

Chapter 1

Noninvasive Brain Imaging

After reading this chapter, you should be able to:

- Compare the relative strengths and limitations of different structural and functional brain imaging techniques
- Explain the physical and physiological basis of MRI/fMRI technology
- Describe the design of a functional brain imaging experiment: formulating a hypothesis, choosing task paradigms, performing the experiment, acquiring and analyzing data, and producing figures

Techniques covered:

- **Structural techniques:** cerebral angiography, computerized tomography (CT), magnetic resonance imaging (MRI), diffusion MR imaging
- **Functional techniques:** functional magnetic resonance imaging (fMRI), positron emission tomography (PET), single-proton emission computerized tomography (SPECT), electroencephalography (EEG), magnetoencephalography (MEG), optical imaging
- **Techniques used to investigate the necessity and sufficiency of a specific brain region for a cognitive function:** transcranial magnetic stimulation (TMS), ultrasonic neuromodulation (USNM), case studies

Modern brain imaging technology can seem like magic. The ability to produce detailed images of the human brain without physically penetrating the skull is a technological marvel that has saved thousands of lives and allowed scientists to study the structure of the human brain throughout development, disease, and aging. Furthermore, the ability to image neural activity in the brain during cognition has provided scientists the opportunity to correlate activity in distinct brain regions with specific mental operations, a truly remarkable achievement. Colorful figures depicting activity in the human brain dazzle scientists and nonscientists alike.

Of course, brain imaging technology is not magic. The technology that produces detailed images of the brain depends on complex physics, expensive equipment, and skilled technicians. As with all scientific experiments, brain imaging studies must be well designed, the data accurately analyzed, and the results carefully interpreted. The purpose of this chapter is to explain the

ostensible magic of noninvasive brain imaging technology and provide insight into how experiments are designed and interpreted.

Noninvasive brain imaging technology can essentially be divided into two categories: structural and functional. Structural techniques produce images of the anatomical architecture of the brain, whereas functional techniques produce visual representations of the physiological processes that underscore neural activity. This chapter will survey both classifications of techniques and describe how they can be used in modern neuroscience research. We focus on MRI and fMRI technology in humans due to the widespread use of these techniques in the neuroscience literature. After reviewing these techniques, we will survey the essential components of a functional imaging experiment: forming hypotheses, choosing appropriate task paradigms, performing experiments, acquiring and analyzing data, and producing figures for publication.

STRUCTURAL BRAIN IMAGING TECHNIQUES

Structural brain imaging techniques are used to resolve the anatomy of the brain in a living subject without physically penetrating the skull. These techniques can be used in combination with **functional brain imaging** techniques to correlate neural activity in specific anatomical regions with behavioral or cognitive functions. Structural techniques can also be used to measure anatomical changes that occur over time, such as a decrease in brain mass that occurs with aging or the progression of disease. Most often, these techniques are used in clinical neuroscience and neurology to diagnose diseases such as tumors and vascular disorders.

Brain imaging technologies take advantage of the different biochemical compositions of brain regions and use these differences to form the basis of an image (Fig. 1.1). Neural cell bodies contain many biomolecules, including proteins and carbohydrates. Axons and fiber tracts are relatively fatty due to the insulation provided by myelin. Cerebrospinal fluid (CSF) in the ventricles and surrounding the brain is essentially a saline solution. The microanatomy and composition of individual neural structures cause distinct regions of the brain to appear differently from each other when examined with the naked eye. For example, when looking at slices of a brain, tissue mostly composed of cell bodies appears gray compared with other areas, and thus is referred to as “gray matter.” Brain tissue mostly composed of axons and fiber tracts appears white, and thus is referred to as “white matter.” Often, the most informative structural images of the brain show the contrast between gray and white matter. Therefore, the ultimate goal of structural imaging technologies is to differentiate between proteins and carbohydrates (cell bodies), fat (axon tracts), and salt water (CSF), as this contrast reveals the most information about brain architecture.

Until the early 1970s, there was no technology that could differentiate between these substances within the brain. Conventional **X-ray** technology is essentially useless for this purpose. During an X-ray procedure, an X-ray beam

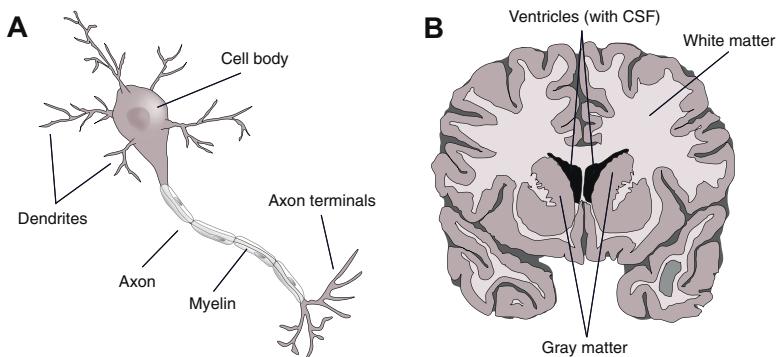


FIGURE 1.1 The composition of the brain. (A) A microscopic view of a neuron. Each neuron is composed of a cell body, dendrites, and an axonal process. The cell bodies and dendrites are rich in proteins and carbohydrates. Axons are surrounded by myelin insulation made of fats. (B) A macroscopic view of the brain. Gray matter is rich in cell bodies and therefore in proteins and carbohydrates. White matter is composed of axon tracts and is therefore rich in fatty myelin. CSF in the ventricles is a saline solution. To produce an image, brain imaging technologies must differentiate between proteins/carbohydrates, fats, and saline.

is passed through an object and then onto a photographic plate (Fig. 1.2A). Each of the molecules through which the beam passes absorbs some of the radiation, so only the unabsorbed portions of the beam reach the photographic plate. X-ray photography is therefore only effective in characterizing internal structures that differ substantially from their surroundings in the degree to which they absorb X-rays, such as bone in flesh (Fig. 1.2B). By the time an X-ray beam passes through the relatively soft consistency of the brain (not to mention the relatively hard consistency of the skull!), little information about individual brain structures can be discerned (Fig. 1.2C). Therefore, technologies that improve upon conventional X-ray technology are necessary to show contrast between neural tissues and form meaningful anatomical images of the brain.

Cerebral Angiography

A **cerebral angiogram** is an enhanced X-ray that uses dyes to make up for the relatively poor soft-tissue contrast of conventional X-rays. A radio-opaque dye that absorbs X-rays better than surrounding tissue is injected into an artery that delivers blood to the brain. This substance heightens the contrast between the cerebral circulatory system and surrounding brain tissue during an X-ray (Fig. 1.3A). Thus, the most prominent aspect of the central nervous system imaged in a cerebral angiogram is the brain vasculature. Angiograms can show vascular damage and indicate the presence of a tumor or aneurysm, an abnormal ballooning of a portion of an artery due to weakening of a blood vessel wall (Fig. 1.3B).

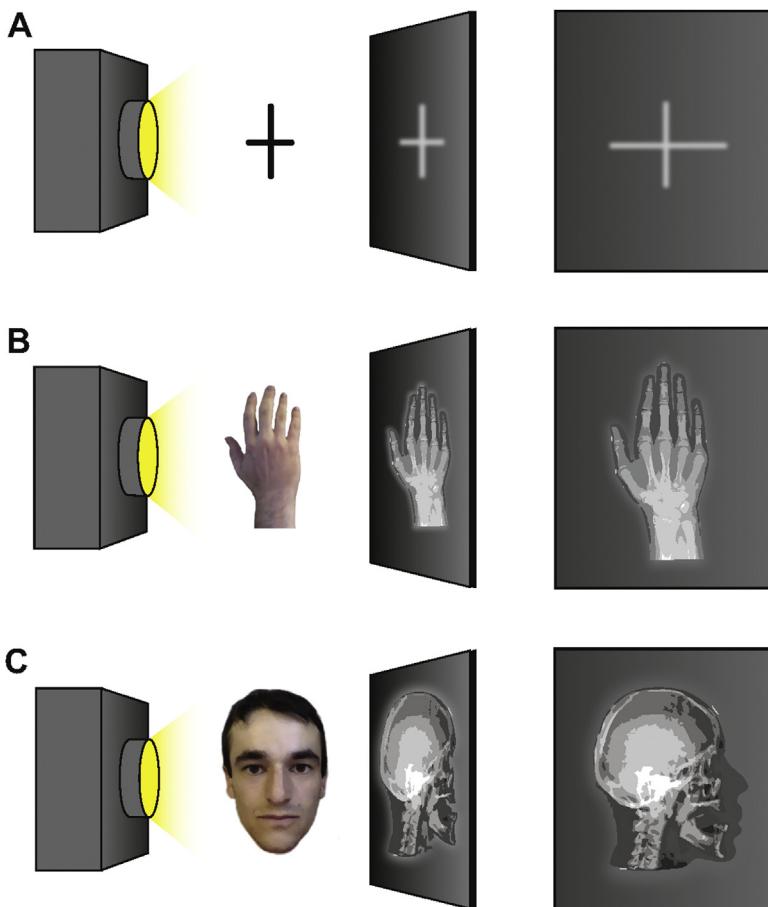


FIGURE 1.2 Standard X-ray technology alone cannot produce detailed images of the brain. (A) During an X-ray procedure, an X-ray beam is passed through an object and onto a photographic plate. Only the unabsorbed portions of the beam reach the plate, creating an image. (B) The contrast between the soft consistency of skin and muscle compared with the hard consistency of bone is sufficient to form a meaningful X-ray image. However, (C) the contrast between the soft consistency of different tissues within the brain is insufficient to form a useful image.

Computerized Tomography

Another method that improves upon conventional X-ray technology to image the brain and body is **computerized tomography** (the “CT scan”—sometimes also called computerized axial tomography, or “CAT scan”). A patient or subject lies with his or her head positioned in the center of a cylinder (Fig. 1.4A). A narrow beam of X-rays is aimed through the person’s head and hits a detector on the opposite side. The beam and detector rotate in a slow arc, taking many individual X-ray scans at the same **axial** plane. As mentioned

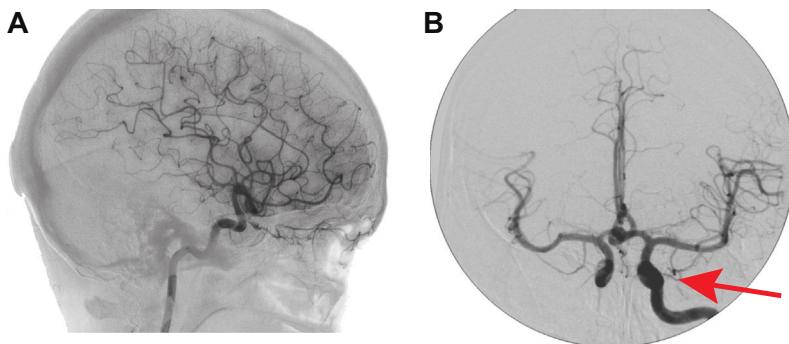


FIGURE 1.3 **Cerebral angiography.** (A) A cerebral angiogram depicting the vasculature of the right hemisphere of the brain. (B) A cerebral angiogram indicating the presence of a brain aneurysm (red arrow). (A), (B) Reprinted from Nolte, J., Angevine, J.B., 2007. *The Human Brain in Photographs and Diagrams*, third ed. with permission from Mosby/Elsevier, Philadelphia. Courtesy of (A) Dr. Joachim F. Seeger and (B) Dr. Raymond F. Carmody.

