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H. RICHARD WINN

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SIXTH EDITION

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YOUMANS
Neurological Surgery

SIXTH EDITION

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PART 1

History

CHAPTER 1

Historical Overview of Neurosurgery

James Tait Goodrich ■ Eugene S. Flamm

In developing this chapter on the history of neurosurgery, our underlying theme is that neurosurgery advanced as surgeons obtained a better understanding of the anatomy and pathophysiology of the nervous system, a goal that continues into the present.

This concept is clearly provided in words written in 1602 by William Clowes, a leading surgeon of the Elizabethan Age. Clowes invokes a challenge that neurosurgeons must still overcome, even in the age of computerized imaging, microsurgery, and functional neurosurgery.

> *Those which are Masters and Professors chosen to performe the like operation, ought indeede to have a Lyons heart, a Ladies hand, and a Haukes eye, for that it is a worke of no small importance.*[1]

The father of medicine even further detailed this concept when he stated: *Nullum capitis vulnus contemnendum est* (No head injury should be considered trivial)—Hippocrates.[2]

NEUROSURGERY IN THE PREHISTORIC PERIOD

Neurosurgery is in many ways one of the most ancient of professions. Early man clearly recognized that to bring down the enemy, a blow to the head was the quickest means. To accomplish this goal a number of weapons, in particular, clubs, were fashioned to inflict these injuries. In a number of anthropologic collections around the world are examples of skulls with head injuries, and more remarkable are a number of skulls with successful trephinations (i.e., meaning that the patient clearly survived surgical opening of the skull) (Fig. 1-1).[3-5] In some cultures, for example, the early Peruvians, there exist many skulls that show evidence of trephinations and in most cases for reasons that remain unknown. There has been speculation that this procedure was performed for religious or medical reasons, but because of the lack of any documentation, we have no understanding today why these procedures were done. Figure 1-1 illustrates examples of trephinated skulls from the early Chimu culture in Peru.

ANCIENT EGYPTIAN NEUROSURGERY

The earliest known written documents that relate to early neurosurgery date from the ancient Egyptian period. This period covers some 30 successive dynasties and produced the earliest known practicing physician—Imhotep (1300 BC). This period of history provided the earliest known medical and surgical material. Today, there are three existing documents available that have relevance to medicine. These papyri, which are named after their early owners, are the Ebers, Hearst, and Edwin Smith papyri.[6-8]

Examination of these Egyptian papyri reveals a practice of medicine that was based largely on magic and superstition. Medical and surgical treatment relied on simple principles, most of which allowed nature to provide restoration of health with little intervention and mostly observation with simple medicants. The Egyptians realized that immobilization for a neck or back injury was important to reduce further injury. They commonly prescribed and applied splints for treatments.

The oldest medical text dates from this early civilization and was written some 500 years after Hammurabi. This text, now called the *Ebers papyrus*, includes 107 pages of hieratic writing and is of interest for its extensive discussions of contemporary surgical practice. Included are discussions of the removal of tumors, as well as recommendations for the surgical drainage of abscesses.[6]

The oldest work that deals extensively with surgical techniques is the Edwin Smith papyrus. This work appears to have been intended to be a surgical textbook. It was originally written sometime after 1700 BC in the time of the New Kingdom. This papyrus scroll is 15 ft in length and 1 ft in width. The surviving text consists solely of a list of 48 cases, including those dealing with injuries to the spine and cranium. The format is such that each case is discussed with a diagnosis, followed by a formulated prognosis. As a result of the scholarly research of James Breasted, this papyrus has now been translated from the original hieroglyphics. The original document is currently in the possession of the New York Academy of Medicine.[8,9] We provide two cases to show the insight and some of the techniques illustrated in this early historical papyrus.

Case Two

Title: Instructions concerning a gaping wound in his head, penetrating to the bone.

Examination: If thou examinest a man having a gaping wound in his head, penetrating to the bone, thou shouldst lay thy hand upon it and thou shouldst palpate his wound. If thou findest his skull uninjured, not having a perforation in it.

Diagnosis: Thou shouldst say regarding him: "One having a gaping wound in his head. An ailment which I will treat."

Treatment: Thou shouldst bind fresh meat upon it the first day; thou shouldst apply for him two strips of linen, and treat afterward with grease, honey, and lint every day until he recovers.

Gloss: As for: "Two strips of linen," it means two bands of linen which one applies upon the two lips of the gaping wound in order to cause that one join to the other.

FIGURE 1-1 Two trephined skulls with well-healed trephinations. The skull on the *right* has a right parietal healed trephination. The skull on the *left* has a well-healed frontal bur hole placed just lateral to the frontal sinus. Between the skulls are three examples of handheld Peruvian tumis, all dating from the period 800 to 1100 AD. These bronze and copper tools were typically used to perform trephinations.*

Case Five

Title: Instructions concerning a gaping wound in his head, smashing his skull.

Examination: If thou examinest a man having a gaping wound in his head, penetrating to the bone, and smashing his skull; thou shouldst palpate his wound. Shouldst thou find that smash which is in his skull deep and sunken under thy fingers, while the swelling which is over it protrudes, he discharges blood from both his nostrils and both his ears, and he suffers with stiffness in his neck, so that he is unable to look at his two shoulders and his breast.

Diagnosis: Thou shouldst say regarding him: "One having a gaping wound in his head, penetrating to the bone, and smashing his skull, while he suffers with stiffness in his neck. An ailment not to be treated."

Treatment: Thou shalt not bind him but moor him at his mooring stakes, until the period of his injury passes by.

Other than the isolated cases found in these remaining papyri, little can be gleaned from them on the actual practice of neurosurgery.[9] It is evident from these writings that the Egyptian physician recognized head injury and would elevate a skull fracture if necessary. At the same time, if the injury appeared too be too severe, no treatment was advocated. The early "neurosurgeon" had to wait until the development of the Greek schools of medicine, for it was then that the management and codification of head injury were first formulized.

CLASSICAL PERIOD—GREEK AND BYZANTINE NEUROSURGERY

Hippocratic School

The intellectual evolution of neurological surgery originated in the golden age of Greece with the founding of the Alexandrian school in 300 BC. For the first time, open anatomic dissection was incorporated into formal lectures,[10] and the concept of a surgeon performing surgery on the head and spine became formalized. Because of both sporting injuries, in particular, gladiator injuries, and wars, head injuries appear to have been plentiful and provided opportunities to develop the early skills of neurosurgery.

*Unless otherwise noted, all figures are courtesy the author, JTC.

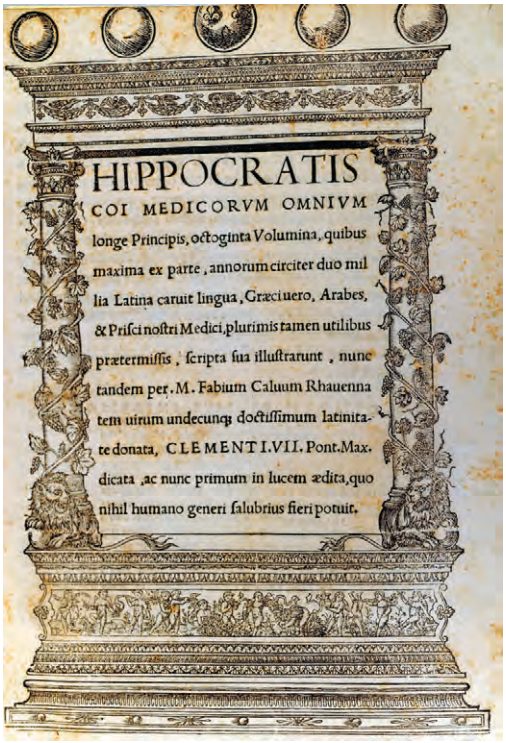

HIPPOCRATIS
COI MEDICORVM OMNIVM
longe Principis, octoginta Volumina, quibus
maxima ex parte, annorum circiter duo mil
lia Latina caruit lingua, Græci uero, Arabes,
& Prisci nostri Medici, plurimis tamen utilibus
prætermissis, scripta sua illustrarunt, nunc
tandem per. M. Fabium Caluum Rhauenna
tem uirum undecunq; doctissimum latinita-
te donata, CLEMENTI. VII. Pont. Max.
dicata, ac nunc primum in lucem ædita, quo
nihil humano generi salubrius fieri potuit.

FIGURE 1-2 Title page from the first printed edition of the writings of Hippocrates—1525. *(From Panourias JG, Skiadas PK, Saklas DE, et al. Hippocrates: a pioneer in the treatment of head injuries.* Neurosurgery. *2005;37:181-189.)*

The earliest medical writings from this period are those of Hippocrates (460-370 BC), the most celebrated of the Asclepiadeans (Fig. 1-2).[11-13] Classical philologists think that many of the writings attributed to Hippocrates, however, were composed by members of the Hippocratic school. The Hippocratic collection presents clinical cases based mainly on observation, and in most cases only the simplest of theories are offered. There are a number of neurological cases within the Hippocratic *corpus*. In reviewing these cases one finds a rather sophisticated understanding of head injury. Hippocrates was the first to describe a number of neurological injuries, most resulting from battlefield injuries. The vulnerability of the brain to injury was categorized by location, from lesser to greater, with injury to the *bregma* being represented as a higher risk than one to the temporal region, which in turn was more dangerous than an injury to the occipital region.[14-16] Hippocrates devoted a full chapter to injuries of the head, *De Capitis Vulneribus*, a work that deals with the diagnosis and management of head injuries. The work divides head injuries into five categories based on details of the skull fracture and is the first systematic work devoted to head injuries. Five types of fractures are described: linear fracture, contusion, depressed fracture, hedra (or dent) occurring with and without fracture, and contrecoup fracture.[14] There is nothing to suggest that the neurological condition of the patient had any bearing on the surgical indications. Surgery was advised according to the type of fracture. The greater the injury to the skull, the less the need for trephination. The technical aspects of the process of trephination are presented in a curious mixture of sound advice and incomprehensible admonitions.

These Hippocratic writings contain numerous anatomic descriptions, even though human dissection appeared to not be routinely practiced. The Greeks were also without an anatomic vocabulary, which was not introduced until Galen standardized

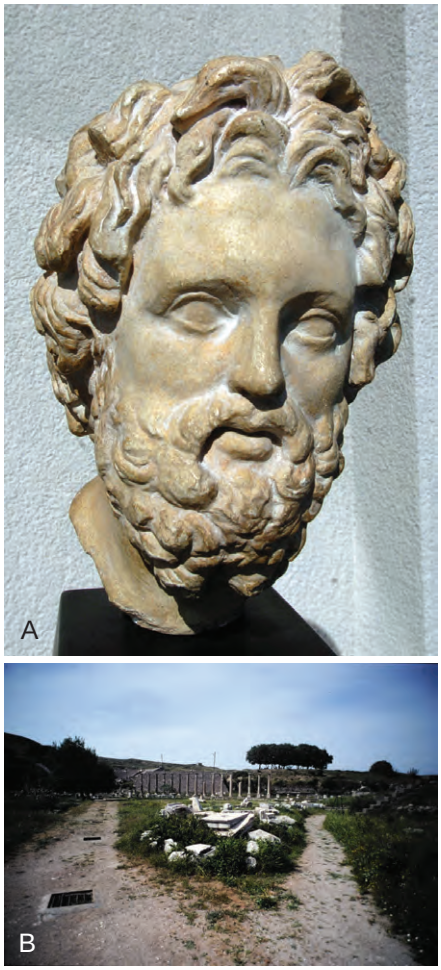


FIGURE 1-3 **A,** Bust of Aesculapius. **B,** The Temple of Aesculapius with the temple in the background. The gate to the bottom left is a "speaking hole" through which the temple priests would communicate to the sick patients in the underground corridors.

use of the Latin language in medicine. These deficiencies retarded the development of standardized anatomic procedures and the practice of surgery. Despite these drawbacks, there are within the Hippocratic writings a number of interesting neurological case studies that reflect a view of the early practice of neurosurgery (Fig. 1-3).

One of earliest descriptions of subarachnoid hemorrhage appears in the *Aphorisms*:

> *When persons in good health are suddenly seized with pains in the head, and straightway are laid down speechless, and breathe with stertor, they die in seven days, unless fever comes on.*[13,17]

Hippocrates and his followers also warned against incising the brain because convulsions can occur on the opposite side and make the prognosis especially serious. Hippocrates advised against making an incision over the temporal artery because this could also lead to contralateral convulsions. In reviewing the writings of the Hippocratic school we find the simple concepts of cerebral localization demonstrated. Also well understood was the concept of a potential critical prognosis in head injury and that sometimes it was best not to operate. In this early era of medicine, the risk for infection, lack of antiseptic technique, and minimal anesthesia prevented generations of surgeons from performing any serious or aggressive surgical intervention for head injury.

Herophilus of Chalcedon (ca. 300 BC)

From the region of the Bosporus, among the crowded schools of Alexandria, came Herophilus, a pupil of Praxagoras and Chrisippus and a member of the educated dynasty of Ptolemy. According to his writing, Herophilus performed dissection on humans, not on animals as was the common practice then.[18-20] Herophilus and Galen later were key figures in the task of developing an anatomic nomenclature and in forming a much needed language of anatomy. In examining the nervous system, Herophilus' neurological contributions included anatomically following the origin of nerves to the spinal cord. He was the first to recognize the difference in motor and sensory tracts. He made a further important differentiation between nerves and tendons, thereby correcting a common earlier error. Herophilus was the first to detail the anatomy of the brain ventricles and venous sinuses. The description of the "confluens of the sinuses," or *torcular Herophili* (torcula means wine press), comes from his early investigations. The first description and naming of the choroid plexus (*plexus choroides)* come from Herophilus, and it was so named because of its resemblance to the vascular membrane of the fetus. He gave the first detailed account of the fourth ventricle and of that peculiar arrangement at its base, which he called the *calamus scriptorius* because it "resembles a pen in writing"—Αναγλυφη τχ χαλαμχ. Among his many other contributions was recognition of the brain as the central organ of the nervous system and the seat of intelligence.[19]

Herophilus was not without errors in his writings. Perhaps the worst was his introduction into the anatomic literature of one of the longest-standing errors—that of the *rete mirabile*, a structure present in ungulates but notably lacking in higher primates. In ungulates this structure acts as an anastomotic network at the base of the brain, a structure that the Greeks incorporated into the early physiologic theories of brain function.[21] The rete mirabile was later elaborated in further detail by Galen of Pergamum and codified by Islamic and medieval writers. This anatomic error remained firmly embedded in the anatomic literature until the 16th century, when it finally became challenged in the accounts of Andreas Vesalius (1514-1564) and Giacomo Berengario da Carpi (1460-1530).[22-24] Both these writers, who performed their own human dissections, clearly recognized that the rete mirabile did not exist in humans. It is possible that the human cavernous sinus confused the early writers and they in turn thought that this represented the rete mirabile.

Aulus Aurelius Cornelius Celsus (25 BC–50 AD)

Celsus (Fig. 1-4A) was neither a physician nor a surgeon, but rather an intellectual patrician and a medical encyclopedist who attempted to compile all the important writings of his time. His writings had an important early influence on medicine and surgery. As counselor to the emperors Tiberius and Caligula, Celsus was held in great esteem. His book on medicine titled *De Medicina*[25,26] (Fig. 1-4B) is now considered one of the most important early medical documents after the Hippocratic writings. As a result of this work originally being lost, Celsus was one of the few major authors not transcribed by the Islamic/Arabic writers. This changed in 1443, when an early Celsus manuscript was uncovered by Thomas Sarazanne (later Pope Nicolas V) and reintroduced to the medical community. With the introduction of movable type, Celsus' work became the first medical manuscript to be printed (1478), even preceding the writings of Hippocrates and Galen. In Book 4, Chapter 10, we find his classic description of inflammation: *notae vero inflammationes sunt quattuor*; *rubor*; *et tumor*; *cum calore et dolore*.

Celsus made a number of interesting early observations related to neurosurgery. Book VIII, Chapter 4 contains one of the earliest descriptions of an epidural hematoma resulting from a

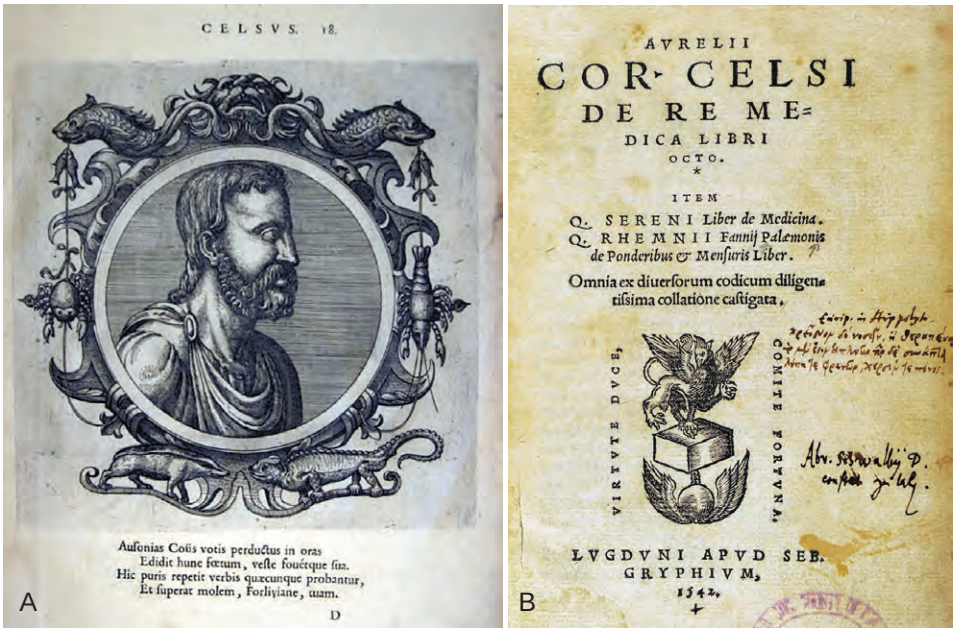


FIGURE 1-4 **A,** Portrait of Celsus. **B,** Title page from a 1542 edition of Celsus' writings.

ruptured middle meningeal artery.[25] In head-injured patients, Celsus recommended that the surgeon always operate on the side of greater pain. The trephine should always be placed at the point where the pain is best localized. Celsus described a technique for a craniotomy that involved drilling a number of holes and then connecting them with a hammer and chisel. The chisel had a protective blade that separated the dura from the bone and prevented injury to it during the surgical dissection. However, he regarded the operation of trephination as the *ultimum refugium*, to be used only when all conservative measures had been exhausted.

Several interesting neurological conditions are described in *De Medicina*, including accurate descriptions of hydrocephalus and facial neuralgia. Following earlier writings, Celsus clearly recognized that a high cervical spine fracture could lead to vomiting and difficulty breathing. Injury to the lower lumbar spine could cause weakness or paralysis of the legs, as well as urinary retention or incontinence.

Galen of Pergamum (129-200 AD)

Galen of Pergamum, whose name comes from "galenos," meaning calm or peaceful, is remembered as a powerful personality, an original investigator, and a leading proponent of the doctrines of the Hippocratic and Alexandrian schools (Fig. 1-5). Galen began his writing career at the age of 13 and continued to add to the literature until his death at the age of 70. In the end, his writings remained the most extensive in size, scope, and influence in early antiquity. Galen's prodigious output accounts for ⅚ of all the surviving medical writings of antiquity (Fig. 1-6).[27]

Galen's life and activity fortunately occurred during the reigns of two of the greatest Roman emperors, Antonius Pius (86-161 AD) and Marcus Aurelius (121-180 AD). Galen became the physician to the gladiators of Pergamum and as a result saw and treated many traumatic injuries. From both his surgical experiences and anatomic studies, he made contributions to the fields of neurology, neurosurgery, and neuroanatomy. In his writings Galen differentiated between the pia mater and the dura mater and gave one of the earliest accurate descriptions of the corpus callosum, the ventricular system, the pineal and pituitary glands, the infundibulum, and what we now call the aqueduct of Sylvius. Nearly 1600 years before the Scottish anatomist Alexander Monro (Secundus) (1733-1817), Galen described the structure now called the *foramen of Monro*. He performed a number of anatomic experiments, including early studies on the effects of transection of the spinal cord.[28,29] From these studies Galen was able to describe the specific loss of function below the level of

FIGURE 1-5 Portrait of Galen.

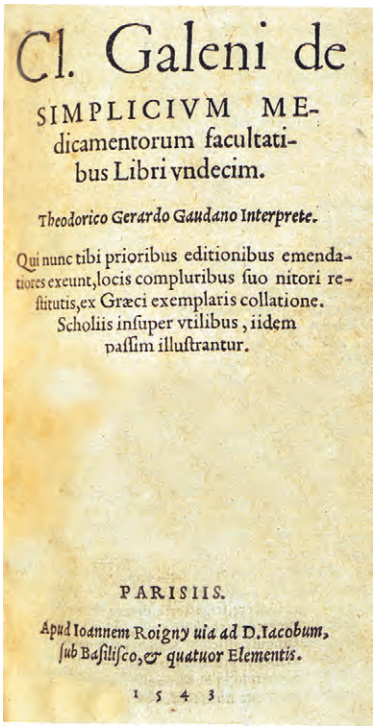

Cl. Galeni de
SIMPLICIVM ME-
dicamentorum facultati-
bus Libri vndecim.
Theodorico Gerardo Gaudano Interprete.
Qui nunc tibi prioribus editionibus emenda-
tiores exeunt, locis compluribus suo nitori re-
stitutis, ex Græci exemplaris collatione.
Scholiis insuper vtilibus, iidem
passim illustrantur.
PARISIIS.
Apud Ioannem Roigny via ad D. Iacobum,
sub Basilisco, & quatuor Elementis.
1543

FIGURE 1-6 Title page from the collected works of Galen. (*From Galen of Pergamum.* Omnia Quae Extant Opera in Latinum Sermonem Conversa (Quinta ed). *Venice: Apud Juntas; 1576-1577.)*

transection. In a now classic experiment he sectioned the recurrent laryngeal nerve in the dog and found that hoarseness occurred (discussed further in Chapter 7, see also *De Usu Partium*, Book VII, Chapters 11 to 18).[28] Galen was the first to provide an early classification of the cranial nerves. In his original classification he described 11 of the 12 cranial nerves; however, by combining several, he arrived at a total of only 7. In his descriptions he regarded the olfactory nerve as merely a prolongation of the brain and hence did not considered it a cranial nerve.[28] Galen published a number of interesting views on higher cortical functions and embraced the concept that the brain was responsible for intelligence, fantasy, memory, and judgment. These views were original and represented an important departure from the cardiocentric teachings of the earlier medical and philosophical schools such as Aristotle's. Galen challenged Hippocrates' view that that the brain was only a gland and instead attributed to the brain the powers of voluntary action and sensation (encephalocentric), this last being a remarkable advance in thinking for the period.

From a series of anatomic studies, Galen provided some of the earliest observations on cervical spine injury and the resulting disturbance in arm function. Further study of spinal cord injury led to his elegant description of what we now call "Brown-Séquard" syndrome (i.e., hemiplegia with contralateral sensory loss in a hemisection of the spinal cord).[29] Galen provided one of the earliest clinical descriptions of hydrocephalus and clearly recognized its poor prognosis. Using his extensive experience with head injuries, he provided innovative arguments for elevation of depressed skull fractures, fractures with hematomas, and comminuted fractures. Galen was more aggressive in his treatment by recommending the removal of bone fragments, particularly those pressing into the brain. In describing surgical techniques, he detailed a safer and more reliable use of the trephine and in particular argued for continuous irrigation during trephination to avoid delivering excessive heat and injury to the underlying brain. Galen, following or adapting earlier Hippocratic views, reiterated the concept the dura should never be violated by the trephine.

Galen was clearly a great physician, surgeon, and early innovator in medicine. Unfortunately, the historical world, in particular the Byzantine world, later accepted Galen's writings as the only true authority. As a result, Galen's writings attained the status of unchallengeable medical dogma. Thus, his work on neurology and neuroanatomy remained essentially unchallenged for the next 1500 years. For all practical purposes, relevant new investigations ceased altogether from Galen's death until the Renaissance. Some writers have pointed out that Galen was literally canonized by Arabic/Islamic writers and later by physicians of the Middle Ages. Consequently, a number of Galen's errors remained part of the anatomic literature (e.g., rete mirabile). Each of these errors or incorrect beliefs was carefully repeated and scribed by subsequent Islamic and medieval physicians and surgeons. Because of the strength of Galen's views, no one was willing to challenge what were clearly anatomic and scientific errors, all of which had a stultifying effect on science and medicine for 1500 years.

Paulus of Aegineta (Paul of Aegina, 625-690?)

Illustrated in Figure 1-7A, Paul of Aegina was a brilliant 7th century Byzantine Greek physician and surgeon who also trained in the Alexandrian school. He was an influential compiler of works in both the Latin and Greek schools; his writings, especially the *Medical Compendium in Seven Books* (Fig. 1-7B), was being consulted until well into the 17th century, with a translation in English appearing in the 19th century.[30,31] His skill as a surgeon, described in Book VI, clearly details an unusual understanding of surgical principles. His skills became contemporary legend, which brought him patients from far and wide. Although Paul venerated the teachings of the ancients as tradition required, he introduced his own techniques with good results. His classic work *The Seven Books of Paulus Aegineta* contains an excellent section on head injury and use of the trephine.[30,31] Paul of Aegina classified skull fractures into several categories: fissure, incision, expression, depression, arched fracture, and in infants, dent (what we now call a ping-pong fracture). In dealing with fractures he used an interesting skin incision: two incisions intersecting one another at right angles and forming the Greek letter χ, with one leg of the incision incorporating the scalp wound. For the comfort of the patient undergoing trephination, he stuffed the patient's ear with wool so that the noise of the trephine would not cause undue distress (see Book VI, Section XC).[31]

In a contemporary discussion on hydrocephalus, Paul of Aegina introduced the intriguing concept of traumatic birth delivery and intraventricular hemorrhage being related; it would appear that he was the first to suggest the possibility that an intraventricular hemorrhage and its "inert fluid" might actually cause hydrocephalus:

> *The hydrocephalic affection occurs in infants, owing to their heads being improperly squeezed by midwives during parturition, or from some other obscure cause; or from the rupture of a vessel or vessels, and the extravasated blood being converted into an inert fluid [see Book VI, Section III, page 250].*[31]

One of the reasons for Paul of Aegina's long-standing influence was that several of his manuscripts survived and were continuously recopied by amanuenses over the centuries. In these manuscripts are offered a number of surgical instruments that he specifically designed for neurosurgical procedures, including elevators, raspatories, and bone biters, among other tools. He also introduced trephine bits with conical styles to reduce the risk of plunging, along with different biting edges. In retrospect, it appears that he probably had better surgical outcomes because

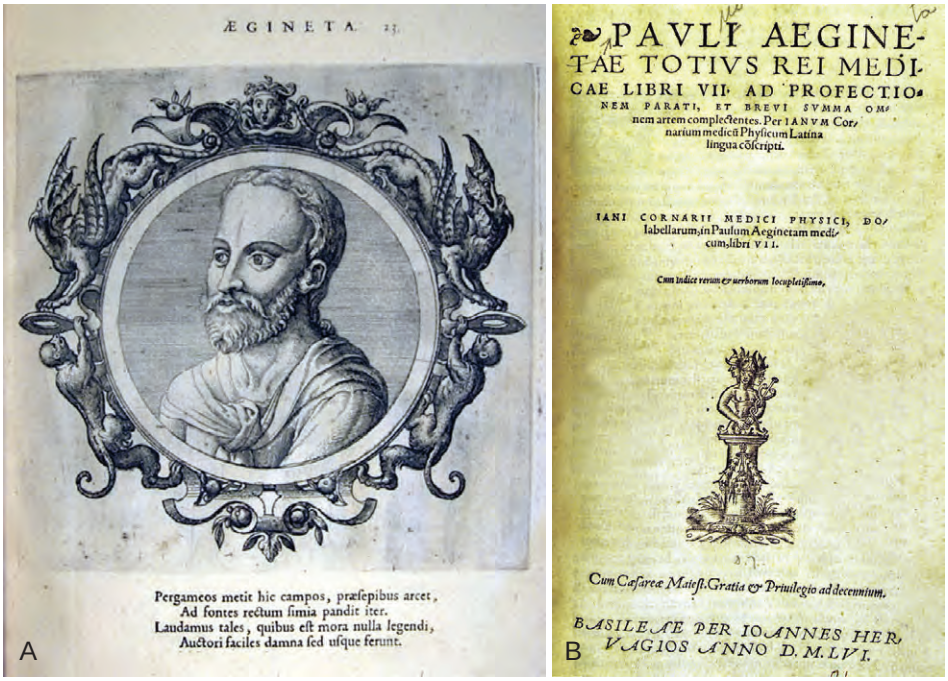


FIGURE 1-7 **A,** Portrait of Paul of Aegina. **B,** Title page of the collected works of Paul of Aegina.

of his sophisticated wound management by making use of wine-soaked dressings (helpful in antisepsis, although a concept then unknown) and by stressing that dressings should be applied with no compression on the brain itself.

The Greek and Byzantine periods proved for medicine and surgery to be eras of intense scholarship and original investigation and produced a group of physicians and surgeons who were deeply interested in better management of their patients. As we have seen, individuals such as Galen of Pergamum, Paul of Aegina, Herophilus, and members of the Hippocratic school all attempted to improve the management of head injuries and at the same time uncover the principles of brain function. Unfortunately, as we shall observe in the next section, during the prescholastic period of the Islamic and late Byzantine era, neurological investigation and the development of new surgical techniques were seriously impaired because of the scholarly ex cathedra elevation of these earlier writers. Although there were some exceptions, they were distinctly uncommon.

ISLAMIC/ARABIC MEDICINE—PRESCHOLASTIC PERIOD

After the great Greek and Roman periods of medicine, the intellectual centers of the discipline shifted to the Islamic/Arabic and Byzantine cultures. This era of influence lasted from approximately 750 AD until 1200 AD, when the medievalist era began. During this same period Europe was intellectually quiescent and unimaginative, having been overrun and ruled by barbarians (Huns, Goths, and Norsemen), individuals not known for endeavors in high scholarship. Unfortunately for neurosurgery, this was a dormant period, a dormancy that prevailed in all facets of surgery. The Islamic/Arabic schools were satisfied to codify the surviving manuscripts from the Greek and Roman period. In some rare cases remarkable insights were offered, but this was a rather rare phenomenon. However, because of their impressive zeal, the best of Greek medicine was made available to Arabic readers by the end of the 9th century and remained available into the Middle Ages. Unfortunately, rigid scholastic dogmatism characterized these learning centers. Rather than offering innovation, the "writers" became copyists of the great works of the antiquities. As a result of their efforts, we now have a surprisingly large number of manuscripts that have been translated from Latin, Greek, and Hebrew into Arabic, and because of their dedication and persistence, they systemized a body of knowledge that could easily have been lost into antiquity. Unfortunately, as copyists these writers frequently added their own "favorite" or contemporary view of the manuscript and, as a result, lost some of the originality in translation. In fairness to them, they had a critically important role in this period of civilization, claiming the only camp of learning; in Europe at this time, having been overrun by barbarians, the lamp remained unlit.

A number of contemporary writers have offered the view that it was the religious influence of the Koran that caused the absence of originality and progress in Islamic/Arabic medicine. It has often been commented on that the Koran forbade dissection; this is only partially correct. Some dissection was allowed and reported by writers of this time. However, as a practical consideration, this part of the world had a hot climate, which led to rapid putrefaction of cadavers and made anatomic dissection undesirable. The opinion of these schools was that the Greeks had already accomplished most of the anatomic studies of interest, so the Islamic student of medicine felt no need to duplicate these earlier and more superior efforts. There were rare exceptions, some of which we will discuss here.

In Islamic/Arabic medicine the concept of a physician doubling as a surgeon was rarely acceptable. The more typical practice was that the physician would confine himself to writing learnedly and assign the "menial tasks of surgery" to an individual of a lower class, typically an apprentice surgeon. As a result of this "declassification" of the surgeon to a mere plebian, the advances in surgery and anatomy developed by the great Alexandrians, among others, were ignored or lost. Fortunately, the writings of men such as Galen of Pergamum and Paul of Aegina were saved and translated into Arabic, but little new was added.

The dominant period for Islamic scholarship in medicine was from the 10th through the 12th centuries. Several medical giants

FIGURE 1-8 Image from a later copy of an Avicenna manuscript demonstrating a physician applying cautery to a patient's leg.

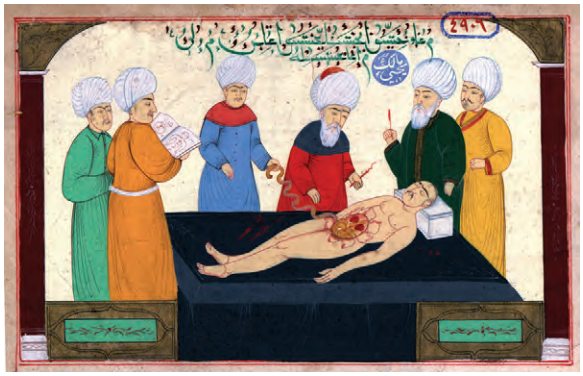


FIGURE 1-9 Unpublished manuscript leaf from a later collection of works by Avicenna showing him participating in an anatomic dissection.

rose to prominence during this period; among the most illustrious scholars were Avicenna (980-1037), Rhazes (865-925), Avenzoars (d. 1162), Albucasis (1013-1106), and Averhose (1126-1198). In reviewing the writings of these great physicians one sees an extraordinary effort to canonize the writings of their Greek and Roman predecessors. Rather than innovation, they became the guardians and academics of what now became the Hippocratic, Greek, and Galenic writings, which became dogma.

One of the most beneficial teaching methods, and a quite modern one, did arise during the Islamic/Arabic period—the concept of bedside medical care and teaching. The paucity or lack of anatomic dissection, along with the prevalent view of the practice of surgery as an occupation done only by individuals of inferior status, reduced any preoccupation with surgical art. Another unfortunate surgical practice that occurred during this period was reintroduction of the Egyptian technique of using hot cautery for control of bleeding. Hot cautery was also used in lieu of the scalpel to create a surgical incision, the results of which often proved unfortunate for the surgical patient (Fig. 1-8).

We will review some of the writings of the significant scholars of this period, starting with those of Rhazes (Abu Bakr Muhammad Ibn Zakariya, 865-925). Rhazes was a scholarly physician, learned in diagnosis and exclusively loyal to Hippocratic teachings. Rhazes developed a considerable reputation that led him to becoming a court physician. He was not a surgeon, although he wrote on surgical topics.[32] Rhazes was an early believer in the concept of "concussion" and advocated surgery for penetrating injuries of the skull in a period when surgical outcomes were almost always fatal. Rhazes believed that head injuries were among the most devastating of all injuries. In the case of skull fractures, which could be permanently damaging to the patient as a result of compression of the brain, his surgical advice would be that depressed fractures needed to be elevated.

Among the most influential physicians of this period was Avicenna (980-1037), physician and philosopher of Baghdad and known as the chief or "second doctor," the first being Aristotle (Figs. 1-9 and 1-10). Avicenna's writings and translations clearly extended the original Greek influence with a force so persuasive and durable that it remained the dominant scholarship until well into the 18th century. His greatest contribution is the detailed translation of Galen's collected works, the *Opera Omnia*. Avicenna's major work, *Canon Medicinae*, was an encyclopedic effort founded and clearly based on the writings of Galen and Hippocrates.[33] The Greek word *Canon* refers to a straight rod, a carpenter rule, or standard of measurement. Accordingly, Avicenna's work became the "rule," the codification of Galen and Greek medicine. In reviewing the *Canon*, a number of interesting neurological discussions are found. Avicenna provides an early and accurate clinical understanding of epilepsy, for which his treatment consisted of administering various medicaments and herbals, with good results. It appears that Avicenna conducted anatomic studies, although he does not discuss this directly. He gives a correct anatomic commentary on the vermis of the cerebellum and the "tailed nucleus," now known as the caudate nucleus. Recent reviews of Avicenna's writings on the treatment of spine injuries and stabilization reveal remarkably modern views for this era.[34-36]

Although Avicenna was clearly the "second doctor," it was Albucasis (Abu Al-Qasim or Al-Zahrawi, 936-1013), a learned Islamic Moorish Spaniard, who was clearly the surgeon of the times (Fig. 1-11). In the Islamic tradition, Albucasis was both a great compiler and a serious scholar whose writings (some 30

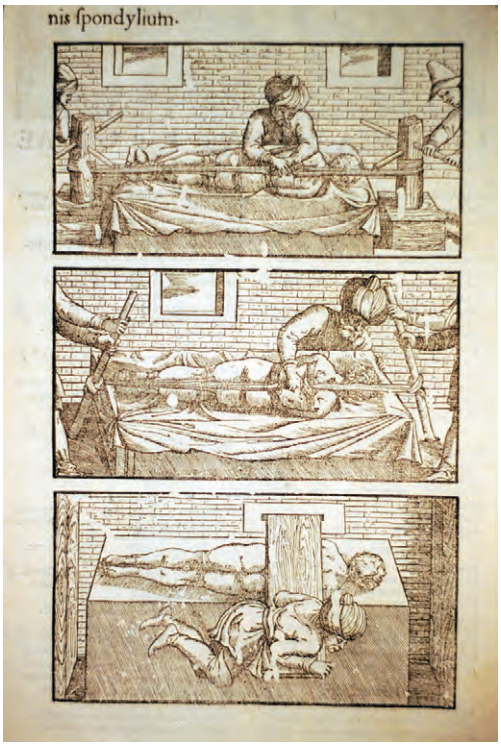


FIGURE 1-10 Title page of Avicenna's *Canon*. (*From Avicenna.* Liber canonis, de medicinis cordialibus, et cantica. *Basel: Joannes Heruagios; 1556.)*

FIGURE 1-11 An allegorical scene of Albucasis operating in the field on an injured soldier—here he is removing an arrow from the chest. It was described in the 17th century Scultetus monograph on surgery. (*From Scultetus J.* Χειροπλοθηκη. Armamentarium Chirurgicum XLIII. *Ulm: Balthasar Kühnen; 1655.)*

volumes) were focused on surgery, dietetics, and materia medica. Albucasis' insights into the importance of surgery are clearly revealed in his introduction to the *Compendium*.[33] In the introduction he discusses the question why the Arabs had made so little progress in surgery. He attributed this lack of progress to a lack of anatomic study and inadequate knowledge of the classics. He clearly believed that anatomic studies were the key to learning and certainly key in performing surgical interventions. Although his thoughts on anatomic studies were excellent, Albucasis unfortunately popularized the frequent use of emetics as prophylaxis against disease, a form of barbaric medical treatment that survived in the form of "purging" until well into the 19th century. So influential were Albucasis' surgical writings that they remained in use in the schools of Salerno and Montpellier for 500 years and had an enormous influence on medicine in the Middle Ages.

In the final section of the *Compendium* there is a lengthy summary of contemporary surgical practice.[38-40] Also included in this part of Albucasis' work is a unique collection of illustrations of surgical instruments. Their appearance here had lasting influence, with his style of instruments being used extensively in the schools of Salerno and Montpellier and later having important influence in the medieval period. Many of the instruments illustrated in the *Compendium* appear to have been designed by Albucasis. He clearly describes their design along with technical aspects of their use. Following on the earlier writings of the Greeks, he provides a novel design for a "nonsinking" trephine. The description of this instrument and others became classic and formed the template for many later instruments. An early and apparently unique technical innovation involved placing a collar on the trephine in a circular fashion, an ingenious design to prevent plunging into the brain. Some of the instruments were clearly copied from those described earlier by Paul of Aegina, but their practical use was further enhanced by their inclusion in the *Compendium*.

In reviewing Albucasis' treatise on surgery, we find an extraordinary work. The text is rational, comprehensive, well illustrated, and designed with the intent to educate the surgeon on the details of each treatment, not even neglecting the types of wound dressings to be used. In reading Albucasis' techniques of brain surgery, not all was well for the patient. In fact, the modern reader can only wonder how patients would allow themselves to undergo some of these surgical practices. For chronic headache, he applied a hot cautery to the occiput and burned through the skin but not the bone. Another headache treatment he described required hooking the temporal artery, twisting it, placing ligatures, and then ripping it out. Albucasis identified and described various types of spinal injury. He recognized the seriousness of spinal injury, particularly dislocation of the vertebrae. In patients with total subluxation, he appreciated that the prognosis was essentially terminal, with patients having involuntary activity (passing urine and stool) and flaccid limbs. Albucasis was innovative in dealing with lesser spinal injuries. He described and illustrated the methods and splints that he used for reduction of such injuries. To the modern reader some of these techniques seem to be dangerous, especially stabilizations that required an aggressive combination of spars and winches and "stretching" of the spinal column. Following earlier Greek and Byzantine views, Albucasis thought that bone fragments in the spinal canal should be removed. In reviewing skull fractures, he has an elegant discussion of the pediatric ping-pong fracture of the skull:

> *This is a fracture due to a fall or a blow from a stone and the like, making a dent in the surface of the bone and a hollow at the site as occurs in a bronze bowl when a blow falls on it and a portion of it is pushed in. This mostly occurs in heads who bones are soft, as those of children.*[38]

The treatment of hydrocephalus was a vexing problem for surgeons and physicians because its outcome was almost always fatal. Albucasis recommended drainage of cerebrospinal fluid (CSF) in patients with hydrocephalus via a series of drains and wicks. He designed a lenticular-shaped surgical tool to make the puncture, which was performed over the anterior fontanelle. Having nicely detailed the technique, he blithely notes that the outcome is almost always fatal. What is interesting to note is that he attributed the poor outcomes not to the surgery but rather to "paralysis" of the brain from relaxation. Albucasis is clever in pointing out to the reader that one must pick the site for drainage carefully. Never cut over an artery because the potential hemorrhage can rapidly lead to death. In recent years, some authors have advocated treatment of hydrocephalus by "binding" the head with tight wraps. Albucasis was advocating this form of treatment more than a thousand years ago. For a child with hydrocephalus, he would "bind" the head with a wrap and then put the child on a "dry diet," with limited fluid intake to dehydrate the child—in looking back, a rather progressive and reasonable treatment plan for this disorder.[38,39]

MIDDLE AGES—THE AGE OF MEDIEVAL MEDICAL SCHOLASTICISM

In the early Middle Ages, the influence of the Islamic/Arabic schools on medicine was beginning to lessen, along with a geographic switch in which intellectual centers for medicine were now returning to Europe. With the advent of medieval scholasticism, a new school of thought developed in which philosophical and metaphysical explanations and dialectic interpretations became prominent. One of the preeminent schools proposing this view was the School of Salerno in what is now Italy (Figs. 1-12 and 1-13).[41] In much of Europe, despite the fact that barbarians were still in control, physicians were being trained and libraries were being built and expanded. At the School of Salerno, an early leader in developing medical scholasticism was

FIGURE 1-12 Constantine the African lecturing at the School of Salerno.

Constantinus Africanus—*magister orientis et occidentis* (1020-1087).[42] Constantine, a *Magistri Salernitani*, provided an important bridge in medicine by introducing the scholarship of Islamic/Arabic medicine there and eventually to all of Europe. Constantine received his medical education in Baghdad, where he learned the prevalent views of Islamic medicine. He moved to a monastery at Monte Cassino, where in the tradition of this period he translated Arabic manuscripts into Latin. Modern scholars believe that his translations included inaccuracies and introduced errors into the medical literature. Recent studies suggest that Constantine was a plagiarist and unreliable translator. Nonetheless, one cannot underestimate his contributions by providing the earliest transfer of Arabic/Islamic medical literature to Europe. In looking back, what we see are Greek texts originally translated into Arabic and now being translated into Latin, with the legacy of Galen and other early writers remaining firmly entrenched as dogma. Rather than providing or developing new ideas, the classical texts in medicine remained fully in control of medical dogma. One can only imagine how much medical and surgical knowledge was lost or distorted by inaccuracies in these successive translations. Constantine did make a key contribution to medieval medicine when he reintroduced anatomic dissection with an annual dissection of a pig, but the findings were compared with those recorded in the Greek classics. If the prosector's findings did not match the ancient texts, they were simply ignored! Constantine was clearly a learned man, but his style of teaching became typical of the Medieval Ages; extensive compilations and translations were undertaken, but original thought or advances in knowledge were absent. In the Middle Ages the School of Salerno lead the way and was followed by the great medical schools at Naples, Bologna, Paris, and Montpellier, the early pillars of medieval medicine.

An unusual and remarkable book was produced during this period—*Regimen Sanitatis Salernitum*, a work that first appeared in the 12th century and was later republished in 140 different editions extending well into the 19th century.[41] This book summarizes the Salernitan school directions for maintenance and care of patients in medicine. In Europe a strong educational system was being developed, but medicine remained cloaked in the literature of the classical Greeks and Islamic writing; for the most part, surgical education and surgical practice continued to be treated as an avocation limited to uneducated barber-surgeons and apprentices. However, there were talented surgeons who escaped the norm and produced original surgical works and practices.

Roger of Salerno (Ruggiero Frugardi, fl. 1170) is considered the first learned medieval European writer on surgery (Figs. 1-14


CONSTANTINI
AFRICANI POST HIPPOCRATEM ET GALENVM, QVO-
rum, Græce linguæ doctus, sedulus fuit lector, medicorũ nulli prorsus, mul
tis doctissimis testibus, posthabendi operã, cõquisita undiq; magno studio,
iam primum typis euulgata, præter paucula quædam quæ impressa fuerũt,
sed & ipsa à nobis ad uetustissimorum exemplarium manuscriptorum ueri
tatem tanta cura castigata, ut huius autoris antehac nihil æditum
censeri possit. Librorum ægrotis & medicis utilissimorum
maximeq; necessariorum catalogum, hæc pa-
gina uersa & prima epistola
demonstrabunt.
BASILEÆ APVD HENRI-
CVM PETRVM.

FIGURE 1-13 Title page from a collection of the works of Constantine the African—1536. (*From Constantinus Africanus.* Constantini Africani Post Hippocratem et Galenum. *Basel: Henricus Petrus; 1536.)*

FIGURE 1-14 Roger of Salerno's demonstration of brain and skull surgery. *(From the Sloane manuscript, 1977—Courtesy of the British Museum.)*

FIGURE 1-15 An early medieval manuscript on the writings of Roger of Salerno in which is demonstrated the "professor and student," a reflection of the educational style of this period. *(From the Sloane manuscript, 1977—Courtesy of the British Museum.)*

and 1-15). Roger was educated in the Salerno tradition and followed many of its teachings. His book on surgical practice, *Practica Chirurgiae*,[43] offers techniques of interest to neurosurgeons. An example was his technique for checking for a tear of the dura and leakage of CSF in a patient with a skull fracture. To detect a leak, Roger would have the patient hold his breath and strain (i.e., Valsalva maneuver) and then look for air bubbles around the fracture site, a clear sign of a leak. He was a pioneer in the techniques of managing peripheral nerve injury. In a severed nerve, he argued for reanastomosis of the nerve ends with close attention paid to their alignment. In dealing with the large bleeding veins of the neck, he urged direct ligation with suture rather than cautery. Several chapters of his text are devoted to the treatment of skull fractures. Much of the technique described mirrors the views of earlier classical writers, but the style is clearer and more succinct. An example of this style is seen in this short description of the management of various skull fractures:

> *When a fracture occurs it is accompanied by various wounds and contusions. If the contusion of the flesh is small but that of the bone great, the flesh should be divided by a cruciate incision down to the bone and everywhere elevated from the bone. Then a piece of light, old cloth is inserted for a day, and if there are fragments of the bone present, they are to be thoroughly removed. If the bone is unbroken on one side, it is left in place, and if necessary elevated with a flat sound (spatumile) and the bone is perforated by chipping with the spatumile so that clotted blood may be soaked up with a wad of wool and feathers. When it has consolidated, we apply lint and then, if it is necessary (but not until after the whole wound has become level with the skin), the patient may be bathed. After he leaves the bath, we apply a thin cooling plaster made of wormwood with rose water and egg.*[43,44]

A 12th century manuscript owned by Harvey Cushing and attributed to Roger of Salerno has recently been translated. It contains an early description of a soporific for pain relief for use in surgery. Roger was particularly fond of citing the writings of Albucasis and Paul of Aegina. He strongly favored therapeutic plasters and salves but was not a strong advocate of the popular application of grease to injuries of the dura. He advocated the use of trephination for the treatment of epilepsy, although he is not clear why this technique would work. Chapters 1 to 13 (capita 1-13) detail contemporary surgical treatment of scalp wounds and fractures of the skull. One of his most significant errors in surgical practice was the concept that provoking suppuration of pus in a wound encouraged healing. This introduced the concept of "laudable pus" in wound healing and delayed good wound care until Lister and 19th century antisepsis.

An unusually talented and inventive medieval surgeon from Bologna was Theodoric of Cervia (Borgognoni, 1205-1298). In contrast to Roger of Salerno, Theodoric was a pioneer in the use of aseptic technique—not what we would refer to as the "clean" aseptic technique of today but rather a method based on avoidance of "laudable pus." Theodoric thought that he had found the ideal principles for good wound healing, which included control of bleeding, removal of contaminated or necrotic material, avoidance of dead space, and careful application of a wound dressing bathed in wine, the last providing a degree of antisepsis. He also argued for primary closure of all wounds when possible and avoiding "laudable pus."

> *For it is not necessary, as Roger and Roland have written, as many of their disciples teach, and as all modern surgeons profess, that pus should be generated in wounds. No error can be greater than this. Such a practice is indeed to hinder nature, to prolong the disease, and to prevent the conglutination and consolidation of the wound.*[45,46]

Theodoric's surgical work, which was first written in 1267, provides one of the best reviews of contemporary medieval surgery.[45] He is also remembered as one of the earliest writers to include illustrations of his techniques. His recommendations called for meticulous (almost Halstedian) techniques with gentle handling of surgical tissues. Theodoric believed that aspiring surgeons should train only under competent masters. In the field of head injury, he argued that parts of the brain could be removed through a wound with little effect on the patient. In the treatment of skull fractures, he strongly argued for elevating depressed fractures. He advocated avoiding punctures of the dura because they could lead to abscess, convulsions, and bad outcomes. For pain relief during surgery, he developed his own "soporific sponge" that contained opium, mandragora, hemlock, and other less important ingredients applied to the nostrils, and once the patient fell asleep, he began surgery. Opium was probably the key ingredient.

William of Saliceto (Guglielo da Saliceto, 1210-1277) was a uniquely skilled Italian surgeon of the 13th century and a professor at the University of Bologna. His book on surgery, *Chirurgia* (or *Cyrurgia*), was completed in 1275, and in it we find highly original concepts that are not totally based on previous classical writings but in which the influence of Galen and Avicenna are clearly present.[47] This book was written by William for his son Bernardino. The observations offered are based on his own surgical cases. Book IV contains the earliest known treatise on surgical and regional anatomy. His most significant contribution for this era was probably his decision to replace cautery with the surgical knife.

> De anathomia in communi et de formis membrorum et figures que sunt considerande in incision et cauterizatione.[47]

He describes interesting and unique techniques for primary peripheral nerve suture repair. In this pre-Harvey era he distinguished arterial from venous bleeding by the "spurting" of blood. He also put forth interesting neurological concepts, such as that the cerebrum governs voluntary motion and the cerebellum involuntary function.

Leonard of Bertapalia (1380?-1460) was a prominent 15th century Italian surgeon and writer. Leonard established an extensive and lucrative practice in the area of Padua and in neighboring Venice. At a time when anatomic dissection was rarely practiced in Europe, he became one of the earliest proponents of the study of anatomy. In 1429 he offered a course of surgery that included the dissection of an executed criminal. He devoted a third of his book to surgery on the nervous system and head injuries.[48,49] He considered the brain the most precious of organs and regarded it as the source of voluntary and involuntary functions. In reviewing his treatment of skull fractures, he would always avoid materials that might generate pus. He argued for never placing a compressive dressing that might drive bone into the brain and proposed that if a piece of bone pierces the brain, the surgeon should remove it.

Leonard put together a set of rules to guide the practice of 15th century surgeons that have modern tones—rules still applicable 5 centuries later.

> *To be the perfect surgeon, you must always bear in mind these eight notations, and remembering them you will be preferred to others.*
>
> *The first task to become a good surgeon should be to use his eyes.*
>
> *Second, you must accompany and observe the qualified physician, seeing him work before you yourself practice.*
>
> *Third, you must command the most gentle touch in operating and treating lest you cause pain to the patient.*
>
> *Fourth, you must insure that your instruments be sharp and unrusted whenever you cut anywhere.*
>
> *Fifth, you must be courageous in operating and cutting but timid to cut in the vicinity of nerves, sinews and arteries, and, so as not to commit error, you should study anatomy, which is the mother of this art perform your surgery cleverly and never operate on human flesh as if you were working on wood or leather.*
>
> *Sixth, you must be kind and sympathetic to the poor, for piety and humility greatly augment your reputation and the sick will more freely commit themselves to your care.*
>
> *Seventh, you must never refuse anything brought you as a fee, for the sick will respect you more.*
>
> *Eighth, you must never argue about fees with the sick, or indeed demand anything unless it be previously agreed upon, for avarice is the most ignoble of vices and should you be so inflicted, you will never achieve the reputation of a good doctor.*[49]

Lanfranchi of Milan (d. 1306) was a pupil of William of Saliceto and often referred to as the father of French surgery. Lanfranchi advocated his teacher's use of the knife in place of the burning cautery. Although born and educated in Italy, he had to leave Italy for France to avoid political strife. In his *Cyrugia Parva* we find a number of interesting surgical techniques. Lanfranchi perfected the use of suture for primary wound repairs.[50] He was among the first to relate the direct effect of head injury to brain function. Hippocrates was the first to articulate the concept of *commotio cerebri*, but it is to Lanfranchi that we owe the first modern characterization of what is now called a *cerebral concussion*. For surgeons he developed a series of guidelines for trephination in skull fractures and "release of irritation" of the dura. Because of the dangers of skull surgery, Lanfranchi argued for using the trephine only when absolutely necessary; otherwise, he evoked the skills of the "Holy Ghost" to provide cure. Among his innovative surgical techniques was the development of esophageal intubation during surgery, a technique not commonly practiced until the 19th century. As an educated surgeon and a "Surgeon of the Long Robe" (i.e., academic), he attempted to elevate the art and science of surgery above the mediocre level of the menial barber-surgeon ("Surgeons of the Short Robe"). Lanfranchi also argued against the separation of surgery and medicine, advocated since the time of Avicenna, for he thought that a good surgeon should also be a good physician.

After Lanfranchi came another important figure in the history of French medicine and surgery—Henri de Mondeville (d. 1317). Educated in Paris and Montpellier, Henri later went on to become a professor at Montpellier. He was strongly motivated to elevate the profession of the surgeon and clearly detested the barber-surgeon: "Most of them were illiterates, debauchees, cheats, forgers, alchemists, courtesans, procuresses, etc."[46]

In 1306 Henri undertook the task of developing a new treatise on surgery for the education of his students at Montpellier. Unfortunately, because of tuberculosis and ill health, the manuscript was never completed. Ironically, the edited portions were not published until 1892, when Professor Julius Pagel of Berlin completed the task.[51] Henri adopted and followed a number of the views of Lanfranchi. He was a believer in clean wounds and avoiding "laudable pus." Unfortunately, Henri would be the last surgeon in this era to argue for avoiding "laudable pus"; after him, surgeons returned to the older belief of pus developing in a wound being a good sign of healing. Henri offered originality in wound management by advocating for healing by primary intention—"*modus novus noster*." In the surgical treatment of wounds, he encouraged the removal of foreign bodies and the use of wine dressings in wound care. Henri was clever in designing a number of surgical instruments. He is remembered for the design of a needle holder and also a forceps-type instrument for extraction of arrowheads. He argued against elevating skull fractures if there was no injury to the overlying soft tissues. He believed that nature would do a better job at healing the fracture by natural union. It was his opinion that unnecessary exploration and probing of the wound would only cause more injury than natural healing—in retrospect a rather brilliant insight into wound care (Fig. 1-16).[46]

No history of surgery can be complete without a discussion of the contributions of Guy de Chauliac (1300-1368) (Fig. 1-17).[52] This surgeon was clearly the most influential European surgeon of the 14th and 15th centuries. He was so highly

FIGURE 1-16 Surgical instruments designed by Lanfranchi are illustrated here in this early 1519 book. (*From Lanfranchi of Milan. Chirurgia. In:* Guy de Chauliac Cyrurgia et Cyrurgia Bruni, Teodorici, Rolandi, Lanfranci, Rogerii, Bertapalie. *Venice: Bernardinus Venetus de Vitalibus; 1519.)*

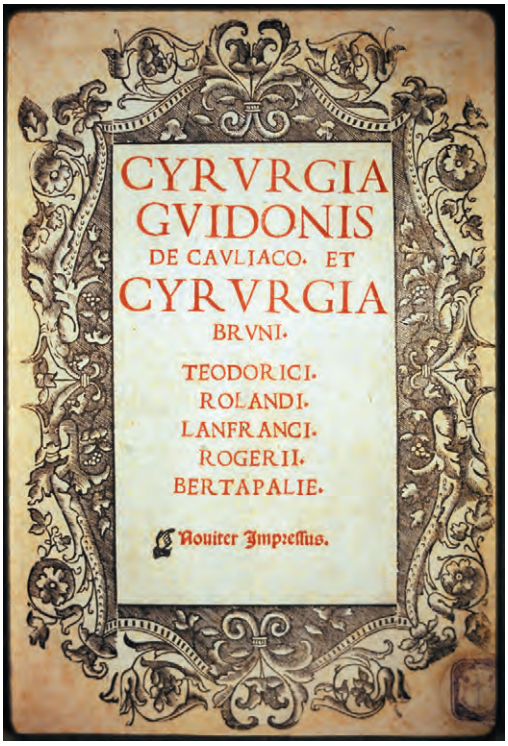


FIGURE 1-17 Title page from collected works dealing with the surgical writings of a number of medieval surgeons, including Guy de Chauliac. (*From Guy de Chauliac. Chirurgia magna. In:* Guy de Chauliac Cyrurgia et Cyrurgia Bruni, Teodorici, Rolandi, Lanfranci, Rogerii, Bertapali. *Venice: Bernardinus Venetus de Vitalibus; 1519. See also Leonardo, RA: History of Surgery. New York, Froben Press, 1943:116.)*

respected that he became the physician for three popes at Avignon (Pope Clement VI, Innocent VI, and Urban V) and leading surgeon and educator at the school of Montpellier. Guy was educated at Toulouse, Paris, Montpellier, and Bologna. He was an early proponent of anatomic dissection of a human cadaver. He states: "In these two ways we must teach anatomy on the bodies of men, apes, swine, and divers other animals, and not from pictures, as did Henri de Mondeville who had 13 pictures for demonstration of anatomy."[53] His writings were popular and continued to exert an influence on surgery until well into the 17th century. His principal didactic surgical text was scribed in 1363 and titled the *Collectorium Cyrurgie*.[53] There are 34 known manuscripts of this work, with the first printed edition appearing in 1478, and more than 70 editions followed. In promoting surgeons as more skilled individuals (versus "mechanics," i.e., barber-surgeons), he stated four conditions that must be satisfied for a practitioner to be a good surgeon: (1) the surgeon should be learned; (2) he should be an expert; (3) he must be ingenious; and (4) he should be able to adapt himself (from the introduction of *Ars Chirurgica*). For the modern neurosurgeon, Guy provides an interesting discussion of techniques that he devised for the treatment of head injuries. Before beginning surgery the head needs to be shaved. Shaving of the hair will prevent hair from getting into the wound and interfering with primary healing. For depressed skull fractures, Guy preferred to put wine-soaked cloths into the injured site to assist healing. He categorized head injuries into seven types and discussed the management of each in detail. Scalp wounds required only cleaning and débridement, whereas a compound depressed skull fracture must be treated by means of trephination and elevation. Skin closure was done by primary repair and for which he claimed good results. To help control excessive bleeding and provide hemostasis, he used egg albumin.

