Chapter 3

Stereotaxic Surgeries

After reading this chapter, you should be able to:

- Describe the process of performing stereotaxic surgeries in rodents and primates, from administering anesthetic agents to monitoring recovery
- Describe common implants that allow permanent access to the brain

Techniques covered:

- **Using brain atlases**
- **Stereotaxic surgeries in animals**: sterile fields, anesthesia, stereotaxic positioning of animals, accessing the brain, implantation, and recovery
- **Implants that allow access to the brain**: sealable chambers, cannulae, electrophysiology implants, and optical imaging implants

A **stereotaxic surgery** is an invasive procedure used to precisely target specific regions of the brain for subsequent injection of a reagent (such as a retrograde tracer, a pharmacological agent, or a virus) and/or implantation of a probe or device. The word *stereotaxic* is derived from the roots *stereos*, meaning "three-dimensional," and *taxic*, meaning "having an arrangement." Thus, a stereotaxic surgery places an animal brain within a three-dimensional coordinate system so an investigator can accurately target discrete brain regions. These surgeries are necessary in many vertebrate model organisms to implant electrodes for electrophysiology (Chapter 4), inject tracers to study neuroanatomy (Chapter 6), implant probes to visualize neural activity or measure neurochemistry (Chapter 7), implant hardware for neuromodulation (Chapter 8), and inject viral vectors for targeted gene delivery (Chapter 11). Therefore, stereotaxic surgeries are incredibly common in neuroscience research.

The purpose of this chapter is to describe the process of performing a stereotaxic surgery in common model organisms. First we will describe how discrete brain regions are localized in three-dimensional coordinates. Then we describe common surgical procedures in rodents, as the majority of stereotaxic surgeries in neuroscience are performed on mice and rats, followed with specialized procedures and techniques in nonhuman primates. These procedures can theoretically be adapted for use in any mammalian organisms,

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including birds and reptiles. Finally, we describe some common implants that provide long-term access to the brain.

DETERMINING COORDINATES OF BRAIN REGIONS

The crucial step in any stereotaxic surgery is to locate a precise brain region of interest in the least physically invasive way possible. Therefore, scientists have developed a method of defining the brain in a three-dimensional coordinate scheme. Just as sailors, travelers, and explorers use maps and astronomical reference points to navigate the globe, neuroscientists use **brain atlases** and anatomical landmarks to navigate the brain. Published atlases provide three-dimensional coordinates of brain structures for a variety of animals, including rodents, primates, and even birds and bats (Fig. 3.1). The coordinates of brain structures are defined in terms of distances to anatomical landmarks visible as seams on the skull: **bregma** and **lambda** (Fig. 3.2). To properly align an animal brain so that its structures are consistent with the coordinates in a brain atlas, a scientist must correctly position the animal's head on a **stereotaxic instrument** (Fig. 3.3). This specialized equipment holds an animal's