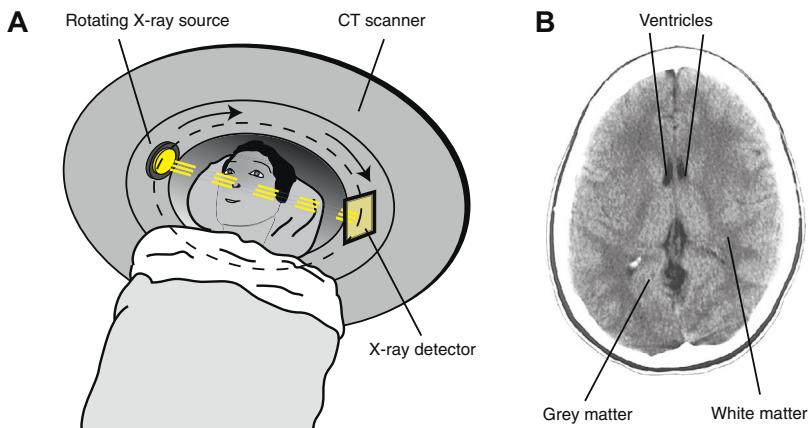


FIGURE 1.4 **Computerized tomography (CT).** (A) During an imaging session, a narrow beam of X-rays is slowly rotated around a subject's head to hit a detector on the opposite side. Signals from around the head are combined using a computer algorithm that constructs a composite picture based on the various X-ray angles. (B) A modern CT scan can distinguish between gray and white matter, differentiate ventricles, and depict structures with a spatial resolution of millimeters. (B) Reprinted from Nolte, J., Angevine, J.B., 2007. *The Human Brain in Photographs and Diagrams*, third ed. with permission from Mosby/Elsevier, Philadelphia. Courtesy of Dr. Raymond F. Carmody.

previously, a single X-ray scan would supply little information about the structure of the brain. However, multiple scans taken from different angles can be combined to provide information about small differences in radiodensity between different brain structures. These data are entered into a computer algorithm that constructs a composite picture based on the X-ray scans from

all the different angles. With this information, a “slice,” or tomogram (*tomo* means “cut” or “slice”), can be generated. Typically, 8–10 images of axial brain sections are obtained for analysis.

The quality of a CT scan depends on the width of the X-ray beam (narrower is better), the sensitivity of the X-ray detector, and the ability of the computer to construct an image from the data. Modern CT scans can distinguish between gray matter, white matter, and ventricles with a spatial resolution of millimeters (Fig. 1.4B). They are particularly useful for identifying fluid boundaries in human patients, such as when blood collects on the brain surface in a hematoma, or detecting hard objects in soft tissue, such as a tumor or calcification. CT scanners are faster, cheaper to operate, and less prone to motion artifacts than MRI scanners; therefore, they tend to be the first tool of choice to diagnose a patient with a potential head injury.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) technology produces highly detailed structural images of the brain and body. The resolution of a modern MR image is far superior to a CT image, typically less than a millimeter. Thus, MRI technology has largely superseded CT as *the* method of imaging the brain in both clinical and research settings. The technology that makes MRI possible is complex, but necessary to understand to fully appreciate MR images of the brain.

The Electromagnetic Basis of MRI Technology

As the name suggests, MRI takes advantage of the magnetic properties of neural tissue to produce an image. Most often, MRI utilizes the magnetic properties of hydrogen protons, as they are highly abundant in the fluids and organic compounds of the brain and body. The main function of an MRI scanner is to artificially excite these hydrogen protons and then measure their relaxation properties over time.

An MRI scanner is composed of a long tube-like chamber in which a subject is placed, surrounded by electric coils hidden within the MRI apparatus (Fig. 1.5). As current passes through the coils in a clockwise rotation, a magnetic field is produced longitudinal to the subject, in the direction of the feet to the head. The purpose of putting the subject in a magnetic field is to affect the hydrogen protons in the subject’s tissues. Protons can be thought of as miniature magnets: they spin about an axis and their rotating positive charge induces a tiny magnetic field (Fig. 1.6A). Normally, the magnetic fields of individual protons orient in random directions (Fig. 1.6B). However, when a subject is placed inside the strong magnetic field of an MRI machine, the magnetic fields of individual protons align in the axis of the field. Some protons align parallel to the magnetic field, toward the subject’s head, while others align in the opposite, “antiparallel” direction, toward the subject’s feet (Fig. 1.6C). It is *slightly* more energetically favorable for the protons to orient

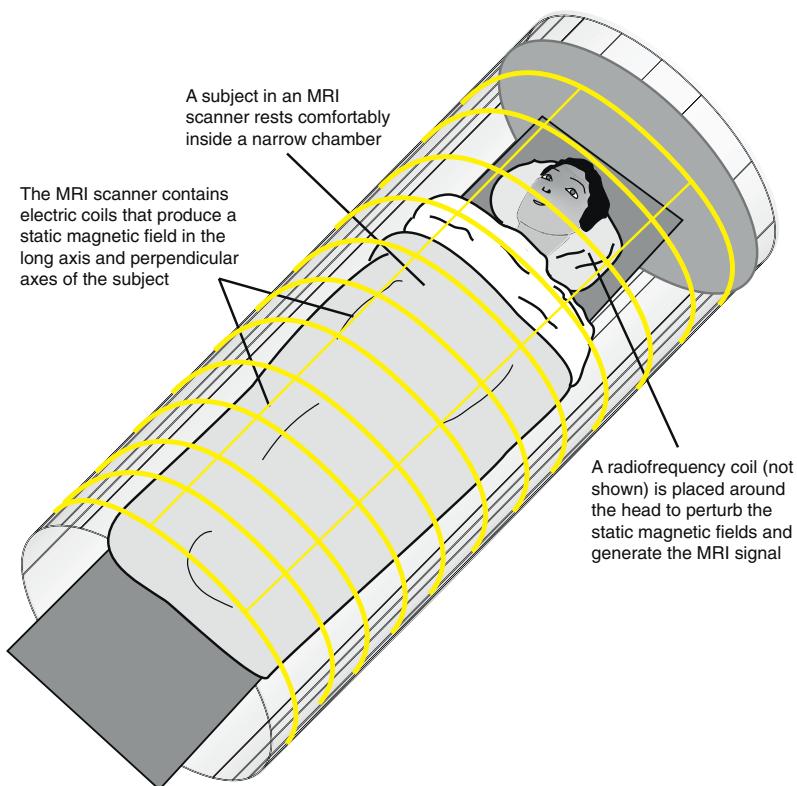


FIGURE 1.5 A human subject/patient in an MRI scanner. The subject lies inside a chamber surrounded by electric coils. Current passing through these coils induces a strong magnetic field.

in the parallel rather than antiparallel direction, so there is a net magnetic field vector in the parallel direction (Fig. 1.6D).

There is one more important detail to know about protons in a magnetic field: they do not simply stay stationary, aligned parallel (or antiparallel) to the magnetic field lines. Instead, they **precess** around their axis, spinning like a top (Fig. 1.7). The frequency with which they spin is dependent on the strength of the external magnetic field (generated by the MRI machine). The larger the external magnetic field, the higher the precession frequency. The strength of a magnetic field is measured in Tesla (T). In the literature, you will see that many conventional MRI machines create external magnetic fields at 1.5–3 T. More powerful MRI scanners create fields at 7 T. Higher magnetic field strengths increase the signal-to-noise ratio and provide higher contrast and spatial resolution. However, these powerful magnets are also more expensive and more likely to cause physiological discomfort in subjects, such as nausea or dizziness.

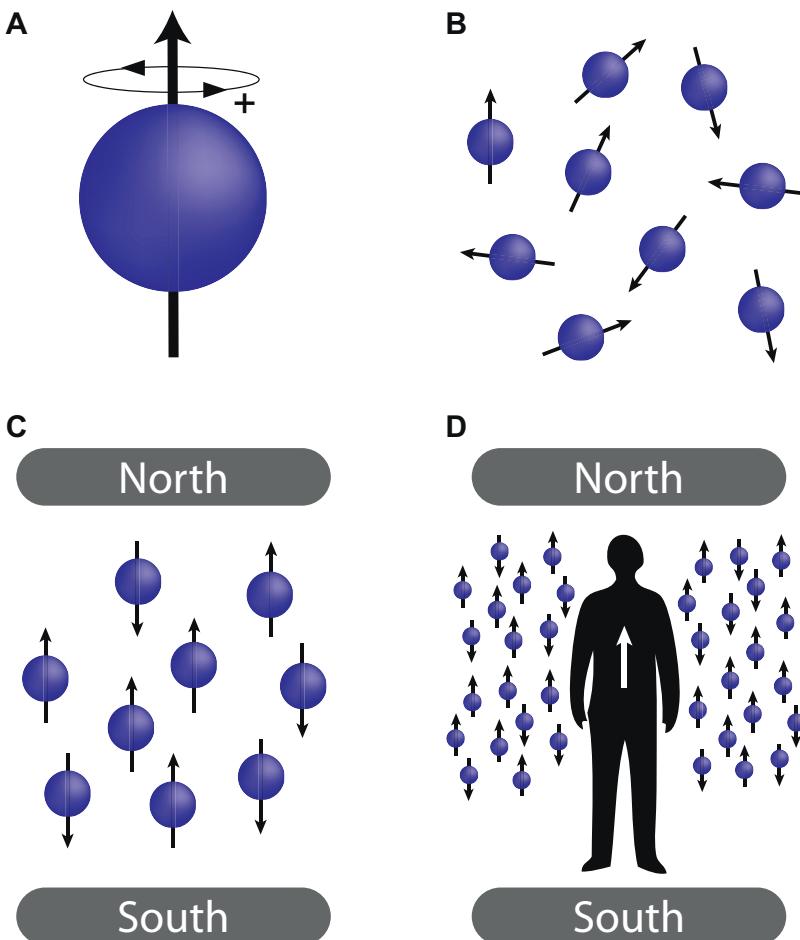


FIGURE 1.6 Protons align with the magnetic field. (A) As a proton spins around its axis, the rotating positive charge induces a magnetic field. Therefore, a proton can be thought of as a tiny magnet. (B) Before an external magnetic field is applied, protons orient in random directions. (C) In the presence of a strong magnetic field, protons align in either a parallel or antiparallel direction. (D) In an MRI scanner, protons aligned in the parallel direction are directed toward the subject's head, while protons aligned in the antiparallel direction are directed toward the subject's feet. Slightly more protons are aligned in the parallel direction, so there is a net magnetic force toward the subject's head.

Generating an Image

Prior to the beginning of an imaging session, there is a net magnetic field vector in the parallel direction, longitudinal to the subject's body (Fig. 1.8A). To collect data for an MR image, the subject is briefly exposed to pulses of electromagnetic energy, referred to as **radiofrequency (RF) pulses**. Applying an RF pulse that has the same frequency as the proton precession frequency

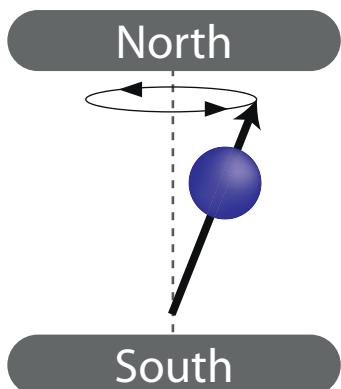


FIGURE 1.7 Protons precess around an axis in a pattern that resembles a spinning top.

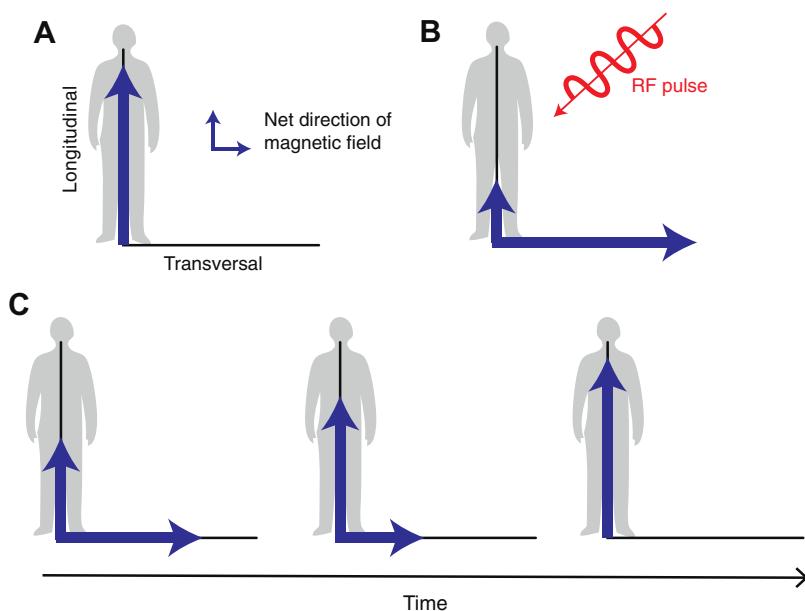


FIGURE 1.8 A radiofrequency pulse alters the net magnetic field. (A) Before the RF pulse is applied, there is a net magnetic force in the longitudinal direction toward the subject's head. (B) After the RF pulse is applied, the net longitudinal field decreases, as some protons reorient to the antiparallel orientation. Also, a new net magnetic field is created in the transverse direction. (C) After the RF pulse is switched off, the longitudinal field increases to normal and the transverse field decreases until the magnetic field returns to the state before the RF pulse was applied.

causes two effects (Fig. 1.8B): (1) Some protons in the parallel phase absorb energy, reverse polarity to the antiparallel phase, and therefore decrease the net longitudinal magnetization; (2) Some protons get in sync and start to precess in phase. Their vectors now add up in a direction that is transverse to the external magnetic field (perpendicular to the subject's body). Thus, a new transversal magnetization is established (Fig. 1.8B).