As England was moving away from the barbarian invasions and into the Middle Ages, university education in England began to become comparable to the European model. The leading surgeon of this period in England was John of Arderne (Arden, 1307-1380), who trained as a military surgeon and saw much war experience. In 1370 he came to London and joined the Guild of Military Surgeons. He adopted the phase "*chirurgus inter medicos*," or a surgeon among physicians. His manuscript on surgery was written about 1412.[54] This manuscript, *De Arte Phisicali et de Cirurgia*, was translated into English by D'Arcy Power in 1922, a valuable addition to the English literature on early surgery.[55] His writings suggest that he was a skilled surgeon and had a number of practical insights into what could or could not be done surgically. He was a firm believer in clean hands and well-shaped nails for surgery, although some writers have thought that this was more for social reasons than for surgery.[56] In addition, he would bathe his open wounds with an irrigation fluid that contained turpentine, a useful surgical antiseptic for keeping wounds clean. Most importantly, John of Arderne was a firm believer in education and learning. The surgeon must also "always be sober during any surgery as drunkenness destroys all virtue and brings it to naught."[46]

In reviewing the late Byzantine/Islamic and Medieval period we see an era of great misguided intellectual activity, an era of innovative somnolence where originality of thought is concerned. Clearly, the educators had more faith in the teachings of antiquity. From the fall of the Roman Empire until the beginning of the 16th century, anatomy and the practice of surgery, with rare exceptions, lay dormant, chained to a staunch Galenic and Hippocratic orthodoxy. The transliteration of medical manuscripts from Latin, Greek, and Hebrew into Arabic and back into Latin resulted in many errors of translation and interpretation. The combination of a lack of anatomic knowledge and poor surgical outcomes naturally led physicians to recommend against operating on the brain, except in simple cases. A review of the work done by the surgical personalities described previously reveals that despite a period of intellectual paralysis, there still existed a number of prominent personalities who did make advances. Monastic recluses in often-inaccessible mountain retreats carefully guarded medical knowledge, yet some surgeons nonetheless succeeded in mastering their art in the midst of intellectual darkness.

> *The history of medicine consists of a successive series of intellectual movements proceeding from different centers and each engulfing its predecessor.*[57]

ORIGINS OF NEUROSURGICAL PRACTICE IN THE RENAISSANCE

With the origins of the Renaissance came innovations in surgical concepts and techniques. Beginning in the mid-15th century, physicians and surgeons introduced basic investigative techniques to learn human anatomy and physiology. Of enormous significance was the introduction of routine anatomic dissection in medical schools. Moving away from subservience to the medievalists, great figures such as Leonardo da Vinci, Berengario da Carpi, Nicholas Massa, Andreas Vesalius, and others explored the human body without being encumbered by the erroneous writings of earlier authors. Codified anatomic errors, many ensconced since the Greco-Roman era, were now being corrected. A better understanding of human anatomy led to a change in epistemological presuppositions and in turn resulted in a great surge of interest in surgery. Putting the teachings of antiquity aside, surgeons went forward with great vigor and enthusiasm to unravel the human fabric. This shift from the somber and somnolent

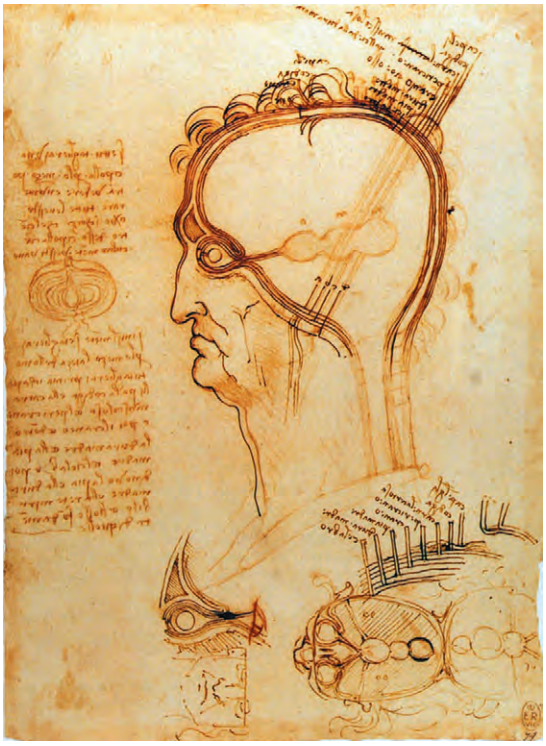


FIGURE 1-18 Leonardo Da Vinci drawing showing the "cell doctrine." *(Courtesy of Windsor Castle Collection, Edinburgh, Scotland—Her Majesty the Queen of England.)*

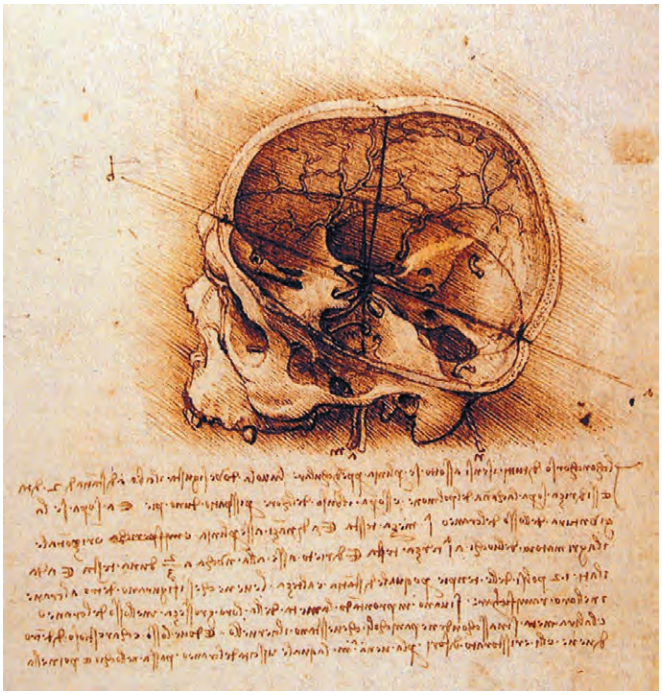


FIGURE 1-19 Leonardo Da Vinci's "three-dimensional" drawing of the anatomy of the skull. *(Courtesy of Windsor Castle Collection, Edinburgh, Scotland—Her Majesty the Queen of England.)*

medieval period to the enlightened, radically inventive Renaissance made it possible to lay the early foundations of modern neurosurgery.

Any discussion of Renaissance surgery and anatomy has to begin with Leonardo da Vinci (1452-1519), the quintessential Renaissance man (Figs. 1-18 to 1-20). Multitalented and recognized as an artist, an anatomist, and a scientist, Leonardo went to the dissection table to better understand surface anatomy and its relationships to art and sculpture. From his studies Leonardo is now recognized as the founder of iconographic and physiologic anatomy.[57-59] For the neurosurgeon, Leonardo provided the earliest, albeit crude, diagrams of the cranial nerves, the optic chiasm, and the brachial and lumbar plexuses. He developed a wax-casting technique that allowed him to understand the anatomy of the ventricular system. To do this he took a fresh brain and poured liquid wax into the ventricles; a hollow tube was inserted to allow egress of the air. Leonardo's experimental studies included sectioning a digital nerve and noting that the affected finger no longer had sensation, even when placed in a fire. Leonardo was not a surgeon, but he gave an important impetus to the study of anatomy and defining correct anatomic relationships—vital concepts for any surgeon. Unfortunately, Leonardo's great opus on anatomy, which was to be published in some 120 volumes, never appeared.[60] His anatomic manuscripts circulated among the artists in Italy throughout the 16th century, only to be lost and then discovered in the 18th century by William Hunter. These anatomic works had a profound influence on artists and physicians and subsequently on the development of modern anatomic studies. Leonardo, as a founder of modern anatomy, provided a creative spark to re-explore the human body by hands-on dissection.

The earliest printed surgical work that contained illustrations was authored by Hieronymus Brunschwig (ca. 1450-1512) in *Buch der Cirurgia* and published in Strasbourg in 1497 (Fig. 1-21).[61] Although the images have nothing to do with specific surgical procedures, the book is the first to discuss the management of gunshot wounds; gunpowder had recently been introduced for the weapons of war. This work was considered to be valuable enough to be plagiarized and published in a pirated edition the same year and appeared in many other editions throughout the 16th century. In the 1513 edition, the first illustration of a patient with a head injury undergoing treatment was added to the work.

An early Renaissance surgeon who incorporated some of the recently revealed anatomic concepts in his publication was Hans von Gersdorff (1455-1529). In his surgical book *Feldtbuch der Wundartzney*, published in 1517, are some of the earliest illustrations on surgical technique (Fig. 1-22).[62] Gersdorff was a military

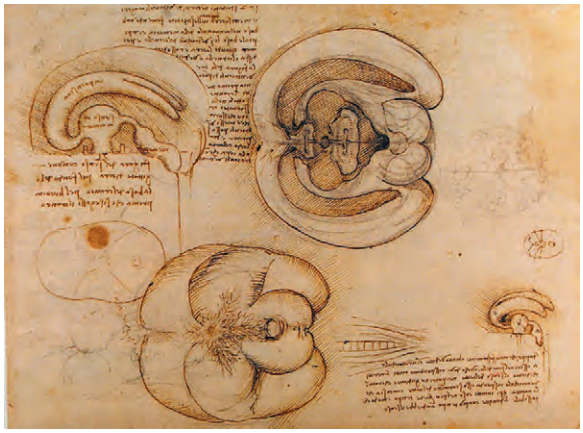


FIGURE 1-20 Leonardo Da Vinci's "wax casting" modeling of the ventricular system. *(Courtesy of Windsor Castle Collection, Edinburgh, Scotland—Her Majesty the Queen of England.)*

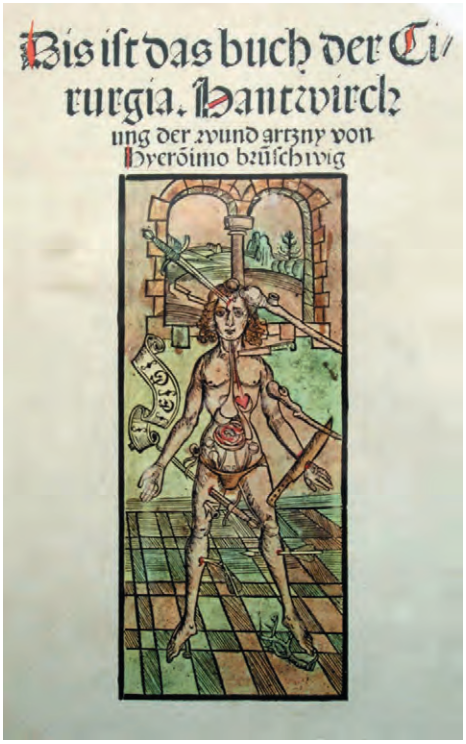


FIGURE 1-21 Title page from the first edition of an important work in surgery showing a "wound man" figure with the various injuries that a surgeon could expect to treat. (*From Brunschwig H.* Dis ist das Buch der Cirurgia. *Strassbourg: J Grüninger; 1497.)*

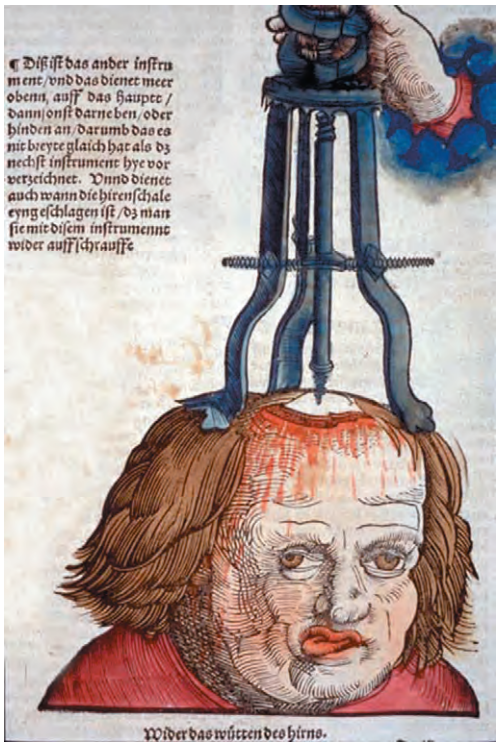


FIGURE 1-22 A rare colored trephination plate from Gerzdorff's 1517 manual on surgery. The victim has clear evidence of a third cranial nerve palsy from a depressed skull fracture. *(From Gersdorff HF.* Feldtbuch der Wundartzney Strassbourg. J Schott. *1517. See also Flamm ES. The dilated pupil and head trauma.* Med Hist. *1972;16:194-199; Image courtesy of the Reynolds Historical Library, Lister Hill Library, University of Alabama, Birmingham.)*

surgeon and, with more than 40 years of war experience, became adept at handling battlefield injuries. This handbook for surgeons was divided into four parts: anatomy, surgery, leprosy, and a glossary of anatomic terms, diseases, and medications. The section on anatomy was based on his own extensive experience, as well as the earlier Islamic writings and the works of Guy de Chauliac. The surgical portion deals with military surgery, mostly on how to extract foreign objects, tourniquet techniques to control bleeding, and amputation techniques. For the neurosurgeon, there are several woodcuts dealing with surgical technique and surgical instrumentation. In one illustration, a third nerve palsy on the side of a depressed skull fracture and a facial paralysis on the opposite side are clearly demonstrated. Included in this work is also the first plate showing dissection of the human brain. This surgical work became very popular and, because of its practical presentation of surgery and the illustrations in the text, went through several editions.

One of the greatest figures in the history of surgery remains Ambroise Paré (1510-1590), a poorly educated humble Huguenot and an individual whom today many historians consider the father of modern surgery (Figs. 1-23 and 1-24). After an extensive military surgical experience, Paré organized and published a substantial body of practical knowledge along with innovative instrument designs. At this time most physicians and surgeons published their writings in Latin. Nevertheless, Paré preferred to publish in the vernacular (i.e., French) rather than in Latin.[63-66] By this transition Paré's books gained wider dissemination and appreciation. As his reputation grew, he became a valued surgeon to the European royal courts. One of Paré's most famous cases was a head injury sustained by Henri II of France. Paré attended the King and was also present at the autopsy. Henri II suffered a subdural hematoma after a joust during the marriage celebration of Elizabeth of France to Philip, King of Spain. When Paré described the clinical findings of Henri II, he noted that the

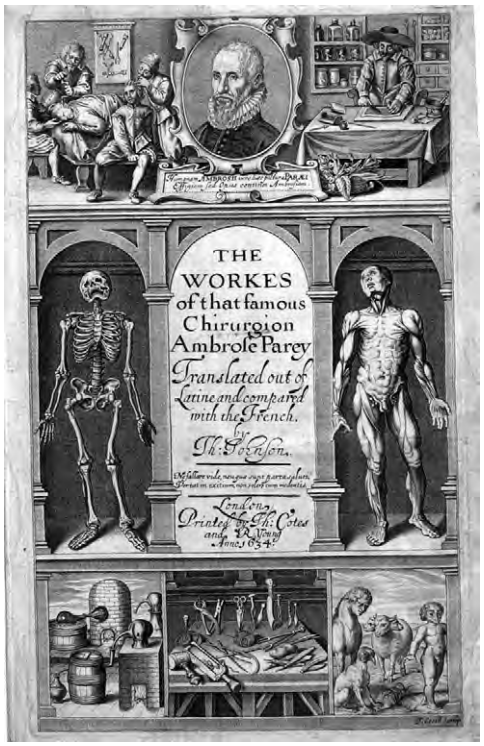


FIGURE 1-23 Title page of Ambroise Paré's collected works on surgery published in English. (*From Paré A, Johnson T, trans.* The Workes of That Famous Chirurgion Ambroise Parey. *London: Richard Coates; 1649.)*

FIGURE 1-24 A, Trephination plate from Paré's surgical works. **B,** Neurosurgical tools from Paré's surgical works. (*From Paré A, Johnson T, trans.* The Workes of That Famous Chirurgion Ambroise Parey. *London: Richard Coates; 1649.)*

FIGURE 1-25 Title page from Berengario's treatise on head injury—1518. (*From Berengario da Carpi J.* Tractatus de Fractura Calvae Sive Cranei. *Bologna: Hieronymus de Benedictus; 1518.)*

patient complained of a headache and blurred vision, with vomiting, lethargy, and signs of decreased respiration eventually developing. Paré postulated that the injury was due to a tear in one of the bridging cortical veins, and he was clearly describing signs of increased intracranial pressure. His remarkable clinical observations and clinical history were confirmed at autopsy. Thus, despite humble beginnings, Paré became one of the giants in this formative period of surgery.

Among Paré's surgical works, his writings on the brain remain the most remarkable.[64,65] Book X is devoted to the diagnosis and management of skull fractures. Although not original to Paré, he popularized the technique of elevating a depressed skull fracture with a Valsalva maneuver:

> *For a breath driven forth of the chest and prohibited passage forth, swells and lifts the substance of the brain and meninges where upon the frothing humidity and sanies sweat forth.*[64]

By doing this, both blood and pus can be expelled from the fracture site. Paré's surgical techniques demonstrated unusual and unique advances over previous writers. He provides extensive discussion on the use of trepans, shavers, and scrapers. He was skilled at removing osteomyelitic bone, incising the dura, and evacuating blood clots and pus, procedures that surgeons had previously done only with trepidation. He advocated débridement of wounds for good healing and emphasized that foreign bodies must be removed from the injury site. Paré's most significant change in contemporary surgical practice was the serendipitous discovery that boiling oil, a then common surgical practice, should not be used in gunshot wounds. Substituting a dressing of egg yolk, rose oil, and turpentine provided improved wound healing and led to a dramatic reduction in morbidity and mortality. Paré also discarded the older Islamic technique of hot cautery for control of bleeding, instead substituting the use of ligatures and thereby reaping the benefits of enhanced healing and reduced blood loss.[65] The improvement in his results was a source of wonder to Paré and gave rise to his famous aphorism:

> Je le pansay, Dieu le guarit
> *(I bandaged him, God cured him)*[65]

Among the Renaissance giants of anatomy and surgery there arose the great Italian surgeon and anatomist Berengario da Carpi (1470-1550). In 1518, Berengario da Carpi wrote the second monograph devoted solely to treating injuries of the head, the first being by Hippocrates (Figs. 1-25 to 1-27).[67,68] This book was motivated by Berengario's successful treatment of a serious head injury in Lorenzo dé Medici, Duke of Urbino. In a dream shortly after he had treated Lorenzo, Berengario was visited by a man wearing a cap adorned with a rooster feather and golden-winged sandals (i.e., Hermes Trismegistus, or the Third Mercury). This individual encouraged Berengario to a write a treatise on skull fractures and head injuries. As a result of this dreamy intervention a marvelous *tractatus* appeared, the first printed work devoted solely to head injuries. In this text we find discussions of original surgical techniques along with the earliest illustrations of cranial instruments designed for the surgical treatment of head injuries. As an anatomist, Berengario, like Leonardo da Vinci, provided one of the earliest and most complete discussions of the ventricular system. Berengario presented some of the earliest descriptions of the pineal gland, choroid plexus, and lateral ventricles.[67] His anatomic illustrations are among the first published from actual anatomic dissections. Berengario was a believer in anatomic dissection because that was the only way one could learn the anatomy; he believed that using only the written word was useless and added that the earlier writings were full of

FIGURE 1-26 Neurosurgical trephine (hand brace) designed by Berengario. (*From Berengario da Carpi J.* Tractatus de Fractura Calvae Sive Cranei. *Bologna: Hieronymus de Benedictus; 1518.)*

anatomic errors. His anatomic writings were among the first to challenge the medieval writings of Galen and others.

A not so well-known writer and anatomist of the Renaissance was a Marburg professor by the name of Johannes Dryander (Johann Eichmann, 1500-1560). In 1536 (expanded version in 1537) Dryander published an illustrated work on the brain and skull (Figs. 1-28 and 1-29).[69,70] Within this remarkable work are 16 plates of the brain showing successive layered dissections of the scalp, dural coverings, and brain. The drawings on the anatomy of the cerebellum and the posterior fossa are particularly striking. There are inaccuracies in the text because of the prevailing influence of Galen and medieval scholasticism, but this book can be considered the first textbook of neuroanatomy. Despite Dryander's allegiance to Galen and his teaching, he advocated public anatomic dissections, the results of which led to these remarkable neuroanatomic drawings.

Military surgery has always been a great educator of surgeons, and one of those particularly influenced by his military service was Volcher Coiter (1534-1576). Coiter was an army surgeon and city physician in Nuremberg who had the good fortune to study under several contemporary giants, including Fallopius, Eustachius, and Aldrovandi. From their teachings and education, Coiter was able to undertake unique and original anatomic and physiologic investigations.[71] Among his anatomic descriptions are the first anatomically correct details of the anterior and posterior spinal roots. He was the first to distinguished gray from white matter in the spinal cord. Coiter had a particularly strong interest in the spine, which led him to conduct a number of anatomic and pathologic studies of the spinal cord, including an early model of decerebrate posturing. Coiter also provided a number of details on how to trephine the skulls of birds, lambs, goats, and dogs.[71] He was the first to associate the pulsation of the brain with the arterial pulse. As an early neurosurgeon and investigator, he

FIGURE 1-27 Title page with allegorical anatomic dissection scene from Berengario's work on anatomy—Isagogae, 1522. (*From Berengario da Carpi J.* Isagogae Breves perlucide ac ubertine in Anatomiam Humani Corporis. *Bononiae, Italy: B. Hectoris; 1522.)*

FIGURE 1-28 Scalp and skull dissection illustrations from Dryander—1537. (*From Dryander J.* Anatomiae. *Marburg: Eucharius Cervicornus; 1537.)*

FIGURE 1-29 "Cell doctrine" theory illustrated here within the ventricular system as illustrated by Dryander—1537. (*From Dryander J.* Anatomiae. *Marburg: Eucharius Ceruicornus; 1537.)*

reported on opening the brain and removing parts of it with no ill effects noted—an early, surprising precursor of cerebral localization.

One of the most skilled of Renaissance surgeons was a Venetian by the name Andreae della Croce (1509?-1580). Croce was a follower and believer in Paré and used many of his techniques (Figs. 1-30 and 1-31). A combination of surgical skill and a Renaissance flair for design led Croce to produce a remarkable book on surgery in 1573.[72] Within this monograph are some of the most beautifully engraved scenes of neurosurgical operations.

FIGURE 1-30 An elegant trephination scene being performed in a noble's bed chambers—from Croce. (*From della Croce GA.* Chirurgiae Libri Septem. *Venice: Jordanus Zilettus; 1573.)*

FIGURE 1-31 Neurosurgical instruments designed and illustrated by Croce. (*From della Croce GA.* Chirurgiae Libri Septem. *Venice: Jordanus Zilettus; 1573.)*

As was typical of the period, surgical operations were performed in family homes, usually in the bedroom with the occasional dog lying at the foot of the bed. Croce textually and in drawings describes techniques for performing trephinations. Several illustrations show the various types of arrows, spears, and bullets used in warfare, and techniques for their removal are detailed. A series of plates are added showing his instrument designs for performing neurosurgical procedures. A concept difficult to comprehend for the modern reader is bloody trephination being performed with minimal anesthesia in a beautifully appointed noble's bedroom.

Croce illustrated a number of trephination instruments in his monograph, some of which were an improvement on their predecessors. Croce's trepanation drill was rotated by means of an attached bow, in the manner of a carpenter's drill. Various trephine bits are proposed and illustrated, many surprisingly modern, with conical designs to avoid plunging. The illustrations of surgical instruments include "Penfield"-style elevators for lifting depressed skull fractures.

A figure in surgery and anatomy that typified the great strides of learning in the Renaissance was Andreas Vesalius (1514-1564). Vesalius was educated at Louvain, Montpellier, and Paris, all staunch schools of Galenic orthodox teaching (Figs. 1-32 and 1-33). Rejecting the views of his Galenic-enthralled professors, Vesalius provided an innovative and dramatic approach to anatomic dissection. Following on the theme of earlier 16th century anatomists such as Leonardo da Vinci and Berengario da Carpi, Vesalius argued that anatomic dissections had to be done by the teacher, not by an ignorant prosector being guided by the professor who sat at the lectern reading from a Galenic monograph on anatomy.

Vesalius was appointed professor of anatomy at Padua at the young age of 23. At the age of 28, in 1543, he produced his great magnum opus, *De Humani Corporis Fabrica*.[73] In Book VII is an extensive discussion on the anatomy of the brain. Included in the chapter are detailed anatomic discussions with excellent illustrations. Following his anatomic caveats, Vesalius noted that the "heads of beheaded men are the most suitable [for study] since they can be obtained immediately after execution with the friendly help of judges and prefects."[74]

FIGURE 1-32 Frontispiece from Vesalius' 1543 work on anatomy showing Vesalius performing a "hands-on" anatomic dissection. (*From Vesalius A.* De Humani Corporis Fabrica Libri Septem. *Basel: Joannes Oporinus; 1543.)*

Vesalius was also trained as a surgeon. In the *Fabrica* is a section of text on the brain and the dural coverings in which Vesalius discusses mechanisms of brain injury and how the various membranes and bone have been designed to protect the brain. Vesalius, in the second edition of his major work, also provided an early case of a child with hydrocephalus and remarked on the pathology as originating from CSF but was unable to offer surgical treatment: "*in ipsius cerebri cavitate, adeoque in dextro sinistroque illius ventriculis: quorum cavitas amplitudoque ita increverat, ipsumque cerebrum ita extensum fuerat, ut novem fere aquae libras continuerint.*"[75] A close examination of several of the illustrated initial letters in the text shows little cherubs performing trephinations!

A contemporary of Vesalius and another leader in Renaissance anatomic studies was a Paris anatomist—Charles Estienne (1504-1564). In 1546, a book with striking neuroanatomic plates appeared in a work on anatomy by Charles Estienne (Figs. 1-34 and 1-35).[76] This book had been completed in 1539, thereby predating Vesalius' work by 4 years, but legal problems delayed its publication until 1546. The book is notable for its wealth of beautiful anatomic plates dealing with neuroanatomy. This work contains representations of a series of anatomic figures with the subjects posed against sumptuous and imaginative Renaissance backgrounds. However, in reviewing the text the anatomic details are not as original as Vesalius'. In addition, in the text many of the errors of Galen and his followers are repeated. Regardless, the plates on the nervous system are graphic and among the most illustrative of this period. An important work, albeit with errors, it details the anatomy of the skull and brain more accurately than in previous works.

In looking back at the contributions of the aforementioned personalities and their works, the remarkable advances made in the Renaissance become clearly evident. Returning to vogue is originality in anatomic research. No surgeon could hope to explore the human body without an accurate understanding of the underlying anatomy. In the Renaissance era several important events occurred: introduction of the printed book and the contribution of the Renaissance artist with accurate anatomic illustrations. To complete the picture, we have the addition of strong personalities such as Berengario da Carpi, Vesalius, Paré, and others.

The overwhelming burden of the Hippocratic emphasis on the skull fracture remained the dominant factor in the management of head injuries, as it had been for the preceding 2000 years.

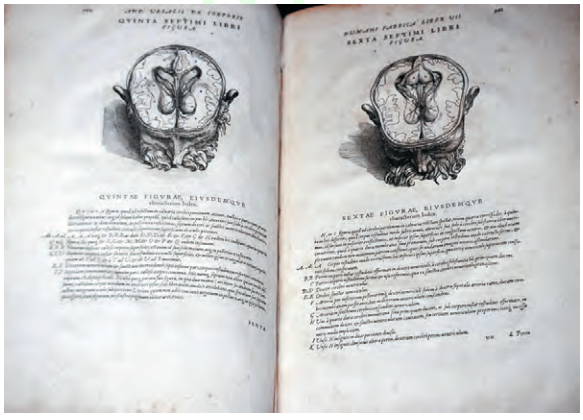


FIGURE 1-33 Two anatomic views of the cross-sectional anatomy of the brain as illustrated by Vesalius in 1543. (*From Vesalius A.* De Humani Corporis Fabrica Libri Septem. *Basel: Joannes Oporinus; 1543.)*

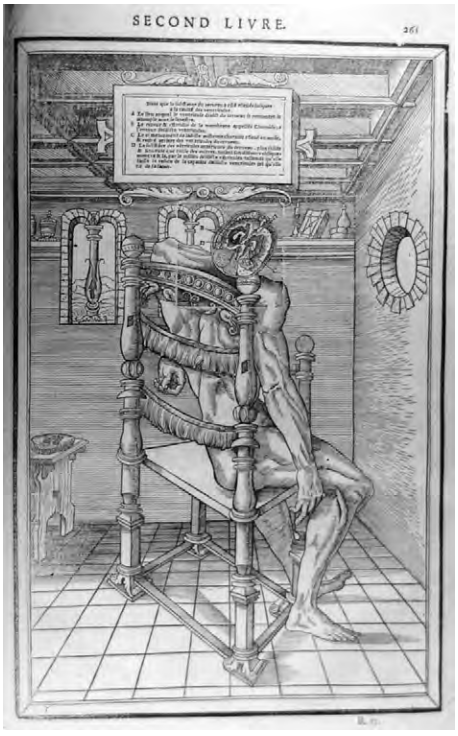


FIGURE 1-34 Estienne's anatomy of the brain. (*From Estienne C.* De Dissectione Partium Corporis Humani Libri Tres. *Paris: Simon Colinaeus; 1546.)*

FIGURE 1-35 Estienne's anatomy of the brain. (*From Estienne C.* De Dissectione Partium Corporis Humani Libri Tres. *Paris: Simon Colinaeus; 1546.)*

FIGURE 1-36 Portrait of Sir Thomas Willis.

With the anatomic period now well under way, the next era was an understanding of the physiology of the human body—the major theme of the 17th century.

SURGEONS OF THE INSURGENCY—17th CENTURY

Sixteenth century medicine and the influence of the Renaissance clearly changed the direction of education and surgical practice for operating on the brain and spinal cord. The 17th century, "the insurgent century," carried these themes even further with spectacular achievements in science and medicine. Some of our historical giants produced their scientific contributions during this century; among them were Isaac Newton (1642-1727), Francis Bacon (1561-1625), William Harvey (1578-1657), and Robert Boyle (1627-1691), and with their ideas and innovations, the introduction of physics, experimental design, discovery of the circulation of blood, and physiologic chemistry. Another critical advance came with the first open public presentation of scientific ideas in the form of scientific societies. Among the most important societies were The Royal Society of London, Académie Des Sciences in Paris, and Gesellschaft Naturforschenden Aerzte in Germany. A firm footing was now available for the advancement of scientific education and exchange of information. For the first time scientific ideas and information could be distributed publically in open dialogue along with discussion of their merit.

A distinctive figure of this period is Thomas Willis (1621-1675), an early describer of the eponymous "circle of Willis" familiar to every physician (Figs. 1-36 and 1-37). Willis was educated at Oxford and became a fashionable London physician. He published a number of important monographs, but the one that stands out is his *Cerebri Anatomie* published in London in 1664.[77,78] With methodical attention to detail, this book became the most accurate anatomic study of the brain to date. Willis was assisted in this work by Richard Lower (1631-1691) in demonstrating that when parts of the "circle" were tied off, the anastomotic network still provided blood to the brain. The superb and anatomically accurate brain engravings were done by the prominent London personality Sir Christopher Wren (1632-1723).

To Willis we owe introduction of the concept of "neurology," or the doctrine of neurons. Willis used the term in a purely

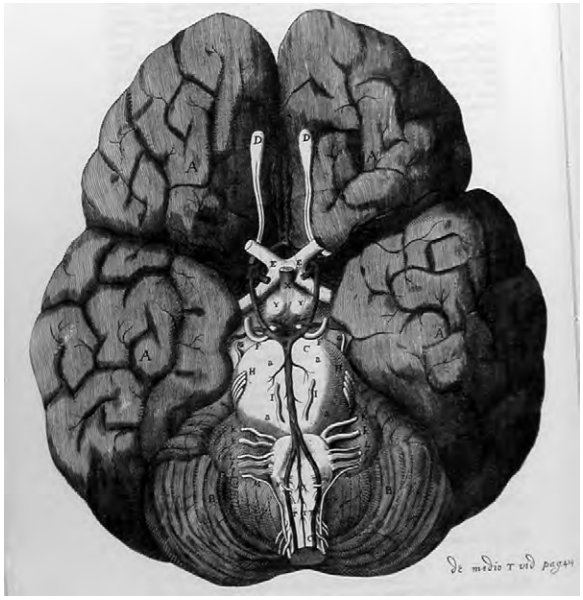


FIGURE 1-37 The "circle of Willis" as described by Willis and drawn by Sir Christopher Wren. (*From Willis T.* Cerebri Anatome: Cui Accessit Nervorum Descriptio et Usus. *London: J Flesher; 1664.)*

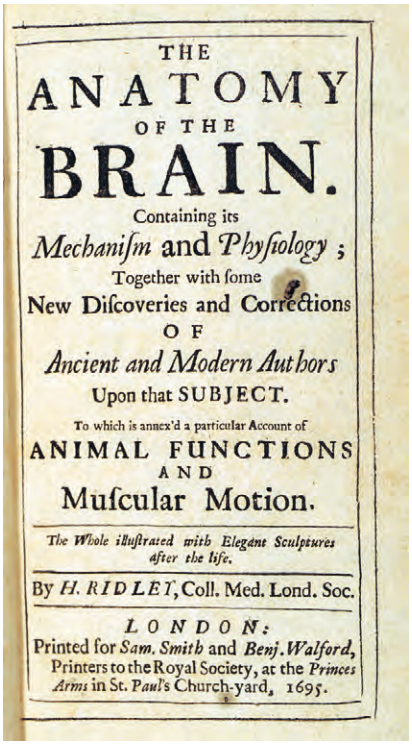

THE
ANATOMY
OF THE
BRAIN.
Containing its
Mechaniſm and *Phyſiology*;
Together with ſome
New Diſcoveries and Corrections
OF
Ancient and Modern Authors
Upon that SUBJECT.
To which is annex'd a particular Account of
ANIMAL FUNCTIONS
AND
Muſcular Motion.
The Whole illuſtrated with Elegant Sculptures after the life.
By *H. RIDLEY*, Coll. Med. Lond. Soc.
LONDON:
Printed for *Sam. Smith* and *Benj. Walford*,
Printers to the Royal Society, at the *Princes Arms* in St. *Paul's* Church-yard, 1695.

FIGURE 1-38 Illustrated here is the title page from Ridley's work on brain anatomy. (*From Ridley H.* The Anatomy of the Brain, Containing Its Mechanisms and Physiology: Together with Some New Discoveries and Corrections of Ancient and Modern Authors upon That Subject. *London: Samuel Smith; 1695.)*

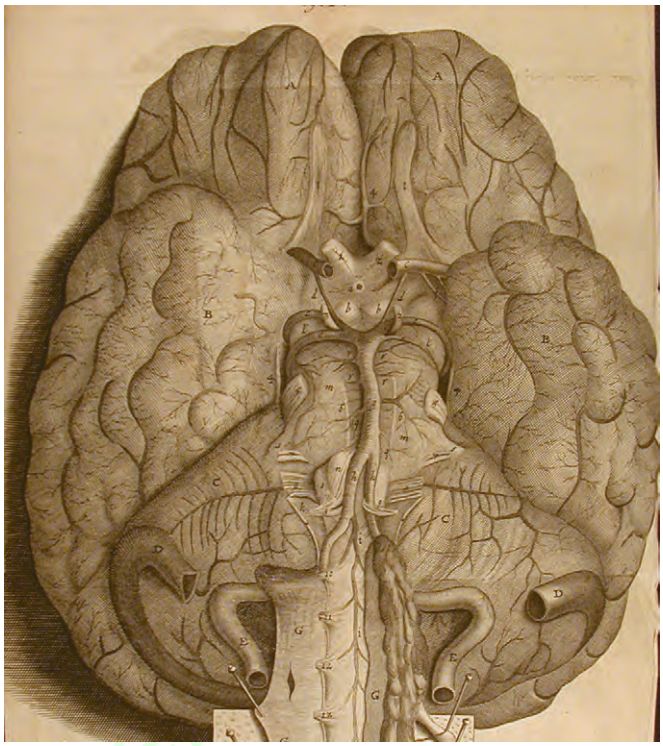


FIGURE 1-39 Ridley's illustration of the anastomotic network at the base of the brain—the "circle of Willis." (*From Ridley H.* The Anatomy of the Brain, Containing Its Mechanisms and Physiology: Together with Some New Discoveries and Corrections of Ancient and Modern Authors upon That Subject. *London: Samuel Smith; 1695.)*

anatomic sense inasmuch as the concept of "neurological" disease had not yet been introduced. Neurology as a noun did not enter general use until Samuel Johnson defined it in his dictionary of 1755.[79] At this point neurology came to be understood as encompassing the entire field of anatomy, function, and physiology. The "circle of Willis" was not uniquely described by Willis; other anatomic descriptions of this circle were provided in other contemporary anatomic publications: Vesling,[80] Casserius,[81] Fallopius,[82] and Humphrey Ridley.[83]

A prominent anatomist at this time and one often overlooked by contemporary writers is Humphry Ridley (1653-1708). Ridley was educated at Merton College, Oxford, England, and at the University of Leyden, where he received his doctorate in medicine in 1679. Ridley produced an important anatomic work on the brain, written in the English vernacular, a work that became widely circulated and influential (Figs. 1-38 and 1-39).

The Anatomy of the Brain. Containing Its Mechanism and Physiology; Together with Some New Discoveries and Corrections of Ancient and Modern Authors upon That Subject was published in London in 1695.[83] As a personality, Ridley remains most elusive. At the time that his work on the brain appeared, many of the classical Greek views of the brain were in vogue. In reviewing 17th century anatomy and medicine we find a movement away from the earlier cell doctrine theory; anatomists were now recognizing the brain as a distinct anatomic entity. By rejecting the "cell doctrine" in which brain function was considered to reside within the ventricles, cerebral function now came to be viewed as a property of the brain.[84,85]

In reviewing Ridley's monograph we find a number of original observations. His description of the "circle of Willis" was even more accurate in details than Willis' and included a more complete anatomic description of both the posterior cerebral artery and the superior cerebellar artery—here described as separate entities. Ridley provided a better demonstration of the principle of anastomotic flow and better elucidated the anastomotic principle of this network with his anatomic studies. To conduct his studies, Ridley had access to recently executed criminals, typically by hanging, which fortuitously caused vascular engorgement of the brain and led to easier identification of the vascular anatomy. Ridley's understanding of the deep nuclei and the anatomy of the posterior fossa was superior to Thomas Willis'. To Ridley we owe one of the earliest descriptions of the arachnoid membrane. It is with interest to note that Ridley still believed that the rete mirabile, a tenacious holdover from Galenic times, was a legitimate anatomic structure and provided a strong argument for it in this monograph. The first accurate description of the fornix and its pathways appears in this monograph. This volume and the work by Willis provided the first scientific anatomic studies of the brain and thereby provided an essential anatomic foundation for future neurosurgeons.

A historical surgical figure often overlooked in neurosurgical history is Wilhelm Fabricius von Hilden (1560-1634). Although Fabricius (Fabry) had received a classical education as a youth, family misfortune did not allow him to obtain a formal medical education. He went on to study in the "lesser field" of surgery and was educated in the apprentice system then prevalent. Fortunately, the teachers whom he trained under were among the finest wound surgeons of the day. Lacking a formal university education but excelling with a surgical apprentice education, he went on to develop a distinguished career in surgery.

Fabricius produced one of the most important surgical works of the 17th century, titled *Observationum et Curationum*, a monograph that included more than 600 surgical cases, along with a number of important and original observations on the brain (Figs. 1-40 and 1-41).[86] Fabricius' observations on the brain and surgery included congenital malformations, skull fractures, and

FIGURE 1-40 Allegorical title page from Fabry's collected works. (*From Fabry W.* Observationum et Curationum Chirurgicarum Centuriae. *Basle: Frankfurt, & Lyons, 1606-1641. A later collected works also contains a number of neurosurgical cases. Opera Observation et Curationum. Frankfurt: Joannis Beyerj; 1646.)*

techniques for bullet extraction, along with original designs for field surgical instruments. He describes operations for intracranial hemorrhage (with cure of insanity), vertebral displacement, congenital hydrocephalus, and an occipital tumor of the newborn (probably an encephalocele). Fabricius carried out trephinations for the treatment of a brain abscess and cure of an old aphasia. He even removed a splinter of metal from the eye with a magnet, a cure that greatly enhanced his reputation.

A monograph in which early and skillfully designed neurosurgical instruments appear is a work by Johann Schultes (Scultetus) of Ulm (1595-1645). With *Armamentarium Chirurgicum XLIII*, Scultetus, also known as Schultes, provides unique and graphic details of neurosurgical instruments, clearly the finest to appear since those published by Berengario in 1518 and Croce in 1573.[37] The illustrations (Figs. 1-42 and 1-43) graphically reveal surgical techniques for treating fractures and dislocations, as well as a variety of bandaging techniques for wounds. The popularity of this surgical work led it to be translated into many languages, including English, and it had a considerable influence on surgery throughout Europe for more than 2 centuries. In reviewing the surgical plates and various operations we find exacting details described, including concepts from antiquity to the present. Interestingly, many of the instruments illustrated by Scultetus remain in use today. His details of surgical operations for injury to the skull and brain are remarkable plebiscite.

Neurosurgical practice continued to evolve in the 17th century. A surgeon who offered interesting technical advances on developing neurosurgical operating skills was John Woodall (ca. 1556?-1643). Woodall was a military surgeon by training and surgeon-general for the East India Company. For the surgeons of the East India Company he compiled a surgical monograph called the *Surgeon's Mate* (1617).[87] In his collected works, published in 1639, he provided a list of surgical instruments and sound advice for a surgical practice.[88] He fabricated a trephine

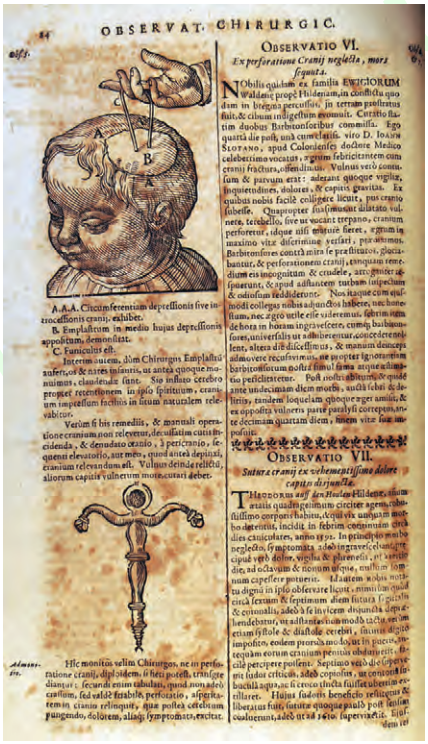


FIGURE 1-41 Illustrations from Fabry's collected works showing a technique for elevating a depressed skull fracture in a child.

FIGURE 1-42 Surgical techniques designed by Scultetus for trephining the skull. (*From Scultetus J.* Χειροπλοθηκη. Armamentarium Chirurgicum XLIII. *Ulm: Balthasar Kühnen; 1655.)*

FIGURE 1-43 Scultetus' techniques for dealing with skull fractures. (*From Scultetus J.* Χειροπλοθηκη. Armamentarium Chirurgicum XLIII. *Ulm: Balthasar Kühnen; 1655.)*

with the unique design of a crown that included a center pin, an innovation that prevented the crown from slipping on a bloody skull (Fig. 1-44). This trephine had a brace added that could be placed against the surgeon's chest for additional support and driving force. This allowed the surgeon to drive the trephine with one hand while the other held the head, all of which could be accomplished on a rolling ship's deck. Woodall, recognizing the ignorance of his contemporary German surgical colleagues, believed that a surgeon should practice trephining on sheep or calf skulls first before performing one on a human head. He comments:

> *The Germane Surgeons use no Trapan, that ever I could see my eight years living among them, though they both speak and write of it. But for as much as it is apparent, the work of a Trapan is very good, I therefore would advise a young Artist to make some experience first upon a calves head, or a sheep's head, till he can well and easily take out a piece of the bone; so shall he the more safely do it to a man without error when occasion is [see page 4].*[88]

An Englishman and Plymouth naval surgeon, James Yonge (1646-1721) was among the first to argue emphatically that "*wounds of the brain are curable*"; Galen had earlier announced, "I have seen the wounded brain heal."[27] Yonge's first surgical text was a small monograph titled *Wounds of the Brain Proved Curable*.[89] He provides a surgical account of a brain operation on a 4-year-old child with extensive compound fractures of the skull from which brain tissue issued forth. The surgery was successful and the child survived, which led Yonge to publish the account. Yonge also reported on more than 60 cases of brain wounds cured that he was able to locate in the older literature, beginning with Galen. The bibliography records the earlier cases that he was able to locate. He comments that this work was written in defense of surgery on the skull and the brain. From the preface,

FIGURE 1-44 **A,** Title page from Woodall's book on military and domestic surgery.[87] **B,** Woodall designed a hand trephine with a series of interchangeable burs along with bone rongeurs. The trephine center pin, Woodall's design, was especially useful on a rolling ship deck when applied to a bloody skull. (*From Woodall J.* The Surgeons Mate. *London: E. Griffin; 1617.)*

I had the good fortune to be a successful chirurgeon to the child, whose case is contained in the following narrative: but I had scarcely wiped my instruments, and put up my plaister-box, before a physician of this town, sneakingly and maliciously endeavor to stifle [my] reputation insinuating that it was impossible to [cure brain wounds] because Wounds of the brain were absolutely mortal.[89]

Yonge is clearly a protagonist of trephination and operating on the skull and brain and demonstrated that it could be done safely.

Other technical innovations for treating head injuries also occurred in this period. Augustin Belloste (1654-1730) describes a technique for repairing "holes in the head" as a result of trauma or trephination with the use of lead plates. Keeping the brain from being exposed to "corrupt air" led to better outcomes in brain surgery.[90]

The 17th century, "the insurgent century," clearly brought a number of advances to the field of brain surgery. Neuroanatomy became an area of intense investigation. Physiologic experimentation, along with the introduction of scientific societies, allowed wide dissemination of the new investigations and scholarly disagreement. Along with this came surgeons with adventurous personalities, individuals who realized that in certain cases brain surgery could be performed safely; not all patients died if you opened the dura mater.

EIGHTEENTH CENTURY—AN ENLIGHTENED PERIOD FOR NEUROSURGERY

The 17th century clearly provided a sound scientific and anatomic basis for neurosurgery and the neurosciences. The 18th century continued this trend and was a period of intense activity in the medical and scientific world. Chemistry as a true science was being propelled forward in the works of Priestley, Lavoisier, Volta, Watt, and others. Clinical bedside medicine, essentially lost since the Byzantine and Islamic era, was reintroduced by Thomas Sydenham (1624-1689), William Cullen (1710-1740), and Herman Boerhaave (1668-1738). With bedside examination came a number of original and new tools for diagnostic examination. Of particular note are the contributions of Leopold Auenbrugger's (1722-1809) introduction of percussion of the chest, William Withering's (1741-1799) pharmacologic introduction of use of digitalis for cardiac problems, and William Jenner's (1749-1823) use of cowpox inoculation for smallpox, which helped eliminate a world scourge. In addition, for the first time the focus for the surgeon is switching from the skull to the brain. This new direction and change in approach to the neurological status of the patient marked a major paradigm shift that represented a very important step toward the origins of a separate surgical discipline of neurosurgery

Judgment in distinguishing, and ability in treating diseases, are not to be attained by a transient cursory view of them; merely running round an Hospital for a few months, or reading a general system of surgery, will not form a compleat practitioner: the man, who aims at that character, must take notice of many little things, which the inattentive pass over, and which cannot be remarked by writers; he must accustom himself to see, and to think for himself; and must regard the rules laid down by authors, as the outlines only of a piece, which he is to fill up and finish: books may give general ideas, but practice, and medication, must make him adroit and discerning; without these, his reading may possibly keep him clear of very gross blunders, but he will still remain injudicious, and inexpert [from preface x-xi, Observations, London 1760].[91]

One of the giants in surgery was Percivall Pott (1714-1788), considered by many historians to be the greatest English surgeon of the 18th century. His list of contributions, several of which apply to neurosurgery, is enormous. His work *Remarks on That Kind of Palsy of the Lower Limbs Found to Accompany a Curvature of the Spine* (London, 1779) describes the disease entity now known as Pott's disease (i.e., tuberculous caries of the spine).[92] His clinical descriptions clearly describe the gibbus and tuberculous infection of the spine. Surprisingly, Pott failed to associate the relationship between the deformity and paralysis. An osteomyelitic infection of the scalp and skull in which pus collects under the pericranium is now called *Pott's puffy tumor*. Pott argued that these lesions should be opened and drained (Figs. 1-45 and 1-46).

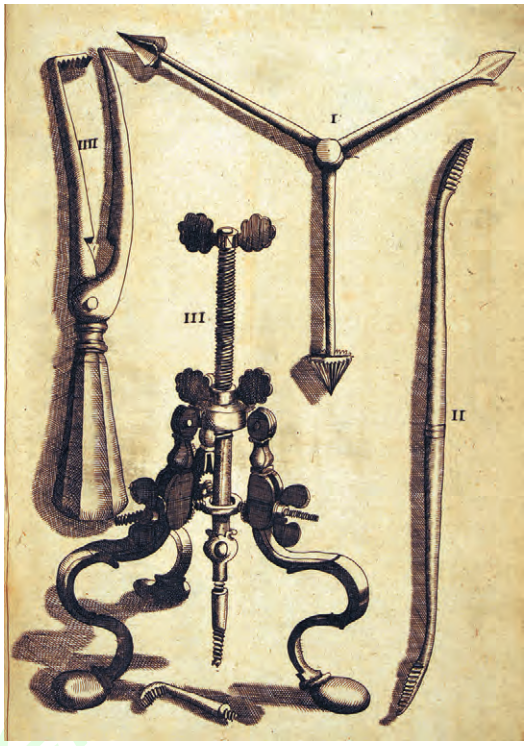


FIGURE 1-45 Pott's trephine for elevating a depressed skull fracture.

Eighteenth century surgeons generated much discussion over the surgical practice of trephination. *To trephine or not to trephine*—Pott was a strong proponent of intervention. In his classic work on head injury (London, 1760), he clearly appreciated that the clinical findings of head injury were due to injury to the brain and not the skull.[93] Pott studied head injuries and began to

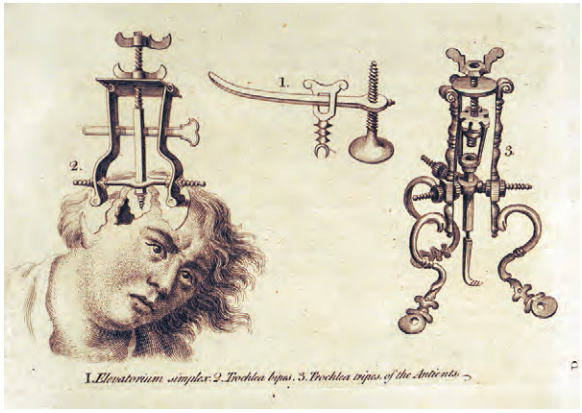


FIGURE 1-46 Trephination instrumentation as designed by Pott. (*From Pott P.* Remarks on That Kind of Palsy of the Lower Limbs Found to Accompany a Curvature of the Spine. *London: J. Johnson; 1779.)*

differentiate between "compression" and "concussion" injury of the brain. The following clinical description from his head injury book outlines some of his views:

> *The reasons for trepanning in these cases are, first, the immediate relief of present symptoms arising from pressure of extravasated fluid; or second, the discharge of matter formed between the skull and dura mater, in consequence of inflammation; or third, the prevention of such mischief, as experience has shown may most probably be expected from such kind of violence offered to the last mentioned membrane.*
>
> *In the mere fracture without depression of bone, or the appearance of such symptoms as indicate commotion, extravasation, or inflammation, it is used as a preventative, and therefore is a matter of choice, more than immediate necessity.*[93]

Pott clearly developed his outstanding reputation by his astute clinical observations and bedside treatment. His management of head injuries makes him the first of the modern neurosurgeons. His caveats, presented in the preface to his work on head injury, hold today.

The most significant development in 18th century writings on neurosurgical topics was the gradual recognition of the effects of trauma on brain function rather than just the skull. Several French surgeons drew a clear-cut distinction between the loss of consciousness accompanying a blow to the head and the drowsiness that appeared later. The former came to be recognized as a direct result of cerebral concussion, and the latter, after a lucid interval, came to be accepted as being due to a collection of blood producing compression of the brain. This idea was introduced by Jean Louis Petit (1674-1750), the leading surgeon in Paris in the first half of the 18th century, in a series of lectures that he gave in Paris.[94] The realization that delayed loss of consciousness could serve as an indication for surgical intervention is one of the epochal events that mark the origins of neurosurgery as a discipline dealing with alterations in brain function and not just superficial injuries to the skull. It was a major conceptual change in an approach that had been followed for 2000 years and marks an important turning point in surgical thinking.

One of the earliest descriptions of the "lucid interval" in head injury was provided by Henri Francosi Le Dran (1685-1770). Le Dran was both an anatomist and surgeon who amassed a large surgical experience by serving as the chief surgeon to the French Army. Le Dran established a very popular school of anatomy in Paris that attracted students from all over Europe. *Observations de Chirurgie* [95] (Fig. 1-47) reveals a skilled surgeon with a wide variety of surgical talents. This work became Le Dran's most popular surgical text and was reprinted several times and translated into English in 1749. It is a broad review of surgery, but most important to us are his views on surgery on the head. Le Dran details the concept of the "lucid interval" after a head injury and then attributes it most commonly to an epidural hematoma.

A remarkable and talented figure in English medicine and surgery and a student of Percivall Pott's was John Hunter (1728-1793). Many writers consider Hunter equally as skilled as Pott, but his additional work in anatomy, pathology, physiology, and surgery led him to make a number of important contributions.[96] Hunter, often referred to as the founder of experimental and surgical pathology, spent most of his career at St. George's Hospital in London. He was trained in the apprentice style and had minimal formal education. He began his training under his older brother William Hunter and spent time with William Cheselden, talented mentors. As a surgeon, Hunter was an atypical figure for this time in that he approached the field of surgery in a more practical manner and at the same time added a bench side experimental touch. In *A Treatise on the Blood, Inflammation, and Gun-Shot Wounds* (London, 1794),[97] Hunter drew on his years of military experience and wrote an important work on the management of gunshot wounds. He did not offer much on neurosurgery; the section on skull fractures occupied only one paragraph. In understanding vascular disorders, Hunter described the concept of collateral circulation. His circulation studies were conducted on a buck whose carotid artery was tied off to see the effect on the antler, but no ill effect was noted; the explanation was the development of collateral circulation, which he had now determined anatomically. Hunter later applied these concepts to the treatment of popliteal aneurysms, previously treated by amputation; he tied off the artery and realized that collateral circulation would develop. He was adroit at posing questions raised by his clinical experience, performing animal experiments to answer the questions, and integrating his clinical and scientific results into the best available treatment. He anatomically dissected a case of craniopagus parasiticus, a set of twins from India in which one child was fully formed and the other twin had only the head. The incomplete twin would show emotion and move the lip and mouth during eating (Fig. 1-48).[98] Hunter is also remembered as a devoted student of anatomic curiosities and would go to great lengths, sometimes nefariously, to obtained specimens. The most famous case was an Irish giant whom Harvey Cushing later determined had acromegaly. The Irish giant knew of Hunter's interest in him and went to great lengths to avoid his laboratory after death. However, the Irish giant became part of the Hunterian museum, which contained more than 13,000 specimens and is now part of the Royal College of Surgeons pathologic collection, a direct donation by Hunter.

Following Hunter was a pupil of his, John Abernethy (1764-1831), who was also a talented anatomist and surgeon. For American surgeons, Abernethy is remembered for publishing the first book in America devoted to a neurosurgical topic.[99] So popular was Abernethy as a lecturer that the governors of St. Bartholomew's Hospital built an anatomic theater for him, a place of training sought out by the brighter students of the period. Abernethy eventually went back to Scotland, his country of birth,

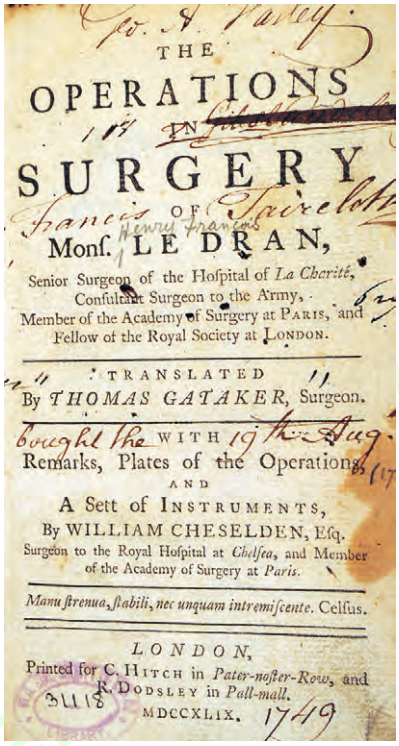

THE
OPERATIONS
IN
SURGERY.
OF
Monſ. LE DRAN,
Senior Surgeon of the Hoſpital of *La Charité*,
Conſultant Surgeon to the Army,
Member of the Academy of Surgery at PARIS, and
Fellow of the Royal Society at LONDON.
TRANSLATED
By THOMAS GATAKER, Surgeon.
WITH
Remarks, Plates of the Operations,
AND
A Sett of INSTRUMENTS,
By WILLIAM CHESELDEN, Eſq.
Surgeon to the Royal Hoſpital at *Chelſea*, and Member of the Academy of Surgery at *Paris*.
Manu ſtrenua, ſtabili, nec unquam intremiſcente. Celſus.
LONDON,
Printed for C. HITCH in *Pater-noſter-Row*, and R. DODSLEY in *Pall-mall*.
MDCCXLIX.

FIGURE 1-47 The title page from the English edition of Le Dran's textbook on surgery, Paris 1749.

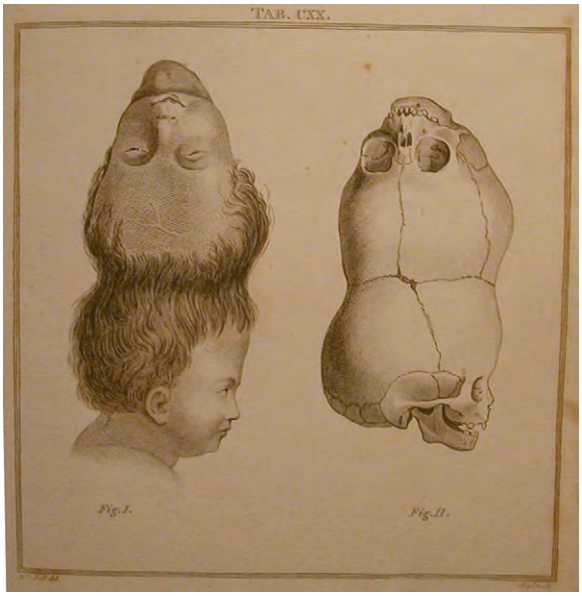


FIGURE 1-48 A set of craniopagus parasiticus twins originally described by John Hunter in manuscript and later published in Evard Home's collected work. The parasitic twin had some emotion and moved the face when the other twin was eating. They died after a venomous snakebite at the age of 4 years. (*From Hunter J.* A Treatise on the Blood, Inflammation, and Gun-Shot Wounds. *London: J. Richards; 1794.)*

FIGURE 1-49 Late 17th century hand trephine set with interchangeable bits, elevators, and a bone brush to remove the accumulated bone dust. The trephines are of two different sizes, one for pediatric and one for adult patients.

and settled in Edinburgh to establish a general practice. He continued to develop a large apprenticeship program with students coming from far and wide. His contributions to neurosurgery included one of the earliest treatments of neuralgia of the arm; he performed a neurectomy in 1793 that provided instant relief to the patient.[100] Later, the patient regained sensation in the hand, thus showing that there had been successful reunion of the nerve. Abernethy was an early advocate of ligating the common carotid artery for a cerebral hemorrhage. He later published his writings on the brain in an important work called *Pathological and Practical Researches on Diseases of the Brain and Spinal Cord* (1828).[101] This work contains more than 150 cases of various neurological and neuropathologic conditions of the brain, spinal cord, and peripheral nerves (Figs. 1-49 and 1-50).

A contemporary of Abernethy was Benjamin Bell (1749-1806), among the most prominent and successful surgeons in 18th-century Edinburgh. Bell was a compassionate surgeon and among the first to emphasize the importance of reducing pain during surgery. Bell published a popular textbook of surgery, *A System of Surgery*.[102] This book was widely read because of its clarity and precision. In reviewing his section on head injury, there is an important discussion on the differentiation of concussion, compression, and inflammation of the brain—each requiring different modes of treatment.[103] Bell was a remarkably aggressive surgeon when it came to the brain; he stressed the importance of relieving compression (i.e., by trephination) of the brain whether it be caused by a depressed skull fracture or pressure from pus or blood. The concept of an epidural hematoma and its symptoms were appreciated by Bell; he argued for rapid and prompt evacuation.

