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Nervous System 20

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Introduction

The central nervous system (brain and spinal cord) controls the rest of the body via the peripheral somatic (sensorimotor or voluntary) and autonomic (involuntary) nervous systems. Functional domains within the system are based on contiguous signaling pathways. The nervous system is, perhaps, anatomically and functionally the organ system that differs most between mouse and human. In delineating what separates man from beast, many philosophers and scientists have argued that it is the highly developed human brain and its unique cognitive capacities for reasoning, language, introspection, and problem solving. Although we continuously learn more about the surprising bounds of animal intelligence, humans remain the only species known to make fire, cook, wear clothes, and surf the Internet.

The nervous system poses unique challenges in morphologic assessment. Relative to other organs and body systems, the elements of the normal central nervous system (CNS) are anatomically diverse within and among species, exhibiting major structural changes at both gross and microscopic levels over very short distances and in all three dimensions. Distinctive processing techniques are required to preserve structural features and to avoid confusing artifacts. Precise regional dissection, special stains, intricate morphological measurements, and/or novel imaging methods are often required if functional information is to be evaluated in the context of its neuroanatomic localization.

Correct nomenclature is essential in describing neuroanatomic structures. The planes describing the orientation of the CNS in space differ between mice (and other quadruped animals) and humans (which are upright bipeds). For example, the "dorsal" and "ventral" surfaces of the horizontal mouse brain are equivalent to the "superior" and "inferior" poles, respectively, of a vertical human brain. In some cases, the same names apply to equivalent structures in mouse and human, such as "rostral" and "caudal" for the "front" and "back" poles of the brain. The most common

TABLE 1 Murine and Human Nervous Tissue

	Tissue	Mouse	Human
		Brain	
Gross	Lobes defined by external landmarks	No	Yes
	Sulci and gyri	No	Yes
	Olfactory bulb/archicortex	Very large	Small
	Cranial lobe of cerebellum	Less lateral spread	More lateral spread
	Paraflocculus lobule of cerebellum	Large	Insignificant
	Middle lobe of cerebellum	Small	Lateral portions larger in humans
Tissue	Cerebral cortex	Primarily paleocortex	Primarily neocortex
	Cortical interneurons	Less significant	Significant role
	Independence of subcortical centers from cerebral cortex control	More independent	Less independent
	Visual cortex	More lateral	More midline
	Functional cortices	Primary	Primary and associative ("higher thought")
	White matter	Relatively scant	Very abundant
	Predominant sensory cortex	Olfactory and face—whisker barrels (motor component to whisker barrels, as well)	Hands and face (lips and tongue)
	Hippocampus	Dorsal aspect of cerebrum	Ventral aspect of cerebrum
	Basal ganglia	Combined caudate nucleus and putamen (rendered as caudate putamen or caudoputamen)	Separate caudate nucleus and putamen
	Cerebellar nuclei	Less discrete	More discrete
	Medial and lateral lemniscus	Small	Large
	Inferior olivary nucleus	Smaller	Large, due to expanded cerebellar hemispheres
	Mineralization with age	Lateral thalamus	Pineal gland
	Meninges	Thin	Thick, well developed
Cells	Substantia nigra cells	Melanin pigment not obvious	Obvious neuromelanin pigment
		Spinal Cord	
Gross	Lumbosacral enlargement	L2–L6	L2–S3
	Spinal nerves	15 dorsal and 15 ventral rootlets per segment (on each side)	6–8 dorsal and 6–8 ventral rootlets per segment (on each side)
	Spinal formula	C7 T13 L6 S4 Cd 28	C7 T12 L5 S5 Cd 4
	Spinal nerves innervating hind limbs/legs	L3–L6	T12–L4
Tissue	Corticospinal tract	Smaller and located in dorsal column; minor role in motor functions	Larger and located in lateral column; major role in motor functions, especially dexterity of distal limbs
	Rubrospinal tract	Larger; major role in locomotion and posture	Smaller; cooperative role with corticospinal tract
	Lateral horn	Less distinct	More prominent
	Segments containing sympathetic nervous system preganglionic cell bodies	T1–L2	T1–L3
	Segments containing parasympathetic nervous system preganglionic cell bodies	L6–S1	S2–S4
	Nucleus of Bishoff (tail afferents)	Possibly	No
		Peripheral Nervous System	
	Peripheral nerve connective tissue	Scant	Abundant
		Endocrine	
	Pituitary gland—pars intermedia	Morphologically distinct; larger than in most species	Poorly developed, thin layer of cells; contains colloid cysts
	Pineal gland	Not lobulated; more homogeneous appearance	Prominently lobulated; cells arranged in rosettes and clusters; age-related mineralization
		Choroid Plexus, Circumventricular Organs, Ependyma, and Vasculature	
Gross	Vasculature	Not yet widely characterized; a few studies provide 3D atlases of deep brain vasculature	Well characterized and more complex due to the sulci, gyri, and lobes of human brain
Tissue	Choroid plexus	Less extensive folds and fronds	More extensive folds and fronds and connective tissue due to increased volume and surface area
	Subcommissural organ	Limited vascularization	Vestigial, with no vascularization
	Callosomarginal artery	No equivalent artery	Runs along cingulate gyrus
	Anterior choroid artery	Supplies choroid plexus and thalamus	Supplies choroid plexus, thalamus, and hippocampus
	Ventral corpus callosum	Medial orbitofrontal artery	Frontopolar branch of anterior cerebral artery

orientation used by neuropathologists in assessing the brain is the coronal plane (or cross section). Sagittal and horizontal planes are seldom used for brain analysis in veterinary neuropathology. In human clinical medicine, however, the increasing role of multiplanar (three-dimensional) neuroimaging requires that neuropathologists also have a thorough knowledge of CNS anatomy in the sagittal and axial planes. The increasing availability and use of three-dimensional mouse brain atlases supports development of similar neuroanatomical expertise in this species.

Neuroanatomic comparisons between mice and humans are complicated by the phylogenetic distance between the two species. Mouse and human brains differ markedly in size (Figure 1) and organization, particularly in the functional and structural arrangement of the cerebral cortex (Table 1). This chapter highlights major differences in the nervous system between mice and humans, emphasizing divergence in their gross and microscopic anatomy as well as their regional organization in adulthood; some functional variations are also noted. References to other species' neuroanatomy are made where informative. Major brain regions discussed are, from rostral to caudal, the cerebral cortex, basal ganglia, hippocampus, diencephalon (thalamus and hypothalamus), mesencephalon (midbrain), cerebellum, and brain stem (pons and medulla). Ancillary brain structures described are the pineal and pituitary glands, circumventricular organs, ventricular structures (choroid plexus and ependyma), and brain vasculature. Spinal cord and the peripheral nervous system (PNS) are also examined.

A complete comparison of neural anatomy and function between mice and humans could easily fill an entire book. Thus, the treatment in this chapter is fairly basic. For more detailed information, the reader is referred to the resources listed in Further Reading.