After the RF pulse is switched off, the high-energy nuclei begin to relax and realign (Fig. 1.8C). Eventually, the longitudinal magnetization increases to its original value, while the transversal magnetization decreases to zero. The time (in milliseconds) required for a certain percentage of the protons to realign in the longitudinal direction is termed **T1**. The transversal relaxation time is termed **T2**.

Both the information acquired in the longitudinal direction (T1), as well as the information acquired in the transversal direction (T2), are measured by an antenna inside the MRI scanner. Recall that the goal of brain imaging technology is to resolve differences in tissues made of proteins and carbohydrates, fat, and salt water. What makes MRI technology useful for producing images of the brain is that these substances exhibit different values for both T1 and T2 relative to each other. For any given point in time during the relaxation phase, the T1 white matter signal is stronger than that of gray matter, and the gray matter signal is stronger than that of CSF (Fig. 1.9A). These differences in

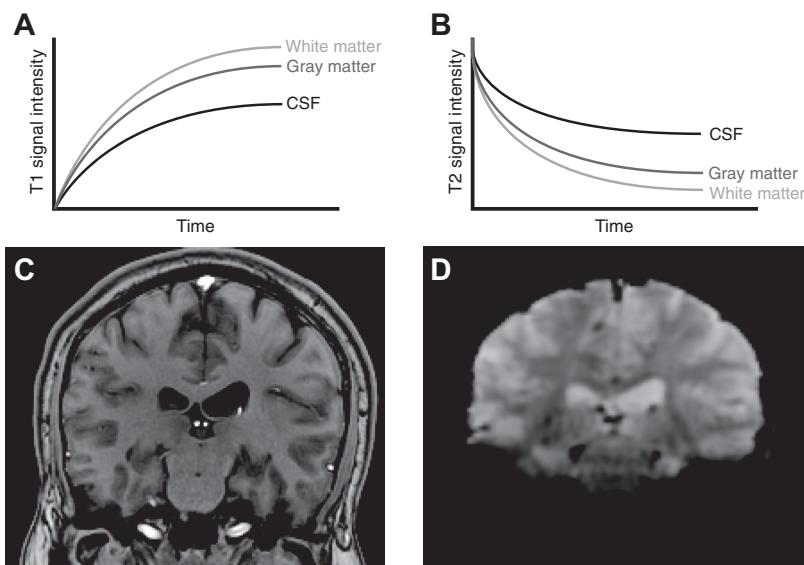


FIGURE 1.9 Different substances exhibit different T1 and T2 time constants. (A) For any given longitudinal magnetization relaxation time (T1), the white matter signal intensity will be greater than that of CSF. (B) In contrast, for any given transversal magnetization relaxation time (T2), the CSF signal intensity is greater than that for white matter. (C) Therefore, white matter will be bright and CSF dark on a T1-weighted image, while (D) white matter will be dark and CSF bright on a T2-weighted image. (C), (D) Courtesy of Dr. Rory Sayres.

signal intensity are exactly opposite for a T2 measurement: the CSF signal is strongest, followed by gray matter and then white matter ([Fig. 1.9B](#)). By examining the contrast in signal from different points in space, it is possible to differentiate between different substances and form an image.

An image of the brain formed from T1 data is referred to as a **T1-weighted** image, while an image formed from T2 data is **T2-weighted**. These images appear differently from one another because of the differences in signal intensity among substances in T1- versus T2-weighted images ([Fig. 1.9C–D](#)). As a rule of thumb, if the CSF is black, you are looking at a T1-weighted image, as CSF has the lowest relative T1 signal intensity. If, on the other hand, the CSF appears bright white, you are looking at a T2-weighted image, as CSF has the highest relative T2 signal intensity. In the literature, most anatomical data are presented as T1-weighted images, as these images usually show better contrast between brain structures. However, this is not always the case—for example, lesions of white matter that occur due to the rupturing of blood vessels are more easily detectable on a T2-weighted image. Therefore, T2-weighted images may be optimal when examining patients following trauma or stroke.

Selecting a “Slice” of Brain to Image

How does an investigator select a **slice** to examine? Recall that an RF pulse will only excite protons with the same precession frequency as the frequency of the pulse. The precession frequency varies with the strength of the external magnetic field, so to select a single slice of the brain to image, an additional magnetic field is applied to the external magnetic field at a gradient ([Fig. 1.10A](#)). Because the field strength is not equal at all planes in the longitudinal direction, an RF pulse at a specific frequency will only affect the protons, and thus the signal, at a specific plane. This is the slice of the brain that will be presented as a two-dimensional image.

To measure signal from individual points within the slice, two additional magnetic gradients are applied in the other two axes ([Fig. 1.10B](#) and [C](#)). Therefore, each point in space will have its own unique magnetic signature. Each point occupies a specific volume and is therefore termed a **voxel**, a three-dimensional version of a pixel that represents a cubic volume of brain space. The resolution of each voxel is determined by the values of the gradients applied to the subject. With greater magnetic field strengths, more dramatic gradients can be established. Therefore, a 7 T MRI scanner can produce an image with a higher **spatial resolution** than a 3 T scanner.

MRI has a number of features that have made it an especially valuable research tool for both diagnostic and research studies:

- It is entirely noninvasive, as no substances need to be injected into human subjects. Sometimes, a contrast agent is injected that enhances the visibility of water-rich regions. However, for most brain imaging procedures, no additional contrast is necessary.

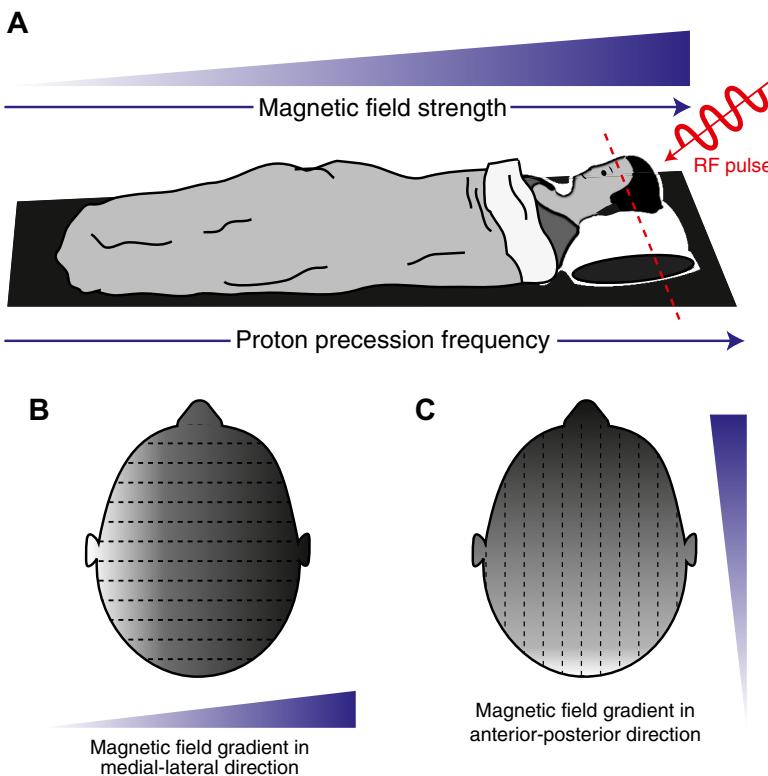


FIGURE 1.10 How a slice is selected in MRI. (A) To image a brain slice, an external magnetic field is applied at a gradient. An RF pulse will only excite protons in a particular slice, as the pulse can only excite atoms with the same precession frequency. To measure the signal intensity for each point within the slice, two additional gradients are applied, one in the medial–lateral direction (B), and one in the anterior–posterior direction (C).

- Slices of the brain can be obtained from any angle. CT scanners can only image slices of the brain based on the axis of rotation of the X-ray emitter and detector within the apparatus.
- By varying the gradient and RF pulse parameters, MRI scanners can be used to generate images that highlight certain brain regions and provide contrast between specific kinds of neural tissue.
- No X-ray radiation is applied to the subject in the scanner.

Even with these relative advantages, MRI has not completely replaced CT imaging. CT imaging is better for visualizing bony or calcified structures in the head, and also remains the imaging technique of choice for subjects who might not be able to enter the high magnetic field (for instance, due to a pacemaker), or subjects with claustrophobia. Additionally, CT scanners have much lower operating costs than MRI scanners, making their use relatively less expensive.

Diffusion Magnetic Resonance Imaging

Diffusion MRI is an application of MRI that is used to examine the structure of axon fiber tracts in the brain. Traditional MRI images present white matter as a homogenous structure (as in Fig. 1.9C–D). In reality, fiber tracts originate from various sources, radiate in different orientations, and travel to distinct regions of the brain. Diffusion MRI provides investigators the opportunity to visualize these different white matter pathways and study the complexities of axonal architecture (Fig. 1.11).

The term **diffusion** refers to the fact that water molecules, like all other molecules, randomly move through a medium over time. For example, in a glass of water, any particular molecule of water will move around randomly in any direction, limited only by the walls of the container. This type of diffusion is referred to as **isotropic**—diffusion in all directions. However, water molecules within brain tissue diffuse quite differently due to the physical environment of the brain. They tend to diffuse most rapidly along parallel bundles of fibers with coherent orientations. This type of diffusion is referred to as **anisotropic**—diffusion that is not equal in all directions but instead tends to move along a single axis.

Diffusion MRI is able to measure the anisotropic diffusion of water along fiber bundles, highlighting the connectivity between brain regions. The physics and mathematics behind this technology are too complex for this text, but the goal is to use MRI technology to analyze the magnitude and direction of the diffusion of water molecules for each voxel of tissue, thus creating a three-dimensional image of fiber tracts. There are various kinds of diffusion MRI methods, the most commonly used called **diffusion tensor imaging (DTI)**.

Diffusion MRI can provide information about which areas of the brain are connected, but it is not able to determine the *direction* of this connectivity (which endpoint is the source and which endpoint is the target). However, it is possible to combine diffusion MRI with functional MRI, allowing researchers to identify temporal correlations between activities in distinct brain regions and therefore to make conclusions about functional connectivity between brain structures.

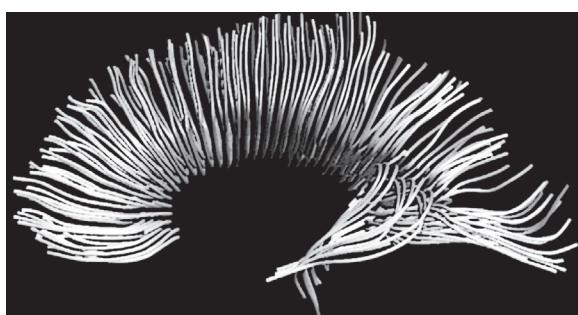


FIGURE 1.11 **Diffusion tensor imaging.** An example of an image of white matter tracts in the corpus callosum. Reprinted from Van Hecke, W., et al., 2008. *On the construction of an intersubject diffusion tensor magnetic resonance atlas of the healthy human brain*. Neuroimage 43 (1), 69–80, with permission from Elsevier.

