Introduction

The human mind has been studied for thousands of years, but the human brain, as well as the brains of other species, have only been studied for about a century. Only 150 years ago, the ability to study the nervous systems of humans and other animals was limited to examining preserved specimens, as well as the effects of brain damage in people and animals. With the advent of histology came the ability to visualize and differentiate between neurons based on morphology. At the end of the 19th century, the great neuroscientist Santiago Ramón y Cajal used a relatively new method called Golgi staining to visualize the morphology and architecture of neurons throughout the brain, propelling the field of neuroscience into its modern state.

Indeed, in the history of neuroscience, each great leap forward in knowledge has been the result of a great leap forward in techniques and technology. Just as Ramón y Cajal used the Golgi stain to advance our understanding of nervous system structure, scientists throughout the 20th century used continually more advanced techniques to advance our understanding of nervous system function: Eccles, Hodgkin, and Huxley used intracellular recording technology to investigate the ionic basis of membrane potentials; Hubel and Wiesel used extracellular recording technology to investigate how information is processed in the visual system; and Neher and Sakmann used patch-clamp technology to investigate the physiology of single ion channels. In the latter half of the 20th century, the explosion of molecular biology techniques and genetic engineering methods allowed neuroscientists the ability to study individual genes, proteins, and cell types. In the first 2 decades of the 21st century, neuroscientists developed strategies of measuring and perturbing neural activity using light, allowing for investigation with unprecedented spatial and temporal precision. Neuroscience methods have progressed so far in the past 100 years that the Golgi stain itself seems to have been reinvented through powerful technologies that allow investigators to turn specific neurons different colors to further investigate the structure and connectivity of the nervous system.

The modern neuroscientist now has hundreds of techniques that can be used to answer specific scientific questions. This book contains 14 chapters that provide an overview of the most commonly used techniques. Although there are dozens of techniques that seem very different at first glance, many of them attempt to study the nervous system in the same way. For example, transcranial magnetic stimulation (Chapter 1), physical lesions (Chapter 8), optogenetic inhibition (Chapter 8), and genetic knockdown or knockouts (Chapter 12) are all attempts to test the effect of a *loss of function* of some aspect of the nervous system on a behavior or phenotype. For each level of investigation (whole brains to individual genes), research strategies can be similar in concept even if the techniques are different in practice.

LEVELS OF INVESTIGATION

Something immediately obvious to all students of neuroscience is that the nervous system is exceptionally complicated and can be examined at multiple levels of investigation. The basic functional unit of the nervous system is the neuron. The human brain is composed of approximately 100 billion neurons that are connected into circuits via approximately 100 trillion synapses. Neural circuits are organized into anatomical structures and larger networks of neurons that can integrate information across modalities from many different parts of the brain. These networks process sensory information from the external and internal environment and provide the neural basis of cognition—learning, memory, perception, decision-making, emotion, and other higher-order processes. The final observable output of the nervous system is a behavior composed of a coordinated motor action. This behavior can either be extremely simple, such as a motor reflex, or incredibly complicated, such as dancing, typing, or playing a musical instrument. Behavior is usually defined not just by what an organism does, but what it chooses to do. Therefore, except in rare circumstances of lesion or disease, cognition and behavior are inseparably linked, and in animals other than humans, behavior is used as a read-out of cognition.

Just as one can start with a neuron and scale up toward circuits, cognition, and behavior, a scientist can also scale down and examine the components that make up a neuron. A neuron is itself defined as having a cell body (soma), axon, and dendrites. These neuronal components contain subcellular specializations that make the neuron unique among other cell types. Specialized organelles in a neuron, such as vesicles containing neurotransmitters, provide the cell with the ability to signal to other neurons. Specialized cytoskeleton processes allow a neural process to extend great distances throughout the brain and body. Several proteins provide neurons with their intercellular signaling abilities and physiological characteristics. For example, biosynthetic enzymes produce neurotransmitters, while other proteins serve as receptors for these signaling molecules. All of these proteins are the products of genes, the functional units of an organism's genome. The human genome contains approximately 25,000 genes, with each neural subtype expressing its own unique gene expression profile.