In many neurological diseases of humans, certain neuroanatomic structures are preferentially involved. Primary examples include the cerebral cortex in Alzheimer's disease, the striatum (caudate nucleus and putamen separated by the intervening internal capsule) in Huntington's disease, substantia nigra in Parkinson's disease, cerebellum in many spinocerebellar ataxias, white matter in multiple sclerosis, and the primary motor cortex/ventral spinal cord in amyotrophic lateral sclerosis. Mice have comparable regions within their nervous systems, but in many instances, murine structures are visibly different from their human counterparts. Understanding the gross and microscopic distinctions in neural structure between humans and mice is a fundamental prerequisite to using genetically engineered, induced, and spontaneous mouse models of disease to model human neurological conditions. This chapter provides a basic overview of neuroanatomic and functional similarities and disparities of these two species.

Brain

Gross Anatomy

Prior to dissection for an internal examination, the whole brain should be examined externally for regional or total alterations in size and shape of grossly visible features. Particular areas to evaluate include the pattern of cerebral gyri (ridges) and sulci (grooves) in humans, the appearance of the cerebellar lobes and folia in both species, and the distribution of blood vessels. The assessment may be performed with the naked eye or using a magnifying glass for human brains, whereas a thorough analysis of the mouse brain will likely require a stereomicroscope.

On gross examination, many striking differences are evident between the brains of an adult mouse and an adult human (Figure 1). The most obvious variations are the human brain's much greater size (approximately 1300 g), readily apparent lobular organization, prominent sulci and gyri, and large amount of white matter relative to the mouse brain. In contrast, the mouse brain is quite small (~0.4 g), is not prominently lobed, and is lissencephalic (does not have sulci or gyri). The pale color of neural tissue is due to its high lipid content, primarily localized to the myelin of the white matter. The amount of white matter increases as a cubic function of an animal's size.

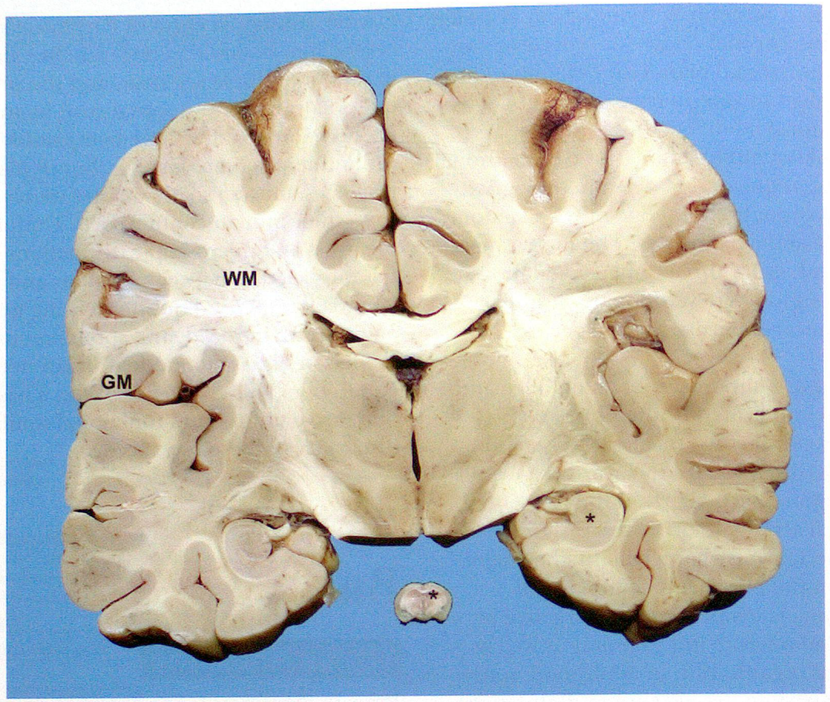


FIGURE 1 Coronal brain slices from an adult human and adult mouse demonstrating the marked differences in organ size and organization. Gray matter (GM) includes neuron cell bodies, glia, and blood vessels. White matter (WM) consists of myelinated axons and myelinating cells. The hippocampal formation on the right side of each slice is marked with an asterisk. Many of the photos and diagrams in this chapter have been adjusted in size to provide optimal anatomical and cellular comparisons.

The quantity of gray matter increases as the square. Although brain weight correlates with body weight, cortical surface area is not directly proportional to brain size. Humans have large brains for their body size due to the expansion of the cerebral cortex and the increased neuronal numbers contained in the bulging gyri.

The brain must be approached carefully to prevent damage to the soft tissue. The usual strategy is to remove the calvaria (skull cap) by splitting the sagittal suture, which follows the midline. The two parietal bones are then removed to expose the delicate brain. The venous drainage system of sinuses located between the reflected skull and the underlying brain follows similar paths in mice and humans. Viewing the mouse brain *in situ*, the cerebri, colliculi, and cerebellum are visible on the dorsal surface (Figure 2A), whereas the same examination in the human would reveal only cerebral cortex. The colliculi in mice are properly designated as "rostral" and "caudal" due to the horizontal orientation of the brain in quadruped animals, but in murine atlases they are sometimes referred to by the human (i.e., biped) planes of orientation as "superior" (rostral) and "inferior" (caudal). Careful reflection of the mouse brain reveals several prominent landmarks on the ventral brain surface, including, but not limited to, the optic chiasm, olfactory tubercles, pituitary gland, piriform cortex, pons, and medulla oblongata (Figure 2B).

Gross comparisons of adult mouse and human brains reveal many distinctions in CNS structures between the two species. Lateral views demonstrate the marked expansion of the cerebral cortex in humans, which is indicated both by the intricate folding of the cerebrum and by its extension over the cerebellum

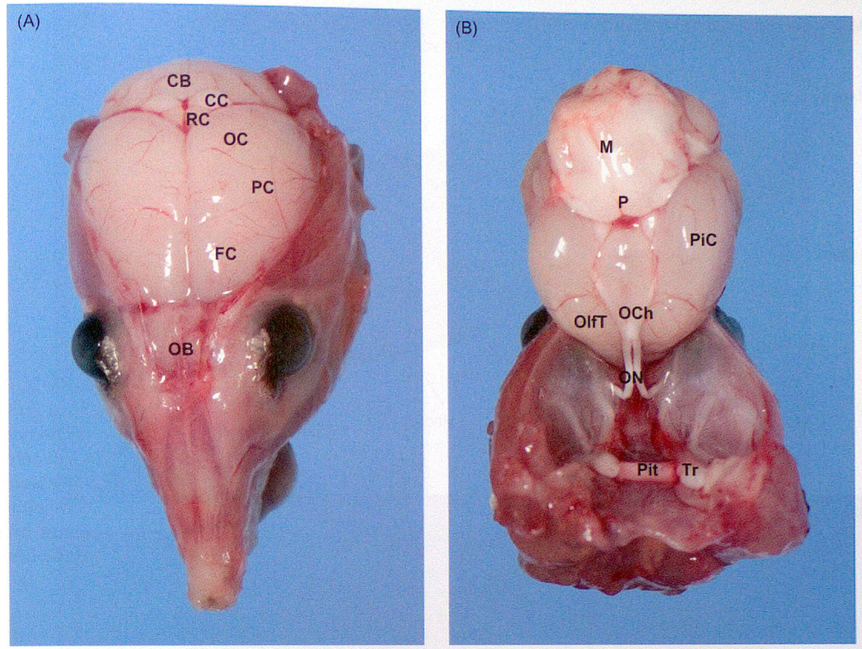
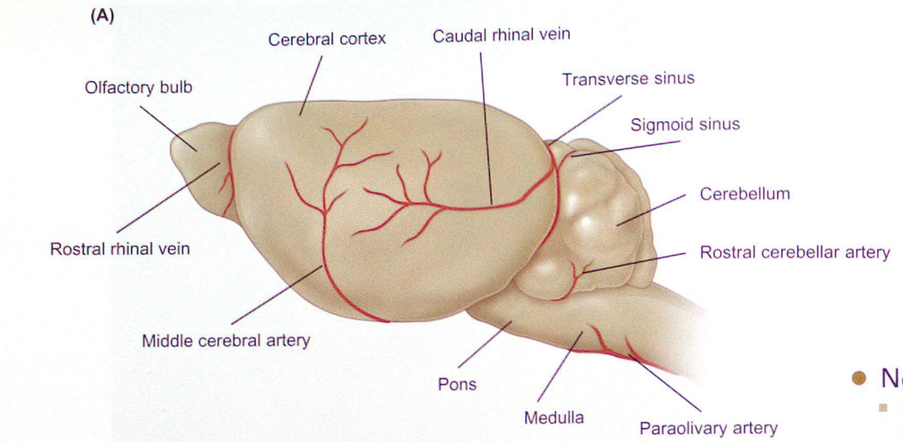