FUNCTIONAL BRAIN IMAGING TECHNIQUES

Functional brain imaging techniques are used to measure neural activity in the central nervous system without physically penetrating the skull. The ultimate goal of these techniques is to determine which neural structures are active during certain mental operations. Although these techniques cannot demonstrate that a brain region *causes* certain actions or is *the* specific structure that regulates a cognitive process, they can demonstrate other useful properties. For example, functional brain imaging techniques can show that activity in specific brain regions is often correlated with a particular stimulus, emotional state, or behavioral task. They can show that information is represented in certain places within the brain, that information may be present in the brain without being consciously represented, and that diseased brains may process information abnormally compared to healthy brains. Importantly, these techniques have allowed neuroscientists the opportunity to study the neural basis of cognition, emotion, sensation, and behavior in *humans*, a feat that cannot be achieved by most other techniques in this book.

Functional Magnetic Resonance Imaging

Functional magnetic resonance imaging (fMRI) uses the same physical principles as MRI to produce high-resolution representations of brain activity over time. As with MRI technology, fMRI detects signals from excited hydrogen protons in a magnetic field. Recall that different substances within the brain exhibit different T1 and T2 values following stimulation with an RF pulse. In a structural imaging experiment, these values are used to measure differences in signal intensity between gray matter, white matter, and CSF. fMRI technology takes advantage of the signal intensity of another substance within the brain: **hemoglobin**, the protein in the blood that carries oxygen to cells. On a T2 image, oxyhemoglobin (hemoglobin that is relatively saturated with oxygen) has a relatively stronger magnetic resonance signal than deoxyhemoglobin (the oxygen-depleted form of hemoglobin). Thus, fMRI allows an investigator the opportunity to examine changes in the oxygenation-state of hemoglobin over time.

The ability to examine changes in oxygen metabolism over time in the brain is useful because it serves as an indirect measure of neural activity. Active neurons will consume more oxygen compared to when they are at rest. Initially, this activity decreases the levels of oxyhemoglobin and increases levels of deoxyhemoglobin. However, within seconds, the brain microvasculature responds to this local oxygen depletion by increasing the flow of oxygen-rich blood to the active area, increasing the local amount of oxyhemoglobin. This is referred to as the **blood oxygen level-dependent (BOLD) effect** (Fig. 1.12), and it forms the basis of the fMRI signal.

fMRI depends on T2-weighted images because the contrast in signal intensity between deoxyhemoglobin and oxyhemoglobin is greatest on these kinds of images. In a typical experiment, a T2-weighted image of the brain is

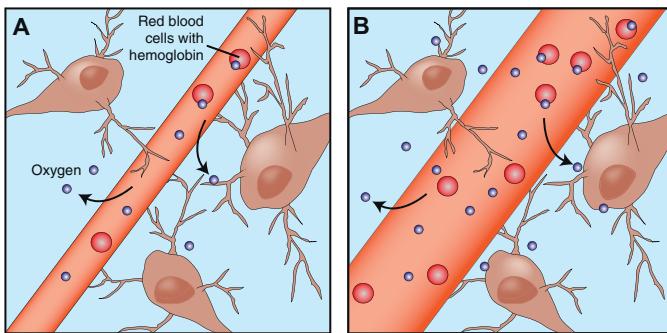


FIGURE 1.12 The BOLD effect. (A) A group of neurons at rest are supplied by blood from capillaries. (B) When these neurons become active, they increase their metabolic demand for oxygen. The microvasculature responds by dilating and supplying more oxygen-rich blood to the local area. The relative increase in the oxygenated form of hemoglobin causes an increase in the T2 signal.

obtained prior to the presentation of a stimulus. After a stimulus is presented or the subject performs a task, additional T2-weighted images are obtained. As neurons become active due to the stimulus or task, the BOLD effect causes a relative increase in oxyhemoglobin to the microenvironment, and thus an increase in T2-weighted signal. The amount of T2 signal is compared between the prestimulus and poststimulus time points and color coded to depict the signal intensity. These data are typically superimposed over a T1-weighted image that more clearly depicts the underlying anatomy of the brain. The end result is a colorful statistical representation of neural activity superimposed on an anatomical image of the brain—a depiction of the BOLD response over time (Fig. 1.13). Therefore, fMRI data in a two-dimensional figure is actually *four* dimensional: the *x*, *y*, *z* coordinate planes for each voxel in space, as well as the fourth dimension of time (the “before and after” time points during which the stimulus is presented).

Although fMRI technology provides a powerful tool to study the neural basis of cognition, there are significant limitations. One of the biggest challenges in fMRI research is that the actual T2 signal change for a given voxel of brain space, before and after BOLD changes, can be as low as 0.2%. This change is very difficult to detect, especially given that the noise of the system can be as high as 0.3%–0.4%. Therefore, fMRI stimuli must be repeated several times for a single subject, and a series of statistical tests must confirm the presence of a reproducible signal. Another significant limitation is the **temporal delay**: it can take 6–10 s after the presentation of a stimulus for oxygenated blood to flow to an active region, so there can be a long time delay between the stimulus or task and the measurement of neural activity. The **temporal resolution**, the ability to resolve neural activity into discrete events, is about 4–8 s, relatively poor compared to other techniques such as EEG or

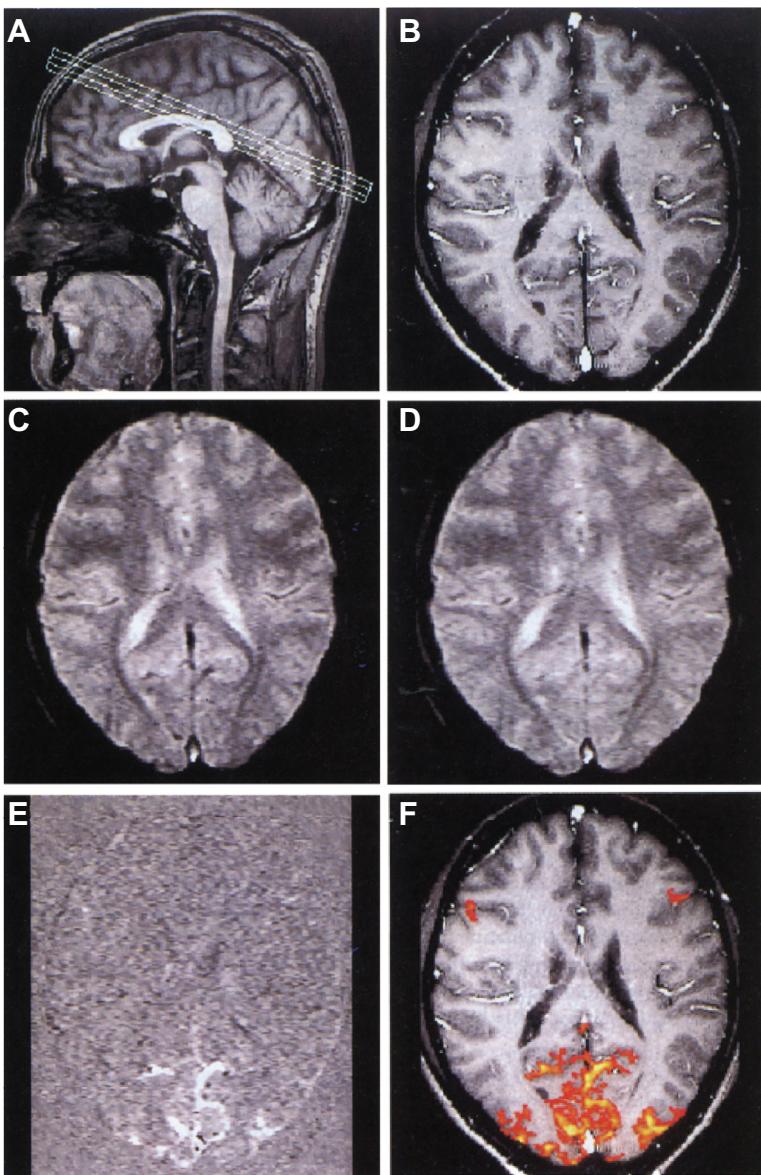


FIGURE 1.13 Example of an fMRI experiment. This experiment examines the difference in BOLD signal intensity between subjects exposed to light or no light. (A) First, the investigators select a slice to examine. (B) A T1-weighted image is obtained that provides structural data. T2-weighted images are used for the actual data collection in the conditions of (C) light and (D) no light. (E) The data analysis compares BOLD signal intensity between (C) and (D). This result is superimposed on the structural data to produce an image (F) suitable for publication. (A)–(F) Reprinted with permission of Springer Science + Business Media and Jens Frahm from Windhorst, U., Johansson, H. (Eds.), 1999. *Modern Techniques in Neuroscience Research*, Ch. 38: Magnetic Resonance Imaging of Human Brain Function, 1064, Fig. 5.

MEG (see below for descriptions of EEG and MEG). Finally, fMRI cannot identify the neurochemistry of neural events, such as which neurotransmitters or neuromodulators mediate a change in neural activity. Inferences about the neurochemical make-up of neural activity must be based on prior knowledge of brain anatomy or other forms of noninvasive brain imaging, such as PET (see the following section).

Even with these relative disadvantages, fMRI remains a powerful technique for correlating neural activity with mental operations. A well-designed experiment can reveal much about the human brain and animal brains (Box 1.1), allowing investigators to noninvasively examine the physiology of cognition. See the last part of this chapter for a thorough discussion of the design of a functional imaging experiment.

BOX 1.1 fMRI Experiments in Animals

A major benefit of functional brain imaging technologies is the ability to noninvasively study neural activity in humans. This technology can also be used with other animals, such as primates and rodents. The major benefits of using animal subjects include the ability to validate the use of animal models and to bridge human fMRI experiments with animal electrophysiology and imaging experiments. The ability to use both fMRI and other techniques in the same animal (and even at the same time) greatly aids our understanding of the functional activity of individual neurons and entire brain regions in the same experiment. Furthermore, fMRI studies in primates that screen neural activity across the entire brain can inform future studies about brain regions containing neurons of interest to an investigator.

fMRI studies using primates present additional limitations and challenges compared to traditional human studies. Primates tend to dislike lying down horizontally while awake, so vertical scanners have been created to accommodate the special chambers that support and brace a conscious primate. The animal's head must be fixed in place with a head post so there is no head movement during an experiment. The animal must also be acclimated to these experimental conditions so it is comfortable in its environment and can perform the task. A further challenge is that during the actual experimental sessions, a primate may lose motivation to perform a task or attend to a stimulus.

MRI scanners with higher magnetic field strengths allow high-resolution, detailed imaging in small animals such as rats and mice. Obviously, these animals cannot perform complicated cognitive tasks and most likely need to be anesthetized during an imaging session. However, the benefit of doing fMRI in rodents is the possibility of injecting psychoactive drugs or performing neuromodulation experiments during an imaging session. Thus, it is possible to study the global effects of a pharmacological agent, such as a receptor antagonist, or the loss-of-function or gain-of function of a brain region over time. Furthermore, a scientist can follow-up functional imaging data with histological studies after the completion of an experiment.

Positron Emission Tomography

Positron emission tomography (PET) provides a representation of neural activity but no information about brain structure. This technology was developed in the 1970s and 1980s as a novel method of functional imaging, but has largely been superseded by fMRI technology for most cognitive experiments. In a PET experiment, an unstable **positron-emitting isotope** is injected into a subject's carotid artery (an artery in the neck that feeds the ipsilateral cerebral hemisphere). As the isotope decays, it emits a **positron**, an antimatter counterpart of an electron. When a positron comes into contact with an electron, an annihilation event occurs, resulting in a pair of gamma photons that move in opposite directions (Fig. 1.14A). These photons pass through the body and can be measured by a gamma-detecting device that circles the subject's head. The detector identifies a pair of gamma photons that arrive at opposite sides of the subject's head at the same time (within a few nanoseconds; Fig. 1.14B). As the detector rotates around the subject's head, these signals can be used to derive the source of the annihilation events within the subject (Fig. 1.14C).