> *Affections of the Brain from external violence, often induce a very complicated set of symptoms; are attended with imminent danger, and give much embarrassment to practitioners: Accordingly, both with respect to the hazard with which they are attended, and the difficulty that we meet with in the cure, there is perhaps no class of diseases to be compared with them.*

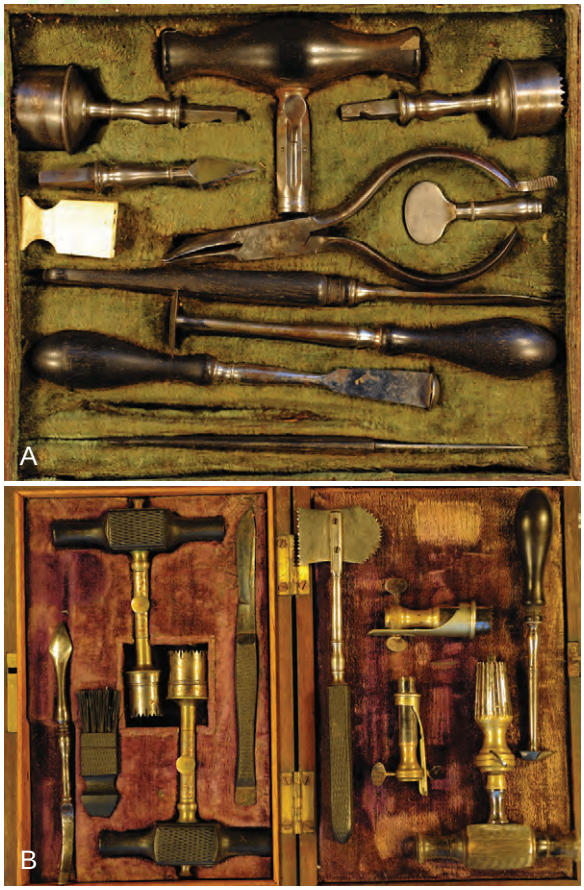


FIGURE 1-50 **A,** An example of an early 19th century traveling trephine set. This set was designed to be compact and easy to carry with changeable burs of different size. This trephine set shows considerable use. **B,** Another example of a traveling trephine set, in this case with additional different styles of hand trephines. On the right side is a Hey saw used for making craniectomies. The trephine bits on the right are more elaborate with adjustable center pins.

Wounds and bruises of the head, which at first exhibit no marks of danger, often induce a train of symptoms which elude the skill of the most experienced practitioner; and, without admitting of any mitigation, proceed to a fatal period, ending only the death of the patient [Volume 3, Chapter X, Section I].[104]

His discussion of the symptoms of brain compression from external violence is classic.

A great variety of symptoms indicating a compressed state of the brain [with] the most frequent, as well as the most remarkable, are the following: Giddiness; dimness of sight; stupefaction; lots of voluntary motion; vomiting; an apoplectic stertor in the breathing; convulsive tremors in different muscles; a dilated state of the pupils, even when the eyes are exposed to a clear light; paralysis of different parts, especially of the side of the body opposite to the injured part of the head; involuntary evacuation of the urine and faeces; an oppressed, and in many case an irregular pulse [Volume 3, Chapter X, Section III].[105]

Bell's discussion of management of head injury by trephination, along with types of incisions, remains among the best from this period. He was also among the first to note that hydrocephalus was often associated with spina bifida. His treatment of a myelomeningocele involved placing a ligature around the base of the myelomeningocele sac and allowing it to slough off; he also noted that the outcome was almost always poor. In reviewing Bell's writings on the brain, one realizes why it was one of the most important and popular surgical works in this era.

In 1709 a small monograph by Daniel Turner (1667-1741) appeared.[106] The book was titled *A Remarkable Case in Surgery: Wherein an Account is Given of an Uncommon Fracture and Depression of the Skull, in a Child about Six Years Old; Accompanied with a Large Abscess or Aposteme upon the Brain.* This monograph provides a contemporary view of an 18th century surgeon and the concerns of trephining the brain (Fig. 1-51).

Turner's case is disturbing to read because it is written in the frank and verbose style of this period. Turner was "*called in much hast, to a Child about the Age of Six Years, wounded by a Catstick* [thrown by a youth who missed his aim] *unfortunately struck the Child over the Head, and knock'd him down. He was taken up for dead and continued speechless for some time.*" On examination of the head, Turner found a considerable depression and thought that the child was in great danger. He sent for the barber to shave the head; while waiting for the barber he performed the common practice of opening a vein in the arm to bleed the child and taking about 6 oz. The patient regained consciousness, complained of a headache, and vomited. With this good response Turner decided to wait on surgery. The next day he found the child still vomiting, restless, and hot, so he decided on exploration of the wound. Through a typical X incision he found bone driven into the brain—"*the Bones were beat thro' both meninges into the substance of the brain.*" Upon elevating the bone he found "*a cavity sufficient to contain near two Ounces of Liquor.*" Postoperatively the patient was awake with "*a quick pulse, thirst and headache but no vomiting. He was very sensible.*" He visited the child the following day and still found him feverish but without other symptoms. He removed the dressings and saw the extent of the fracture, which he now realized had been only partially elevated. Turner pulled out a trepan, surveyed the situation, and decided where it was safest to trephine. He removed what bone he thought was safe to remove and applied a clyster. The next day Turner called in a consultant, a Mr. Warden Herenden, to whom he showed the wound and the magnitude of the operation. Herenden was impressed with the extent of surgery and the condition of the wound and noted that despite all this the patient complained of only a headache and was able "*to walk about the Chamber.*"

This child was to undergo several explorations for removal and drainage of pus. Surgical cannulas were placed for drainage, the wound was carefully attended to, but the patient nonetheless died: "*Thus did this little Hero, of truly Manly Courage, who had struggled under, and got thro' so many Difficulties, at last decease, after Fourscore and four days.*"

So despite 3 months of aggressive medical treatment and multiple explorations, the child expired. This poignant treatise perhaps gives the best example of an 18th century effort at dealing with a head injury. Turner concluded

That wounds of the brain, are not always mortal [see page 52][106]

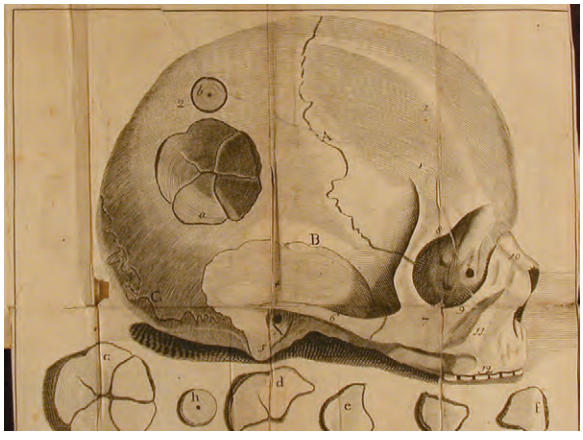
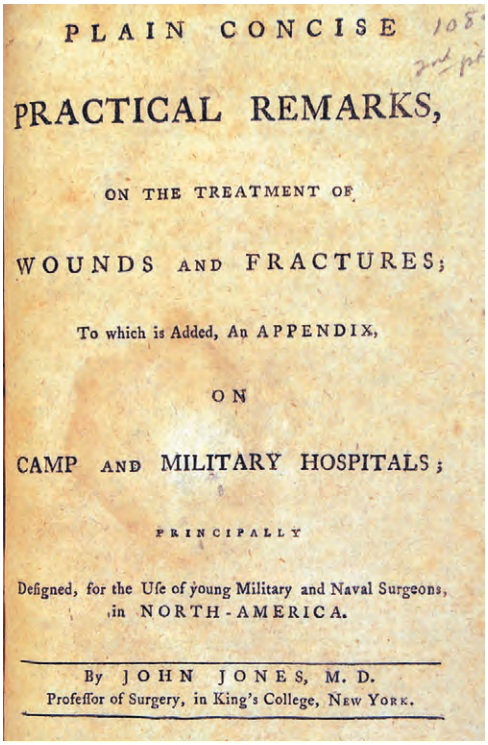


FIGURE 1-51 An illustration from Turner's book on "a remarkable case in surgery," where he demonstrates the skull fracture and the elevation of the injury described in the text. (*From Turner D.* A Remarkable Case in Surgery: Wherein an Account is Given of an Uncommon Fracture and Depression of the Skull, in a Child about Six Years old; Accompanied with a Large Abscess or Aposteme upon the Brain. With Other Practical Observations and Useful Reflections Thereupon. Also an exact Draught of the Case, annex'd. And for the Entertainment of the Senior, but Instruction of the Junior Practitioners, Communicated. *London: R. Parker; 1709.)*

An American surgeon who made an interesting contribution to neurosurgery was John Jones (1729-1791). In his monograph published in New York in 1775, this Revolutionary War surgeon provided the first American textbook on surgery (Fig. 1-52).[107] Jones was educated in Europe; studied under Pott, Hunter, Monro, Petit, and Le Dran; and carried this education back to the United States. Jones was among the first faculty to form the first medical school in America, the University of Pennsylvania, Philadelphia. Jones was also one of the founders of New York Hospital. His monograph on surgery became the handbook of surgery for Revolutionary War surgeons. His views and techniques on trephination clearly reflect the views of his European teachers, especially Pott, Le Dran, and Petit.

In Europe there were a number of important figures refining the art and skills of surgery. These surgical figures were important in leading surgical treatment away from the more common itinerant charlatan and barber-surgeon, mostly ignorant charm and relic dispensers.

One of the most popular surgical textbooks of this century was published by a German surgeon—Lorenz Heister (1683-1758) (Fig. 1-53). Heister was educated as both a surgeon and anatomist, a now common theme. He began his lectures in Latin, but because his students were so ignorant, he changed them to German. He went on to publish his first textbook in the vernacular German.[108] So popular was the text that it was subsequently translated into a number of languages, including English, and circulated widely in Europe and England.[109] The wide range of surgical knowledge that it communicated and its many practical surgical illustrations, bandaging techniques, and surgical techniques made it a well-used text. Heister would treat a head



PLAIN CONCISE

PRACTICAL REMARKS,

ON THE TREATMENT OF

WOUNDS AND FRACTURES;

To which is Added, An APPENDIX,

ON

CAMP AND MILITARY HOSPITALS;

PRINCIPALLY

Designed, for the Use of young Military and Naval Surgeons, in NORTH-AMERICA.

By JOHN JONES, M. D.
Professor of Surgery, in King's College, New York.

FIGURE 1-52 Title page from the first American textbook printed on surgery in the colonies—a book that became the handbook for American Revolutionary War surgeons. (*From Jones J.* Plain Concise Practical Remarks, on the Treatment of Wounds and Fractures; to Which Is Added, an Appendix, on Camp and Military Hospitals; Principally Designed, for the Use of Young Military and Naval Surgeons, in North-America. *New York: John Holt; 1775.)*

injury, but in accordance with earlier, more conservative views, he thought that trephination should be restricted to cases of fracture associated with depression. In wounds involving only concussion and contusion, he thought that trephination was too dangerous. When one realizes that Heister was practicing during the pre-Lister era, a period of very high risk for infection and injury to the brain, he might have been the more pragmatic surgeon.

FIGURE 1-53 Illustration from Heister's popular 18th century textbook on surgery illustrating trephination instruments and tools for elevating depressed skull fractures. (*From Heister L.* Chirurgie in welcher alles was zur Wund-Artzney gehöret, nach der neuesten und besten Art. *Nümberg: J. Hoffmann; 1718.)*

> *XXVII. But when the Cranium is so depressed, whether in Adults or Infants, as to suffer a Fracture, or Division of its Parts, it must instantly be relieved: the Part depressed, which adheres, after cleaning the Wound, must be restored to its Place, what is separated must be removed, and the extravasated Blood be drawn off through the Aperture [he goes on to argue against the use of "sneezing", i.e., the Valsalva maneuver, in elevating depressed fractures]—the ill Consequences that attend this Practice are so grievous, that in my Opinion it ought to be rejected [Book I, Chapter XIV, page 100].*[110]

Heister popularized a number of techniques that proved helpful to contemporary surgeons. To control scalp hemorrhage, he used a "crooked needle and thread" that was weaved in and out of the scalp and then drawn tight. An astute observer, he pointed out that when the assistant applied pressure to the edges of the skin, bleeding could be markedly reduced. He was aggressive in the management of spine injuries. He would operate and expose the fractured vertebra and then remove the fragments that had damaged the spinal marrow (the spinal cord); he recognized that grave outcomes of such attempts were not uncommon and that the surgeon should be prepared for that.

An early and successful treatment of a brain abscess was accomplished by François-Sauveur Morand (1697-1773). In Morand's patient, a monk, otitis and subsequently mastoiditis developed and led to a temporal brain abscess.[111] Morand trephined over the carious bone and discovered pus. He placed a catgut wick into the open surgical wound, but it continued to drain. He reopened the wound, opened the dura through a cruciate incision, and found a brain abscess. He explored the abscess with his finger, removed as much of the contents as he could, and then instilled balsam and turpentine into the cavity. He placed a silver tube for drainage, and as the wound healed, he slowly withdrew the tube. The abscess healed, the patient survived, and he reported this case as successful treatment of a brain abscess.

The Neapolitan physician Domenico Cotugno (1736-1822) published a small monograph of 100 pages, *De Ischiade Nervosa Commentarius* (Naples, 1764), in which are given the first descriptions of CSF and sciatica (Fig. 1-54).[112] Cotugno performed a number of experiments on the bodies of 20 adults. Using a lumbar puncture technique, he was able to demonstrate the characteristics of CSF. In *De Ischiade Nervosa Commentarius*, he demonstrated the "nervous" origin of sciatica and differentiated it from arthritis, the common explanation prevalent at that time. He discovered the pathways of CSF and showed that it circulated in the pia-arachnoid interstices and flowed throughout the brain and spinal cord via the aqueducts and convexities. Cotugno also described hydrocephalus ex vacuo, the type of hydrocephalus seen with cerebral atrophy.

A popular and skilled French military surgeon, Louis Sebastian Saucerotte (1741-1814) (also listed as Nicolas) was at one time surgeon for the King of Poland and then a surgeon in various French Army units. As has often been the case in the history of neurological surgery, the occasion to deal with war injuries provided the most training and insight into the management of head injury. Saucerotte reintroduced the concept of the contrecoup injury, lost since antiquity. In his surgical textbook *Mélanges de Chirurgie* (Paris 1801),[113] he describes in detail a series of intracranial injuries and their symptoms, including compression of the brain by blood clot. Saucerotte described a case of ataxia caused by a cerebellar lesion, including opisthotonos and rolling of the eyes. He divided the brain into "areas" of injury and pointed out that areas of severe injury are those at the base of the brain whereas injuries to the forebrain are the best tolerated. He also contributed one of the earliest clinical descriptions of acromegaly.

The close of the 18th century brings remarkable change in philosophies of surgery on the brain. Surgeons are being much more aggressive in the management of head injury. The clinical symptoms associated with brain injury are better recognized.

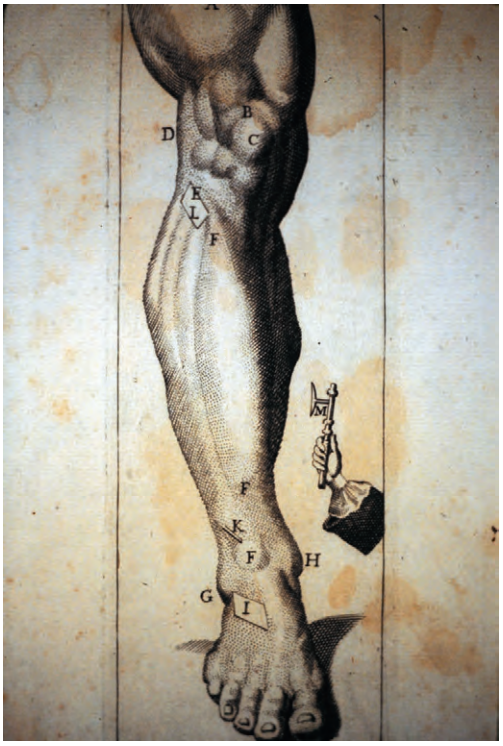


FIGURE 1-54 Cotugno was the first to describe cerebrospinal fluid and the first to demonstrate the "nervous" origins of sciatica—differentiating it from the then common view that sciatica was secondary to arthritis. In this plate from his work Cotugno is demonstrating the distribution of "sciatica." (*From Cotugno D.* De Ischiade Nervosa Commentarius. *Neapoli, apud Frat. Simonios. 1764.)*

Anatomic concepts such as the circulation of CSF are being understood. However, the surgeon still lacked understanding of cerebral localization, methods to treat surgical infection, and the ability to provide insensibility to pain during surgery. The role of trephination for head injuries was being fiercely debated. There was a backlash against it because of the inability to distinguish between the lack of efficacy of the operation and the introduction of infection by the surgical procedure. As a result of iatrogenic infection, the outcomes without surgery often seemed better than those with intervention. With the 19th century we see several important events: cerebral localization, anesthesia, and antisepsis, critical developments for the origins of modern neurosurgery.

NINETEENTH CENTURY—INCUNABULA PERIOD OF MODERN NEUROSURGERY

The origins of what we would now call modern neurosurgery began with three important developments in the 19th century. The first was the introduction of anesthesia, which provided patients freedom from pain during surgery. The second was the introduction of cerebral localization (neurological signs and symptoms), which helped strengthen the framework in which the surgeon could reach a diagnosis and plan the operative approach. The third was the introduction of antisepsis and aseptic technique, which enabled the surgeon to operate with a reduced risk for perioperative complications as a result of infection.

A medical and surgical giant in this period was Sir Charles Bell (1774-1842), a Scottish surgeon and anatomist. Bell was educated at the University of Edinburgh and spent most of his professional career in London. He is remembered for his many

FIGURE 1-55 This hand-drawn illustration by Bell demonstrates a severe open head injury with a depressed skull fracture. A series of trephinations have been performed, and the extracted bone can be seen in the lower left of the image. (*From Bell C.* Illustrations of the Great Operations of Surgery. *London: Printed for Longman, et. al.; 1821. see preface leaf iv.)*

contributions to the neurosciences, including differentiation of the motor and sensory components of the spinal nerve root. Bell wrote a number of works on surgery, many of which were beautifully illustrated with his own drawings. These surgical drawings remain unrivaled in detail, accuracy, and beauty (Figs. 1-55 and 1-56).

> *If a drawing of all that we see in an operation, be an imperfect demonstration, so is the lesson of an operation performed on the dead body imperfect, for the circumstances most essential to know, cannot be presented there: so is the actual operation on the living body an imperfect demonstration, from the partial and rapid view which the spectator obtains. And, finally, as to description, words alone will never inform the young Surgeon of the things most necessary to a safe operation.*[114]

Bell provided a skillful contemporary account of trephination as practiced in 1821:

> *Let the bed or couch on which the patient is lying be turned to the light—have the head shaved—put a wax-cloth on the pillow—let the pillow be firm, to support the patient's head. Put tow [sic] or sponge by the*

FIGURE 1-56 Bell thought that there were areas where one could trephine safely and areas that were dangerous. In this skull Bell has outlined areas that are safe; the opinions he offers here differ little from Hippocrates' original writings. (*From Bell C.* Illustrations of the Great Operations of Surgery. *London: Printed for Longman, et. al.; 1821. see preface leaf iv.)*

side of the head—let there be a stout assistant to hold the patient's head firmly, and let others put their hands on his arms and knees. The surgeon will expect the instruments to be handed to him in this succession—the scalpel; the rasparatory; the trephine; the brush, the quill, and probe, from time to time; the elevator, the forceps, the lenticular.[115]

Combined with his detailed description of trephination is a discussion of the techniques and pitfalls to avoid. The hand-colored illustrations that accompany the text are dramatic in their detail and are designed to assist the surgeon in mastering the techniques. Bell's work is important and unique in providing illustrations on detailed neurosurgical technique.

Over the previous centuries surgeons tried various methods of reducing sensibility to pain but had minimal success. The use of mandrake, *Cannabis*, opium and other narcotics, the "soporific sponge" (saturated with opium), and alcohol had all been tried. In 1844, Horace Wells (1815-1848), a dentist in Hartford, Connecticut, introduced the use of nitrous oxide for dental procedures and for the first time had a good anesthetic result.[116] Unfortunately, the death of one of his patients from what was probably an overdose of the anesthetic stopped him from investigating further. In Boston, another early investigator, W. T. G. Morton (1819-1868), also a dentist and early collaborator with Wells, persuaded a surgeon, Dr. J. C. Warren (1778-1856), to use ether to induce anesthesia. On October 16, 1846, Warren did so and produced a state of insensibility in a patient during which a vascular tumor of the submaxillary region was removed.[117] In the United Kingdom, another surgeon, James Y. Simpson (1811-1870), was using chloroform, which had just been introduced in 1847 as an anesthetic agent.[118] The contemporary literature was full of arguments on which was the best agent. Morton patented the ether technique and then approached the U.S. Congress to seek compensation for his discovery of ether and its use in surgery. The result of this advance was the first opportunity for a surgeon to operate on a patient without the need for heavy restraints or the necessity of operating at breakneck speed. Patients gained freedom from pain during the procedure, as well as now a lack of fear of surgery—developments whose importance cannot be overestimated in a surgical practice and in particular in the treatment of brain lesions.

One can easily appreciate the great trepidation with which the early surgeon approached either a skull or brain injury. Even with the best surgical technique, the surgeon would have the patient die postoperatively of suppuration and infection. Fevers, purulent material, brain abscesses, and draining wounds all led the best surgeons to suffer defeat. No surgeon could hope to invade or open the dura mater without inviting disaster until the risk for operative infection could be reduced. The first significant change came about when Lord Lister (1827-1912), using concepts developed by medical practitioners, introduced antisepsis into the operating room.[119,120] In a different operating arena, Oliver Wendell Holmes (1809-1894) and I. G. Semmelweis (1818-1865) first showed that it was the contaminated hands of the obstetrician that spread puerperal fever, a devastating infection occurring in women during delivery.[121,122] Holmes and Semmelweiss argued for hand washing between cases, a concept that was bitterly debated at the time. It is beneficial to examine the typical mid-19th century obstetrician. The obstetrician typically entered an operating room wearing with pride a black cloth coat soaked in old blood and the grime from earlier deliveries. The table on which the soon to be born baby was to be delivered was rarely cleaned, must less sterilized. These conditions led to the spread of multiple organisms on hands, instruments, and table surfaces, and many women died in childbirth of puerperal fever. The infection and contagion concepts developed by Louis Pasteur (1822-1895) and Robert Koch (1843-1910) and their introduction of antisepsis and aseptic technique revolutionized surgery. By adopting these sterile techniques, a surgeon operating on the brain or the skull, or both, with aseptic technique in a clean operating theater could complete the surgery with a significant reduction in surgical infection.

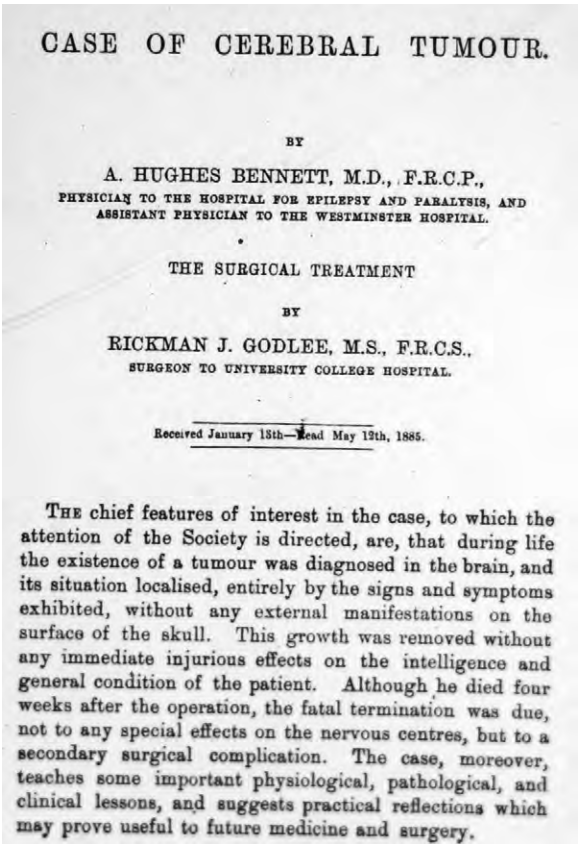
To diagnose a brain lesion or to localize a brain injury was not meaningful until the concept of neurological localization was formulated. In the 1860s, several investigators, including G. T. Fritsch (1838-1891) and E. Hitzig (1838-1907), as well as Paul Broca (1824-1880), first introduced the concept that each part of the brain corresponded to a particular function.[123-125] In monitoring a patient with an expressive aphasia, Broca clarified localization of speech in the brain at an autopsy in 1861.[124] Later, Carl Wernicke (1848-1904) identified a different area of the brain where speech was associated with conduction defects.[126] These studies led to an explosion of research on the brain, with brain function being further investigated with the use of electrical stimulation in work pioneered by David Ferrier (1843-1928)[127] and John Hughlings Jackson (1835-1911).[128] Jackson is considered the founder of modern neurology. Both these physicians demonstrated important anatomic areas of brain function by electrical studies and developed an understanding of epilepsy. The field of neurology received its greatest impetus in this period. The neurological examination now became a rigorous study designed to uncover subtle anatomic and physiologic findings, which in turn provided a surgical map for surgeons to plan incisions and exploration.

The surgical personalities of the 19th century were varied and talented. Until the end of the 19th century, neurosurgery was not specialized, with operations still being performed by general surgeons. By the middle of the 19th century, we find the distinction between brain concussion and compression gradually being accepted. In 1841, William Sharp (1805-1896) published a short monograph titled *Practical Observations on Injuries of the Head*.[129] He provides a modern definition of concussion as "a loss of function without change in structure." Sharp advises against trephining in patients with concussion because there is no extravasation of blood to remove and it will not prevent inflammation. He notes that the middle meningeal artery is the usual source of an epidural hematoma and concludes his monograph with a review of Percivall Pott's earlier surgical experience with head injuries; in Pott's 43 reported cases, 29 were operated on with 17 recoveries and 12 deaths, whereas in the 14 patients who were not operated on, 2 recovered and 12 died.

Sir Jonathan Hutchinson (1828-1913) provided an important chapter in the acceptance of neurological signs and symptoms as indicators for surgical intervention. In 1867, the same year that Lister published his first papers on the role of antisepsis in surgery, Hutchinson published a series of papers on brain compression that introduced a new diagnostic sign for head injury.[130] His recognition of third nerve paralysis remains one of the most useful signs of head injury and increased intracranial pressure. Coupled with the recognition of a lucid interval after head trauma, it provided an important neurological sign that enabled surgeons to recognize the need for trephining. Hutchinson also argued that the finding of a fixed and dilated pupil was likely to be on the side of the hematoma. For the first time in 350 years, since a 16th century artist (Gerzdorf,[62] 1517) recorded this observation, the mechanism and significance of this finding were established.

Hutchinson wrote: "from the position of the clot there can be little doubt that the third nerve is compressed and thus, the dilatation of the pupil is explained. These two cases, so exactly parallel, seem to supply us with a new and very valuable symptom indicative of effusion of blood in this situation."

He went on modestly to note: "nor can we boast of having learnt much which may aid us in the diagnosis of future cases, with the one exception of having discovered the meaning of the one dilated pupil. This point we will store up carefully for future use."[130]



CASE OF CEREBRAL TUMOUR.

BY

A. HUGHES BENNETT, M.D., F.R.C.P.,
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THE SURGICAL TREATMENT

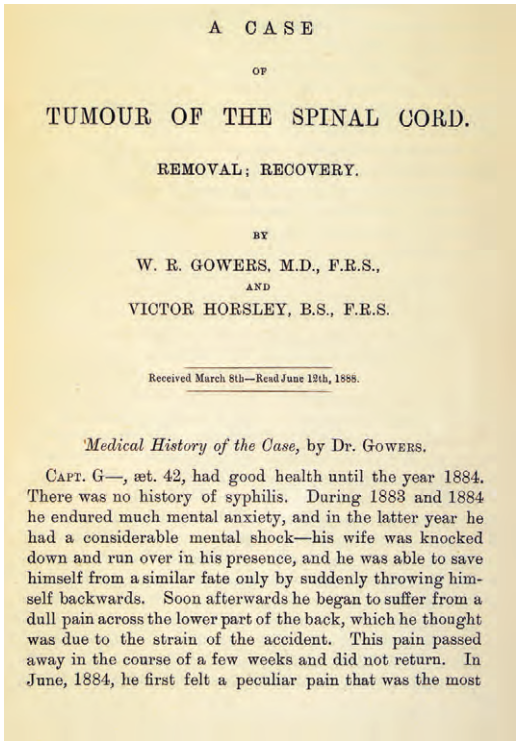
BY

RICKMAN J. GODLEE, M.S., F.R.C.S.,
SURGEON TO UNIVERSITY COLLEGE HOSPITAL.

Received January 13th—Read May 13th, 1885.

THE chief features of interest in the case, to which the attention of the Society is directed, are, that during life the existence of a tumour was diagnosed in the brain, and its situation localised, entirely by the signs and symptoms exhibited, without any external manifestations on the surface of the skull. This growth was removed without any immediate injurious effects on the intelligence and general condition of the patient. Although he died four weeks after the operation, the fatal termination was due, not to any special effects on the nervous centres, but to a secondary surgical complication. The case, moreover, teaches some important physiological, pathological, and clinical lessons, and suggests practical reflections which may prove useful to future medicine and surgery.

FIGURE 1-57 A celebrated case in the history of neurosurgery. In this publication a neurologist describes using cerebral localization techniques to determine where the tumor might lie in the brain. Under the direction of a neurologist, the prominent British surgeon Rickman Godlee performed the craniectomy, located the tumor, and successfully removed it. (*From Bennett AH, Godlee RJ. Case of cerebral tumor.* Medical-Chirurgical Trans. *1885;68:243-245.)*



A CASE

OF

TUMOUR OF THE SPINAL CORD.

REMOVAL; RECOVERY.

BY

W. R. GOWERS, M.D., F.R.S.,
AND
VICTOR HORSLEY, B.S., F.R.S.

Received March 8th—Read June 12th, 1888.

Medical History of the Case, by Dr. GOWERS.

CAPT. G—, æt. 42, had good health until the year 1884. There was no history of syphilis. During 1883 and 1884 he endured much mental anxiety, and in the latter year he had a considerable mental shock—his wife was knocked down and run over in his presence, and he was able to save himself from a similar fate only by suddenly throwing himself backwards. Soon afterwards he began to suffer from a dull pain across the lower part of the back, which he thought was due to the strain of the accident. This pain passed away in the course of a few weeks and did not return. In June, 1884, he first felt a peculiar pain that was the most

FIGURE 1-58 Gowers and Horsley reported the first diagnosis, localization, and successful removal of a spinal cord tumor in 1888. (*From Gowers WR, Horsley V. A case of tumour of the spinal cord. Removal, recovery.* Med Chirurg Trans. *1888;71:377-428.)*

Sir Rickman Godlee (1849-1925) removed one of the most celebrated brain tumors, the first to be successfully diagnosed by cerebral localization, in 1885.[131] The patient had suffered for 3 years from focal motor seizures (Fig. 1-57). They started as focal seizures of the face and proceeded to involve the arm and then the leg. For 3 months before surgery the patient also experienced weakness and eventually had to give up his work. Working with a neurologist, Alexander H. Bennett (1848-1901), Godlee was able to localize the tumor and remove it. This case was an important landmark in neurosurgery. For the first time a neurologist, basing his conclusions on the findings from a neurological examination, localized a brain tumor and recommended removal to a surgeon. Godlee made an incision over the rolandic area and through a small cortical incision removed the tumor. The patient survived the surgery with mild weakness and did well only to die of a wound infection 1 month later. Added to the importance of the surgery itself was the presence in the operation room of three important gentlemen: Hughes Bennett, a prominent English physician, and J. Hughlings Jackson and David Ferrier, two local neurologists. These gentlemen were extremely interested in whether cerebral localization studies could provide good results in the operating theater. This operation was the impetus that truly moved neurosurgery forward, a landmark operation.

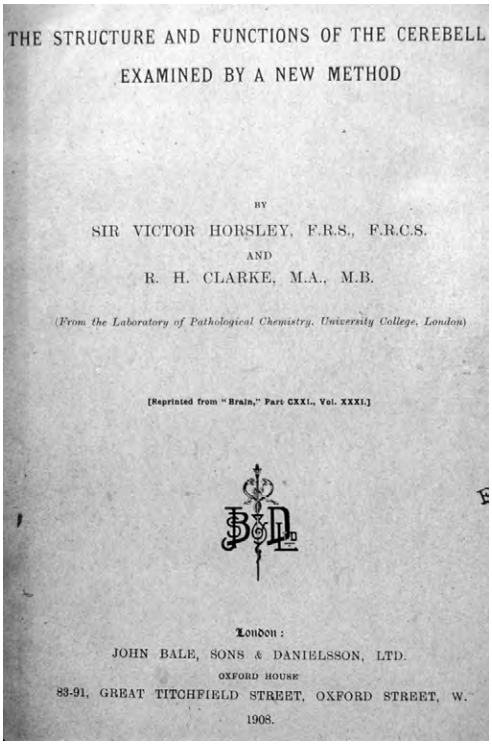
Three years later, in 1888, Victor Horsley (1857-1916) performed the first removal of a spinal cord tumor, a tumor that had been diagnosed and localized by William Gowers (1845-1915) (Fig. 1-58).[132] Horsley performed a laminectomy on Gower's patient, Captain Golby. Golby was slowly losing function in his legs from a spinal cord tumor. Gowers localized the tumor by examination and suggested to Horsley where to operate; the tumor was successfully removed. A postoperative photograph of the patient with a healed midline thoracic scar is included in the original paper.

William Gowers (1845-1915) was one of an extraordinary group of English neurologists of that era. Using some of the recently developed techniques in physiology and pathology, he made great strides in refining the concept of cerebral localization. Gowers was noted for the clarity and organization of his writing, works that remain classics in the field.[133,134] Studies such as these allowed surgeons to consider operating on the central nervous system (CNS) for other than heroic circumstances. Godlee and Horsley were trained general surgeons who had the ambition and fortitude to surgically explore the CNS now that their neurology colleagues could localize the lesion.

The successful removal of a spinal tumor brought Sir Victor Alexander Haden Horsley (1857-1916) to the forefront in the development of neurosurgery during its birthing period (Fig. 1-59). Horsley began his experimental studies on the brain in the early 1880s, at the height of the cerebral localization controversies. Using faradic stimulation he worked with Sharpey-Schäfer in analyzing and localizing motor functions in the cerebral cortex, internal capsule, and spinal cord of primates.[135] In a classic study with Gotch (1891) using a string galvanometer, he showed that electrical currents originate in the brain.[136] These experimental studies showed Horsley that localization was possible and that operations on the brain could be conducted safely by using techniques adapted from general surgery.

Horsley made a number of technical contributions to neurosurgery, including the use of beeswax to stop bone bleeding.[137,138]

FIGURE 1-59 A photograph of Victor Horsley that dates from his World War 1 military experience, taken shortly before he died during the war as a result of a severe desert fever.


THE STRUCTURE AND FUNCTIONS OF THE CEREBELL
EXAMINED BY A NEW METHOD

BY

SIR VICTOR HORSLEY, F.R.S., F.R.C.S.

AND

R. H. CLARKE, M.A., M.B.

(From the Laboratory of Pathological Chemistry, University College, London)

[Reprinted from "Brain," Part CXXI., Vol. XXXI.]

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JOHN BALE, SONS & DANIELSSON, LTD.

OXFORD HOUSE

83-91, GREAT TITCHFIELD STREET, OXFORD STREET, W.

1908.

FIGURE 1-60 Horsley provided a number of original scientific contributions. He and R. H. Clarke were the first to develop a stereotactic frame for performing localized brain lesions in animals. Although this frame was never used on humans or for human studies, it was the model for the human stereotactic frames developed in the 1940s. (*From Horsley V, Clarke RH. The structure and functions of the cerebellum examined by a new method.* Brain. *1908;31:45-124.)*

He performed one of the earliest operations for craniostenosis and relief of increased intracranial pressure. He pioneered the technique of sectioning the posterior root of the trigeminal nerve for trigeminal neuralgia, the first effective treatment of this relentless condition.[139] Using his technical gifts, he helped Clarke design the first useful stereotactic unit for brain surgery. Although the unit was used only on animals, the Horsley-Clark stereotactic frame remains the standard from which all subsequent designs have derived (Fig. 1-60).[140]

With the breakout of World War I, Horsley was sent to Mesopotamia to help develop hygienic procedures in a desert outpost. Ironically, he died within 2 days of arrival after contracting a severe desert fever, a tragic loss of a brilliant mind and surgeon. Horsley was one of those remarkable talents who were able to combine experimental research with clinical practice, which in turn provided remarkable advances for neurosurgery.

William Macewen (1848-1924), a Scottish surgeon and pioneer in the field of neurosurgery, successfully accomplished one of the early brain operations on July 29, 1879.[141] Macewen operated on a 14-year-old child and removed a periosteal tumor over the right eye (Figs. 1-61 and 1-62). Using meticulous technique and the recently developed neurological examination, he localized the tumor and removed it. After surviving for 8 years, Bright's disease developed and the patient died; at autopsy no tumor was detected. By 1888, Macewen had operated on 21 neurosurgical cases with only 3 deaths and 18 successful recoveries—a remarkable turnabout from earlier experience. When Macewen published his monograph in 1893 on pyogenic infections of the brain and their surgical treatment, a revolution occurred in neurosurgery.[142] This monograph was the earliest to deal with the successful treatment of brain abscess. His morbidity and mortality statistics, reflecting the application of localization techniques and effective antisepsis, were not inferior to those in

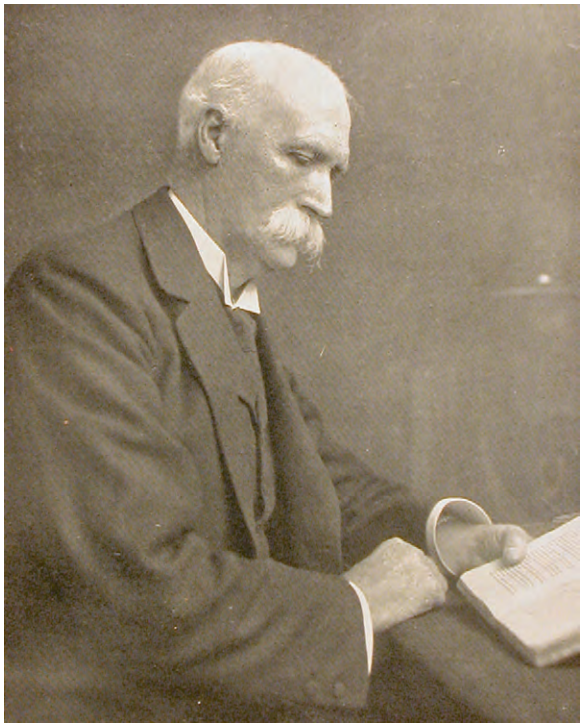


FIGURE 1-61 Photograph of William Macewen, who developed sterile surgical techniques that produced results that remained among the best in the literature for nearly 50 years.

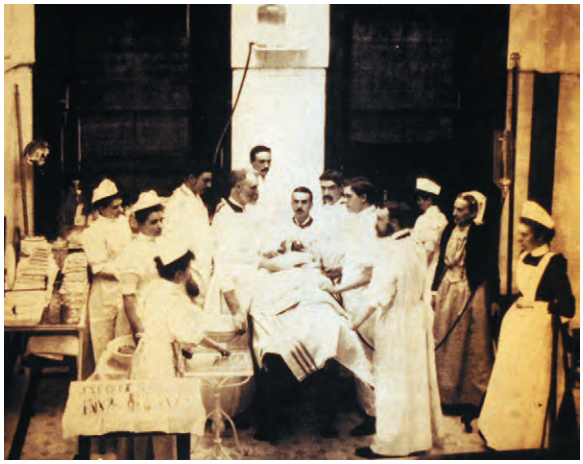


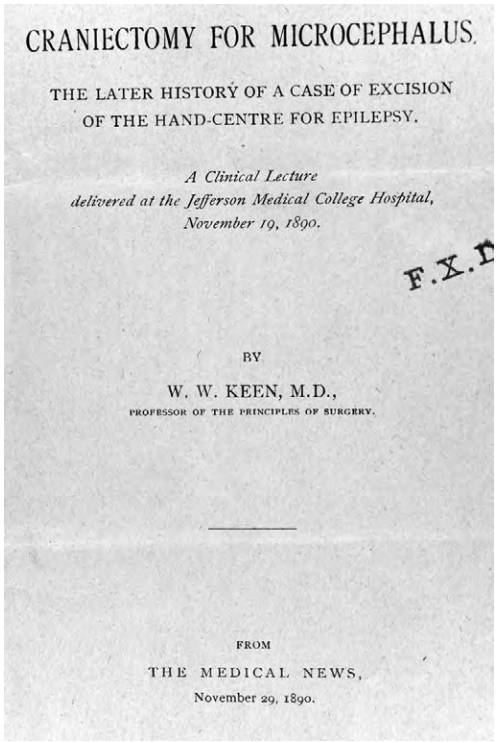
FIGURE 1-62 Macewen in the operating room, the bearded gentleman on the patient's right. He is surrounded by his staff, who although not gloved and masked, are using sterile principles in the operating room, including the Lister carbolic sprayer, clean gowns, and hands.

any series reported today. Without good surgical results, the neurologist of that era was hesitant to recommend surgery; Macewen helped immensely to make the case for soundly conducted operations on the brain.

> *Though not sharing the hopelessness of the opinion expressed in 1883 by a distinguished neurologist as to the inutility of operations on the brain undertaken for abscess, the author was then inclined to take a more sombre view of the prospects of recovery from such operations than his subsequent experience has proven to be necessary. He now regards an uncomplicated cerebral abscess, early recognized, accurately localized, and promptly operated on, as one of the most satisfactory of all intracranial lesions, the patient being at once relieved from a perilous condition, and usually restored to sound health [from the preface to Pyogenic Infective Diseases of the Brain and Spinal Cord].*[142]

In the United States, among the earliest pioneers in neurosurgery was William W. Keen (1837-1924), professor of surgery at Jefferson Medical College in Philadelphia. Keen was one of the strongest American advocates for use of the recently introduced Listerian antiseptic techniques in surgery (Fig. 1-63).[143] The concept of surgical bacteriology, along with those of asepsis and antisepsis, was aggressively discussed in his writings.[144] He prepared one of the earliest American monographs on neurosurgery, a book titled *Linear Craniotomy*.[143] He developed a technique for the treatment of spastic torticollis involving division of the spinal accessory nerve and the posterior roots of the first, second, and third spinal nerves (Fig. 1-64).[145] For treatment of the excoriating pain associated with trigeminal neuralgia he devised a technique for resection of the gasserian ganglion.[146] Keen exercised a rare inventiveness in surgical technique; he used bent spoons from his kitchen as brain retractors! Keen was also the first to introduce the Gigli saw to American surgeons, a technical advance in performing a craniotomy.[147]

A professor of surgery in Berlin, Fedor Krause (1857-1937) was an early general surgeon who developed a keen interest in neurosurgery. Krause's three-volume atlas on neurosurgery, published in the first decade of the 20th century, was among the first to graphically detail the techniques of neurosurgery. Digital extirpation of a meningioma is described.[148] A number of neurosurgical techniques are reviewed, including resection of scar tissue for the treatment of epilepsy. Krause was an early pioneer in the extradural approach to the gasserian ganglion for the treatment of trigeminal neuralgia (Figs. 1-65 and 1-66).



CRANIECTOMY FOR MICROCEPHALUS.

THE LATER HISTORY OF A CASE OF EXCISION OF THE HAND-CENTRE FOR EPILEPSY.

A Clinical Lecture
delivered at the Jefferson Medical College Hospital,
November 19, 1890.

F.X.D

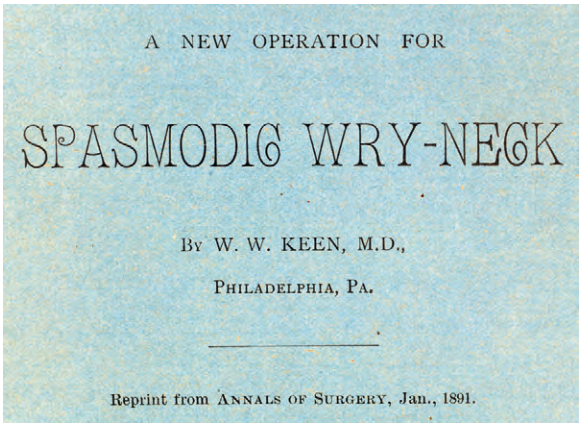
BY

W. W. KEEN, M.D.,
PROFESSOR OF THE PRINCIPLES OF SURGERY.

FROM
THE MEDICAL NEWS,
November 29, 1890.

FIGURE 1-63 One of the great pioneers in early American neurosurgery and little recognized today is William W. Keen. An early advocate of Listerian aseptic techniques, he made important contributions to surgery and particularly in a small subsurgical specialty—neurosurgery. Illustrated here is his paper on the treatment of microcephaly. (*From Keen WW.* Linear Craniotomy. *Philadelphia: Lea Bros; 1891 Published a year earlier as a paper. Keen WW. Craniectomy for microcephalus.* Med News. *November 29, 1890.)*

The first American monograph devoted to brain surgery was written not by a neurosurgeon but by a New York neurologist, Allen Starr (1854-1932).[149] Starr was professor of nervous diseases at Columbia University and an American leader in neurology. He trained in Europe and worked in the laboratories of Erb, Schultze, Meynert, and Nothnagle—experiences that



A NEW OPERATION FOR

SPASMODIC WRY-NECK

By W. W. KEEN, M.D.,

PHILADELPHIA, PA.

Reprint from ANNALS OF SURGERY, Jan., 1891.

FIGURE 1-64 Keen's original contribution on the treatment of severe torticollis or "wry-neck" in which he discusses the technique of transecting the nerves supplying the neck muscles. (*From Keen WW. A new operation for spasmodic wry neck, namely, division or exsection of the nerves supplying the posterior rotator muscles of the head.* Ann Surg. *1891;13:44-52.)*

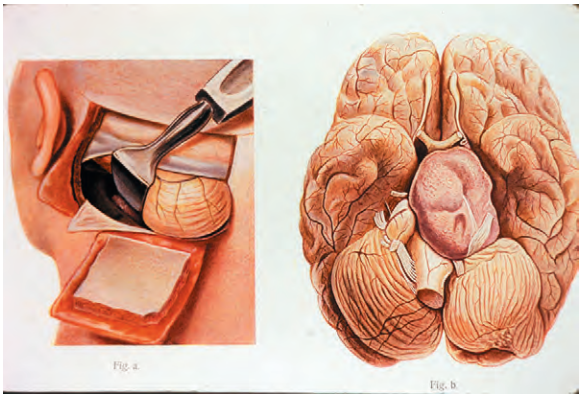


FIGURE 1-65 From Krause's monograph on brain surgery showing one of the earliest cerebellopontine angle approaches for an acoustic neuroma. Both the surgical approach and the anatomy of the tumor in relation to the seventh and eighth cranial nerves are clearly outlined. (*From Krause F.* Surgery of the Brain and Spinal Cord Based on Personal Experiences. Translated by H. Haubold and M. Thorek. *New York: Rebman Co; 1909-1912.)*

provided him with a strong foundation in neurological diagnosis (Fig. 1-67). Working closely with Charles McBurney (1845-1913), a New York City general surgeon, he came to the realization that not only could brain surgery be done safely but also was clearly necessary in the treatment of certain neurological problems.[149-151] Starr summarized his views in the preface:

> *Brain surgery is at present a subject both novel and interesting. It is within the past five years only that operations for the relief of epilepsy and*

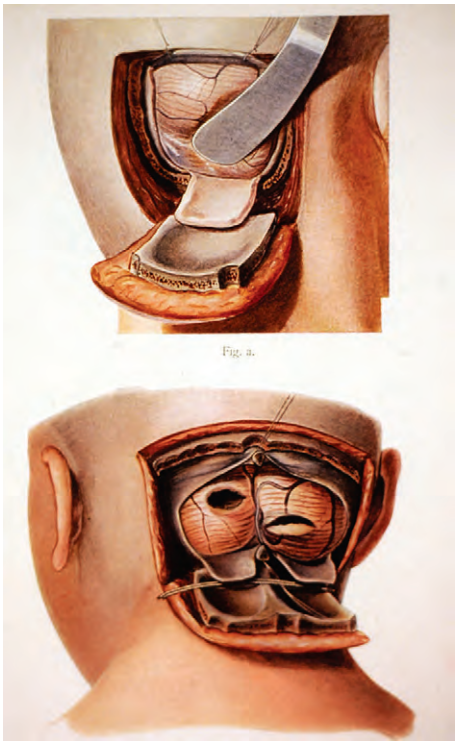
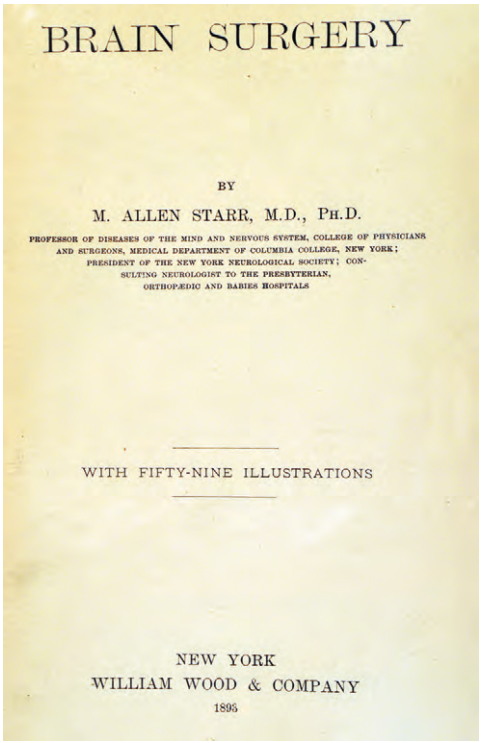


FIGURE 1-66 Krause was an advocate of the "osteoplastic" flap in which the bone was removed with the overlying muscle and scalp. In these images Krause outlines a unilateral and a bilateral craniotomy to expose the cerebellum. (*From Krause F.* Surgery of the Brain and Spinal Cord Based on Personal Experiences. Translated by H. Haubold and M. Thorek. *New York: Rebman Co; 1909-1912.)*


BRAIN SURGERY

BY

M. ALLEN STARR, M.D., PH.D.

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WITH FIFTY-NINE ILLUSTRATIONS

NEW YORK
WILLIAM WOOD & COMPANY
1893

FIGURE 1-67 A neurologist, not a neurosurgeon, authored the first monograph on brain surgery published in the United States. Allen Starr, a neurologist, was one of the earliest and strongest advocates of exploration of the brain based on a thorough neurological examination at the bedside. (*From Starr MA.* Brain Surgery. *New York: William Wood and Co; 1893.)*

> *of imbecility, for the removal of clots from the brain, for the opening of abscesses, for the excision of tumors, and the relief of intra-cranial pressure have been generally attempted. Brain surgery has as its essential basis the accurate diagnosis of cerebral lesions, which was impossible until the localization of cerebral functions had been determined. And this diagnosis must be made by the physician before the surgeon is called in to remove the disease. It is the object of this book to state clearly those facts regarding the essential features of brain disease which will enable the reader to determine in any case both the nature and situation of the pathological process in progress, to settle the question whether the disease can be removed by surgical interference, and to estimate the safety and probability of success by operation. The facts have been reached by a careful study of the literature of the subject and by a considerable personal experience. It is my hope that this work may aid the physician to diagnosticate brain diseases with more accuracy, and to select such cases as are properly open to surgical treatment by trephining, and also that may enable the surgeon to perform his delicate task with more precision and with a fuller knowledge of those principles of local diagnosis which should form this constant guide.*[149]

Harvey William Cushing (1869-1939) was the founder of American neurosurgery (Figs. 1-68 and 1-69). Cushing had the good fortune to be alive and in training during the formative years of neurosurgery. Educated at Johns Hopkins under one of the premier general surgeons, William Halsted (1852-1922), Cushing learned meticulous surgical technique from his mentor. As was standard then, Cushing spent time in Europe; he worked in the laboratories of Theodore Kocher in Bern, where he investigated the physiology of CSF. These studies led to his important monograph in 1926 on the third circulation.[152] It was during this period of experimentation that the cerebral phenomenon of increased intracranial pressure in association with hypertension and bradycardia was defined; it is now referred to as the *Cushing*

FIGURE 1-68 Photograph of a young dapper Cushing taken during his early period at Johns Hopkins. This portrait comes from a rare album issued in the early part of the 20th century that illustrated some of the prominent figures of the Johns Hopkins University and Hospital. Cushing's personality is evident in this early image of him.

phenomenon. While traveling through Europe, he met several important surgical personalities, including Macewen and Horsley. These individuals provided the impetus for Cushing to consider neurosurgery as a full-time endeavor.

Cushing's contributions to the literature of neurosurgery are too extensive for this brief chapter. Among his most significant is a monograph on pituitary surgery published in 1912.[153] This monograph was to inaugurate a sterling career in pituitary studies. Cushing's syndrome was defined in his final monograph on the pituitary gland published in 1932.[154] In a monograph written with Percival Bailey in 1926, Cushing introduced the first rational approach to the classification of brain tumors.[155] Cushing's monograph on meningioma, written in collaboration with Louise Eisenhardt in 1938, remains the standard for the profession.[156]

Cushing retired as Moseley Professor of Surgery at Harvard in 1932. When he completed his 2000th brain tumor operation, he had unquestionably made one of the most important contributions to the field of neurosurgery—a contribution comprising meticulous, innovative surgical techniques and a career-long attempt to understand brain function from both a physiologic and a pathologic perspective.[157] An ardent bibliophile, Cushing spent his final years in retirement as Sterling Professor of Neurology at Yale, where he put together his extraordinary monograph on Andreas Vesalius.[158] Cushing's life was faithfully recorded by his close friend and colleague John F. Fulton (1946)[159] and in a recent biography by Michael Bliss.[160]

If Harvey Cushing is the father of American neurosurgery, his prodigal son is Walter Dandy (1886-1946) (Fig. 1-70), who trained under Cushing at Johns Hopkins Hospital. Dandy made a number of important contributions to neurosurgery. Using the serendipitous finding of Luckett,[161] the presence of air in the ventricles after a skull fracture, Dandy developed the technique of pneumoencephalography (PEG).[162-164] The introduction of PEG provided the neurosurgeon, for the first time, the opportunity to localize a tumor by analyzing the displacement of air in the ventricles. Dandy was an innovative neurosurgeon, considerably more aggressive in style and technique than Cushing. Dandy was the first to show that acoustic neuromas could be removed in their totality.[165,166] He devoted much effort to the treatment of hydrocephalus.[167,168] He first introduced the technique of ablating and removing the choroid plexus to reduce the production of CSF.[169] Dandy was among the first to surgically deal with

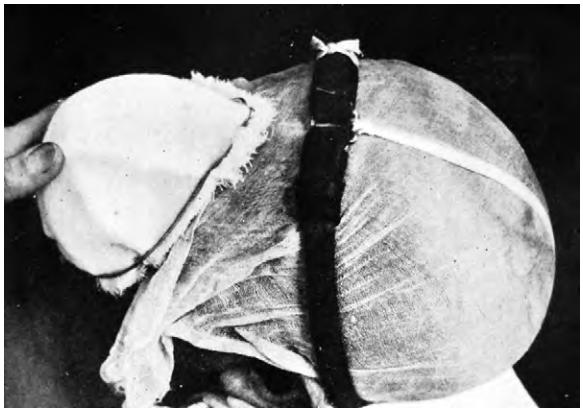

FIGURE 1-69 A key to Cushing's success as a surgeon was a number of inventive techniques designed to reduce morbidity and mortality during neurosurgical procedures. Illustrated here is one of Cushing's numerous innovations, a pneumatic tourniquet placed on the scalp before skin incision to reduce blood loss.

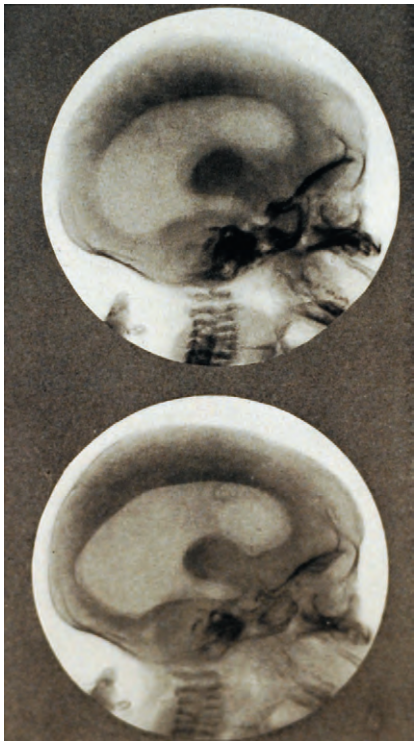

FIGURE 1-70 One of the landmark contributions to neurosurgery made by Dandy (and Blackfan), introduction of the pneumoencephalogram—a technique in which air was introduced into the ventricles and with a radiograph the ventricular system could be seen in outline.

cerebral aneurysms by obliterating them with snare ligatures or metal clips.[170] His monograph on the third ventricle and its anatomy remains a textbook standard to this day, with illustrations that are among the best ever produced.[171]

In the field of spine surgery, two important American figures appeared in the first quarter of the 20th century: Charles Elsberg (1871-1948), professor of neurosurgery at the New York Neurological Institute, and Charles Frazier (1870-1936), professor of surgery at the University of Pennsylvania. Work in the 19th century by J. L. Corning (1885) had shown that lumbar puncture can be safely performed.[172] This procedure was popularized by H. Quincke, who used it for the treatment of hydrocephalus, and from this procedure spine surgery developed.[173,174] When Charles Frazier's book on spine surgery appeared in 1918, the most comprehensive work on spine surgery yet to be written became available.[175] Frazier summarized much of the spine surgery literature to that point. He established that spine surgery could be performed with minimal morbidity and mortality. Frazier's experience in World War I led him to devote his career to neurosurgery. A gracious person, he followed a heavy work schedule. It was not uncommon for Frazier to sweep the operating room at the completion of a case just to relax his shoulder muscles, only then discussing the operation just completed with his colleagues.

Charles Elsberg (1871-1948), a pioneer in spine surgery, had surgical technique that was described as impeccable and consistently led to excellent outcomes. In 1912 Elsberg published a landmark paper in which he reported on a series of 43 laminectomies.[176] In 1916 he published the first of what were to be three monographs on surgery on the spine.[177] One of Elsberg's seminal contributions was a staged technique to allow the delivery of an intramedullary spinal cord tumor.[176] It consisted of first a myelotomy, which in theory allowed an intramedullary tumor to deliver itself over time into the laminectomy. Then at a second operation the tumor could be removed after having extruded through myelotomy. Elsberg was known as a driven worker who approached the practice of neurosurgery with a fierce intensity, always looking for new techniques. Working with Cornelius Dyke (1900-1943), a neuroradiologist at the New York Neurological Institute, he treated spinal glioblastomas with directed radiation in the operating room after the tumor had been exposed! Procedures such as these were performed with the patients receiving only local anesthesia. During the ½-hour therapy, while the radiation was being delivered, the surgeon and assistants stood off in the distance behind a glass shield.[178]

CONCLUSION

The 19th century brought the introduction of anesthesia, antisepsis, and cerebral localization. The later half of the 19th century produced strong surgical personalities, surgeons adventurous enough to perform surgery on the formidable cranial vault and spine. In the first half of the 20th century, formalization of the field of neurosurgery occurred. Besides the pioneering techniques of Dandy, Cushing, and others, a number of diagnostic techniques were introduced that made it easier for the neurosurgeon to localize lesions. One technique, myelography with opaque substances, was brought forward by Jean Athanase Sicard (1872-1929).[179] Using radiopaque iodized oil, the spinal cord and its elements could be outlined on radiographs. Antonio Caetano de Egas Moniz (1874-1955), professor of neurology in Lisbon, Portugal, perfected arterial catheterization techniques and the cerebral angiogram in animal studies.[180,181] This procedure, in combination with PEG, offered the neurosurgeon a detailed view of the intracranial contents. Moniz was awarded the Nobel Prize in 1949 for his work on prefrontal lobotomy for psychiatric disorders.

In 1929 Alexander Fleming (1881-1955) published a report on the first observation of a substance that appeared to block a bacterium from growing.[182] This substance, identified as penicillin, introduced a new era of medicine and surgery. With the World War II experience, antibiotics for the treatment of bacterial infection were perfected, thereby even further reducing the risk for infection during brain and spine surgery.

As a result of our surgical forebearers, surgeons can now complete a neurosurgical procedure with the patient suffering no pain and minimal risk for infection. Our 19th century ancestors provided us pioneering techniques in cerebral localization that have led to the introduction of frameless guidance systems. The surgical fear of operating on the wrong area is no longer an issue; for this we can thank our historical giants, on whose shoulders and studies the field of neurosurgery has developed.