FIGURE 2 Gross view of the adult mouse brain *in situ*. (A) The dorsal surface of the brain includes, from rostral (front) to caudal, the olfactory bulbs (OB), frontal cortex (FC), parietal cortex (PC), occipital cortex (OC), rostral colliculus (RC), caudal colliculus (CC), and cerebellum (CB). The pineal gland (located near the RC) is closely associated with the dura mater, and it usually remains attached to the skull during removal of the brain. (B) Careful reflection of the brain reveals its ventral surface with such major structures as, from rostral to caudal, optic nerves (ON (cranial nerve II)), olfactory tubercles (OlfT), optic chiasm (OCh), piriform cortex (PiC), pons (P), and medulla (M). The pituitary gland (Pit) is attached to the center of the base of the skull and is bracketed by the trigeminal ganglia (Tr (cranial nerve V)).

(Figure 3). Whereas the neuraxis of the mouse brain is linear from rostral to caudal, the human upright posture has resulted in ventral angulation of the caudal aspects of the brain and brain stem (Figure 3). This, combined with the proportionally larger cerebral cortical and white matter volumes in humans, has resulted in dramatic differences in positioning of some neuroanatomic structures in humans compared to mice. Side-by-side comparisons of the dorsal (superior) and ventral (inferior) brain surfaces of mice and humans highlight other significant differences (Figure 4). The most pronounced divergence is the absence of fissures, gyri, and sulci on the mouse cerebrum, with the exception of the longitudinal fissure that divides the two hemispheres. Thus, it is impossible to divide the mouse brain on the basis of surface topography. The ventral surface of the brain harbors the olfactory elements. Smell is a critical sensory modality in mice, so they have extremely large olfactory bulbs and bulging olfactory tubercles. Humans, who rely far less on olfaction for sensory cues, have only a small olfactory nerve. In mice, the optic chiasm is rostral to the tuber cinereum of the hypothalamus. The tuber cinereum surrounds the infundibulum, which connects the hypothalamus to the neurohypophysis (pars nervosa (posterior region)) of the pituitary gland, and also borders the rostral limit of the mammillary bodies. In mice, the infundibulum is disrupted with removal of the brain from the skull because the pituitary gland is firmly attached to the skull by a fibrous membrane. In contrast, in humans, the mammillary bodies are superior to the optic chiasm, somewhat recessed between the frontal and temporal lobes, and the infundibulum sits posterior to the optic chiasm (i.e., it is not visible). The cerebellum can be divided into lateral hemispheres and a central vermis (Figure 4). In both species, the brain stem is



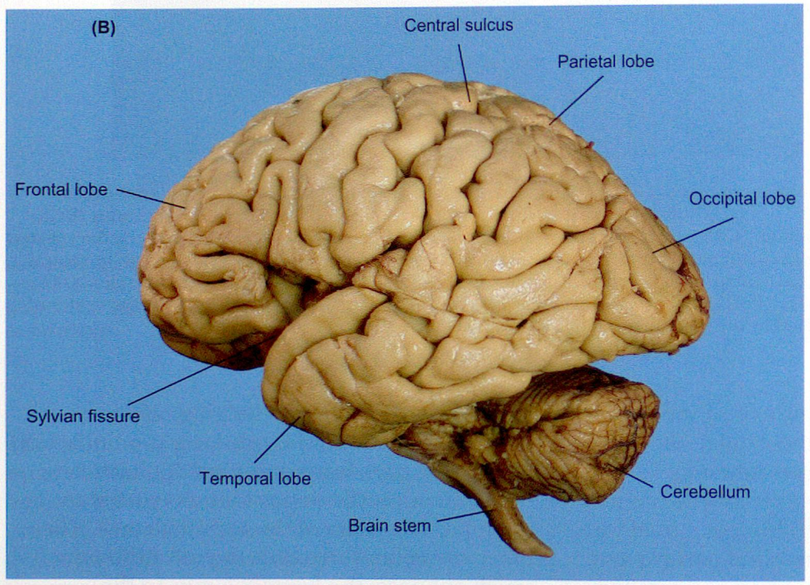


FIGURE 3 Lateral views of gross brain features in the adult mouse (A) and adult human (B) showing major differences in anatomical organization. Meninges have been removed from the human cerebrum but were left intact on the cerebellum. Source: For Panel A © Elsevier, Inc., www.nettersimages.com.

- **Need-to-know**
  - Main gross structural differences are the small cerebrum (forebrain) and large cerebellum of the mouse brain relative to the human brain.

composed of the broad pons rostrally before tapering gradually to the medulla oblongata. The medulla is connected with the spinal cord; in life, the junction of brain stem and spinal cord occupies the foramen magnum. The pyramids of the medulla oblongata, formed of descending motor axons, can be seen in both species on the ventral surface of the brain stem (Figure 4).

Understanding the inputs and outputs among CNS regions can facilitate localization and identification of brain lesions, and knowing the functions of different regions can help guide what CNS sites to examine most closely based on clinical signs. Coronal (cross) sections are a standard plane for examining the brain because they allow related functional regions to be followed longitudinally while permitting bilateral