A PET experiment can use a variety of positron-emitting isotopes. One of the most commonly used is **fluorodeoxyglucose (FDG)**, a radioactive form of glucose. As metabolically active neurons require an increase in the uptake of glucose from the blood, the presence of FDG can be used as an indirect

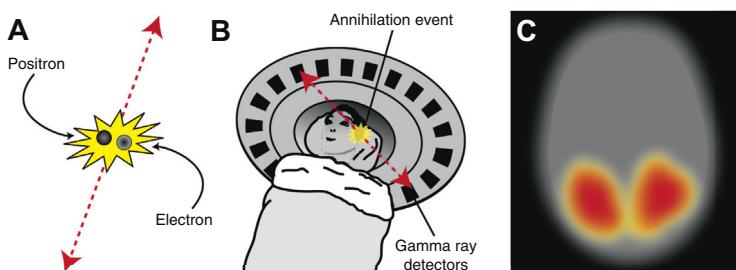


FIGURE 1.14 PET imaging. (A) An unstable positron-emitting isotope will decay over time and emit a positron. When a positron comes into contact with an electron, an annihilation event occurs and a pair of gamma photons are released. (B) If this unstable isotope is injected into a subject, the annihilation event can be detected by a PET scanner. This scanner consists of a series of gamma ray detectors arranged around the subject's head. (C) Unstable isotopes for many metabolic substances, such as glucose or neuropeptide metabolic proteins, can be imaged in a PET experiment. The increase in signal over time can mark sites of increased metabolic activity that correlate with a specific neural stimulus or task.

marker of neural activity. In addition to FDG, radioactive water can be injected into the brain's circulatory system. Because there is an increase in blood flow to active areas of the brain, the PET scan will indicate the areas in which blood flow is increased during activity.

A particularly useful aspect of PET imaging is the ability to use positron-emitting isotopes that can bind to specific receptors in the brain. For example, a radioactive ligand that binds to serotonin receptors can indicate the locations and binding potential of these receptors in the brain, providing information about the relative metabolism of serotonin in human subjects. The ability to image the metabolism of specific bioactive molecules makes PET imaging unique among functional imaging techniques and provides a utility that fMRI cannot.

There are a number of limitations to PET. Compared to fMRI, PET has about the same temporal resolution (4–8 s), a lower spatial resolution, and cannot generate anatomical data. PET images are often combined with CT or MRI images to present functional data in an anatomical context. Performing a PET scan is also very expensive. Because most isotopes used for PET studies have extremely short half-lives, positron-emitting isotopes must be synthesized on site using a room-sized device called a **cyclotron**, which itself is expensive to purchase and maintain. For example, the half-life of FDG is 110 min, so this compound cannot be ordered and delivered from a remote location. Finally, PET requires the injection of these radioactive substances into subjects. Therefore, multiple PET-imaging sessions are not recommended for a single subject. Because of these limitations, most modern functional imaging experiments utilize fMRI technology instead of PET. However, PET is advantageous over fMRI to study the metabolism of bioactive substances, such as neurotransmitters. It is also possible to diagnose some diseases using PET, such as identifying tau and amyloid plaques and other markers of neuroinflammation in Alzheimer's disease.

Single-Proton Emission Computerized Tomography

Single-proton emission computerized tomography (SPECT) imaging is very similar to PET imaging, producing functional images of neural activity but no structural data. Like PET, a radioactive probe is injected (or inhaled) into the circulatory system. The probes bind red blood cells to be carried throughout the body. Because blood flow is increased in active brain structures, the radioactive signal is used to assess an increase in neural metabolism. As the label undergoes radioactive decay, it emits high-energy photons that can be detected using a gamma camera. The camera is rapidly moved around the head of a subject to collect photons from many different angles, permitting a three-dimensional reconstruction.

Although SPECT is very similar to PET in concept, it is not as costly because the radiolabeled probes are usually commercially available and do not

require an on-site cyclotron. Therefore, SPECT can be thought of as a cheaper alternative to PET. The disadvantages to using SPECT as an imaging technique are similar to the disadvantages of using PET: the technology has a relatively low spatial resolution compared to fMRI (about 8 mm), and radioactive substances must be injected into a subject.

Electroencephalography

Electroencephalography (EEG) is a measure of the gross electrical activity of the surface of the brain. It is not truly a brain *imaging* technique, as no meaningful images of the brain can be produced using this technique alone. However, EEG is noninvasive and can be used to ascertain particular states of consciousness with a temporal resolution of milliseconds. EEG combined with other imaging techniques, such as fMRI, can provide an excellent temporal and spatial representation of neural activity.

To produce an electroencephalogram, several disk-shaped electrodes, about half the size of a dime, are placed on the scalp (Fig. 1.15). The scalp EEG reflects the sum of electrical events at the surface of the brain. These events include action potentials and postsynaptic potentials, as well as electrical signals from scalp muscles and skin. A good way to think about EEG technology is to imagine what it would be like to place a microphone above a large crowd of people, such as above Times Square during New Year's Eve. It would be impossible to make out the signal from an individual person in the crowd; however, it would be possible to determine when a meaningful event occurred, such as when the crowd counted down to midnight. Likewise, it is impossible to record the electrical activity from a single neuron with EEG, but it is possible to ascertain when a meaningful event occurs in the brain, such as when a subject detects a salient stimulus during an experiment. Combining the temporal resolution of EEG with the spatial resolution of fMRI provides a powerful method to detect the precise timing and location of neural activity within the brain.

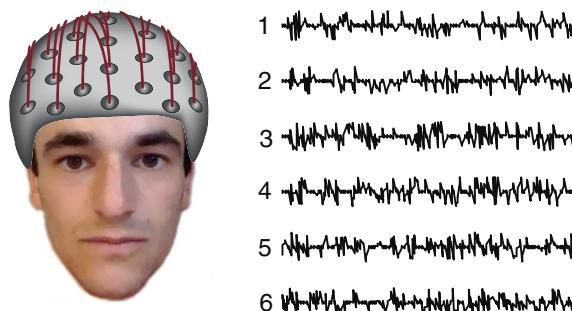


FIGURE 1.15 A human subject wearing electrodes for EEG recordings. The electrodes measure the global electrical activity of millions of neurons in the brain. Each electrode placed on the scalp records a unique trace of activity based on its location.

A powerful application of EEG is the study of **event-related potentials (ERPs)** in humans and animals. An ERP is a distinct, stereotyped waveform in the EEG that corresponds to a specific sensory, cognitive, or motor event. For example, if a human subject abruptly hears an alarm, the perception of sound may be represented as a larger-amplitude deflection within the ERP (known as the P300 component) as compared to the deflections in response to less-startling background sounds. Thus, neuroscientists can study the moment that the brain processes a stimulus with subsecond temporal resolution, allowing a noninvasive means of evaluating brain function in experimental subjects or patients with cognitive diseases.

Magnetoencephalography

Magnetoencephalography (MEG) measures changes in magnetic fields on the surface of the scalp that are produced by changes in underlying patterns of neural electrical activity (Fig. 1.16). About 50,000 neurons are required to produce a detectable signal with MEG, a number that may seem large but is actually much smaller than what is required for an EEG signal, which may require millions of neurons. MEG offers relatively poor spatial resolution but excellent temporal resolution compared with PET, SPECT, and fMRI. Therefore, MEG can be thought of as a compromise technique: it offers excellent temporal resolution and much better spatial resolution compared with EEG, but not as good spatial resolution as other imaging techniques. In

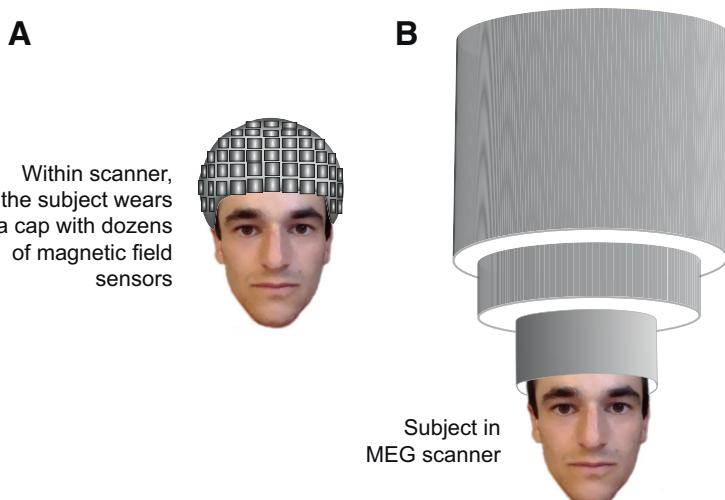


FIGURE 1.16 A magnetoencephalography setup. (A) A subject wears a cap with magnetic field sensors and (B) is placed inside an imaging chamber. MEG equipment is expensive and requires a room with strong insulation.

particular, MEG in combination with fMRI allows for excellent temporal and spatial resolution of neural activity. Unfortunately, MEG is a very expensive technique, requiring a room that can obstruct magnetic fields from outside sources; even something as small as a coffee machine in a neighboring building can be detected if the room is not adequately insulated.

Like EEG, the temporal precision of MEG allows for the study of event-related changes in brain activity. The MEG equivalent of an ERP is referred to as an **event-related field (ERF)**.

Optical Imaging

Optical imaging techniques produce images of neural activity by measuring changes in blood flow and metabolism from the surface of the brain. Rather than detecting changes in the magnetic or electrical properties of neurons, as in fMRI, EEG, or MEG, optical imaging detects changes in light reflectance from the surface of the brain due to changes in the amount of blood flowing to neural tissue. In animal preparations or during human surgery, light is shined on an exposed portion of the brain. Any light that is reflected off the surface is detected by a sensitive camera and recorded by a computer. When neurons are more active, changes in blood volume, blood oxygenation, and the light-scattering properties of neural tissue (resulting from ion and chemical movements) all cause small (0.1%–3.0%) changes in the reflectance of light from the brain's surface. For each experiment, the investigator images a baseline amount of light reflectance, and then compares this baseline parameter to changes in light reflectance that arise due to the presentation of a stimulus. Optical imaging technologies allow for spatial resolutions of <1 mm and temporal resolutions of 2–8 s. These technologies have been used to produce high-resolution functional maps of visual cortex in both animals and humans.

Earlier in this chapter, we defined functional imaging techniques as methods that allowed investigators to measure changes in neural activity over time without physically penetrating the skull. Many optical imaging techniques are an exception to this definition, as the brain surface must be exposed to allow light to penetrate and reflect back to a camera. However, **diffuse optical imaging (DOI)** and **near-infrared spectroscopy (NIRS)** are noninvasive alternatives that utilize the same basic principles as invasive optical imaging but record light reflectance through the scalp (Fig. 1.17). The signal is much weaker than invasive optical imaging, as light must pass through the superficial layers of the head to the brain, and then from the brain to optical electrodes, known as **optrodes**, placed on the surface of the scalp. However, these techniques are sensitive enough to detect large changes in neural activity and can be useful in clinical applications as an alternative to fMRI or PET because of their low cost and portability. For example, long-term monitoring of cerebral oxygenation in a patient following a stroke is a potential use of DOI that would be practically impossible with fMRI or PET.

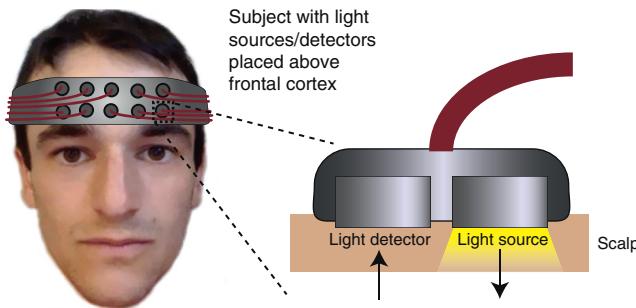


FIGURE 1.17 Optical imaging. Light is shined onto the surface of the brain. A portion of this light is reflected off the brain and detected by multiple optrodes. Changes in neural activity produce changes in the amount of light that is absorbed and reflected by the brain. Therefore, optical imaging can be used to indirectly detect changes in neural activity. These techniques can be either invasive (the skull is opened to reveal the surface of the brain) or noninvasive.