The complexity of the nervous system is awesome in scope. It is fascinating that a mutation in a single gene, such as a gene that codes for a transmembrane ion channel, can produce effects that alter the electrical properties of a neuron, in turn altering the normal firing patterns of a neural circuit and thus causing an abnormal behavior.

A neuroscientist can approach the study of the nervous system through any of these levels of organization. The 14 chapters of this book provide a guide to the types of experiments that can be performed at each level. However, irrespective of technique, the basic scientific approach one can use to study the nervous system is consistent from level to level, whether the subject is human cognition or axon guidance in cell culture.

APPROACHES TO STUDYING THE NERVOUS SYSTEM

There are four general approaches for studying the nervous system: (1) Examining case studies—identifying interesting events that have occurred naturally and using these events to develop hypotheses that can be tested in future experiments; (2) Screens—performing unbiased searches for anatomical structures, neurons, proteins, or genes that could play a role in a behavior or phenotype of interest; (3) Description—using techniques that allow a scientist to observe the nervous system without manipulating any variables; and (4) Manipulation—testing hypotheses by determining the effect of an independent variable on a dependent variable. Each of these four methods is described in more detail below.

Examining Case Studies

A **case study** is an example of an event that happened to a subject (most often a human or group of humans) that retrospectively demonstrates an important finding about the nervous system. The circumstances surrounding the event are usually nonrepeatable and cannot be precisely recreated in a laboratory setting. Such demonstrations are, therefore, not true experiments in that no variables are deliberately controlled by a scientist. However, these events can often reveal substantial information about an aspect of neural function that was previously unknown.

For example, consider the case of Phineas Gage, a railroad worker who was involved in an accident in 1848 that caused an iron rod to pass through his skull. The rod entered the left side of his face, passed just behind his left eye, and exited through the top of his head, completely lesioning his frontal lobes. This is an amazing event, not only because Gage survived (and lived for another 12 years), but also because it informed scientists about the function of the frontal lobe of the brain. The event allowed investigators to retrospectively ask the question "What is the effect of removing the frontal lobe on consciousness and behavior?" According to Gage's friends, family, and co-workers, he was "no longer Gage." He retained the ability to learn, remember, sense, and perceive his environment, to execute motor functions, and to live a fairly normal life, but it seemed to people who knew him that his personality had changed completely. After the accident, Gage was described as impolite, erratic, unreliable, and offensive to others. This incident therefore provided evidence that the frontal lobe is not necessary for sensation, movement, or memory, but instead likely plays a role in impulsivity and perhaps gating actions.

This case study is not a true experiment, as no scientist decided to test the removal of the frontal lobe on personality. But the incident, and others like it, allow neuroscientists to form hypotheses based on naturally occurring events. Because of Gage's story, neuroscientists could hypothesize about the contribution of the frontal lobe to human personality. Future experiments tested hypotheses on humans in noninvasive imaging experiments, as well as in animal models that model human personality traits.

Screens

A **screen** is a method that allows an investigator to identify which genes, proteins, neurons, or circuits may be involved in a particular biological process. Such experiments are not necessarily driven by a hypothesis, but the experiment identifies candidates that can form the basis for future hypothesis-driven research. For example, a neuroscientist who wants to identify genes involved in body weight regulation may compare gene expression profiles in central feeding centers of the brain in both fed and starved animals: genes expressed in starved animals relative to fed animals may be important for generating the motivation to eat.

Screens can be performed at multiple levels of investigation. When a cognitive neuroscientist places a human subject into an fMRI scanner and examines which brain areas show increased activation in response to a specific stimulus or task, the scientist is essentially performing a screen of different brain regions. When a fly geneticist examines thousands of mutagenized flies for deficiency in a behavioral task, the scientist is attempting to identify genes necessary for that behavior to occur. Such genes can then be tested in future experiments. Thus, a screen is often the first step towards discovering a new element in the nervous system for subsequent descriptive and manipulation studies.

Description

Descriptive science is the act of observing properties of the nervous system without manipulating them. For example, an investigator could describe the sequence of a gene and where in the brain the gene is expressed. Likewise, in the case of proteins, an investigator could describe the amino acid sequence of the protein and where in the brain the protein is expressed. Neurons can be described in terms of what genes/proteins they express, their morphology, how many neurons make up a population of neurons, their electrophysiological properties, and their projection patterns.