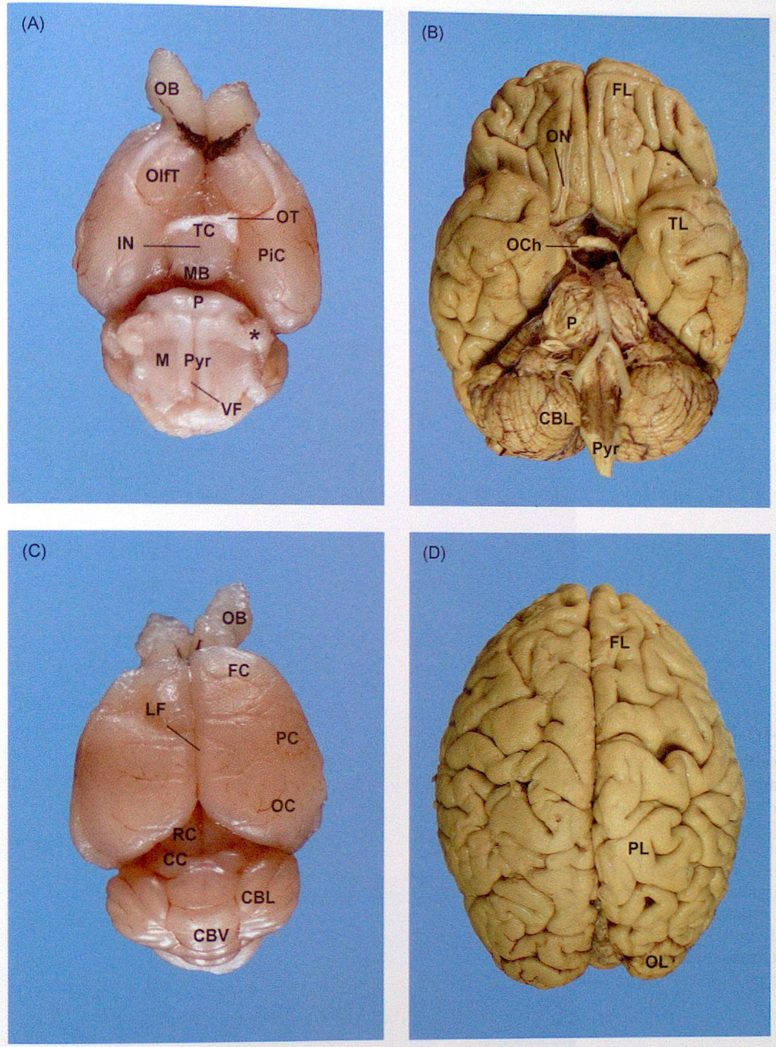
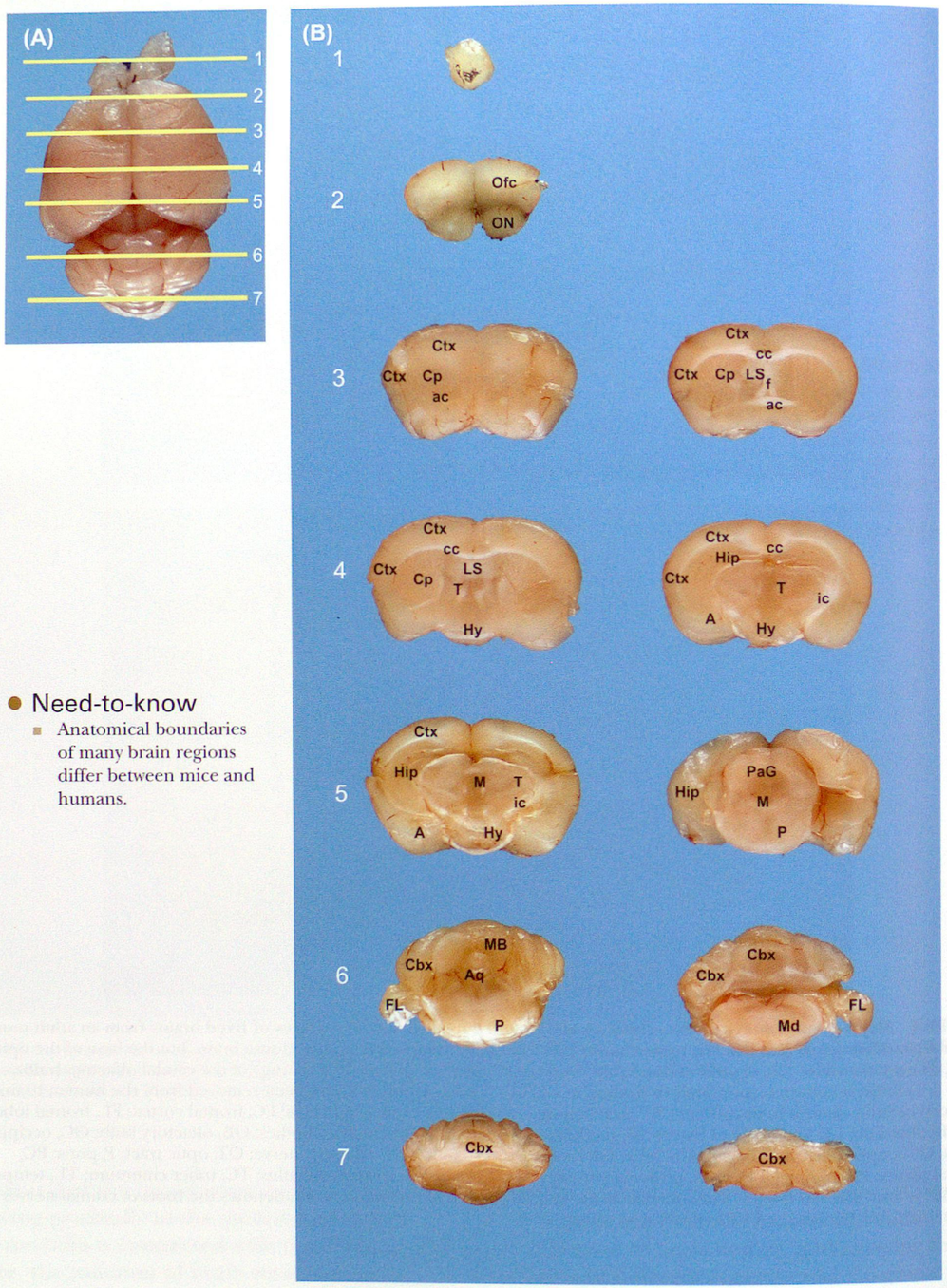


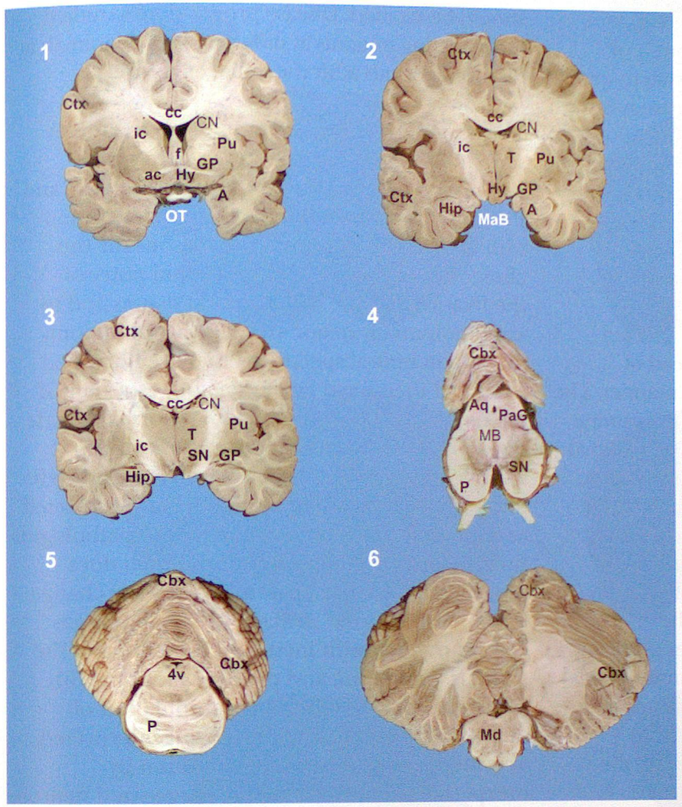
FIGURE 4 Major gross features of the ventral (A and B) and dorsal (C and D) surfaces of fixed brains from an adult mouse (A and C) and human (B and D). The optic chiasm (OC) has been removed from the mouse brain, but the base of the optic tract (OT) is still visible. The ventral mouse brain shows meningeal melanosis (blackening) of the caudal olfactory bulbs, which is a relatively common spontaneous finding in C57BL/6 mice. Meninges have been removed from the human brain. CBL, cerebellum—lateral hemisphere; CBV, cerebellum—vermis; CC, caudal colliculus; FC, frontal cortex; FL, frontal lobe; IN, infundibulum; LF, longitudinal fissure; M, medulla oblongata; MB, mammillary bodies; OB, olfactory bulb; OC, occipital cortex; OCh, optic chiasm; OL, occipital lobe; OlfT, olfactory tubercle; ON, olfactory nerve; OT, optic tract; P, pons; PC, parietal cortex; PL, parietal lobe; PiC, piriform cortex; Pyr, pyramids; RC, rostral colliculus; TC, tuber cinereum; TL, temporal lobe; VF, ventral median fissure. The asterisk on the ventral surface of the mouse brain denotes the roots of cranial nerves V (trigeminal), VII (facial), and VIII (vestibulocochlear).

