PROLOGUE | Heart Repaired, Brain Damaged

All her life, Mrs. H. had been active. She had never been particularly athletic, but she and her husband often went hiking and camping with their children when they were young, and they continued to hike and go for bicycle rides after their children left home. Her husband died when she was 60, and even though she no longer rode her bicycle, she enjoyed gardening and walking around the neighborhood with her friends.

A few years later, Mrs. H. was digging in her garden when a sudden pain gripped her chest. She felt as if a hand were squeezing her heart. She gasped and dropped her spade. The pain crept toward her left shoulder and then traveled down her left arm. The sensation was terrifying; she was sure that she was having a heart attack and was going to die. But after a few minutes the pain melted away, and she walked slowly back to her house.

Her physician examined her, performed some tests, and later told her that she had not had a heart attack. Her pain was that of angina pectoris, caused by insufficient flow of blood to the heart. Some of her coronary arteries had become partially obstructed with atherosclerotic plaque—cholesterol-containing deposits on the walls of the blood vessels. Her efforts in her garden had increased her heart rate, and as a consequence, the metabolic activity of her heart muscle had also increased. Her clogged coronary arteries simply could not keep up with the demand, and the accumulation of metabolic by-products caused intense pain. Her physician cautioned her to avoid unnecessary exertion and prescribed nitroglycerine tablets to place under her tongue if another attack occurred.

Mrs. H. stopped working in her garden but continued to walk around the neighborhood with her friends. Then one evening, while climbing the stairs to get ready for bed, she felt another attack grip her heart. With difficulty, she made her way to her bathroom cabinet, where she found her nitroglycerine tablets. Fumbling with the childproof cap, she extracted a tablet and placed it under her tongue. As the tablet dissolved and the nitroglycerine entered her bloodstream, she felt the tightness in her chest loosen, and she stumbled to her bed.

Over the next year the frequency and intensity of Mrs. H.'s attacks increased. Finally, the specialist to whom the physician had referred her recommended that she consider having a coronary artery bypass performed. She readily agreed. The surgeon, Dr. G., replaced two of her coronary arteries with sections of vein that he had removed from her leg. During the procedure, an artificial heart took over the pumping of her blood so that the surgeon could cut out the diseased section of the arteries and delicately sew in the replacements.

Several days later, Dr. G. visited Mrs. H. in her hospital room. "How are you feeling, Mrs. H?"

"I'm feeling fine," she said, "but I'm having trouble with my vision. Everything looks so confusing, and I feel disoriented. I can't. . . ."

"Don't worry," he cut in. "It's normal to feel confused after such serious surgery. Your tests look fine, and we don't expect a recurrence of your angina. You should be good for many years!" He flashed a broad smile at her and left the room.

But Mrs. H.'s visual problems and her confusion did not get better. Although the surgeon's notes indicated a successful outcome, her family physician saw that something was wrong and asked Dr. J., a neuropsychologist, to evaluate her. Dr. J.'s report confirmed the physician's fears: Mrs. H. had Balint's syndrome. She could still see, but she could not control her eye movements. The world confused her because she saw only fleeting, fragmentary images. She could no longer read, and she could no longer locate and grasp objects in front of her. In short, her vision was almost useless. Her heart was fine, but she would henceforth have to live in a nursing home, where others could care for her.

Study of the physiology of behavior involves the efforts of scientists in many disciplines, including physiology, neuroanatomy, biochemistry, psychology, endocrinology, and histology. Pursuing a research project in behavioral neuroscience requires competence in many experimental techniques. Because different procedures often produce contradictory results, investigators must be familiar with the advantages and limitations of the methods they employ. Scientific investigation entails a process of asking questions of nature. The method that is used frames the question. Often we receive a puzzling answer, only to realize later that we were not asking the question we thought we were. As we will see, the best conclusions about the physiology of behavior are made not by any single experiment, but by a program of research that enables us to compare the results of studies that approach the problem with different methods.

An enormous—and bewildering—array of research methods is available to the investigator. If I merely presented a catalog of them, it would not be surprising if you got lost—or simply lost interest. Instead, I will present only the most important and commonly used procedures, organized around a few problems that researchers have studied. This way, it should be easier to see the types of information provided by various research methods and to understand their advantages and disadvantages. It will also permit me to describe the strategies that researchers employ as they follow up the results of one experiment by designing and executing another one.

Experimental Ablation

One of the most important research methods used to investigate brain functions involves destroying part of the brain and evaluating the animal's subsequent behavior. This method is called **experimental ablation** (from the Latin word *ablatus,* a "carrying away"). In most cases experimental ablation does not involve the removal of brain tissue; instead, the researcher destroys some tissue and leaves it in place. Experimental ablation is the oldest method used in neuroscience, and it remains in common use today.

Evaluating the Behavioral Effects of Brain Damage

A *lesion* is a wound or injury; a researcher who destroys part of the brain usually refers to the damage as a *brain lesion.* Experiments in which part of the brain is damaged and the animal's behavior is subsequently observed are called **lesion studies.** The rationale for lesion studies is that the function of an area of the brain can be inferred from the behaviors that the animal can no longer perform after the area has been damaged. For example, if, after part of the brain is destroyed, an animal can no longer perform tasks that require vision, we can conclude that the animal is blind—and that the damaged area plays some role in vision.

Just what can we learn from lesion studies? Our goal is to discover what functions are performed by different regions of the brain and then to understand how these functions are combined to accomplish particular behaviors. The distinction between *brain function* and *behavior* is an important one. Circuits within the brain perform functions, not behaviors. No one brain region or neural circuit is solely responsible for a behavior; each region performs a function (or set of functions) that contributes to performance of the behavior. For example, the act of reading involves functions required for controlling eye movements, focusing the lens of the eye, perceiving and recognizing words and letters, comprehending the meaning of the words, and so on. Some of these functions also participate in other behaviors; for example, controlling eye movement and focusing are required for any task that involves looking, and brain mechanisms used for comprehending the meanings of words also participate in comprehending speech. The researcher's task is to understand the functions that are required for performing a particular behavior and to determine what circuits of neurons in the brain are responsible for each of these functions.

experimental ablation The removal or destruction of a portion of the brain of a laboratory animal; presumably, the functions that can no longer be performed are the ones the region previously controlled.

lesion study A synonym for experimental ablation.

excitotoxic lesion (*ek sigh tow* ***tok*** *sik*) A brain lesion produced by intracerebral injection of an excitatory amino acid, such as kainic acid.

Producing Brain Lesions

How do we produce brain lesions? Usually, we want to destroy regions that are hidden away in the depths of the brain. Brain lesions of subcortical regions (regions located beneath the cortex) are usually produced by passing electrical current through a stainless steel wire that is coated with an insulating varnish except for the very tip. We guide the wire stereotaxically so that its end reaches the appropriate location. (Stereotaxic surgery is described in the next subsection.) Then we turn on a lesion-making device, which produces radio frequency (RF) current—alternating current of a very high frequency. The passage of the current through the brain tissue produces heat that kills cells in the region surrounding the tip of the electrode. (See ***Figure 5.1.***)

Lesions produced by these means destroy everything in the vicinity of the electrode tip, including neural cell bodies and the axons of neurons that pass through the region. A more selective method of producing brain lesions employs an excitatory amino acid such as *kainic acid,* which kills neurons by stimulating them to death. (As we saw in Chapter 4, kainic acid stimulates glutamate receptors.) Lesions produced this way are referred to as **excitotoxic lesions.** When an excitatory amino acid is injected through a cannula (a small metal tube) into a region of the brain, the chemical destroys neural cell bodies in the vicinity but spares axons that belong to different neurons that happen to pass nearby. (See ***Figure 5.2.***) This selectivity permits the investigator to determine whether the behavioral effects of destroying a particular brain structure are caused by the death of neurons located there or by the destruction of axons that pass nearby.

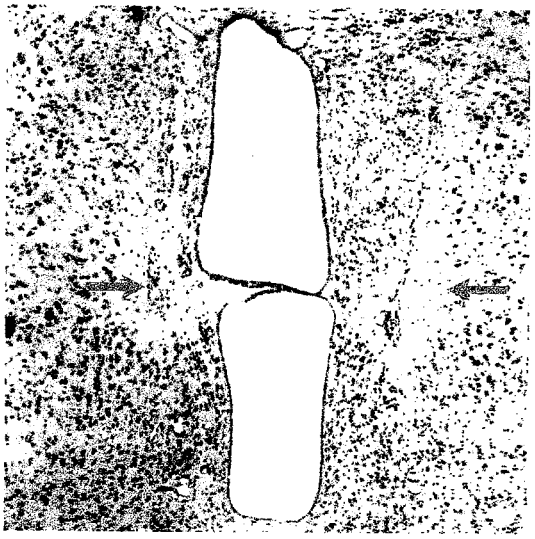
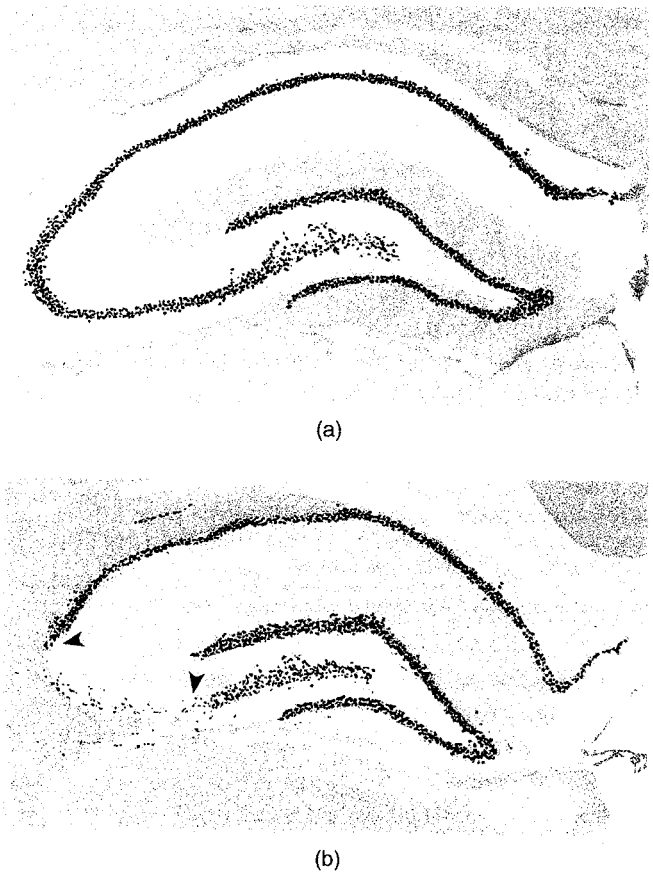


FIGURE 5.1 Radio Frequency Lesion. The arrows point to very small lesions produced by passing radio frequency current through the tips of stainless steel electrodes placed in the medial preoptic nucleus of a rat brain. The oblong hole in the middle of the photograph is the third ventricle. (Frontal section, cell-body stain.)

Turkenburg, J. L., Swaab, D. F., Endert, E., et al. *Brain Research Bulletin,* 1988, 21, 215–224.



(a)

(b)

FIGURE 5.2 **Excitotoxic Lesion.** Slices through the hippocampus of a rat brain. (a) Normal hippocampus. (b) Hippocampus with a lesion produced by infusion of an excitatory amino acid. Arrowheads mark the ends of the region in which neurons have been destroyed.

Based on research from Benno Roozendaal/University of Groningen.

Even more specific methods of targeting and killing particular types of neurons are available. For example, molecular biologists have devised ways to attach toxic chemicals to antibodies that will bind with particular proteins found only on certain types of neurons in the brain. The antibodies target these proteins, and the toxic chemicals kill the cells to which the proteins are attached.

Note that when we produce subcortical lesions by passing RF current through an electrode or infusing a chemical through a cannula, we always cause additional damage to the brain. When we pass an electrode or a cannula through the brain to get to our target, we inevitably cause a small amount of damage even before turning on the lesion maker or starting the infusion. Therefore, we cannot simply compare the behavior of brain-lesioned animals with that of unoperated control animals; the incidental damage to the brain regions above the lesion may actually be responsible for some of the behavioral deficits we see. What we do is operate on a group of animals and produce **sham lesions**. To do so, we anesthetize each animal, put it in the stereotaxic apparatus (described in the following text), cut open the scalp, drill the holes, insert the electrode or cannula, and lower it to the proper depth. In other words, we do everything we would do to produce the lesion except turn on the lesion maker or start the infusion. This group of animals serves as a control group; if the behavior of the animals with brain lesions is different from that of the sham-operated control animals, we can conclude that the lesions caused the behavioral deficits. (As you can see, a sham lesion serves the same purpose as a placebo does in a pharmacology study.)

Most of the time, investigators produce permanent brain lesions, but sometimes it is advantageous to disrupt the activity of a particular region of the brain temporarily. The easiest way to do so is to inject a local anesthetic or a drug called *muscimol* into the appropriate part of the brain. The anesthetic blocks action potentials in axons from entering or leaving that region, thus effectively producing a temporary lesion (usually called a *reversible* brain lesion). Muscimol, a drug that stimulates GABA receptors, inactivates a region of the brain by inhibiting the neurons located there. (You will recall that GABA is the most important inhibitory neurotransmitter in the brain.)

Stereotaxic Surgery

So how do we get the tip of an electrode or cannula to a precise location in the depths of an animal's brain? The answer is **stereotaxic surgery**. *Stereotaxis* literally means "solid arrangement"; more specifically, it refers to the ability to locate objects in space. A *stereotaxic apparatus* contains a holder that fixes the animal's head in a standard position and a carrier that moves an electrode or a cannula through measured distances in all three axes of space. However, to perform stereotaxic surgery, one must first study a *stereotaxic atlas.*

sham lesion A "placebo" procedure that duplicates all the steps of producing a brain lesion except for the one that actually causes the brain damage.

stereotaxic surgery (*stair ee oh* ***tak*** *sik*) Brain surgery using a stereotaxic apparatus to position an electrode or cannula in a specified position of the brain.

bregma The junction of the sagittal and coronal sutures of the skull; often used as a reference point for stereotaxic brain surgery.

THE STEREOTAXIC ATLAS

No two brains of animals of a given species are completely identical, but there is enough similarity among individuals to predict the location of particular brain structures relative to external features of the head. For instance, a subcortical nucleus of a rat might be so many millimeters ventral, anterior, and lateral to a point formed by the junction of several bones of the skull. Figure 5.3 shows two views of a rat skull: a drawing of the dorsal surface and, beneath it, a midsagittal view. (See ***Figure 5.3.***) The skull is composed of several bones that grow together and form *sutures* (seams). The heads of newborn babies contain a soft spot at the junction of the coronal and sagittal sutures called the *fontanelle.* Once this gap closes, the junction is called **bregma**, from the Greek word meaning "front of head." We can find bregma on a rat's skull, too, and it serves as a convenient reference point. If the animal's skull is oriented as shown in the illustration, a particular region of the brain is found in a fairly constant position relative to bregma.

A **stereotaxic atlas** contains photographs or drawings that correspond to frontal sections taken at various distances rostral and caudal to bregma. For example, the page shown in Figure 5.4 is a drawing of a slice of the brain that contains a brain structure (shown in red) that we are interested in. If we wanted to place the tip of a wire in this structure (the fornix), we would have to drill a hole through the skull immediately above it. (See ***Figure 5.4.***) Each page of the stereotaxic atlas is labeled according to the distance of the section anterior or posterior to bregma. The grid on each page indicates distances of brain structures ventral to the top of the skull and lateral to the midline. To place the tip of a wire in the fornix, we would drill a hole above the target and then lower the electrode through the hole until the tip was at the correct depth, relative to the skull height at bregma. (Compare ***Figures 5.3*** and ***5.4.***) Thus, by finding a neural structure (which we cannot see in our animal) on one of the pages of a stereotaxic atlas, we can determine the structure's location relative to bregma (which we can see). Note that because of variations in different strains and ages of animals, the atlas gives only an approximate location. We always have to try out a new set of coordinates, slice and stain the animal's brain, see the actual location of the lesion, correct the numbers, and try again. (Slicing and staining of brains are described later.)