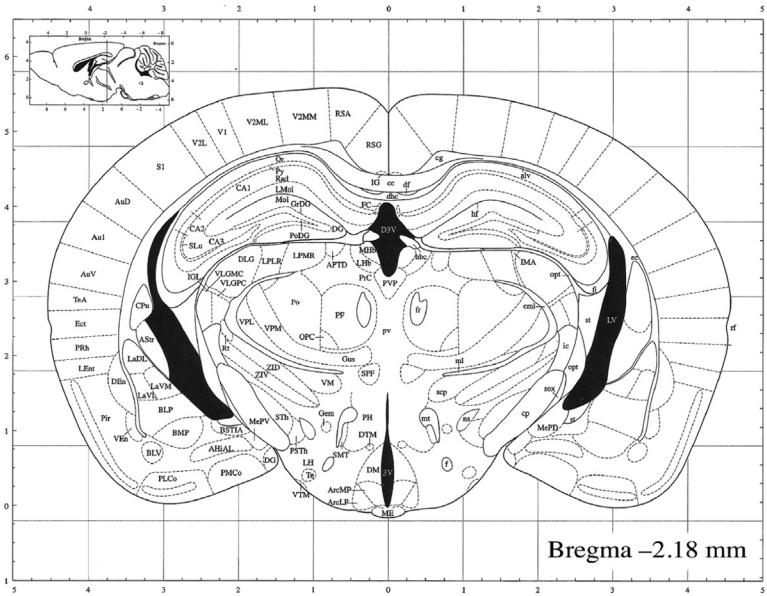

FIGURE 3.1 A stereotaxic brain atlas diagram. A stereotaxic atlas presents the locations of distinct brain structures in three-dimensional coordinates relative to an anatomical landmark on the skull. This example diagram from a mouse brain atlas presents a single coronal section of the brain at a distance −2.18 mm from bregma. The top left diagram shows the location of the coronal image on a sagittal brain diagram. *Reprinted with permission from Paxinos, G., Franklin, K., 2006. The Mouse Brain in Stereotaxic Coordinates, second Ed. Academic Press/Elsevier.*

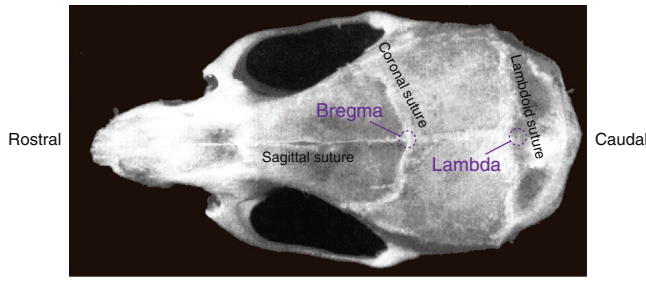

FIGURE 3.2 **Bregma and lambda landmarks on the skull.** Bregma is generally defined as the intersection between the sagittal and coronal sutures of the skull. Lambda is defined as the intersection between the lines of best fit through the sagittal and lambdoid sutures. The coordinates for these landmarks are generally consistent across animals, allowing for reproducible targeting of brain structures in relation to these coordinates. *Reprinted and modified with permission from Paxinos, G., Franklin, K., 2006. The Mouse Brain in Stereotaxic Coordinates, second Ed. Academic Press/Elsevier.*

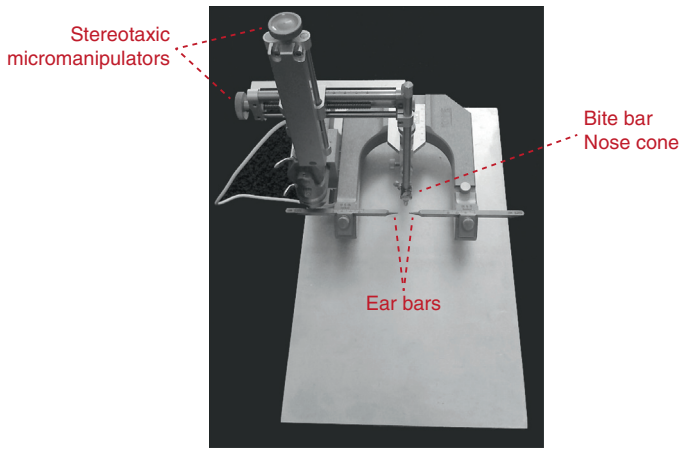

FIGURE 3.3 **A rodent stereotaxic instrument.** A rodent's head is held firmly in place between two ear bars, while a bite bar and/or nose cone ensures the head is precisely oriented in the forward direction. The nose cone also allows for delivery of a gas anesthetic. Micromanipulators in the X, Y, and Z directions correctly move instruments and probes in precise increments relative to bregma or lambda.

head in place and uses fine-scale micromanipulators to precisely target distances in 10-μm increments in the X, Y, and Z planes.

Although brain atlases provide a useful three-dimensional guide for targeting discrete brain regions, different animal strains and individuals vary, creating inherent variability in targeting coordinates. Therefore, scientists must validate the most optimal coordinates for a region of interest before any experiments take place. Scientists can verify the correct targeting of brain

regions by examining histological sections of the brain after experiments have ended. In larger animals such as primates, it is also possible to examine the position of brain structures and implants using magnetic resonance imaging (Chapter 1).