evaluation of potential targets. The locations of major brain regions in coronal sections of the adult mouse shown in Figure 5 are compared with their corresponding levels in coronal sections of cerebrum and axial (horizontal) sections of cerebellum for the adult human in Figure 6. The same gross differences between mice and humans that may be observed when evaluating the brain surface are even more readily appreciated when assessing cross sections. In particular, the



- Need-to-know
  - Anatomical boundaries of many brain regions differ between mice and humans.

FIGURE 5 Gross images of coronal (cross) sections of an adult mouse brain. The numbered levels shown in Panel A correspond to the labeled levels in Panel B. The sections on the left column show the rostral side of each slice, whereas the right column shows the caudal side. Note that only one olfactory lobe is shown for Level 1. A, amygdala; ac, anterior commissure; Aq, mesencephalic aqueduct; Cbx, cerebellar cortex; cc, corpus callosum; Cp, caudate/putamen; Ctx, cerebral cortex (neocortex); f, fornix; FL, flocculus lobe of the cerebellum; Hip, hippocampus; Hy, hypothalamus; ic, internal capsule; LS, lateral septum; M, mesencephalon (midbrain); Md, medulla oblongata; Ofc, orbitofrontal cortices; ON, olfactory nucleus; P, pons; PaG, periaqueductal gray matter; T, thalamus.



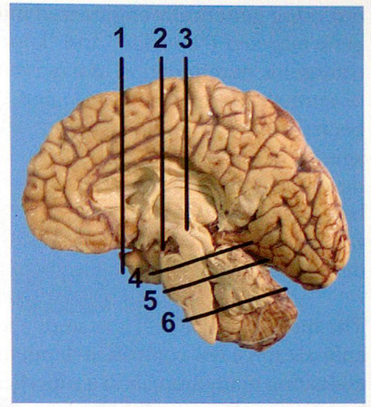


FIGURE 6 Gross images of coronal (cross) sections of an adult human brain. The approximate levels of each image relative to the human neuraxis are depicted on the gross lateral image of human brain (right). The human neuraxis is unique in that it has a 90° bend. Levels may be distinguished by major external and internal features: 1, striatum, composed of the caudate nucleus (CN), putamen (Pu), and globus pallidus (GP); 2, mammillary bodies (MaB); 3, substantia nigra (SN); 4, mesencephalon (midbrain), with the aqueduct of Sylvius (Aq) surrounded by the periaqueductal gray matter (PaG); 5, pons (P); and 6, medulla oblongata (Md). 4v, fourth ventricle; A, amygdala; ac, anterior commissure; Cbx, cerebellar cortex; cc, corpus callosum; Ctx, cerebral cortex (neocortex); f, fornix; Hip, hippocampus; Hy, hypothalamus; ic, internal capsule; OT, optic tract; P, pons; T, thalamus. Images 4–6 are oriented to facilitate comparison with the mouse levels in Figure 5.

enormous extent of the cerebral cortex and, to a lesser degree, the cerebellar cortex of humans relative to the sizes of the mesencephalon (midbrain) and brain stem is quite evident.

Histology

Microscopic assessment is a valuable method for assessing nervous system structural abnormalities in mice as well as humans, and it provides excellent correlation with gross pathology. This approach is a well-validated means for detecting and defining regional and cytoarchitectural anomalies, provides unquestionable evidence of anatomic abnormality, and offers a high level of sensitivity to certain changes. Proper handling, sampling, and sectioning of tissue are critical for an effective and efficient assessment of the nervous system. In all instances, the astute morphologist will have made the effort to gain a thorough understanding of basic neurobiological tenets and will guide the neuropathology assessment using any of numerous age-appropriate atlases of brain and spinal cord neuroanatomy (see Further Reading).

Conventional neurohistological methods for the brain

Histologic evaluation in the context of mouse models of human disease is typically carried

out using genetically engineered mice (e.g., gene targeted ("knockin" and "knockout") or transgenic manipulation of a nervous system gene) or animals in which a neurological disease has been induced (e.g., by treatment with infectious agents, neurotoxicants, or tissue xenografts) or develops spontaneously. Regardless of the model, based on our experience and literature reports, we recommend the following guidelines when designing mouse neurological experiments.

The most critical aspect is to study a few affected animals and a few controls in-depth instead of evaluating many animals in a superficial manner. The best means of accomplishing this task is to examine a full spectrum of organs (40–50) from each animal and, for organs with heterogeneous structure (e.g., the brain), to inspect multiple sites for each organ. A basic mouse neuroanatomy examination should assess the following brain structures (generally in coronal ("transverse") sections): rostral cerebrum (frontal cortex with basal ganglia), middle cerebrum (parietal and temporal cortices overlying hippocampus, thalamus, and hypothalamus), caudal cerebrum (occipital cortex with subjacent midbrain), and cerebellum with both pons and medulla oblongata (Figures 7–9). It should also assess the spinal cord (lumbar intumescence, where the motor neurons supplying the hind limb are located) and sciatic nerve. Some neuropathologists prefer acquiring brain sections in the sagittal or horizontal planes to facilitate identification and evaluation of neural circuits; some scientists mix the methods, dividing the brain in half and taking coronal sections from one side and sagittal sections from the other. The most important consideration in choosing the orientation is that a researcher consistently produce sections in the same orientation through the same sets of neural structures. Important ancillary structures that should be evaluated include the retina and pituitary gland. Where warranted, a more extensive neurohistological examination may be performed in which additional neural structures (e.g., olfactory mucosa and bulbs, colliculi (in the midbrain), and dorsal root ganglia) are reviewed. The key in designing mouse neurological experiments is to assess as many potential sites as feasible to (1) ensure that potential changes/phenotypes are not missed and (2) strengthen any eventual relationship between the mouse model and the human disease. The common practice of evaluating only "tissues of interest" to the laboratory should be avoided. Disease progression is studied by evaluating mutants at different ages or animals at different periods after the initial exposure.