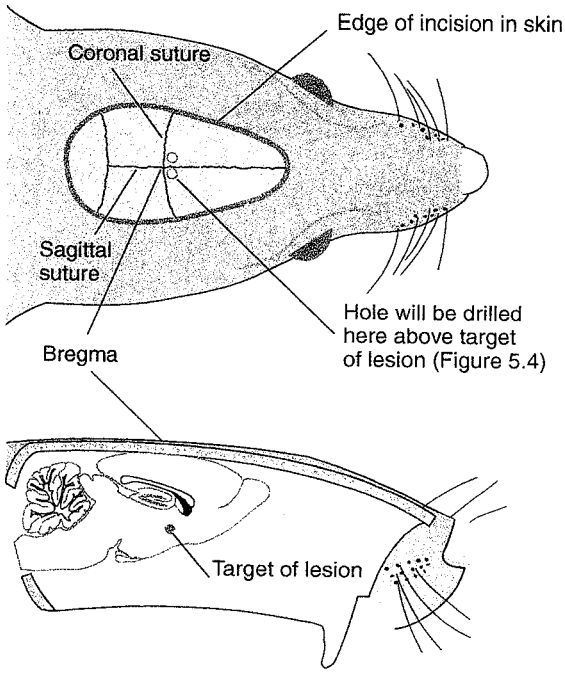


FIGURE 5.3 **Rat Brain and Skull.** The figure shows the relation of the skull sutures to a rat's brain, and the location of a target for an electrode placement. *Top:* Dorsal view. *Bottom:* Midsagittal view.

THE STEREOTAXIC APPARATUS

A **stereotaxic apparatus** operates on simple principles. The device includes a head holder, which maintains the animal's skull in the proper orientation; a holder for the electrode; and a calibrated mechanism that moves the electrode holder in measured distances along the three axes: anterior–posterior, dorsal–ventral, and lateral–medial. Figure 5.5 illustrates a stereotaxic apparatus designed for small animals; various head holders can be used to outfit this device for such diverse species as rats, mice, hamsters, pigeons, and turtles. (See ***Figure 5.5.***)

Once we obtain the coordinates from a stereotaxic atlas, we anesthetize the animal, place it in the apparatus, and cut the scalp open. We locate bregma, dial in the appropriate numbers on the stereotaxic apparatus, drill a hole through the skull, and lower the device into the brain by the correct amount. Now the tip of the cannula or electrode is where we want it to be, and we are ready to produce the lesion.

Of course, stereotaxic surgery may be used for purposes other than lesion production. Wires placed in the brain may be used to stimulate neurons as well as destroy them, and drugs can be

stereotaxic atlas A collection of drawings of sections of the brain of a particular animal with measurements that provide coordinates for stereotaxic surgery.

stereotaxic apparatus A device that permits a surgeon to position an electrode or cannula into a specific part of the brain.

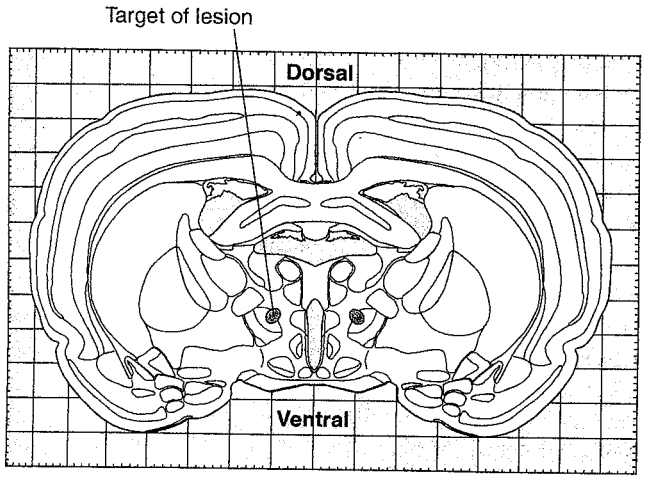


FIGURE 5.4 **Stereotaxic Atlas.** This sample page from a stereotaxic atlas shows a drawing of a frontal section through the rat brain. The target (the fornix) is indicated in red. Labels have been removed for the sake of clarity.

Adapted from Swanson, L. W. *Brain Maps: Structure of the Rat Brain.* New York: Elsevier, 1992.

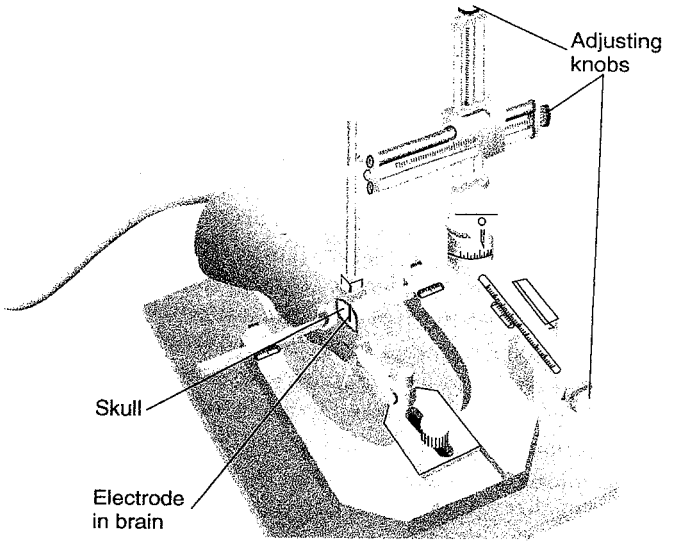


FIGURE 5.5 **Stereotaxic Apparatus.** This apparatus is used for performing brain surgery on rats.

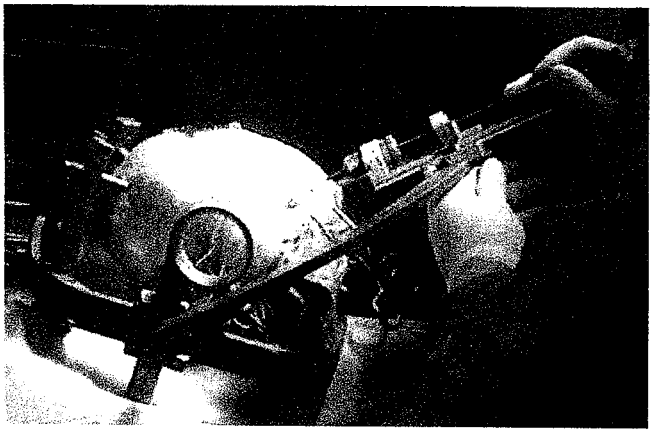


FIGURE 5.6 **Stereotaxic Surgery on a Human Patient.**

John W. Snell, University of Virginia Health System.

injected that stimulate neurons or block specific receptors. We can attach cannulas or wires permanently by following a procedure that will be described later in this chapter. In all cases, once surgery is complete, the wound is sewn together, and the animal is taken out of the stereotaxic apparatus and allowed to recover from the anesthetic.

Stereotaxic apparatuses are also made for humans, by the way. Sometimes a neurosurgeon produces subcortical lesions—for example, to reduce the symptoms of Parkinson's disease. Usually, the surgeon uses multiple landmarks and verifies the location of the wire (or other device) inserted into the brain by taking brain scans or recording the activity of the neurons in that region before producing a brain lesion. (See ***Figure 5.6***.)

Histological Methods

After producing a brain lesion and observing its effects on an animal's behavior, we must slice and stain the brain so that we can observe it under a microscope and see the location of the lesion. Brain lesions often miss the mark, so we have to verify the precise location of the brain damage after testing the animal behaviorally. To do so, we must fix, slice, stain, and examine the brain. Together, these procedures are referred to as *histological methods.* (The prefix *histo-* refers to body tissue.)

FIXATION AND SECTIONING

If we hope to study the tissue in the form it had at the time of the organism's death, we must destroy the autolytic enzymes (*autolytic* means "self-dissolving"), which will otherwise turn the tissue into mush. The tissue must also be preserved to prevent its decomposition by bacteria or molds. To achieve both of these objectives, we place the neural tissue in a **fixative**. The most commonly used fixative is **formalin**, an aqueous solution of formaldehyde, a gas. Formalin halts autolysis, hardens the very soft and fragile brain, and kills any microorganisms that might destroy it.

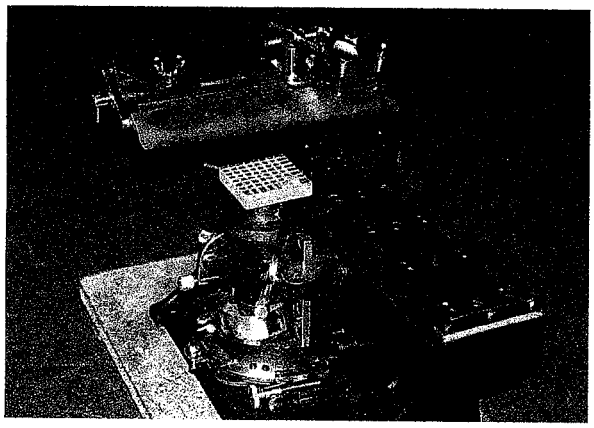


FIGURE 5.7 **A Microtome.**

Simulate Histological Methods in **MyPsychLab**

Once the brain has been fixed, we must slice it into thin sections and stain various cellular structures to see anatomical details. Slicing is done with a **microtome** (literally, "that which slices small"). (See ***Figure 5.7***.) Slices prepared for examination under a light microscope are typically 10 to 80 μm in thickness; those prepared for the electron microscope are generally cut at less than 1 μm. (A μm, or *micrometer,* is one-millionth of a meter, or one-thousandth of a millimeter.) For some reason, slices of brain tissue are usually referred to as *sections.*

After the tissue is cut, we attach the slices to glass microscope slides. We can then stain the tissue by putting the entire slide into various chemical solutions. Finally, we cover the stained sections with a small amount of a transparent liquid known as a *mounting medium* and place a very thin glass coverslip over the sections. The mounting medium keeps the coverslip in position. (Simulate *Histological Methods* in **MyPsychLab**, which shows videos of slicing, staining, and mounting of brain tissue on slides for microscopic examination.)

fixative A chemical such as formalin; used to prepare and preserve body tissue.

formalin (*for ma lin*) The aqueous solution of formaldehyde gas; the most commonly used tissue fixative.

microtome (*my krow tome*) An instrument that produces very thin slices of body tissues.

STAINING

If you looked at an unstained section of brain tissue under a microscope, you would be able to see the outlines of some large cellular masses and the more prominent fiber bundles. However, no fine details would be revealed. For this reason the study of microscopic neuroanatomy requires special histological stains. Researchers have developed many different stains to identify specific substances within and outside of cells. For verifying the location of a brain lesion, we will use one of the simplest: a cell-body stain.

In the late nineteenth century Franz Nissl, a German neurologist, discovered that a dye known as methylene blue would stain the cell bodies of brain tissue. The material that takes up the dye, known as the *Nissl substance,* consists of RNA, DNA, and associated proteins located in the nucleus and scattered, in the form of granules, in the cytoplasm. Many dyes besides methylene blue can be used to stain cell bodies found in slices of the brain, but the

most frequently used is cresyl violet. Incidentally, the dyes were not developed specifically for histological purposes but were originally formulated for use in dyeing cloth.

The discovery of cell-body stains made it possible to identify nuclear masses in the brain. Figure 5.8 shows a frontal section of a cat brain stained with cresyl violet. Note that you can observe fiber bundles by their lighter appearance; they do not take up the stain. (See ***Figure 5.8.***) The stain is not selective for *neural* cell bodies; all cells are stained, neurons and glia alike. It is up to the investigator to determine which is which—by size, shape, and location.

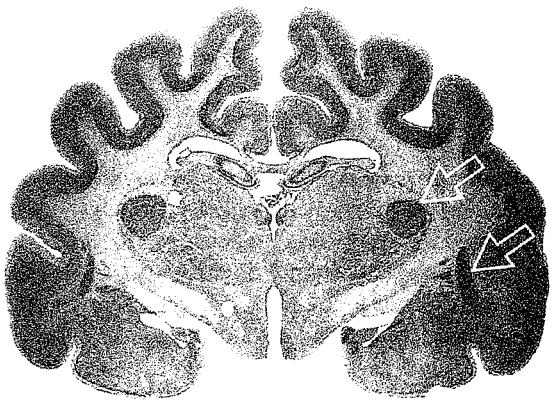


FIGURE 5.8 **Cell-Body Stain.** The photomicrograph shows a frontal section through a cat brain, stained with cresyl violet, a cell-body stain. The arrowheads point to *nuclei*, or groups of cell bodies.

Mary Carlson.

ELECTRON MICROSCOPY

The light microscope is limited in its ability to resolve extremely small details. Because of the nature of light itself, magnification of more than approximately 1500 times does not add any detail. To see such small anatomical structures as synaptic vesicles and details of cell organelles, investigators must use an electron microscope. A beam of electrons is passed through the tissue to be examined. A shadow of the tissue is then cast onto a sheet of photographic film, which is exposed by the electrons. Electron photomicrographs produced in this way can provide information about structural details on the order of a few tens of nanometers. (See ***Figure 5.9.***)

A **scanning electron microscope** provides less magnification than a standard transmission electron microscope, which transmits the electron beam through the tissue. However, it shows objects in three dimensions. The microscope scans the tissue with a moving beam of electrons. The information received from the reflection of the beam is used to produce a remarkably detailed three-dimensional view. The scanning electron micrograph of a cut section of a nerve that you saw in Chapter 2 illustrates the detail that can be revealed by this method. (Refer to ***Figure 2.30.***)

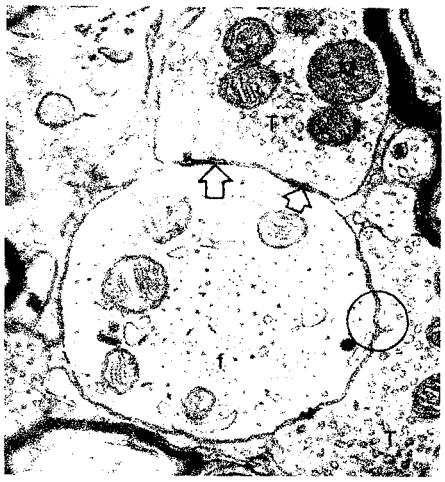


FIGURE 5.9 **Electron Photomicrograph.** The electron photomicrograph shows a section through an axodendritic synapse. Two synaptic regions are pointed out by arrows, and a circle indicates a region of pinocytosis in an adjacent terminal button, presumably representing recycling of vesicular membrane. T = terminal button; f = microfilaments; M = mitochondrion.

Rockel, A. J., and Jones, E. G. *Journal of Comparative Neurology*, 1973, 147, 61–92. John Wiley & Sons, Inc., Journals.

Tracing Neural Connections

Let's suppose that we were interested in discovering the neural mechanisms responsible for reproductive behavior. To start out, we wanted to study the physiology of sexual behavior of female rats. On the basis of some hints we received by reading reports of experiments by other researchers published in scientific journals, we performed stereotaxic surgery on two groups of female rats. We made a lesion in the ventromedial nucleus of the hypothalamus (VMH) of the rats in the experimental group and performed sham surgery on the rats in the control group. After a few days' recovery we placed each animal with a male rat. We found that the females in the control group responded positively to the males' attention; they engaged in courting behavior followed by copulation. However, the females with the VMH lesions rejected the males' attention and refused to copulate with them. We confirmed with histology that the VMH was indeed destroyed in the brains of the experimental animals. (One experimental rat did copulate, but we discovered later that the lesion had missed the VMH in that animal, so we discarded the data from that animal.)