STEREOTAXIC SURGERIES IN RODENTS

Scientists routinely perform stereotaxic surgeries on mice and rats because of the widespread use of these model organisms and their tractability for genetic manipulation experiments. Additionally, brain atlases for mice and rats have been developed and continuously refined over several decades, providing coordinates for any brain region an investigator wishes to target.

As with any procedures involving vertebrate animals, an Institutional Animal Care and Use Committee must approve procedures before any experiments take place. This committee evaluates survival surgery procedures to ensure that pain and distress are minimized. This is important for the well-being of the animals, as well as for the experiments themselves, as pain and distress can alter physiology and behavior. See Box 2.2, Chapter 2 for ethical considerations for animal use.

Creating a Sterile Environment

The first step in any invasive surgical procedure is to prepare sterile working conditions. **Sterile** does not simply mean clean, but also aseptic—free from microorganisms that can invade the brain and cause infection. Some aspects of the surgical environment can be made clean, but not sterile. For example, a scientist's lab coat, scrubs, face mask, and gloves can decrease the possibility of contamination but can never be 100% sterile. Cleaning the surgical environment with alcohols and specialized disinfectant solutions can also decrease the possibility of contamination but is also not 100% sterile. The only aspects of the surgical environment that must be kept completely sterile are the instruments and probes that will come in contact with the brain, as well as a dedicated surface to place these tools when they are not in use.

There are two common pieces of equipment to sterilize implants and tools that come into contact with an animal's internal environment: an autoclave and a tabletop heat sterilizer. An **autoclave** is a large appliance that generates a high pressure to heat water above its natural boiling point (Fig. 3.4A). The high-temperature steam kills any microorganisms growing on tools and reagents placed inside the autoclave chamber. A glass **bead sterilizer** is a smaller device that can sterilize tools or fine instruments when an autoclave is not convenient, such as sterilization between surgeries (Fig. 3.4B). These sterilizers are filled with thousands of tiny glass beads that allow the heat to evenly surround the instruments.

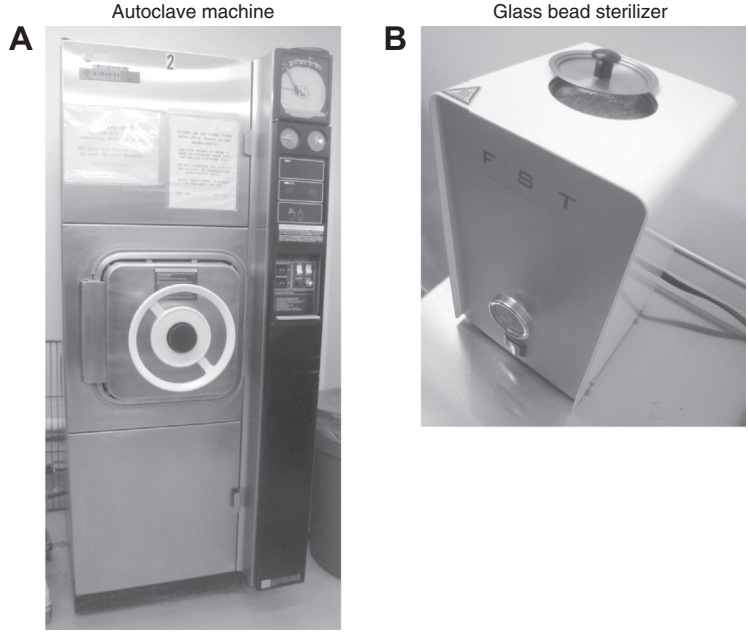

FIGURE 3.4 Equipment for sterilization. (A) An autoclave uses high-temperature steam to sterilize contents. (B) A tabletop bead sterilizer uses high temperatures to sterilize small instruments and surgical tools before and between surgeries.