The second essential requirement is to employ suitable methods for fixing and processing nervous tissues. For the best results, the CNS and PNS should be fixed by intravascular perfusion with the fixative solution. The reasons for this choice are to provide the most rapid entry of the fixative into the dense neural tissues and to avoid potentially major artifacts that commonly develop in neural specimens that are harvested fresh and then fixed by immersion, especially if the fixative is 10% formalin (3.7% formaldehyde in water). One prominent artifact in white matter is perivascular retraction spaces and cleft formation, which are occasionally misinterpreted as a spongiform change. Some mouse pathologists prefer fixing in Bouin's solution, which does not cause this artifact. Another common change is the "dark neuron" artifact, which appears as cell contraction with increased basophilia of the nucleus, cytoplasm, and processes (Figure 10). "Dark neurons" result from manipulation of unfixed gray matter and may be misinterpreted as evidence of neuronal degeneration by inexperienced morphologists. Large-sized neurons most frequently show the dark neuron alteration, although any population may be affected; some areas seem to show the artifact more frequently, such as the deep layers of the cerebral cortex (especially in mice), the pyramidal layer of the hippocampus, and the Purkinje cells of the cerebellum. For specific details on the preparation and processing of CNS and PNS specimens, see Further Reading.

In both humans and mice, neural organs must be sampled in a consistent manner. The reasons are that (1) direct comparisons between subjects are made feasible in multi-individual experiments, especially in conventional mouse studies, and (2) reproducible sampling of neuroanatomic patterns in normal neural specimens greatly facilitates identification of altered structures. The simplest means of acquiring repeatable section orientations is to employ gross landmarks (external and internal) to guide tissue trimming (Figure 5). Blocks may be separated by "freehand" (unguided) cutting or, for the mouse, by using a metal or plastic brain matrix with prepositioned slots for positioning the blades. The matrix size will vary with the size of the rodent.

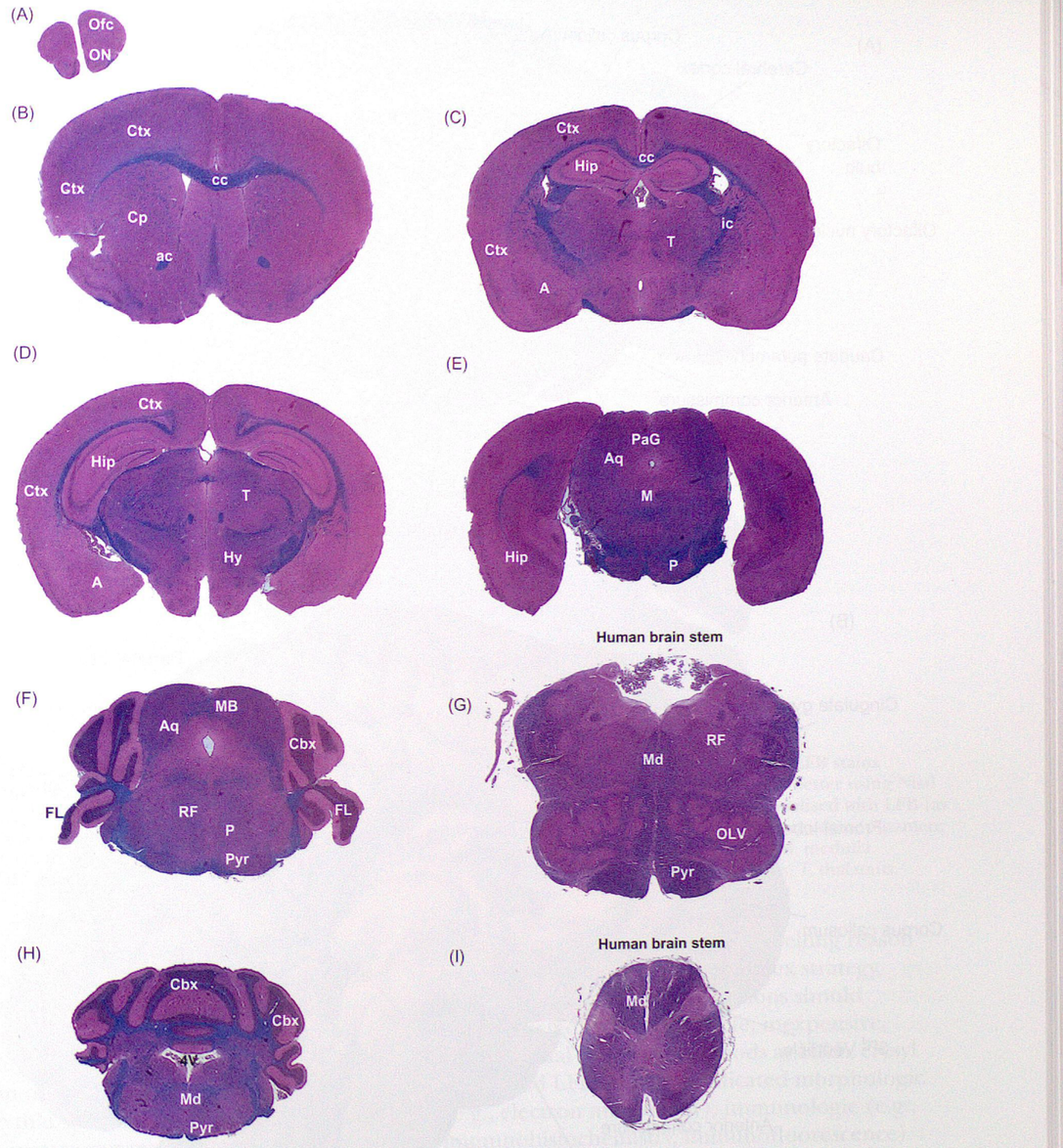


FIGURE 7 Coronal (cross) sections of adult mouse brain (A–F and H) and human brain stem (G and I) for histologic evaluation, stained with H&E and LFB. Levels are taken at approximately the same levels as shown in Figure 5. 4v, fourth ventricle; A, amygdala; ac, anterior commissure; Aq, mesencephalic aqueduct; Cbx, cerebellar cortex; cc, corpus callosum; Cp, caudate/putamen; Ctx, cerebral cortex (neocortex); FL, flocculus lobe of the cerebellum; Hip, hippocampus; Hy, hypothalamus; ic, internal capsule; M, mesencephalon (midbrain); Md, medulla oblongata; Ofc, orbitofrontal cortices; OLV, olivary nucleus; ON, olfactory nucleus; P, pons; PaG, periaqueductal gray matter; Pyr, pyramids; RF, reticular formation; T, thalamus.