The results of our experiment indicate that neurons in the VMH appear to play a role in functions required for copulatory behavior in females. (By the way, it turns out that these lesions do not affect copulatory behavior in males.) So where do we go from here? What is the next step? In fact, there are many questions that we could pursue. One question concerns the system of brain structures that participate in female copulatory behavior. Certainly, the VMH does not stand alone; it receives inputs from other structures and sends outputs to still others. Copulation requires the integration of visual, tactile, and olfactory information and the organization of patterns of movements in response to those of the partner. In addition, the entire network must be activated by the appropriate sex hormones. What is the precise role of the VMH in this complicated system?

Before we can hope to answer this question, we must know more about the connections of the VMH with the rest of the brain. What structures send their axons to the VMH, and to what structures does the VMH, in turn, send its axons? Once we know what the connections are, we can investigate the role of these structures and the nature of their interactions. (See ***Figure 5.10.***)

scanning electron microscope A microscope that provides three-dimensional information about the shape of the surface of a small object.

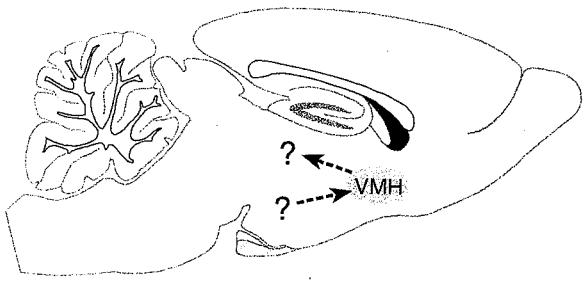


FIGURE 5.10 **Tracing Neural Connections.** Once we know that a particular brain region is involved in a particular function, we may ask what structures provide inputs to the region and what structures receive outputs from it.

How do we investigate the connections of the VMH? The question cannot be answered by means of histological procedures that stain all neurons, such as cell-body stains. If we look closely at a brain that has been prepared by these means, we see only a tangled mass of neurons. But in recent years researchers have developed very precise methods that make specific neurons stand out from all of the others.

TRACING EFFERENT AXONS

Because neurons in the VMH are not directly connected to muscles, they cannot affect behavior directly. Neurons in the VMH must send axons to parts of the brain that contain neurons that are responsible for muscular movements. The pathway is probably not direct; more likely, neurons in the VMH affect neurons in other structures, which influence those in yet other structures until, eventually, the appropriate motor neurons are stimulated. To discover this system, we want to be able to identify the paths followed by axons leaving the VMH. In other words, we want to trace the *efferent axons* of this structure.

We will use an **anterograde labeling method** to trace these axons. (*Anterograde* means "moving forward.") Anterograde labeling methods employ chemicals that are taken up by dendrites or cell bodies and are then transported through the axons toward the terminal buttons.

Over the years neuroscientists have developed several different methods for tracing the pathways followed by efferent axons. For example, to discover the destination of the efferent axons of neurons located within the VMH, we inject a minute quantity of **PHA-L** (a protein found in kidney beans) into that nucleus. (We would use a stereotaxic apparatus to do so, of course.) The molecules of PHA-L are taken up by dendrites and are transported through the soma to the axon, where they travel by means of fast axoplasmic transport to the terminal buttons. Within a few days the cells are filled in their entirety with molecules of PHA-L: dendrites, soma, axons and all their branches, and terminal buttons. Then we kill the animal, slice the brain, and mount the sections on microscope slides. A special *immunocytochemical* method is used to make the molecules of PHA-L visible, and the slides are examined under a microscope. (See ***Figure 5.11***.)

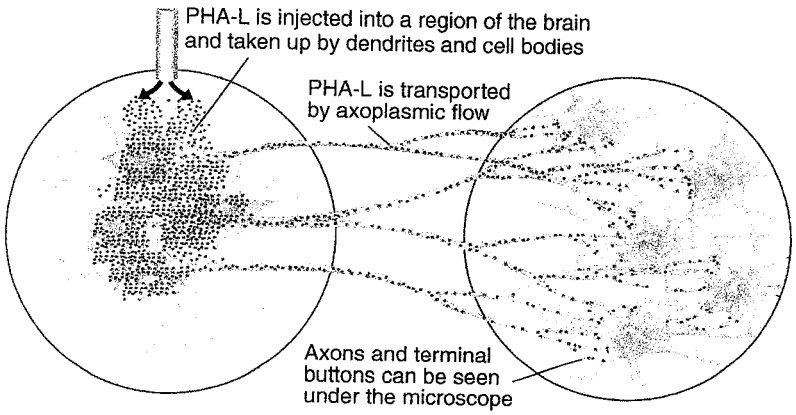


FIGURE 5.11 **Tracing Efferent Axons.** The diagram illustrates the use of PHA-L to trace efferent axons.

Immunocytochemical methods take advantage of the immune reaction. The body's immune system has the ability to produce antibodies in response to antigens. *Antigens* are proteins (or peptides), such as those found on the surface of bacteria or viruses. *Antibodies,* which are also proteins, are produced by white blood cells to destroy invading microorganisms. Antibodies either are secreted by white blood cells or are located on their surface, in the way neurotransmitter receptors are located on the surface of neurons. When the antigens that are present on the surface of an invading microorganism come into contact with the antibodies that recognize them, the antibodies trigger an attack on the invader by the white blood cells.

Molecular biologists have developed methods for producing antibodies to any peptide or protein. The antibody molecules are attached to various types of dye molecules. Some of these dyes react with other chemicals and stain the tissue a brown color. Others are fluorescent; they glow when they are exposed to light of a particular wavelength. To determine where the peptide or protein (the antigen) is located in the brain, the investigator places fresh slices of brain tissue in a solution that contains the antibody/dye molecules. The antibodies attach themselves to their antigen. When the investigator examines the slices with a microscope (under light of a particular wavelength in the case of fluorescent dyes), he or she can see which parts of the brain—even which individual neurons—contain the antigen.

Figure 5.12 shows how PHA-L can be used to identify the efferents of a particular region of the brain. Molecules of this chemical were injected into the VMH. Two days later, after the PHA-L

anterograde labeling method (***ann ter oh grade***) A histological method that labels the axons and terminal buttons of neurons whose cell bodies are located in a particular region.

PHA-L Phaseolus vulgaris leukoagglutinin; a protein derived from kidney beans and used as an anterograde tracer; taken up by dendrites and cell bodies and carried to the ends of the axons.

immunocytochemical method A histological method that uses radioactive antibodies or antibodies bound with a dye molecule to indicate the presence of particular proteins of peptides.

had been taken up by the neurons in this region and transported to the ends of their axons, the animal was killed. Slices of the brain were treated with an antibody to PHA-L and then attached to a dye that stains the tissue a reddish brown color. Figure 5.12 shows a photomicrograph of the periaqueductal gray matter (PAG). As you can see, this region contains some labeled axons and terminal buttons (gold color), which proves that some of the efferent axons of the VMH terminate in the PAG. (See *Figure 5.12.*)

To continue our study of the VMH's role in female sexual behavior, we would find the structures that receive information from neurons in the VMH (such as the PAG) and see what happens when each of them is destroyed. Let's suppose that damage to some of these structures also impairs female sexual behavior. We will inject these structures with PHA-L and see where *their* axons go. Eventually, we will discover the relevant pathways from the VMH to the motor neurons whose activity is necessary for copulatory behavior. (In fact, researchers have done so, and some of their results are presented in Chapter 9.)

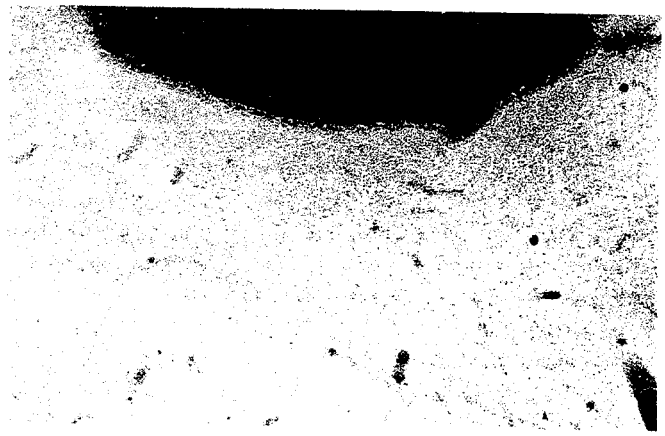


FIGURE 5.12 **Anterograde Tracing Method.** PHA-L was injected into the ventromedial nucleus of the hypothalamus (VMH), where it was taken up by dendrites and carried through the cells' axons to their terminal buttons. Labeled axons and terminal buttons are seen in the periaqueductal gray matter (PAG).

Kirsten Nielsen Ricciardi and Jeffrey Blaustein, University of Massachusetts.

TRACING AFFERENT AXONS

Tracing efferent axons from the VMH will tell us only part of the story about the neural circuitry involved in female sexual behavior: the part between the VMH and the motor neurons. What about the circuits *before* the VMH? Is the VMH somehow involved in the analysis of sensory information (such as the sight, odor, or touch of the male)? Or perhaps the activating effect of a female's sex hormones on her behavior act through the VMH or through neurons whose axons form synapses there. To discover the parts of the brain that are involved in the "upstream" components of the neural circuitry, we need to find the inputs of the VMH—its afferent connections. To do so, we will employ a **retrograde labeling method.**

Retrograde means "moving backward." Retrograde labeling methods employ chemicals that are taken up by terminal buttons and carried back through the axons toward the cell bodies. The method for identifying the afferent inputs to a particular region of the brain is similar to the method used for identifying its efferents. First, we inject a small quantity of a chemical called **fluorogold** into the VMH. The chemical is taken up by terminal buttons and is transported back by means of retrograde axoplasmic transport to the cell bodies. A few days later we kill the animal, slice its brain, and examine the tissue under light of the appropriate wavelength. The molecules of fluorogold fluoresce under this light. We discover that the medial amygdala is one of the regions that provides input to the VMH. (See ***Figure 5.13.***)

FIGURE 5.13 **Retrograde Tracing Method.** Fluorogold was injected in the VMH, where it was taken up by terminal buttons and transported back through the axons to their cell bodies. The photograph shows these cell bodies, located in the medial amygdala.

Yvon Delville, University of Texas.

The anterograde and retrograde labeling methods that I have described identify a single link in a chain of neurons—neurons whose axons enter or leave a particular brain region. **Transneuronal tracing methods** identify a series of neurons that form serial synaptic connections with each other. The most effective transneuronal tracing method uses various strains of weakened rabies viruses or herpes viruses. The virus is injected directly into a brain region, is taken up by neurons there, and infects them. The virus spreads throughout the infected neurons and is eventually released, passing on the infection to other neurons that form synaptic connections with them. Depending on the type and strain of the virus, the infection is preferentially transmitted in an anterograde or a retrograde direction. After the animal is killed and the brain is sliced, immunocytochemical methods are used to localize a protein produced by the virus.

Together, anterograde and retrograde labeling methods—including transneuronal methods—enable us to discover circuits of interconnected neurons. Thus, these methods help to provide us with a "wiring diagram" of the brain. (See ***Figure 5.14.***) Armed with other research methods (including some to be described later in this chapter), we can try to discover the functions of each component of this circuit.

retrograde labeling method A histological method that labels cell bodies that give rise to the terminal buttons that form synapses with cells in a particular region.

fluorogold (***flew*** *roh gold*) A dye that serves as a retrograde label; taken up by terminal buttons and carried back to the cell bodies.

transneuronal tracing method A tracing method that identifies a series of neurons that form serial synaptic connections with each other, either in an anterograde or retrograde direction; involves infection of specific neurons with weakened forms of rabies or herpes viruses.

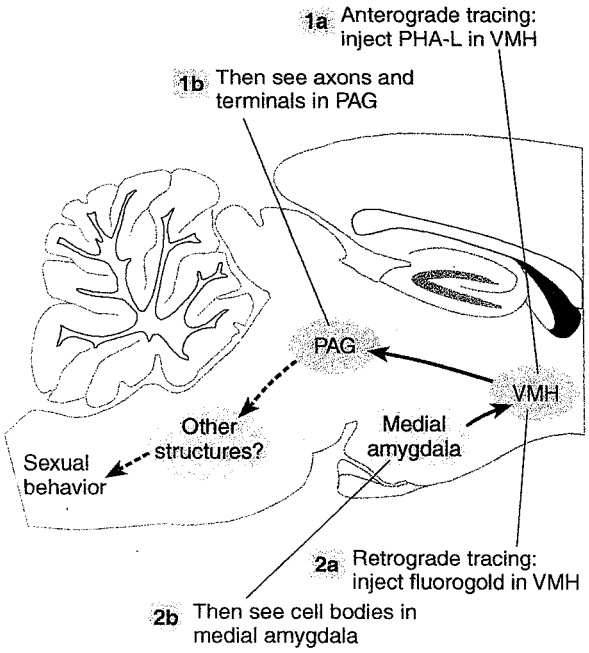


FIGURE 5.14 **Results of Tracing Methods.** One of the inputs to the VMH and one of the outputs, as revealed by anterograde and retrograde labeling methods.

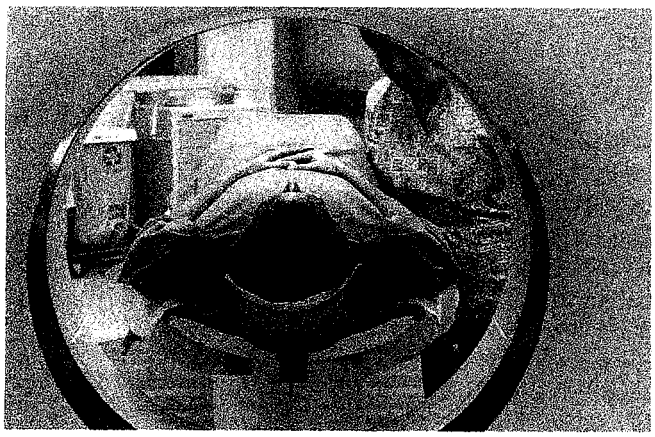


FIGURE 5.15 **Computerized Tomography (CT) Scanner.**

Studying the Structure of the Living Human Brain

There are many good reasons to investigate the functions of brains of animals other than humans. For one thing, we can compare the results of studies made with different species in order to make some inferences about the evolution of various neural systems. Even if our primary interest is in the functions of the human brain, we certainly cannot ask people to submit to brain surgery for the purposes of research. But diseases and accidents do occasionally damage the human brain, and if we know where the damage occurs, we can study the people's behavior and try to make the same sorts of inferences we make with deliberately produced brain lesions in laboratory animals. The problem is, where is the lesion?

In past years a researcher might study the behavior of a person with brain damage and never find out exactly where the lesion was located. The only way to be sure was to obtain the patient's brain when he or she died and examine slices of it under a microscope. But it was often impossible to do so. Sometimes the patient outlived the researcher. Sometimes the patient moved out of town. Sometimes (often, perhaps) the family refused permission for an autopsy. Because of these practical problems, study of the behavioral effects of damage to specific parts of the human brain made rather slow progress.

