abilities in those with relatively normal function. These applications in humans are obviously highly controversial and hotly debated among neuroethicists.

Which neuromodulation techniques have immediate potential for use in humans? Physical methods are still in use, primarily for trying to limit epileptic electrical activity from spreading by surgically removing the seizure-generating regions or disrupting the connections through which the activity spreads. Electrical stimulation is used to treat severe symptoms in intractable human diseases including rigidity in Parkinson's disease and even serious cases of depression.

Pharmacological techniques have been used over several decades to treat pain, depression, anxiety, attention deficit disorders, and other neurological causes of human suffering. Most psychoactive agents were developed many years ago, and new pharmacological treatments for neuropsychiatric conditions are relatively rare.

Chapter 1 described two methods of manipulating neural activity in humans without penetrating the skull. **Transcranial magnetic stimulation (TMS)** is capable of altering neural activity by applying a magnetic field outside the skull, inducing weak electric currents at a specific focal point on the surface of the brain. Depending on the brain region and the strength of the magnetic field impulse, the electrical activity may either stimulate neural activity or cause a hyperpolarized state in which neural activity is temporarily inactivated. This technique has a spatial resolution of millimeters but can only be applied to superficial neurons near the surface of the skull, such as cortical neurons. Another technique, **ultrasonic neuromodulation (USNM)**, uses focused sound waves to heat the brain in a defined area. Like TMS, this technique is currently only efficient in superficial neurons and has a spatial resolution of millimeters. Both techniques have been applied in clinical, as well as experimental settings.

Neuromodulation tools that require genetically encoded transgenes, such as optogenetics, have not been applied to humans, mostly due to the need of delivering DNA to human cells in vivo. The only gene delivery strategy that can efficiently target transgenes to human neurons is viral gene delivery (Chapter 11), and although commonly used AAV and lentiviral vectors are relatively safe in animal models, scientists have been reasonably reluctant to inject these vectors into human subjects. Perhaps in the future, other methods of gene delivery will be possible so that cell type−specific modulation of neural activity in humans will become a possibility.

CONCLUSION

The methodological toolkit used to manipulate neural activity in model organisms has dramatically expanded in the past several years. There are multiple methods of stimulating (Table 8.2) and inhibiting (Table 8.3) neurons in a

TABLE 8.2 Methods of Stimulating Neural Activity.

Category	Tool	Invasiveness	Spatial Precision	Temporal Precision
Electrical	Microelectrode	Surgical implantation	Cannot distinguish between cell types or fibers of passage	Milliseconds
Pharmacological	Various agonists and antagonists of membrane-bound receptors	Intraperitoneal/subcutaneous injection or surgical implantation of cannula	Depends on specificity of receptor expression	Effects can last minutes to hours
Genetic	Modulation of endogenous membrane-bound receptors	Not invasive unless viral gene delivery	Depends on specificity of receptor expression	Permanent effects
Chemogenetic	Ligand-gated receptors (e.g., hM3Dq, TrpV1)	Not invasive unless viral gene delivery; need to inject drugs intraperitoneally	High cell-type specificity	Effects can last minutes in vitro or hours in vivo
Optogenetic	Channelrhodopsin-2 (ChR2)	Surgical implantation; attachment to fiber optic cable	High cell-type specificity	Milliseconds

TABLE 8.3 Methods of Inhibiting Neural Activity.

Category	Tool	Invasiveness	Spatial Precision	Temporal Precision
Electrical	Microelectrode (causes electrolytic lesion)	Surgery necessary	Cannot distinguish between cell types or fibers of passage	Permanent lesion
Pharmacological	Various agonists and antagonists of membrane-bound receptors	Intraperitoneal/subcutaneous injection or surgical implantation of cannula	Depends on specificity of receptor expression	Effects can last minutes to hours
Genetic	Modulation of endogenous membrane-bound receptors	Not invasive unless viral gene delivery	Depends on specificity of receptor expression	Permanent effects
Chemogenetic	Ligand-gated receptors (e.g., hM4Di, AlstR)	Not invasive unless viral gene delivery; need to inject drugs intraperitoneally	High cell-type specificity	Effects can last minutes in vitro or hours in vivo
Optogenetic	Halorhodopsin (NpHR), archaerhodopsin (Arch), Anion-conducting channelrhodopsins (e.g., GtACR)	Surgical implantation; attachment to fiber optic cable	High cell-type specificity	Milliseconds

wide variety of model organisms, each with their own advantages and disadvantages. The relatively recent development of genetically encoded tools that can reversibly affect neural activity with unparalleled spatial and temporal resolution has allowed scientists to probe many previously intractable questions about the role of specific neural systems and circuits in physiology and behavior.

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