Numerous histological stains are available for evaluating neural tissues. Standard methods include hematoxylin and eosin (H&E), which permits a global assessment of basic architecture; cresyl violet (CV; a Nissl stain) for evaluating neuronal cell body numbers and features; Luxol fast blue (LFB), which labels myelin and thus

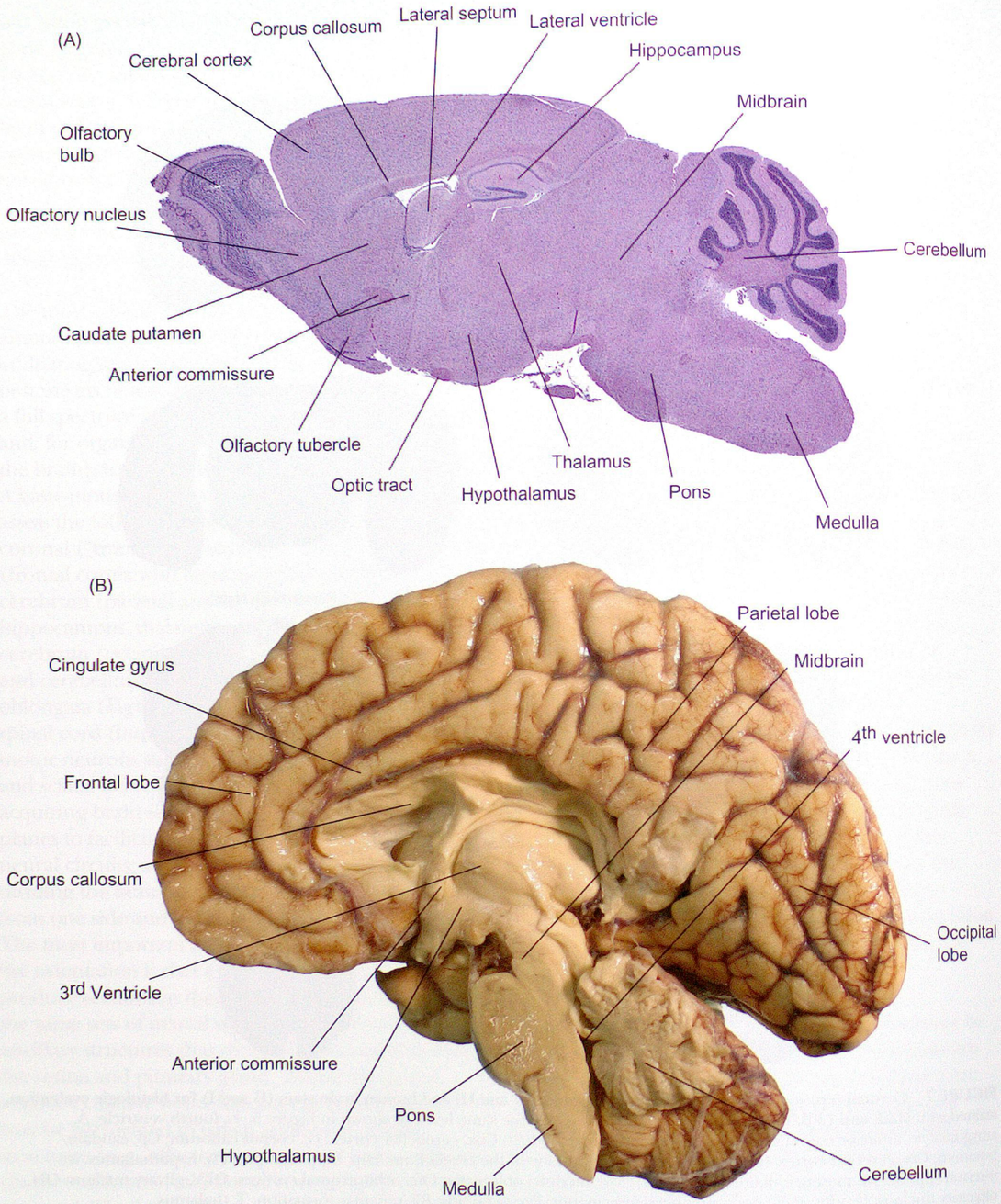


FIGURE 8 Parasagittal section of adult mouse brain (A; H&E) and mid-sagittal gross preparation of adult human brain (B). The hippocampus is not visible in the sagittal plane of human brain. The asterisk denotes the approximate position of the pineal gland in the mouse (although the actual gland is not shown in the section).

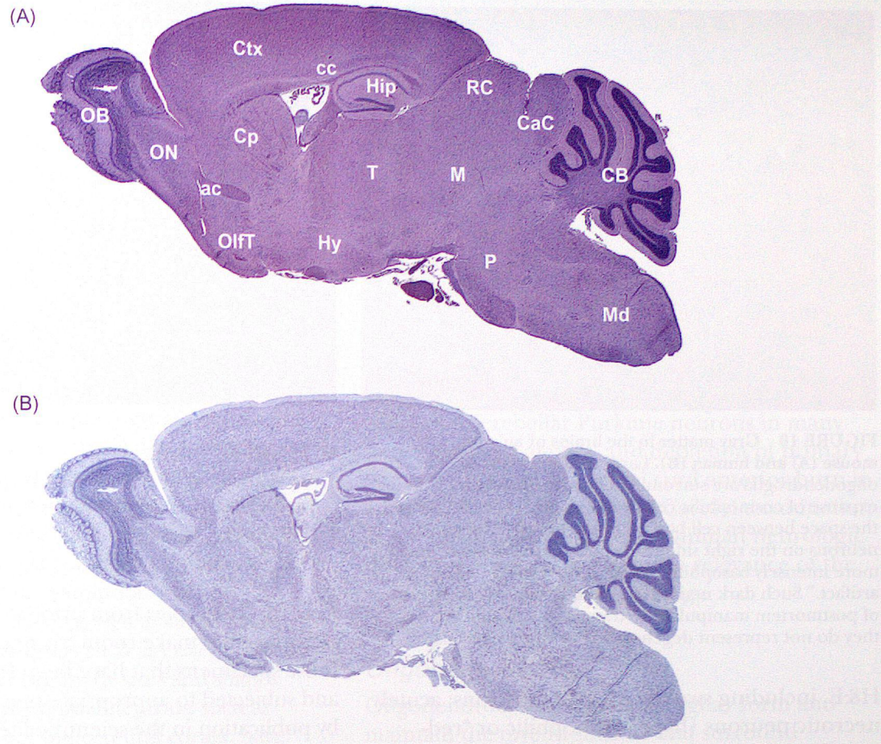


FIGURE 9 Parasagittal sections of adult mouse brain contrasting anatomic features visible using H&E and LFB stains (A; labeled) and Nissl stain (B; unlabeled). Collections of neuronal cell bodies (brain nuclei) are delineated better using Nissl stain (which enhances the cell bodies of RNA-rich, metabolically active neurons), whereas myelin is best visualized with LFB (as dark blue tracts). ac, anterior commissure; CB, cerebellum; cc, corpus callosum; CaC, caudal colliculus; Cp, caudate/putamen; Ctx, cerebral cortex (neocortex); Hip, hippocampus; Hy, hypothalamus; M, mesencephalon (midbrain); Md, medulla oblongata; OB, olfactory bulb; OlfT, olfactory tubercle; ON, olfactory nucleus; P, pons; RC, rostral colliculus; T, thalamus.

white matter; Bielchowsky's or Bodian's silver stains, which emphasize axons; and Fluoro Jade dyes, which delineate degenerating neurons. Some stains may be combined on the same section, such as H&E and LFB (Figure 7). The application of such special stains highlights the difference that specific visualization of neurons and white matter can make in examining the brain anatomy in the mouse (Figures 7A–AF, 7H, 8, and 9) and human (Figures 7G and 7I). Immunohistochemical alternatives exist for some stains—anti-neurofilament protein (NFP) detects neurons and their processes, whereas anti-myelin basic protein (MBP) labels myelin—and other immunohistochemical methods specifically show certain cell populations (e.g., anti-glial fibrillary acidic protein (GFAP) for astrocytes, or anti-CD68 or anti-ionized calcium-binding adaptor molecule 1 (Iba-1) for activated microglia in humans and mice, respectively). Unless a compelling reason exists for choosing a more complex strategy, the initial search for neural lesions should be conducted with such simple, inexpensive, conventional histologic methods as H&E, cresyl violet, and LFB. More complicated morphologic (e.g., electron microscopy), immunologic (e.g., immunohistochemistry, immunofluorescence) and molecular (e.g. *in situ* hybridization) methods should typically be deferred until the need arises to correlate gross and basic histomorphologic abnormalities. Be wary of any "lesion" that is only observed with immunohistochemical staining because such findings are often spurious in the absence of another structural alteration.