Recent advances in X-ray techniques and computers have led to the development of several methods for studying the anatomy of the living brain. These advances permit researchers to study the location and extent of brain damage while the patient is still living. The first method that was developed is called **computerized tomography (CT)** (from the Greek words *tomos,* "cut," and *graphein,* "to write"). This procedure, usually referred to as a *CT scan,* works as follows: The patient's head is placed in a large doughnut-shaped ring. The ring contains an X-ray tube and, directly opposite it (on the other side of the patient's head), an X-ray detector. The X-ray beam passes through the patient's head, and the detector measures the amount of radioactivity that gets through it. The beam scans the head from all angles, and a computer translates the numbers it receives from the detector into pictures of the skull and its contents. (See ***Figure 5.15.***)

Figure 5.16 shows a series of these CT scans taken through the head of a patient who sustained a stroke. The stroke damaged a part of the brain involved in bodily awareness and perception of space. The patient lost her awareness of the left side of her body and of items located on her left. You can see the damage as a white spot in the lower left corner of scan 5. (See ***Figure 5.16.***)

An even more detailed, high-resolution picture of what is inside a person's head is provided by a process called **magnetic resonance imaging (MRI)**. The MRI scanner resembles a CT scanner, but it does not use X-rays. Instead, it passes an extremely strong magnetic field through the patient's head. When a person's body is placed in a strong magnetic field, the nuclei of some atoms in molecules in the body spin with a particular orientation. If a radio frequency wave is then passed through the body, these nuclei emit radio waves of their own. Different molecules emit energy at different frequencies. The MRI scanner is tuned to detect the radiation from hydrogen atoms. Because these atoms are present in different concentrations in different tissues, the scanner can use the information to prepare pictures of slices of the brain. Unlike CT scans, which are generally limited to the horizontal plane, MRI scans can be taken in the sagittal or frontal planes as well. (See ***Figure 5.17.***)

As you can see in Figure 5.17, MRI scans distinguish between regions of gray matter and white matter, so major fiber bundles (such as the corpus callosum) can be seen. However, small fiber bundles are not visible on these scans. A special modification of the MRI scanner permits the visualization of even small bundles of fibers and the tracing of fiber tracts. Above absolute zero, all molecules move in random directions because of thermal agitation: the higher the temperature, the faster the random movement. **Diffusion tensor imaging (DTI)** takes advantage of the fact that the movement of water molecules in bundles of white matter will not be random,

computerized tomography (CT) The use of a device that employs a computer to analyze data obtained by a scanning beam of X-rays to produce a two-dimensional picture of a "slice" through the body.

magnetic resonance imaging (MRI) A technique whereby the interior of the body can be accurately imaged; involves the interaction between radio waves and a strong magnetic field.

diffusion tensor imaging (DTI) An imaging method that uses a modified MRI scanner to reveal bundles of myelinated axons in the living human brain.

but will tend to be in a direction parallel to the axons that make up the bundles. The MRI scanner uses information about the movement of the water molecules to determine the location and orientation of bundles of axons in white matter. Figure 5.18 shows a sagittal view of some of the axons that project from the thalamus to the cerebral cortex in the human brain, as revealed by diffusion tensor imaging. The computer adds colors to distinguish different bundles of axons. (See *Figure 5.18.*)

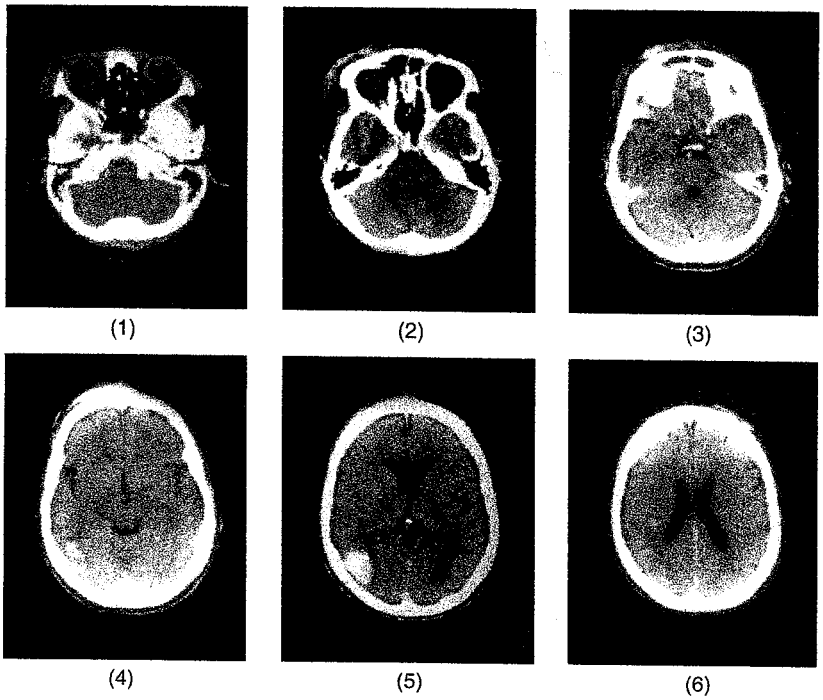


FIGURE 5.16 **CT Scans of a Lesion.** The patient had a lesion in the right occipital-parietal area (scan 5). The lesion appears white because it was accompanied by bleeding; blood absorbs more radiation than does the surrounding brain tissue. Rostral is up, caudal is down; left and right are reversed. Scan 1 shows a section through the eyes and the base of the brain.

J. McA. Jones.

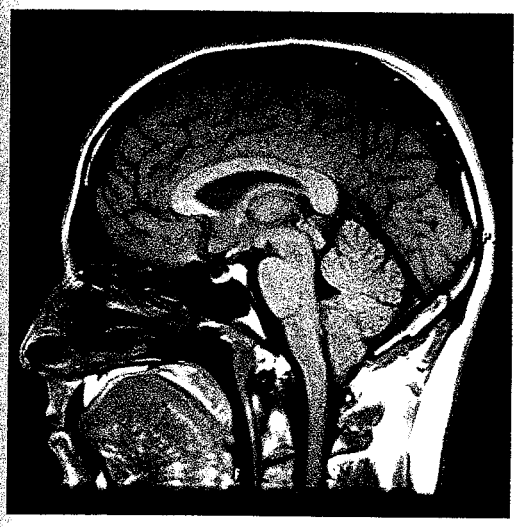


FIGURE 5.17 **Magnetic Resonance Imaging (MRI).** The figure shows a midsagittal MRI scan of a human brain.

Living Art Enterprises/Science Source/Photo Researchers, Inc.

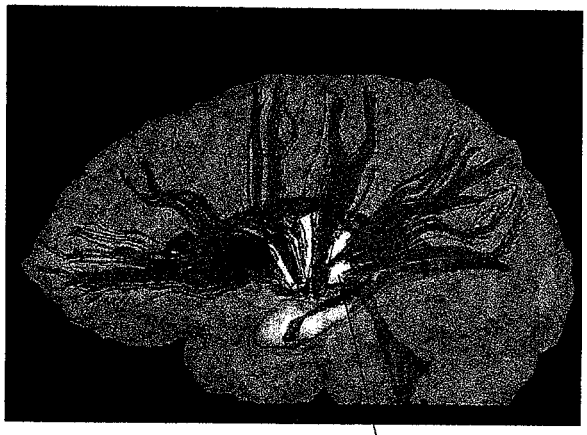


FIGURE 5.18 **Diffusion Tensor Imaging (DTI).** A sagittal view of some of the axons that project from the thalamus to the cerebral cortex in the human brain is revealed by diffusion tensor imaging.

From Wakana, S., Jian, H., Nagae-Poetscher, L. M., van Zijl, P. C. M., and Mori, S. *Radiology*, 2004, 230, 77–87. Reprinted with permission.

SECTION SUMMARY

Experimental Ablation

The goal of research in behavioral neuroscience is to understand the brain functions required for the performance of a particular behavior and then to learn the location of the neural circuits that perform these functions. The lesion method is the oldest one employed in such research, and it remains one of the most useful. A subcortical lesion is made under the guidance of a stereotaxic apparatus. The coordinates are obtained from a stereotaxic atlas, and the tip of an electrode or cannula is placed at the target. A lesion is made by passing radio frequency current through the electrode or infusing an excitatory amino acid through the cannula, producing an excitotoxic lesion. The advantage of excitotoxic lesions is that they destroy only neural cell bodies; axons passing through the region are not damaged.

The location of a lesion must be determined after observation of the animal's behavior. The animal is killed by humane means, and the brain is removed and placed in a fixative such as formalin. A microtome is used to slice the brain, which is usually frozen to make it hard enough to cut into thin sections. These sections are mounted on glass slides, stained with a cell-body stain, and examined under a microscope.

Light microscopes enable us to see cells and their larger organelles, but an electron microscope is needed to see small details, such as individual mitochondria and synaptic vesicles. Scanning electron microscopes provide a three-dimensional view of tissue, but at a lower magnification than transmission electron microscopes.

The next step in a research program often requires the investigator to discover the afferent and efferent connections of the region of interest with the rest of the brain. Efferent connections (those that carry information from the region in question to other parts of the brain) are revealed with anterograde tracing methods, such as the one that uses PHA-L. Afferent connections (those that bring information to the region in question from other parts of the brain) are revealed with retrograde tracing methods, such as the one that uses fluorogold. Chains of neurons that form synaptic connections are revealed by anterograde or retrograde transneuronal tracing methods, which utilized weakened forms of rabies and herpes viruses.

Although brain lesions are not deliberately made in the human brain for the purposes of research, diseases and accidents can cause brain damage, and if we know where the damage is located, we can study people's behavior and make inferences about the location of the neural circuits that perform relevant functions. If the patient dies and the brain is available for examination, ordinary histological methods can be used. Otherwise, the living brain can be examined with CT scanners and MRI scanners. Diffusion tensor imaging (DTI) uses a modified MRI scanner to visualize bundles of myelinated axons in the living human brain.

Table 5.1 summarizes the research methods presented in this section. (See ***Table 5.1***.)

Thought Questions

1. In the subsection "Tracing Neural Connections," I wrote that "one experimental rat did copulate, but we discovered later that the lesion had missed the VMH in that animal, so we discarded the data from that animal." Should the person who looks at the stained brain sections containing the lesions and decides whether the target was destroyed or missed know which animal each of these sections belonged to? Explain.
2. Would you like to see an MRI of your own brain? Why or why not?

TABLE 5.1 **Research Methods: Part I**

Goal of Method	Method	Remarks
Destroy or inactivate specific brain region	Radio frequency lesion	Destroys all brain tissue near the tip of the electrode
	Excitotoxic lesion; uses excitatory amino acid such as kainic acid	Destroys only cell bodies near the tip of the cannula; spares axons passing through the region
	Infusion of local anesthetic or drug that produces local neural inhibition	Temporarily inactivates specific brain region; animal can serve as its own control
	Infusion of a toxin attached to an antibody	Destroys neurons that contain the antibody; produces very precise brain lesions
Place electrode or cannula in a specific region within the brain	Stereotaxic surgery	Consult stereotaxic atlas for coordinates
Find the location of the lesion	Fix brain; slice brain; stain sections	
Identify axons leaving a particular region and the terminal buttons of these axons	Anterograde tracing method, such as PHA-L	
Identify the location of neurons whose axons terminate in a particular region	Retrograde tracing method, such as fluorogold	
Identify chain of neurons that are interconnected synaptically	Transneuronal tracing method; uses pseudorabies virus	Can be used for both anterograde and retrograde tracing
Find location of lesion in living human brain	Computerized tomography (CT scanner)	Shows "slice" of brain; uses X-rays
	Magnetic resonance imaging (MRI scanner)	Shows "slice" of brain; better detail than CT scan; uses a magnetic field and radio waves

Recording and Stimulating Neural Activity

The first section of this chapter dealt with the anatomy of the brain and the effects of damage to particular regions. This section considers a different approach: studying the brain by recording or stimulating the activity of particular regions. Brain functions involve activity of circuits of neurons; thus, different perceptions and behavioral responses involve different patterns of activity in the brain. Researchers have devised methods to record these patterns of activity or to artificially produce them.

Recording Neural Activity

Axons produce action potentials, and terminal buttons elicit postsynaptic potentials in the membrane of the cells with which they form synapses. These electrical events can be recorded (as we saw in Chapter 2), and changes in the electrical activity of a particular region can be used to determine whether that region plays a role in various behaviors. For example, recordings can be made during stimulus presentations, decision making, or motor activities.

Recordings can be made *chronically,* over an extended period of time after the animal recovers from surgery, or *acutely,* for a relatively short period of time during which the animal is kept anesthetized. Acute recordings, made while the animal is anesthetized, are usually restricted to studies of sensory pathways. Acute recordings seldom involve behavioral observations, since the behavioral capacity of an anesthetized animal is limited, to say the least.

If we want to record the activity of a particular region of the brain of an animal that is awake and free to move about, we would implant the electrodes by means of stereotaxic surgery. We would attach the electrodes to miniaturized electrical sockets and bond the sockets to the animal's skull, using plastics that were originally developed for the dental profession. Then, after recovery from surgery, the animal can be "plugged in" to the recording system. Laboratory animals pay no heed to the electrical sockets on their skulls and behave quite normally. (See ***Figure 5.19***.)

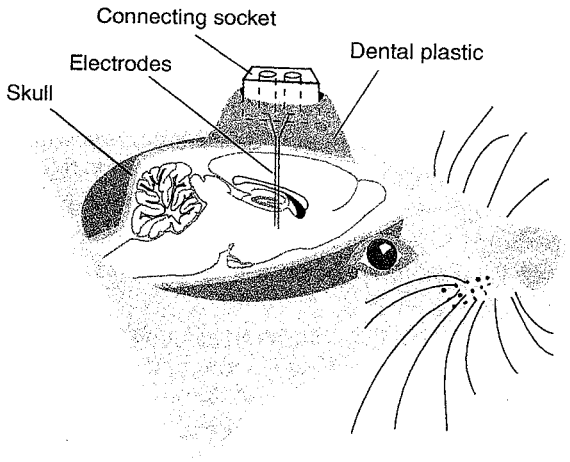


FIGURE **5.19** **Implantation of Electrodes.** This schematic shows a permanently attached set of electrodes, with a connecting socket cemented to the skull.

RECORDINGS WITH MICROELECTRODES

Drugs that affect serotonergic and noradrenergic neurons also affect REM sleep. Suppose that, knowing this fact, we wondered whether the activity of serotonergic and noradrenergic neurons would vary during different stages of sleep. To find out, we would record the activity of these neurons with microelectrodes. **Microelectrodes** have a very fine tip, small enough to record the electrical activity of individual neurons. This technique is usually called **single-unit recording** (a unit refers to an individual neuron).

Because we want to record the activity of single neurons over a long period of time in unanesthetized animals, we want more durable electrodes. We can purchase arrays of very fine wires, gathered together in a bundle. The wires are insulated so that only their tips are bare.

Researchers often attach rather complex devices to an animal's skull when they implant microelectrodes. These devices include screw mechanisms that permit the experimenter to move the electrode—or array of electrodes—deeper into the brain so that they can record from several different parts of the brain during the course of their observations.

The electrical signals detected by microelectrodes are quite small and must be amplified. Amplifiers used for this purpose work just like the amplifiers in a stereo system, converting the weak signals recorded at the brain into stronger ones. These signals can be displayed on an oscilloscope and stored in the memory of a computer for analysis at a later time.

What about the results of our recordings from serotonergic and noradrenergic neurons? As you will learn in Chapter 8, if we record the activity of these neurons during various stages of sleep, we will find that their firing rates fall almost to zero during REM sleep. This observation suggests that these neurons have an *inhibitory* effect on REM sleep. That is, REM sleep cannot occur until these neurons stop firing.

microelectrode A very fine electrode, generally used to record activity of individual neurons.

single-unit recording Recording of the electrical activity of a single neuron.