Just before a surgery takes place, a scientist cleans the surgical environment and sterilizes tools and probes in an autoclave or tabletop sterilizer. Some delicate implants cannot handle the high heat of sterilization and instead can be soaked in 70% ethanol or another disinfectant prior to use. It is usually necessary to create a **sterile field**, a dedicated surface that can serve as a resting place for tools when they are not in use. To create a sterile field, a scientist can purchase sterile cloths/papers commercially or can autoclave a large strip of gauze. If the tips of probes or instruments come in contact with gloves or other nonsterile surfaces, they must be considered nonsterile.

Anesthesia

Once the environment is clean and ready for a surgery to take place, a rodent can be anesthetized with either pharmacological agents or gas anesthetics. A widely used pharmacological agent is a cocktail of ketamine/xylazine. **Ketamine** is an anesthetic that acts by inhibiting N-methyl D-aspartate (NMDA) and hyperpolarization-activated cyclic nucleotide–modulated (HCN1) ion channels. **Xylazine** is a sedative and analgesic that serves as an agonist for β2 adrenergic receptors. A ketamine/xylazine cocktail typically anesthetizes an

animal for 1−2 h. Reinjection of the cocktail is not recommended, as long-term exposure can lead to bradycardia (abnormally slow heart rate), blindness, seizures, or death.

Gas anesthetics are especially useful for longer (>60 min) surgeries. A common gas anesthetic is **isoflurane**, an inhalable ether that likely works by disrupting synaptic transmission. To anesthetize the animal, the scientist places the rodent in a small, ventilated chamber. Gas is released into the chamber and the scientist waits 1−3 min until the rodent ceases all movements. Then the animal is quickly placed on a bite bar and/or provided with a nose cone (Fig. 3.3) that receives a steady supply of oxygen mixed with the gas anesthetic.

After an animal is anesthetized, a scientist must verify that the animal is indeed unconscious by testing for muscle responses to stimuli. For example, pinching the animal's tail produces a leg withdrawal response in an animal that is not fully unconscious. Only after the animal shows no signs of motor responses should the scientist begin a surgical procedure.

Positioning a Rodent on a Stereotaxic Instrument

After cleaning the surgical area and properly anesthetizing an animal, the scientist places the animal on a stereotaxic instrument (Fig. 3.3). The apparatus is designed to position the animal's head in a precise orientation so that three-dimensional stereotaxic coordinates remain consistent from animal-to-animal.

First, the scientist inserts ear bars into the animal's ear canals. This placement allows the head to be tilted up and down but not side to side. To restrict movement up and down, the animal's teeth are positioned on a bite bar and the nose is held firmly in a nose cone. It is crucial that the head is precisely fixed in place so that the scientist can accurately traverse the three-dimensional brain.

To ensure proper orientation of the brain, the scientist must place the head in the **flat skull position**, such that the top surface of the skull is flat from bregma to lambda in the rostral−caudal direction, as well as from left and right lateral points in the medial−lateral direction. To achieve this position, the skull is exposed by a careful incision down the midline of the skin. The skin is gently moved aside and the skull is cleaned. Micromanipulators on the stereotax are used to measure three-dimensional coordinates in the X, Y, and Z axes. The scientist uses the micromanipulators to ensure the top surface of the skull is flat. If the skull is not flat, the scientist can reposition the head, or raise, or lower the bite bar/nose cone until the flat skull position is achieved.

Accessing the Brain

Once the animal's head is in the flat skull position, the investigator can target a discrete brain region using three-dimensional coordinates. Starting from bregma or lambda, the scientist orients the micromanipulator over the appropriate area of the skull. A dental drill is used to perform a craniotomy, a small hole in the skull that exposes the brain. Sometimes bleeding occurs at this stage, and the investigator must remove excess blood with a cotton swab or thin tissue. If a scientist intends to insert a delicate probe into the brain, such as a glass electrode, it is often necessary to also remove a section of dura, the thin meningeal layer of tissue surrounding the brain, a procedure known as a durotomy.