Table 1.1 compares the spatial and temporal resolutions, cost, and invasiveness of the various functional brain imaging techniques. As mentioned previously, many imaging laboratories now combine multiple techniques to make up for the inadequacies of any single technique. For example, fMRI offers excellent spatial resolution and is noninvasive, but it does not offer good temporal resolution of neural activity during experiments because the BOLD effect occurs over a period of 2–8 s. Therefore, some laboratories combine fMRI with MEG or EEG, in which signals can be detected within a fraction of a second.

So far, this chapter has focused on the technology of structural and functional brain imaging. Of course, the utility of these technologies depends on

TABLE 1.1 Comparison of Functional Imaging Techniques.

| | Spatial resolution | Temporal resolution | Cost | Invasiveness |
|-----------------|--------------------|---------------------|----------------|-----------------------|
| fMRI | 1 mm | 2–8 s | Expensive | Noninvasive |
| PET | 4 mm | 1 min | Very expensive | Radioactive injection |
| SPECT | 8 mm | 2–8 s | Expensive | Radioactive injection |
| EEG | 1 cm | 1 ms | Moderate | Noninvasive |
| MEG | 1 mm | 1 ms | Very expensive | Noninvasive |
| Optical imaging | 1 mm | 2–8 s | Inexpensive | Can be invasive |

how they are used: the research hypotheses, experimental designs, and methods of data analysis. In the last part of this chapter, we will examine the approaches that can be taken in the design and analysis of functional imaging experiments.

FUNCTIONAL IMAGING EXPERIMENTAL DESIGN AND ANALYSIS

Functional imaging technologies are particularly well suited to **cognitive neuroscience**, the field of neuroscience dedicated to elucidating the neural basis of thought and perception. Cognitive operations routinely studied in the literature include attention, learning and memory, executive function, language, emotion, and higher-order cognitive phenomena, such as the enjoyment of art or music. Determining the neural basis of cognitive processes, especially processes that seem unique to humans, absolutely depends on brain imaging techniques.

In addition to answering scientific questions about cognitive neuroscience, functional imaging methods have added important information to other neuroscience fields, such as sensory or motor **systems neuroscience**. These studies identify regions of the human brain that are active in the presence of certain sensory stimuli or motor actions. Some studies reproduce findings in humans that were previously demonstrated in other animals, as well as add important insights that enhance our understanding of these systems. For example, fMRI research has demonstrated that visual pathways in humans are anatomically consistent with visual pathways found in other mammals. However, our understanding of the visual system has been greatly enhanced by the fact that human subjects can report experiences and receive verbal instructions that other animals cannot. For example, activity in visual cortex increases not only when human subjects are exposed to visual stimuli, but also when subjects are told to *imagine* visual stimuli. Furthermore, fMRI research has demonstrated that different areas of the visual cortex process different aspects of visual stimuli. Thus, fMRI contributes meaningfully to our understanding of both cognitive as well as systems neuroscience.

Unfortunately, the experimental design of functional imaging experiments is not always appreciated by scientists who use other methods. This lack of understanding is probably due to the fact that these technologies are unlike most other techniques used in neuroscience, as well as the fact that they are usually only performed by the dedicated researchers who use them regularly. A common grievance among brain imaging specialists is the misconception that you can simply place a human subject into a scanner, tell them to look at a stimulus, and then publish the results. Like any other technique, noninvasive brain imaging experiments must be carefully designed and interpreted, more than nonspecialists may sometimes appreciate. The next section describes some of the scientific considerations in the design and execution of a

functional imaging experiment. We will focus on fMRI, as this technique dominates the field, but the same principles can be applied to other functional imaging techniques as well.

Planning the Experiment

Before any human subject is placed in a scanner, an investigator conceives of an experiment months or even years in advance. There are many practical considerations that must be taken into account in the design of the experiment that may affect the ambitions of the investigator. Once these obstacles are overcome, the investigator designs the experiment to answer a specific question or test a specific hypothesis. Finally, the investigator designs a proper task paradigm and tests efficacy of the stimuli so that the results are appropriate and accurate.

Practical Considerations

Perhaps the most important practical consideration of an fMRI experiment is its cost. The actual scanning machine, as well as its support apparatus, costs millions of dollars and is almost always shared by multiple labs. Routine maintenance of the scanner is also expensive, with specialized technicians on call to fix any potential physical problems that may arise. Most research institutions charge an individual lab based on the number of hours the scanner is used. These institutions may also charge money based on the time of day, with scanner use during the day costing more than scanner use late at night. It is not unusual for scanner time to cost between \$100 and \$1000 per hour, depending on the institution's operating costs. Therefore, an fMRI experiment must be well designed so that scanner time is not wasted. fMRI is too expensive to simply "play around with" and put humans in the scanner for no defined purpose. Finally, scanner time is usually reserved well in advance due to the number of scientists who wish to perform experiments.

Another important practical concern of fMRI is that a subject must keep their head completely still during a scanning session. Even small movements can disrupt the magnetic field, causing artifacts in the images. Also, if a subject moves during a scan, the acquired images will not line up with each other, making them uninterpretable. This immobility means the subject will be unable to perform certain activities while inside the scanner, such as speaking, exercising, or moving facial muscles. However, subjects can be trained to perform simple hand movements to complete a task or respond to a stimulus, such as pressing a button on a hand device. Some specialized devices have been invented to deliver olfactory or gustatory stimuli to subjects.

A practical concern for an investigator who wishes to study sleep, attention, or the auditory system is that an fMRI scanner can be very noisy. The RF pulse sequences are loud and last the entire duration of the experiment. Therefore, many subjects do not sleep normally in a scanner, and the changes in attention due to a surprising stimulus may actually be due to the surprise of

a noisy scanner turning on and off. Auditory stimuli can also be very difficult to work with, for obvious reasons. These limitations are not insurmountable, but require extra planning by the scientist to make sure that a sporadically loud environment does not bias the results.

Experimental Paradigm of a Functional Imaging Experiment

Like any other experiment in neuroscience, functional imaging experiments examine the effect of an **independent variable** on a **dependent variable**. The independent variable is the experimental variable that is intentionally manipulated by the researcher and is hypothesized to cause a change in the dependent variable. In a functional imaging experiment, the independent variable can be a stimulus, task, or even a difference in the subjects being tested such as age, gender, or disease state. The dependent variable is the quantifiable variable measured by the researcher to determine the effect of the independent variable. This variable is different for each brain imaging technique. In the case of fMRI research, the dependent variable is the BOLD signal intensity for a particular part of the brain. In the case of PET or SPECT technology, the dependent variable is the intensity of gamma radiation. All of these dependent variables are used as proxies for neural activity.

The specific hypotheses tested during a functional imaging experiment are usually framed in the following manner:

- Activity in brain region X is correlated with stimulus/task Y.
- For a given stimulus or task, activity in brain region X precedes activity in brain region Y.
- Activity in brain region X is higher under condition Y than condition Z.
- Activity in brain region X is higher in human population Y than human population Z.
- Activity in brain region X changes across time as a subject learns a task.

These hypotheses depend upon the investigator knowing of the existence of “brain region X” and having a good rationale for studying that region. The amount of data generated during an fMRI experiment is quite large, and processing fMRI data can be easier if the investigator has a specific idea about where to examine.

Despite large amounts of data, fMRI can also be used to screen the entire brain to identify brain regions that increase neural activity in response to a stimulus/condition, or to examine distributed networks of neural activity during a resting state. The increasing availability of powerful computational resources and techniques has enabled these more data driven, exploratory approaches to analyzing the large quantities of data produced during brain imaging. Machine learning-based techniques, such as multivariate pattern analysis, can be used to extract patterns from large datasets, allowing the advancement of studies that look at brain-wide functional networks.

It is also important to differentiate between studies that are **between subjects** versus **within subjects**. In a study that is between subjects, the independent variable is the different populations of subjects, such as male versus female, old versus young, healthy versus diseased, genotype A versus genotype B, etc. These differences in subjects can potentially introduce confounding variables that make analysis more difficult. For example, it is known that the BOLD response can change with age; therefore, comparing a younger group of subjects with an older group of subjects using fMRI may require additional controls to ensure that changes in brain activation are not simply due to age-related changes in the BOLD effect. In a study that is within subjects, each subject participates in all experimental conditions and the independent variable is something other than the identity of the particular subject.

Task Paradigms

After developing a specific research hypothesis, an investigator determines an appropriate **task paradigm**, a strategy for presenting stimuli to subjects during an experiment. These paradigms are usually divided into two major categories: **blocked designs** or **event-related designs**.

In a blocked design, a subject is presented with two or more categories of stimuli that alternate every 1–2 min. For example, consider an experiment that features two categories of stimuli, X and Y. Category X might represent something like “smiling faces,” while category Y could represent “neutral faces.” In the first block of stimuli, the subject is presented with different images made up entirely of category X: X1, X2, X3, X4, and so on. A typical block may last between 10 and 60 s, with each individual stimulus presented from 1 to 10 s. After the end of the block, there may or may not be a brief period when no stimuli are presented. Then, a second block of stimuli are presented made up entirely of category Y: Y1, Y2, Y3, Y4, and so on. These blocks of stimuli are repeated in cycles, alternating X and Y, until a sufficient amount of data is collected from a subject. Some blocked designs contain a third category, alternating between blocks of X, Y, and Z.

Blocked designs are useful for obtaining relatively high signal-to-noise information from active brain regions. Because stimuli of the same experimental category are presented repeatedly for 1–2 min, the hemodynamic BOLD response has sufficient time to increase and a strong signal is produced. These paradigms are often chosen for experiments involving the determination of which brain regions are active for a given stimulus or task. They are also good for neural processes that last a relatively long time, such as changes in cognitive or emotional state. A blocked design is not as useful for examining a change in brain activity during relatively short events that last 2–5 s, such as when a subject is required to make a decision inside a scanner.

In an event-related design, stimuli are presented as isolated, individual events of short duration. This paradigm is useful for discrete tasks in which a subject

must recognize an event, detect a novel stimulus, or make a decision, all processes that take place in short time intervals. The hemodynamic properties of the BOLD effect may return to baseline between events, or for fast-paced stimuli, the BOLD response may not return to baseline and so the effects of two stimuli on neural activity can be examined within a small time window. For example, stimulus A may indicate the nature of stimulus B, and the BOLD signal can be measured for B with and without the presence of A. Event-related designs are not usually used in experiments that affect a subject's emotional state over time, as these states usually last over relatively longer time periods.

Blocked designs and event-related designs are not mutually exclusive, and indeed many studies take advantage of both designs and employ what is referred to as a **mixed design**. The relative advantages and disadvantages of all three task paradigms are compared in [Table 1.2](#).

Pilot Experiments

Because MRI scanner time is so expensive, it is often necessary to test the delivery of stimuli or characterize the behavior of subjects during a task *before* any imaging experiments take place. These pilot experiments take place outside of the MRI facility and demonstrate the efficacy of stimulus delivery in producing an appropriate human response. Statistical analysis of how subjects

TABLE 1.2 Relative Advantages and Disadvantages of Different fMRI Task Paradigms.

| | Advantages | Disadvantages |
|---------------|--|--|
| Blocked | <ul style="list-style-type: none"> • More statistical power for detecting subtle differences across different conditions • Tend to be simpler to implement and analyze • Good for examining state changes | <ul style="list-style-type: none"> • Grouping and predictability of stimuli may confound results • Information about the time course of the activation response is lost within a block • Not applicable to certain types of tasks (e.g., novelty) |
| Event related | <ul style="list-style-type: none"> • Reduces confounds of predictable stimulus order, since stimuli can be presented randomly • Can sort trials after the experiment according to specific behavioral outcomes • Useful for examining temporal characteristics of responses • Flexible analysis strategies | <ul style="list-style-type: none"> • More complex design and analyses than blocked design • Lower signal-to-noise ratio than block designs • Must perform more scans to compensate for loss in statistical power |
| Mixed | <ul style="list-style-type: none"> • Can compare short-term transient activity with long-term sustained activity | <ul style="list-style-type: none"> • Most complicated analyses |

perform their tasks is sometimes necessary to gain approval before the actual experiments take place in the scanner. Only after the investigator characterizes a cognitive task in terms of the efficacy of the stimuli and behavior of human subjects will it be appropriate to begin to associate neural correlates with that behavior.