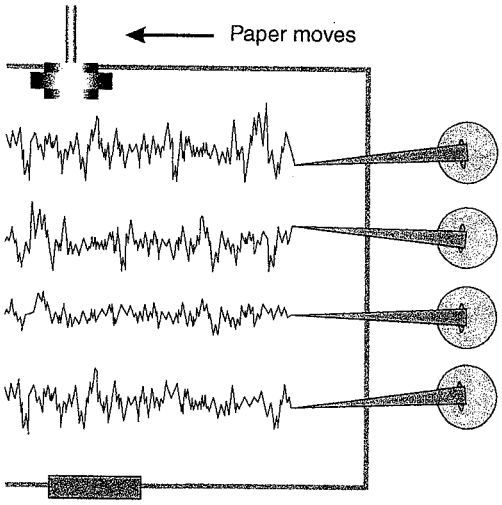


FIGURE 5.20 **A Record from a Polygraph.**

RECORDINGS WITH MACROELECTRODES

Sometimes, we want to record the activity of a region of the brain as a whole, not the activity of individual neurons located there. To do this, we would use macroelectrodes. **Macroelectrodes** do not detect the activity of individual neurons; rather, the records that are obtained with these devices represent the postsynaptic potentials of many thousands—or millions—of cells in the area of the electrode. These electrodes can consist of unsharpened wires inserted into the brain, screws attached to the skull, or even metal disks attached to the human scalp with a special paste that conducts electricity. Recordings taken from the scalp, especially, represent the activity of an enormous number of neurons, whose electrical signals pass through the meninges, skull, and scalp before reaching the electrodes.

Occasionally, neurosurgeons implant macroelectrodes directly into the human brain. The reason for doing so is to detect the source of abnormal electrical activity that is giving rise to frequent seizures. Once the source has been determined, the surgeon can open the skull and remove the source of the seizures—usually scar tissue caused by brain damage that occurred earlier in life. Usually, the electrical activity of a human brain is recorded through electrodes attached to the scalp and displayed on a *polygraph.*

A polygraph contains a mechanism that moves a very long strip of paper past a series of pens. These pens are essentially the pointers of large voltmeters, moving up and down in response to the electrical signal sent to them by the biological amplifiers. Figure 5.20 illustrates a record of electrical activity recorded from macroelectrodes attached to various locations on a person's scalp. (See ***Figure 5.20.***) Such records are called **electroencephalograms (EEGs)**, or "writings of electricity from the head." They can be used to diagnose epilepsy or study the stages of sleep and wakefulness, which are associated with characteristic patterns of electrical activity.

Another use of the EEG is to monitor the condition of the brain during surgical procedures that could potentially damage it, such as the one we encountered in the prologue to this chapter. The use of EEG monitoring during blood-vessel surgery is described in the epilogue to this chapter.

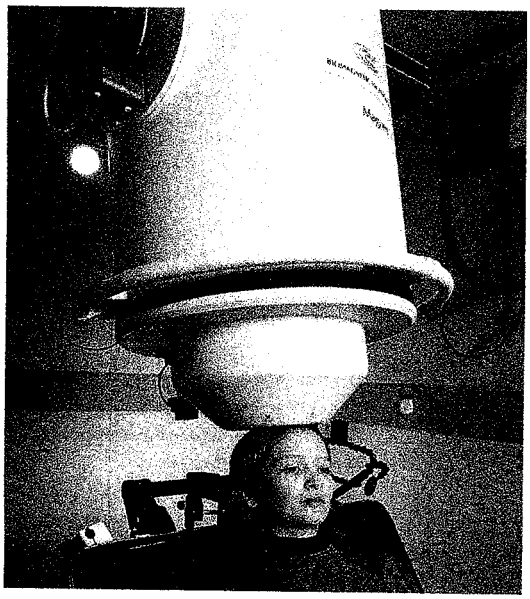


FIGURE 5.21 **Magnetoencephalography.** An array of SQUIDs in this neuromagnetometer detects regional changes in magnetic fields produced by electrical activity of the brain.

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MAGNETOENCEPHALOGRAPHY

As you undoubtedly know, when electrical current flows through a conductor, it induces a magnetic field. This means that as action potentials pass down axons or as postsynaptic potentials pass down dendrites or sweep across the somatic membrane of a neuron, magnetic fields are also produced. These fields are exceedingly small, but engineers have developed superconducting detectors (called superconducting quantum interference devices, or SQUIDs) that can detect magnetic fields that are approximately one-billionth of the size of the earth's magnetic field. **Magnetoencephalography (MEG)** is performed with *neuromagnetometers,* devices that contain an array of several SQUIDs, oriented so that a computer can examine their output and calculate the source of particular signals in the brain. The neuromagnetometer shown in Figure 5.21 contains 275 SQUIDs. These devices can be used clinically—for example, to find the sources of seizures so that they can be removed surgically. They can also be used in experiments to measure regional brain activity that accompanies the perception of various stimuli or the performance of various behaviors or cognitive tasks. (See ***Figure 5.21.***)

An important advantage of magnetoencephalography is its temporal resolution. Functional MRI (described later) provides excellent spatial resolution, but relatively poor temporal resolution. That is, the image can accurately measure differences in activity of closely spaced regions of the brain, but the acquisition of an fMRI image takes a relatively long time compared with the rapid flow of information in the brain. The image produced by means of magnetoencephalography is much more crude than an fMRI, but it can be acquired much more rapidly, and can consequently reveal fast-moving events.

macroelectrode An electrode used to record the electrical activity of large numbers of neurons in a particular region of the brain; much larger than a microelectrode.

electroencephalogram (EEG) An electrical brain potential recorded by placing electrodes on the scalp.

magnetoencephalography (MEG) A procedure that detects groups of synchronously activated neurons by means of the magnetic field induced by their electrical activity; uses an array of superconducting quantum interference devices (SQUIDs).

Recording the Brain's Metabolic and Synaptic Activity

Electrical signals are not the only signs of neural activity. If the neural activity of a particular region of the brain increases, the metabolic rate of this region increases, too, largely as a result of increased operation of transporters in the membrane of the cells. This increased metabolic rate

can be measured. The experimenter injects radioactive **2-deoxyglucose** (2-DG) into the animal's bloodstream. Because this chemical resembles glucose (the principal food for the brain), it is taken into cells. Thus, the most active cells, which use glucose at the highest rate, will take up the highest concentrations of radioactive 2-DG. But unlike normal glucose, 2-DG cannot be metabolized, so it stays in the cell. The experimenter then kills the animal, removes the brain, slices it, and prepares it for *autoradiography.*

Autoradiography can be translated roughly as "writing with one's own radiation." Sections of the brain are mounted on microscope slides. The slides are then taken into a darkroom, where they are coated with a photographic emulsion (the substance found on photographic film). Several weeks later, the slides, with their coatings of emulsion, are developed, just like photographic film. The molecules of radioactive 2-DG show themselves as spots of silver grains in the developed emulsion because the radioactivity exposes the emulsion, just as X-rays or light will do.

The most active regions of the brain contain the most radioactivity, showing this radioactivity in the form of dark spots in the developed emulsion. Figure 5.22 shows an autoradiograph of a slice of a rat brain; the dark spots at the bottom (indicated by the arrow) are nuclei of the hypothalamus with an especially high metabolic rate. Chapter 8 describes these nuclei and their function. (See ***Figure 5.22.***) (Simulate *Autoradiography* in **MyPsychLab**, which shows videos of preparation of brain tissue for examination of regions that were activated by an experimental procedure.)

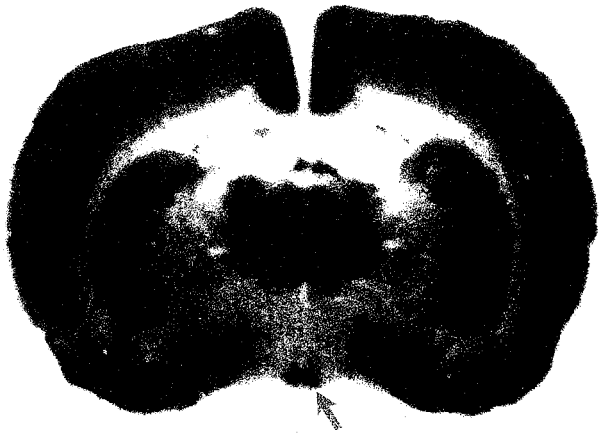


FIGURE **5.22** **2-DG Autoradiography.** This 2-DG autoradiogram of a rat brain (frontal section, dorsal is at top) shows especially high regions of activity in the pair of nuclei in the hypothalamus, at the base of the brain.

American Association for the Advancement of Sciences.

Simulate Autoradiography in **MyPsychLab**

Another method of identifying active regions of the brain capitalizes on the fact that when neurons are activated (for example, by the terminal buttons that form synapses with them), particular genes in the nucleus called *immediate early genes* are turned on and particular proteins are produced. These proteins then bind with the chromosomes in the nucleus. The presence of these proteins indicates that the neuron has just been activated.

One of the nuclear proteins produced during neural activation is called **Fos.** You will remember that earlier in this chapter we began an imaginary research project on the neural circuitry involved in the sexual behavior of female rats. Suppose we want to use the Fos method in this project to see what neurons are activated during a female rat's sexual activity. We place female rats with males and permit the animals to copulate. Then we remove the rats' brains, slice them, and follow a procedure that stains Fos protein. Figure 5.23 shows the results: Neurons in the medial amygdala of a female rat that has just mated show the presence of dark spots, indicating the presence of Fos protein. Thus, these neurons appear to be activated by copulatory activity—perhaps by the physical stimulation of the genitals that occurs then. As you will recall, when we injected a retrograde tracer (fluorogold) into the VMH, we found that this region receives input from the medial amygdala. (See ***Figure 5.23.***)

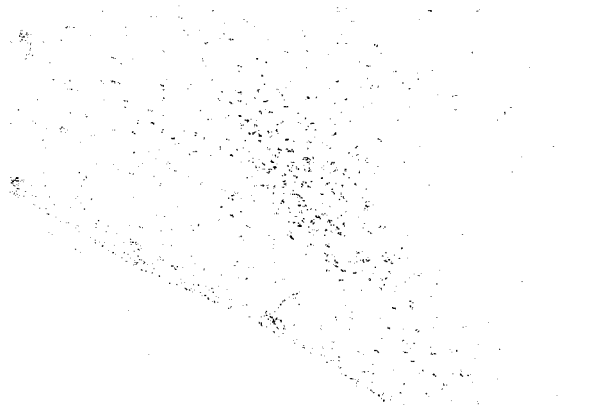


FIGURE **5.23** **Localization of Fos Protein.** The photomicrograph shows a frontal section of the brain of a female rat, taken through the medial amygdala. The dark spots indicate the presence of Fos protein, localized by means of immunocytochemistry. The synthesis of Fos protein was stimulated by permitting the animal to engage in copulatory behavior.

Dr. Marc Tetel, Wellesley College.

The metabolic activity of specific brain regions can be measured in human brains, too, by means of **functional imaging**—a computerized method of detecting metabolic or chemical changes within the brain. The first functional imaging method to be developed was **positron emission tomography (PET).** First, the patient receives an injection of radioactive 2-DG. (The chemical soon breaks down and leaves the cells. The dose given to humans is harmless.) The person's head is placed in a machine similar to a CT scanner. When the radioactive molecules of 2-DG decay, they emit subatomic particles called positrons, which meet nearby electrons. The particles annihilate each other and emit two photons, which travel in directly opposite paths. Sensors arrayed around the person's head detect these photons and the scanner plots the locations from which these photons are being emitted. From this information, the computer produces a picture of a slice of the brain, showing the activity level of various regions in that slice. (See ***Figure 5.24.***)

One of the disadvantages of PET scanners is their operating cost. For reasons of safety the radioactive chemicals that are administered have very short half-lives; that is, they decay and lose

2-deoxyglucose (2-DG) (*dee ox ee* ***gloo*** *kohss*) A sugar that enters cells along with glucose but is not metabolized.

autoradiography A procedure that locates radioactive substances in a slice of tissue; the radiation exposes a photographic emulsion or a piece of film that covers the tissue.

Fos (*fahs*) A protein produced in the nucleus of a neuron in response to synaptic stimulation.

functional imaging A computerized method of detecting metabolic or chemical changes in particular regions of the brain.

positron emission tomography (PET) A functional imaging method that reveals the localization of a radioactive tracer in a living brain.

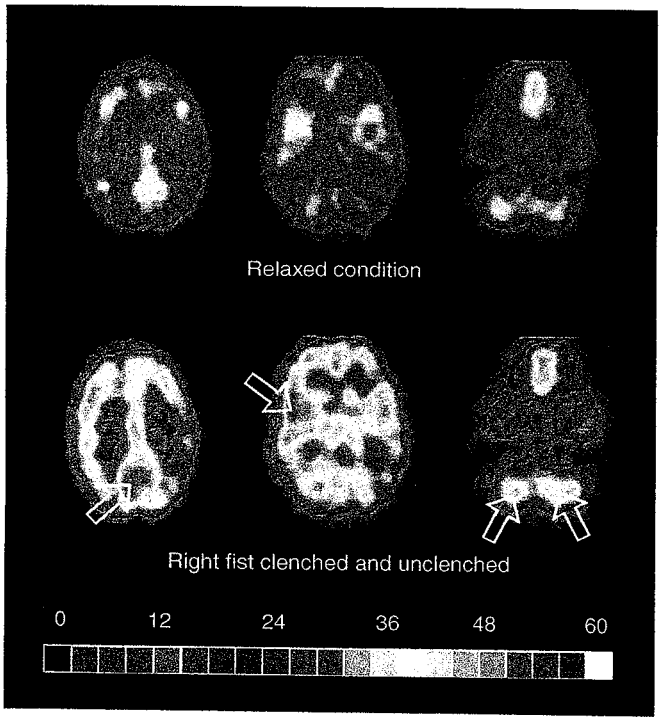


FIGURE 5.24 **PET Scans.** In the top row of these human brain scans are three horizontal scans from a person at rest. The bottom row shows three scans from the same person while he was clenching and unclenching his right fist. The scans show increased uptake of radioactive 2-deoxyglucose in regions of the brain that are devoted to the control of movement, which indicates increased metabolic rate in these areas. Different computer-generated colors indicate different rates of uptake of 2-DG, as shown in the scale at the bottom.

Brookhaven National Laboratory and the State University of New York, Stony Brook.

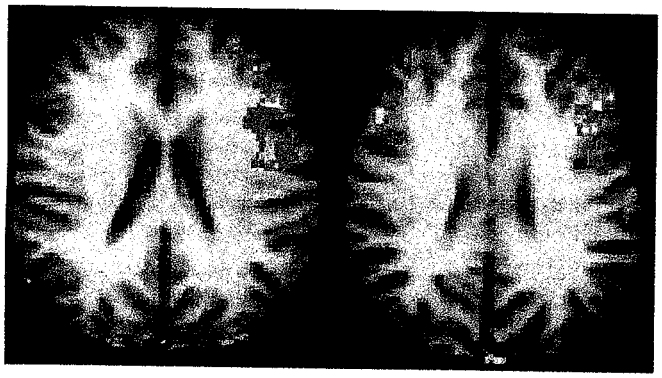


FIGURE 5.25 **Functional MRI Scans.** These scans of human brains show localized average increases in neural activity of males (left) and females (right) while they were judging whether pairs of written words rhymed.

Shaywitz, B. A., et al., *Nature*, 1995, 373, 607–609.

their radioactivity very quickly. For example, the half-life of radioactive 2-DG is 110 minutes; the half-life of radioactive water (also used for PET scans) is only 2 minutes. Because these chemicals decay so quickly, they must be produced on site, in an atomic particle accelerator called a *cyclotron*. Therefore, the cost of the PET scanner must be added to the cost of the cyclotron and the salaries of the personnel who operate it.

The functional brain imaging method with the best spatial and temporal resolution is known as **functional MRI (fMRI)**. Engineers have devised modifications to existing MRI scanners and their software that permit the devices to acquire images that indicate regional metabolism. Brain activity is measured indirectly, by detecting levels of oxygen in the brain's blood vessels. Increased activity of a brain region stimulates blood flow to that region, which increases the local blood oxygen level. The formal name of this type of imaging is *BOLD*—blood oxygen level-dependent signal. Functional MRI scans have a higher resolution than PET scans, and they can be acquired much faster. Thus, they reveal more detailed information about the activity of particular brain regions. You will read about many functional imaging studies that employ fMRI scans in subsequent chapters of this book. (See ***Figure 5.25***.)