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PART 2

Basic Science

CHAPTER 2

Surgical Anatomy of the Brain

Hung Tzu Wen ■ Albert L. Rhoton Jr. ■ Antônio C. M. Mussi

It is our belief that observation of the anatomy of the brain from different angles is the key to assemble an authentic tridimensional knowledge. As important as knowledge of the surface anatomy, or the anatomy of deeply located structures, is establishment of correlation between them. Such correlation will empower us to have "x-ray" vision that will enable us to "see" the depths of the brain through its surface.

In this chapter, the surgical anatomy of the neural and vascular structures of both the cerebrum and cerebellum is reviewed in stepwise dissection by following the logical sequence based on the three surfaces that each one of them presents.

CEREBRUM

Lateral Surface: Neural Structures

Superficial Anatomy

The cerebrum is arbitrarily divided into five lobes: frontal, parietal, temporal, occipital, and the hidden insula. On the lateral surface, they are limited by the central sulcus, the posterior ramus of the sylvian fissure, the lateral parietotemporal line (from the impression of the parieto-occipital sulcus to the preoccipital notch), and the temporo-occipital line (from the posterior end of the posterior ramus of the sylvian fissure to the midpoint of the lateral parietotemporal line). The cerebrum has four main sulci that are 100% continuous—the sylvian fissure and the callosal, parieto-occipital, and collateral sulci—and two almost continuous (92%) sulci—the central and calcarine sulci. There are two 100% interrupted sulci: the precentral and inferior temporal sulci.[1] The central sulcus starts from the medial surface of the hemisphere above the cingulate sulcus and extends on the lateral surface of the hemisphere in a medial-to-lateral, superior-to-inferior, and posterior-to-anterior direction. It does not usually intercept the posterior ramus of the sylvian fissure and leaves a "bridge" connecting the precentral to the postcentral gyrus, known as *pli de passage frontoparietal inferior*, *opercule rolandique*, or the *subcentral gyrus* (Fig. 2-1A).

Frontal Lobe

The two main sulci are the superior and inferior frontal sulci, which are anteroposteriorly oriented and extend from the precentral sulcus to the frontal pole. At their posterior end, these two sulci are intercepted perpendicularly by the precentral sulcus, which has a direction very similar to that of the central sulcus. The precentral sulcus forms the anterior limit of the precentral gyrus. These two frontal sulci divide the lateral surface of the frontal lobe into three gyri: the superior, middle, and inferior frontal gyri (Fig. 2-1A). The anterior horizontal, the anterior ascending, and the posterior rami of the sylvian fissure divide the inferior frontal gyrus into three parts: the pars orbitalis, triangularis, and opercularis. The apex of the pars triangularis is usually retracted superiorly and leaves a space in the sylvian fissure that is generally the largest space in the superficial compartment of the sylvian fissure. The apex of the pars triangularis is directed inferiorly toward the junction of three rami of the sylvian fissure; this junctional point coincides with the anterior limiting sulcus of the insula in the depth of the sylvian fissure. It marks the anterior limit of the basal ganglia and the location of the anterior horn of the lateral ventricle. At the intercepting point between the superior frontal and precentral sulci, the precentral gyrus often has the morphology of the Greek letter "Ω" (omega), with its convexity pointing posteriorly. This is the most easily identifiable landmark of the motor strip and corresponds to the hand area (Fig. 2-1B).

Parietal Lobe

The parietal lobe is limited anteriorly by the central sulcus, medially by the interhemispheric fissure, inferolaterally by the sylvian fissure and the temporo-occipital line, and posteriorly by the lateral parietotemporal line. Its two main sulci are the postcentral and intraparietal sulci. The postcentral sulcus is very similar to the central sulcus, except for its variable continuity. The postcentral sulcus is the posterior limit of the postcentral gyrus, and it can sometimes be double. The intraparietal sulcus starts at the postcentral sulcus and is directed posteriorly and inferiorly toward the occipital pole; its direction is often parallel and 2 to 3 cm lateral to the midline. The bottom of the intraparietal sulcus is related to both the roof of the atrium and the occipital horn. The intraparietal sulcus divides the lateral surface of the parietal lobe into two parts: the superior and inferior parietal lobules. The superior parietal lobule, which is the superomedial and smaller part, continues as the precuneus on the medial surface of the parietal lobe. The inferior parietal lobule is constituted by the supramarginal and angular gyri. The supramarginal gyrus, the posterior continuation of the superior temporal gyrus, turns around the posterior ascending ramus of the sylvian fissure. The

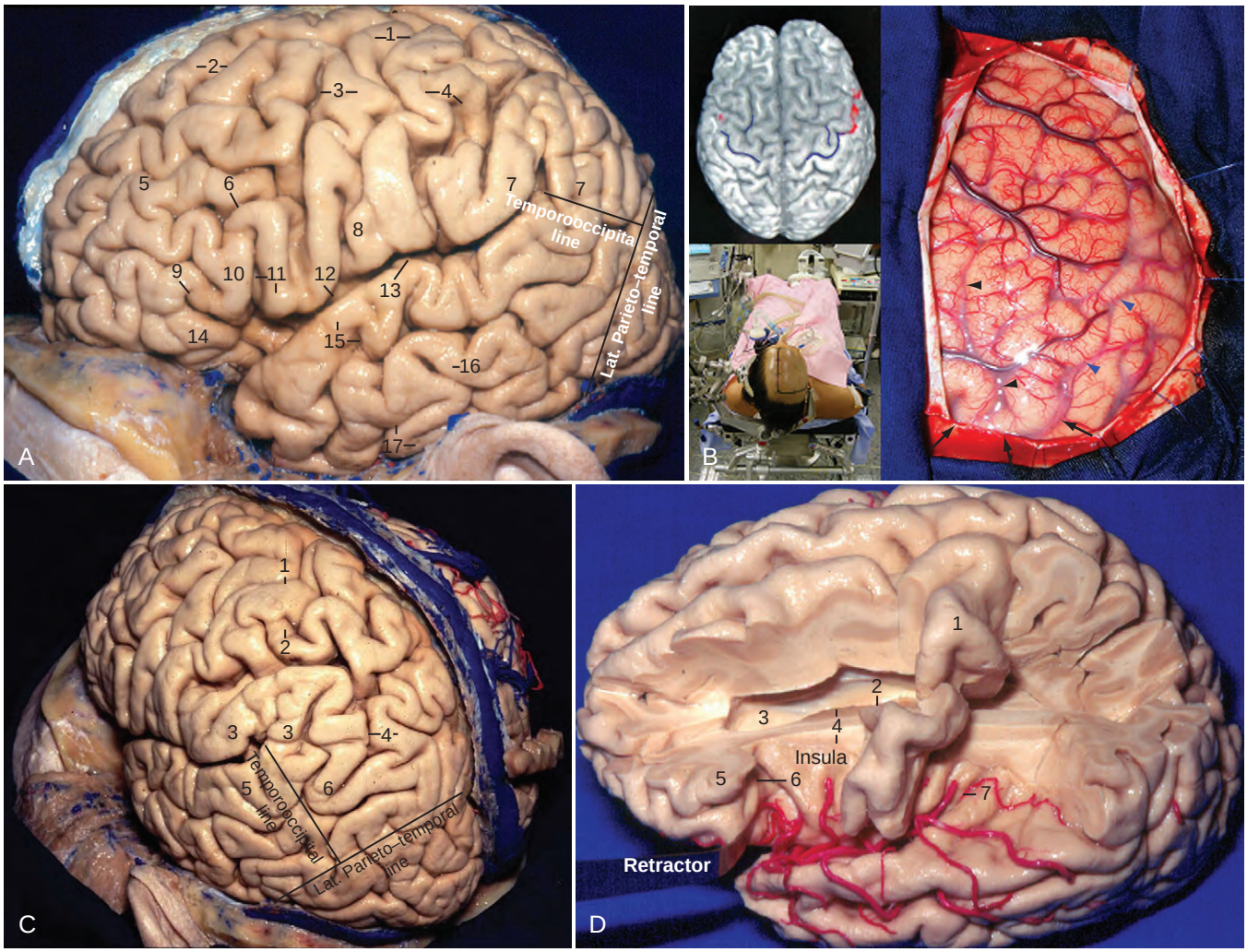

FIGURE 2-1 **A,** Lateral view of the left hemisphere. 1, "Omega" (motor hand area) and central sulcus; 2, superior frontal sulcus and gyrus; 3, precentral sulcus and gyrus; 4, postcentral sulcus and gyrus; 5, middle frontal gyrus; 6, inferior frontal sulcus; 7, supramarginal gyrus; 8, pli de passage; 9, anterior horizontal ramus; 10, pars triangularis; 11, ascending ramus and pars opercularis; 12, posterior ramus; 13, Heschl's gyrus; 14, pars orbitalis; 15, superior temporal gyrus and sulcus; 16, middle temporal gyrus; 17, inferior temporal sulcus and gyrus. **B,** *Upper left*, "omega"sign. *Lower left*, surgical positioning. *Right*, the *arrows* indicate the "omega," the *black arrowheads* indicate the superior frontal sulcus, and the *blue arrowheads* indicate the central sulcus. **C,** Posterolateral view of the left hemisphere. 1, Central sulcus; 2, postcentral gyrus and sulcus; 3, supramarginal gyrus; 4, intraparietal sulcus and superior parietal lobule; 5, superior temporal gyrus; 6, angular gyrus. **D,** Superolateral view of the left hemisphere. 1, Precentral gyrus; 2, foramen of Monro; 3, frontal horn; 4, head of the caudate nucleus and the superior limiting sulcus (insula); 5, pars orbitalis; 6, anterior limiting sulcus (insula); 7, inferior limiting sulcus (insula).

angular gyrus is the posterior continuation of the middle temporal gyrus and turns superiorly and medially behind the posterior ramus of the sylvian fissure up to the intraparietal sulcus; it is sometimes limited between the two posterior terminations of the superior temporal sulcus, the angular and anterior occipital rami (Fig. 2-1C).

The postcentral and intraparietal sulci and the superior parietal lobule are a "mirror image" of the precentral and superior frontal sulci and the superior frontal gyrus, with the central sulcus being the "mirror."

Temporal Lobe

The temporal lobe is limited superiorly by the posterior ramus of the sylvian fissure and posteriorly by the temporo-occipital and lateral parietotemporal lines. It has two main sulci, the superior and inferior temporal sulci, that divide the lateral surface of the temporal lobe into three gyri, the superior, middle, and inferior temporal gyri. The inferior temporal gyrus occupies the lateral and basal surfaces of the cerebrum. The superior and inferior temporal gyri converge anteriorly to form the temporal pole (Fig. 2-1A).

Occipital Lobe

The occipital lobe is located behind the lateral parietotemporal line and is composed of a number of irregular convolutions that are divided by a short horizontal sulcus, the lateral occipital sulcus, into the superior and inferior occipital gyri.

The "x-ray" vision concept can be demonstrated by the precentral gyrus, which begins on the medial surface of the cerebrum, above the level of the splenium of the corpus callosum, and passes above the body of the lateral ventricle, thalamus, posterior limb of the internal capsule, and posterior part of the lentiform nucleus to reach the sylvian fissure approximately midway between the anterior and posterior limits of the insula (Fig. 2-1D).

Sylvian Fissure

The sylvian fissure is the space between the frontal, parietal, and temporal opercula and the insula and extends from the basal to the lateral surface of the brain. It is composed of a superficial and a deep part. The superficial part has a stem and three rami; the stem extends medially from the semilunar gyrus of the uncus to the lateral end of the sphenoid ridge, where the stem divides into the anterior horizontal, anterior ascending, and posterior rami (Fig. 2-1A). The deep part is divided into a "sphenoidal compartment" and an "operculoinsular compartment." The sphenoidal compartment, which arises in the region of the limen insulae lateral to the anterior perforated substance (APS), is a narrow space posterior to the sphenoid ridge between the frontal and temporal lobes that communicates medially with the carotid cistern, also called *sylvian vallecula* (see Fig. 2-4D).[2] The operculoinsular compartment is formed by two narrow clefts, the opercular cleft between the opposing lips of the frontoparietal and temporal opercula and the insular cleft, which has a superior limb located between the insula and the frontoparietal opercula and an inferior limb between the insula and the temporal operculum (Fig. 2-2A).[3] The gyri that constitute the frontal and parietal opercula of the sylvian fissure are, from posterior to anterior, the supramarginal, postcentral, and precentral gyri and the pars opercularis, triangularis, and orbitalis (see Fig. 2-1A); the gyri that constitute the temporal operculum of the sylvian fissure are, from posterior to anterior, the planum temporale, Heschl's gyrus, and the planum polare (Fig. 2-2B, *left*). Each gyrus of the frontoparietal operculum is related to its counterpart on the temporal side; the supramarginal gyrus is in contact with the planum temporale, the postcentral gyrus is in contact with Heschl's gyrus, and the precentral gyrus and pars opercularis, triangularis, and orbitalis are related to the planum polare. The site on the posterior ramus of the sylvian fissure where the postcentral gyrus meets Heschl's gyrus is projected in the same coronal plane as the external acoustic meatus. The medial wall of the sylvian fissure is the insula or island of Reil, which can be seen only when the lips of the sylvian fissure are widely separated. The insula has the shape of a pyramid with its apex directed inferiorly and has an anterior and a lateral surface.

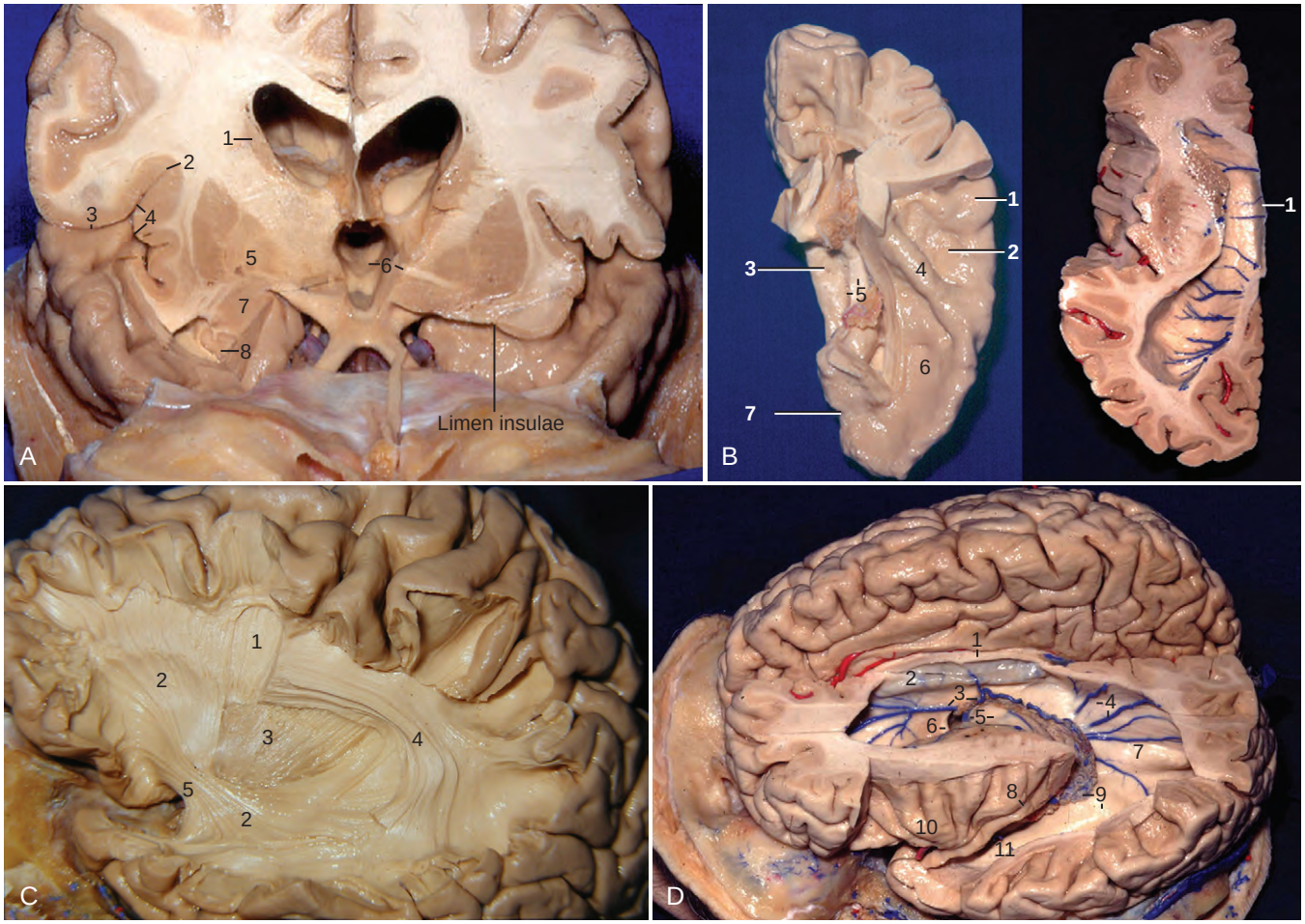

FIGURE 2-2 **A,** Coronal view. 1, Body of the caudate nucleus; 2, superior limiting sulcus; 3, opercular compartment; 4, insular compartment; 5, globus pallidus; 6, floor of the third ventricle and anterior commissure; 7, amygdala; 8, head of the hippocampus. **B,** *Left*, Anterosuperior view of the left temporal lobe. 1, Posterior transverse temporal gyrus; 2, middle transverse temporal gyrus; 3, parahippocampal gyrus; 4, Heschl's gyrus; 5, fornix and dentate gyrus; 6, planum polare; 7, rhinal incisura. *Right*, Basal view of the roof of the lateral ventricle. 1, Septum pellucidum. The veins of the roof of the lateral ventricle drain toward the midline. **C,** Fiber dissection of the left hemisphere. 1, Corona radiata; 2, inferior occipitofrontal fascicle; 3, putamen; 4, superior longitudinal fascicle; 5, uncinate fascicle. **D,** Lateral view of the left lateral ventricle. 1, Corpus callosum; 2, septum pellucidum; 3, anterior septal and superior choroidal veins; 4, bulb of the callosum and medial atrial vein; 5, thalamostriate vein and thalamus (anterior tubercle); 6, column of the fornix and foramen of Monro; 7, calcar avis; 8, central sulcus of the insula; 9, choroid plexus and atrium; 10, apex of the insula; 11, temporal horn.

The anterior surface is triangular in shape and is constituted by the transverse and accessory gyri and the insular pole. The medial portion of the insular pole is marked by an arched ridge of variable prominence, the limen insulae, which is composed of fibers of the uncinate fasciculus covered by a thin layer of gray matter that extends from the anterior end of the long gyrus, passes through the medial part of the insular pole, and ends at the middle of the posterior orbital gyrus. "Limen" means threshold, and the limen insulae is the threshold between the carotid cistern medially and the sylvian fissure laterally (Fig. 2-2A). The insula is encircled and separated from the opercula by a deep furrow called the *circular* or *limiting sulcus of the insula*, which has three parts, the superior, anterior, and inferior parts (see Fig. 2-1D). From the limen insulae, the sulci and gyri of the insula are directed superiorly in a radial manner. The deepest sulcus, the central sulcus of the insula, is a constant sulcus that extends upward and backward across the insula, in the general line of the central sulcus of the cerebrum. It divides the lateral surface of the insula into a large anterior zone that is divided by several shallow sulci into three to five short gyri and a posterior zone that is formed by the anterior and posterior long gyri (Fig. 2-2D). From microsurgical and radiologic viewpoints, the insula represents the external covering of the central core and is constituted by the extreme, external, and internal capsules, the claustrum, the basal ganglia, and the thalamus (Fig. 2-3A, *left*; also see Fig 2-2A). The anterior, inferior, and posterior limits of the insula on the lateral projection correspond to the anterior, inferior, and posterior limits of the central core. The upper limit of the central core (caudate nucleus) is higher than the upper limit of the insula (Fig. 2-2A).

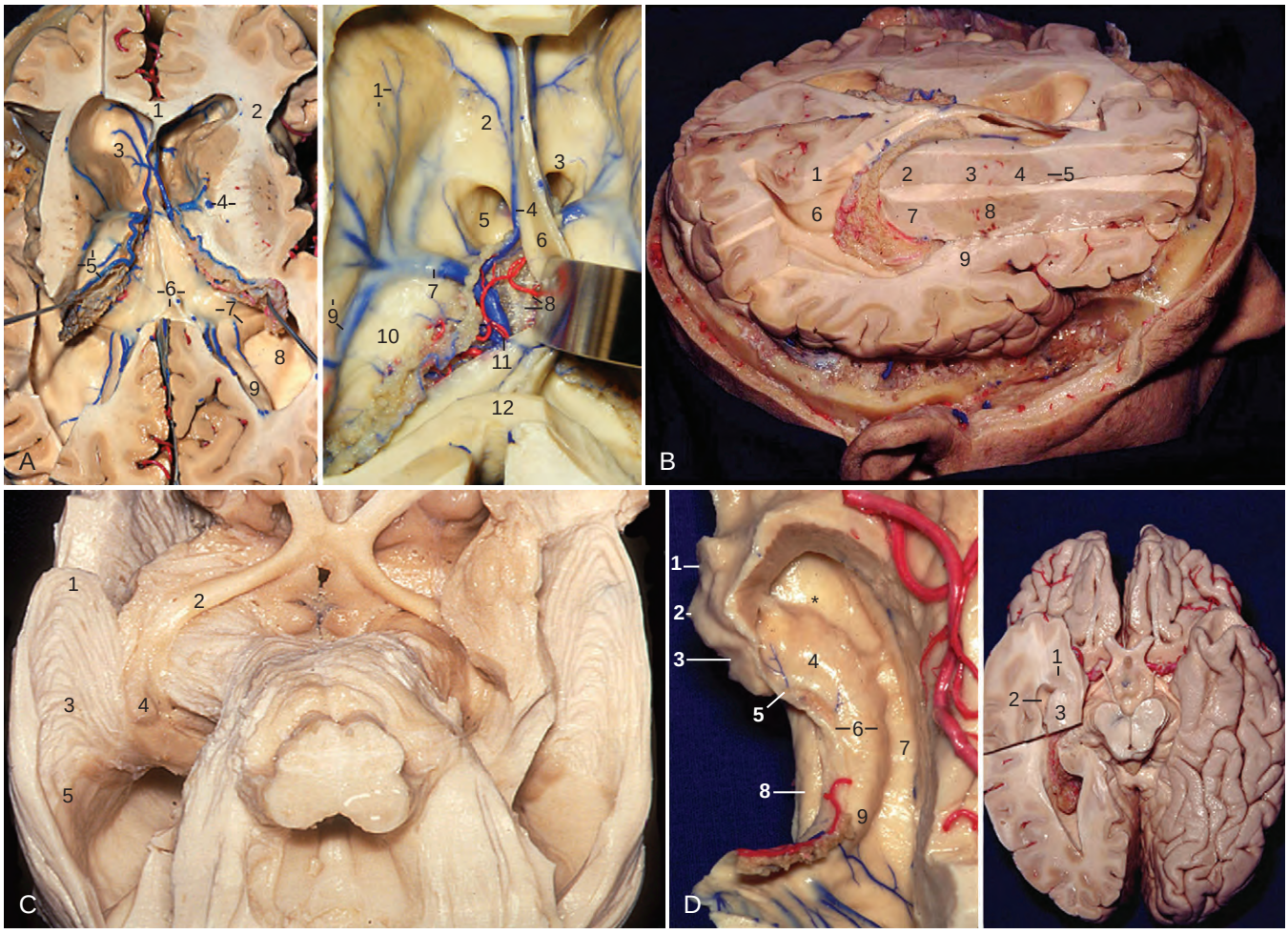

FIGURE 2-3 **A,** *Left*, Superior view. 1, Genu of the corpus callosum; 2, anterior limb of the internal capsule; 3, frontal horn; 4, genu of the internal capsule and thalamostriate vein; 5, striothalamic sulcus, thalamus, and superior choroidal vein; 6, bodies of the fornix and hippocampal commissure; 7, crus of the fornix and tail of the hippocampus; 8, collateral trigone; 9, calcar avis. *Right*, Roof of the third ventricle through a transchoroidal approach. 1, Head of the caudate nucleus and anterior caudate vein; 2, rostrum of the corpus callosum; 3, column of the fornix; 4, anterior septal vein; 5, foramen of Monro; 6, body of the fornix; 7, thalamostriate vein; 8, inferior membrane of the tela choroidea and choroid plexus of the third ventricle (the superior membrane of the tela has been removed); 9, body of the caudate nucleus and thalamostriate vein; 10, dorsal surface of the thalamus; 11, internal cerebral vein and medial posterior choroidal artery; 12, splenium of the corpus callosum. **B,** Superolateral view of the right hemisphere. 1, Bulb of the callosum; 2, thalamus; 3, internal capsule (genu); 4, internal capsule (anterior limb); 5, caudate nucleus; 6, calcar avis, 7, internal capsule (retrolenticular portion); 8, lentiform nucleus; 9, internal capsule (sublenticular portion). **C,** Basal view of the optic radiation. 1, Meyer's loop; 2, optic tract; 3, middle part; 4, lateral geniculate body; 5, posterior part. **D,** *Left*, Superior view of the floor of the right temporal horn. 1, Uncus (anterior segment); 2, uncus (apex); 3, uncus (posterior segment); 4, head of the hippocampus; 5, inferior choroidal point; 6, body of the hippocampus and fimbria; 7, collateral eminence; 8, parahippocampal gyrus; 9, tail of the hippocampus; *, uncal recess. *Right*, Basal view. 1, amygdala; 2, temporal horn; 3, hippocampus.

Association Fibers of the Cerebrum

The association fibers are tracts of myelinated fibers that connect cortical areas of different lobes in the same hemisphere; they may be divided into short and long association fibers. The short association fibers connect adjacent gyri, whereas the long association fibers (fasciculi) connect distant gyri and form distinct compact bundles. The main fasciculi are (1) the superior longitudinal fasciculus, which is the largest, arches around the insula, and connects parts of the frontal, parietal, and temporal lobes; (2) the uncinate fasciculus, which lies in the depth of the limen insulae, has a marked curvature, and connects the basal parts of the frontal lobe with the temporal lobe; (3) the inferior occipitofrontal fasciculus, which connects the frontal and occipital lobes, as well as the posterior part of the temporal and parietal lobes; these fibers converge from the frontal lobe as a single bundle that runs lateral to the lentiform nucleus, where they are closely associated with the uncinate fasciculus (Fig. 2-2C); and (4) the cingulum, the fibers of which lie within the cingulate gyrus from below the rostrum of the corpus callosum to the parahippocampal gyrus.

Lateral Ventricles

Wrapping around the central core of the hemisphere are the lateral ventricles (Fig. 2-2D). Each ventricle has five components: a frontal horn, body, atrium, and occipital and temporal horns.[4] The frontal horn is located in front of the foramen of Monro and has a roof, floor, and anterior, lateral, medial, and posterior walls. The transition between the genu and the body of the corpus callosum forms the roof, the rostrum of the corpus callosum forms the narrow floor, the septum pellucidum forms the medial wall, and the thalamus forms the posterior wall. The head of the caudate nucleus forms the majority of the lateral wall, but the most anterior part is constituted by the most anterior portion of the anterior limb of the internal capsule, and it is in close relation to the anterior limiting sulcus of the insula. The body of the lateral ventricle is located behind the foramen of Monro and extends to the point where the septum pellucidum, corpus callosum, and fornix meet. It has a roof, floor, and lateral and medial walls. The body of the corpus callosum forms the roof, the septum pellucidum above and the body of the fornix below form the medial wall, the body of the caudate nucleus forms the lateral wall, and the thalamus forms the floor. The caudate nucleus and the thalamus are separated by the striothalamic sulcus, the groove in which the stria terminalis and the thalamostriate vein course. The atrium has a roof, floor, and anterior, medial, and lateral walls. The roof is formed by the body, splenium, and tapetum of the corpus callosum. The floor is formed by the collateral trigone, a triangular area that bulges upward over the posterior end of the collateral sulcus. The medial wall is formed by two roughly horizontal prominences: the upper prominence, or the bulb of the callosum, is formed by a large bundle of fibers called the *forceps major* that connects the two occipital lobes; the lower prominence, or the calcar avis, overlies the deepest part of the calcarine sulcus. The lateral wall has an anterior portion formed by the caudate nucleus as it wraps around the lateral margin of the pulvinar, as well as a posterior portion formed by the fibers of the tapetum as they sweep anteroinferiorly along the lateral margin of the ventricle and separate the ventricular cavity from the optic radiation. The anterior wall has a medial part composed of the crus of the fornix as it wraps around the posterior portion of the pulvinar and a lateral part formed by the pulvinar of the thalamus. The occipital horn extends posteriorly into the occipital lobe from the atrium. It varies in size from being absent to extending far posterior in the occipital lobe. The bulb of the callosum and the calcar avis form its medial wall, the tapetum forms the roof and the lateral wall, and the collateral trigone forms the floor (Fig. 2-3A and B).[4] The temporal horn extends forward and inferiorly from the atrium into the medial part of the temporal lobe and has a roof, floor, and anterior, lateral, and medial walls. The tapetum, the tail of the caudate nucleus, part of the retrolentiform and sublentiform components of the internal capsule, and the amygdaloid nucleus form the roof. The retrolentiform component is the posterior thalamic radiation that includes the optic radiation (Fig. 2-3C); the sublentiform component is formed mainly by the acoustic radiation. The amygdaloid nucleus constitutes the most anterior portion of the roof of the temporal horn and is located above and in front of the head of the hippocampus (Fig. 2-3D, *right*), anterior to the inferior choroidal point, which is the most anterior site of attachment of the choroid plexus in the temporal horn.[5] There is no clear separation between the roof of the temporal horn and the thalamus because all fibers of the optic radiation come from the lateral geniculate body. Therefore, it is reasonable to consider the roof of the temporal horn a lateral extension of the thalamus.[5] The attachment site of the choroid plexus can be a surgical landmark to separate the thalamus from the roof of the temporal horn (see Fig. 2-6D, *right*). The tapetum and the optic radiation form the lateral wall, the amygdaloid body forms the anterior wall, the head of the hippocampus forms the anterior third of the medial wall, and the choroidal fissure forms the posterior two thirds of the medial wall.[5] The floor is formed medially by the hippocampus and laterally by the collateral eminence (Fig. 2-3D, *left*). The temporal horn is projected onto the middle temporal gyrus on the lateral view.

The structures related to the lateral ventricle are the foramen of Monro, internal capsule, corpus callosum, fornix, thalamus, caudate nucleus, hippocampus, temporal amygdala, and choroidal fissure.

Foramen of Monro

The foramen of Monro is a passage through which the lateral ventricle communicates with the third ventricle. It usually has a crescent shape and is bounded anteriorly and superiorly by the columns of the fornix and posteriorly by the thalamus[6]; the elements that run close to the foramen of Monro are the anterior septal vein superiorly and medially, the choroidal plexus posterior and medially, and the thalamostriate vein laterally and posteriorly (see Figs. 2-2D and 2-3A).

Internal Capsule

The internal capsule has five parts: the anterior and posterior limbs, the genu, and the retrolentiform and sublentiform parts. The anterior limb is located between the head of the caudate nucleus and the anterior half of the lentiform nucleus and contains frontopontine fibers; the posterior limb is located between the thalamus and the posterior half of the lentiform nucleus and contains corticospinal tract, frontopontine, and corticorubral fibers and fibers of the superior thalamic radiation (somesthetic radiation). The genu comes to the ventricular surface immediately lateral to the foramen of Monro in the interval between the caudate nucleus and the thalamus, where the thalamostriate vein usually drains into the internal cerebral vein; the genu contains corticonuclear fibers and anterior fibers of the superior thalamic radiation. The retrolentiform part is located posterior to the lentiform nucleus and contains mainly parietopontine, occipitopontine, occipitocollicular, and occipitotectal fibers and the posterior thalamic radiation that includes the optic radiation. The sublentiform part is located below the lentiform nucleus and contains temporopontine and parietopontine fibers and acoustic radiation from the medial geniculate body to the superior temporal gyrus and the transverse temporal gyri (Fig. 2-3A, *left*, and B).

Corpus Callosum

The corpus callosum is the largest transverse commissure connecting the cerebral hemispheres. It contributes to the wall of each of the five parts of the lateral ventricle (see Fig. 2-2B, *right*). The corpus callosum is divided in four parts: rostrum, genu, body, and splenium. The rostrum is the floor of the frontal horn. The genu gives rise to a large fiber tract, the forceps minor, that forms the anterior wall of the frontal horn, and it connects the frontal lobes. The splenium gives rise to a large tract, the forceps major, that forms a prominence called the *bulb* in the upper part of the medial wall of the atrium and occipital horn as it sweeps posteriorly to connect the occipital lobes. Another fiber tract, the tapetum, arises in the posterior part of the body and splenium and sweeps laterally and inferiorly to form the roof and lateral wall of the atrium and the temporal and occipital horns.

Optic Radiation

The optic radiation is a bundle of fibers that extend from the lateral geniculate body to the visual area in the occipital lobe. The optic radiation may be divided into three parts: anterior, middle, and posterior. In the anterior part, the fibers initially take an anterior direction along the roof of the temporal horn, usually reach as far anteriorly as the tip of the temporal horn, and then loop backward in the lateral and inferior aspects of the atrium and the occipital horn to end in the lower lip of the calcarine fissure; this anterior loop is called *Meyer's loop*. The anterior part represents the upper quadrants of the visual field. In the middle part, the fibers take a lateral direction initially, course along the roof of the temporal horn, and then proceed posteriorly along the lateral wall of the atrium and the occipital horn; the middle part contains the macular fibers. The fibers of the posterior part course directly backward along the lateral wall of the atrium and the occipital horn to end in the upper lip of the calcarine fissure; these fibers are responsible for the lower quadrants of the visual field (Fig. 2-3C).

Fornix

The fornix is a C-shaped structure that wraps around the thalamus in the wall of the lateral ventricle. The initial portion of the fornix, the fimbria, arises from the alveus, which is the subcortical white matter of the hippocampal allocortex, and thickens along the medial edge of the hippocampus; it is separated from the dentate gyrus by the fimbriodentate sulcus. The fimbria then passes posteriorly to become the crus of the fornix, which is the subcortical radiation of the hippocampal allocortex. In the atrium the crus wraps around the posterior surface of the pulvinar of the thalamus and arches superomedially toward the lower surface of the splenium of the corpus callosum; at the junction between the atrium and body of the lateral ventricle, the paired crura meet to form the body of the fornix. At the anterior margin of the thalamus, the body of the fornix separates into two columns that arch along the superior and anterior margins of the foramen of Monro. The columns of the fornix then split, pass predominantly posterior to the anterior commissure, and are directed inferiorly and posteriorly through the lateral wall of the third ventricle to reach the mamillary bodies at the floor of the third ventricle. In the area below the splenium, the two crura of the fornix are united by the hippocampal commissure (Fig. 2-4A; also see Fig. 2-3A and D, *left*).

Basal Ganglia

Although macroscopically fused and gathered into a "core" that is covered laterally by the insula, the basal ganglia and the thalamus are embryologically and functionally distinct structures. The basal ganglia are telencephalic structures, whereas the thalamus is a diencephalic structure. The basal ganglia consist of four nuclei: (1) the striatum (caudate nucleus, putamen, and nucleus accumbens), (2) globus pallidus, (3) substantia nigra, and (4) subthalamic nucleus.

The caudate nucleus is a C-shaped structure that wraps around the thalamus; it has a head, body, and tail. The head and the body are the lateral walls of the frontal horn and the body of the lateral ventricle. The tail extends from the atrium into the roof of the temporal horn and is continuous with the amygdaloid nucleus (see Figs. 2-2A and 2-3A).

Thalamus

The thalamus is located in the center of the lateral ventricle. Each lateral ventricle wraps around the superior, inferior, and posterior surfaces of the thalamus. The anterior tubercle of the thalamus is the posterior limit of the foramen of Monro; the posterior part, called the *pulvinar* (pillow) *of the thalamus*, is the wall of three different compartments in the cerebrum. The posterolateral part of the pulvinar is the lateral half of the anterior wall of the atrium, the posteromedial part is covered by the crus of the fornix and is part of the superolateral wall of the quadrigeminal cistern, and the inferolateral part of the pulvinar is the roof of the wing of the ambient cistern. The medial part of the thalamus is the lateral wall of the third ventricle (see Figs. 2-3A and 2-4B).

Hippocampus

The hippocampus occupies the medial portion of the floor of the temporal horn and is divided into three parts: head, body, and tail. The head of the hippocampus, the anterior and largest part, is directed anteriorly and inferiorly and then medially. At the medial end of the tip of the temporal horn, it turns up vertically and bends over laterally to form the medial wall of the tip of the temporal horn, ahead of the choroidal fissure. The head of the hippocampus is free of the choroid plexus and features three or four hippocampal digitations; its overall shape resembles a feline paw, and it is directed toward the posterior segment of the uncus. Its posterior limit is the initial segment of the fimbria and the choroidal fissure. Superiorly, the head of the hippocampus is related to the posteroinferior portion of the amygdala. Anteriorly, it is related to the uncal recess of the temporal horn, which is the anterior continuation of the collateral eminence. The emergence of the choroid plexus, fimbria, and choroidal fissure marks the beginning of the body of the hippocampus. The body of the hippocampus takes an anteroposterior and inferosuperior direction and narrows as it approaches the atrium of the lateral ventricle. Posterior to the head of the hippocampus, the medial wall of the temporal horn is the choroidal fissure. At the atrium of the lateral ventricle, the body of the hippocampus changes direction and has its longitudinal axis oriented transversely to become the tail of the hippocampus. The tail of the hippocampus is slender and constitutes the medial part of the floor of the atrium; medially, the tail of the hippocampus fuses with the calcar avis. Histologically, the terminal segment of the hippocampal tail continues as the subsplenial gyrus, which covers the inferior splenial surface (see Figs. 2-3D, *left*, and 2-4A).

Amygdala

The amygdala and the hippocampus constitute the core of the limbic system. The temporal amygdala is composed of a series of gray matter nuclei classified into three main groups: basolateral, corticomedial, and central. From a neurosurgical viewpoint, the temporal amygdala can be considered to be located entirely within the boundaries of the uncus: superiorly, the amygdala

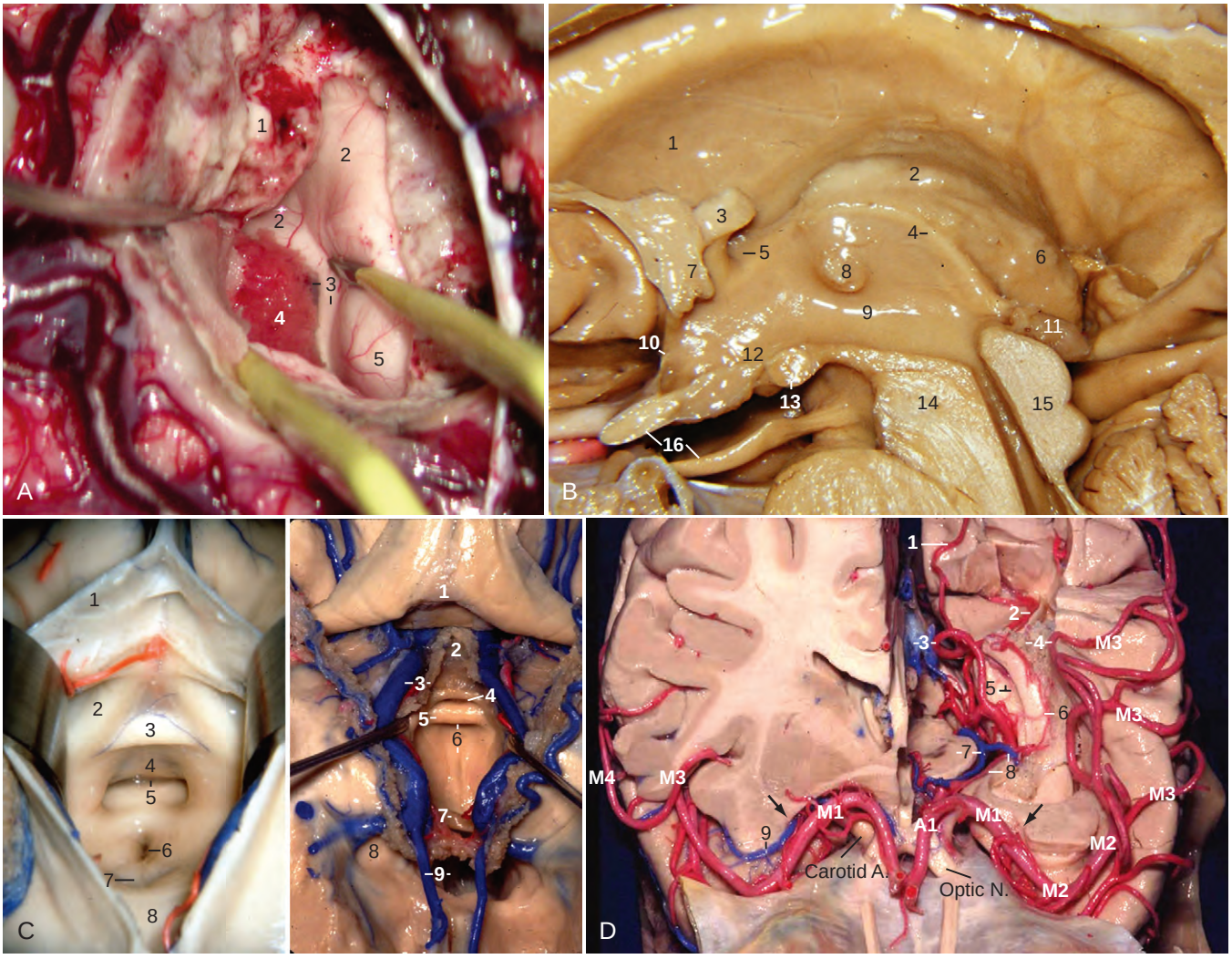

FIGURE 2-4 **A,** Intraoperative view of the right temporal horn. 1, Amygdala; 2, head of the hippocampus; 3, fimbria and taenia fimbriae; 4, choroid plexus; 5, body of the hippocampus. **B,** Medial view of the right thalamus. 1, Caudate nucleus; 2, thalamus (dorsal surface); 3, fornix (column); 4, taenia thalami; 5, foramen of Monro; 6, thalamus (pulvinar); 7, anterior commissure; 8, massa intermedia; 9, hypothalamic sulcus; 10, lamina terminalis; 11, pineal gland; 12, hypothalamus; 13, mamillary body; 14, midbrain; 15, quadrigeminal plate; 16, optic nerve and oculomotor nerve. **C,** *Left*, Posterosuperior view of the anterior wall and floor of the third ventricle. 1, Fornix (column); 2, fornix (column); 3, anterior commissure; 4, lamina terminalis; 5, optic recess; 6, infundibular recess; 7, tuber cinereum; 8, midbrain. *Right*, Anterosuperior view of the posterior wall of the third ventricle. 1, Fornix (reflected); 2, suprapineal recess and pineal gland; 3, internal cerebral vein and choroid plexus; 4, habenular commissure; 5, posterior commissure; 6, aqueduct and midbrain; 7, massa intermedia; 8, thalamostriate vein; 9, anterior septal vein. **D,** Frontal view. 1, Parieto-occipital artery; 2, calcarine artery; 3, vein of Galen and P3; 4, sylvian point and atrium; 5, parahippocampal and dentate gyri; 6, lateral posterior choroidal artery; 7, crus cerebri and basal vein; 8, anterior choroidal artery and inferior ventricular vein; 9, deep middle cerebral vein. *Arrows* indicate the limen insulae.

blends into the globus pallidus; inferiorly, the temporal amygdala bulges inferiorly into the most anterior portion of the roof of the temporal horn above the hippocampal head and the uncal recess; and medially, it is related to the anterior and posterior segments of the uncus. It also forms the anterior wall of the temporal horn (see Figs. 2-2A, 2-3D, *right*, and 2-4A).

Choroidal Fissure

The choroidal fissure is a cleft located between the thalamus and the fornix and is the site of attachment of the choroid plexus in the lateral ventricle. It is a C-shaped arc that extends from the foramen of Monro through the body and atrium to the temporal horn.[7] The body portion of the choroidal fissure lies between the body of the fornix and the thalamus,[8] the atrial portion is located between the crus of the fornix and the pulvinar of the thalamus (see Fig. 2-3A), and the temporal portion lies between the fimbria of the fornix and the stria terminalis of the thalamus. The choroid plexus is attached to the fornix and the thalamus by an ependymal covering called the *taenia fornicis* and *taenia choroidea*, respectively; in the temporal part, the taenia fimbriae attaches the choroid plexus to the fimbria. The choroidal fissure is one of the most important landmarks in microneurosurgery involving the temporal lobe in that it separates temporal structures that can be removed from thalamic structures that should be preserved (Fig. 2-4A).

Third Ventricle

The third ventricle is a narrow, funnel-shaped, unilocular midline cavity. It communicates at its anterosuperior margin with each lateral ventricle through the foramen of Monro and posteriorly

with the fourth ventricle through the aqueduct of Sylvius (Fig. 2-4B). It has a roof, a floor, and an anterior, posterior, and two lateral walls.[9] The roof extends from the foramen of Monro anteriorly to the suprapineal recess posteriorly and is constituted superiorly to inferiorly by five layers (see Fig. 2-3A). The first layer is the fornix; the body of the fornix is the anterior portion of the roof of the third ventricle, and the crura and the hippocampal commissure are the roof of the posterior portion. The second layer is the superior membrane of the tela choroidea, which is the part of the tela choroidea that passes thorough the forniceal side of the choroidal fissure to cover the choroid plexus of the lateral ventricle. The third layer is a vascular layer located in a space between the superior and inferior membranes of the tela choroidea called the *velum interpositum;* it contains the internal cerebral veins and branches of the medial posterior choroidal arteries. The fourth layer, the inferior membrane of the tela choroidea, forms the floor of the velum interpositum. It is attached anterolaterally to the taenia thalami, a small ridge on the free edge of a fiber tract, the striae medullaris thalami, that extends along the superomedial border of the thalamus from the foramen of Monro to the habenular commissure (Fig. 2-4B). The posterior part of the inferior membrane of the tela choroidea is attached to the superior surface of the pineal body. The fifth layer is the choroidal plexus of the third ventricle and is usually represented by two parallel strands of choroid plexus projecting backward on each side of the midline. The floor extends from the optic chiasm, anteriorly, to the orifice of the aqueduct of Sylvius posteriorly, and it is constituted, from anterior to posterior, by the optic and infundibular recesses, the tuber cinereum, the mamillary bodies, the posterior perforated substance, the midbrain, and the aqueduct (Fig. 2-4B). The anterior wall is formed by the lamina terminalis and the posterior wall is represented, from inferior to superior, by the posterior commissure, pineal recess, habenular commissure, pineal gland, and suprapineal recess (Fig. 2-4C). At the inner angle formed by the roof and the anterior wall is the anterior commissure.[10] Frequently, there is another commissure in the cavity of the third ventricle located posterior to the foramen of Monro called the *massa intermedia*, which connects both thalami. The lateral wall of the third ventricle is constituted by the thalamus above and by the hypothalamus below, both separated by the hypothalamic sulcus, a shallow groove extending from the foramen of Monro to the aqueduct. The hypothalamic sulcus is the rostral continuation of the sulcus limitans of the brainstem (Fig. 2-4B).

Lateral Surface: Arterial Relationships

The *middle cerebral artery* (MCA) is divided into four segments: the *M1* or *sphenoidal segment* extends from the bifurcation of the internal carotid artery (ICA) to the limen insulae and is discussed in the section on the basal surface.[11,12] The *M2* or *insular segment* extends from the limen insulae to the superior and inferior circular sulci of the insula; it runs in the insular compartment of the sylvian fissure and is constituted by the superior and inferior trunks and their branches. After reaching the superior or inferior circular sulcus of the insula, the M2 branches enter the opercular compartment and are called the *M3 segment*. The *M3* or *opercular segment* runs in the opercular compartment and is related to the frontal and parietal opercula superiorly and to the temporal operculum inferiorly. The loop of the most posterior M3 segment branch that exits from the sylvian fissure is called the *M point* or the *sylvian point.*[13] Anatomically, the sylvian point is located behind the insula, above the medial end of Heschl's gyrus (Fig. 2-4D). The angiographic sylvian point or M point is the location of the medial end of Heschl's gyrus, the posterior end of the insula, and the central core, atrium, and pulvinar of the thalamus (Fig. 2-13A). On a lateral projection, the M2 and M3 segments form the "sylvian triangle," which depicts the shape of the insula and represents the anterior, inferior, and posterior limits of the central core (Figs. 2-5A and 2-13B). The caudate nucleus is projected above the superior level of the sylvian triangle on a lateral projection (Figs. 2-2A and 2-13B). The fourth segment is the *M4* or *cortical segment*; it extends from the sylvian fissure to the lateral surface of the cerebrum.

Lateral Surface: Venous Relationships

The superficial venous system drains the superficial fifth of the thickness of the cerebrum, whereas the deep venous system drains the remaining four fifths of the depth of the cerebrum. On the lateral surface of the cerebrum, the superficial venous drainage system is directed to venous channels adjacent to the lobes. On the frontal and parietal lobes, venous drainage may be directed superiorly toward the superior sagittal sinus or inferiorly toward the superficial sylvian vein. On the temporal lobe, the veins can drain superiorly toward the superficial sylvian vein or inferiorly toward the dural sinuses below the temporal lobe.[14] There are three main anastomotic veins on the lateral surface of the cerebrum. The *superficial sylvian vein* begins at the posterior part of the posterior ramus of the sylvian fissure, runs inferiorly and anteriorly along the fissure, and commonly anastomoses with the veins of Trolard and Labbé. It may arise as two trunks or have several variations. In the region of the pterion, it enters the dura, runs along the lesser wing of the sphenoid in the sphenoparietal sinus or sinus of the lesser wing of the sphenoid, enters the anterior end of the cavernous sinus via the medial end of the superior orbital fissure, and then drains into the basilar sinus and the inferior petrosal sinus. The *vein of Trolard*, or the superior anastomotic vein, is the largest anastomotic vein crossing the lateral surface of the brain between the superior sagittal sinus and the sylvian fissure. It is more frequently located at the parietal lobe. The *vein of Labbé*, or the inferior anastomotic vein, is the largest anastomotic vein that crosses the temporal lobe between the sylvian fissure and the transverse sinus. It usually arises from the middle portion of the sylvian fissure and is directed posteriorly and inferiorly toward the anterior part of the transverse sinus, at the level of the preoccipital notch (Fig. 2-5B).

The deep part of the sylvian fissure is related to the deep sylvian or middle cerebral vein and its tributaries. The tributaries of the deep sylvian vein come mainly from the sulci of the insula. The *deep middle cerebral vein* begins as a vein in the central sulcus of the insula and runs anteriorly and inferiorly toward the limen insulae, where it joins other insular veins to form a common trunk (Fig. 2-6B).[15]

The deep venous system is divided into ventricular and cisternal groups; the cisternal group is discussed in the section on the basal surface. The ventricular veins are named mainly according to the location where they course: *frontal horn veins*—anterior caudate and anterior septal veins; *veins of the body of the lateral ventricle*—thalamostriate, thalamocaudate, posterior caudate, and posterior septal veins; *atrium* and *occipital horn veins*—medial and lateral atrial veins; *temporal horn veins*—inferior ventricular, amygdalar, and transverse hippocampal veins; *deep thalamic veins*—anterior and superior thalamic veins; *superficial thalamic veins*—anterior, superior, and posterior superficial thalamic veins; and *choroidal veins*—superior and inferior choroidal veins.[15]

Basal Surface: Neural Relationships

The basal surface is composed of the frontal, temporal, and occipital lobes. The olfactory tract and sulcus divide the basal surface of the *frontal lobe* into two uneven parts: the smaller and medial part is the rectus gyrus, whereas the larger and lateral part, the orbital surface, is located above the orbit and is composed of the orbital gyri. The orbital surface is divided by the orbital sulcus, a complex sulcus that exhibits a rough configuration of

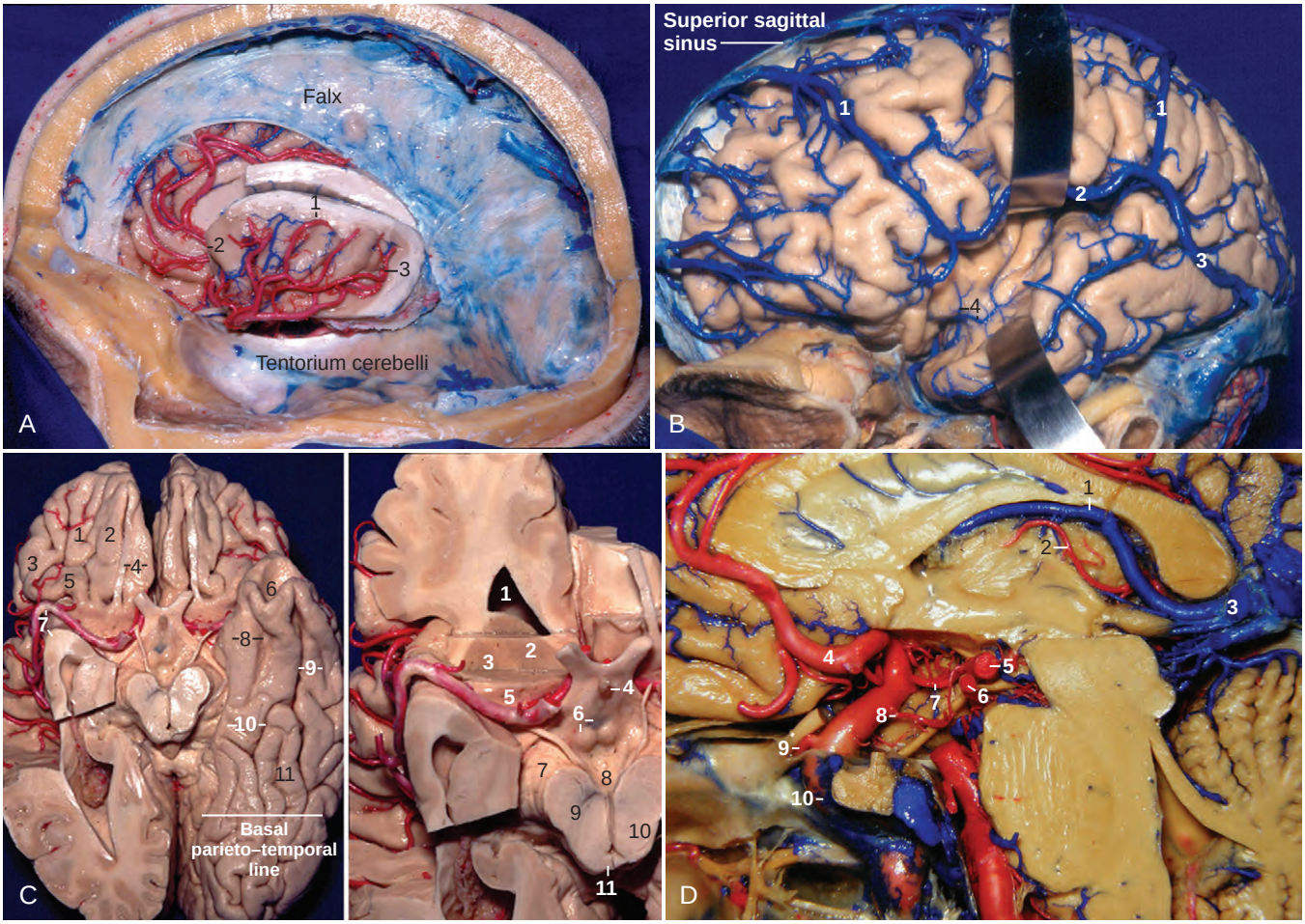

FIGURE 2-5 **A,** Lateral view of the M2 branches over the left insula. 1, Superior limiting sulcus; 2, anterior limiting sulcus; 3, inferior limiting sulcus. **B,** Lateral view. 1, Superior anastomotic vein; 2, superficial sylvian vein; 3, vein of Labbé; 4, insular veins. **C,** Basal view. *Left*, 1, Anterior orbital gyrus; 2, medial orbital gyrus; 3, lateral orbital gyrus; 4, rectus gyrus and olfactory tract; 5, posterior orbital gyrus; 6, temporal pole; 7, genu of the middle cerebral artery and insular pole; 8, uncus and rhinal sulcus; 9, occipitotemporal sulcus and inferior temporal gyrus; 10, parahippocampal gyrus and collateral sulcus; 11, fusiform gyrus. *Right*, 1, Frontal horn; 2, caudate nucleus (head); 3, lentiform nucleus; 4, pituitary stalk; 5, anterior perforated substance; 6, tuber cinereum and mamillary body; 7, crus cerebri; 8, posterior perforated substance; 9, substantia nigra; 10, tegmentum; 11, tectum. **D,** Medial view. 1, Internal cerebral vein; 2, medial posterior choroidal artery; 3, vein of Galen; 4, anterior cerebral artery; 5, P2A; 6, superior cerebellar artery; 7, anterior choroidal artery; 8, posterior communicating artery; 9, ophthalmic artery and optic nerve; 10, intracavernous carotid artery.

the letter "H," into four quadrants: the anterior, medial, posterior, and lateral orbital gyri. The pars orbitalis of the inferior frontal gyrus is continuous with the posterior part of the lateral orbital gyrus and with the lateral part of the posterior orbital gyrus. The *temporal lobe* is separated posteriorly from the occipital lobe by the basal parietotemporal line (from the preoccipital notch to the junction between the parieto-occipital and calcarine fissures) and, laterally to medially, is composed of the inferior temporal gyrus, occipitotemporal sulcus, fusiform gyrus, collateral sulcus, and parahippocampal gyrus (Fig. 2-5C, *left*). The collateral sulcus is oriented inferiorly to superiorly and medially to laterally and bulges into the lateral part of the floor of the temporal horn (collateral eminence) and the atrium (collateral trigone). The collateral sulcus separates the allocortical parahippocampal gyrus medially from the mesocortical fusiform gyrus laterally. These gyri are kept separated anteriorly by the rhinal sulcus, which separates the uncus medially from the temporal pole laterally. The rhinal sulcus can be considered an anterior continuation of the collateral sulci, and it continues superiorly on the surface of the planum polare and separates it from the uncus medially (Figs. 2-2A and 2-5C, *left*).

The *interpeduncular region* is bounded by two oculomotor nerves and the posteromedial surface and apex of the uncus laterally; by the diencephalic membrane of the Liliequist membrane (the membrane that extends from the dorsum sellae to the mamillary bodies), pituitary stalk, and dorsum sellae anteriorly; by the tuber cinereum, mamillary bodies, and the posterior perforated substance superiorly; and by the inner surface of both crura cerebri posteriorly. The prepontine cistern forms the inferior limit of the interpeduncular fossa (Fig. 2-5C and D).