Planning an Experiment that Uses Human Subjects

Once an experiment is designed, a lab must be given permission to use humans as research subjects by an **Institutional Review Board (IRB)**. An IRB is a committee of about 10 individuals, typically composed of physicians and research faculty, as well as nonfaculty members such as a nurse, minister, graduate student, or lawyer. The purpose of the IRB is to review studies that use human subjects and determine if the study meets ethical, safety, and scientific obligations. An IRB typically meets anywhere from 2 to 12 times a year to review and vote on the submitted applications. Additionally, any research project involving human subjects must meet the standards of the Health Insurance Portability and Accountability Act's (HIPAA) "Privacy Rule," which regulates the protection, security, and confidentiality of private health information.

Conducting the Experiment

After an experiment is designed, discussed, and approved, a scientist proceeds with recruiting human subjects and collecting data in the scanner. These scientists must be familiar with the scanner technology and experimental procedures before any actual data collection begins. The difficulty in recruiting human subjects and the high cost of scanner time makes it essential that experiments are conducted properly and without any mistakes.

Working With Human Subjects

Though the exact number varies based on the experimental design, usually about 10–20 subjects are necessary for a typical imaging experiment to reach an appropriate, statistically significant conclusion. Recruiting human subjects can be relatively easy or difficult depending on the type of subjects needed. If the only requirement for a subject is that they are an average, healthy adult, then recruiting subjects can be relatively straightforward. At academic institutions, undergraduates and graduate students are often targeted for recruitment, with compensation given in the form of cash payment or extra credit for a course. When subjects must represent a particular population of individuals (e.g., individuals with posttraumatic stress disorder, elderly subjects, or individuals diagnosed with depression), it can be much harder to recruit a necessary number of subjects. Consider a study in which the hypothesis is that depressed individuals have a significantly different level of activity in a particular brain region than nondepressed individuals. In this case,

the investigator must find individuals who have been formally diagnosed with depression. Additionally, because psychotropic drugs can confound the results, it is necessary that none of these subjects received medication after their diagnosis. Therefore, the investigator must identify 10–20 subjects who have been diagnosed with depression but chose not to undergo pharmacological treatment (or who have not yet started treatment). In studies like this, recruiting subjects can be the bottleneck in the time it takes to complete an experiment. Investigators might need to formally recruit individuals from other institutions, such as nursing homes, hospitals, or secondary schools. It might be necessary to formally collaborate with clinical neurologists to recruit patients with various mental health disorders.

Once a subject is recruited, the investigator schedules a specific session time in the scanner. Prior to the start of an experiment, the investigator must inform the subject about the nature of the experiment (although details of the experiment can be withheld for experimental purposes). The subject must sign a release form to indicate that they are a willing participant in the study and that any data gathered can be used for publication. Finally, the subject must complete a brief survey about his or her personal health to ensure that there are no confounding variables such as history of mental health disease, cardiovascular disorders, or heavy use of alcohol or other drugs. The subject is also requested to remove any metal objects before entering the scanner, such as loose change, belt buckles, earrings, etc. Often the investigators ask the subjects to change into a disposable gown to ensure that there are no metal objects placed inside the scanner.

During the experiment, the investigator must ensure that the subject is as comfortable as possible inside the scanner. To reduce head movement, the subject's head is surrounded by soft padding to hold it firmly in place inside a head coil. The investigator may provide the subject with a squeeze ball that, when pressed, can stop the scans at any time if the subject becomes uncomfortable. For experiments that require the subject to make a choice or respond to a stimulus, the investigator also provides a nonmetallic handheld device with buttons that the subject can press to provide feedback. Visual stimuli can be projected through goggles or to a mirror inside the scanner to allow for video presentations from a nonmetallic surface.

After the experiment is complete, an investigator usually debriefs the subject about the experiment and compensates the subject for their time. Depending on the experiment, follow-up sessions can be necessary and these are also scheduled well in advance.

Data Acquisition

In a typical experiment, it is common for about 10–20 human subjects to participate. Each subject usually participates in 1–3 **sessions**, the scheduled time when an actual experiment is conducted. Each session contains many **runs** in which the brain is repeatedly scanned and the hypothesis is tested.

Within each run, functional data is accumulated and stored as **volumes**—three-dimensional constructions of brain space. A complete volume is typically acquired every 1–3 s. Each volume contains a complete set of **slices**, two-dimensional representations of a plane of the brain that are only one voxel thick. A **voxel** is the smallest functional unit of brain space that can be analyzed for changes in signal intensity over time. In fMRI, a voxel is usually 1–5 mm³. Remember that a voxel of fMRI data is essentially four dimensional: three dimensions are the locations of the voxel in space (*x*, *y*, *z*) and the fourth dimension is the time in which the signal intensity was recorded. Thus, an fMRI experiment generates huge quantities of data: 10–20 subjects, each with 1–3 sessions, each with several experimental runs, each with several volumes of brain space, each with several brain slices, each with a grid of voxels that represent the data! Therefore, computers with ample storage space are required to record and store data.

Manipulating Neural Activity During an Experiment

One of the limitations of studying the human nervous system is that it is very difficult or even impossible to functionally perturb the brain during an experiment. For example, if activity in a specific part of the brain is correlated with performance on a task, it would be interesting to determine if a loss of activity in that part of the brain caused a deficit in the ability of the subject to perform the task. Likewise, stimulating neural activity in a particular brain region might be hypothesized to improve performance on a task. Such **loss-of-function** or **gain-of-function** experiments could be performed in model organisms using a variety of methods (see [Chapter 8](#)), but are relatively difficult to achieve in noninvasive imaging experiments in humans.

Two technologies make it possible to noninvasively and reversibly modulate neural activity in humans. **Transcranial magnetic stimulation (TMS)** reversibly activates or inactivates regions of the human brain. In this technique, a coil that generates magnetic field pulses is positioned near a subject's head ([Fig. 1.18](#)). The changing magnetic fields induce weak electric currents at a specific focal point on the surface of the brain. Depending on the brain region and the strength of the magnetic field impulse, the electrical activity may either stimulate neural activity or cause a hyperpolarized state in which neural activity is temporarily inactivated. Therefore, it is theoretically possible to perform a loss-of-function or gain-of-function experiment using this technology. One of the limitations of TMS is that it can only induce electrical activity on the outer surface of the brain—it is not possible to stimulate or inactivate deep brain structures that may be of interest to an investigator. However, there are thousands of publications that have used TMS to selectively activate and inactivate activity in the human cerebral cortex. This technology can also potentially be used for therapeutic treatment of diseases and is being heavily researched in the treatment of depression and motor diseases.

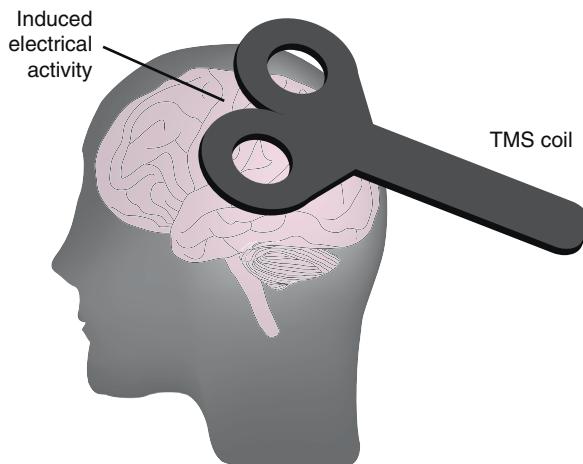


FIGURE 1.18 Transcranial magnetic stimulation. A coil is held near a person’s head, and a magnetic field stimulates electrical activity in a superficial brain region. Depending on the strength of the magnetic field and the brain region, this electrical activity can cause either a temporary loss-of-function or gain-of-function in that brain region.

Another approach, **ultrasonic neuromodulation (USNM)**, reversibly activates brain regions of interest through the delivery of transcranial ultrasound waves. These waves subtly increase the temperature of the brain, causing a transient increase in neural activity at a greater spatial resolution than TMS technologies.

An additional strategy for investigators to perform loss-of-function experiments is to attempt to find patients with lesions (caused by an accident or stroke) in specific brain regions. Because many institutions that utilize fMRI scanners are associated with hospitals, identifying and recruiting patients with lesions can be coordinated between researchers and neurologists. However, these patients may present lesions that are not tightly localized to the brain area of interest, there may be compensatory mechanisms that develop over time, and it may be more difficult for these patients to perform specific tasks. All of these caveats must be considered before including these patients in experiments, as well as when evaluating these experiments in the literature.

Postexperimental Data Analysis

The end result of most fMRI experiments is a colorful figure depicting the activation or inactivation of a particular region of the brain that is correlated with a stimulus or task. These figures may seem deceptively simple to produce, yet require rigorous methods of data analysis and interpretation.

Data Analysis

What does an investigator do with the huge quantity of data acquired from multiple research subjects? In most analyses, the data from each subject is analyzed

separately first and then pooled together into a common subject pool. Because there is variability in every subject's brain structure, data must first be fit into a common three-dimensional brain template. Structural data from each individual's brain is therefore stretched and warped to fit specific anatomical landmarks in a precise mapping system. One of the most widely used coordinate systems for normalizing fMRI data is called **Talairach space**, based on the stereotaxic measurements of a single postmortem brain. This coordinate system is ubiquitous in brain imaging research as it defines brain regions and **Brodmann's areas** into stereotaxic coordinates, allowing investigators to make anatomical comparisons among different brains. Another commonly used coordinate system is the **MNI template** (MNI stands for Montreal Neurological Institute, the institution that established this system). This template is a probabilistic mapping system based on the averages of hundreds of individual brain scans and scaled to match the landmarks within the Talairach atlas.

After an individual subject's brain is adjusted to either the Talairach or MNI templates, signal intensity is compared at different time points according to the task paradigm. There are two general strategies of analyzing signal intensity in the brain: **voxelwise analysis** or **region-of-interest (ROI)** analysis. Most fMRI studies present data using voxelwise analysis, in which each voxel is analyzed for significant differences in signal intensity in time between two experimental conditions. In ROI analysis, the brain is divided into a set of discrete regions assigned by the investigator. Rather than voxel-by-voxel comparisons, entire brain regions are compared for significant differences in signal intensity. ROI analysis can provide information about specific structures, but can initially be more time consuming, as each voxel has to be assigned to a specific ROI. Sometimes an investigator will first analyze data using a voxelwise analysis and then analyze discrete ROIs.

Each individual's fMRI data is analyzed for significant differences, and then these data are pooled together for all the individuals within a certain subject pool. For a within-subjects study, the subject pool is the same and the investigator determines if there is a significant difference in signal intensity between two different stimuli or tasks. For a between-subjects study, the subject pools are different and the investigator determines if there is a significant difference in signal intensity between the two groups for a stimulus or task. Following this data analysis, the statistically significant differences in the pooled subject data are presented as figures for publication.