Stimulating Neural Activity

So far, this section has been concerned with research methods that measure the activity of specific regions of the brain. But sometimes we may want to artificially change the activity of these regions to see what effects these changes have on the animal's behavior. For example, female rats will copulate with males only if certain female sex hormones are present. If we remove the rats' ovaries, the loss of these hormones will abolish their sexual behavior. We found in our earlier studies that VMH lesions disrupt this behavior. Perhaps if we *activate* the VMH, we will make up for the lack of female sex hormones and the rats will copulate again.

ELECTRICAL AND CHEMICAL STIMULATION

How do we activate neurons? We can do so by electrical or chemical stimulation. Electrical stimulation simply involves passing an electrical current through a wire inserted into the brain, as you saw in Figure 5.19. Chemical stimulation is usually accomplished by injecting a small amount of an excitatory amino acid, such as kainic acid or glutamic acid, into the brain. As you learned in Chapter 4, the principal excitatory neurotransmitter in the brain is glutamic acid (glutamate), and both of these chemicals stimulate glutamate receptors, thus activating the neurons on which these receptors are located.

Injections of chemicals into the brain can be done through an apparatus that is permanently attached to the skull so that the animal's behavior can be observed several times. We place a metal cannula (a guide cannula) in an animal's brain and cement its top to the skull. At a later date we place a smaller cannula of measured length inside the guide cannula and then inject a chemical into the brain. Because the animal is free to move about, we can observe the effects of the injection on its behavior. (See ***Figure 5.26***.) (Simulate *Cannula Implantation* in **MyPsychLab**, which shows the insertion of a metal cannula in a rodent's brain and later observation of the animal's behavior after a chemical has been infused into the brain.)

Simulate Cannula Implantation in **MyPsychLab**

functional MRI (fMRI) A functional imaging method; a modification of the MRI procedure that permits the measurement of regional metabolism in the brain.

The principal disadvantage of chemical stimulation is that it is slightly more complicated than electrical stimulation; chemical stimulation requires cannulas, tubes, special pumps or syringes, and sterile solutions of excitatory amino acids. However, it has a distinct advantage over electrical stimulation: It activates cell bodies but not axons. Because only cell bodies (and their dendrites, of course) contain glutamate receptors, we can be assured that an injection of an excitatory amino

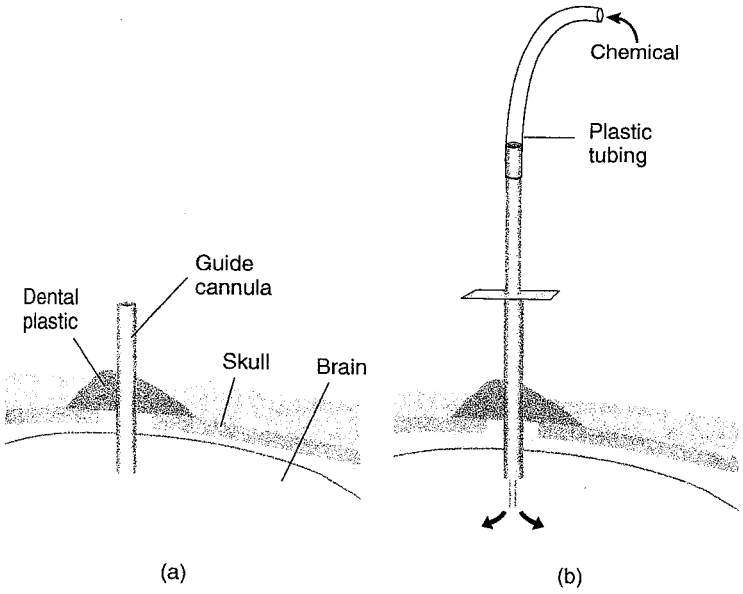


FIGURE 5.26 **Intracranial Cannula.** A guide cannula is permanently attached to the skull (a), and at a later time a thinner cannula can be inserted through the guide cannula into the brain (b). Chemicals can be infused into the brain through this device.

acid into a particular region of the brain excites the cells there, but not the axons of other neurons that happen to pass through the region. Thus, the effects of chemical stimulation are more localized than the effects of electrical stimulation.

You might have noticed that I just said that kainic acid, which I described earlier as a neurotoxin, can be used to stimulate neurons. These two uses are not really contradictory. Kainic acid produces excitotoxic lesions by stimulating neurons to death. Whereas large doses of a concentrated solution kill neurons, small doses of a dilute solution simply stimulate them.

What about the results of our imaginary experiment? In fact (as we shall see in Chapter 9), VMH stimulation does substitute for female sex hormones. Perhaps, then, the female sex hormones exert their effects in this nucleus. We will see how to test this hypothesis later in this chapter.

OPTOGENETIC METHODS

When chemicals are injected into the brain through cannulas, molecules of the chemicals diffuse over a region that includes many different types of neurons: excitatory neurons, inhibitory neurons, interneurons that participate in local circuits, projection neurons that communicate with different regions of the brain, and neurons that release or respond to a wide variety of neurotransmitters and neuromodulators. Stimulating a particular brain region with electricity or an excitatory chemical affects all of these neurons, and the result is unlikely to resemble normal brain activity, which involves coordinated activation and inhibition of many different neurons. Ideally, we would like to be able to stimulate or inhibit selected populations of neurons in a given brain region.

Recent developments are providing the means to do just this. **Optogenetic methods** can be used to stimulate or inhibit particular types of neurons in particular brain regions (Boyden et al., 2005; Zhang et al., 2007; Baker, 2011). Photosensitive proteins have evolved in many organisms—even single-celled organisms such as algae and bacteria. Researchers have discovered that when blue light strikes one of these proteins *(ChR2)*, the channel opens and the rush of positively charged sodium and calcium ions depolarizes the membrane, causing excitation. When yellow light strikes a second photosensitive protein, *(NpHR)*, a transporter moves chloride into the cell causing inhibition. The action of both of these photosensitive proteins begins and ends very rapidly when light of the appropriate wavelength is turned on and off. (See *Figure 5.27*.)

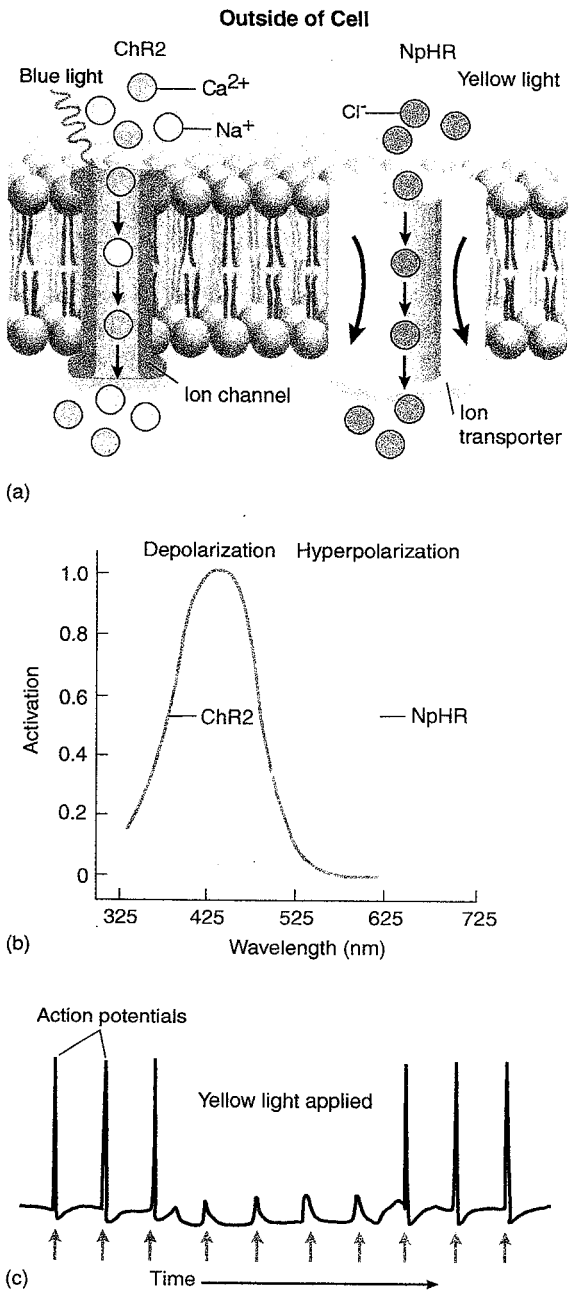


FIGURE 5.27 **Photostimulation.** (a) Photosensitive proteins can be inserted into neural membranes by means of genetically modified viruses. Blue light causes ChR2 ion channels to depolarize the membrane, and yellow light causes NpHR ion transporters to hyperpolarize it. (b) The graph shows the effects on the membrane potential of different wavelengths of light acting on ChR2 or NpHR proteins. (c) The graph shows action potentials elicited by pulses of blue light (blue arrows) and the inhibitory effects of the hyperpolarization caused by yellow light.

Part (a) based on Hausser, M., and Smith, S. L. *Nature*, 2007, *446*, 617–619, and parts (b) and (c) based on Zhang, F., Wang, L. P., Brauner, M., et al. *Nature*, 2007, *446*, 633–639.

optogenetic methods The use of a genetically modified virus to insert light-sensitive ion channels into the membrane of particular neurons in the brain; can depolarize or hyperpolarize the neurons when light of the appropriate wavelength is applied.

FIGURE 5.28 **Transcranial Magnetic Stimulation (TMS).** Pulses of electricity through the coil produce a magnetic field that stimulates a region of the cerebral cortex under the crossing point in the middle of the figure 8.

The Kastner Lab/Princeton University.

ChR2 and NpHR can be introduced into neurons by attaching the genes that code for them into the genetic material of harmless viruses. The viruses are then injected into the brain, where they infect neurons and begin expressing the proteins, which are inserted into the cell membrane. The genes can be modified so that the proteins will be expressed only in particular types of neurons. In this way, researchers can observe the effects of turning on or off particular types of neurons in a particular region of the brain. To activate photosensitive proteins in the membranes of neurons deep within the brain, optical fibers can be implanted by means of stereotaxic surgery, just like electrodes or cannulas, and light can be transmitted through these fibers.

The development of these procedures has caused much excitement among neuroscientists because they promise ways to study the functions of particular neural circuits in the brain. Some investigators are also exploring possible clinical uses of photosensitive proteins. For example, retinitis pigmentosa is a genetic disease that causes blindness in humans. People with this disease are born with normal vision, but they gradually become blind as the photoreceptor cells in their retinas degenerate. The retina contains two major categories of photoreceptors: *rods,* which are responsible for night vision, and *cones,* which are responsible for daytime vision. The rods of people with retinitis pigmentosa die, but although the cones lose their sensitivity to light, their cell bodies survive. Busskamp et al. (2010) used an optogenetic method to try to re-establish vision in mice with a genetic modification that caused them to develop retinitis pigmentosa. The investigators targeted the animals' cones with NpHR. (Because the membranes of photoreceptors are normally hyperpolarized by light, they chose to use this protein.) Electrical recording and behavioral studies found that the treatment at least partially re-established the animals' vision. Furthermore, the same treatment re-established light sensitivity in retinal tissue removed from deceased people who had suffered from retinitis pigmentosa. These findings provide hope that further research may develop a treatment for this form of blindness.

TRANSCRANIAL MAGNETIC STIMULATION

As we saw earlier in this chapter, neural activity induces magnetic fields that can be detected by means of magnetoencephalography. Similarly, magnetic fields can be used to stimulate neurons by inducing electrical currents in brain tissue. **Transcranial magnetic stimulation (TMS)** uses a coil of wires, usually arranged in the shape of the numeral 8, to stimulate neurons in the human cerebral cortex. The stimulating coil is placed on top of the skull so that the crossing point in the middle of the 8 is located immediately above the region to be stimulated. Pulses of electricity send magnetic fields that activate neurons in the cortex. Figure 5.28 shows an electromagnetic coil used in transcranial magnetic stimulation and its placement on a person's head. (See ***Figure 5.28.***)

transcranial magnetic stimulation (TMS) Stimulation of the cerebral cortex by means of magnetic fields produced by passing pulses of electricity through a coil of wire placed next to the skull; interferes with the functions of the brain region that is stimulated.

The effects of TMS are very similar to those of direct stimulation of the exposed brain. For example, as we shall see in Chapter 6, stimulation of a particular region of the visual association cortex will disrupt a person's ability to detect movements in visual stimuli. In addition, as we will see in Chapter 15, TMS has been used to treat the symptoms of mental disorders such as depression. Depending on the strength and pattern of stimulation, TMS can either excite the region of the brain over which the coil is positioned or interfere with its functions.

SECTION SUMMARY

Recording and Stimulating Neural Activity

When circuits of neurons participate in their normal functions, their electrical activity and metabolic activity increase. Thus, by observing these processes as an animal perceives various stimuli or engages in various behaviors, we can make some inferences about the functions performed by various regions of the brain. Microelectrodes can be used to record the electrical activity of individual neurons. Chronic recordings require that the electrode be attached to an electrical socket, which is fastened to the skull with a plastic adhesive. Macroelectrodes record the activity of large groups of neurons. In rare cases macroelectrodes are placed in the depths of the human brain, but most often they are placed on the scalp and their activity is recorded on a polygraph or computer. Such records can be used in the diagnosis of epilepsy or the study of sleep.

Section Summary (*continued*)

Metabolic activity can be measured by giving an animal an injection of radioactive 2-DG, which accumulates in metabolically active neurons. The presence of the radioactivity is revealed through autoradiography: Slices of the brain are placed on microscope slides, covered with a photographic emulsion, left to sit a while, and then are developed like photographic negatives. When neurons are stimulated, they synthesize the nuclear protein Fos. The presence of Fos, revealed by a special staining method, provides another way to discover active regions of the brain. The metabolic activity of various regions of the living human brain can be revealed by the 2-DG method, but a PET scanner is used to detect the active regions. Other noninvasive methods of measuring regional brain activity are provided by functional MRI, which detects localized changes in oxygen levels, and magnetoencephalography (MEG), which detects magnetic fields produced by the electrical activity of neurons.

Researchers can stimulate various regions of the brain by implanting a macroelectrode and applying mild electrical stimulation. Alternatively, they can implant a guide cannula in the brain; after the animal has recovered from the surgery, they insert a smaller cannula and inject a weak solution of an excitatory amino acid into the brain. The advantage of this procedure is that only neurons whose cell bodies are located nearby will be stimulated; axons passing through the region will not be affected. Viruses can be used to deliver genes for photosensitive proteins that produce depolarizations or hyperpolarizations of the membranes of specific neurons when the proteins are stimulated by light. Transcranial magnetic stimulation induces electrical activity in the human cerebral cortex, which temporarily disrupts the functioning of neural circuits located there.

Table 5.2 summarizes the research methods presented in this section. (See ***Table 5.2***.)

Thought Questions

1. Suppose that you had the opportunity to record an fMRI from a person (perhaps yourself) while the person was performing a behavior, thinking about something, or attending to a particular stimulus. Describe what you would have the person do.
2. Can you think of some ways that you could use optogenetic methods to investigate neural mechanisms involved in the control of a behavior or sensory system?