Anterior Perforated Substance

The APS is the entry site for perforating arteries from the ICA, the anterior choroidal artery (AChA), the anterior cerebral artery (ACA), and the MCA to the basal ganglia, the anterior portion of the thalamus, the genu, and the anterior and posterior limbs of the internal capsule. It is also the exit site for the inferior striate veins. The APS is a convex cavity extending upward at the posterior end of the basal surface of the frontal lobe; it is bounded anteriorly by the lateral and medial olfactory striae, posteromedially by the optic tract, posterolaterally by the anteromedial

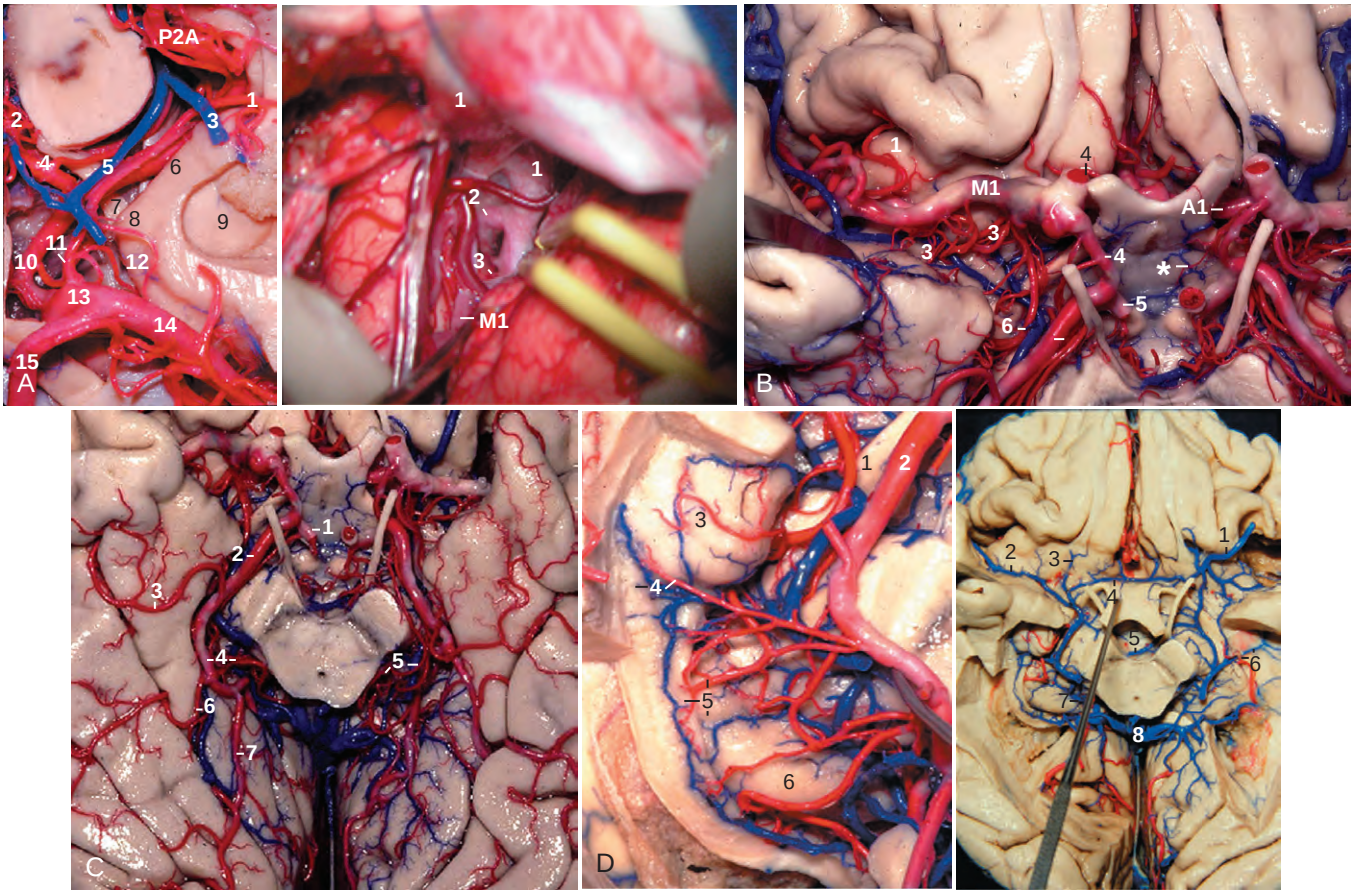

FIGURE 2-6 **A,** *Left*, 1, Inferior choroidal point; 2, posterior perforating arteries; 3, inferior ventricular vein; 4, P1 and medial posterior choroidal artery; 5, basal vein; 6, uncus (posterior segment); 7, oculomotor nerve; 8, uncus (apex); 9, hippocampus (head); 10, posterior communicating artery; 11, anterior choroidal artery; 12, uncus (anterior segment); 13, internal carotid artery; 14, M1; 15, A1. *Right*, Left trans-sylvian approach. 1, Supraclinoid carotid artery; 2, fetal posterior communicating artery; 3, anterior choroidal artery. **B,** Basal view. 1, Insula; 2, supraclinoid carotid artery; 3, lateral lenticulostriate arteries; 4, posterior communicating artery; 5, P1; 6, anterior choroidal artery; 7, P2A; *, premamillary artery. **C,** Basal view. 1, P1; 2, P2A; 3, anterior inferior temporal artery; 4, P2P and long circumflex arteries; 5, short circumflex arteries; 6, middle inferior temporal artery; 7, posterior inferior temporal artery. **D,** Basal view. *Left*, 1, Optic tract; 2, P2A; 3, uncus (inferior surface); 4, hippocampal artery and dentate gyrus; 5, lateral posterior choroidal artery, fornix, and lateral geniculate body; 6, thalamus (pulvinar). *Right*, Basal view. 1, Fronto-orbital vein; 2, deep middle cerebral vein; 3, olfactory vein; 4, anterior cerebral vein; 5, peduncular vein; 6, inferior ventricular vein and inferior choroidal point; 7, posterior mesencephalic segment; 8, vein of Galen. The choroid plexus separates the roof of the temporal horn from the thalamus.

surface of the uncus, and laterally by the limen insulae. Medially, the APS extends above the optic chiasm to the interhemispheric fissure. The APS and the carotid bifurcation can be identified intraoperatively by following the olfactory tract posteriorly. The APS can be considered the "floor" of the anterior half of the basal ganglia (Fig. 2-5C, *right*).

Basal Surface: Arterial Relationships

The *internal carotid artery* is divided into five parts: the cervical, petrous, cavernous, clinoid, and supraclinoid portions. The supraclinoid portion has been divided into three segments based on the origin of its major branches: the *ophthalmic segment* extends from the origin of the ophthalmic artery to the origin of the posterior communicating artery (PCom), the *communicating segment* extends from the origin of the PCom to the origin of the anterior choroidal artery (AChA), and the *choroidal segment* extends from the origin of the AChA to the bifurcation of the ICA (Fig. 2-5D). The *ophthalmic artery* arises under the optic nerve, usually from the medial third of the superior surface of the ICA, passes anteriorly and laterally to become superolateral to the carotid, and enters the optic canal and the orbit. The perforating arteries from this segment arise from the posterior, medial, or posteromedial aspect of the ICA and are distributed to the stalk of the pituitary gland, the optic chiasm, and less commonly the optic nerve, premamillary portion of the floor of the third ventricle, and the optic tract. The *superior hypophysial arteries*, which can range from 1 to 5 in number, pass medially to supply the pituitary stalk and the anterior lobe of the pituitary gland. The *inferior hypophysial artery* from the meningohypophysial trunk of the cavernous ICA supplies the posterior lobe. The *infundibular arteries* are another group of arteries that arise from the PCom and supply the same area as the superior hypophysial artery. The PCom arises from the posteromedial or the posterior or posterolateral aspect of the ICA and passes posteromedially to join the posterior cerebral artery (PCA) (Fig. 2-6A, *left*). In the embryo, the PCom continues as PCA, but in adults the PCA becomes part of the basilar system. If the PCom remains the major origin of the PCA, the configuration of the PCom is termed *fetal* (Fig. 2-6A, *right*). In 60% of individuals there are no perforating arteries arising from the communicating segment of the ICA; when present, the perforating arteries from the PCom

range from 4 to 14 in number, arise predominantly from the proximal half of the artery, course superiorly, and terminate in the floor of the third ventricle. The largest branch from the PCom is the *premamillary artery* or "*anterior thalamoperforating artery*" (Fig. 2-6B).

The *anterior choroidal artery* arises from either the posterolateral or posterior aspect of the ICA. The AChA courses posteriorly below the optic tract toward the temporal horn by passing through the choroidal fissure (Fig. 2-6A, *left*). The AChA sends off branches to the optic tract, crus cerebri, lateral geniculate body, and uncus and supplies the optic radiation, globus pallidus, midbrain, thalamus, and the retrolenticular and posterior portions of the posterior limb of the internal capsule.

The choroidal segment of the ICA is the most frequent site of perforating arteries (range, one to nine) arising from the posterior aspect of the ICA. They terminate in the posterior half of the central region of the APS, optic tract, and uncus.[16]

The anterior perforating arteries are those arising from the ICA, MCA, AChA, and ACA, and they enter the brain through the APS (Fig. 2-6B).

The M1, or *sphenoidal segment* of the MCA, extends from the bifurcation of the ICA to the limen insulae. It courses first in the carotid cistern and then continues in the sphenoidal compartment. The proximal half of M1 is related posteriorly and inferiorly to the anteromedial surface of the uncus, anteriorly to the lesser wing of the sphenoid, and superiorly to the APS; the distal half is related inferiorly to the planum polare, anteriorly to the lesser wing of the sphenoid, and superiorly and posteriorly to the insular pole. M1 has two types of branches: the lateral lenticulostriate arteries, which arise mostly from the superior or posterosuperior aspect of M1 and penetrate the middle and posterior portions of the lateral half of APS, and the early branches, which course toward the temporal lobe to supply the temporal pole. The bifurcation of the MCA occurs before the limen insulae in 86% of individuals (see Figs. 2-4D and 2-6B and C).[3]

Embryologically, the *posterior cerebral artery* arises as a branch of the ICA, but up to birth its most frequent origin is the basilar artery.[17] The PCA is divided into four segments: *P1* extends from the basilar bifurcation to the site where the PCom joins the PCA. *P2* extends from the PCom to the posterior aspect of the midbrain. P2 is further divided into *P2A* (*anterior*) and *P2P* (*posterior*) *segments*. P2A begins at the PCom and courses around the crus cerebri, inferior to the optic tract, AChA, and basal vein and medial to the posteromedial surface of the uncus, up to the posterior margin of the crus cerebri. P2P begins at the posterior margin of the crus cerebri; runs lateral to the tegmentum of the midbrain within the ambient cistern, parallel and inferior to the basal vein, inferolateral to the geniculate bodies and pulvinar, and medial to the parahippocampal gyrus; and enters the quadrigeminal cistern. *P3* begins under the posterior part of the pulvinar in the lateral aspect of the quadrigeminal cistern and ends at the anterior limit of the anterior calcarine sulcus. P3 often divides into its major terminal branches, the calcarine and parieto-occipital arteries, before reaching the anterior limit of the anterior calcarine sulcus. The point where the PCAs from each side are closer to each other is called the *collicular* or *quadrigeminal point*. It marks the posterior limit of the midbrain on angiograms (see Fig. 2-14A). The *P4 segment* is the cortical branches of the PCA (Fig. 2-6C).

The main branches arising from the PCA are the posterior thalamoperforating, the direct perforating, the short and long circumflex, the thalamogeniculate, the medial and lateral posterior choroidal, the inferior temporal, the parieto-occipital, the calcarine, and the posterior pericallosal arteries. The *posterior thalamoperforating arteries*, which arise from P1 and enter the brain through the posterior perforated substance, interpeduncular fossa, and medial crus cerebri, supply the anterior and part of the posterior thalamus, hypothalamus, subthalamus, substantia nigra, red nucleus, oculomotor and trochlear nuclei, oculomotor nerve, mesencephalic reticular formation, pretectum, rostromedial floor of the third ventricle, and the posterior portion of the internal capsule. The *direct perforating arteries* to the crus cerebri arise mainly from the P2A segment and supply the crus cerebri. The *short* and *long circumflex arteries* to the brainstem arise mainly from P1 and less frequently from P2A; the short circumflex artery courses around the midbrain and terminates at the geniculate bodies, whereas the long circumflex artery courses around the midbrain and reaches the colliculi. The *thalamogeniculate arteries* arise equally from the P2A or P2P segments, perforate the inferior surface of the geniculate bodies, and supply the posterior half of the lateral thalamus, posterior limb of the internal capsule, and the optic tract (Fig. 2-6C). The *medial posterior choroidal arteries* (MPChAs) arise mainly from P2A and less frequently from the P2P and P1 segments, course around the midbrain medial to the main trunk of the PCA, turn around the pulvinar of the thalamus and proceed superiorly at the lateral side of the colliculi and pineal gland, enter the roof of the third ventricle through the velum interpositum, and finally course through the foramen of Monro to enter the choroid plexus in the lateral ventricle (see Figs. 2-3A, *right*, and 2-5D). The MPChA supplies the crus cerebri, tegmentum, geniculate bodies (mainly the medial one), colliculi, pulvinar, pineal gland, and medial thalamus. Angiographically on a lateral projection, the MPChA describes the shape of the number "3." The inferior curve of the "3" is the point where it turns around the pulvinar, and the superior curve is the point where it contours the colliculi before entering the roof of the third ventricle (Fig. 2-14B). The *lateral posterior choroidal arteries* (LPChAs) arise mainly from P2P and less frequently from the P2A segment and pass laterally and enter the ventricular cavity directly through the choroidal fissure to supply the choroid plexus in the atrium and the temporal horn. It anastomoses with the AChA (see Figs. 2-4D and 2-6D, *left*). The *inferior temporal arteries* are distributed to the basal surface of the temporal and occipital lobes. They include the hippocampal artery and three groups of temporal arteries, namely, the anterior, middle, and posterior temporal arteries (Fig. 2-6C). The anterior temporal artery arises mainly from P2A, whereas the middle and posterior temporal arteries arise mainly from the P2P segment. The *parieto-occipital* and *calcarine arteries* are usually terminal branches of the PCA; they arise predominantly from P3 but may sometimes also arise from the P2P segment and course into the parieto-occipital and calcarine fissures, respectively. As the calcarine fissure reaches laterally and bulges into the medial wall of the atrium and the occipital horn, the calcarine artery also follows laterally into the depth of the calcarine fissure (see Fig. 2-4D). The *splenial* or *posterior pericallosal artery* supplies the splenium of the corpus callosum and arises from the parieto-occipital artery in 62% of individuals, but it can also arise from the calcarine artery, MPChA, posterior temporal artery, P2P, P3, and LPChA.

Basal Surface: Venous Relationships

The inferior frontal veins drain the basal surface of the *frontal lobe*; they either drain anteriorly to the superior sagittal sinus (anterior group) or drain posteriorly to join the deep sylvian vein in the sylvian fissure (posterior group). The *anterior group* is composed of the anterior fronto-orbital and frontopolar veins, whereas the *posterior group* is composed of the olfactory and the posterior fronto-orbital veins. The inferior temporal veins drain the *temporal lobe*; they are divided into a lateral group that drains into the sinuses in the anterolateral part of the tentorium and a medial group that empties into the basal vein. The *lateral group* is composed of the anterior, middle, and posterior temporobasal veins. The temporobasal veins appear to radiate from the preoccipital notch across the inferior surface of the temporal lobe. The *occipital lobe* is drained by the *occipitobasal vein*, which courses

anterolaterally toward the preoccipital notch and frequently joins the posterior temporobasal vein before emptying into the lateral tentorial sinus.

The most important deep venous channel on the basal surface is the basal vein of Rosenthal. The *basal vein* originates below the APS and is divided into three segments (Fig. 2-6D, *right*): the *first*, or *anterior* or *striate segment*, originates from the junction of the anterior cerebral, inferior striate, olfactory, fronto-orbital, and deep middle cerebral veins under the APS and runs posteriorly under the optic tract, medial to the anterior portion of the crus cerebri. This point corresponds to the most medial (before its termination into the vein of Galen) and usually most inferior part of the basal vein and laterally indicates the location of the apex of the uncus. The *second*, or *middle* or *peduncular segment*, starts from the most medial point in the course of the basal vein, usually corresponding to the site where the peduncular vein joins the basal vein. It runs laterally between the upper part of the posteromedial surface of the uncus and the upper part of the crus cerebri and under the optic tract to reach the most lateral part of the crus cerebri, which corresponds to the most lateral point of the vein as it turns around the crus cerebri, generally where the inferior ventricular vein joins the basal vein; this is called the *anterior peduncular segment* by Huang and Wolf.[18] It then turns medially, superiorly, and posteriorly to the plane of the lateral mesencephalic sulcus behind the crus cerebri to constitute the posterior peduncular segment. The main tributaries of the second segment are the peduncular or interpeduncular, inferior ventricular, inferior choroidal, hippocampal, and anterior hippocampal veins. The *third*, or *posterior* or *posterior mesencephalic segment*, runs medially, superiorly, and posteriorly from the lateral mesencephalic sulcus and under the pulvinar of the thalamus to penetrate the quadrigeminal cistern and generally drains into the vein of Galen. The main tributaries of the third segment are the lateral mesencephalic, posterior thalamic, posterior longitudinal hippocampal, medial temporal, and medial occipital veins. Sometimes, the precentral cerebellar, superior vermian, internal occipital, splenial, medial atrial, and direct lateral and lateral atrial subependymal veins may drain into the third segment of the basal vein. In the angiographic frontal view, the overall shape of both basal veins resembles the legs of a frog lying on its back with its toes directed anterolaterally. The foot corresponds to the striate segment and is related superiorly to the APS, laterally to the anterior segment of the uncus, medially to the optic tract, and inferiorly to the contents of the carotid cistern. The ankle corresponds posteriorly to the anterior aspect of the crus cerebri, laterally to the apex of the uncus, and superiorly to the optic tract; the leg corresponds to the anterior peduncular segment and is related superiorly to the optic tract, laterally to the upper portion of the posteromedial surface of the uncus, and medially to the upper portion of the crus cerebri. The knee corresponds to the most lateral aspect of the crus cerebri and to the posterior edge of the posterior segment of the uncus. It is related laterally to the inferior choroidal point, superiorly to the optic tract just before it reaches the lateral geniculate body, and inferiorly to the contents of the ambient cistern. The thigh, which includes the posterior peduncular and the posterior mesencephalic segments, is related medially to the tegmentum of the midbrain, laterally to the parahippocampal gyrus, superiorly to the medial aspect of the pulvinar of the thalamus, which is the roof of the wing of the ambient cistern, and inferiorly to the contents of the wing of the ambient cistern (see Fig. 2-6D).[17] In the angiographic lateral view, the basal and the internal cerebral veins delimit the thalamus and hypothalamus (Fig. 2-7A; also see Fig. 2-13D).

Medial Surface: Neural Relationships

The medial surface of the cerebrum contains the sulci and gyri of the frontal, parietal, occipital, and temporal lobes. The general organization of the gyri of the frontal, parietal, and occipital lobes on this surface can be compared with that of a three-layer roll: the inner layer is represented by the corpus callosum, the intermediate layer by the cingulate gyrus, and the outer layer by the medial frontal gyrus, paracentral lobule, precuneus, cuneus, and lingual gyrus. The cingulate gyrus is separated inferiorly from the corpus callosum by the callosal sulcus and superiorly from the outer layer by the cingulate sulcus. Several secondary rami ascend from the cingulate sulcus in a radiating pattern and divide the outer layer into several sections. There are two secondary rami of particular importance: the *paracentral ramus*, which ascends from the cingulate sulcus at the level of the midpoint of the corpus callosum and separates the medial frontal gyrus anteriorly from the paracentral lobule posteriorly, and the *marginal ramus*, which ascends from the cingulate sulcus at the level of the splenium of the corpus callosum and separates the paracentral lobule anteriorly from the precuneus posteriorly. The *marginal ramus* intercepts the postcentral gyrus in almost 100% of individuals and is an important landmark to determine the location of the sensory or motor areas in the lateral convexity on midsagittal magnetic resonance images. The parieto-occipital sulcus separates the precuneus superiorly from the cuneus inferiorly, and the calcarine sulcus separates the cuneus superiorly from the lingual gyrus inferiorly. The paracentral ramus and the marginal ramus form the *paracentral lobule*, which is concerned with movements of the contralateral lower limb and perineal region and is involved in voluntary control of defecation and micturition. The paracentral lobule comprises the anterior portion of the postcentral and precentral gyri and the posterior portion of the superior frontal gyrus. The *precuneus* and the part of the paracentral lobule behind the central sulcus form the medial part of the parietal lobe; the precuneus corresponds to the superior parietal lobule on the lateral surface. The precuneus presents the *subparietal sulcus*, a vaguely H-shaped sulcus where the vertical arm of the H tends to align with the marginal ramus, and the parieto-occipital sulcus, which separates the precuneus above from the cingulate gyrus below (Fig. 2-7A). The parieto-occipital and calcarine sulci define the *cuneus*; the cuneus and medial part of the lingual gyrus are the medial portion of the occipital lobe. The *calcarine sulcus* starts at the occipital pole and is directed anteriorly; it has a slightly curved course with a characteristic upward convexity. The calcarine sulcus joins the parieto-occipital sulcus (only superficially) at an acute angle behind the isthmus of the cingulate gyrus and continues anteriorly to intercept the isthmus of the cingulate gyrus. The portion of the calcarine sulcus anterior to the junction is called *anterior calcarine sulcus*; it is crossed by a buried *anterior cuneolingual gyrus* and bulges into the medial wall of the atrium of the lateral ventricle as the calcar avis. It contains the visual cortex only on its lower lip. The part of the calcarine sulcus posterior to the union is called the *posterior calcarine sulcus* and includes the striate (visual) cortex on its upper and lower lips (Fig. 2-7A and B). Anteriorly, the cingulate and medial frontal gyri wrap around the genu and rostrum of the corpus callosum. At the inferior end of these two gyri, under the rostrum of the corpus callosum and in front of the lamina terminalis, is a narrow triangle of gray matter, the *paraterminal gyrus*, separated from the rest of the cortex by a shallow *posterior paraolfactory sulcus*. Slightly anterior to this sulcus, a short vertical sulcus may occur, the *anterior paraolfactory sulcus*; the cortex between the posterior and anterior paraolfactory sulci is the *subcallosal area* or paraolfactory gyrus. Frequently, two anteroposteriorly directed sulci, the *superior* and *inferior rostral sulci*, which are parallel to the floor of the anterior fossa, divide the inferior portion of the medial frontal gyrus into three parts. Posteriorly, the cingulate gyrus continues inferiorly with the parahippocampal gyrus through the isthmus of the cingulate gyrus. The *mesial portion of the temporal lobe* contains intraventricular and extraventricular elements. The intraventricular

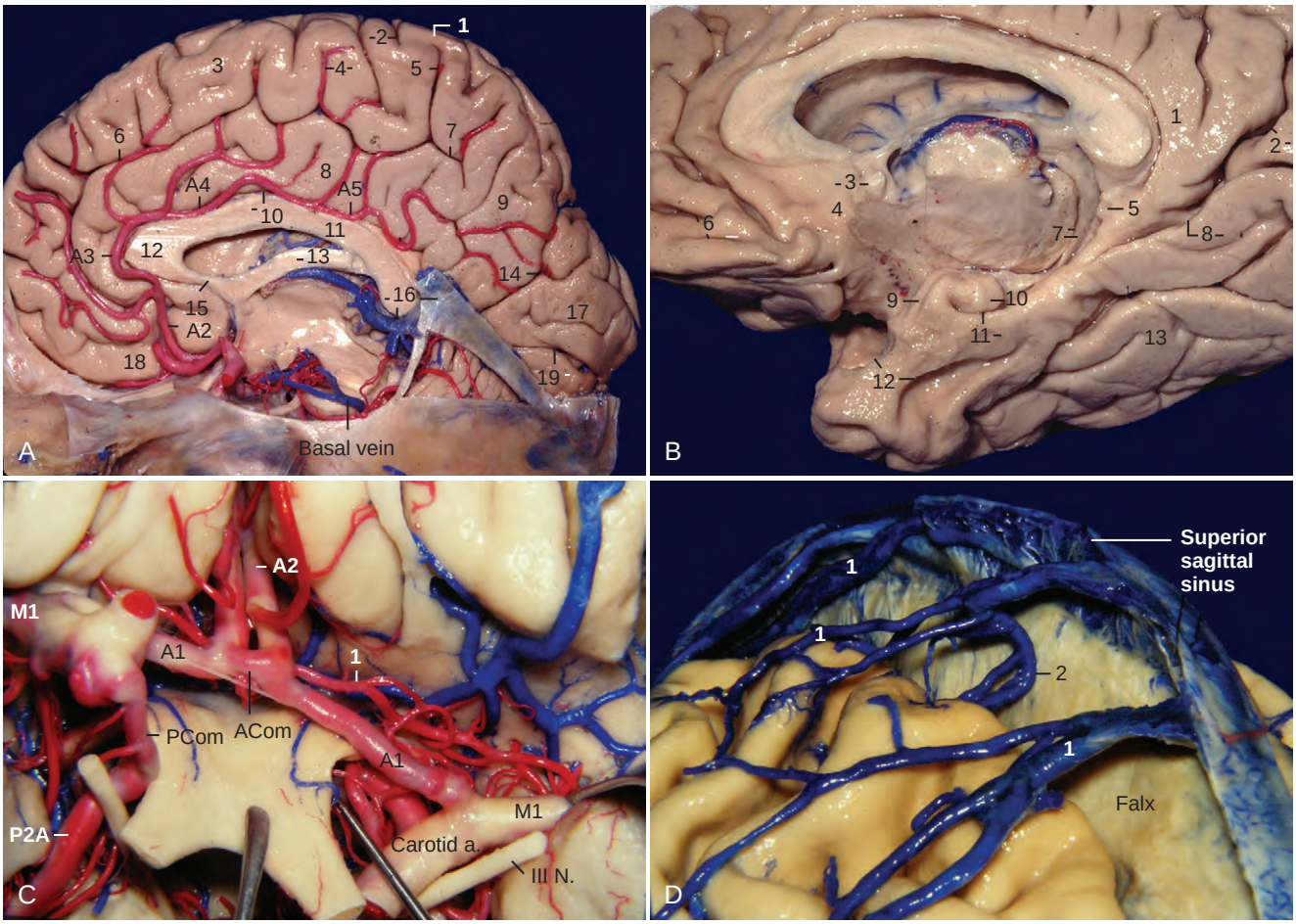

FIGURE 2-7 **A,** Medial view. 1, Postcentral gyrus; 2, precentral gyrus and central sulcus; 3, medial frontal gyrus; 4, paracentral ramus and paracentral lobule; 5, marginal ramus; 6, cingulate sulcus; 7, intraparietal sulcus; 8, cingulate gyrus; 9, precuneus; 10, corpus callosum (body) and callosal sulcus; 11, corpus callosum (isthmus); 12, corpus callosum (genu); 13, fornix and internal cerebral vein; 14, parieto-occipital sulcus; 15, corpus callosum (rostrum); 16, corpus callosum (splenium), vein of Galen, and straight sinus; 17, cuneus; 18, rectus gyrus; 19, posterior calcarine sulcus and lingual gyrus. **B,** Medial view. 1, Cingulate gyrus (isthmus); 2, parieto-occipital sulcus and cuneus; 3, anterior commissure and subcallosal area; 4, paraterminal gyrus; 5, dentate gyrus; 6, superior and inferior rostral sulci; 7, choroidal fissure and fornix; 8, anterior calcarine sulcus and lingual gyrus; 9, uncus (anterior segment); 10, uncus (posterior segment); 11, uncal notch; 12, rhinal incisura and rhinal sulcus; 13, fusiform gyrus. **C,** Basal view. 1, Recurrent artery. ACom, anterior communicating artery; III n., cranial nerve III, PCom, posterior communicating artery; **D,** Anterolateral view of the right parasagittal area. 1, Vein from the lateral surface; 2, vein from the medial surface.

elements are the hippocampus, fimbria, amygdala, and choroidal fissure; the extraventricular elements are the parahippocampal gyrus, uncus, and dentate gyrus. The *parahippocampal gyrus* extends anteriorly to posteriorly, and at its anterior extremity, it deviates medially and bends posteriorly to constitute the uncus. Posteriorly, just bellow the splenium of the corpus callosum, the parahippocampal gyrus is often intersected by the anterior calcarine sulcus, which divides the posterior portion of the parahippocampal gyrus into the isthmus of the cingulate gyrus superiorly and the parahippocampal gyrus inferiorly; the parahippocampal gyrus continues posteriorly as the lingual gyrus. Superiorly, the parahippocampal gyrus is separated from the dentate gyrus by the hippocampal sulcus. Laterally, the parahippocampal gyrus is limited by the collateral sulcus posteriorly and the rhinal sulcus anteriorly. The rhinal sulcus marks the lateral limit of the entorhinal area of the parahippocampal gyrus; the parahippocampal gyrus is separated from the inferior surface of the posterior segment of the uncus by the *uncal notch*. Medially, the parahippocampal gyrus is related to the edge of the tentorium and to the contents of the ambient cistern. The various components of the parahippocampal gyrus are the subiculum, presubiculum, parasubiculum, and entorhinal area; the subiculum is the medial round edge of the parahippocampal gyrus. The name *uncus* means "hook." It is formed by the anterior portion of the parahippocampal gyrus, which has deviated medially and folded posteriorly. Inferiorly, the uncus is separated from the parahippocampal gyrus by the uncal notch. Anteriorly, the uncus continues with the anterior portion of the parahippocampal gyrus without a sharp boundary; superiorly, the uncus is continuous with the globus pallidus. At the basal surface, the uncus is separated laterally from the temporal pole by the rhinal sulcus, and its medial part is normally herniated medially to the edge of the tentorium. When viewed from its basal surface, the uncus has the shape of an arrowhead with its apex pointing medially; it features an apex, an anterior segment, and a posterior segment (see Fig. 2-3D). The anterior segment of the uncus has one surface, the anteromedial surface, whereas the posterior segment has two surfaces, the posteromedial and inferior surfaces. Both segments converge superiorly at the junction between the amygdala and the globus pallidus. The uncus is composed of five small gyri and a small part of the entorhinal area, which occupies the anterior portion of the anteromedial surface. *The anterior segment* or *anteromedial surface* is part of the parahippocampal gyrus and contains the semilunar and ambient gyri. The semilunar gyrus occupies the

superior portion of the anteromedial surface and is bordered inferiorly by the sulcus annularis, and the ambient gyrus is medial and inferior to the semilunar gyrus; the anteroinferior area of this surface is occupied by the entorhinal area, which continues anteriorly and inferiorly with the entorhinal area of the parahippocampal gyrus (Fig. 2-7B). The anteromedial surface is related to the proximal sylvian fissure and carotid cistern and is the posterolateral limit of the APS. *The posterior segment* is related to the hippocampus and has two surfaces: a posteromedial and inferior surface (see Figs. 2-3D, *left*, and 2-6D, *left*). The posterior segment is occupied by three small gyri; from anterior to posterior, they are the uncinate gyrus, the band of Giacomini, and the intralimbic gyrus. The superior and inferior portions of the posteromedial surface of the uncus are related, respectively, to the crural and ambient cisterns. Posterior and superior to the uncus is the inferior choroidal point, where the choroid plexus of the temporal horn begins. The inferior choroidal point corresponds to the site where the AChA enters and the inferior ventricular vein leaves the temporal horn through the choroidal fissure (see Fig. 2-6A, *left*, and 2-6D, *right*). The inferior surface is the superior lip of the uncal notch, and it is visible only from below when the parahippocampal gyrus is removed. The *dentate gyrus* bears this name because of its characteristic tooth-like elevations; the margo denticulatus is prominent mainly in its anterior and middle portions. The dentate gyrus continues anteriorly with the band of Giacomini, also called the *tail of the dentate gyrus*, and continues posteriorly with the fasciolar gyrus, a smooth grayish band that is located posterior to the splenium of the corpus callosum; the fasciolar gyrus continues above the corpus callosum as the indusium griseum and finally ends as the paraterminal gyrus. The fimbriodentate and hippocampal sulci separate the dentate gyrus, respectively, from the fimbria superiorly and the parahippocampal gyrus inferiorly (Fig. 2-7B).

The extraventricular and intraventricular structures of the mesial temporal lobe are intimately related. The anterior segment of the uncus is related to M1, the carotid artery, and the amygdala. The apex of the uncus passes above the oculomotor nerve and is related to the uncal recess and the amygdala laterally (see Figs. 2-5D and 2-6A); the posterior segment is related to the head of the hippocampus and the amygdala laterally, to P2A inferomedially, and to the AChA superomedially.

Medial Surface: Arterial Relationships

The *anterior cerebral artery* has five segments. The *A1 segment* extends from the bifurcation of the ICA to the anterior communicating artery (ACom). The *A2 segment* extends from the ACom to the junction between the rostrum and the genu of the corpus callosum. The *A3 segment* extends from the genu of the corpus callosum to the point where the artery turns sharply and posteriorly above the genu of the corpus callosum. The A2 and A3 segments together are also called the *ascending segment*. The *A4* and *A5 segments* extend above the corpus callosum, from the genu to the splenium. These two segments together are also called the *horizontal segment*, and a point bisected in the lateral view close behind the coronal suture separates them. The segment of the ACA distal to the ACom (A2 to A5) has also been called the *pericallosal artery* (see Fig. 2-7A). The junction of the ACom with the A1 segment occurs above the chiasm in 70% of individuals and above the nerve in 30%. The shorter A1 segments are usually stretched tightly over the chiasm; the longer ones pass anteriorly over the optic nerve and can be elongated and tortuous and reach either the tuberculum sellae or the planum sphenoidale (Fig. 2-7A). The medial lenticulostriate perforators, ranging from 1 to 11 branches (average of 6.4), arise from the superior, posterior, or posterosuperior aspect of the proximal half of the A1 segment and pursue a direct posterior and superior course to enter the medial half of the APS.[19] Embryologically, the ACom develops from a multichanneled vascular network that coalesces to a variable degree by the time of birth.[19] Only in 20% of individuals does the ACom communicate with two A1 segments of equal size. The ACom complex probably exists as a single channel in about 75% of individuals.[19] The perforators from the ACom, ranging from 0 to 4 (average of 1.6), usually arise from its posteroinferior aspect and supply the infundibulum, the APS, the optic chiasm, the subcallosal area, and the preoptic areas of the hypothalamus. The *recurrent artery of Heubner* of the ACA arises in 78% of individuals from the proximal A2 segment, and it doubles back on its parent vessel, courses anterior to the A1 segment in 60% of individuals, and can be seen on elevating the frontal lobe before visualization of the A1 segment; it is the largest and longest branch directed to the APS. After its origin, it passes above the carotid bifurcation and accompanies the M1 segment into the medial part of the sylvian fissure before entering the anterior and middle portions of the full mediolateral extent of the APS (see Fig. 2-7C). The *A2 segment* is also the source of the central or the basal perforating arteries, which pass posteriorly and enter the optic chiasm, the lamina terminalis, and the anterior forebrain, below the corpus callosum. The two first cortical branches of the ACA supplying the medial surface, the *orbitofrontal* and the *frontopolar* arteries, usually arise from the A2 segment. The segments A3 to A5 give rise to other cortical branches and supply the medial surface of the hemisphere. All the cortical branches arise more frequently from the pericallosal than from the callosomarginal artery.

Medial Surface: Venous Relationships

The *medial frontal veins* drain the medial surface of the *frontal lobe*. They can empty either superiorly into the superior sagittal sinus or inferiorly into the inferior sagittal sinus or into the veins that pass around the corpus callosum and drain into the anterior end of the basal vein. The medial parietal veins drain the medial surface of the *parietal lobe*. They can either empty superiorly into the superior sagittal sinus or course around the splenium of the corpus callosum and drain inferiorly into the vein of Galen or its tributaries. On both lobes, the veins commonly curve over the superior margin of the hemisphere onto the upper part of the lateral surface, where they join the terminal end of the veins from the lateral surface before emptying into the superior sagittal sinus (see Fig. 2-7D). The *posterior pericallosal veins*, one on each side, arise from tributaries that drain the posterior part of the cingulate gyrus and the precuneus and course side by side around the splenium of the corpus callosum to terminate in either the vein of Galen or the internal cerebral vein. The anterior and posterior calcarine veins drain the *occipital lobe*. The *anterior calcarine* or *internal occipital vein* arises from tributaries that drain the anterior portion of the cuneus and lingual gyrus and passes forward to join the posterior pericallosal vein near the splenium before terminating in either the internal cerebral vein or the vein of Galen. The *posterior calcarine vein* arises from tributaries that drain the area bordering the posterior part of the calcarine fissure and then curves sharply upward on the cuneus to reach the superior sagittal sinus.

The deep venous system of the *mesial temporal region* drains into the basal vein of Rosenthal.

POSTERIOR FOSSA

The posterior fossa is characterized by the "rule of three" whereby the brainstem has three parts (midbrain, pons, and medulla) and the cerebellum has three surfaces (petrosal, tentorial, and suboccipital), three cerebellar peduncles (superior, middle, and inferior), three fissures (cerebellomesencephalic, cerebellopontine, and cerebellomedullary), three main arteries (superior

cebellar artery [SCA], anterior inferior cerebellar artery [AICA], and posterior inferior cerebellar artery [PICA]), and three main venous draining groups (petrosal, galenic, and tentorial).

Brainstem

The brainstem is divided into three parts: midbrain, pons, and medulla.

The *midbrain* is divided by a midline sagittal plane into two cerebral peduncles. Each peduncle is further divided into three parts: an anterior part, the crus cerebri or basis pedunculi; an intermediate part, the tegmentum; and a posterior part located behind the aqueduct, the tectum. The substantia nigra and lateral mesencephalic sulcus separate the crus cerebri from the tegmentum. The oculomotor nerves emerge from the medial side of the crura cerebri in the interpeduncular fossa (see Fig. 2-5C). The pontomesencephalic sulcus, which separates the midbrain from the pons, originates in the depth of the interpeduncular fossa and runs around the inferior margin of the crus cerebri to join the lateral mesencephalic sulcus behind the crus cerebri. The posterior aspect of the midbrain contains the superior and inferior colliculi (quadrigeminal plate). The trochlear nerve exits the brainstem below the inferior colliculus.

The *pons* or *protuberance* has a prominent anterior surface that is considerably convex from side to side, and it consists of transverse fibers that cross the median plane and converge on each side to form the middle cerebellar peduncles. The basilar sulcus is a shallow median groove on the anterior surface of the pons and usually lodges the basilar artery; this sulcus is bounded on each side by an eminence caused by descent of the corticospinal fibers through the substance of the pons. The *middle cerebellar peduncle* is separated from the belly of the pons by a vertical shallow groove, the lateral pontine sulcus. Just lateral to the lateral pontine sulcus is the emergence of the trigeminal nerve, with its smaller superomedial motor root and a larger inferolateral sensory root. From a microneurosurgical standpoint, the apparent origin of the trigeminal nerve can be considered as the limit between the pons and the middle cerebellar peduncle. Posteriorly, the pons constitutes the upper portion of the floor of the fourth ventricle.

The *medulla* has three longitudinal fissures at its anterior aspect, one median and two paramedian; the median one is the anterior median fissure, which continues inferiorly as the anterior median fissure of the spinal cord. The paramedian sulci of the anterior aspect of the medulla are the anterolateral sulci. At the medulla, the anterolateral sulcus is situated medial to the olive; consequently, it is also called *preolivary sulcus.* The preolivary sulcus is the upper continuation of the anterolateral sulcus of the spinal cord. The rootlets of the hypoglossal nerve, which exit from the preolivary sulcus, are analogous to the ventral motor rootlets that exit from the anterolateral sulcus of the spinal cord. The pyramid characterizes the *anterior region*, which is located between the anterior median fissure and the preolivary sulcus.

The rootlets of the accessory, vagus, and glossopharyngeal nerves exit from the postolivary sulcus, the continuation of the posterolateral sulcus of the spinal cord in the medulla; these cranial nerve rootlets are analogous to the dorsal spinal rootlets. The rootlets emerge from the brainstem and extend almost straight laterally to the jugular foramen. The pontomedullary sulcus separates the pons from the medulla, and its junction with the preolivary sulcus marks the apparent origin of the abducens nerve.

The *supraolivary fossette* is a triangular depression located behind and above the olive, anteromedial to the flocculus, and corresponds to the junction of the pons, the medulla, and the middle and inferior cerebellar peduncles. It is limited superiorly by the inferior aspect of the pons and the middle cerebellar peduncle and posteriorly by the inferior cerebellar peduncle. The fossette resembles a right-angled triangle with its right angle located between the superior pole of the olive and the inferior aspect of the pons, the superior leg corresponds to the inferior border of the pons and the middle cerebellar peduncle, the vertical leg corresponds to the posterior border of the olive, and the hypotenuse corresponds to the inferior cerebellar peduncle. Cranial nerves VI, VII, and VIII exit from the brainstem at the superior leg, and nerves IX, X, and XI exit from the brainstem at the hypotenuse (Fig. 2-8A).

Cerebellum

The cerebellum has three surfaces: the petrosal, tentorial, and suboccipital surfaces. The petrosal surface is related anteriorly to the petrous part of the temporal bone, the tentorial surface is related superiorly to the tentorium cerebelli and inferiorly to the upper part of the roof of the fourth ventricle, and the suboccipital surface is related inferiorly to the squamosal part of the occipital bone and anteriorly to the inferior part of the roof of the fourth ventricle. Because the fourth ventricle and cerebellum are closely related, their anatomy is considered together.

The *fourth ventricle* is often described as a tent-shaped midline structure surrounded mainly by the vermian components of the cerebellum. A regular tent has a roof that is divided into two halves, a floor, and two lateral walls; the fourth ventricle resembles a turned-over tent with its base facing forward and two open lateral walls. The floor is represented by the pons and medulla; the superior cerebellar peduncles, superior medullary velum, and the adjacent lingula constitute the superior part of the roof; the inferior part of the roof is composed of the inferior medullary velum, tela choroidea, choroid plexus, uvula, and the nodule; and the two open lateral walls are represented by lateral recesses through which the fourth ventricle communicates with the cerebellopontine angle (Fig. 2-8B and C).

Petrosal Surface of the Cerebellum and Fourth Ventricle

Each half of the petrosal surface is intersected by the great horizontal fissure, or petrosal fissure, that circumscribes the cerebellum.[20] At the level of the flocculus, the petrosal fissure bifurcates into a larger supraflocculus portion and a smaller infraflocculus portion of the *posterolateral fissure*, which separates the flocculonodule lobule from the rest of the cerebellum and communicates with the cerebellomedullary fissure at the cerebellopontine angle. The folia that constitute the upper half of the petrosal surface are the folia of the tentorial surface that have folded over the middle cerebellar peduncle and over the core of the cerebellum. These folia are the wing of the central lobule and the quadrangular, simple, and superior semilunar lobules. The folia that constitute the lower half of the petrosal surface are the folia derived from the suboccipital surface that have folded over the inferior cerebellar peduncle and over the core of the cerebellum and correspond to the inferior semilunar and biventral lobules (Fig. 2-8A and D).

The choroid plexus and the rhomboid lip of the foramen of Luschka are located anterior and inferior to the flocculus. The glossopharyngeal nerve is the most superior rootlet; a single nerve, it is located immediately in front of the choroid plexus. The flocculus is located below the lateral extension of the pontomedullary sulcus, and it is the hemispheric counterpart of the nodule.

The *upper half of the roof* of the fourth ventricle consists of neural elements: the superior cerebellar peduncles, the superior medullary velum, and the lingula. The lingula can be visualized behind the relatively transparent superior medullary velum. The

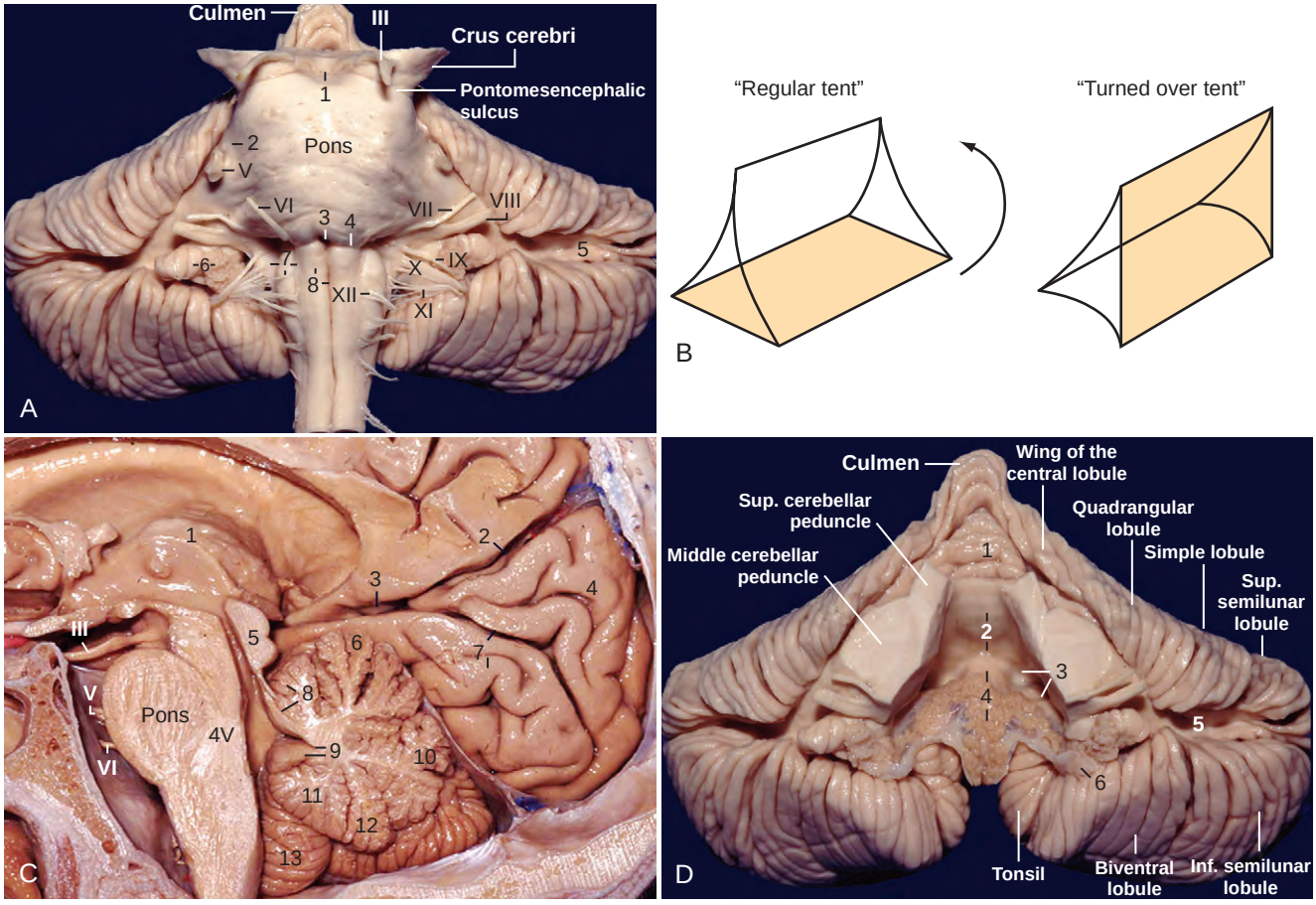

FIGURE 2-8 **A,** Frontal view. 1, Interpeduncular fossa; 2, lateral pontine sulcus; 3, inferior foramen caecum; 4, pontomedullary sulcus; 5, greater horizontal or petrosal fissure; 6, flocculus and choroid plexus; 7, supraolivary fossette, olive, and anterolateral sulcus; 8, pyramid and anterior median fissure. **B,** A regular tent is shown on the *left*, with its floor in *yellow* and the two portions of the roof transparent. The fourth ventricle resembles a turned-over tent with its floor facing forward. **C,** Sagittal view. 1, Thalamus; 2, parieto-occipital sulcus; 3, anterior calcarine sulcus; 4, cuneus; 5, quadrigeminal plate; 6, culmen; 7, posterior calcarine sulcus and lingual gyrus; 8, lingula and central lobule; 9, fastigium and nodule; 10, folium; 11, uvula; 12, pyramid; 13, tonsil. **D,** Frontal view. The brainstem has been removed to display the roof of the fourth ventricle. 1, Central lobule; 2, superior medullary velum (overlying lingula) and fastigium; 3, superolateral recess and inferior medullary velum (overlying tonsil); 4, inferior medullary velum (overlying nodule), choroid plexus, and tela choroidea; 5, petrosal fissure; 6, rhomboid lip.

lower half of the roof is composed of non-neural elements and has a horizontal portion, the inferior medullary velum, that covers the nodule and the superior pole of the tonsils, as well as a vertical portion, the tela choroidea and the choroid plexus, that covers the anterior aspect of the nodule, the uvula, and part of the tonsils. At the midline, the upper and lower halves of the roof converge at the fastigium.

The *lateral recess* is the lateral extension of the fourth ventricle, and it connects the fourth ventricle to the cerebellopontine angle. It is directed in a medial-to-lateral, slightly superior-to-inferior, and posterior-to-anterior direction and forms an angle of about 45 degrees with the sagittal plane. The lateral recess has anterior, superior, and posterior walls and a floor. The anterior and superior walls are formed by the inferior cerebellar peduncle as it runs upward and then turns backward toward the cerebellum. The floor of the lateral recess consists of the tela choroidea anteriorly, the choroid plexus in the middle, and the inferior medullary velum posteriorly; at the foramen of Luschka the inferior medullary velum becomes thicker and is called the *peduncle of the flocculus* and forms the posterior wall of the foramen of Luschka. The *superolateral recess* is the space in the fourth ventricle limited medially by the nodule and inferiorly by the superior pole of the tonsil and covered by the inferior medullary velum. Above the superolateral recess, the superior cerebellar peduncle has a prominence, the dentate tubercle, where the dentate nucleus comes to the surface (Fig. 2-8D).

The morphology of the choroid plexus of the fourth ventricle resembles the letter "T" with two vertical bars. The horizontal part of the choroid plexus, which starts from the fourth ventricle and protrudes into the cerebellopontine angle, resembles the horns of a bull. The vertical part and the proximal half of the horizontal part of the choroid plexus of the fourth ventricle are usually supplied by the PICA; the lateral half of the horizontal part and the choroid plexus located at the cerebellopontine angle are generally supplied by the AICA.[21]

The *tonsils* are two reniform structures that are the hemispheric components of the uvula and are attached to the cerebellum through the peduncles of the tonsil, which are located at the superolateral aspect of each tonsil. The superior, medial, anterior, posterior, and most of the lateral surfaces of the tonsils are free. The spaces around the tonsils are the supratonsillar space between its superior pole and the inferior medullary velum, the vallecula between the medial surfaces of the two tonsils, the cerebellomedullary fissure between the anterior

surface of the tonsil and the medulla, and the retrotonsillar space between the posterior surface of the tonsil and the adjacent vermis. The furrowed band of Reil connects the uvula to the tonsil, and the copula pyramidis connects the pyramid to the biventral lobule (Fig. 2-9A). The copular point is the angiographic landmark at which the retrotonsillar veins unite to form the inferior vermian vein; the copular point denotes the location of the copula pyramidis. The copular point can also be defined as the lowest part of the inferior vermian artery, the vermian branch of the PICA surrounding the copula pyramidis.

Tentorial Surface of the Cerebellum and Fourth Ventricle

The tentorial surface faces the tentorium and consists of two cerebellar incisurae, three margins, and two angles. The cerebellar incisurae are the anterior and posterior cerebellar incisurae; the brainstem fits into the *anterior cerebellar incisura* and the falx cerebelli fits into the *posterior cerebellar incisura*. The margins are the *anterosuperior margin*, or the posterior wall of the cerebellomesencephalic fissure that extends from the top of the culmen downward, forward, and laterally to reach a point above and behind the middle cerebellar peduncle; the *anterolateral margin*, which separates the tentorial from the petrosal surface; and the *posterolateral margin*, which separates the tentorial from the suboccipital surface. The junction between the anterosuperior and anterolateral margins forms the *anterior angle*, and the junction between the anterolateral and posterolateral margins forms the *lateral angle* (see Figs. 2-11C and 2-14D). Angiographically on the lateral projection, the lateral angle is located just below the knee between the transverse and sigmoid sinuses, and the outer portion of the anterolateral margin runs somewhat below the superior petrosal sinus (Fig. 2-15A). Anteriorly to posteriorly, the vermis and the hemispheric counterpart of the tentorial surface are the lingula (without the hemispheric counterpart), the central lobule (wing of the central lobule), the culmen (quadrangular

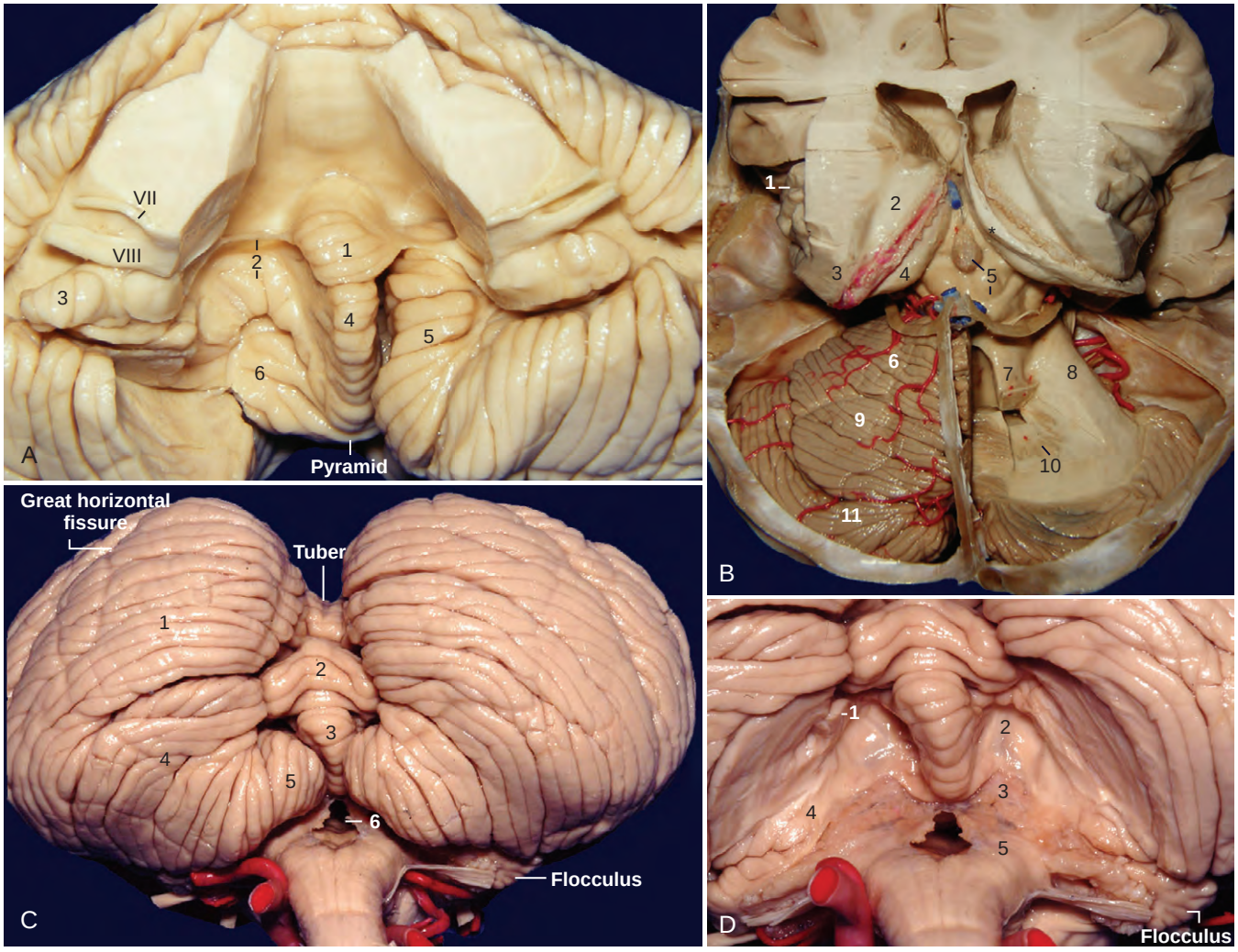

FIGURE 2-9 **A,** Frontal view of the roof of the fourth ventricle. The right tonsil has been removed to display the retrotonsillar space. 1, Nodule; 2, inferior medullary velum and furrowed band of Reil; 3, flocculus; 4, uvula; 5, tonsil; 6, copula pyramidis. **B,** Posterosuperior view. 1, Insula; 2, thalamus (dorsal surface); 3, thalamus (pulvinar, atrial surface); 4, thalamus (pulvinar, cisternal surface); 5, pineal gland and superior colliculus; 6, quadrangular lobule; 7, superior cerebellar peduncle; 8, middle cerebellar peduncle; 9, simple lobule; 10, dentate nucleus; 11, superior semilunar lobule; *, fornix. **C,** Suboccipital view. 1, Inferior semilunar lobule; 2, pyramid; 3, uvula; 4, biventral lobule; 5, tonsil; 6, foramen of Magendie. **D,** Suboccipital view. The tonsils and biventral lobules have been removed to display the inferior portion of the roof of the fourth ventricle. 1, Peduncle of the tonsil; 2, inferior medullary velum; 3, tela choroidea and choroid plexus; 4, peduncle of the flocculus; 5, inferior cerebellar peduncle.

lobule), the declive (simple lobule), and the folium (part of the superior semilunar lobule). The primary fissure is located between the quadrangular and simple lobules; the most prominent fissure, the postclival fissure, is located between the simple and superior semilunar lobules. The tentorial surface contains the *cerebellomesencephalic* or *precentral cerebellar fissure*, which is situated between the cerebellum and the midbrain. Posteriorly, it is limited by the culmen and quadrangular lobule above and the central lobule and its wing below. Anteriorly, it is limited from midline to laterally by the lingula and the superior and middle cerebellar peduncles. The *interpeduncular* or *interbrachial sulcus*, which separates the superior from the middle cerebellar peduncles, ascends from the bottom of the cerebellomesencephalic fissure toward the lateral aspect of the pons, where it is joined by the pontomesencephalic sulcus and proceeds superiorly as the lateral mesencephalic sulcus to the medial geniculate body; the *lateral mesencephalic sulcus* separates the crus cerebri from the tegmentum (see Fig. 2-9B).

Among the cerebellar nuclei (fastigial, globose, emboliform, and dentate), the *dentate nucleus* is the most laterally located and the largest one. The majority of fibers that constitute the superior cerebellar peduncle arise from the dentate nucleus, which is located at the posterior projection of the superior cerebellar peduncle. The dentate nucleus can be considered the roof of the superolateral recess. The superior pole of the tonsils, covered by the inferior medullary velum, is the floor of the superolateral recess.

Suboccipital Surface of the Cerebellum and Fourth Ventricle

The suboccipital surface of the cerebellum and the fourth ventricle are located below the transverse sinuses and between the sigmoid sinuses. Therefore, for better visualization of this surface either during surgery or for anatomic studies, the head has to be bent forward.

The suboccipital surface contains the posterior cerebellar incisura and the vermohemispheric or paravermian fissure, which separates the inferior vermis from the cerebellar hemisphere. The components of the inferior vermis and its hemispheric counterparts are the folium (superior semilunar lobule), tuber (inferior semilunar lobule), pyramid (biventral lobule), uvula (tonsil), and nodule (flocculus). In the anatomic position, the most inferior part of the inferior vermis is the pyramid. The most prominent fissure on the suboccipital surface is the *great horizontal fissure*, which is a circumferential fissure that begins in the posterior cerebellar notch between the folium and the tuber and runs forward and slightly downward on the suboccipital surface, between the superior and inferior semilunar lobules, and then onto the petrosal surface as the petrosal fissure. The *secondary fissure* is located between the tonsils and the biventral lobule (Fig. 2-9C).

After removal of the tonsils, the inferior portion of the roof of the fourth ventricle comes into view (Fig. 2-9D). After removal of the inferior portion of the roof of the fourth ventricle, the floor of the fourth ventricle is exposed.

The *floor of the fourth ventricle* has a rhomboid shape and consists of a strip between the lower margin of the cerebellar peduncles and the site of attachment of the tela choroidea; called the *junctional part*, this strip is formed by the medullary striae, which extend into the lateral recesses. The junctional part divides the floor of the fourth ventricle into two unequal triangles: the superior and larger one, with its apex directed toward the aqueduct, is the pontine part, and the inferior and smaller one, with its apex directed toward the obex, is the medullary part of the floor. These three parts of the floor are also divided longitudinally into two symmetrical halves by the median sulcus. The sulcus limitans, another longitudinal sulcus, divides each half of the floor into a raised median strip called the *median eminence* and a lateral strip called the *area vestibularis*. The motor nuclei of the cranial nerves are located medial to the sulcus limitans, and the sensory nuclei are situated lateral to it. The pontine part is characterized by two rounded prominences, the *facial colliculi*, located on the median eminence, one on each side of the median sulcus. The facial colliculi are limited laterally by the superior fovea, a dimple formed by the sulcus limitans. The medullary part has the configuration of a feather, or pen nib, and is called the *calamus scriptorius*, with three triangular areas overlying the hypoglossal and vagus nuclei (hypoglossal and vagal trigones) and the area postrema; just lateral to the hypoglossal trigone, the sulcus limitans has another dimple called the *inferior fovea*. At the junctional part the sulcus limitans is discontinuous (Fig. 2-10A).

Veins of the Posterior Fossa

The posterior fossa venous system is divided into three groups: the anterior or petrosal group, which drains into the superior and inferior petrosal sinuses; the superior or galenic group, which drains into the vein of Galen; and the posterior or tentorial group, which drains into the sinuses near the torcula.[22] There is a tendency for the veins to drain into the nearest draining system.

The veins running on the *petrosal surface* of the cerebellum and the anterior surface of the brainstem tend to drain into the petrosal sinuses via the superior petrosal vein, except for the veins running on the surface of the midbrain, which drain into the galenic system. The superior petrosal vein is usually formed by the junction of the transverse pontine and pontotrigeminal (brachial) veins and the vein of the cerebellopontine fissure (great horizontal fissure) (Fig. 2-10B).