Penetrating the Brain and Attaching Implants

Once the brain is exposed, an investigator can deliver a substance into the brain using a syringe mounted on the stereotaxic frame and controlled by a precise injection pump. For example, an investigator can inject an anterograde or retrograde tracer (Chapter 6), a pharmacological compound (Chapter 8), or a viral vector (Chapter 11) into a discrete brain region.

An investigator may also introduce a permanent implant for long-term access to the brain (Fig. 3.5). Permanent implants can be held in place with dental acrylic and/or cement that is specifically designed to adhere small objects to the surface of bone. If properly applied, implants can remain

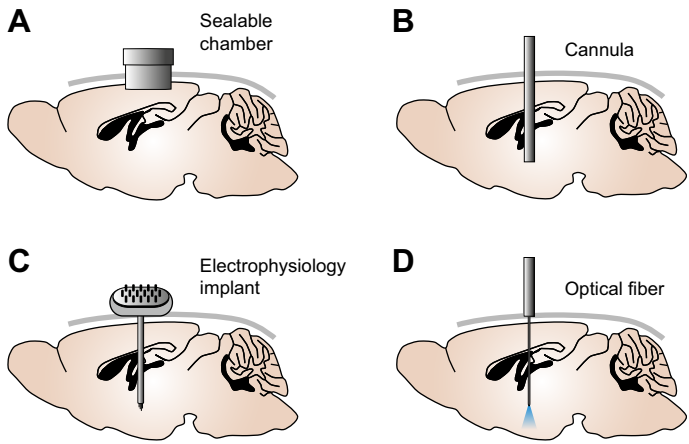

FIGURE 3.5 Implants for long-term access to brain structures. (A) A sealable chamber. (B) A cannula. (C) An implanted electrophysiological device. For a more detailed description of implantable recording devices, see Fig. 4.6. (D) An optic fiber. For a more detailed description of optical implants, see Fig. 7.6 (visualizing neural activity) and/or Fig. 8.4 (optically manipulating neural activity).

securely adhered to the skull throughout the life of the animal. The most common implants include sealable chambers, cannulae, electrophysiological implants, and optical implants, described below.

Sealable Chambers

A sealable chamber is a round, hollow well with one end affixed to the skull and the other end capped with a screw top (Fig. 3.5A). This implant allows an investigator chronic access to a specific region of the brain for electrophysiology. Care must be taken to keep the chamber as clean and sterile as possible. Whenever not in use, the chamber must always be closed to prevent outside contaminants from penetrating the brain.

Cannulae

A **cannula** is a hollow, narrow, cylindrical tube used to provide continual access to deep structures within the brain (Fig. 3.5B). Cannulae can be made of plastic, glass, or steel of various sizes. Once the animal recovers, a scientist can repeatedly deliver substances such as pharmacological compounds over the subsequent days or weeks of an experiment. If a cannula is implanted in the lateral ventricles of the brain, scientists can achieve **intracerebroventricular (i.c.v.)** delivery of compounds that can spread throughout the entire brain. At the end of experiments, scientists verify the correct placement of cannulae by histological examination of cannula tracks within brain sections.

Electrophysiology Implants

Recording the electrical activity of neurons using electrodes is discussed in Chapter 4. There are numerous ways of inserting an electrode into specific brain regions. A scientist could implant a sealable chamber (Fig. 3.5A) to provide access for the insertion of electrodes over many weeks. A scientist could also insert a narrow recording electrode into a cannula (Fig. 3.5B) to repeatedly target the same brain region.

More sophisticated electrophysiology implants (including tetrodes, microelectrode arrays, silicone neuropixels probes, etc.) can record from many neurons at once and are typically permanently implanted within the brain (Fig. 3.5C). These devices are discussed in greater detail in Chapter 4.

Optical Implants

In addition to measuring neural activity using electrophysiology techniques, it is also possible to visualize neural activity using fluorescent voltage sensors and calcium sensors (Chapter 7). Using these voltage and calcium sensors depends on being able to deliver light at an excitation wavelength to neurons

of interest and simultaneously measure emitted light at an emission wavelength. There are numerous ways to gain optical access to the brain (including optical fibers, microendoscopes with GRIN lenses, and cranial windows), discussed in greater detail in Chapter 7.