TABLE 5.2 **Research Methods: Part II**

Goal of Method	Method	Remarks
Record electrical activity of single neurons	Glass or metal microelectrodes	Metal microelectrodes can be implanted permanently to record neural activity as animal moves
Record electrical activity of regions of the brain	Metal macroelectrodes	In humans, usually attached to the scalp with a special paste
Record magnetic fields induced by neural activity	Magnetoencephalography; uses a neuromagnetometer, which contains an array of SQUIDs	Can determine the location of a group of neurons firing synchronously
Record metabolic activity of regions of brain	2-DG autoradiography	Measures local glucose utilization
	Measurement of Fos protein	Identifies neurons that have recently been stimulated
	2-DG PET scan	Measures regional metabolic activity of the human brain
	Functional magnetic resonance imaging (fMRI) scan	Measures regional metabolic activity of the human brain; better spatial and temporal resolution than PET scan
Stimulate neural activity	Electrical stimulation	Stimulates neurons near the tip of the electrode and axons passing through the region
	Chemical stimulation with excitatory amino acid	Stimulates only neurons near the tip of the cannula, not axons passing through the region
	Transcranial magnetic stimulation	Stimulates neurons in the human cerebral cortex with an electromagnet placed on the head

Neurochemical Methods

Sometimes we are interested not in the general metabolic activity of particular regions of the brain, but in the location of neurons that possess particular types of receptors or produce particular types of neurotransmitters or neuromodulators. We might also want to measure the amount of these chemicals secreted by neurons in particular brain regions during particular circumstances.

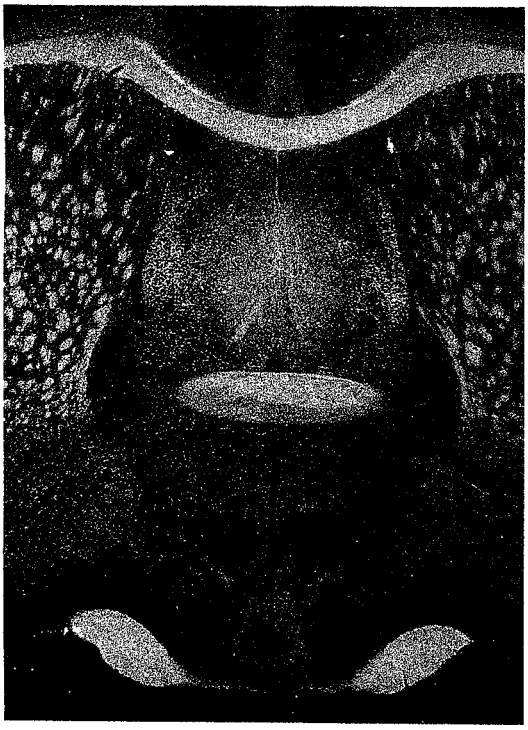


FIGURE 5.29 **Localization of a Peptide.** The peptide is revealed by means of immunocytochemistry. The photomicrograph shows a portion of a frontal section through the rat forebrain. The gold- and rust-colored fibers are axons and terminal buttons that contain vasopressin, a peptide neurotransmitter.

Geert De Vries, Georgia State University.

Finding Neurons That Produce Particular Neurochemicals

Suppose we learn that a particular drug affects behavior. How would we go about discovering the neural circuits that are responsible for the drug's effects? To answer this question, let's take a specific example. Physicians discovered several years ago that farm workers who had been exposed to certain types of insecticides (the organophosphates) had particularly intense and bizarre dreams and even reported having hallucinations while awake. A plausible explanation for these symptoms is that the drug stimulates the neural circuits responsible for REM sleep—the phase of sleep during which dreaming occurs. (After all, dreams are hallucinations that we have while sleeping.)

The first question to ask relates to how the organophosphate insecticides work. Pharmacologists have the answer: These drugs are acetylcholinesterase inhibitors. As you learned in Chapter 4, acetylcholinesterase inhibitors are potent acetylcholine agonists. By inhibiting AChE, the drugs prevent the rapid destruction of ACh after it is released by terminal buttons and thus prolong the postsynaptic potentials at acetylcholinergic synapses.

Now that we understand the action of the insecticides, we know that these drugs act at acetylcholinergic synapses. What neurochemical methods should we use to discover the sites of action of the drugs in the brain? First, let's consider methods by which we can localize particular neurochemicals, such as neurotransmitters and neuromodulators. (In our case we are interested in acetylcholine.) There are at least two basic ways of localizing neurochemicals in the brain: localizing the chemicals themselves or localizing the enzymes that produce them.

Peptides (or proteins) can be localized directly by means of immunocytochemical methods, which were described in the first section of this chapter. Slices of brain tissue are exposed to an antibody for the peptide and linked to a dye (usually, a fluorescent dye). The slices are then examined under a microscope using light of a particular wavelength. For example, Figure 5.29 shows the location of axons in the forebrain that contain vasopressin, a peptide neurotransmitter. Two sets of axons are shown. One set, which forms a cluster around the third ventricle at the base of the brain, shows up as a rusty color. The other set, scattered through the lateral septum, looks like strands of gold fibers. (As you can see, a properly stained brain section can be beautiful. See ***Figure 5.29.***)

But we are interested in acetylcholine, which is not a peptide. Therefore, we cannot use immunocytochemical methods to find this neurotransmitter. However, we can use these methods to localize the enzyme that produces it. The synthesis of acetylcholine is made possible by the enzyme choline acetyltransferase (ChAT). Thus, neurons that contain this enzyme almost certainly secrete ACh. Figure 5.30 shows acetylcholinergic neurons in the pons that have been identified by means of immunocytochemistry; the brain tissue was exposed to an antibody to ChAT attached to a fluorescent dye. In fact, research using many of the methods described in this chapter indicate that these neurons play a role in controlling REM sleep. (See ***Figure 5.30.***)

FIGURE 5.30 **Localization of an Enzyme.** An enzyme responsible for the synthesis of a neurotransmitter is revealed by immunocytochemistry. The photomicrograph shows a section through the pons. The orange neurons contain choline acetyltransferase, which implies that they produce (and thus secrete) acetylcholine.

Courtesy of David Morilak, Ph.D. and Roland Ciaranello, M.D.

Localizing Particular Receptors

As we saw in Chapter 4, neurotransmitters , neuromodulators, and hormones convey their messages to their target cells by binding with receptors located on or in these cells. The location of these receptors can be determined by two different procedures.

The first procedure uses autoradiography. We expose slices of brain tissue to a solution containing a radioactive ligand for a particular receptor. Next, we rinse the slices so that the only radioactivity remaining in them is that of the molecules of the ligand bound to

their receptors. Finally, we use autoradiographic methods to localize the radioactive ligand—and thus the receptors. Figure 5.31 shows an example of the results of this procedure. We see an autoradiogram of a slice of a rat's brain that was soaked in a solution that contained radioactive morphine, which bound with the brain's opiate receptors. (See ***Figure 5.31***.)

The second procedure uses immunocytochemistry. Receptors are proteins; therefore, we can produce antibodies against them. We expose slices of brain tissue to the appropriate antibody (labeled with a fluorescent dye) and look at the slices with a microscope under light of a particular wavelength.

Let's apply the method for localizing receptors to the first line of investigation we considered in this chapter: the role of the ventromedial hypothalamus (VMH) in the sexual behavior of female rats. As we saw, lesions of the VMH abolish this behavior. We also saw that the behavior does not occur if the rat's ovaries are removed but that it can be activated by stimulation of the VMH with electricity or an excitatory amino acid. These results suggest that the sex hormones produced by the ovaries act on neurons in the VMH.

This hypothesis suggests two experiments. First, we could use the procedure shown in Figure 5.26 to place a small amount of the appropriate sex hormone directly into the VMH of female rats whose ovaries we had previously removed. As we shall see in Chapter 9, this procedure works; the hormone *does* reactivate the animals' sexual behavior. The second experiment would use autoradiography to look for the receptors for the sex hormone. We would expose slices of rat brain to the radioactive hormone, rinse them, and perform autoradiography. If we did so, we would indeed find radioactivity in the VMH. (And if we compared slices from the brains of female and male rats, we would find evidence of more hormone receptors in the females' brains.) We could also use immunocytochemistry to localize the hormone receptors, and we would obtain the same results.

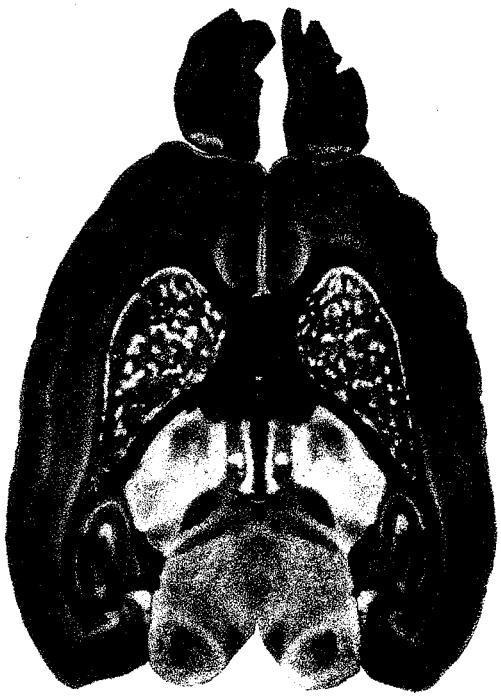


FIGURE 5.31 Autoradiogram. The photograph shows an autoradiogram of a rat brain (horizontal section, rostral is at top) that was incubated in a solution containing radioactive morphine, a ligand for opiate receptors. The receptors are indicated by white areas.

Herkenham, M. A., and Pert, C. B. *Journal of Neuroscience*, 1982, 2, 1129–1149. Reprinted with permission.

Measuring Chemicals Secreted in the Brain

The previous two subsections described methods that permit researchers to identify the location of chemicals within cells or in cell membranes. But sometimes we might want to measure the concentration of particular chemicals secreted in particular regions of the brain. For example, we know that cocaine—a particularly addictive drug—blocks the reuptake of dopamine, which suggests that the extracellular concentration of dopamine increases in some parts of the brain when a person takes cocaine. To measure the amount of dopamine in particular regions of an animal's brain, we would use a procedure called **microdialysis**.

Dialysis is a process in which substances are separated by means of an artificial membrane that is permeable to some molecules but not others. A microdialysis probe, as shown in Figure 5.32, consists of a small metal tube that introduces a solution into a section of dialysis tubing—a piece of artificial membrane shaped in the form of a cylinder, sealed at the bottom. Another small metal tube leads the solution away after it has circulated through the pouch. (See ***Figure 5.32***.)

We use stereotaxic surgery to place a microdialysis probe in a rat's brain so that the tip of the probe is located in the region we are interested in. We then pump a small amount of a solution similar to extracellular fluid through one of the small metal tubes into the dialysis tubing. The fluid circulates through the dialysis tubing and passes through the second metal tube, from which it is taken for analysis. As the fluid passes through the dialysis tubing, it collects molecules from the extracellular fluid of the brain, which are pushed across the membrane by the force of diffusion.

We analyze the contents of the fluid that has passed through the dialysis tubing by an extremely sensitive analytical method. This method is so sensitive that it can detect neurotransmitters (and their breakdown products) that have been released by the terminal buttons and have

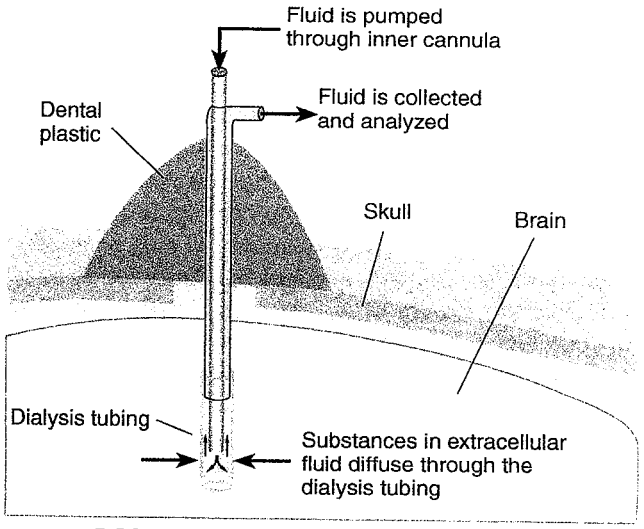
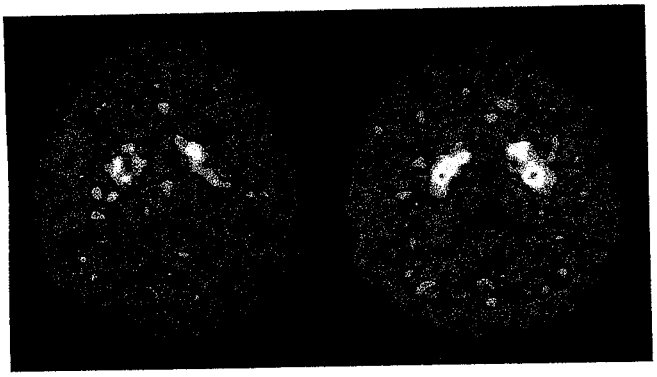


FIGURE 5.32 Microdialysis. A dilute salt solution is slowly infused into the microdialysis tube, where it picks up molecules that diffuse in from the extracellular fluid. The contents of the fluid are then analyzed.

microdialysis A procedure for analyzing chemicals present in the interstitial fluid through a small piece of tubing made of a semipermeable membrane that is implanted in the brain.



(a) (b)

FIGURE 5.33 **PET Scans of Patient with Parkinsonian Symptoms.** The scans show uptake of radioactive L-DOPA in the basal ganglia of a patient with parkinsonian symptoms induced by a toxic chemical before and after receiving a transplant of fetal dopaminergic neurons. (a) Preoperative scan. (b) Scan taken 13 months postoperatively. The increased uptake of L-DOPA indicates that the fetal transplant was secreting dopamine.

Adapted from Widner, H., Tetrud, J., Rehncrona, S., Snow, B., Brundin, P., Gustavii, B., Björklund, A., Lindvall, O., and Langston, J. W. *New England Journal of Medicine*, 1992, 327, 1556–1563.

escaped from the synaptic cleft into the rest of the extracellular fluid. We find that the amount of dopamine present in the extracellular fluid of the nucleus accumbens, located in the basal forebrain, *does* increase when we give a rat an injection of cocaine. In fact, we find that the amount of dopamine in this region increases when we administer any addictive drug, such as heroin, nicotine, or alcohol. We even see an increased dopamine secretion when the animal participates in a pleasurable activity such as eating when hungry, drinking when thirsty, or engaging in sexual activity. Such observations support the conclusion that the release of dopamine in the nucleus accumbens plays a role in reinforcement.

In a few special cases (for example, in monitoring brain chemicals of people with intracranial hemorrhages or head trauma), the microdialysis procedure has been applied to study of the human brain, but ethical reasons prevent us from doing so for research purposes. Fortunately, there is a noninvasive way to measure neurochemicals in the human brain. Although PET scanners are expensive machines, they are also versatile. They can be used to localize *any* radioactive substance that emits positrons.

As we saw in the prologue to Chapter 4, several years ago, several people injected themselves with an illicit drug that was contaminated with a chemical that destroyed their dopaminergic neurons. As a result they suffered from severe parkinsonism. Recently, neurosurgeons used stereotaxic procedures to transplant fetal dopaminergic neurons into the basal ganglia of some of these patients. Figure 5.33 shows PET scans of the brain of one of them. The patient was given an injection of radioactive L-DOPA one hour before each scan was made. As you learned in Chapter 4, L-DOPA is taken up by the terminals of dopaminergic neurons, where it is converted to dopamine; thus, the radioactivity shown in the scans indicates the presence of dopamine-secreting terminals in the basal ganglia. The scans show the amount of radioactivity before (part a) and after (part b) the patient received the transplant, which greatly diminished his symptoms. (See ***Figure 5.33***.)

I wish I could say that the fetal transplantation procedure has cured people stricken with Parkinson's disease and those whose brains were damaged with the contaminated drug. Unfortunately, as we will see in Chapter 14, the therapeutic effects of the transplant are often temporary, and with time, serious side effects often emerge.