The *tentorial surface* and the posterior aspect of the brainstem are served by three draining systems: the midline portion of the cerebellomesencephalic fissure, the veins near the central lobule and culmen (superior vermian veins), and the veins draining the intermediate portion of the wing of the central lobule and the quadrangular lobule (superior hemispheric veins, anterior group), which tend to drain into the vein of Galen. The veins draining the lateral portion of the wing of the central, quadrangular, and simple lobules and the tentorial part of the superior semilunar lobule (superior hemispheric veins, lateral group) tend to drain into the superior petrosal sinus. The veins draining the declive, folium (declival vein), and the intermediate portion of the simple and superior semilunar lobules (superior hemispheric veins, posterior group) tend to drain into the torcula or transverse or tentorial sinus in the tentorium cerebelli (Fig. 2-10C).

The posterior inferior hemispheric veins drain the *suboccipital surface* of the cerebellar hemispheres. Drainage of the inferior vermis is via the inferior vermian veins, which are formed by the junction of the superior and inferior retrotonsillar veins running in the retrotonsillar space (Fig. 2-10D).

The inferior portion of the roof of the fourth ventricle and the lateral recess are drained by the vein of the lateral recess of the fourth ventricle, also called the *vein of the cerebellomedullary fissure*. It courses laterally under the lateral recess toward the cerebellopontine angle, passes above or below the flocculus, joins the vein of the middle cerebellar peduncle or the vein of the cerebellopontine fissure, and finally empties into the superior petrosal sinus via the superior petrosal vein. The vein of the lateral recess of the fourth ventricle can also anastomose with the retrotonsillar veins at the retrotonsillar space to establish communication between the petrosal and the tentorial groups of venous drainage (Fig. 2-10D).

The brachial veins running in the cerebellomesencephalic fissure can also establish communication between the petrosal and galenic groups via the pontotrigeminal and precentral cerebellar veins (Fig. 2-10C).

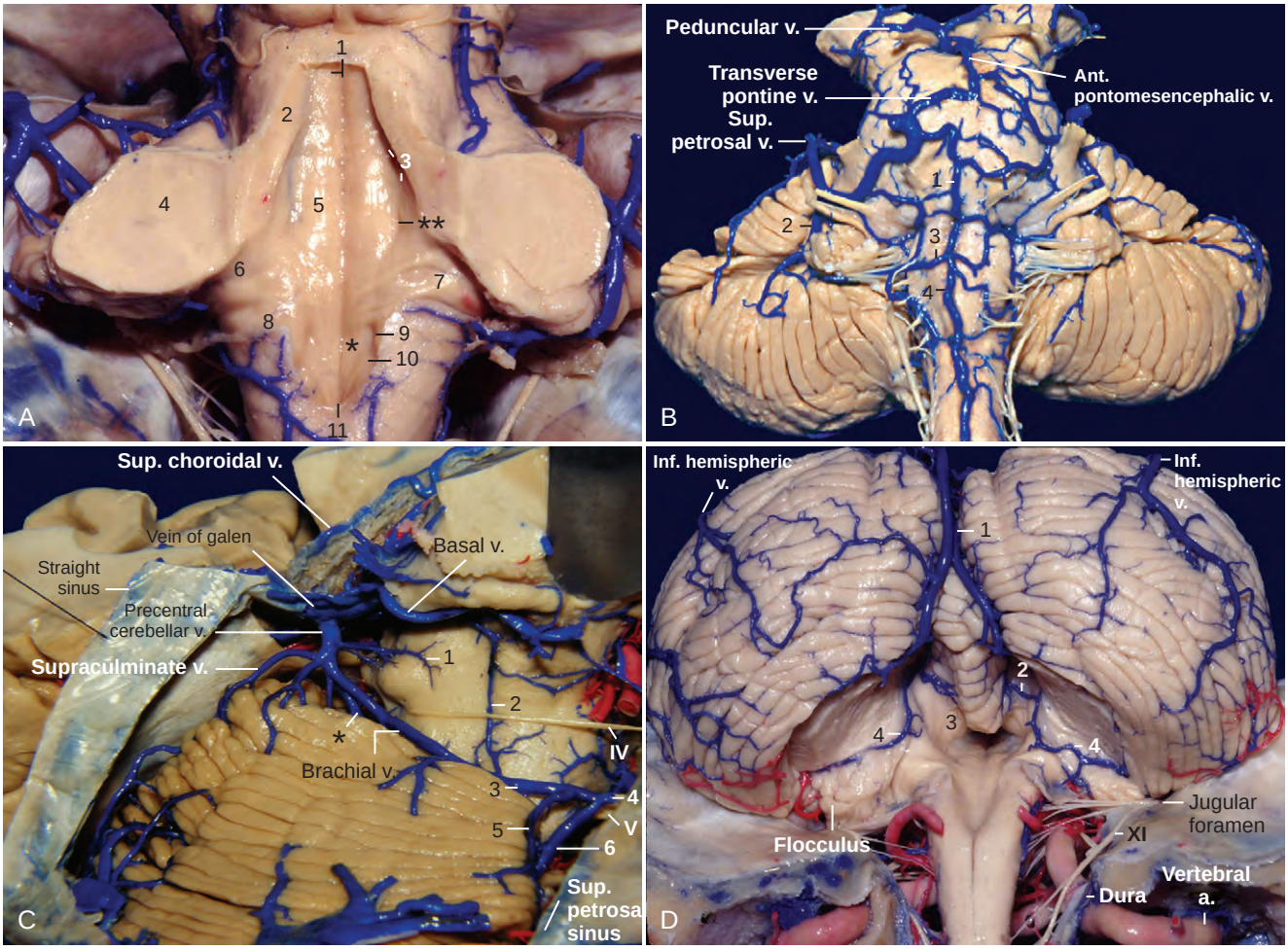

FIGURE 2-10 **A,** Floor of the fourth ventricle. 1, Median sulcus and median eminence; 2, superior cerebellar peduncle; 3, sulcus limitans and vestibular area; 4, middle cerebellar peduncle; 5, facial colliculus; 6, cochlear area; 7, striae medullaris; 8, inferior cerebellar peduncle; 9, inferior fovea; 10, vagal trigone; 11, obex and area postrema; *, hypoglossal trigone; **, superior fovea. **B,** Frontal view. 1, Anterior pontomesencephalic vein; 2, vein of the great horizontal fissure; 3, transverse medullary vein; 4, anterior medullary vein. **C,** Right posterolateral view of the tentorial surface. 1, Tectal veins; 2, lateral mesencephalic vein; 3, pontotrigeminal vein; 4, superior petrosal vein; 5, superior hemispheric vein; 6, vein of the great horizontal fissure; *, vein of the cerebellomesencephalic fissure. **D**, Suboccipital view. 1, Inferior vermian vein; 2, superior retrotonsillar vein; 3, superior cerebellar peduncle; 4, vein of the lateral recess of the fourth ventricle.

The veins of the posterior fossa can be differentiated into the petrosal group, the superior or galenic group, and the posterior or tentorial group (see Figs. 2-14D and 2-15A to C):

The *petrosal group* may be divided into (1) veins related to the anterior aspect of the brainstem—the anterior pontomesencephalic, transverse pontine, lateral pontine, anterior medullary, and parenchymal perforating veins; (2) veins in the wing of the precentral cerebellar fissure—the brachial veins; (3) veins on the superior and inferior surfaces of the cerebellar hemispheres—the superior and inferior hemispheric veins, including the veins of the great horizontal fissure; (4) veins on the cerebellar side (the medial tonsillar vein) and medullary side (the retro-olivary vein and vein of the inferior cerebellar peduncle of the cerebellomedullary fissure); and (5) the vein of the lateral recess of the fourth ventricle.

The *superior* or *galenic group* includes (1) the mesencephalic tributaries—the median anterior pontomesencephalic, lateral anterior pontomesencephalic, lateral pontomesencephalic, lateral mesencephalic, peduncular, posterior mesencephalic, and tectal veins—and (2) the cerebellar tributaries—the precentral cerebellar vein and its variants and the superior vermian vein.

The *posterior* or *tentorial group* includes the inferior vermian vein and its superior and inferior retrotonsillar tributaries and the superior and inferior hemispheric veins.

Arteries of the Posterior Fossa

The *vertebral artery* (VA) arises from the subclavian artery, enters the transverse foramen of C6, and then ascends through the transverse foramina of the upper cervical vertebrae up to C2. After exiting from the transverse foramen of C2, the VA deviates laterally and enters the laterally placed transverse foramen of C1. The VA then turns behind the lateral mass and above the posterior arch of C1, courses medially and superiorly, and pierces the dura at the foramen magnum. At this level the VA usually gives off the posterior spinal and posterior meningeal arteries. The intradural segment of the VA is divided into lateral medullary and anterior medullary segments before joining its contralateral mate to form the basilar artery (Figs. 2-10D and 2-11B; also see Fig. 2-12B).

The lateral medullary segment of the VA extends from its entrance into the posterior fossa to the preolivary sulcus. From its entrance the VA courses anteriorly, medially, and superiorly

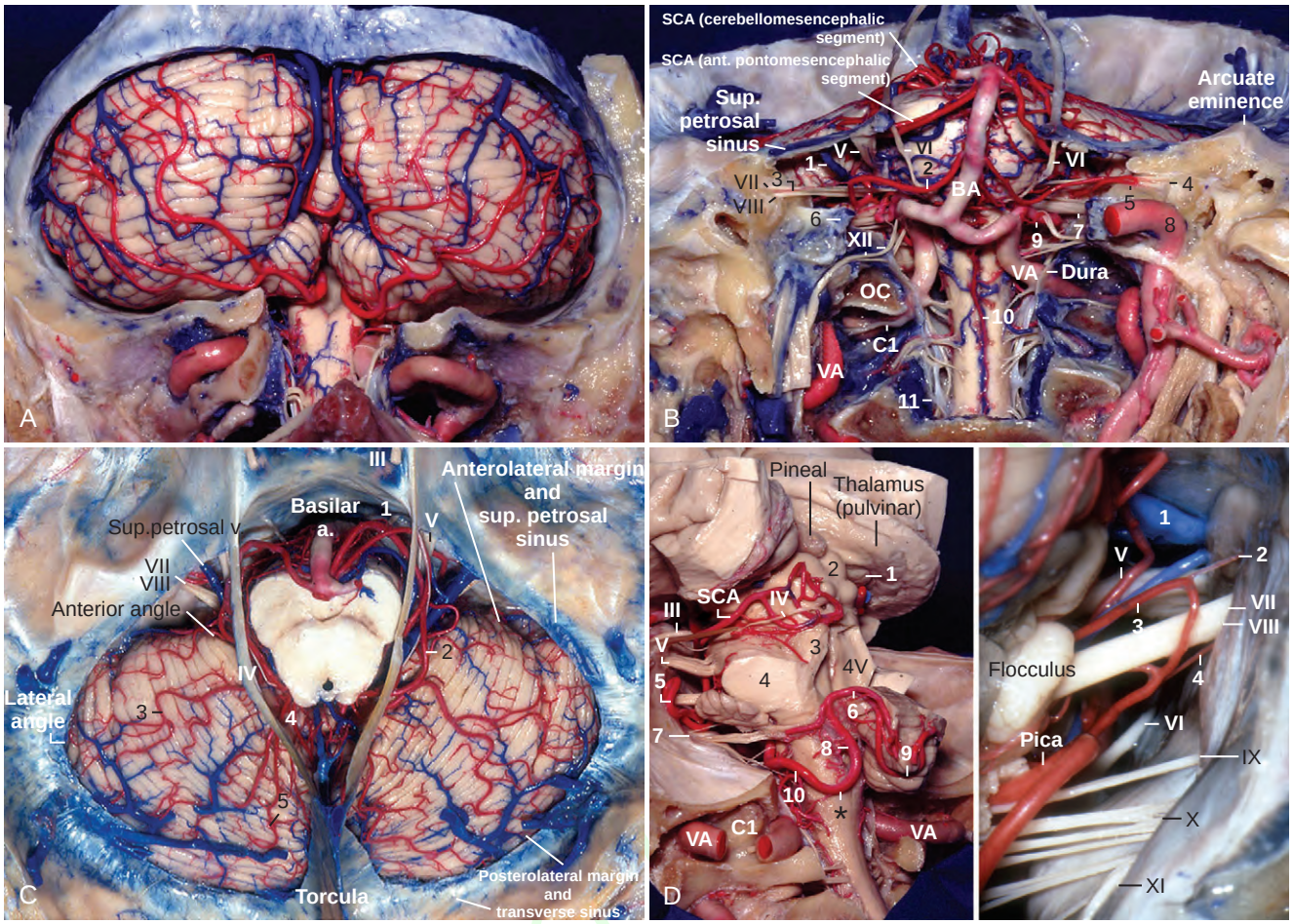

FIGURE 2-11 **A,** Suboccipital view to display the cortical branches of the posterior inferior cerebellar artery (PICA). **B,** Frontal view. 1, Superior petrosal vein; 2, anterior inferior cerebellar artery (AICA); 3, internal auditory artery; 4, internal acoustic meatus; 5, meatal loop of the AICA; 6, inferior petrosal sinus; 7, vagus nerve; 8, petrous carotid artery; 9, PICA; 10, anterior spinal artery; 11, triangular process of the dentate ligament. BA, basilar artery; OC, occipital condyle; SCA, superior cerebellar artery; VA, vertebral artery. **C,** Superior view of the tentorial surface. 1, Anterior pontomesencephalic segment of the SCA; 2, lateral pontomesencephalic segment of the SCA; 3, superior hemispheric branches of the SCA; 4, cerebellomesencephalic segment of the SCA; 5, superior hemispheric branches of the SCA. **D,** *Left*, Posterolateral view. 1, Medial geniculate body; 2, superior colliculi; 3, superior cerebellar peduncle; 4, middle cerebellar peduncle; 5, AICA, VII and VIII; 6, PICA (supratonsillar segment); 7, jugular foramen; 8, PICA (retrotonsillar segment); 9, PICA (pyramidal loop); 10, PICA (lateral medullary segment); *, caudal loop. *Right*, Retromastoid view of the cerebellopontine angle. 1, Superior petrosal vein; 2, subarcuate artery (AICA); 3, AICA; 4, internal auditory artery.

through the lower cranial nerve rootlets, lateral to the medulla, to reach the preolivary sulcus. The anterior medullary segment begins at the preolivary sulcus, courses in front of or between the hypoglossal rootlets, and crosses the pyramid to join with the other VA at or near the pontomedullary sulcus to form the basilar artery. The main branches of the VA are the posterior spinal artery, anterior spinal artery, PICA, and anterior and posterior meningeal arteries. The VA also sends off branches to supply the lateral and anterior parts of the medulla along its way around the medulla (see Figs. 2-11B, 2-12B, and 2-14A).

The *posterior inferior cerebellar artery* arises from the VA and supplies the medulla, the inferior vermis, the inferior portion of the fourth ventricle, the tonsils, and the inferior aspect of the cerebellum. The "regular" PICA has the most complex and variable course of the cerebellar arteries and is divided into five segments.[23] The *anterior medullary segment* lays in front of the medulla and extends from the origin to the level of the inferior olive. The *lateral medullary segment* courses beside the medulla and extends from the inferior olive to the origin of the glossopharyngeal, vagus, and accessory nerves. The *tonsillomedullary* or *posterior medullary segment* begins at the level of the nerves and loops below the inferior pole of the cerebellar tonsil and upward along the medial surface of the tonsil toward the inferior medullary velum (caudal loop). The *telovelotonsillar* or *supratonsillar segment* courses in the cleft between the tela choroidea and the inferior medullary velum rostrally and the superior pole of the cerebellar tonsil caudally. It begins below the fastigium, where the PICA turns posteriorly over the medial side of the superior pole of the tonsil. This segment forms the "cranial loop." It sometimes passes posteriorly before reaching the superior pole of the tonsil, thus giving the cranial loop a variable relationship to the fastigium. The junction of the posterior medullary and supratonsillar segments is called the *choroidal point.* The fifth segment is the *cortical segment*; after a short distance distal to the apex of the cranial loop, the PICA continues posteriorly downward in the retrotonsillar fissure, where it usually bifurcates into the tonsillohemispheric branch, which supplies the under aspect of the cerebellar hemisphere, and the inferior vermian branch, which lies on the lower aspect of the inferior vermis and forms a convex loop around the

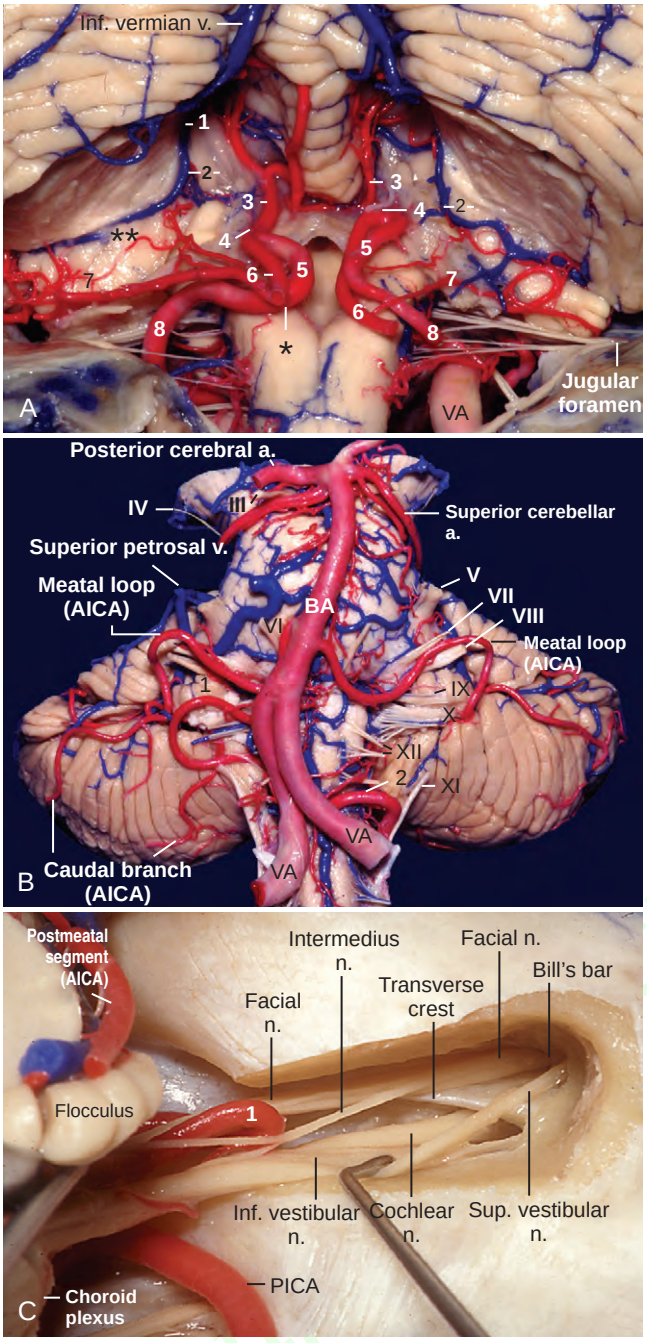

FIGURE 2-12 **A,** Suboccipital view. The tonsils and the biventral lobule have been removed. 1, Superior retrotonsillar vein; 2, vein of the lateral recess of the fourth ventricle and inferior medullary velum; 3, posterior inferior cerebellar artery (PICA, vermian branch); 4, PICA (supratonsillar segment, cranial loop); 5, PICA (posterior medullary segment); 6, PICA (tonsillohemispheric branch); 7, PICA (choroidal branches); 8, PICA (lateral medullary segment); VA, vertebral artery; *, caudal loop; **, peduncle of the flocculus. **B,** Anterior view. 1, Flocculus; 2, PICA (anterior medullary segment); AICA, anterior inferior cerebellar artery; BA, basal artery. **C,** Posterior view of the contents of the right internal acoustic meatus. 1, AICA (meatal segment).

copula pyramidis (pyramidal loop). The most anterior point of the pyramidal loop is called the *copular point*. The terminal portion of the vermian branch curves around the tuber in the posterior cerebellar notch (see Figs. 2-11A and B; 2-11, *left*; 2-12A; 2-14A and B; 2-15C and D; 2-16A and B).

The *anterior inferior cerebellar artery* and the PICA are defined according to their origin rather than by the portions of cerebellum that they supply. The AICA arises more frequently from the lower third and less frequently from the middle third of the basilar artery. It courses posteriorly, laterally, and usually downward on the belly of the pons, in contact with either the superior or inferior aspect of the abducens nerve. In this course it supplies the lateral aspect of the lower two thirds of the pons and the upper medulla. Either immediately before or after crossing the roots of the facial, intermedius, and acoustic nerves within the cerebellopontine angle, the AICA bifurcates into its two major branches, the *rostrolateral* and the *caudomedial* arteries. The main or *rostrolateral trunk* has been divided into three segments according to their relationship to cranial nerves VII and VIII[24]: the premeatal, meatal, and postmeatal segments. The *premeatal segment* begins at the basilar artery and courses around the brainstem to reach cranial nerves VII and VIII and the region of the meatus, usually anteroinferior to the nerves. Seventy-seven percent of internal auditory arteries and 49% of recurrent perforating arteries to the brainstem arise from this segment. The *meatal segment* is located in the vicinity of the internal auditory meatus, where the nerve-related vessels turn toward the brainstem; this segment often forms a laterally convex loop, the meatal loop, directed toward or through the meatus. It usually stays medial to the meatus, but it sometimes protrudes into the canal. The *postmeatal segment* begins distal to the nerves and courses medially to supply the brainstem and the cerebellum. The subarcuate artery generally arises from this segment (see Figs. 2-11B and D, *right*; 2-12B; 2-14A and C; 2-15C; 2-16A and B).

The *caudomedial artery* originates on the lateral aspect of the pons in the vicinity of the sixth nerve and courses posterosuperiorly toward the pontomedullary sulcus; it has a caudal loop on the lateral aspect of the pons and medulla. This lateral loop can course on the anteroinferolateral aspect of the flocculus or on the petrosal aspect of the biventral lobule. Multiple small arteries to the choroid plexus of the lateral recess often arise from the inner aspect of this lateral loop. Distal to the loop, the biventral segment turns posteroinferiorly on the lateral edge of the inferior surface of the biventral lobule or within the cerebellomedullary fissure to reach the posterior surface of the cerebellum, where it anastomoses with branches of the PICA (Fig. 2-12B).

The *superior cerebellar artery* is the most rostral of the infratentorial vessels, and it arises near the apex of the basilar artery and encircles the pons and the lower midbrain. It supplies the tentorial surface of the cerebellum, the upper brainstem, the deep cerebellar nuclei, and the inferior colliculi. The SCA is divided into four segments. The *anterior pontomesencephalic segment* courses laterally under the oculomotor nerve on the anterior aspect of the upper pons, often in an arcuate convex curve inferiorly; the configuration of the anterior pontomesencephalic segment is related to the height of the basilar bifurcation. With a low basilar bifurcation (anterior to the pons), this segment tends to pass upward, whereas with a high basilar bifurcation (anterior to the midbrain), this segment pursues an anterior and inferior course. The *lateral pontomesencephalic segment* begins at the anterolateral margin of the brainstem and follows caudally onto the lateral side of the upper pons in the infratentorial portion of the ambient cistern, where it terminates at the anterior margin of the cerebellomesencephalic fissure; it is related medially to the brainstem, laterally to the wing of the central lobule, and inferiorly to the middle cerebellar peduncle. The anterior part of this segment is often visible above the free edge of the tentorium, whereas its caudal loop projects toward and often reaches the root entry zone of the trigeminal nerve. Bifurcation of the SCA into rostral and caudal trunks often occurs in this segment; the rostral trunk supplies the vermis and a variable portion of the adjacent tentorial surface, and the caudal trunk supplies the surface lateral to the area supplied by the rostral trunk. The *cerebellomesencephalic*

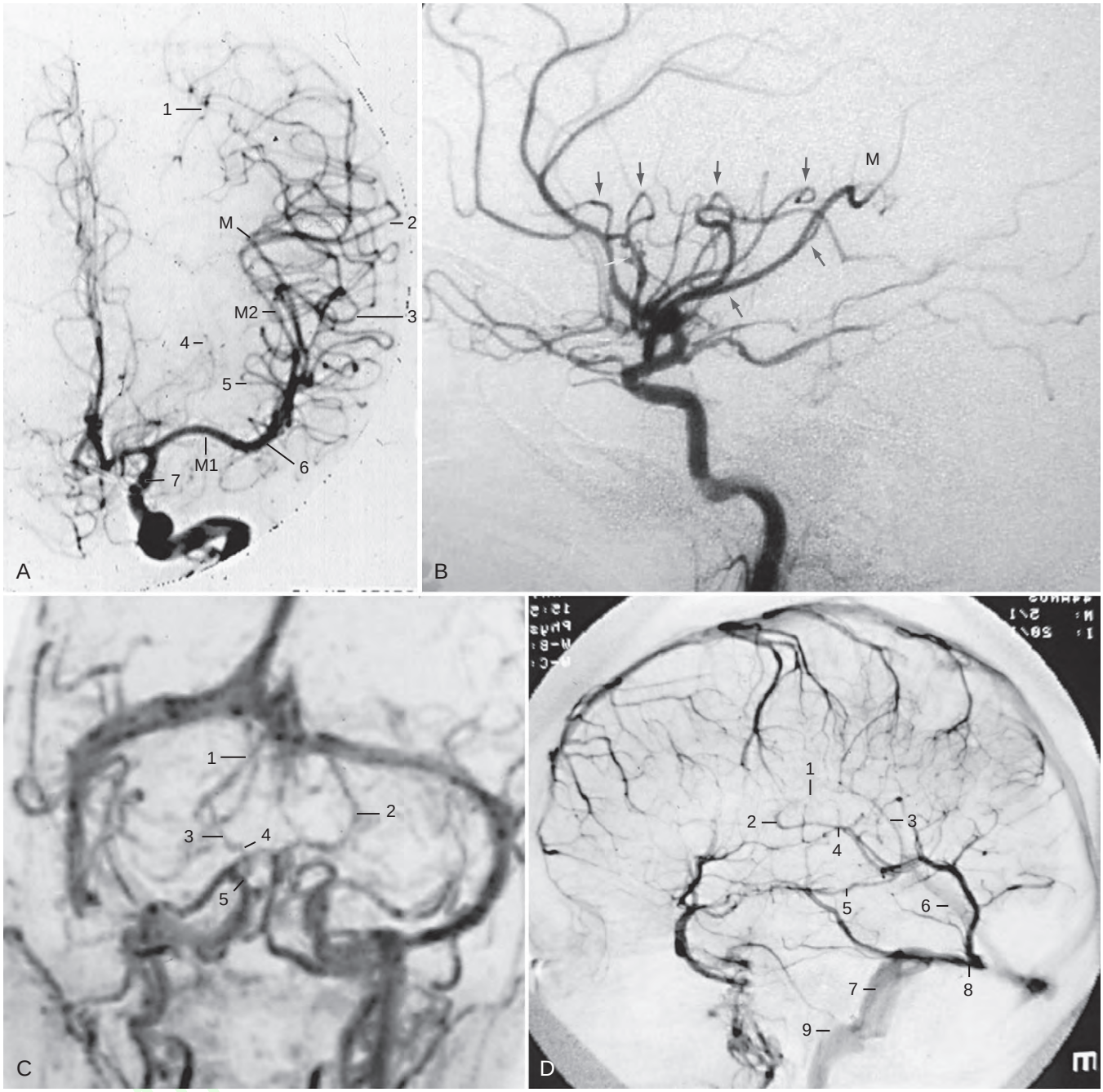

FIGURE 2-13 **A,** Frontal view of a right carotid angiogram. 1, Intraparietal sulcus; 2, M3 branches on the planum temporale; 3, M3 branches in the central sulcus region; 4, lateral lenticulostriate arteries; 5, M3 branches in the anterior limiting sulcus of the insula; 6, genu of the middle cerebral artery; 7, internal carotid artery (supraclinoid segment); M, "M point" or "sylvian point." **B,** Lateral view of a carotid angiogram. The *blue arrows* indicate the superior limiting sulcus of the insula, the *red arrows* indicate the inferior limiting sulcus of the insula, and the *yellow arrow* indicates the anterior limiting sulcus of the insula. **C,** Frontal view of a venous magnetic resonance angiogram to display the basal vein. 1, "Thigh" (posterior mesencephalic segment); 2, "knee" (junction between the anterior and posterior peduncular segments); 3, "leg" (anterior peduncular segment); 4, "ankle" (junction between the striate and peduncular segments); 5, "foot" (striate segment). **D,** Lateral view of a venous angiogram. 1, Thalamostriate vein; 2, "venous angle"; 3, inferior sagittal sinus; 4, internal cerebral vein; 5, basal vein; 6, straight sinus; 7, sigmoid sinus; 8, transverse sinus and the "vein of Labbé complex"; 9, bulb of the jugular vein.

segment courses in the cerebellomesencephalic fissure through a series of hairpin-like curves and then passes upward to reach the anterosuperior margin of the cerebellum. Inside the cerebellomesencephalic fissure, the rostral and caudal trunks send off small precentral branches. These precentral branches arising from the rostral trunk supply the inferior colliculi (the superior colliculi are supplied by the PCA) and the superior medullary velum, and those arising from the caudal trunk supply the deep cerebellar nuclei. Finally, the *cortical segment* is represented by the hemispheric and the vermian branches, which supply the tentorial surface of the cerebellum. Among these cortical branches, the *marginal* or *lateral branch* deserves special attention: it is present in 62% of individuals, it is the first large cortical branch of the SCA, and it arises from the lateral pontomesencephalic segment

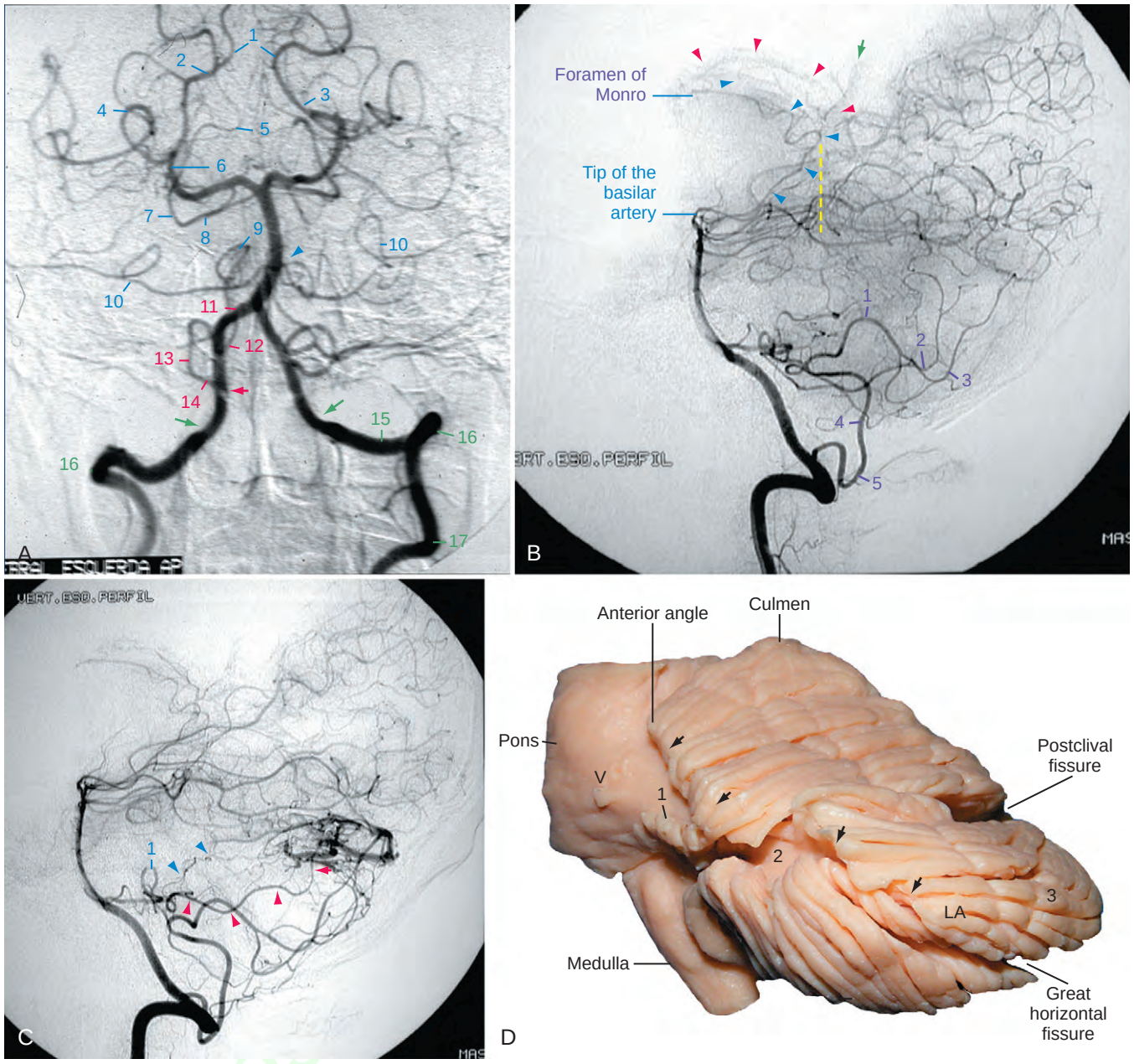

FIGURE 2-14 **A,** Frontal view of a vertebrobasilar angiogram. 1, Collicular or quadrigeminal point; 2, P3 segment; 3, beginning of the P2A segment; 4, collateral sulcus; 5, beginning of the cerebellomesencephalic segment of the superior cerebellar artery (SCA); 6, P2A segment; 7, lateral pontomesencephalic segment of the SCA; 8, anterior pontomesencephalic segment of the SCA; 9, supratonsillar segment of the posterior inferior cerebellar artery (PICA, cranial loop); 10, meatal loop of the anterior inferior cerebellar artery (AICA); 11, posterior medullary segment of the PICA; 12, caudal loop of the PICA; 13, lateral medullary segment of the PICA; 14, anterior medullary segment of the PICA; 15, extradural vertebral artery behind the lateral mass of C1; 16, vertebral artery in the foramen transversarium of C1; 17, vertebral artery in the foramen transversarium of C2. The *blue arrowhead* indicates the origin of the AICA from the basilar artery, the *red arrowhead* indicates the origin of the PICA from the vertebral artery, and the *green arrows* indicate the probable transition between the extradural and intradural segments of the vertebral artery. Note the constriction in the vertebral artery. **B,** Lateral view of the late arterial phase of a vertebrobasilar angiogram. 1, "Cranial loop" of the PICA; 2, vermian division of the PICA (pyramidal loop); 3, hemispheric branch of the PICA; 4, posterior medullary segment of the PICA; 5, "caudal loop" of the PICA. The *red arrowheads* indicate the lateral posterior choroidal arteries in the lateral ventricle (indicate the location of the posterior wall of the pulvinar of the thalamus), the *green arrow* indicates the posterior pericallosal artery, and the *blue arrowheads* indicate the medial posterior choroidal artery (MPChA). The most posterior point of the trajectory of the MPChA indicates the posterior limit of the quadrigeminal plate and consequently the posterior limit of the brainstem in the lateral view (*yellow dashed line*). For the anatomic location of the lateral posterior choroidal artery and MPChA, refer to Figures 2-4B and 2-6D. Note that the foramen of Monro is located above the tip of the basilar artery (in the same coronal plane). **C,** Lateral view of the arterial phase of a vertebrobasilar angiogram. 1. "Meatal loop" of the AICA. The *blue arrowheads* indicate small branches to the lateral recess of the fourth ventricle through the foramen of Luschka, and the *red arrowheads* indicate the main trunk of the AICA in the great horizontal fissure, which is supplying an arteriovenous malformation located in the superior semilunar lobule. **D,** Lateral view of the cerebellum. Compare this illustration with Figures 2-8B and C and 2-12A. 1, Flocculus; 2, petrosal fissure or great horizontal fissure; 3, superior semilunar lobule; LA, lateral angle. The *arrows* indicate the anterolateral margin.

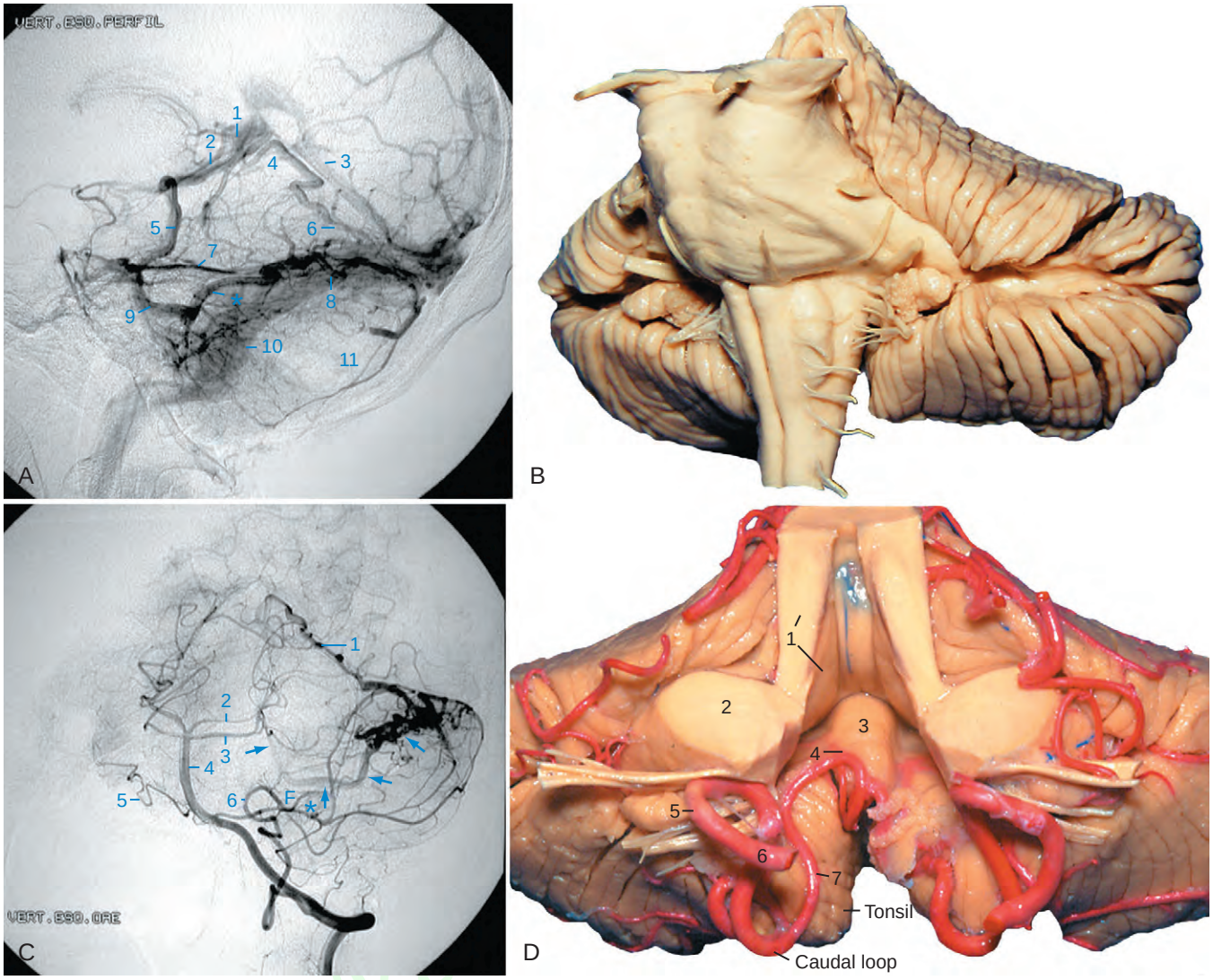

FIGURE 2-15 **A,** Lateral view of the venous phase of a vertebrobasilar angiogram. Compare with Figure 2-8D. 1, Vein of Galen; 2, cerebellomesencephalic fissure; 3, straight sinus; 4, culmen; 5, vein running on the middle cerebral peduncle that continues with a vein in the petrosal fissure (vein of the great horizontal fissure)[9]; 6, vein running on the tentorial surface of the cerebellum (superior hemispheric vein) toward the vein of Galen; 7, superior petrosal sinus; 8, transverse sinus; 9, vein running in the petrosal fissure or great horizontal fissure (vein of the great horizontal fissure); 10, sigmoid sinus; 11, suboccipital surface of the cerebellum; *, a superior hemispheric vein draining an arteriovenous malformation that runs on the tentorial surface, descends toward the petrosal surface, and joins the vein of the great horizontal fissure. **B,** Left anterior oblique view of the cerebellum and brainstem. Compare this illustration with Figure 2-12C. **C,** Left anterior oblique view of a vertebrobasilar angiogram. 1, Vein running on the tentorial surface toward the vein of Galen (superior hemispheric vein); 2, posterior cerebral artery; 3, superior cerebellar artery; 4, basilar artery; 5, meatal loop of the anterior inferior cerebellar artery (AICA); 6, posterior inferior cerebellar artery (PICA). The *arrows* indicate the vein shown in Figure 2-12A, which originates from the tentorial surface (superior hemispheric vein) and descends toward the petrosal surface to join the vein in the great horizontal fissure. F, approximate location of the flocculus; it was estimated by the location meatal loop of the AICA.* **D,** Anterior view of the PICA and the roof of the fourth ventricle. Compare this illustration with Figure 2-8A. 1, Superior cerebellar peduncle; 2, middle cerebellar peduncle; 3, nodule (covered by the inferior medullary velum); 4, supratonsillar segment of the PICA (cranial loop); 5, lateral medullary segment of the PICA; 6, anterior medullary segment of the PICA; 7, posterior medullary segment of the PICA.

and courses anteriorly and laterally to reach the anterolateral margin of the cerebellum. It is an important arteriographic landmark for locating the anterolateral margin and the anterior angle of the cerebellum (see Figs. 2-11A and C, 2-14A, 2-15C, 2-16A and B).[25]

Because of its surgical importance, the cerebellopontine angle regions deserves special mention: it is a region limited superiorly by the infratentorial portion of the ambient cistern (SCA, cranial nerve IV), medially by the prepontine cistern (basilar artery, cranial nerve VI, origin of the AICA and the transverse pontine vein), inferiorly by the lateral cerebellomedullary cistern (cranial nerves IX, X, XI and XII; the VA; and the first segment of the PICA), and laterally by the petrous portion of the temporal bone. The cerebellopontine angle contains cranial nerves V, VII, and VIII; the AICA; the auditory artery; branches of the petrosal vein and the vein of the middle cerebellar peduncle; the vein of the lateral recess of the fourth ventricle; and the transverse pontine vein.

After their origin from the brainstem, the facial and intermedius nerves pass anterolaterally with the vestibulocochlear nerve; in this location, the facial nerve lies in an anterosuperior groove on the vestibulocochlear nerve, with the intermedius nerve

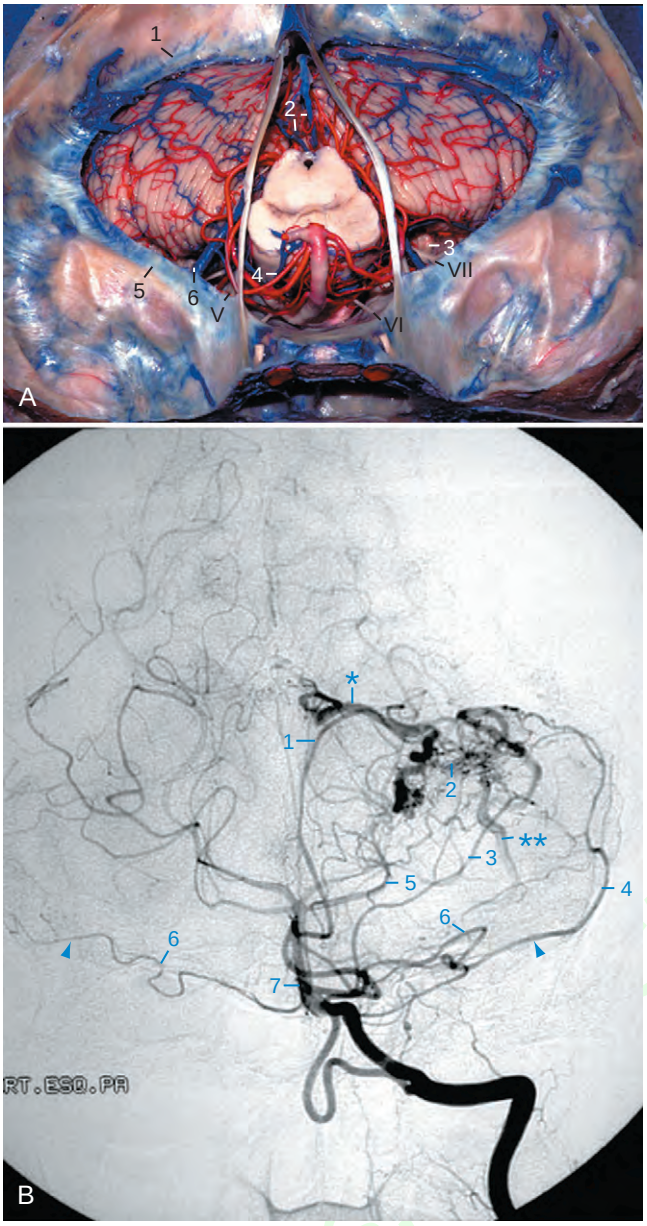

FIGURE 2-16 **A,** Anterosuperior (Towne) view of the posterior fossa. Compare this illustration with **B.** 1, Transverse sinus; 2, precentral cerebellar vein and cerebellomesencephalic fissure; 3, flocculus; 4, superior cerebellar artery (SCA); 5, superior petrosal sinus; 6, superior petrosal vein. **B,** Towne view of a vertebrobasilar angiogram. Compare this illustration with **A.** 1, Vermian branch of the posterior inferior cerebellar artery (PICA); 2, arteriovenous malformation located at the superior semilunar lobule; 3, inferior hemispheric branch from the PICA; 4, branch from the anterior inferior cerebellar artery (AICA) in the great horizontal fissure; 5, lateral pontomesencephalic segment of the SCA; 6, meatal loop of the AICA; 7, basilar artery; *, a superior hemispheric vein draining toward the vein of Galen; **, a superior hemispheric vein coursing initially on the tentorial surface and then descending to the great horizontal fissure on the petrosal surface of the cerebellum. The *arrowheads* indicate the location of the great horizontal fissure or petrosal fissure.

between them. At the lateral end of the internal acoustic meatus, the vertical Bill bar and the transverse crest divide the fundus of the meatus into four quadrants: the facial nerve is located in the anterosuperior quadrant, the cochlear nerve in the anteroinferior quadrant, the superior vestibular nerve in the posterosuperior quadrant, and the inferior vestibular nerve in the posteroinferior quadrant (see Fig. 2-12C).

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CHAPTER 3

Molecular Biology Primer for Neurosurgeons

Kevin Y. Miyashiro ■ James Eberwine

Commenting on the structure and connections of neurons during his acceptance speech for the Nobel Prize in Medicine, Ramon y Cajal proposed that

> *These fibres, ramifying several times, always proceed towards the neuronal body, or towards the protoplasmic expansions around which arise plexuses or very tightly bound and rich nerve nests [these] morphological structures, whose form varies according to the nerve centres being studied, confirm that the nerve elements possess reciprocal relationships in contiguity but not in continuity. It is confirmed also that those more or less intimate contacts are always established, not between the nerve arborizations alone, but between these ramifications on the one hand, and the body and protoplasmic processes on the other.*

With these observations in support of the neuron doctrine in 1906, so began one of the most abiding lines of scientific pursuit in biology—understanding the principal mechanisms by which the fate of central neurons are specified during development at the correct time and place to facilitate the quadrillion synaptic connections that are established, maintained, and remodeled. These arrays of neural networks codify our perceptions and other cognitive functions. To do so, synapses bring together in apposition specialized morphologic structures of the presynaptic, usually axonal, and postsynaptic, typically dendritic, subcellular neuronal domains often ensheathed by the end-feet of astrocytes. The precision and strength of these connections rely on the pinpoint placement of gene products in each of these cellular compartments. Our understanding of neuroscience in these molecular terms has been one of the fundamental challenges in the past several decades but has been complicated by the varying intrinsic properties of neurons—including morphology, types of neurotransmitter release, projection targets, and basic input/output characteristics—that exist along a wide spectrum of neuronal phenotypes even within the same neuroanatomic region.

Over the past several decades, neuroscientists have embraced a rapidly evolving set of molecular biologic techniques to gain insight into understanding these dynamics of gene expression. These findings have been critical in understanding not only the mechanistic underpinnings of normal development but also the role that some genes play in neurological diseases from the developmental to the degenerative. Our purpose here is to introduce a series of core methods in the contemporary molecular neuroscientist's toolbox. We do so in the broader context of the traditional candidate gene approach and the more recent rise of functional genomics.

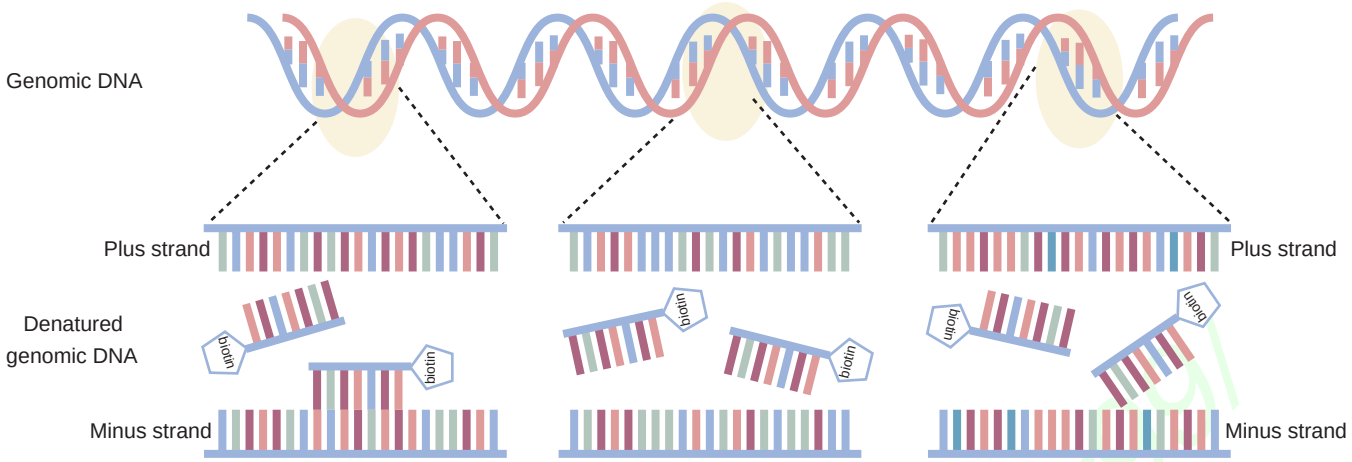
THE CANDIDATE GENE APPROACH

Lacking any biochemical basis that would aid in purifying gene products associated with a disease state, the candidate gene approach was largely an outgrowth of the efforts of positional cloning strategies in the early 1980s. Using linkage analysis to look at differences in chromosome structure in diseased versus nondiseased individuals often in tandem with linkage disequilibrium mapping to define broad (i.e., <10 centimorgans) and much finer respective chromosomal intervals associated with a priori knowledge of their etiologic role in a disease state enabled molecular geneticists to narrow the number of candidate causative genes. Before completion of the Human Genome Project, identifying these loci was an incredibly arduous and time-consuming task, in large part because of the lack of high-resolution genetic and physical chromosomal maps. The unprecedented efforts to surmount these struggles were most famously recorded in the midst of the pioneering work of Louis Kunkel and Ronald Worton[1] in identifying dystrophin as the gene responsible for Duchenne's muscular dystrophy. In the past several decades, numerous genes with high relative risk for similar "simple" mendelian diseases have been successfully identified with these methods. More than 30% of mendelian disease has neurological manifestations.[2] By applying the tools of molecular biology to a gene or the small set of candidate genes identified within a quantitative trait loci it has become possible to facilitate a systematic approach to answering some very basic questions about the organization of a gene and the expression of its gene products.

Emerging from the sequence of methods conceptualized first by Sol Spiegelman[3] and most effectively by Edwin Southern,[4] molecular biologists have exploited the now well-worn principle of molecular hybridization in which a single-stranded nucleic acid probe (or primer) forms a stable hybrid molecule, as a result of nucleotide complementarity, with a single-stranded target sequence immobilized on a solid support (i.e., nitrocellulose or nylon membranes) or in solution (Fig. 3-1). Under the appropriate experimental conditions, the stability and biochemical kinetics of the hybrid are directly proportional to the length and degree of nucleotide complementarity.

As first applied in Southern blotting whereby genomic DNA was separated according to size by agarose gel electrophoresis, transferred to nitrocellulose, and annealed to complementary DNA probes labeled with a detectable tag hybridized to target genomic DNA, the dosage or deletion analysis, or both, of candidate genes was affirmed. Indeed, Southern blot analysis of the dystrophin gene has identified duplications as well as mapped various exon deletion mutants. Recently, variation in the copy number of genes has received new consideration inasmuch as several new genomic disorders have been shown to be manifested in a gene dosage–dependent manner.[5] Such disorders include dup7 (q11.23) syndrome, methyl-CPG-binding protein 2 (MECP2), and adult-onset autosomal dominant leukodystrophy. By reducing the stringency of hybridization conditions, however, differences in hybridization patterns may reveal the existence of certain fragments that are not able to hybridize to the probe under the most stringent hybridization conditions. These data are often the first clues that the gene is part of a larger multigene family that shares significant but not complete nucleotide sequence identity. By using a modification of the Southern blotting method to screen a complementary DNA library at moderate stringency, the dystrophin-like sequence utrophin was identified.[6]

Traditional Southern blotting has largely been supplanted by the advent of polymerase chain reaction (PCR) protocols. The ability to replicate short fragments of DNA with an enzymatic



Technique	Hybridization	Maximum sensitivity	Target sequence	Probe selection	Uses
Southern blot	Solid support	~ 0.5–1.0 pg	DNA	DNA/oligonucletode	Chromosomal mapping, copy number variation, multigene families, gene mutants
Northern blot	Solid support	~ 25 pg	RNA	RNA	Messenger RNA abundance, degradation, and stability, alternative mRNA splicing
RNAse protection assay	Solution	~ 0.1–1.0 pg	RNA	RNA	Messenger RNA abundance
Polymerase chain reaction	Solution	~ 1.0 pg	DNA	DNA	Cloning, messenger RNA abundance, genotyping
In situ hybridization	Solid support (cell matrix)	~ 1–10 copies/cell	RNA	RNA/DNA/ oligonucleotide	Subcellular localization
	Solid support (chromosomes)		DNA	DNA/oligonucleotide	Chromosomal mapping, karyotype analysis

FIGURE 3-1 The fundamental tools of molecular biology are based on molecular hybridization. A cartoon schematic of molecular hybridization shows the basic principle in practice illustrated with a portion of genomic DNA. When genomic DNA is transferred to a solid-phase support such as nylon or nitrocellulose in a Southern blot, the DNA is denatured so that labeled probes may hybridize to the single-strand genomic DNA sequence. We highlight three representative areas of the genomic DNA, each having a different sequence. The length of these sequences, as well as the probes, is not drawn to scale and would contain many more base pairs than depicted. A probe, labeled here with biotin, that has nucleotide complementarity to the genomic sequence will bind with specificity. Using this principle in some form, a table of the basic tools of molecular biology is given to provide a cursory overview of the methods and their uses. The maximal sensitivity of the technique is not ordinarily the success that will be found with every experiment; it is the best observed sensitivity.

assay first described by Kleppe and colleagues[7] and transformed by Mullis and associates' use of a thermostable DNA polymerase[8,9] has dramatically reduced lead times in comparison to conventional Southern blotting methods. As with Southern blotting, there must be some knowledge of the target DNA sequence for PCR. In practice, PCR typically requires two primers, one of which is complementary to the 5′ DNA region of interest and the other to the 3′ end of the DNA region. The DNA regions can be any part of a gene, exonic or intronic, although amplifications longer than 10 kilobases become increasingly difficult to isolate. Replication of the DNA fragment, or amplicon, occurs processively as part of a series of 20 to 40 repeated temperature cycles in which one cycle consists of denaturation of the DNA target, annealing of the primers to the single-stranded DNA, and enzymatically catalyzed elongation of complementary DNA by a thermostable DNA polymerase. One noteworthy improvement in the past 15 years has been the addition of thermostable DNA polymerases with separate 3′ to 5′ exonuclease proofreading activity, which has resulted in higher sequence fidelity of the amplified product. Among the multiple variants of the basic PCR methodology that have been developed, identification of allele-specific, single nucleotide polymorphisms (SNPs)[10] and foci of methylated CpG islands[11] in genomic DNA are just two. Because of the dependability and efficiency of amplifying a specific locus within the genome, PCR and its basic multiplex versions, which can amplify several amplicons simultaneously,[12] continue to be workhorses for the preparation of genomic samples.

A parallel set of approaches for studying expression of the primary products of gene transcription that involve messenger RNA (mRNA) has enabled neuroscientists to assign critical spatiotemporal information to gene function. The first technique developed to do so, the Northern blot,[13] differs from the Southern blot in that the target molecules separated according to size by agarose gel electrophoresis under denaturing conditions and transferred to a membrane support for hybridization consist of RNA, whether total RNA or poly A^+ mRNA. At the organ or

tissue level, it remains unmatched in its ability to provide an accurate size of the transcript and quantifiable patterns of gene expression during development or disease states, or both. Furthermore, differences in the size of the transcript within or among samples suggest any number of possibilities, including but not limited to alterative splicing, alternative start sites, or differences in polyadenylation. Typically, medium- and high-abundance messages are readily visualized with labeled probes. However, low-abundance messages can be difficult to detect.

The low-throughput methodology, long lead times, and limits of detection dictated by RNA immobilization on solid-phase supports have largely been overcome by the development of solution-based hybridization techniques that provide at least an order of magnitude more sensitive for detecting mRNA transcripts. Detection of mRNA in its native state by the ribonuclease protection assay adapts the Northern blot approach by hybridizing a labeled complementary RNA probe (usually 100 to 500 base pairs) with high specific activity to unlabeled cellular RNA that is freely suspended in solution.[14] The resulting RNA duplexes, which are highly stable, are protected from digestion by single-strand–specific ribonucleases and separated by polyacrylamide gel electrophoresis (PAGE). Detection of signal by the isotopic or nonisotopic labels offer sensitive and reliable detection and quantitation of mRNA that is up to 50 times more sensitive than Northern blotting.[15] A second solution-based alternative requires conversion of the native mRNA to complementary DNA by reverse transcriptase followed by PCR. Although gene-specific primers can be used for the reverse transcription, a polythymidine oligonucleotide (oligo-dT), random hexamers, or combination of them are most frequently used. Oligo-dT primers readily hybridize to the translationally important poly A^+ tails present in most mRNA and thereby bias the start site of reverse transcription toward the 3′ end of the mRNA. Conversely, random hexamers are often used when attempting to obtain a target template closer to the 5′ end of long mRNA sequences. Detection of the target sequence can be achieved by standard PCR protocols, whereas multiplex PCR protocols allow the simultaneous detection of several amplicons.[12] However, when quantitation is required, quantitative real-time PCR (qRT-PCR) protocols are necessary.[16,17] qRT-PCR requires primers with specific design characteristics and amplicons with no more than 250 base pairs. Before quantitation, it is essential that the primers be exhaustively tested with the target template to ensure that the gene of interest is amplified and is the expected size. Two versions of qRT-PCR are now in use. The most simple qRT-PCR methodology uses a fluorescent dye that emits short wavelengths of ultraviolet spectrum light as a function of the number of amplicons created during each successive cycle when it intercalates with the double-stranded DNA formed during the thermocycling process. The generation of nonspecific, double-stranded PCR products, often referred to as primer-dimers, can interfere with or completely prevent quantitation. A more reliable but more expensive qRT-PCR route uses the addition of a separate sequence-specific RNA- or DNA-based probe modified with a fluorescence reporter at one end of the molecule and a quencher of fluorescence at the other end. In this version of qRT-PCR, the fluorescent reporter probe anneals to the target template somewhere in the target amplicon between the qRT-PCR primers. In intact reporter probes, the fluorescence is quenched. Detection and quantitation occur only as signal is emitted because the fluorescent reporter is physically cleaved from the quencher by the 5′-3′ exonuclease activity of the thermostable polymerase during the elongation step. To ensure accuracy with any of these solution-based mRNA quantitation assays, it is necessary to normalize expression of the target transcript with a stably expressed control gene. These methods are most favored when dealing with low-abundance transcripts and when the cellular RNA material is limited or, in a worst-case scenario, partially degraded.