Preparation of Figures

The most common functional imaging figures presented in the literature take the form of color-coded brain activation data superimposed on a structural image of a brain slice (Fig. 1.19). As mentioned previously, the brain slice is almost always a T1-weighted image because these images present the greatest contrast between brain regions. These brain slices can be taken from either one

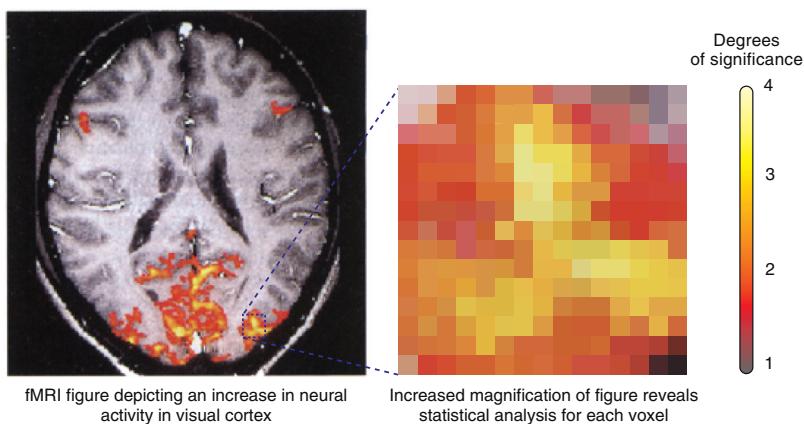


FIGURE 1.19 Preparation of an fMRI figure for publication. A typical fMRI figure contains a representation of the changes of BOLD signal intensity over time superimposed over a T1-weighted structural image. It is important to remember that the colored representation of signal intensity is essentially a grid of numbers, with each number representing a measure of statistical significance. *fMRI image reprinted with permission of Springer Science + Business Media and Jens Frahm from Windhorst, U., Johansson, H. (Eds.), 1999. Modern Techniques in Neuroscience Research, Ch. 38: Magnetic Resonance Imaging of Human Brain Function, 1064, Fig. 5.*

of the subjects during the experiment or from a stock set of images from the Talairach or MNI templates. Sometimes, investigators superimpose imaging data on an “inflated brain” in which the **sulci** and **gyri** of the brain are expanded into a balloon-like shape to better show neural activity in the sulci (Fig. 1.20).



FIGURE 1.20 An inflated representation of the right hemisphere of the human brain used to show activity within sulci. The dark gray regions represent the exposed sulci of the inflated brain, while the light gray regions represent gyri. *Courtesy of Dr. Rory Sayres.*

It is important to recognize that these fMRI figures are statistical comparisons in signal intensity over time for a grid of voxels, and that color-coded activation does not just “light up” during a scanning session. The color-coded voxels superimposed over the structural brain image are essentially a grid of numbers (Fig. 1.19). Each voxel is assigned a statistical value that represents the difference in signal intensity for that region of brain space between two conditions. The exact color scheme is chosen by the investigator to reflect the statistical magnitude of signal intensity: usually bright colors represent high differences in intensity, while darker colors represent more subtle differences in intensity.

CONCLUSION

The goal of this chapter was to provide a thorough introduction to the technologies that currently exist to image the structure and function of the brain, as well as the approaches an investigator may take when designing and analyzing functional imaging experiments (Box 1.2). These technologies are more

BOX 1.2 Walkthrough of an fMRI Experiment

Suppose you work in a laboratory that uses fMRI to study cognition in humans. Your research group becomes interested in the neural basis of how a person recognizes and identifies familiar people. One way to frame this question is “Are there specific brain regions that are active in response to familiar individuals versus nonfamiliar individuals?” How would you go about designing an fMRI study to answer this question?

A good starting point would be to identify the independent and dependent variables in this experiment. The independent variable, the variable that varies from trial to trial, is a person who is familiar or unfamiliar to the subject. This immediately leads to another decision to make as an investigator: the choice of stimuli. Will the stimulus be a picture of an individual? The sound of the individual’s voice? The individual’s name? In this example, let’s make the independent variable a person’s name that is visually displayed on a screen. Therefore, in each trial, the subject will see a name on a screen. Because the independent variable is the stimulus and not the identity of the subject, this is a “within subjects” study.

The dependent variable is the change in BOLD signal intensity in the brain. We might hypothesize that a specific brain region will show a significant difference in signal intensity between the two conditions, but in this experiment such a specific hypothesis is unnecessary, as we can scan major regions of the brain (if not the entire brain) to identify all regions that significantly differ between the two stimulus conditions.

What will be the subject’s task during the experiment? If all we ask the subjects to do is stare at the screen, they may become bored, distracted, and even fall asleep. Therefore, we must provide the subjects with a specific task so that they

Continued

BOX 1.2 Walkthrough of an fMRI Experiment—cont'd

fully attend to the stimuli. In this example, a good task may be to indicate whether the name on the screen is traditionally male or female. Each subject could be presented with a handheld device with two buttons, one for male and one for female. At the end of the imaging session, we can measure the accuracy of the responses to ensure that the subject was fully attentive throughout the experiment.

Obviously, we will need to generate a list of names for both the “familiar” and “unfamiliar” categories of stimuli. Each list should contain a balanced list of males and females, old and young, and so forth, to ensure that there are no confounding variables in the study. Familiar names could include famous celebrities and politicians, while the unfamiliar names could include combinations of first and last names that are not well-established celebrities. How can we ensure that the subject knows all of the “familiar” names and none of the “unfamiliar” names? This is impossible to confirm before the experiment begins, but after the experiment is over, each subject could complete a brief survey in which they indicate the level of familiarity of each name, perhaps on a scale of 1–10. This survey can then be utilized when analyzing the functional imaging data to ensure that each stimulus is adequately categorized.

The next major decision will be the best task paradigm to present stimuli to subjects. A blocked design will allow us to present multiple stimuli of the same category (familiar or unfamiliar names) to a subject over a 1- or 2-min period, a condition that may allow for maximal signal intensity in active brain regions. However, we may wish to evaluate the degree of signal intensity for each name presented to the subject. In this case, we could pursue an event-related design, which will allow us to correlate the degree of signal intensity with the degree of familiarity with a famous name, as indicated by the subject on a survey after the experiment is over.

Once the IRB approves the study and our proposal to use human subjects, we are ready to reserve scanner time and start collecting data! We will need 10–20 subjects for this experiment, but we should recruit at least 20–40 because some of the data will need to be discarded. For example, some subjects may fail to show up, some may fall asleep in the scanner, or some may turn out to have medical conditions or treatments that may preclude them from participation in the study. To recruit subjects, the lab could offer \$25/hour for their time. After the imaging session ends, we will provide them with the familiarity survey and then start to analyze the data.

For each subject, we morph the structural coordinates from their brain scans to match a normalized template system, such as the MNI template. Then we can proceed with a voxelwise comparison in signal intensity between the “familiar name” trials and the “unfamiliar name” trials. Individual brains can then be pooled across all subjects for each condition. The final figure will be the difference in signal intensity between the two conditions, voxel-for-voxel, superimposed on a T1 image from the MNI template. Because the template contains coordinates for discrete brain regions, we can interpret the specific regions activated in response to familiar or unfamiliar names.

complicated than the brief descriptions found here, and readers interested in more detailed explanations should consult the Suggested Readings section below. However, this survey of techniques hopefully provides an appreciation for the ingenuity of scientists and engineers in developing and improving these techniques over the past few decades. Imaging neural function can be performed based on the electrical, magnetic, metabolic, and light-scattering properties of neural tissue. Producing so many different methods of imaging the brain is a remarkable achievement, and it will be exciting to observe, either as fellow scientists or participants, how these methods are combined and improved over the next several decades.

SUGGESTED READING AND REFERENCES

Books

- Bandettini, P.A., 2020. fMRI. The MIT Press Essential Knowledge Series.
- Filippi, M. (Ed.), 2016. fMRI Techniques and Protocols. Humana Press.
- Huettel, S.A., Song, A.W., McCarthy, G., 2014. Functional Magnetic Resonance Imaging, third ed. Sinauer.
- Mori, S., Tournier, J.D., 2013. Introduction to Diffusion Tensor Imaging: and Higher Order Models. Academic Press.
- Schild, H.H., 1990. MRI Made Easy ... Well Almost. Schering AG.
- Sahakian, B.J., Gottwald, J., 2020. Sex, Lies, and Brain Scans: How fMRI Reveals what Really Goes on in Our Minds. Oxford University Press.

Review Articles

- Aguirre, G.K., 2014. Functional neuroimaging: technical, logical, and social perspectives. Hastings Cent. Rep. 44, S8–S18.
- Carmichael, O., et al., 2018. The role of fMRI in drug development. Drug Discov. Today 23, 333–348.
- Kim, S.G., 2018. Biophysics of BOLD fMRI investigated with animal models. J. Magn. Reson. 292, 82–89.
- Logothetis, N.K., 2008. What we can do and what we cannot do with fMRI. Nature 453, 869–878.
- Mezer, A., et al., 2013. Quantifying the local tissue volume and composition in individual brains with magnetic resonance imaging. Nat. Med. 19, 1667–1672.
- Mori, S., Zhang, J., 2006. Principles of diffusion tensor imaging and its applications to basic neuroscience research. Neuron 51, 527–539.
- Turk-Browne, N.B., 2013. Functional interactions as big data in the human brain. Science 342, 580–584.
- Wang, K.S., Smith, D.V., Delgao, M.R., 2016. Using fMRI to study reward processing in humans: past, present, and future. J. Neurophysiol. 115, 1664–1678.
- Watanabe, T., Sasaki, Y., Shibata, K., Kawato, M., 2017. Advances in fMRI real-time neurofeedback. Trends Cognit. Sci. 21, 997–1010.

Primary Research Articles—Interesting Examples From the Literature

- Cui, Z., et al., 2020. Individual variation in functional topography of association networks in youth. Neuron 106, 340–353.

- Dagher, A., Leyton, M., Gunn, R.N., Baker, G.B., Diksic, M., Benkelfat, C., 2006. Modeling sensitization to stimulants in humans: an [¹¹C]raclopride/positron emission tomography study in healthy men. *Arch. Gen. Psychiatr.* 63, 1386–1395.
- deCharms, R.C., et al., 2005. Control over brain activation and pain learned by using real-time functional MRI. *Proc. Natl. Acad. Sci. USA* 102, 18626–18631.
- Duff, E.P., et al., 2015. Learning to identify CNS drug action and efficacy using multistudy fMRI data. *Sci. Transl. Med.* 7, 274ra16.
- Gil-da-Costa, R., Martin, A., Lopes, M.A., Muñoz, M., Fritz, J.B., Braun, A.R., 2006. Species-specific calls activate homologs of Broca's and Wernicke's areas in the macaque. *Nat. Neurosci.* 9, 1064–1070.
- Glasser, M.F., Rilling, J.K., 2008. DTI tractography of the human brain's language pathways. *Cerebr. Cortex* 18, 2471–2482.
- Gomez, J., Barnett, M., Grill-Spector, K., 2019. Extensive childhood experience with Pokémon suggests eccentricity drives organization of visual cortex. *Nat. Hum. Behav.* 3, 611–624.
- Hariri, A.R., et al., 2002. Serotonin transporter genetic variation and the response of the human amygdala. *Science* 297, 400–403.
- Laquaitaine, S., Gardner, J.L., 2018. A switching observer for human perceptual estimation. *Neuron* 97, 462–474.
- Logothetis, N.K., Pauls, J., Augath, M., Trinath, T., Oeltermann, A., 2001. Neurophysiological investigation of the basis of the fMRI signal. *Nature* 412, 150–157.
- McClure, S.M., Li, J., Tomlin, D., Cyphert, K.S., Montague, L.M., Montague, P.R., 2004. Neural correlates of behavioral preference for culturally familiar drinks. *Neuron* 44, 379–387.
- Nishimoto, S., Vu, A.T., Naselaris, T., Benjamin, Y., Yu, B., Gallant, J.L., 2011. Reconstructing visual experiences from brain activity evoked by natural movies. *Curr. Biol.* 21, 1641–1646.
- Parks, N.A., 2013. Concurrent applications of TMS and near-infrared optical imaging: methodological considerations and potential artifacts. *Front. Hum. Neurosci.* 7, 592.
- Tsurugizawa, T., et al., 2020. Awake functional MRI detects neural circuit dysfunction in a mouse model of autism. *Sci. Adv.* 6, eaav4520.

Websites

fMRI 4 Newbies. <http://fmri4newbies.com/>.

fMRI Methods Wiki. <http://www.fmrimethods.org/>.

The Basics of MRI. <http://www.cis.rit.edu/htbooks/mri/>.

Neuroimaging Informatics Tools and Resources Clearinghouse (NITRC). <http://www.nitrc.org/>.