SECTION SUMMARY

Neurochemical Methods

Neurochemical methods can be used to determine the location of an enormous variety of substances in the brain. They can identify neurons that secrete a particular neurotransmitter or neuromodulator and those that possess receptors that respond to the presence of these substances. Peptides and proteins can be directly localized, through immunocytochemical methods; the tissue is exposed to an antibody that is linked to a molecule that fluoresces under light of a particular wavelength. Other substances can be detected by immunocytochemical localization of an enzyme that is required for their synthesis.

Receptors for neurochemicals can be localized by two means. The first method uses autoradiography to reveal the distribution of a radioactive ligand to which the tissue has been exposed. The second method uses immunocytochemistry to detect the presence of the receptors themselves, which are proteins.

The secretions of neurotransmitters and neuromodulators can be measured by implanting the tip of a microdialysis probe in a particular region of the brain. A PET scanner can be used to perform similar observations of the human brain. People are given an injection of a radioactive tracer such as a drug that binds with a particular receptor or a chemical that is incorporated into a particular neurotransmitter, and a PET scanner reveals the location of the tracer in the brain.

Table 5.3 summarizes the research methods presented in this section. (See ***Table 5.3.***)

Thought Question

Using methods you learned about in this chapter so far, describe a behavior, cognitive process, or perceptual ability that you would like to study. Describe the kinds of experiments you would perform and say what kinds of information each method might provide.

TABLE 5.3 **Research Methods: Part III**

Goal of Method	Method	Remarks
Measure neurotransmitters and neuromodulators released by neurons	Microdialysis	A wide variety of substances can be analyzed
Measure neurochemicals in the living human brain	PET scan	Can localize any radioactive substance taken up in the human brain
Identify neurons producing a particular neurotransmitter or neuromodulator	Immunocytochemical localization of peptide or protein	Requires a specific antibody
	Immunocytochemical localization of enzyme responsible for synthesis of substance	Useful if substance is not a peptide or protein
Identify neurons that contain a particular type of receptor	Autoradiographic localization of radioactive ligand	
	Immunocytochemical localization of receptor	Requires a specific antibody

Genetic Methods

All behavior is determined by interactions between an individual's brain and his or her environment. Many behavioral characteristics—such as talents, personality variables, and mental disorders—seem to run in families. This fact suggests that genetic factors may play a role in the development of physiological differences that are ultimately responsible for these characteristics. In some cases the genetic link is very clear: A defective gene interferes with brain development, and a neurological abnormality causes behavioral deficits. In other cases the links between heredity and behavior are much more subtle, and special genetic methods must be used to reveal them.

Twin Studies

A powerful method for estimating the influence of heredity on a particular trait is to compare the *concordance rate* for this trait in pairs of monozygotic and dizygotic twins. Monozygotic twins (identical twins) have identical genotypes—that is, their chromosomes, and the genes they contain, are identical. In contrast, the genetic similarity between dizygotic twins (fraternal twins) is, on the average, 50 percent. Investigators study records to identify pairs of twins in which at least one member has the trait—for example, a diagnosis of a particular mental disorder. If both twins have been diagnosed with this disorder, they are said to be *concordant.* If only one has received this diagnosis, the twins are said to be *discordant.* Thus, if a disorder has a genetic basis, the percentage of monozygotic twins who are concordant for the diagnosis will be higher than the percentage of dizygotic twins. For example, as we will see in Chapter 15, the concordance rate for schizophrenia in twins is at least four times higher for monozygotic twins than for dizygotic twins, a finding that provides strong evidence that schizophrenia is a heritable trait. Twin studies have found that many individual characteristics, including personality traits, prevalence of obesity, incidence of alcoholism, and a wide variety of mental disorders, are influenced by genetic factors.

Twin studies provide a powerful method for estimating the relative roles of heredity and environment in the development of particular behavioral traits.

Michael Schwartz/The Image Works.

Adoption Studies

Another method for estimating the heritability of a particular behavioral trait is to compare people who were adopted early in life with their biological and adoptive parents. All behavioral traits are affected to some degree by hereditary factors, environmental factors, and an

interaction between hereditary and environmental factors. Environmental factors are both social and biological in nature. For example, the mother's health, nutrition, and drug-taking behaviors during pregnancy are prenatal environmental factors, and the child's diet, medical care, and social environment (both inside and outside the home) are postnatal environmental factors. If a child is adopted soon after birth, the genetic factors will be associated with the biological parents, the prenatal environmental factors will be associated with the biological mother, and most of the postnatal environmental factors will be associated with the adoptive parents.

Adoption studies require that the investigator know the identity of the parents of the people being studied and be able to measure the behavioral trait in the biological and adoptive parents. If the people being studied strongly resemble their biological parents, we conclude that the trait is probably influenced by genetic factors. To be certain, we will have to rule out possible differences in the prenatal environment of the adopted children. If, instead, the people resemble their adoptive parents, we conclude that the trait is influenced by environmental factors. (It would take further study to determine just what these environmental factors might be.) Of course, it is possible that both hereditary and environmental factors play a role, in which case the people being studied will resemble both their biological and adoptive parents.

Genomic Studies

The human **genome** consists of the DNA that encodes our genetic information. Because of the accumulation of mutations over past generations of our species, no two people, with the exception of monozygotic twins, have identical genetic information. The particular form of an individual gene is called an **allele** (from the Greek *allos*, "other"). For example, different alleles of the gene responsible for the production of iris pigment produce pigments with different colors. Genomic studies attempt to determine the location in the genome of genes responsible for various physical and behavioral traits.

Linkage studies identify families whose members vary with respect to a particular trait—for example, the presence or absence of a particular hereditary disease. A variety of *markers*, sequences of DNA whose locations are already known, are compared with the nature of an individual person's trait. For example, the gene responsible for *Huntington's disease*, a neurological disorder discussed in Chapter 15, was found to be located near a known marker on the short arm of chromosome 4. Researchers studied people in an extended family in Venezuela that contained many members with Huntington's disease and found that the presence or absence of the disease correlated with the presence or absence of the marker.

Genome-wide association studies have been made possible by the development of methods to obtain the DNA sequence of the entire human genome. These studies permit researchers to compare all or portions of the genomes of different individuals to determine whether differences in the people's genomes correlate with the presence or absence of diseases (or other traits). As we will see in Chapters 15 and 16, these studies are beginning to reveal the location of genes that control characteristics that contribute to the development of various mental disorders.

Targeted Mutations

A genetic method developed by molecular biologists has put a powerful tool in the hands of neuroscientists. **Targeted mutations** are mutated genes produced in the laboratory and inserted into the chromosomes of mice. In some cases, the genes (also called knockout genes) are defective and fail to produce a functional protein. In many cases the target of the mutation is an enzyme that controls a particular chemical reaction. For example, we will see in Chapter 12 that lack of a particular enzyme interferes with learning. This result suggests that the enzyme is partly responsible for changes in the structure of synapses required for learning to occur. In other cases the target of the mutation is a protein that itself serves useful functions in the cell. For example, we will see in Chapter 16 that a particular type of cannabinoid receptor is involved in the reinforcing and analgesic effects of opiates. Researchers can even produce *conditional knockouts* that cause the animal's genes to stop expressing a particular gene when the animal is given a particular drug. This permits the targeted gene to express itself normally during the animal's development and then be knocked out at a later time.

genome The complete set of genes that compose the DNA of a particular species.

allele The nature of the particular sequence of base pairs of DNA that constitutes a gene; for example, the genes that code for blue or brown iris pigment are different alleles of a particular gene.

targeted mutation A mutated gene (also called a "knockout gene") produced in the laboratory and inserted into the chromosomes of mice; fails to produce a functional protein.

Investigators can also use methods of genetic engineering to insert new genes into the DNA of mice. These genes can cause increased production of proteins normally found in the host species, or they can produce entirely new proteins.

Antisense Oligonucleotides

Another genetic method involves the production of molecules that block the production of proteins encoded by particular genes by injecting **antisense oligonucleotides**. The most common type of antisense oligonucleotide consists of modified strands of RNA or DNA that will bind with specific molecules of messenger RNA and prevent them from producing their protein. Once the molecules of mRNA are trapped this way, they are destroyed by enzymes present in the cell. The term *antisense* refers to the fact that the synthetic oligonucleotides contain a sequence of bases complementary to those contained by a particular gene or molecule of mRNA. Table 5.4 summarizes the research methods presented in this section. (See *Table 5.4.*)

antisense oligonucleotide (*oh li go **new** klee oh tide*) A modified strand of RNA or DNA that binds with a specific molecule of messenger RNA and prevents it from producing its particular protein.

TABLE 5.4 **Research Methods: Part IV**

Goal of Method	Method	Remarks
Genetic methods	Twin studies	Comparison of concordance rates of monozygotic and dizygotic twins estimates heritability of trait
	Adoption studies	Similarity of offspring and adoptive and biological parents estimates heritability of trait
	Genomic studies	Use of genomic analysis (linkage studies or genome-wide association studies) to identify genes that are associated with particular traits
	Targeted mutations	Inactivation, insertion, or increased expression of a gene
	Antisense oligonucleotides	Bind with messenger RNA; prevent synthesis of protein

SECTION SUMMARY

Genetic Methods

Because genes direct an organism's development, genetic methods are very useful in studies of the physiology of behavior. Twin studies compare the concordance rates of monozygotic (identical) and dizygotic (fraternal) twins for a particular trait. A higher concordance rate for monozygotic twins provides evidence that the trait is influenced by heredity. Adoption studies compare people who were adopted during infancy with their biological and adoptive parents. If the people resemble their biological parents, evidence is seen for genetic factors. If the people resemble their adoptive parents, evidence is seen for a role of factors in the family environment. Linkage studies and genome-wide association studies make it possible to identify the locations of genes that are responsible for a variety of behavioral and physical traits.

Targeted mutations permit neuroscientists to study the effects of the presence or absence of a particular protein—for example, an enzyme, structural protein, or receptor—on an animal's physiological and behavioral characteristics. Genes that cause the production of foreign proteins or increase production of native proteins can be inserted into the genome of strains of animals. Antisense oligonucleotides can be used to block the production of particular proteins.

Thought Questions

1. You have probably read news reports about studies of the genetics of human behavioral traits or seen them on television. What does it really mean when a laboratory reports the discovery of, say, a "gene for shyness"?
2. Most rats do not appear to like the taste of alcohol, b[illegible]ow, graded have bred some rats that will drink alcohol in lar[illegible]roduced by a you think of ways to use these animals to i[illegible]esponse to a physical role of genetic factors in susceptibility t

EPILOGUE | Watch the Brain Waves

What went wrong? Why did Mrs. H.'s "successful" surgery cause a neurological problem? And can anything be done for her?

First, let's consider the cause of the problem. As you will recall, an artificial heart circulated Mrs. H.'s blood while the surgeon was removing two of her coronary arteries and replacing them with veins taken from her leg. The output of the machine is adjustable; that is, the person operating it can control the patient's blood pressure. The surgeon tries to keep the blood pressure just high enough to sustain the patient but not so high as to interfere with the delicate surgery on the coronary arteries. Unfortunately, Mrs. H.'s coronary arteries were not the only blood vessels to be partially blocked; the arteries in her brain, too, contained atherosclerotic plaque. When the machine took over the circulation of her blood, some parts of her brain received an inadequate blood flow, and the cells in these regions were damaged.

If Mrs. H.'s blood pressure had been maintained at a slightly higher level during the surgery, her brain damage might have been prevented. For most patients, the blood pressure would have been sufficient, but in her case it was not. Mrs. H.'s brain damage is irreversible. But are there steps that can be taken to prevent others from sharing her fate?

The answer is yes. The solution is to use a method described in this chapter: electroencephalography. What we need is a warning system to indicate that the brain is not receiving a sufficient blood flow so that the surgeon can adjust the machine and increase the patient's blood pressure. That warning can be provided by an EEG. For many years, clinical electroencephalographers (specialists who perform EEGs to diagnose neurological disorders) have known that diffuse, widespread brain damage caused by various poisons, anoxia, or extremely low levels of blood glucose produces slowing of the regular rhythmic pattern of the EEG. Fortunately, this pattern begins right away, as soon as the damage commences. Thus, if EEG leads are attached to a patient undergoing cardiac surgery, an electroencephalographer can watch the record coming off the polygraph and warn the surgeon if the record shows slowing. If it does, the patient's blood flow can be increased until the EEG reverts to normal, and brain damage can be averted.

Mrs. H. was operated on over twenty years ago, at a time when only a few cardiac surgeons had their patient's brain waves monitored. Today, the practice is common, and it is used during other surgical procedures that may reduce blood flow to the brain. For example, when the carotid arteries (the vessels that provide most of the brain's blood supply) become obstructed by atherosclerotic plaque, a surgeon can cut open the arteries and remove the plaque. During this procedure, called *carotid endarterectomy,* clamps must be placed on the carotid artery, completely stopping the blood flow. Some patients can tolerate the temporary clamping of one carotid artery without damage; others cannot. If the EEG record shows no slowing while the artery is clamped, the surgeon can proceed. If it does, the surgeon must place the ends of a plastic tube into the artery above and below the clamped region to maintain a constant blood flow. This procedure introduces a certain amount of additional risk to the patient, so most surgeons would prefer to do it only if necessary. The EEG provides the essential information.

KEY CONCEPTS

EXPERIMENTAL ABLATION

1. Neuroscientists produce brain lesions to try to infer the functions of the damaged region from changes in the animals' behavior.
2. Brain lesions may be produced in the depths of the brain by passing electrical current through an electrode placed there or by infusing an excitatory amino acid; the latter method kills cells but spares axons that pass through the region.
3. The behavior of animals with brain lesions must be compared with that of a control group consisting of animals with sham lesions.
4. A stereotaxic apparatus is used to place electrodes or cannulas in particular locations in the brain. The coordinates are obtained from a stereotaxic atlas.
5. The location of a lesion is verified by means of histological procedures that include fixation, slicing, staining, and examination under a microscope.
6. Special histological methods have been devised to trace the afferent and efferent connections of a particular brain region.
7. The structure of the living human brain can be revealed through CT scans or MRI scans.

RECORDING AND STIMULATING NEURAL ACTIVITY

8. The electrical activity of single neurons can be recorded with microelectrodes, and that of entire regions of the brain can be recorded with macroelectrodes. EEGs are recorded on polygraphs with data from macroelectrodes pasted on a person's scalp.
9. Metabolic activity of particular parts of animals' brains can be assessed by means of 2-DG autoradiography or by measurement of the production of Fos protein. The metabolic activity of specific regions of the human brain can be revealed through PET scans or functional MRI scans.

iris
particular brain region.
targeted mutation (also called a "knockout gene") is created in the laboratory and inserted into the chromosomes of mice; fails to produce a functional protein.

10. Neurons can be stimulated electrically, through electrodes, or chemically, by infusing dilute solutions of excitatory amino acids through cannulas.

NEUROCHEMICAL METHODS

11. Immunocytochemical methods can be used to localize peptides in the brain or localize the enzymes that produce substances other than peptides.
12. Receptors can be localized by exposing the brain tissue to radioactive ligands and assessing the results with autoradiography or immunocytochemistry.
13. Microdialysis permits a researcher to measure the secretion of particular chemicals in specific regions of the brain. PET scans can be used to reveal the location of particular chemicals in the human brain.

GENETIC METHODS

14. Twin studies, adoption studies, and genomic studies enable investigators to estimate the role of hereditary factors in a particular physiological characteristic or behavior.
15. Targeted mutations are artificially produced mutations that interfere with the action of one or more genes, which enables investigators to study the effects of the lack of a particular gene product.
16. Genes that cause the production of foreign proteins or increase production of native proteins can be inserted into the genome of strains of animals, and antisense oligonucleotides can be used to block the production of particular proteins.

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