In the past several years there has been a renaissance in Northern blotting methods for detecting a specific set of small RNA molecules, microRNAs (miRNAs). miRNAs are abundant, single-stranded, 21- to 23-nucleotide RNA molecules processed from endogenous 70-nucleotide pre-miRNAs by two enzymes, Drosha and Dicer.[18-20] These small noncoding RNAs are recruited to unique ribonucleoprotein complexes, RNA-induced silencing complexes,[21,22] where they mediate the translational suppression or degradation of nascent mRNA transcripts bearing homologous antisense sequence in their 3′ untranslated regions.[23] miRNA function appears to have an essential role in neuronal development,[24,25] and recent indications suggest that dysregulation of miRNA networks contributes to neurodegenerative disease.[26-28] As an analytic tool, the popularity of the Northern blot lies in its continued accuracy in estimating the size of the mature miRNA molecule and the ability to detect the pre-miRNA product simultaneously. Enhanced detection advances consisting of cross-linking small RNA molecules (<100 nucleotides) to nylon membranes,[29] as well as the use of locked nucleic acids,[30,31] to increase probe specificity are among the most recent contributions.

Gene expression observations at the organ or tissue level provide only an aggregate view of gene expression because the robust, cell-specific controls that create numerous subpopulations of individual neurons and nonneuronal cell types with considerable heterogeneity in mRNA expression phenotypes within the same anatomic region are lost. In addition, de novo expression, induction, and repression are rarely observed in the mature nervous system, so the dynamic range of expression is often modest. To determine the cellular resolution of gene expression, in situ hybridization is most often used. As a method for detecting and localizing specific mRNA transcripts in morphologically preserved tissue or cells, in situ hybridization uses a single-stranded complementary DNA or RNA probe that hybridizes to the target endogenous mRNA transcripts. Detecting mRNA transcripts within the cellular cytoarchitecture presents a unique balancing act. Because the mRNA population has not been diluted by mRNA from other cell types, the target mRNA of interest is present at its individual steady-state level. Many times, it is present at higher levels of abundance in specific subpopulations of neurons than one would detect within the whole tissue. However, mRNAs within the normal cellular matrix are but one constituent of a multi-megadalton ribonucleoprotein complex, and consequently their primary sequence is often masked or sterically inaccessible to a probe. Thus, one must fix the mRNA in place while balancing the ability to permeabilize the architecture so that the DNA probes, which are typically oligonucleotides of 20 to 50 bases, PCR-generated probes of several hundred bases, or complementary RNA probes (i.e., riboprobes) that can be routinely made with in vitro transcription kits up to one kilobase long, have access to the target mRNA sequences. However, care must be taken when using longer complementary riboprobes because cross-hybridization with similar sequences in other genes may occur. It is important to isolate gene-specific sequences to use as riboprobes. Typically, two or three separate gene-specific complementary DNA probes or riboprobes targeting the same transcript are used to cross-validate the subcellular distribution. There are two usual controls for specificity. The primary control is a competition control with excess unlabeled probe hybridization followed by labeled probe hybridization and subsequent detection. The DNA probes or riboprobes are complementary to the target mRNA transcripts and thus antisense. Sense controls are also used to show the degree of nonspecific background.

Originally, detection schemes for in situ hybridization used isotopic labels (^{33}P or ^{35}S) incorporated into the complementary probes. Quantitative methods for converting radioactive signal with silver grain density via photographic emulsion have been widely used since the mid-1990s.[32] Nonisotopic detection methods have multiplied over the past 2 decades because they

take a considerably shorter time, can have greater signal resolution, and allow the simultaneous detection of multiple different targets by combining various detection methods. Complementary probes labeled with biotin or digoxigenin allow a number of different detection options. Some use colorimetric substrates consisting of horseradish peroxidase or alkaline phosphatase conjugated to streptavidin beads or primary antidigoxigenin antibodies to facilitate detection. Data from the Allen Brain Atlas use just such a colorimetric detection strategy, which provides an increasingly comprehensive data set of expressed gene at the cellular level.[33] One continuing technical concern with enzyme-linked amplification schemes is diffusion of the colorimetric signal from the site of localization. Research laboratories have used fluorescence detection schemes within the past several decades that involve new generations of fluorophores with long-term photostability, such as Alexa dyes or quantum dot (Qdot) nanocrystals; these fluorophores have been key components in illuminating the trafficking dynamics of mRNA molecules within the subcellular compartments of dendrites[34] and axons[35,36] via conventional epifluorescent microscopy or single-photon confocal laser scanning microscopy. Quantitative data analysis of these fluorescent images requires the use of image acquisition and analysis software such as Metamorph or IP Lab. In a typical sample, the total fluorescence intensity for a region of interest, normalized against background noise and any differences in the area of the region of interest, is compared across experiments or among samples and subjected to statistical analysis. In contemporary molecular cytogenetics, fluorescence in situ hybridization (FISH)-based karyotyping and banding methods refer to a rubric of techniques used for both clinical genetics and tumor cytogenetics that can simultaneously characterize several chromosomes or chromosomal subregions (Box 3-1).

As intermediaries in the continuum between genotype and phenotype, levels of mRNA should be taken as a surrogate for corresponding protein expression or their functional activity with caution. Early data sets attempting to establish a correlation between protein and mRNA levels found varying degrees of concordance. Although a significant positive correlation has been observed in human transitional cell carcinomas,[57] more marginal grading was observed in a comparative examination of 19 genes in the human liver.[58] Conversely, in a more limited study of three matrix metalloproteinase–related genes expressed in benign and neoplastic prostate tissue,[59] no correlative relationships were identified. When these data are placed in the context of the vast regulatory networks that monitor the various posttranscriptional events, this wide variability is not entirely unexpected. A nearly universal theme among the traditional tools for assaying protein expression is the use of affinity between an antibody and an epitope on the target protein to facilitate protein detection. The specificity of the primary antibody is the central determinant in the accuracy of protein recognition. Much like assays for detecting nucleic acids, methods to identify protein expression can be accomplished with the use of blots, free in solution or in situ.

Box 3-1 Array of Fluorescence In Situ Hybridization–Based Methods in Molecular Cytogenetics

Over the past several decades, G-banding has served as one of the routine standards for chromosome banding. It relies on successful culture of the tissue of investigation, often fetal or tumor tissue, and preparation of metaphase cells. It should be noted that product-of-conception samples in particular suffer relatively high rates of failure (10% to 40%) during the tissue-culturing process[37] and poor chromosome morphology.[38] Monochrome changes in the visible karyotype of morphologically optimal samples produced by Giemsa staining can provide low but sufficient chromosomal resolution to distinguish changes in chromosome number and large structural rearrangements whether translocations or macrodeletions. Chromosomes with small translocations, cryptic aberrations, microdeletions, and inversions or more complex karyotypes are often beyond the limits of conventional G-banding analysis.[39] As a result, complementary fluorescence in situ hybridization (FISH)-based karyotyping and banding methods have been developed to overcome the intrinsic morphologic and technical obstacles in karyotyping associated with G-banding protocols.

In its most basic form, FISH-based karyotyping uses DNA probes hybridizing to a specific gene or chromosome locus in metaphase or, less commonly, interphase chromosome preparations for the straightforward detection of deletion/duplication syndromes and gene fusions or rearrangements. In contrast to metaphase FISH, interphase FISH does not require the growth of viable cells for the preparation of a chromosomal spread. Interphase FISH makes use of preserved tissue and cellular material such as that found in paraffin-embedded biopsy samples. Perhaps the most significant advance in the past decade or so has been the development of multicolor FISH-based karyotyping and banding techniques (Fig. 3-2). The various iterations of multicolor FISH karyotyping methods are all based on the availability of chromosome-specific probe sets developed from degenerate oligonucleotide–primed polymerase chain reaction of flow-sorted chromosomal libraries[40,41] labeled in tandem with four to seven spectrally separable fluorochrome labels[42] that simultaneously "paint" and distinguish each whole chromosome.[43,44] Chromosome painting with chromosome-specific probes can be done combinatorially (e.g., multiplex FISH or spectral karyotyping) or by using an additional ratiometric approach[45] (i.e., combined binary ratio–FISH) and has proved to be a powerful adjunct to conventional cytogenetic techniques in the diagnostic analysis of interchromosomal events and characterization of the cytogenetic evolution of tumors, even in complex aberrations. The sensitivity (i.e., whether a translocation can be detected) and specificity (i.e., whether it can classified with assurance) of the analysis are vitally dependent on the fluorochrome combinations used in the target chromosomes.[46] The resolution of these whole chromosome–painting techniques is limited by the lack of any additional spatial delineation within a chromosome. For this reason, multicolor karyotyping alone is often not sensitive enough to precisely determine chromosomal breakpoints, subtle chromosome rearrangements, or intrachromosomal aberrations (e.g., inversions, duplications, terminal deletions). Differential hybridization signals obtained with other FISH-based karyotype techniques, such as comparative genomic hybridization, have similar resolving capabilities.[47] Efforts to improve the resolution of these assays have used chromosome arm–specific,[44,48] region-specific,[49,50] centromeric,[51] and subtelomeric probes.[52,53] To address the latter issue of intrachromosomal aberrations, FISH-based banding patterns obtained by using differentially labeled and pooled subregional DNA probes were designed to produce high-resolution chromosomal karyotypes with identifiable fluorescent banding patterns within a single chromosome via spectral color banding,[54] cross-species color banding[49,55] or multicolor banding.[50,56]

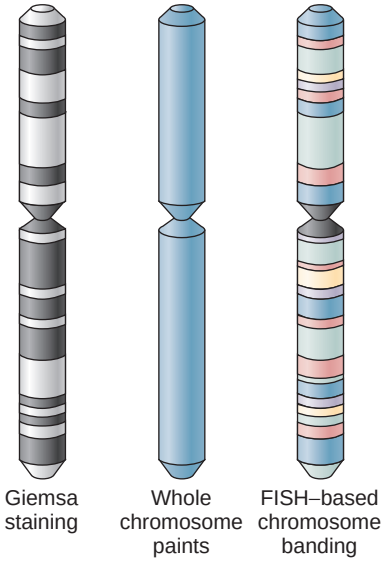


FIGURE 3-2 An idealized graphic comparison of a Giemsa-stained, whole chromosome–painted, and multibanded chromosome. FISH, fluorescence in situ hybridization.

Protein blots, more commonly referred to as Western blots,[60] immobilize size-separated protein lysates on membranes (e.g., nitrocellulose or polyvinylidene difluoride [PVDF]), where they are detected with a monoclonal or polyclonal antibody.[61,62] In short, cellular proteins solubilized in a lysis buffer containing one or more detergents, protease inhibitors, and more often than not, a strong reducing agent are separated by PAGE. Separation is most commonly accomplished by molecular weight. However, separation in this single dimension may be further refined by prior separation in an immobilized pH gradient gel, and these gels are often referred to as two-dimensional (2D) gels. Although protein lysates can be run under nondenaturing conditions, usually when separating by molecular weight, sodium dodecyl sulfate (SDS) is incorporated in the polyacrylamide gels and the running buffers to maintain the protein lysates in a denatured state. Strong reducing agents such as β-mercaptoethanol or dithiothreitol in the lysis or loading buffer (or in both) eliminate any secondary or tertiary folding that would interfere with protein migration within the polyacrylamide gel. Changes in the percentage of acrylamide within the gel determine how well low- or high-molecular-weight proteins separate within the gel. Gels with a higher percentage of acrylamide resolve lower-molecular-weight proteins better and vice versa. The gel-immobilized proteins are transferred in a glycine-based buffer to nitrocellulose or PVDF membranes. The addition of a primary antibody recognizing the target protein facilitates detection of the protein, most commonly in a multistep (i.e., indirect) detection scheme using fluorescently labeled secondary antibodies or enzyme-linked secondary antibodies and various colorimetric, chemifluorescent, or chemiluminescent substrates. Advances in antibody production offer not only a growing selection of primary immunoreagents but also novel innovations in antibody development that combine the most attractive properties of mouse monoclonal (e.g., uniformity, purity, indefinite availability) with rabbit polyclonal (higher affinity) antibodies.[63,64] The choice of primary antibody is not without a caveat. A single primary antibody will rarely share equal affinity for the target proteins in nondenaturing and denaturing conditions and often has to be determined empirically. Signal detection reveals the experimental molecular weight of the protein, which can be compared against the calculated molecular weight of the open reading frame. Although aberrant migration can occur as a result of the intrinsic properties of the protein sequence, significant differences in molecular weight are also suggestive of cotranslational or posttranslational protein modifications. The ability to obtain quantitative information from signal detection is quite limited regardless of the detection scheme, but with proper normalization for loading errors[65] and effort to increase the dynamic range of signal[66] by using cameras with charge-coupled devices, there has been considerable improvement. A far more sensitive technique for determining protein abundance is the sandwich enzyme-linked immunosorbent assay (ELISA).[67] Sandwich-ELISA methodology requires a capture antibody that recognizes the protein of interest. It is adsorbed to the surface of a microtiter plate and flooded with the presence of protein lysate. Any antigenic sequence within the protein of interest binds to the capture antibody. A detecting antibody that also recognizes the protein of interest, although at a different epitope not occluded by the capture antibody, is added to facilitate detection of the protein. Once an enzyme-linked secondary antibody is bound to the detecting antibody, an inert substrate of the enzyme is cleaved to create fluorescent or chemiluminescent signal that can be quantified to determine the concentration of the antigen.

Another widely used protein detection method is immunoprecipitation.[68] The principal methodology is very simple. An antibody recognizing the target protein is incubated with cell lysates in solution in the presence of protease inhibitors and allowed to form an immune complex, which can then be "precipitated" out of the solution by using protein A/G–coupled agarose beads. Proteins not captured by the primary antibody and sequestered within the immune complex are washed away. The immune complexes (antibody and antigen) are eluted from the protein A/G–coupled beads, separated by SDS-PAGE, and analyzed by Western blot to verify the identity and quantity of the target protein. One important variable is the composition of the immunoprecipitation lysis buffer. Ideally, the lysis buffer is able to balance the solubilization of proteins from the original tissue or cell matrix while leaving their native conformation intact.[69] By so doing, immunoprecipitation protocols seek to physically isolate the protein of interest from the rest of the cell lysate while retaining the protein-protein interactions of the target antigen. One criticism of this methodology has focused on the potential for identifying false-positive co-immunoprecipitation protein partners because of the lysis step, which allows all the solubilized cellular constituents to mix in solution. This mixing may produce nonphysiologic interactions because proteins normally compartmentalized within the cellular milieu or expressed in different cell types are now allowed to interact. As a result, validation of such protein-protein interactions by parallel methods will be required (Box 3-2).

Determining the subcellular distribution of protein expression from the results of Western blotting or immunoprecipitation is constrained by the type of lysate used. Differential centrifugation protocols are the simplest way to fractionate various organelles, membranes, and subcellular structures as starting material for these protein assays, but residual contaminants interfere with detection of the nuanced changes in subcellular localization and intercompartmental translocations that are thought to be crucial to cellular information-processing networks.[86] In situ visualization of protein expression in sections of frozen or paraffin-embedded fixed tissue (i.e., immunohistochemistry) and its cognate technique in fixed cells in culture (i.e., immunocytochemistry) are robust and dynamic methods for identifying and quantifying alterations in patterns of localization in the various compartments and subcellular structures intrinsic to nerve cells. They apply a common methodology based on the ability of a primary antibody to bind to endogenous proteins expressed within its native cytoarchitectural matrix. Primary antibodies can

Box 3-2 Protein Domains and Selected Parallel Methodologies for Assessing Protein-Protein Interactions

The three-dimensional structure of a protein is often scattered, with various protein modules that can fold and, in some cases, autonomously retain the function of the rest of the protein chain.[70] Numerous protein-protein interaction domains and their sequence boundaries have been identified by structural data from x-ray crystallography and nuclear magnetic resonance, but obtaining these data is time-consuming and labor-intensive. By extrapolating from these data, mathematical algorithms have been constructed to query the primary amino acid sequence for the presence of and boundaries for various types of protein-protein–interacting domains and interfaces. Meanwhile, curated databases have been assembled to identify and catalog many of the physical interactions among pairs or larger groups of proteins.[71] Identification of direct interacting partners will require multiple ways of verifying any interaction. A selected set of protein-protein interaction methodologies that are, to varying degrees, complementary with immunoprecipitation are presented in the following sections.

PROTEIN AFFINITY CHROMATOGRAPHY/ PULL-DOWN ASSAY

A protein is covalently or noncovalently coupled to a solid-phase matrix, such as cyanogen bromide (CNBr), and protein lysates are run through the column.[72] Weakly retained and strongly retained protein can be eluted under differing salt conditions. These columns can be incredibly sensitive with detectable binding constants as weak as 10^{-5} M. However, retaining the native structure, activity, and proper orientation of the protein when directly immobilized to a CNBr matrix can be difficult and result in contradictory results when compared with other methods.[73] As an alternative, the target protein is expressed with a suitable affinity tag (e.g., poly-His, biotin, GST, FLAG, c-myc) and immobilized on agarose-Sepharose supports by its respective ligand (e.g., Ni^{2+}, streptavidin, glutathione, or monoclonal antibodies specific to FLAG or c-myc). The interacting partner can be identified by Western blots, direct sequencing, or mass spectrometry.

TANDEM AFFINITY PURIFICATION

This protein affinity assay requires placing two tags such as protein A and the calmodulin-binding protein on a bait protein separated by a TEV protease cleavage site. It reduces nonspecific pull-down of proteins through successive rounds of purification, but it does so at the expense of transient protein-protein interactions.[74,75] A particularly successful application of the tandem affinity purification system in combination with mass spectrometry was used for the systematic analysis of yeast protein complexes.[76]

IN VIVO CROSS-LINKING

False-positive results generated by the loss of spatial organization during the lysis step are mitigated by chemical and photo cross-linking to covalently "freeze" the protein-protein interactions in situ before isolation. Chemical cross-linking[77,78] typically uses the exogenous introduction of a variety of homo- or hetero-bifunctional cross-linking reagents. In contrast, photo cross-linking uses modified versions of leucine (photo-leucine) and methionine (photo-methionine) containing a photoactivatable diazirine ring incorporated into growing peptide chains by the nascent translation apparatus.[79] The addition of either amino acid does not alter the cell's metabolism, but when the cells are exposed to ultraviolet light, highly reactive intermediates enable identification of protein-protein interactions when used in combination with immunoprecipitation, Western blotting, or mass spectrometry.

LABEL TRANSFER

Label transfer is a variation of the cross-linking methodologies that is especially useful for detecting weak or transient protein-protein interactions. It involves coupling of an isotopic or nonisotopic label transfer reagent into the bait protein and incubating it with an unlabeled protein lysate. When exposed to ultraviolet light, any interacting proteins are cross-linked by the label transfer reagent. The actual label transfer occurs when the cross-linker is cleaved so that the label is left attached to the interacting protein, where it can be detected by separation via sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western analysis, sequence analysis, or mass spectrometry.[80,81]

YEAST TWO-HYBRID SCREENING

Pioneered by Fields and Song,[82] the yeast two-hybrid screening technique uses transcriptional activity to assess protein-protein interactions. The premise of the technique is activation of a downstream reporter gene (e.g., β-galactosidase, secreted alkaline phosphatase, luciferase) that is easily assayed by binding of the Gal4 binding domain (BD) and Gal4 activating domain (AD) proteins to an upstream activating sequence. The BD and AD do not need to directly bind but are brought together in apposition with suspected interacting proteins fused with the AD or BD. Although useful as a complementary tool, this technique has a notoriously high false-positive rate.

FLUORESCENCE RESONANCE ENERGY TRANSFER

Intermolecular fluorescence resonance energy transfer (FRET) is a spectroscopic method that assesses the proximity and relative angular orientation of two different proteins—one having a donor fluorophore such as cyan fluorescent protein and the other having an acceptor fluorophore such as yellow fluorescent protein—by monitoring the emission spectra when laser excitation of the donor protein is applied.[83-85] When the fluorophores are separated, only the emission spectra of the donor protein is observed (Fig. 3-3). When in close enough proximity, laser excitation of the donor fluorophore transfers the excited energy state to the acceptor fluorophore and generates a peak in its emission spectra. The two principal reasons why this technique is proving to be an extremely valuable tool for probing protein-protein interactions are that (1) the efficiency of this transfer is extremely sensitive to the separation in distance between the two fluorophores and (2) the range over which the transfer in the excited energy state can occur is spatially delimited to approximately 10 nm.

be directly conjugated to enzymes or fluorophores to facilitate detection. More often, because of reduced cost, labeled secondary antibodies are used with colorimetric or indirect immunofluorescence visualization schemes to provide quantifiable patterns of protein distribution. Indirect immunolabeling of multiple primary antibodies, which is most easily accomplished when the primary antibodies are raised in different species, can be used to correlate the colocalization of additional proteins when the emission spectra of the fluorophore-conjugated secondary antibody are separable. Because the maximal optical spatial resolution defined by Rayleigh scattering is approximately 200 nm for conventional wide-field fluorescence microscopic techniques, colo-

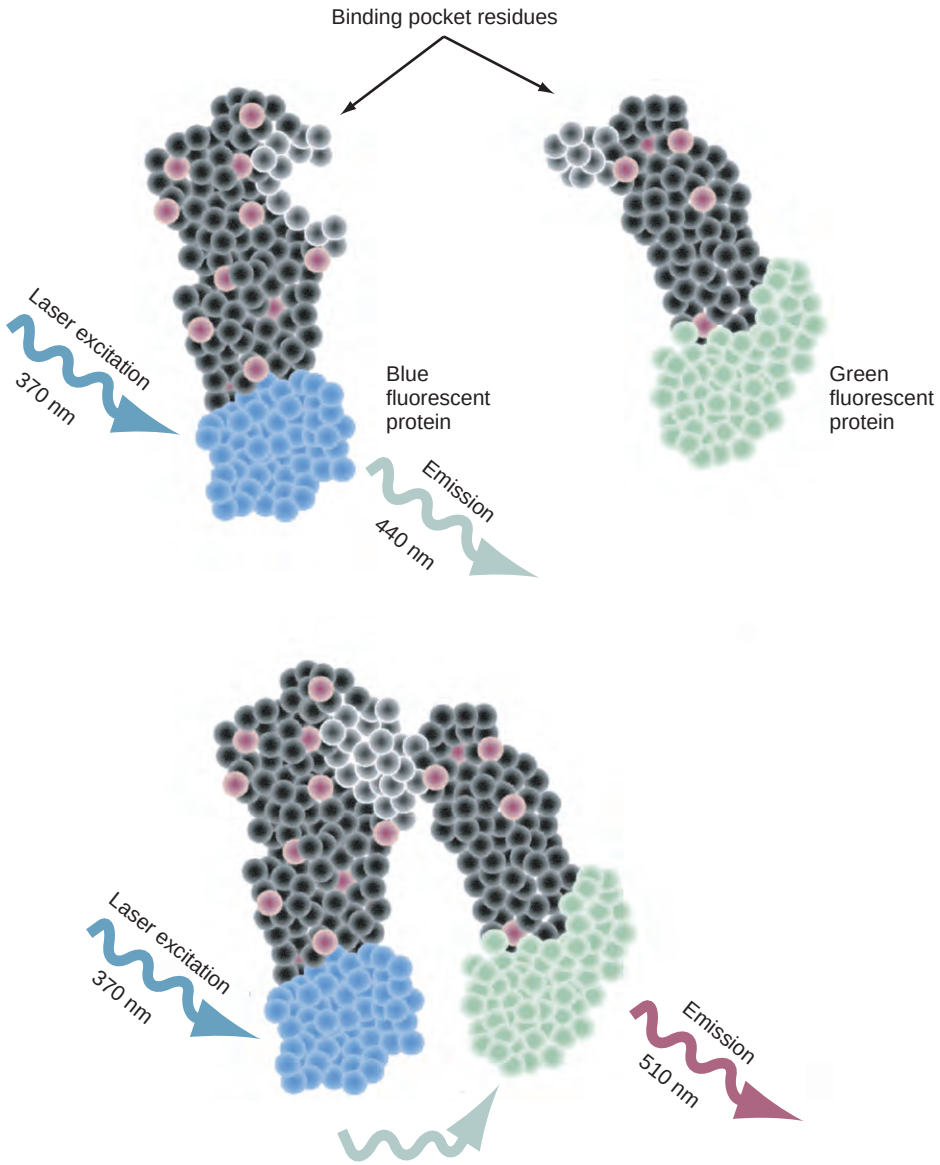


FIGURE 3-3 Intermolecular fluorescence resonance energy transfer. Two hypothetical fusion proteins are depicted. One is fused in frame with blue fluorescent protein (BFP) and the other with green fluorescent protein (GFP). The binding pocket amino acid residues responsible for the protein-protein interaction are highlighted by *white shading*. On laser excitation at a wavelength of 370 nm, only the BFP would emit a signal in the range of 440 nm. Very little or no GFP emission in the 510-nm range would be observed. However, if the protein-protein interaction brings the fusion protein to within 10 nm of each other, laser excitation would excite the BFP excitation is not seen as emission spectra in the 440-nm range. Rather, the energy of excitation is transferred to the GFP, emission is observed at a wavelength of 510 nm.

calization is suggestive of, although not formal proof of a protein-protein interaction.

The microinjection of a directly conjugated, high-quality antibody presents an opportunity to visualize protein expression dynamics in live cells. Recent improvements in neuronal transfection techniques frequently make heterologous expression of DNA constructs a more consistent option. A chimera of the target protein fused with a fluorescent protein (FP),[87,88] such as GFP, DsRed, or their variants, with increased brightness and folding efficiency[89] has the enviable characteristic of requiring no cofactors other than O_2. When directly imaged, changes in the subcellular distribution of the chimeric protein can be quantified in largely the same manner described for FISH-based mRNA trafficking dynamics in neuronal processes. There persist three experimental concerns, only one of which is technical, that limit the usefulness of this type of transfection approach. The technical limitation is the size of the chimera sequence itself. Ordinarily, mammalian expression constructs are only efficient at driving overexpression of DNA constructs that are less than about 10 kilobases in size. The additional concerns are biologic and twofold. First, does overexpression of the DNA construct lead to ectopic expression of the target chimera? Second, does expression of the FP tag interfere in any way with the functional activity of the target protein? Most autofluorescent fusion proteins have been constructed by placing the coding sequence of the target protein upstream or downstream of the FP sequence. When either of these locations has a negative impact on target protein function, the alternative is to insert the FP sequence within the open reading frame. Lacking any previous knowledge of where to place the FP within the open reading frame, finding

a permissive site is guesswork at best. One strategy to overcome the pitfalls of performing sequential insertions to create a functional FP fusion uses the bacterial Tn5 transposon to create libraries of FPs that can then be individually isolated and tested.[90]

When interfering with the activity of the target protein is the desired goal, there is no equivalent instrument in the molecular neurobiologist's toolbox to genetic techniques that precisely and completely eliminate gene function.[91] One now widely disseminated nucleic acid–based gene-specific silencing method to partially silence gene function uses the RNA interference (RNAi) pathway.[92] Small interfering RNAs (siRNAs) are 20- to 25-nucleotide, double-stranded effector molecules of RNAi. They are integrated into the RNA-induced silencing complex in the same fashion as miRNA (Fig. 3-4),[93-95] where they become juxtaposed with endogenous mRNA, base-pair with perfect homology to the mRNA, and cause it to be cleaved by argonaute-2 (Ago-2), the catalytic component of the RNA-induced silencing complex (RISC). In primary neuronal or astrocyte culture, transient transfection with the siRNA duplexes or with plasmid-encoded short-hairpin RNA (shRNA), which expresses the siRNA duplex as a hairpin suitable for processing with the enzyme Dicer, is the most common course of action. In vivo delivery of siRNA has proved successful with plasmid- or viral vector–encoded shRNA via a number of local[96-100] or systemic[96,101-103] applications. Although the empirical rules for rational siRNA design and selection prediction algorithms improve specificity,[104] the efficacy of the knockdown can vary as a function of whether individual or pooled

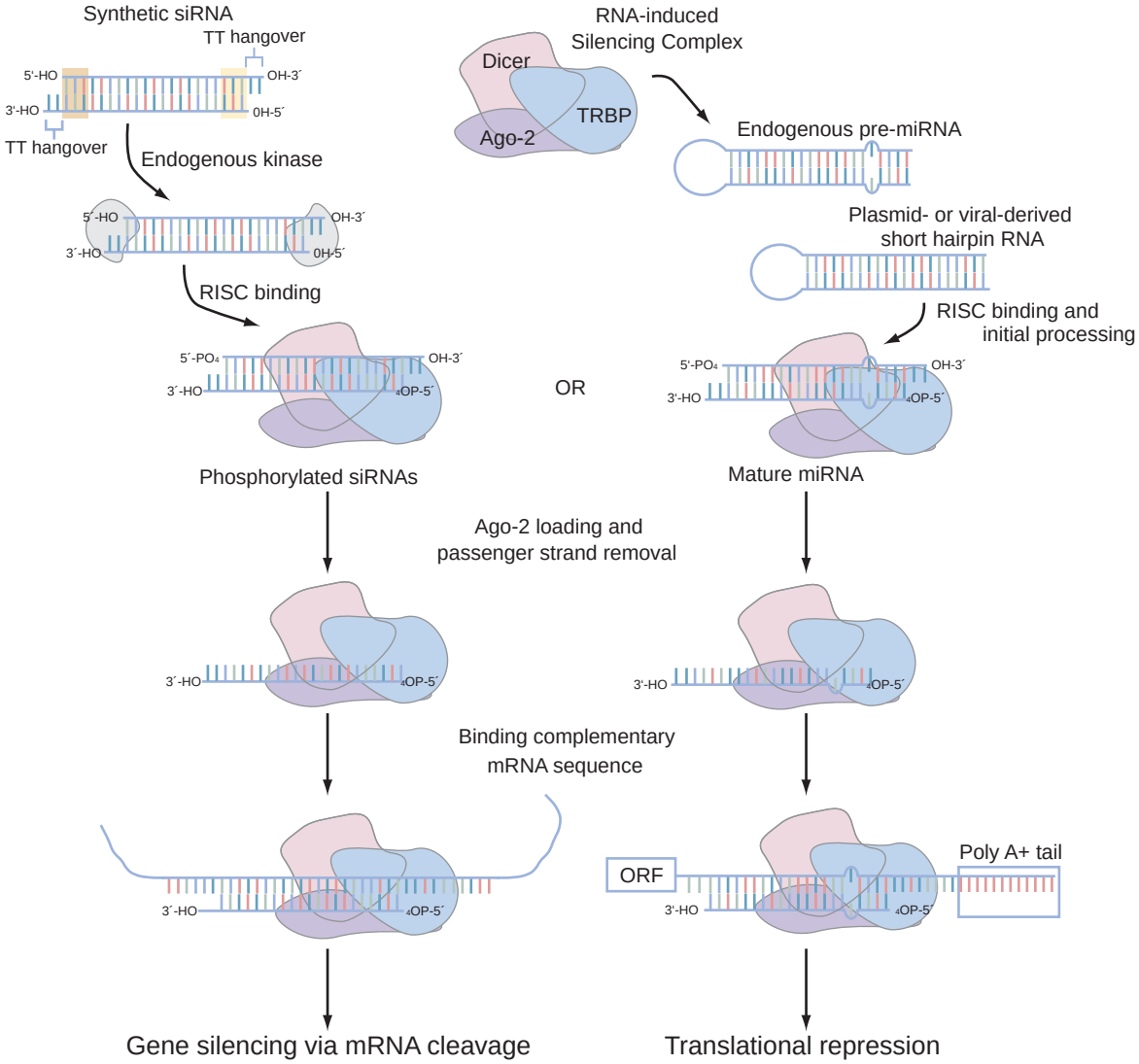


FIGURE 3-4 Small interfering RNA (siRNA) and microRNA (miRNA) processing pathway using the RNA-inducing silencing complex (RISC). RISC mediates the functional activity of both siRNA and miRNA. Short-hairpin versions of siRNA or endogenous miRNA are recruited to the RISC, which in mammals is composed of three core proteins (e.g., Dicer, TRBP, and argonaute-2 [Ago-2]) and associate with each other in the absence of double-stranded RNA (dsRNA).[93,94] Synthetic siRNA duplexes, which are initially processed by an endogenous kinase, have a number of structural features that we highlight. The 21- to 23-nucleotide complexes have areas of high (highlighted by the *green shaded box*) and low (highlighted by the *yellow shaded box*) thermodynamic stability that dictates which strand is eventually incorporated into the RISC complex as the guide strand. These siRNA duplexes typically also incorporate a pair of thymidine base pairs as 3′ overhangs of each strand. After incorporation into the RISC, the dsRNA of both siRNA and miRNA is then unwound; only the guide strand is loaded onto Ago-2, whereas the passenger strand is released.[95] On binding of mRNA with complete complementarity (siRNA) or with nucleotide mismatches (miRNA or, not pictured, siRNA), this dsRNA is able to influence gene function. siRNA can bind anywhere in the messenger RNA (mRNA), whereas miRNA is thought to bind primarily in the 3′ untranslated region.

sets of siRNA are used and the potency of this siRNA. The most attractive potential of RNAi is the flexibility that it allows in controlling the spatial and temporal effects of inhibition. With the development of inducible siRNA whose expression is controlled by tetracycline- or doxycycline-regulated promoters,[105-109] photoactivated versions of "caged" siRNA,[110] and focal transfection methods,[111] stepwise advances to this promise are being realized. Although the low to moderate concentrations of siRNA typically used to produce significant knockdown tend to evade interferon response–mediated changes in global gene expression,[112,113] a secondary effect has on occasion been noted.[114,115] A more acute concern is the possibility of an siRNA modulating the expression of a closely related sequence[116-118] and resulting in observable changes in phenotype.[119] Chemical modifications of the siRNA can mitigate some of these off-target effects,[120] but the exact nature of siRNA specificity remains unclear (see Elbashir and colleagues[121] and Miller and associates,[122] but compare with Semizarov and coworkers[123]). Until a better consensus of siRNA specificity is reached, current siRNA design suggests allowing for at least two nucleotide mismatches with all off-target genes. A recent editorial suggests that the ideal control is to rescue the siRNA phenotype by using an siRNA-resistant gene with a silent mutation in the 3′ nucleotide of a codon in the middle of the siRNA binding site.[124]

THE RISE OF FUNCTIONAL GENOMICS

Candidate gene studies take advantage of two lines of evidence that dovetail to increase success: the increased efficiency of association studies in selected population-based samples and an a priori understanding of the clinical phenotypes and how it might be affected by candidate gene function. However, this approach has met with mixed success when assessing complex diseases in which multiple genes, as well as their sequence and functional variants, probably initiate small individual contributions and relative risk for a cumulative phenotype that varies in the severity of symptoms and age at onset and evolves over time. Lacking the tools of scale to perform the simultaneous analyses required, continuing efforts toward miniaturization and scalability epitomize the new "omics" technologies that are transforming nervous system studies by allowing data-rich and detailed characterization of the molecular mechanisms underlying cell physiology. Ironically, it does so by using the very same methods of biochemistry, molecular biology, and cell biology worked out decades earlier. At its core, functional genomics aspires to integrate data from the study of different molecular strata—the genome, transcriptome, proteome, metabolome, and their regulatory mechanisms—into a systems-level model of cell biology. The ostensible goal is to obtain a richly detailed, global understanding of the nervous system's emergent properties through the interactions among all its constituent elements.[125] In so doing, it promises to expand our insight into the root problems of complex diseases and transform the current predictive power of our diagnostic and therapeutic regimens.[126]

Transcriptomics

Evolving technologic innovations fostering miniaturization, increased scalability and efficiency, and decreased cost born from the Human Genome Project now exploit this wealth of genomic data. Gene expression profiling[127,128] is the most widely used functional genomics technology due in equal parts to its early development and the ease with which it can be performed. High-density DNA microarrays anchor cDNA[129] or oligonucleotides[130,131] of different genes in massively parallel arrays of up to approximately 10,000 spots/cm^2 and greater than 1,000,000 spots/cm^2, respectively, on a glass surface. Using the principle of molecular hybridization, several micrograms of fluorescently labeled RNA or cDNA probes hybridize with the target DNA and are analyzed with high-resolution scanners that optically detect the strength of fluorescent signal from the bound probes. Raw data are normalized and processed through a series of statistical approaches to determine whether any gene is differentially expressed. The probes are constructed from abundant sources of high-integrity mRNA, which is reverse-transcribed in the presence of fluorophore-coupled deoxyribonucleoside triphosphates (dNTPs) or amino-allyl–labeled dNTPs that can be coupled to a fluorophore such as Cy3 or Cy5. In postmortem tissue, even with extended postmortem intervals of up to 30 hours, high-integrity mRNA can be isolated for microarray sample preparation. However, it is not the postmortem interval inasmuch as it is the pH of the tissue (great than 6.25) that determines mRNA integrity.[132] Alternatively, when confronted with small amounts of starting material, it may be necessary to amplify the mRNA population so that there will be enough material to drive the hybridization reaction when labeled without skewing the complexity of the original mRNA population.[16] Because exponential amplification techniques, such as PCR, do not offer this capability, a linear amplification technique, aRNA amplification, enzymatically converts the mRNA population into a single-stranded cDNA with reverse transcriptase by using a specialized oligo-dT primer containing a 3′ T7 RNA polymerase promoter sequence. When a complementary second strand is synthesized, it serves as a transcription template for T7 RNA polymerase to produce RNA oriented in the antisense direction, which can be labeled directly or converted into labeled cDNA as a probe. Amplification of mRNA by manual harvesting of individual live neurons[128,133,134] or dendrites,[135,136] as well as neurons in fixed tissue,[137,138] has been equally as successful as automated approaches such as laser capture microdissection.[139]

Experimental artifacts can be introduced by sample preparation (e.g., differences in the integrity of mRNA or in the efficiency of labeling), the array (e.g., DNA spotting or printing errors), or processing (e.g., variable fluorescence scanner performance). Careful experimental design (e.g., checking the integrity of the mRNA before use, dye swaps and parallel processing of samples to control for labeling efficiency, quality control experiments for each lot of custom and commercial microarrays) can largely eliminate these issues. Reducing systemic biases in the results requires the optical data to be normalized at the global level to facilitate comparisons across microarray experiments and at the local level to account for individual variations in signal intensity that are unique to the surface of that microarray.[140,141] Common approaches on how best to apply these normalization procedures to distribution of the data are available in the form of open source and open development software offered by the Bioconductor project. When analyzing data, the most conservative treatment of it uses a Bonferroni correction to reduce possible false-positive errors. However, this correction for multiple measurements can also lead to increases in the number of false-negative errors. Various data analysis software packages try to balance the discovery rates of these two types of errors. As with all high-throughput assays, these data should be verified with other techniques. Although the current generation of high-throughput, low-cost cDNA or oligonucleotide microarrays is the primary laboratory workhorse for quantifying the transcriptome, next-generation technology is already on the horizon (Box 3-3).

There are two principal areas where highly paralleled mRNA expression technology has proven utility. One is as a comparative expression profile or signature profile. This genome-wide molecular fingerprint provides a distinctive pattern of gene expression that can be used as a comprehensive framework for assessing differences in classes of neurons[134] and astrocytes,[152] during development in myoblasts,[153] and in genetic mutants in model systems.[154] It has also been used to evaluate the secondary effects

Box 3-3 Next-Generation Technology for mRNA Gene Expression

Sources of noise within the experiment can be controlled, in part, by the normalization techniques discussed earlier. However, there are several notable technical limitations of DNA microarrays at present: high background levels as the result of cross-hybridization by multiple targeting probes[142] and low concordance (≈30% to 40%) of transcript detection between platforms.[143-145] In the former circumstance, the probe will normally bind with high specificity to an arrayed target sequence. Off-target effects such as cross-hybridization occur when a flanking, unbound sequence of the same probe binds weakly to an adjacent arrayed sequence. The resulting background noise contributes to the relatively small dynamic range (≈2 orders of magnitude) of signal detection in microarrays, although in the latter circumstance the weak overlap in mRNA expression profiles across different microarray platforms is a consequence of unpredictable intramolecular folding events in some long probes[146] and hybridization differences driven by the use of different sequences for the same target gene on various platforms. The cross-platform differences can be improved by using the RefSeq database for gene matching[147] and still further when expression patterns are analyzed only when target sequences between platforms overlap.[148]

Next-generation technologies determine the identity of the mRNA transcript with the use of highly paralleled, direct sequencing methods.[149] Although expensive at the moment, RNA sequencing (RNA-Seq) has several clear advantages over the current array-based methods, including low background noise, a large dynamic range (up to ≈3.5 orders of magnitude), and single–base pair resolution, which allows the ability to distinguish different isoforms (i.e., splice variants) and allelic expression (i.e., single nucleotide polymorphisms or structural variants, including insertion-deletions and copy number variations) of the same mRNA without subsequent need for any specialized normalization. Briefly, the total or poly A^+ mRNA population is converted into a library of short, adaptor-modified cDNA (200 to 500 base pairs) that is compatible with deep-sequencing instrumentation. In the case of a population enriched in poly A^+ mRNA, there are two general paths for processing. The poly A^+ mRNA can be fragmented first, usually by hydrolysis or nebulization, and then ligated to adaptors and reverse-transcribed into cDNA. Conversely, the poly A^+ mRNA can be ligated to the adaptor, reverse-transcribed into cDNA, and then fragmented with DNAse I or sonication. When fragmented at the RNA level, there is limited bias over the length of the transcript.[150] However, fragmentation at the cDNA level greatly biases the readable sequence toward the 3′ end.[151] Depending on the amount of input mRNA, amplification of the population may or may not be necessary. These libraries can generate millions of short reads that typically vary from 30 to 250 base pairs by 454, Solexa, or SOLid sequencing and can be compared against the genomic sequence or the coding sequencing of a gene.

of drug compounds on regulation of gene expression.[155] Signature profile comparisons of cells under different stimulation conditions[141] or environmental influences[156] that expand the complexity of the mRNA populations, or "expression space," have colloquially been referred to as "exercising the genome."[157] The Human Genome Project estimated the total number of human genes expressed in the central nervous system to be approximately 25,000 to 30,000.[158] However, alternative splicing, which is thought to occur in 92% to 94% of genes[159] and is often subverted in disease,[160,161] generates further heterogeneity in the mature mRNA population from a single pre-mRNA transcript.[162] The patterns of expression of alternative splice forms are strongly correlated across different tissues, thus suggesting the presence of tissue-specific regulatory mechanisms.[159,163,164] Because classic microarray designs do not incorporate this additional level of mRNA complexity, filling this gap in signature profile data is a series of new alternative splicing arrays[165-167] with promising insight into transformation of Hodgkin's lymphomas[168] and gliomagenesis.[169] Adaptations of DNA oligonucleotide microarray methods have also been made to signature-profile the expression of miRNA in low-density microarrays.[170,171]

Most highly paralleled gene expression studies make no a priori hypotheses about which individual genes are regulated among comparison sample sets. It is done within the framework of a systems biology approach in which it is assumed that transcription occurs with a finite set of resources. Thus, a change in the mRNA transcription of one gene will have collateral, sometimes seemingly stochastic influences on other mRNA. A second application of gene expression attempts to mine the comparative expression profiles for information on transcriptional regulatory networks. Data mining of signature profile results can identify clusters of mRNA to be transcriptionally active or silent. The genomic sequences of this mRNA are then analyzed for the presence of shared promoter elements that might contribute to the levels of expression. This sequence analysis is usually paired with direct analysis of promoter occupation by the suspected transcription factor via chromatin immunoprecipitation (ChIP).[172,173] In a typical ChIP assay, the DNA-protein interaction is cross-linked by formaldehyde in situ to fix the interaction, although this step can be omitted when analyzing histone-DNA interactions (referred to as a native-ChIP). The DNA is then fragmented into approximately 500–base pair stretches by sonication or enzymatic digestion. The cross-linked transcription factor is used as an epitope to immunoprecipitate the complex. Antibodies for this purpose are often prequalified by commercial suppliers because they must be of very high quality. The cross-linking in the isolated complex is reversed wherein the DNA sequence of the chromatin fragment is identified by direct sequencing (ChIP-seq), a PCR-based method (ChIP-display),[174] or most commonly, hybridization to a tiling array (ChIP-on-chip or ChIP-chip) for genome-wide detection. Tiling arrays are a relatively recent variation of microarrays with many design considerations that contain short (≈25 base pairs) oligonucleotides, or tiles, of non-repetitive regions of genomic sequences that are arrayed linearly (i.e., contiguous sequence end to end or separated by five nucleotides) or with a fractional offset (i.e., overlapping genomic sequence tiles) for higher resolution studies.[175] One important adjunctive function of ChIP-chip studies is the ability to establish the presence of the possible epigenetic effects of histone modifications and, by extension, nongermline DNA methylation.[176-178] It is important to note that these descriptive studies of transcription factor occupancy alone do not indicate the efficacy of the interaction on transcription. However, when integrated with mRNA expression profiling, it is possible to identify functional regulatory network motifs, which is the aim of the ENCODE (Encyclopedia of DNA Elements) Project.[179]

This type of analysis, often enhanced by expression profile comparisons with genetic methods (Box 3-4) that create overexpression or null mutation phenotypes of the transcription factors that bind to the promoter loci, are more likely to reveal the presence of multitiered regulatory networks. A good example of this is maintenance of a human embryonic stem (ES) cell phenotype. ES cells maintain their pluripotency and ability to self-renew by maintaining a feedforward transcriptional regulatory network

Box 3-4 Genetic Tools for Modifying Gene Function

RNA INTERFERENCE

The processing of hairpin microRNA (miRNA) or plasmid-derived short-hairpin RNA by the enzymes Drosha and Dicer leads to the generation of small interfering RNA (siRNA)—short, double-stranded 21– to 23–base pair RNA with symmetrical 2- to 3-nucleotide 3′ overhangs. Synthetic siRNA gains functional activity by an endogenous kinase that modifies the 5′ hydroxyl groups to phosphate groups. These RNA duplexes are recruited into the RISC complex, where they are guided to endogenous mRNA. On base pairing, the transcript is translationally silenced or cleaved by the catalytic component of the RISC. Although commonly used in cell culture models to reduce gene expression, it has been applied in embryonic stem cells to inactivate genes in a heritable fashion, but without the complete functional reduction in gene expression.

INSERTIONAL MUTAGENESIS

In contrast to chemical mutagenesis, insertional mutagenesis is a transposon-based technique for generating gene disruptions by inserting a molecular tag randomly[180] or, more recently, by using targeted methods that combine the transposon with a DNA-binding domain.[181] In the randomized version, mapping the site of insertion is required to determine where it occurred and whether the insertion site will generate any dysfunction and, if so, the severity of dysfunction in protein activity.

HOMOLOGOUS RECOMBINATION

This most precise and elegant method for altering gene function requires a DNA construct to align with the targeted gene of interest, by mechanisms still poorly understood but probably similar to the alignment of homologous chromosomes during meiosis and mitosis (Fig. 3-5). The recombination event, which is most efficient in yeast and mice but much less common than random insertion events, takes place anywhere in the flanking homologous sequence. The DNA construct contains both a positive (i.e., neomycin) and a negative (i.e., thymidine kinase gene) selection marker to select for homologous recombination events and against nonhomologous recombination, respectively. The neomycin selectable marker by itself, in traditional knockout strategies, causes a significant disturbance in gene function when introduced into an intron. Over the past decade, site-specific recombinase (SSR) systems have allowed geneticists to conditionally express or silence targeted genes, which can be exogenously engineered transgenes encoding reporter, sensor, or effector molecules.[91,182] In approximately 15% of conventional transgenics, embryonic lethality is an issue. The basic concept of conditional transgenes evolved from this obstacle. The most commonly used SSRs are Cre (causes recombination of the bacteriophage P1 genome) and Flp (named for its ability in *Caenorhabditis elegans* to invert a gene). Three versions of Flp are currently in use (e.g., enhanced Flp, Flp-wt, and low-activity Flp) and have a dynamic range of activity across them of more than 1 order of magnitude. Each of the SSRs catalyze recombination events at specific DNA target motifs built into the DNA construct before homologous recombination. For Cre, that site is *loxP*. The cognate site for wild-type Flp or any of its variants is *FRT*. These SSRs possess a combination of fortuitous characteristics: neither of their DNA target motifs are found naturally in mice, and they catalyze the recombination between these target sequences with efficiency and reliability and do so without the need for any additional cofactors. In traditional gene-targeting deletions, the neomycin marker can interfere with the phenotype by influencing the expression of nearby genes.[183] Removal of the selectable marker is one of the most obvious applications of the SSR system and requires only inserting *loxP* sites flanking the neomycin cassette.[184,185] Conditional transgenesis can remove or repair the gene of interest. In the former, a single SSR strategy has *loxP* sites that remove both the neomycin selectable marker and the exon to be deleted. Partial Cre excision occurs by transient expression of the recombinase in recombinant cells after selection, thereby leaving a conditional null allele. The same situation can be accomplished by using both the Cre and Flp recombinases in tandem. For repairing gene functions, we show two hypothetical approaches. The relative strength of perturbing gene function with a neomycin cassette can be enhanced significantly when the cassette is oriented in the reverse direction of the target gene. Excision of the reverse-orientation neo^r marker is accomplished by flanking *loxP* sites. An alternative tactic uses a synthetic stop sequence and a positive selection marker placed between the 5′ untranslated region and the start codon[186] with dual SSRs.

that requires the OCT4-SOX2 complex to autoregulate its own expression, as well as initiate expression of NANOG.[187,188] These transcription factors interact to maintain ES cells in the undifferentiated state by repressing the activation of a host of other transcription factors, including key homeodomain proteins, while activating the transcription of another set of transcription factors, including REST, SKIL, and STAT3.[189]

Although these types of analysis were most easily performed with microarray-based signature profiles in model systems such as yeasts early on,[190-192] these methods have gained traction in mammalian models for identifying transcriptional regulatory networks in dopaminergic neurons of the midbrain[193] and in ES cells of neural[194,195] and hematopoietic[196] origin. Additionally, a recent publication has illustrated the power of next-generation RNA-Seq technology when applied to this analysis.[197]

Some epigenetic modifications (i.e., genomic imprinting) or other genetic variations, such as SNPs, that are potential sources of variation in transcript abundance are not likely to be accounted for. Because sequencing results of the human genome estimate that SNPs are the most prevalent class of common genetic variations (i.e., variants with a minor allele frequency of >1%), they may require additional consideration. Although the vast majority of these genetic variants introduce silent mutations and neutral phenotypes, there has been much focus on determining the relative ratio of neutral, near-neutral,[198] and non-neutral SNPs within populations of different ancestry.[199-201] These genetic polymorphisms are naturally occurring, evolutionarily stable differences thought to confer a predisposition, susceptibility, or resistance to disease and influence individual responses to curative regimens, perhaps by altering the three-dimensional local DNA topography.[202]

There are a number of highly paralleled methods for assaying SNPs across the genome.[203,204] Both the mass spectrometry (MS)-based assay and fluorescence polarization–based assay are allele-specific primer extension methods in which the genomic region is amplified by PCR and used as a template for the annealing of an oligonucleotide primer immediately upstream of the polymorphism. A DNA polymerase then adds only a single nucleotide, because chain-terminating dideoxynucleotide triphosphates (ddNTPs) are used, as dictated by the target DNA sequence at the polymorphic site. Multiplex MS versions rely on the natural differences in molecular weight of the DNA for detection by

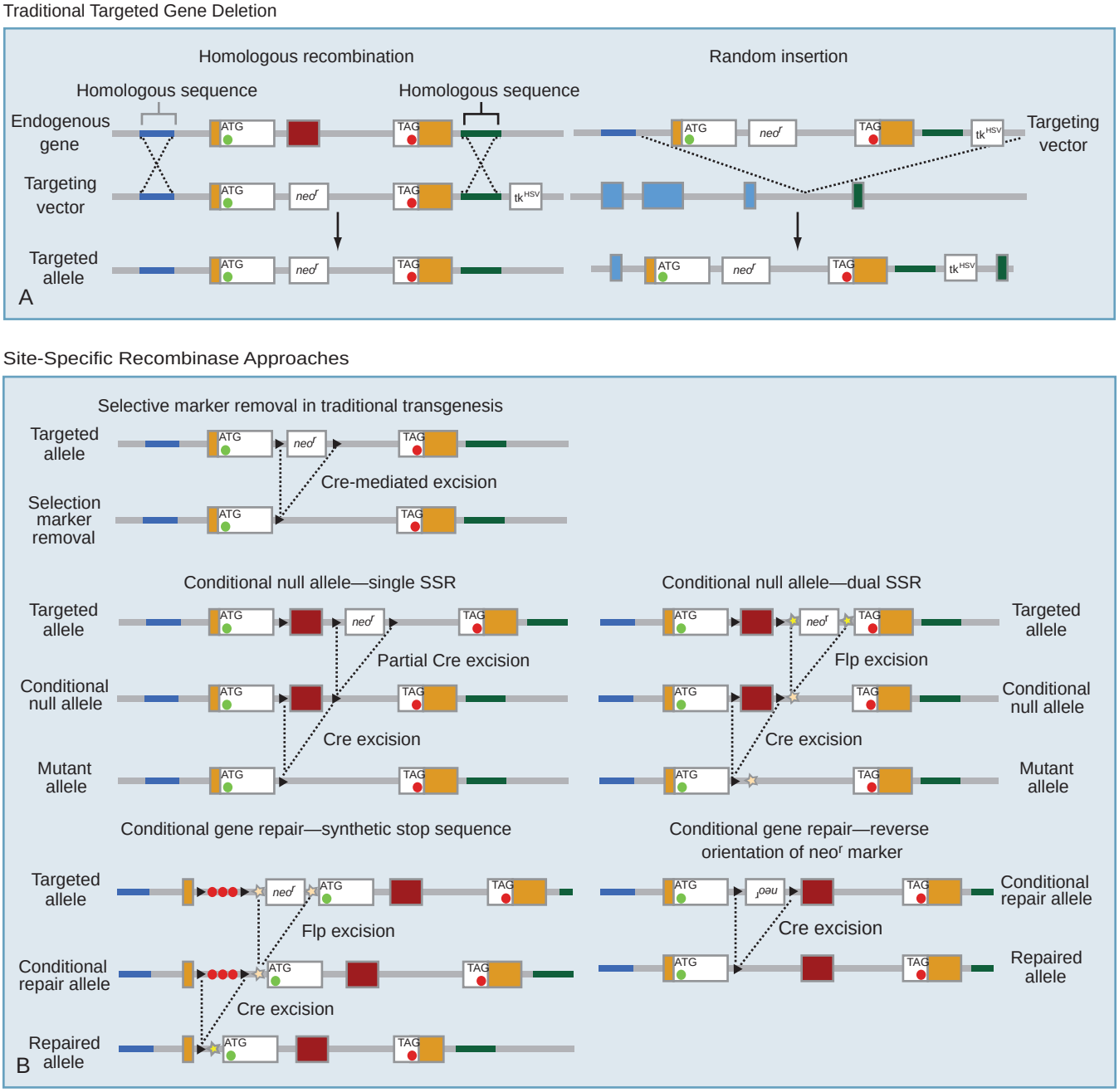


FIGURE 3-5 Mechanism of homologous recombination and the utility of site-specific recombinase approaches. **A,** Homologous recombination uses the presence of both a positive (neomycin [neo^r]) and a negative (herpes simplex virus thymidine kinase gene [tk^{HSV}]) selection marker to identify recombinant cells. Recombinant cells in which one allele is disrupted are conferred resistance to G-418 as a result of the neo^r marker. Unlike its mouse counterpart, tk^{HSV} can convert the nucleotide analogue ganciclovir. Nonhomologous insertions will include the tk^{HSV} gene, thus making only these cells sensitive to ganciclovir. Untranslated regions are represented in *orange boxes* before the start codon (ATG) or after the termination codon (TAG). An intervening exon is denoted in *red* and targeted for replacement with the neo^r cassette. **B,** Various site-specific recombinase strategies are shown starting with the structure of the targeted allele after a homologous recombination event. These strategies entail removing the selection marker cassette used during normal knockout transgenesis, as well as genetic manipulation to allow the conditional disruption or repair of gene function. *Arrowheads* represent *loxP* sites, whereas *stars* represent *FRT* sites.

matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) MS for efficiently assigning genotype.[205] In the fluorescence polarization–based version,[206] the ddNTPs are labeled with different fluorophores. For detection, the labeled ddNTP is incorporated into the primer, which causes it to rotate more slowly within the plane of laser polarization and thereby emit more signal than the unincorporated ddNTPs, which rotate more quickly. A more robust fluorescence polarization assay uses PCR with a universal fluorescence resonance energy transfer (FRET) reporter system for detection of SNPs.[207] An alternative set of highly multiplexed SNP assays incorporate universal PCR. One example of this is the molecular inversion probe assay,[208] in which a single oligonucleotide simultaneously binds a complementary genomic DNA sequence that flanks either side of the

SNP. The single base being flanked is filled in with a DNA polymerase and ligated to circularize the oligonucleotide. The circularized molecular inversion probe is isolated from the linear, nonbinding probes and amplified. The highly paralleled, multiplexing capability comes from a unique bar code in each set of SNP-specific oligonucleotides that can be detected on a high-density array containing sequence complementary to the bar code tags. In contrast, the GoldenGate assay uses allele-specific primer extension through the SNP to which it can be ligated to a second oligonucleotide (specific only for the locus and not the polymorphism), amplified, and detected with bar code tags.[209] Genome-wide studies of SNP analysis have also used SNP arrays, which provide relatively comprehensive coverage (≈80%) of the genome currently mapped. Apart from the previously discussed sources of limitations for any array-based platform, it was originally thought that SNP array artifacts may arise from an additional bias. Detailed position mapping of SNP sites suggest that they are enriched in the 250–base pair sequences upstream of transcription start sites. Because of the close proximity of SNPs to each other at this loci, it was estimated that approximately15% of the microarray probes for any given gene will overlap with SNPs that are polymorphic in the population study. However, in a large-scale human study, no such systemic artifacts arose.[210] Studies integrating the simultaneous effect of genome-wide DNA polymorphisms and global effects on gene expression suggest that transcript abundance can be a quantitative trait that can be mapped.[211]

Proteomics

Complementing global gene expression analysis with studies examining the intrinsic variations in global protein expression, state of posttranslational modification, and subcellular localization within the functionally important networks with which they interact is the focus of high-throughput proteomics techniques. By definition, the proteome is the expression of the entire complement of proteins in a cell, tissue, or organism produced from a particular genome at a single static point in time.

Highly paralleled analysis of protein expression and abundance is the most widely featured form of analysis (Table 3-1). Some forms approach the experimental design from a so-called top-down approach in which naturally occurring proteins can be analyzed. The most cost-effective form of these techniques is conventional 2D gel electrophoresis.[213,245] Determining protein levels by 2D gel electrophoresis requires separating a protein lysate by isoelectric point in the first dimension followed by SDS-PAGE in the second. Comparison of Coomassie brilliant blue, Sypro Ruby, or silver-stained gels differentially exposes expressed spots that can be excised and enzymatically digested from the preparation after identification of the peptides by MS. Peptide mass mapping by MALDI-TOF MS and peptide sequencing by electron spray ionization MS are highly efficient at identifying gel-separated proteins. However, various technical issues, such as labor-intensive image analysis for gel matching, bias in protein representation, and small dynamic range of resolution (≈1 order of magnitude), are responsible for the high coefficient of variation (20% to 30%) that has limited its wider use.[246] Using methods with sufficient dynamic range in proteomics is important because the rather modest differences observed in the changes in mRNA abundance in microarray studies are in sharp contrast to the range of protein expression (≈5 to 8 orders of magnitude).

A multiplexing fluorescent 2D difference in gel electrophoresis (2D-DIGE)[247] method directly labels lysine groups of proteins with mass- and charge-matched cyanine (Cy) dyes before resolving them in the first isoelectric dimension. Up to three samples,[248,249] each with a spectrally separable Cy dye, can be run on the same 2D gel and optically overlayed, thereby providing a significant increase in confidence during spot matching among samples. Addition of the Cy dye adds approximately 500 Da to the labeled protein, but it is also the basis for the large dynamic range (≈4 orders of magnitude) of 2D-DIGE[250,251] and its ability to detect relatively small changes in protein expression.[248] Titrating the Cy dye labeling limits the tagging to one lysine on each protein and prevents precipitation of the protein because of its increased hydrophobicity and visualization artifacts associated with the added mass of each additional fluorophore. As with standard 2D gels, hydrophobic membrane proteins are underrepresented in the sample despite the use of various chaotropic agents.[212,252] Similarly, proteins with high molecular weight are difficult to resolve in the first dimension, low-abundance proteins (<1000 copies per cell) are not detected,[253] and spots may contain more than one protein.