Optical implants are also necessary to deliver light for optogenetic experiments, discussed in Chapter 8. To deliver light to a specific brain region, an optical fiber is implanted above a brain region of interest (Fig. 3.5D). The optical fiber consists of a very thin fiber optic wire connected to a shaft implanted on the skull. During an experiment, the shaft is connected to a fiber optic cable connected to a laser. Alternatively, the fiber optic wire implanted in the brain can be attached to a light emitting diode, directly implanted on the skull to avoid the need to connect the rodent to a wire.

Finishing the Surgery

After reagents have been injected into the brain and/or adhesives have hardened around implants, the final tasks are to seal the incision, clean the affected area, and help the animal recover. Depending on the length of the incision, a scientist can use sterile **sutures** or glues designed for tissues to seal the skin around an implant. After the skin is sealed, the top of the animal's head can be lightly treated with an iodine solution to clean the affected area and help prevent infection.

During surgery, an animal can lose body heat due to blood loss and low heart rate. Therefore, recovering animals should be placed in a recovery cage on a heat pad or under a warm light. As the animal recovers, a scientist should inject further analgesic and/or antibiotic agents and should always monitor the animal until it becomes conscious before returning it to an animal storage room. It will take the animal from a few days to a full week to recover from the surgery. During this time, the animal should be inspected for any signs of pain or discomfort, including little movement, hunched posture, and a lack of eating or drinking. Further injections of an analgesic and/or antibiotic may be necessary to aid in the recovery of the animal.

STEREOTAXIC SURGERIES IN NONHUMAN PRIMATES

Stereotaxic surgeries in primates are conceptually similar to surgeries in rodents: the investigator anesthetizes the animal, exposes the skull, performs a craniotomy, applies dental acrylic to implant probes, sutures the skin, and carefully monitors the animal throughout the recovery process. The major difference between primate and rodent surgeries is that primates are more precious resources and individual investigators may only use one to three

monkeys over the course of single studies lasting 4–6 years. Therefore, investigators have no room for error and take extra steps to ensure correct targeting of brain regions. For example, although primate brain atlases exist, scientists often produce structural MR images (Chapter 1) for each individual animal weeks before the actual surgery takes place so they can target specific regions more accurately.

The purpose of most primate surgeries is to implant devices for future electrophysiology experiments. In these experiments, the scientist trains the monkey to perform a task and then records neural activity in a specific part of the brain. To perform these experiments, a scientist usually implants one of three devices:

- **Sealable chamber**. After performing a craniotomy, a scientist implants a chamber around the hole with a screw cap. This allows the surface of the brain to be exposed at any time after the surgery for the insertion of a recording electrode, but protected by the cap at other times.
- **Headpost**. The purpose of this post is to keep the primate's head fixed in place during experiments. It is especially important to stabilize a monkey's head for the presentation of visual stimuli when the monkey must fix its gaze upon a target and make decisions about complicated visual scenes. A headpost can be as simple as a bolt that attaches to the top of the apparatus where the monkey sits during experiments.
- **Eye coil**. The eye coil is also used to ensure that the monkey correctly fixes its gaze during the presentation of visual stimuli. A coil consists of a wire loop that is implanted around the outer circumference of the eye. This coil is usually hidden beneath the eyelids after the surgery. The wire is threaded beneath the skin from the eye to the top of the head, where it can be attached to additional cables to measure eye movements during experiments. An eye coil is not necessary for experiments in which there is no need to monitor the animal's eye movements. Many laboratories implant and adjust these devices over two or three surgical sessions so that their placement is accurate, firm, and precise.

CONCLUSION

Stereotaxic surgeries provide the ability to target specific brain regions to study neuroanatomy, or to measure or manipulate neural activity. Although this chapter focuses on rodent and primate surgeries, scientists can theoretically target brain regions in any animals provided they have accurate stereotaxic coordinates (including a reliable anatomical landmark, such as lambda and bregma).

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