It is important to note that just because a study may be descriptive does not mean that it is necessarily easier than other types of experiments. Observation and description form the foundation for understanding the relationship between structure and function, as well as providing insight about what elements to manipulate in future experiments. Thus, descriptive neuroscience plays just as important a role in modern research as when Ramón y Cajal observed the structure of neurons 100 years ago.

Manipulation

Manipulating an aspect of the nervous system and examining the effects on a behavior or phenotype allows scientists the ability to test hypotheses, the hallmark of the scientific method. A manipulation experiment tests the effect of X on Y. The variable that is manipulated, X, is the **independent variable**. The part of the system that is measured, Y, is the **dependent variable**.

Two of the most common types of manipulation experiments are loss-of-function and gain-of-function experiments. In a **loss-of-function** (also called "**necessity**") experiment, a part of the nervous system is diminished or removed in an attempt to determine if it is necessary for a certain process to occur. The following questions are all loss-of-function questions:

- Is a normal copy of the gene Fezf2 necessary for the proper development of the cerebral cortex?
- Is the receptor for the hypocretin neuropeptide necessary for normal sleep/wake transitions in mammals?
- Is electrical activity in the medial geniculate nucleus required for sound cue-driven spikes in auditory cortex?
- Can human patients with damage to the cerebellar vermis perform as well as healthy controls on a verbal-memory task?

In all of these experiments, an aspect of the nervous system is partially or totally disrupted, whether it is a gene, a protein, neural activity, or an entire brain structure. The independent variable is the loss of the structure, and the dependent variable is a behavior or phenotype. Sometimes, a good follow-up for a loss-of-function experiment is a **rescue experiment** in which the aspect of the nervous system that is lost is deliberately returned. For example, if it is found that a certain line of fruit flies lacks a gene that is necessary for proper development of an eye, the specific gene can be reintroduced to determine if the functional gene can rescue the aberrant phenotype.

In a **gain-of-function** (also call "**sufficiency**") experiment, an aspect of the nervous system is increased relative to normal. This may include an increased expression of a gene or protein, or an increase in electrical activity in a population of neurons. The aspect of the nervous system that is increased is the independent variable, and the effect on another part of the nervous system is the dependent variable. The following questions are all gain-of-function questions:

- Can an increase in the gene TrpA1 cause mice to be hypersensitive to cold temperatures?
- Can an intracerebroventricular injection of Neuropeptide Y cause an increase in feeding behavior in rats?
- Can optogenetic stimulation of the lateral geniculate nucleus increase spike frequencies over time in area V4 of visual cortex?
- Can stimulation of the motor cortex using transcranial magnetic stimulation (TMS) induce motor behaviors?

In both loss-of-function and gain-of-function experiments, it is important not to overstate the conclusions of the experiments. For example, consider a loss-of-function experiment in which a mouse that lacks a gene is unresponsive to painful stimuli. The investigator could conclude that this gene is necessary for proper performance on an assay for pain detection. However, an inappropriate conclusion would be that this gene regulates pain detection. Perhaps this gene is responsible for normal development of the spinal cord and the mouse lacks all peripheral sensation. Alternatively, this gene may code for a protein that is necessary for normal development of the thalamus; if improper development of the part of the thalamus that receives information about painful stimuli causes the stimuli not to reach somatosensory cortex, the animal will not perform normally on the pain detection task. Careful controls are necessary to reach appropriate conclusions.

UNDERSTANDING TECHNIQUES IN NEUROSCIENCE

We hope that these 14 chapters serve as a useful guide to studying the nervous system. All of the techniques described throughout this book depend on the principles described above. For each level of investigation that a scientist may choose to study, the same four general approaches can be used to study the nervous system: examining case studies, screens, description, and manipulation. The same principles that are used to study brain activity in awake, human subjects are used to study genes and proteins in tissue samples. The methods may vary, but the principles remain the same. It is also important to remember that techniques and methods should never be the guiding force behind doing experiments in research. Ideally, experiments should be performed to answer an interesting question, not the other way around. A technique should not be used for its own sake but because it is the best technique available to answer a particular research question. Therefore, we hope this book answers your questions about what techniques are available in modern neuroscience research, but more importantly, we hope these techniques answer your specific research questions as well!