Current global biomarker discovery strategies attempt to generate differential maps of small peptide markers (<30 kD) that can correctly classify masked serum or cerebrospinal fluid (CSF) samples to a specific disease. To do so, most of these recent studies have relied on surface-enhanced laser desorption/ionization (SELDI) MS.[254] A sample of serum or CSF is directly applied to the surface of a gold-plated chip with a modified chromatographic matrix (e.g., weak positive ion exchange [CM10], metal binding surface [IMAC30], or strong anion exchange [Q10]) that will bind only certain sets of proteins within the serum or CSF. With the use of TOF MS, patterns of peptide biomarkers in ovarian,[255] breast,[214] and prostate cancer[256] have shown promising correlative strength.

In contrast to this trio of techniques, bottom-up proteomic methods consistently use enzymatic digestion to generate peptide fragments as the primary input for mass measurements by MS coupled with efficient multidimensional liquid chromatographic separation. Isotope-coded affinity tags (ICAT) are the most popular method for quantifying the relative expression levels of individual proteins.[257] By specifically labeling cysteine residues with a reagent that contains nine ^{12}C (light) or nine ^{13}C (heavy) atoms[258] and a biotin tag, samples of control proteins derivatized with the [^{12}C]-ICAT reagent or experimental proteins with the [^{13}C]-ICAT reagent are combined, digested with trypsin, and separated on an avidin column. All the cysteine-containing peptides tagged with biotin are selectively separated on an avidin column, subjected to reverse-phase chromatography, and identified by liquid chromatography–MS or MS. The selective enrichment of cysteine-containing peptides, which are thought to constitute 10% to 20% of the peptides from a whole cell extract,[217,259] does significantly decrease the complexity of the peptide mixture. Quantifying the $^{12}C/^{13}C$ ratio provides a relative, not absolute expression ratio of individual proteins within the sample set. A variation of ICAT uses a combination of four isobaric labels that can label multiple lysine-containing peptides per protein. This feature of the isobaric tag for relative and absolute quantitation (iTRAQ)[260] has the effect of increasing the confidence interval of identification and quantitation. iTRAQ-labeled peptides are normally separated by reverse-phase chromatography, but OFFGEL fractionation has also been used as another option.[216] A recent comparison of ICAT and iTRAQ with 2D-DIGE supports the notion that the global-tagging approach of iTRAQ is more sensitive than either ICAT or 2D-DIGE,[215] although all are likely to have dynamic ranges of resolution of at least 4 orders of magnitude. A more important point elucidated in this study was the limited overlap of proteins characterized by each of the techniques, thus suggesting the complementary quality of their data sets.

A third MS-based assay, multidimensional protein identification technology (MudPit),[261] separates complex peptide mixtures in two dimensions, as opposed to 2D gels, which fractionate in two dimensions. Peptide mixtures are separated in the first dimension on the basis of electrostatic charge and eluted by using

TABLE 3-1 Proteomics Methodologies and Detection of Posttranslational Modifications*

TECHNIQUE	LABELING METHOD	IN VITRO/IN VIVO	AMINO ACIDS LABELED	MULTIPLEXING	POSTTRANSLATIONAL DETECTION MODIFICATION	REFERENCES
BASIC OVERVIEW OF SELECTED PROTEOMICS METHODOLOGIES						
2D gel electrophoresis	Silver stain/Sypro Ruby	N/A	N/A	Possible with other gel stains	Yes	210, 211
2D-DIGE	Cy2, Cy3, or Cy5 dyes	In vitro	Primary amines	Yes	Yes	213
SELDI	None	None	None	No	Yes	212
ICAT	[^{12}C]- and [^{13}C]- ICAT reagent	In vitro	Cysteine	Yes	Yes	214
iTRAQ	Isobaric reagents 114, 115, 116, and 117	In vitro	Lysine	Yes	Yes	217
MudPit	[^{14}N] and [^{15}N]	In vitro	Nitrogen	Yes	Yes	216
SILAC	[^{12}C] and [^{13}C], [^{14}N] and [^{15}N]	In vivo	Lysine/arginine	Yes	Yes	215

REAGENT—DIRECT TOOLS	POSTTRANSLATIONAL MODIFICATION	DETECTION METHOD	REFERENCES
TOOLS AND ENRICHMENT METHODOLOGIES FOR POSTTRANSLATIONAL MODIFICATION DETECTION			
Pro-Diamond Q Stain	Phosphorylation	PAGE	220, 221
Pro-Q Emerald 300 Stain	Glycosylation	PAGE	218, 219
REAGENT—SELECTIVE ENRICHMENT			
Immobilized metal affinity chromatography (IMAC)	Nitration	2D-DIGE followed by MS	228-230
Strong cation exchange (SCX)	Phosphorylation	MS	226, 227
Context-independent motif Abs	Phosphorylation	MS or microarray	
Antiphosphotyrosine Abs	Phosphorylation	MS	222-225
Antiphosphoserine/antithreonine Abs	Phosphorylation	MS or microarray	235-238
Anti–3-nitrotyrosine Abs	Phosphorylation	MS or microarray	231-234
Anti-monomethyl and anti-dimethyl arginine Abs	Methylation	MS	244
Phosphodependent binding domains	Glycosylation	MS	239
Lectin-affinity protein-specific phosphospecific Abs	Phosphorylation	Microarray	241
Lectin-affinity	Glycosylation	MS	244
Nitrotyrosine chemical labeling	Nitration	MS	242, 243

*A generalized overview of each of the major proteomic techniques is discussed here, as well as the current approaches used to detect several types of posttranslational modification, including phosphorylation,[220-230,235-239,241] glycosylation,[218,219,240] methylation,[244] and nitration.[231-234,242,243]
Abs, antibodies; 2D, two-dimensional; DIGE, difference in gel electrophoresis; ICAT, isotope-coded affinity tags; iTRAQ, isobaric tag for relative and absolute quantitation; MS, mass spectrometry; MudPit, multidimensional protein identification technology; N/A, not applicable; PAGE, polyacrylamide gel electrophoresis; SELDI, surface-enhanced laser desorption/ionization; SILAC, stable isotope labeling with amino acids in cell culture.

a step gradient of increasing salt concentrations directly onto a tandemly coupled column that separates on the basis of hydrophobicity. The advantage of this unbiased approach is that it allows integral membrane proteins to be identified among the complex peptide mixture. Typically, these proteins are lost by 2D gel separation.

Stable isotope labeling with amino acids in cell culture (SILAC)[262] is a simple approach for the in vivo labeling of proteins for MS-based detection and quantitation of protein expression and posttranslational modifications.[263] Isotopically labeled amino acids, usually lysine and arginine, are added directly to the growth media, where cells directly incorporate the light (e.g., ^{12}C or ^{14}N) or heavy (e.g., ^{13}C or ^{15}N) label into newly synthesized protein chains that can be prepared for peptide identification and quantitation by MS. SILAC has been coupled with various enrichment techniques to monitor phosphorylation,[264-266] as well as methylation,[244] in protein lysates.

The large data sets that are generated by these techniques do not directly address the state of posttranslational modification. Phosphorylation events are common and affect approximately 30% of all proteins[267] at one time, and they are well-known regulatory switches in neuronal development,[268] synaptic plasticity,[269,270] and neuron-generated biologic rhythms.[271] The current tools for the study of phosphoproteomics place heavy emphasis on the direct use of phospho-specific reagents in array-based platforms or various enrichment techniques to isolate phosphoproteins for subsequent analysis with MS (see Table 3-1). Selective phosphopeptide enrichment can be achieved by chromatographic separation, such as with immobilized metal affinity chromatographic (IMAC) columns[228-230] and antiphosphotyrosine affinity columns[222-225] or phospho-dependent binding domains.[239] As a result of the negative charge of the phosphopeptides and their hydrophilic nature, specialized MS protocols have been used in some circumstances for analysis of phosphorylation

sites.[272,273] The one exception to the dependence on a phosphoprotein enrichment scheme is the fluorescent Pro-Q Diamond stain for direct use in isoelectric focusing gels, SDS-PAGE, and 2D gels for the detection of all types of phosphorylated amino acids,[220] although greater proteome coverage can be achieved when Pro-Q Diamond staining is coupled with liquid chromatography–MS approaches.[274] In ICAT, phosphorylated residues within a cysteine-containing peptide can be detected as a normal consequence of the experiment.[275] Tools for detection or enrichment of other posttranslational modifications, such as glycosylation and nitration, have also been used (see Table 3-1).

The protein expression profiles generated by antibody array–based detection methods have matured considerably in surface designs of solid supports, coupling chemistries, on-chip probe stability, and fabrication.[276] First-generation high-density arrays printing approximately 1000 unique antibodies with current fabrication platforms, with expectations of up to 10,000 in the near future, are beginning to provide an alternative to other proteomic methodologies for generating highly paralleled data in disease biomarker discovery signatures,[277,278] phosphoproteomics,[279,280] and oncoproteomics.[281,282] One distinct advantage of this approach is the minute amounts of sample consumed, usually less than 1 μL, with multispotting techniques potentially providing significant improvement.[283,284] In DNA microarrays, multiple housekeeping genes act as an internal control during the data normalization process. Lacking a comparable standard, a variety of data normalization approaches have been devised for antibody-based arrays.[285] Other complementary microarray formats include binding domain–based arrays, such as phospho-dependent Src homology 2 domains,[239] and reverse-phase protein microarrays,[286] which are the inverse of antibody-based arrays (i.e., cells, serum, or tissue is spotted on a nitrocellulose slide and probed with a single antibody) and have been used to show disease progression in cancer.

CONCLUSION

The molecular biology revolution was initiated by discovery of the structure of DNA and became a full-fledged revolution in the 1970s with the advent of cDNA cloning. This revolution has made it possible to observe changes in, quantify, and manipulate DNA and RNA biology in neurological illnesses. The development of genomics-level high-throughput data acquisition and functional genomics methodologies has now taken us to the next step, that of assessing the association and coregulation of multiple RNA molecules and proteins simultaneously. It is the simultaneous responsiveness of multiple genes and their mRNA and protein products that underlies all of normal and abnormal cellular functioning. This idea, coupled with the development of technology, has heralded the age of genomics-informed medicine.

It has been more than a century since Ramon y Cajal commented in his Nobel Prize lecture that "nature seems unaware of our intellectual need for convenience and unity, and very often takes delight in complication and diversity."

It seems that we are now on the precipice of truly starting to understand the workings of cells, and with this knowledge the hope for better therapeutic development and intervention strategies is becoming manifest.

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CHAPTER 4

Neuroembryology

Harvey B. Sarnat ■ Laura Flores-Sarnat ■ Joseph D. Pinter

Classic neuroembryology dealt primarily with documentation of the timing and location of morphologic changes in embryonic development, both gross and microscopic, but an acceleration of genetic, molecular, and radiologic (i.e., neuroimaging) advances in recent years has fundamentally changed the way that we perceive and understand both normal formation and malformation of the brain. Completion of the first draft of the sequence of the human genome will have profound implications on our understanding of the genetics of brain development.[1-4] An accelerating pace of genetic discovery is accompanied by the risk that reviews become obsolete before their publication. We now speak of genetics and genomics, but since the human genome project met its initial goals, we will necessarily move onto proteomics (i.e., study of all proteins expressed by the genome)[5] and an ever-growing list of "omics" as we delve deeper into the complex molecular genetic interactions required for creating the brain.

Although we do not dispute the critical role of these advances in elucidating development, we strongly believe that this information is best used within the framework of a solid understanding of the timing of structural changes during brain development. In this chapter we offer a unified vision of neuroembryology, with molecular genetic details presented along with classic structural information. It is beyond the scope of this chapter to review the principles of genetics or the revolutionary techniques of gene cloning, polymerase chain reaction, and positional cloning or to attempt to catalog the genes responsible for neurological abnormalities.[6] More information is available in review articles or book chapters on neurogenetics.[7,8] We do provide examples of important genes relevant to neurodevelopment and extensive references that can aid in understanding basic concepts.

Because much of our knowledge about human brain development has resulted from searching for human homologues to developmental genes discovered in animals from *Drosophila* to mice, we include examples from these studies along with our discussions of the stages, processes, and abnormalities of human neuroembryology. Table 4-1 lists known gene mutations responsible for several important human central nervous system (CNS) malformations.[9-44] When specifically referring to a human gene, we use the convention of denoting the gene symbol in italicized, capital letters.

We once thought of brain insults arising from either environmental or genetic factors, but we now recognize that these causes are interconnected and inseparable. Environmental factors act by influencing gene regulation and expression, and genetic differences determine responses to environmental agents, including toxins and transcription factors. The molecular details of how thousands of genes and the proteins that they encode work together to determine normal or abnormal structure and function are becoming clearer daily but are still overwhelmingly complex. Although the estimated number of genes in the human genome is only about 30,000 (far less than the previously estimated 100,000 and only 2.5 times larger than the fly genome), the human proteome is estimated to contain between 130,000 and 400,000 distinct proteins. Each has many potential ways of interacting with other proteins or genes and of being posttranslationally modified.[4]

Improvements in neuroradiologic techniques are helping to uncover relationships between genetic abnormalities and structural malformations and to provide another justification for learning more about the basics of embryologic and fetal development. As imaging has improved, so has our appreciation that many developmental disorders represent a spectrum of abnormalities much more complex than previously appreciated. The quality of the image and the speed of acquisition of fetal magnetic resonance imaging (MRI) are constantly improving, and it is already capable of very good anatomic definition of fetal brain malformations.[45] The next major step, which will almost certainly occur in the near future, will be the development of MRI techniques for evaluating functional gene expression. In combination with the other molecular genetic advances described, improved imaging will provide an unprecedented opportunity to understand brain development and its disorders.

The basic details that we provide are meant to be an introduction to the concepts that we believe are fundamental for a broad understanding of normal and abnormal neurodevelopment. Because of space limitations, we have focused on a small number of brain malformations and chosen to review certain developmental processes but not others. We hope this overview will serve as a useful starting point for exploration of these important ideas.

CLASSIC NEUROEMBRYOLOGY

The technical meaning of the term *embryology* should restrict the study of development to the first 6 postconceptional weeks in humans, the embryonic period proper, but traditional use extends the term to include fetal life until birth, and this is the application that we use in this chapter. Although nonhuman embryologic studies have long been important to our understanding of brain development, meticulous descriptive morphogenic studies of serially sectioned human embryos over the past several decades have provided unique and invaluable information. Based on studies of internal and external morphology that originated from the Carnegie collection of embryos, the 8 weeks of embryonic development have been subdivided into 23 morphologic (Carnegie) stages.[46] Stages 8 to 23 are relevant to neuroembryology, with the neural groove and folds first appearing in stage 8, which occurs at about 23 days' gestation. At this time, the embryo is only about 1 mm long. No accepted morphologic staging system has been developed for the fetal period. These studies remain valuable in using known milestones in structural development to identify the *termination period*, or the gestational day beyond which the onset of a specific malformation could not have occurred. Knowledge of the exact times when defects such as anencephaly or meningomyelocele may occur is critical for molecular genetic and epidemiologic investigations and can provide important clues to pathophysiologic mechanisms. Further detail can be obtained from O'Rahilly and Muller's updated classic atlas of developmental stages.[46] From careful studies such as these, the adult derivatives of embryonic structures were determined long before we began to understand the signals and processing underlying their formation (Fig. 4-1).

TABLE 4-1 Known Gene Mutations Causing Human Central Nervous System Malformations

MALFORMATION	INHERITANCE	CHROMOSOMAL LOCATION	GENE OR TRANSCRIPTION PRODUCT	REFERENCES
Cerebrohepatorenal syndrome (Zellweger's)*	AR	Xq22.3-q23	*DCX*	9
Hemimegalencephaly	AR	Xq28	*L1CAM*	10
Holoprosencephaly†	AD; AR	7q36-qter	*SHH*	11-13
Holoprosencephaly	AR; sporadic	13q32	*ZIC2*	14
Holoprosencephaly	AR; sporadic	2q21	*SIX3*	15
Holoprosencephaly	AD; sporadic	18p11.3	*TGIF*	16
Kallmann's syndrome	XR	Xp22.3	*KAL1*	17, 18
Lissencephaly type 1 (isolated and Miller-Dieker syndrome)	AR	17p13.3	*LIS1*	19-21
Lissencephaly (Fukuyama's congenital muscular dystrophy)	AR	9q31	*FCMD*, fukutin	22
Lissencephaly with cerebellar hypoplasia	AR	7q22	*RELN*	23
Midbrain agenesis and cerebellar hypoplasia	?AR; sporadic	7q36	*EN2*	24
Periventricular heterotopia	XD	Xq28	*FLNA*, filamin	25, 26
Rett's syndrome	XD	Xq28	*MECP2*	27
Sacral agenesis‡	AD	7q36.1-qter	*SHH*	28-30
Schizencephaly	AR	10q26.1	*EMX2*	31
Septo-optic pituitary dysplasia	AR; sporadic	3p21.1-p21.2	*HESX1*	32
Subcortical laminar heterotopia (band heterotopia; double cortex)	XD	Xq22.3-q23	*DCX*	33-35
Tuberous sclerosis	AD	9q34.3	*TSC1*, hamartin	36-38
		16p13.3	*TSC2*, tuberin	39-41
X-linked hydrocephalus (X-linked aqueductal stenosis and pachygyria)	XR	Xq28	*L1CAM*	42-44

*The *DCX* (doublecortin) mutation is primary in subcortical laminar heterotopia but is also described in Zellweger's syndrome, although it is probably only a secondary defect in this lysosomal disease associated with major neuroblast migratory defects; *DCX* is localized on the X chromosome, and Zellweger's syndrome is an autosomal recessive condition.
†Holoprosencephaly is associated with many chromosomal defects in addition to those listed, but the gene products associated with the others have not been identified.
‡Sacral agenesis (autosomal dominant form) maps to the same locus at 7q36 as one form of holoprosencephaly and is associated with defective *SHH* expression, the same genetic defect expressed at opposite ends of the neural tube. Sacral agenesis and holoprosencephaly also occur with a high incidence in infants born to mothers with diabetes mellitus. Agenesis of more than two vertebral bodies is generally associated with dysplasia of the spinal cord in that region during fetal development, fusion of the ventral horns, and a deformed central canal with heterotopic ependyma, consistent with defective neural induction. A second gene with a locus at 1q41-q42.1 has been identified as another cause of autosomal dominantly transmitted sacral agenesis.
AD, autosomal dominant; AR, autosomal recessive; CAM, cell adhesion molecule; SHH, Sonic Hedgehog; XD, X-linked dominant; XR, X-linked recessive.
Adapted from Sarnat HB. Central nervous system malformations; locations of known human mutations. *Eur J Paediatr Neurol.* 2000;4:289-290.

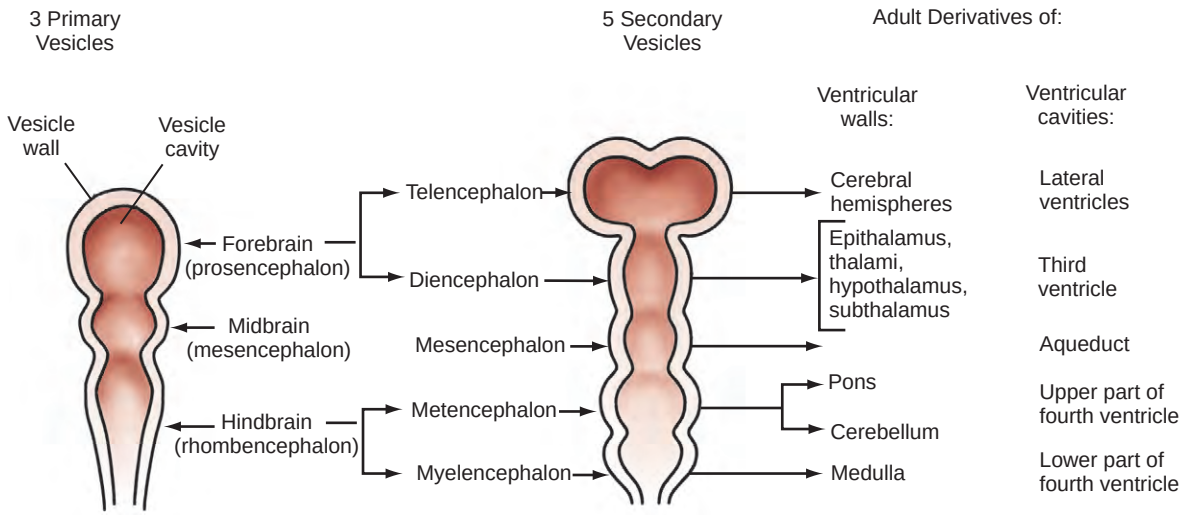

FIGURE 4-1 Embryonic vesicles and their adult derivatives are shown schematically in the progression from three primary vesicles (i.e., neuromeres) during the fourth week of gestation (just after neural tube formation) to five secondary vesicles in the fifth week. (From Jinkins JR. *Atlas of Neuroradiologic Embryology, Anatomy, and Variants.* Philadelphia: Lippincott Williams & Wilkins; 2000:9.)

Along with careful work over the past several decades that resulted in this embryonic staging system, similar analysis of the development of the cerebral vasculature led to a staging system usually referred to as *Padgett stages*.[47-49] Knowledge of how the vascular system evolves leads to a clearer understanding of vascular malformations and common vascular anomalies (covered elsewhere in this book) and the patterns of secondary embryonic and fetal brain injuries and malformations. The arterial system essentially achieves an adult pattern by the end of the embryonic period, whereas the venous system develops much later in the fetal period. Although neither the seven stages of arterial evolution developed by Padgett (and still used) nor cerebral venous development will be considered further here, more information can be found in any of several excellent reviews of vascular development.[50-53]

DEVELOPMENTAL ORGANIZATION: STAGES, GENES, AND REGULATORY FACTORS

Gastrulation

Gastrulation is the birthday of the nervous system. It is not only the time that bilateral symmetry and the three axes are established in the body of all vertebrates but also the time when a neuroepithelium can first be identified and distinguished from primitive germinal tissues. The traditional concept of three germ layers dates from gastrulation as well, but the convenient conception of all mature tissues having been derived from one of the three layers is probably more arbitrary than biologic because the neural crest forms tissues assigned to all three germinal layers and the expression of many families of genes does not respect these germinal boundaries and mediates the development of structures corresponding to all three.

In simple chordates, such as *Amphioxus* and amphibians, gastrulation is the invagination of a spherical blastula. In birds and mammals, the blastula is collapsed as a flattened, bilayered disk, and gastrulation appears not as an invagination but as a groove between two ridges on one surface of this disk, called the *primitive streak* on the *epiblast*. In each embryo, the primitive streak establishes the basic body plan of all vertebrates: a midline axis, bilateral symmetry, rostral and caudal ends, and dorsal and ventral surfaces.

As the primitive streak extends forward, cells aggregate at one end, a collection designated the *primitive node* or *Hensen's node*. Hensen's node defines rostral. Cells of the epiblast on either side move toward the primitive streak, stream through it, and emerge beneath it to pass into the narrow cavity between the two sheets of cells, with the epiblast above and the hypoblast below; these migratory cells give rise to the mesoderm and endoderm internally, and some then replace the hypoblast.[54]

After extending about halfway across the blastoderm (epiblast), the primitive streak with Hensen's node reverses the direction of its growth and retreats, moving posteriorly as the head fold and neural plate form anterior to Hensen's node. As the node regresses, a *notochordal process* develops in the area rostral to it, and somites begin to form on either side of the notochord, with the more caudal somites differentiating first and successive ones differentiating anterior to the somites already formed. The notochord induces epiblast cells to form neuroectoderm (see "Induction"). Several genes essential in creating the fundamental architecture of the embryo and its nervous system are already expressed in the primitive node,[55] and many reappear later to influence more advanced stages of ontogenesis.

Induction

Induction refers to the influence of one embryonic tissue on another such that both the inducer and the induced differentiate as different mature tissues. In the case of the nervous system, neural tube development may be defined in terms of gradients of inductive influences. Induction usually occurs between germ layers, as with the notochord (mesoderm) inducing the floor plate of the neural tube (ectoderm), although induction also may occur within a single germ layer. An example is the optic cup (neuroectoderm) inducing the formation of a lens and cornea from the overlying epithelium (surface ectoderm) that otherwise would have differentiated as more epidermis. *Neural induction* is the differentiation or maturation of neural structures from undifferentiated ectodermal cells as a result of the influence of surrounding embryonic tissues.

Induction was discovered in 1924, when Hans Spemann and Hilde Mangold demonstrated that the dorsal lip of the newt gastrula was capable of inducing the formation of an ectopic second nervous system when transplanted to another site in a host embryo, into another individual of the same species, or to a ventral site of the same embryo.[56] This *dorsal lip* of the amphibian gastrula, also called the *Spemann organizer*, is homologous with the *Hensen node* of embryonic birds and mammals.

The first gene isolated from the Spemann organizer was *Gsc* (goosecoid), which encodes a *homeodomain* protein (see the later section "Transcription Factors and Homeoboxes") able to recapitulate transplantation of the dorsal lip tissue when injected into an ectopic site. It also normally induces the prechordal mesoderm and contributes to prosencephalic differentiation.[55,57,58] In Hensen's node in the chick, even before the primitive streak is fully formed, *Wnt8c* is expressed and is essential for the regulation of axis formation and later for hindbrain patterning in the region of the future rhombomere 4 (r4).[59] The regulatory gene *Cnot*, with major domains in the primitive node, notochord, and prenodal and postnodal neural plate, is also involved in the induction of prechordal mesoderm and in formation of the notochord in particular.[60]

The specificity of induction lies not in the inductive molecule but rather in the receptor in the induced cell. This distinction is important because foreign molecules similar in structure to the natural inductor molecule may sometimes be erroneously recognized by the receptor as identical; such foreign molecules may act as teratogens if the embryo is exposed to such a toxin. Induction occurs during a very precise temporal window; the period of responsiveness of the induced cell is designated its *competence*, and the cell is incapable of responding before or after that precise time.[61]

Induction receptors are not necessarily in or on the plasma membrane of the cell but may be in the cytoplasm or in the nucleus. Retinoic acid is an example of a nuclear inducer. In some cases, the stimulus acts exclusively at the plasma membrane of target cells and does not require actual penetration of the cell.[61,62] The receptors that represent the specificity of induction are also genetically programmed. *Notch* is a particularly important gene in regulating the competence of a cell to respond to inductive cues from within the neural tube and from surrounding embryonic tissues.[63] Some mesodermal tissues, such as smooth muscle of the fetal gut, can act as *mitogens* on the neuroepithelium by increasing the rate of cellular proliferation,[64,65] but this phenomenon is not true neural induction because the proliferating cells do not differentiate or mature. Some organizer and regulatory genes of the nervous system, such as *Wnt1*, also exhibit mitogenic effects,[66] and *insulin-like growth factor* and *basic fibroblast growth factor* act as mitogens as well.[67-69]

Early formation of the neural plate is not accomplished exclusively by mitotic proliferation of neuroepithelial cells; surrounding cells are also converted to a neural fate. In amphibians, a gene known as *Xash* (achaete-scute) is expressed very early in the dorsal part of the embryo from the time of gastrulation and acts as a molecular switch to change the fate of undifferentiated cells to become neuroepithelium rather than surface ectodermal or

mesodermal tissues.[70] Some cells differentiate as specific types because they are actively inhibited from differentiating into others. All ectodermal cells are preprogrammed to form neuroepithelium, and neuroepithelial cells are preprogrammed to become neurons if not inhibited by genes that direct them along a different lineage, such as epidermal, glial, or ependymal.[71-73]

The neural tube induces craniofacial development and mediates it through the neural crest, which migrates rostrally from the prosencephalon, at the dorsal part of the lamina terminalis, and from the dorsal midline of the mesencephalon. The prosencephalic neural crest migrates as a vertical sheet of cells in the midline of the future nose and forehead and forms, among other structures, the intercanthal ligament that hold the orbits together so that the eyes are directed forward in the face instead of being located at the sides of the head. This program is genetically determined in some families of mammals, including primates, felines, canines, bears, and koalas, as well as in one family of birds only, the owls. Other animals have laterally placed eyes, which provide better panoramic, but not stereoscopic vision.

Neurulation

Bending of the neural placode to form the neural tube requires extrinsic and intrinsic mechanical forces in addition to dorsalizing and ventralizing genetic influences, which are discussed in detail later in this chapter.

These forces arise in part from growth of the surrounding mesodermal tissues on either side of the neural tube, the future somites (Table 4-2).[74] After surgical removal of mesoderm and endoderm from one side of the neuroepithelium in experimental animals, the neural tube still closes, but it is rotated and becomes asymmetric.[75] The mesoderm appears to be important for orientation but not for closure of the neural tube. Expansion of the surface epithelium of the embryo is the principal extrinsic force for folding of the neuroepithelium to form the neural tube.[76] Cells of the neural placode are mobile and migrate beneath the surface ectoderm, which causes the lateral margins of the placode to become raised toward the dorsal midline. Growth of the whole embryo itself does not appear to be an important factor because neurulation proceeds equally well in anamniotes (e.g., amphibians), which do not grow during this period, and in amniotes (e.g., mammals), which grow rapidly at this time.[77]

Among the intrinsic forces of the neuroepithelium, the cells of the floor plate have a wedge shape—narrow at the apex and broad at the base—that facilitates bending.[78] Although the width of the floor plate is small, its site in the ventral midline is crucial and sufficient to allow a significant influence. It represents yet another aspect of induction of the floor plate by the notochord, apart from its influence on the differentiation of neural cells.[79] The ependymal cells that form the floor plate are the first neural cells to differentiate, and they induce growth of the parenchyma of the ventral zone more than the dorsal regions.[80,81] This mechanical effect may also facilitate curving of the neural placode. The direction of proliferation of new cells in the mitotic cycle, determined in part by the orientation of the mitotic spindle, becomes another mechanical force shaping the neural tube.[77,78] Adhesion molecules are also probably an important mechanical factor for neurulation. In later stages, the ependymal cell–lined central canal, which is much larger in the fetus than in the newborn, may have a role in exerting a centrifugal force to create the tubular shape, although in early spinal cord development the central canal is a tall, narrow, midline slit and only later in fetal life does it assume a rounded contour as seen in transverse sections.[80]

Neuroepithelial cells of the neural placode or plate downregulate the polarity of their plasma membrane so that the apical and basilar surfaces are not as distinct before neural tube closure. Cell differentiation in general involves such changes in cell polarity.[82] The rostrocaudal orientation of most mitotic spindles of the neuroepithelium and the direction in which they push by the mass of daughter cells that they form also influence the shape of the neural tube (Fig. 4-2).[83]

The neural tube closes in the dorsal midline first in the cervical region, with the closure then extending rostrally and caudally such that the anterior neuropore of the human embryo closes at 24 days and the posterior neuropore closes at 28 days, with the distances from the cervical region being unequal. This traditional view of a continuous zipper-like closure is an oversimplification. In the mouse embryo, the neural tube closes in the cranial region at four distinct sites, with the closure proceeding bidirectionally or unidirectionally and in general synchrony with somite formation.[84,85] An intermittent pattern of anterior neural tube closure involving multiple sites has also been described in human embryos.[86] In this closure pattern, the principal rostral neuropore closes bidirectionally[87] to form the lamina terminalis, an essential primordium of the forebrain.[80]

Bending of the neural plate to form the neural tube is termed *primary neurulation*. Failure of the anterior neuropore to close by 24 days results in anencephaly. Because the lamina terminalis does not form, its derivatives (including the basal ganglia and other forebrain structures) do not develop. The lack of forebrain neuroectoderm results in failure of induction of the overlying mesoderm, and the cranium, meninges, and scalp fail to close in the midline.[88] The term *secondary neurulation* refers only to the most caudal part of the spinal cord (i.e., conus medullaris), which develops from neuroepithelium caudal to the site of posterior neuropore closure. More details on abnormalities that occur because of problems with secondary neurulation are offered in other chapters in this textbook.

Neural crest cells arise from the dorsal midline of the neural tube at or shortly after the time of closure and migrate extensively along prescribed routes through the embryo to differentiate as the peripheral nervous system. This includes the dorsal root and sympathetic ganglia, adrenal medulla and carotid body chromaffin cells, melanocytes, and a few other cell types of ectodermal and mesodermal origin.[89,90]

TABLE 4-2 Factors Involved in Closure of Neuroepithelium to Form the Neural Tube

Extrinsic mechanical forces
Surrounding mesodermal tissues
Surface epithelium
Intrinsic mechanical forces
Wedge shape of floor plate cells
Differential growth in the dorsal and ventral zones
Adhesion molecules
Orientation of mitotic spindles of the neuroepithelium
Large fetal central canal
Molecular genetic programming
Induction of the floor plate by Sonic Hedgehog
Ventralizing gene transcription products
Dorsalizing gene transcription products
Genetic transcription products that regulate axonal guidance (attraction and repulsion) across the midline and in the longitudinal axis
Separation of the neural crest

From Menkes JH, Sarnat HB. *Child Neurology*, 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2000:289.

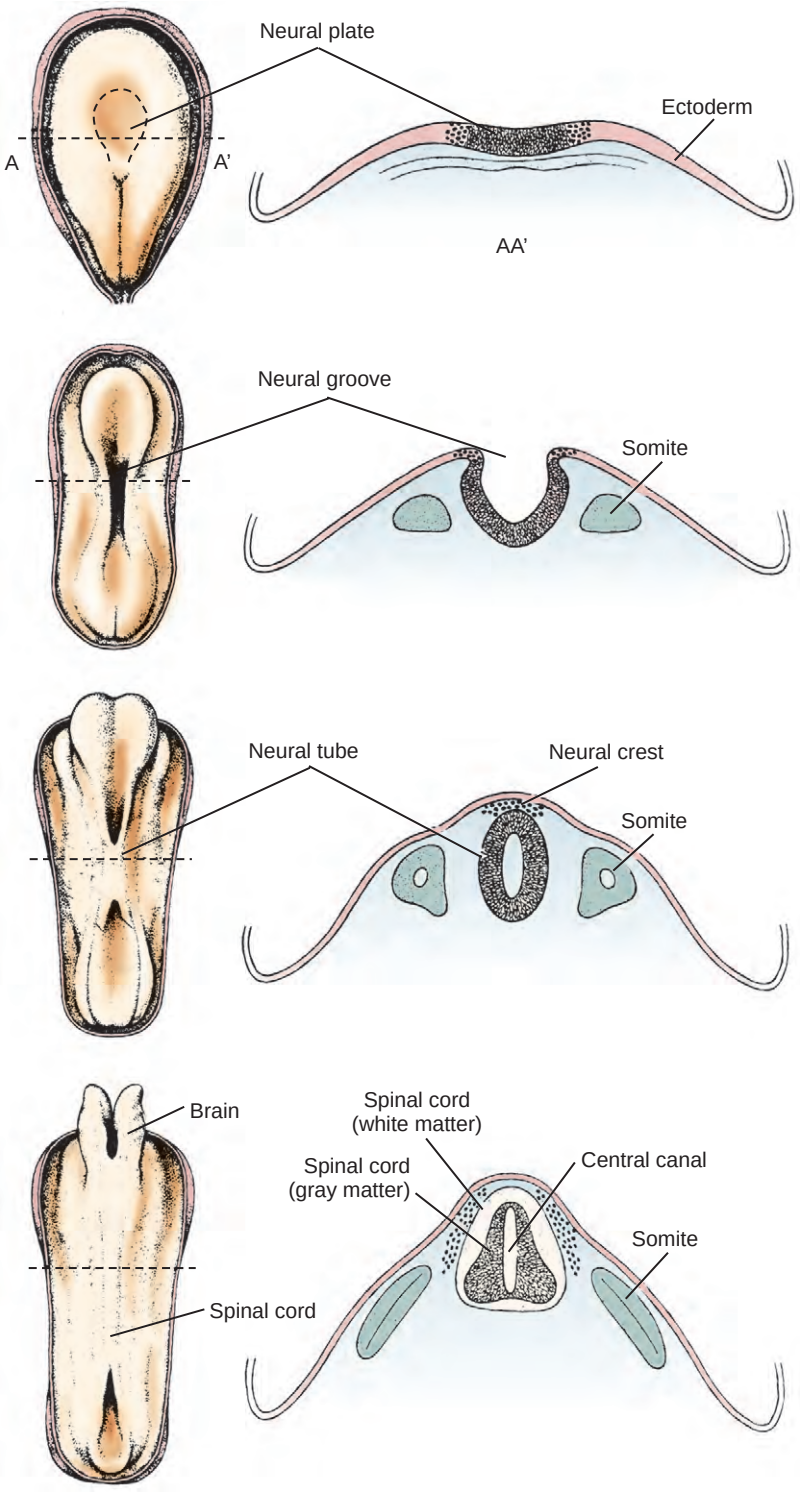

FIGURE 4-2 Primary neurulation: schematic illustration of formation of the neural tube during the third and fourth weeks of gestation. (From Cowan WM. The development of the brain. *Sci Am.* 1979;241:113.)

Segmentation and Regionalization

Segmentation of the neural tube creates intrinsic compartments that restrict the movement of cells by physical and chemical boundaries between adjacent compartments. These embryonic compartments are known as *neuromeres*. The spinal cord has the appearance of a highly segmented structure; however, it is not intrinsically segmented in the embryo, fetus, or adult but rather corresponds in its entirety to the most caudal of the eight neuromeres that create the hindbrain. The apparent segmentation of the spinal cord results from clustering of nerve roots imposed by true segmentation of surrounding tissues derived from the mesoderm, tissues that form the neural arches of the vertebrae, somites, and associated structures. Neuromeres of the hindbrain are designated *rhombomeres*.[91-94] The entire cerebellar cortex, vermis, flocculonodular lobe, and lateral hemispheres develop from rhombomere 1 (r1), with a small contribution to the anterior vermis from the mesencephalic neuromere, but the dentate and other deep cerebellar nuclei are formed in rhombomere 2 (r2).[95,96] The rostral end of the neural tube forms a

mesencephalic neuromere and probably six forebrain neuromeres (i.e., two diencephalic and four telencephalic prosomeres), although these may be subdivided further.[97-99] The segmentation of the human embryonic brain into neuromeres is summarized in Table 4-3.

The segments of the embryonic neural tube are distinguished by physical barriers formed by processes of early specializing cells that resemble the radial glial cells that appear later in development[100,101] and by chemical barriers from secreted molecules that repel migratory cells. Cell adhesion is increased in the boundary zones between rhombomeres, which also contributes to the creation of barriers against cellular migration in the longitudinal axis. Limited mitotic proliferation of the neuroepithelium occurs in the boundary zones between rhombomeres. Although cells still divide in this zone, their nuclei remain near the ventricle during the mitotic cycle and do not move as far centrifugally within the elongated cell cytoplasm during the interkinetic gap phases as they generally do.[101] The rhombomeres of the brainstem may also be visualized as a series of transverse ridges and grooves on the dorsal surface, the future floor of the fourth ventricle; these ridges are gross morphologic markers of the hindbrain compartments.[94,102]

The first evidence of segmentation is a boundary that separates the future mesencephalic neuromere from r1 of the hindbrain. More genes play a role in this initial segmentation of the neural tube than in any boundaries that subsequently form to separate other neuromeres. The mesencephalic-metencephalic region appears to develop early as a single, independent unit or "organizer" for other neuromeres rostral and caudal to that zone.[103,104] The organizer genes recognized at the mesencephalic-metencephalic boundary for this earliest segmentation of the neural tube include *Pax2*, *Wnt1*, *En1*, *En2*, *Pax5*, *Pax8*, *Otx1*, *Otx2*, *Gbx2*, *Nkx2-2*, and *Fgf8*.

The earliest known gene with regional expression in the mouse is *Pax2*, and it is expressed even before the neural plate forms. It is the earliest gene recognized in the presumptive region of the midbrain-hindbrain boundary.[105,106] In invertebrates, *Pax2* is important for the activation of *Wg* (wingless) genes; this relationship is relevant because the first gene definitely associated with an identified midbrain-hindbrain boundary in vertebrates is *Wnt1*, a homologue of *Wg*. Regulation of *Wnt1* may be divided into two phases. In the early phase (1 or 2 somites), the mesencephalon broadly expresses the gene throughout; in the later phase (15 to 20 somites), expression is restricted to the dorsal regions, the roof plate of the caudal diencephalon, the mesencephalon, the myelencephalon, and the spinal cord, but it is also expressed in a ring that extends ventrally just rostral to the midbrain-hindbrain boundary and in the ventral midline of the caudal diencephalon and mesencephalon.[107-109] *Wnt1* is essential in activating and preserving the function of the mouse engrailed genes *En1* and *En2*. *En1* is coexpressed with *Wnt1* at the 1-somite stage in a domain only slightly caudal to *Wnt1*, which includes the midbrain and r1, the rostral half of the pons, and the cerebellar cortex but excludes the diencephalon.[110] Activation of *En2* begins at the 4-somite stage, and its function in mesencephalic and r1 development is similar, with differences in some details, particularly their roles in cerebellar development.[111,112] The homeobox gene *Otx2* appears early in the initial boundary zone of the midbrain-hindbrain, and as with *Wnt1*, it appears to be essential for the later expression of *En1*, *En2*, and *Wnt1*.[113,114]

The creation of neuromeres allows the development of structures within regions of the brain without the wandering of neuroblasts that form these nuclei to other parts of the neuraxis where they would not be able to later establish their required synaptic relationships. The interaction of genes with one another is a complexity that makes analysis of single-gene expression more difficult in interpreting programmed malformations of the brain.

TABLE 4-3 Segmentation of the Neural Tube

NEUROMERE	DERIVED STRUCTURES IN MATURE CENTRAL NERVOUS SYSTEM
Rhombomere 8 (r8)	Entire spinal cord; caudal medulla oblongata; cranial nerves XI, XII
Rhombomere 7 (r7)	Medulla oblongata; cranial nerves IX, X; neural crest
Rhombomere 6 (r6)	Medulla oblongata; cranial nerves VIII, IX
Rhombomere 5 (r5)	Medulla oblongata; cranial nerves VI, VII; no neural crest
Rhombomere 4 (r4)	Medulla oblongata; cranial nervess VI, VII; neural crest
Rhombomere 3 (r3)	Caudal pons; cranial nerve V; no neural crest
Rhombomere 2 (r2)	Caudal pons; cranial nerves IV, V; cerebellar nuclei
Rhombomere 1 (r1)	Rostral pons; cerebellar cortex
Mesencephalic neuromere	Midbrain; cranial nerve III; neural crest
Diencephalic prosomere 2	Dorsal diencephalons
Diencephalic prosomere 1	Ventral diencephalon
Prosencephalic prosomere 2	Telencephalic nuclei; olfactory bulb
Prosencephalic prosomere 1	Cerebral cortex; hippocampus; corpus callosum

From Menkes JH, Sarnat HB. *Child Neurology*, 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2000:280.

Patterning of the Neural Tube

Development of the basic characteristics of the body plan is called *patterning*.[91] These patterns are the anatomic expression of the genetic code within the nuclear DNA of every cell, but they may also result from signals from neighboring cells carried by molecules that are secretory translation products of various families of organizer genes, each in a highly precise and predictable temporal and spatial distribution.

Early development of the CNS in all vertebrates, even before closure of the neural placode or plate to form the neural tube, requires the establishment of a fundamental body plan of bilateral symmetry, with cephalization, or the identity of head and tail ends, and determination of the dorsal and ventral surfaces. These axes of the body itself and the CNS require the expression of genes that impose gradients of differentiation and growth. The genes that determine the polarity and gradients of the anatomic axes are called *organizer genes*. Many express themselves in the CNS and in other organs and tissues.[54,109] The bilateral symmetry of many organs and programmed asymmetries, probably including neural structures such as the different targets of the left and right vagal nerves and left-right asymmetries in the cerebral cortex, is determined in large part by *Pitx2*, a gene expressed as early as in the primitive node.[115] Some genes function to stimulate or inhibit the expression of others, or there is an antagonism or equilibrium between certain families of genes, as exemplified by those that exert dorsoventral or ventrodorsal gradients. The difference between an organizer gene and a regulator gene is its function, and the same gene often subserves both roles at

TABLE 4-4 Programs of Developmental Genes

ORGANIZER GENES
Cell proliferation
Identity of organs or tissues (e.g., neural, renal)
Axes of polarity and growth
Ventrodorsal
Dorsoventral
Rostrocaudal
Mesiolateral
Segmentation
Left-right symmetry or asymmetry
REGULATOR GENES
Differentiation of structures within organs
Cell lineage: differentiation and specialization of individual cells
Inhibition of other genetic programs to change a cell lineage

From Menkes JH, Sarnat HB. *Child Neurology*, 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2000:281.

different stages of development. The definitions and programs of these two groups are summarized in Table 4-4.

Transcription Factors and Homeoboxes

Transcription factors are proteins expressed by regulatory genes that bind to the regulatory regions of other genes and control transcription. These transcription factors are essential for functional expression of the gene, and several different protein structural motifs have been discovered to be highly evolutionarily conserved. One such motif is the *basic helix-loop-helix* structure, which is so fundamental to the evolution of life that it appears for the first time in certain bacteria even before evolution of a cell nucleus to concentrate the DNA.[116]

The *zinc finger* is another DNA-binding, gene-specific transcription factor motif. It consists of 28 amino acid repeats with pairs of cysteine and histidine residues and with each sequence folded around a zinc ion.[117] *Krox20* (this gene name is applied to the mouse; the human form is designated *EGR2*) is a zinc finger gene expressed in alternating rhombomeres, especially r3 and r5; neural crest tissue does not differentiate from these two rhombomeres, although it does in adjacent segments, including r4.[118] *Krox20* serves an additional function in the peripheral nervous system, where it regulates myelination by Schwann cells,[119] and it also regulates the expression of some other genes, most notably those of the *Hox* family.[118,120-123] Another developmentally important zinc finger gene is *PLZF* (human promyelocytic zinc finger). Studies of mouse and chick homologues of this gene have revealed that it is expressed in a restricted zone surrounding hindbrain rhombomere boundaries, thus suggesting an important functional role for it and other zinc finger genes in vertebrate hindbrain regionalization.[124]

Some transcription factor genes include *homeoboxes*. These restricted DNA sequences of 183 base pairs of nucleotides encode a class of proteins sharing a common or very similar 60–amino acid motif called the *homeodomain*.[91] Homeoboxes or *homeotic genes* are classified into various families with common molecular structures and similar general expression during ontogenesis. They are especially associated with genes that program segmentation and the rostrocaudal gradients of the neural tube. Some of the families of homeobox genes important in development of the vertebrate nervous system are *Gsc*, *Hox*, *En*, *Wnt*, *Shh*, *Nkx*, *Lim*, and *Otx*.

Growth factors may also influence the pattern of the neural tube by behaving biologically as transcription factors: *basic fibroblast growth factor* behaves as an auxiliary inductor of the longitudinal axis with a rostrocaudal gradient during formation of the neural tube.[125]

Developmental Gene Families of the Central Nervous System

The genes that program the axes and gradients of the neural tube may be classified as *families* based on their similar nucleic acid sequences and their similar general functions, although important differences occur within a family in the site or neuromere where each gene is expressed and the anatomic structures that they form. A dorsalizing gene has a dorsal territory of expression and causes the ventral parts of the neural tube to differentiate as dorsal structures if influences from ventralizing genes do not antagonize them sufficiently and vice versa. In development of the somite, the sclerotome (which forms the cartilage and bone of the vertebral body) is normally situated ventral to the myotome (which forms muscle cells) and the dermatome. Ectopic cells of the floor plate or notochord implanted next to the somite of the chick embryo cause ventralization of the somite such that excess cartilage and bone are formed and there is a deficiency of muscle and dermis.[126,127] The floor plate, or notochord in this instance, is the ventralizing inductor of the mesodermal somite, and this is caused by expression of *Shh* (Sonic Hedgehog), which also serves as a strong ventralizing gradient force in the neural tube.[128-131] If a section of notochord is ectopically implanted dorsal or lateral to the neural tube, a second floor plate forms opposite the notochord, and motor neurons differentiate on either side of it despite the presence of a normal floor plate and motor neurons in the normal position.[132] *Shh*, which is expressed as early as in the primitive node, induces ventralization of a dorsal region of the neural tube or duplicates the neural tube. Such an influence in the human fetus, the so-called split notochord, could be an explanation for the rare cases of diplomyelia or diastematomyelia.[80] Excessive *Shh* expression, particularly its amino-terminal cleavage product, upregulates floor plate differentiation at the expense of motor neuron formation[129] and may induce duplication of the neuraxis. *Shh* exerts a strong influence on differentiation of the ventral and medial structures of the prosencephalon,[133] and defective expression of this gene has been found to be one molecular basis of human holoprosencephaly.[134]

To establish an equilibrium with genes with ventralizing influence, other genes exercise a dorsalizing influence. The *Pax* family is an example of genes that cause differentiation of the dorsal structures of the neural tube.[135,136] The *Wnt* family is also dorsalizing in the hindbrain; in situ hybridization shows its transcription products to be expressed diffusely only in the early neuroepithelium and to be restricted to dorsal regions as the neural tube develops.[137] The zinc finger gene *Zic2* has a dorsalizing gradient in the forebrain. The rostrocaudal axis of the neural tube and segmentation, or the formation of neuromeres, are directed in large part by a family of 38 homeobox genes that are divided into four groups called *Hox* genes.[92,93,138-141] Each of 13 *Hox* genes is expressed in certain rhombomeres and not in others (Table 4-5). *Hox* genes are not expressed in the forebrain. In addition to their functions in establishing the compartments or rhombomeres of the brainstem and effecting the differentiation of certain anatomic structures, *Hox* genes guide the growth cones forming the long descending and ascending pathways between the brain and spinal cord.[158]

Genes that direct the specific differentiation of structures are called *regulator genes*, and in many cases they have served as organizer genes in an earlier period. The most important families for development of the brainstem and midbrain in vertebrates are

TABLE 4-5 Organizer and Regulator Genes of the Embryonic and Fetal Nervous System

GENE*	REGIONS	FUNCTIONS	REFERENCES
Ash3a, Ash3b (*Xenopus* homologues of *Drosophila* achaete-scute)	Epiblast	Changes the fate of undifferentiated cells to form neuroepithelium	70
Bmp4 (bone morphogenetic protein)	Hensen's node; neural plate	Inhibits cells from forming neural tissue; dorsalizing to the neural tube; in the TGF-β family	72, 73
Cart1	Head mesenchyme	Organizes head mesoderm before arrival of the neural crest	142
Cnot	Hensen's node	Induces the primitive node to form the notochordal process; induces the neural placode	60
Delta		Antagonizes *Notch*; inhibits neural differentiation	
Dab1 (disabled-1)	Laminated cortices	Acts downstream of *Reln* for terminal neuroblast migration and cortical lamination	143
Dkk1 (dickkopf-1)	Primitive node	Head induction	144
Dlx1, Dlx2 (distal-less)	Prosomeres; ventral thalamus; anterior hypothalamus; corpus striatum	Subcortical neuroblast migration; interneuron migration from the basal forebrain to the neocortex	145
Dsl1 (dorsalin-1)	Neural tube	Dorsalizing; in the TGF-β family	146
DCX (doublecortin)	Telencephalon	Neuroblast migration; Xq22.3-q23 locus; defective in subcortical laminar heterotopia (band heterotopia, double-cortex syndrome)	147-149
EMX1	Telencephalon	Cell proliferation; corrects errors in cortical lamination	
EMX2	Telencephalon	Neuroblast migration; defective in schizencephaly	150
En1, En2 (engrailed)	Mesencephalon, r1	Formation of the mesencephalon and metencephalon, including the entire cerebellar cortex	95, 96, 103, 107, 109-112, 151-153
FLNA (previously *FLN1* [filamin-1])	Telencephalon	Neuroblast migration; defective in X-linked dominant periventricular heterotopia	154, 155
Foxb1 (previously *Fkh5*, forkhead)	Mesencephalon, r1-r7	Lamination of the superior colliculus; somatic afferent zone of the hindbrain; dorsalizing gradient	156
Gbx2 (unplugged)	r1-r3	Specification of the anterior hindbrain; contributes to formation of the cerebellum, motor trigeminal nerve	104
Gsc (goosecoid)	Hensen's node, neural plate	Induces the prechordal mesoderm and prosencephalon; ectopically duplicates the neural tube	56-58
HESX1	Prosencephalon	Defective in septo-optic dysplasia	32
Foxa2 (previously *Hnf3b*, winged helix)	Notochord	Regulates floor plate development; suppresses the dorsalizing influence of *Pax3*	157
Hox 1.5	r3, r5	Segmentation; formation of the parathyroid, thymus	93, 94
Hox 1.6 (*Hoxa1*)	r4-r7	Rostrocaudal gradient and segmentation	92-94, 138, 140, 141, 158
Hox 2.1	r8	Rostrocaudal gradient of the spinal cord	
Hox 2.6	Border r6/7-r8	Rostrocaudal gradient and segmentation	
Hox 2.8	Border r2/3-r8	Rostrocaudal gradient and segmentation; regulates axonal projections from r3	
Hox 2.9 (*Hoxb1*)	r4	Formation of the neural crest	122, 138
Islet1	Ventral neural tube	Motor neuroblast differentiation	159
Islet3	Neural plate	Floor plate differentiation; regulates development of the optic vesicle and tectal and cerebellar primordia	160, 161
EGR2 (*Krox20*)	r3, r5	Zinc finger; neural crest formation in r3 and r5; regulates expression of *HOX* genes; regulates myelination by Schwann cells	118-123
L1CAM	Mesencephalon; telencephalon	Formation of the aqueduct; cerebral neuroblast migration and corticospinal axon guidance; defective in X-linked hydrocephalus with aqueductal stenosis and also reported in hemimegalencephaly	162-164

Continued

TABLE 4-5 Organizer and Regulator Genes of the Embryonic and Fetal Nervous System—cont'd

GENE*	REGIONS	FUNCTIONS	REFERENCES
Lhx2	Prosomeres	*LIM* family homeobox; development of the hippocampus and cellular proliferation for neocortex; development of the eye before formation of the optic cup	165
Lhx9	Subplate neurons of the cortical plate; cerebellar nuclei	Expressed in pioneer axons of the cerebral cortex and in cerebellar nuclei	166
Lim	Neural plate; prechordal mesenchyme	Organizer of cephalic mesenchyme before migration of the neural crest; organizer of the neural placode	160, 161, 167-171
Lis1	Cortical plate	Neuroblast migration; 17p13.3 locus; defective in lissencephaly type 1	172-176
Mash1	Telencephalon; neural crest	Regulates differentiation of the ventral telencephalon; in achaete-scute family; requires *Phox2* for expression	177
Math1 (human homologue is *ATOH1*)	r1, cerebellum	Differentiation of cerebellar granule cells	178
Math5 (human homologue is *ATOH7*)	Optic cup	Retinal differentiation	179
Mnr2	Motor neuroblasts	Motor neuron identity	180-187
Neuro D	Ectodermal cells	Neuronal differentiation; three subtypes on human chromosomes 2, 5, 17; related to gene regulating transcription of insulin; retinal development	188-190
Ngn1 (neurogenin)	Ectodermal cells	Neuronal differentiation in the central and peripheral nervous systems; expressed earlier than *Neuro D*; interacts with *Delta* and *Notch*; family of subtypes	191
Nkx2-1	Prosomeres	Differentiation of the hypothalamus; induced by *Shh*	191, 192
Nkx2-2	All neuromeres	Specifies diencephalic neuromeric boundaries; interacts with *Dlx1* and transcription factor Ttf1 for prosencephalic differentiation	191
Nkx6-1	Diencephalon–r8; motor neurons	Induced by *Shh* and repressed by *Bmp7*; coexpressed with Islet-1 in motor neurons	191
Nkx6-2	Diencephalon–r8	Glial cell differentiation	72, 73, 193, 194
Nog (noggin)	Hensen's node	Inhibits *Bmp4* to allow neural plate differentiation	193, 195, 196
Notch	Neural plate; neuroepithelium	Regulates the competence of cells to respond to inductive signals; differentiation of neural placode; asymmetric distribution in cytoplasm during mitotic cycle; *Notch3* mutation in CADASIL syndrome in adults	193, 197
Numb	Neural plate; fetal neuroepithelium	Antagonizes *Notch* by preventing neural differentiation	
Otx1 (orthodenticle)	Mesencephalon/r1 boundary; telencephalon; sensory nerves	Onset of neuromere formation; corticogenesis; sense organ development	113, 114
Otx2 (orthodenticle)	Prestreak blastomere; neural plate	Gastrulation; specification and maintenance of anterior neural plate	
Pou1f1 (previously *Pit1*, pituitary specific)	Adenohypophysis	Differentiation of anterior pituitary	198-201
Ptc (patched)	Cerebellar cortex	Regulates granule cell proliferation; tumor suppressor gene	103, 105-107, 136
Pax2 (paired)	Primitive streak; r2-r8; prosomeres	Dorsalizing polarity gradient; segmentation regulated by the notochord and floor plate; formation of the ventral half of the optic cup, retina, and optic nerve; overlaps and partially redundant with *PAX5*	202
Pax3 (paired)	r1; r8	Identity of Bergmann glia; active spinal cord dorsalizing gradient; Waardenburg's syndrome	136

TABLE 4-5 Organizer and Regulator Genes of the Embryonic and Fetal Nervous System—cont'd

GENE*	REGIONS	FUNCTIONS	REFERENCES
Pax5 (paired)	r1	Partially redundant with *PAX2* for differentiation of the cerebellar cortex; dorsalizing gradient	136, 202
Pax6 (paired)	r1; r8; prosomeres	Identity of cerebellar granule cells; active in the spinal cord as a dorsalizing gradient; neuroblast migration to the cerebral cortex and deep telencephalic nuclei; iris	135, 136
Arix (*Phox2a*, *Phox2b*)	Neural crest	Differentiation of autonomic ganglia; in achaete-scute family	173-176
Pitx	Primitive streak	Determines right-left asymmetries of internal organs	115
RELN (reelin)	Laminar cortices	Extracellular matrix glycoprotein product secreted by Cajal-Retzius neurons and cerebellar granule cells; essential for terminal neuroblast migration and laminar architecture	143, 146, 203-205
RhoB	Dorsal neural tube	Delamination of neural crest cells; expression induced by *BMP* products	206
SHH (Sonic Hedgehog)	Notochord; floor plate; prechordal mesoderm	Induces floor plate; ventralizing influence of the neural tube; ventral midline of the prosencephalon; induction of motor neurons; mitogen to cerebellar granule cells	127-131, 133, 134, 198, 199, 207
SIX3	Prechordal mesoderm	Differentiation of the rostral neural plate and retina; 2p21 locus; mutation in humans is one cause of holoprosencephaly; overexpression causes ectopic retinas	208, 209
SMN (survival motor neuron)	Motor neuroblasts	Arrests apoptosis of motor neuroblasts	210, 211
TSC1, *TSC2*	Neuraxis	Encode the proteins hamartin (*TSC1* at the 9q34 locus) and tuberin (*TSC2* at 16p13); defective in tuberous sclerosis	212-217
Twist	Hensen's node	Organizer of cephalic mesenchyme before migration of the neural crest; cranial neural tube morphogenesis	218
Toad64 (unc33)	Growth cones	Promotes axonal outgrowth	219
Wnt1 (wingless)	r1, r3-r8	Formation of the mesencephalic-metencephalic boundary; formation of the mesencephalon, rostral pons, and cerebellum; essential for expression of *EN1*; weak dorsal polarizing influence in r3-r8; mitogen	95, 103, 107, 110, 137, 220-222
Wnt3 (wingless)	Mesencephalon; r1; r3-r8	Overlaps and redundant with *WNT1* in the mesencephalic neuromere and r1; strong dorsal polarizing influence in r3-r8, including the spinal cord; differentiation of brainstem nuclei; identity of Purkinje cells	137, 223
Wnt7 (wingless)	Prosomeres	Differentiation of structures of the diencephalon and telencephalon	99
Wnt8 (wingless)	Epiblast; primitive streak; r1-r8	Primitive streak formation; segmentation	59
Zic1	Cerebellum	Zinc finger; differentiation of granule cells	224, 225

*An *organizer gene* is one that programs differentiation of the neural placode and axes, gradients, and segmentation of the neural plate and neural tube; a *regulator gene* is one that programs the differentiation of specific structures and cellular types in the developing nervous system, conserves their identity, and mediates developmental processes such as neuroblast migration or synaptogenesis. The genes are listed alphabetically rather than by function because many of the same genes serve various functions at different stages, such as being organizer genes in early ontogenesis and regulator genes at later periods. Developmental genes recognized in invertebrates such as *Drosophila*, but for which the vertebrate homologue has not yet been identified, are excluded. CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; r1, rhombomere 1; TGF-β, transforming growth factor-β.

Modified from Menkes JH, Sarnat HB. *Child Neurology*, 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2000:283-285.