Different staining modalities can be used to optimally visualize certain neuropathologic processes. Many lesions show well with routine

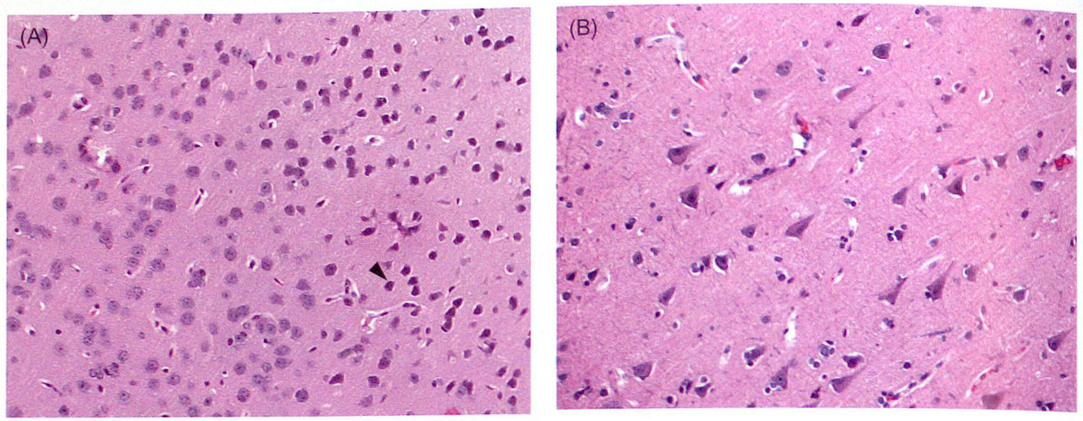


FIGURE 10 Gray matter in the brains of an adult mouse (A) and human (B). Large neurons, astrocytes, and oligodendroglia are surrounded by neuropil, an amorphous expanse of eosinophilic cell processes ("sea of pink") filling the space between cell bodies. In the mouse image, the neurons on the right side (arrowhead) exhibit the contracted, more intensely basophilic appearance of "dark neuron artifact." Such dark neurons are thought to be a consequence of postmortem manipulation/trauma in ischemic brain tissue; they do not represent degenerating or dying neurons.

H&E, including swollen, dystrophic axons; acutely necrotic neurons (hypereosinophilic or "red-dead" cells); and hypertrophic reactive astrocytes. In contrast, CV or Nissl primarily stains RNA, highlighting the cellular rough endoplasmic reticulum (RER) in metabolically active neurons, so CV staining in degenerate or dead neurons (e.g., acute or subacute hypoxia–ischemia) is prominently reduced as a consequence of ribosomal disassociation from the RER. Damage to white matter, such as in mouse models of autoimmune leukoencephalomyelitis, is best seen with LFB or other myelin stains.

A final and often forgotten point is to seldom if ever discard any tissues. If you habitually fail to retain all samples, you will almost certainly encounter one or more occasions in which you will wish that you had not. Formalin-fixed neural tissues may be retained for an extended period in the "wet" state in formalin-filled bags. Bouin's-fixed specimens must be trimmed and embedded within a few days of fixation to avoid excessive hardening; we recommend that formalin-fixed tissue slated for later molecular analysis also be completely trimmed and embedded to provide for easier storage (tissue blocks do not leak, and they take less space than fluid-filled bags) and better retention of molecular integrity. If samples from prior studies must be thrown out to make room for new material, only those specimens that have been fully analyzed and subjected to appropriate peer review (e.g., by publication in the scientific literature and/or by another neuropathologist as part of a product registration package for a regulatory submission) should be considered for disposal.

Need-to-know

- Mouse cerebrocortical neurons appear more compact and are more densely bunched compared to human neurons.

Cell types

Cells of the CNS can be divided into two categories based on their embryonic origin. Cells arising from the neuroectodermal layer include neurons, astrocytes, oligodendrocytes, and ependymal cells. Cells of mesenchymal origin include the meninges, blood vessels, and microglia. The catchall term "glia" refers to astrocytes, oligodendrocytes, ependyma, and choroid plexus. Microglia are sometimes included in this category as well, but they are not true "glia" because they are thought to arise from yolk sac progenitors and/or circulating precursors of the macrophage lineage. In the CNS, each neuron is sustained by approximately 10–50 glial cells. More detailed descriptions of CNS histology and cytoarchitecture, including common lesions and artifacts, may be found in standard histology and neuropathology texts and also reviews listed in Further Reading and Relevant Websites.

Neurons

The functional unit of the nervous system is the neuron. Neurons have one or more dendrites through which they receive input from other neurons and one axon that synapses on other neurons or non-neural tissues, such as the musculature. Within the adult human brain, there are approximately 130 billion neurons forming 150 trillion synapses.

Neurons can be categorized in a number of ways, but the principal features used to distinguish populations of neurons are their neurotransmitter phenotype and their morphologic appearance. Neurons have a high metabolic rate, which makes them extremely vulnerable to certain global toxic insults that impair intracellular energy metabolism. Neurons typically secrete a single small molecule neurotransmitter, most commonly glutamate (in excitatory cells) or γ-aminobutyric acid (GABA; inhibitory cells), as well as certain small peptide neurotransmitters such as enkephalin or parvalbumin. There are many sizes and shapes of neurons. Large pyramidal neurons, such as projection neurons of the cerebral cortex, have relatively large cell bodies, nuclei with a single prominent nucleolus, and prominent Nissl substance (rough endoplasmic reticulum) in the peripheral soma. These features may not be apparent in smaller neurons, such as the granule neurons of the cerebellar cortex. Interneurons are usually smaller than projection neurons (so called because their long axons innervate distant central nuclei or peripheral tissues). However, there are clear exceptions to this pattern; in the striatum, some interneurons are larger than the prevalent GABAergic medium spiny projection neurons. The variety of neuronal appearances can help pathologists identify specific brain regions and nuclei. Generally, neurons have multiple dendrites surrounding their cell bodies and a single axon. Neurons can also be categorized by the number of processes extending from the cell body. Unipolar neurons have one axon. Bipolar neurons have an axon and one dendrite extending from the cell body toward opposite poles. Multipolar neurons have multiple dendrites and a single axon. Generally, in the nervous system of mice, neurons are smaller and populate the neuropil more densely than is evident in the same structures of human neural tissues (Figure 10). The most common markers for neurons are NFP, neuronal nuclei (NeuN), and protein gene product 9.5 (PGP 9.5) for general detection of cells, as well as neurotransmitters or transmitter-producing enzymes for specific neuronal populations.

Many neurologic insults can affect any part of the CNS, including stroke, infection, and trauma. A subset of neurologic disorders, particularly chronic neurodegenerative diseases, are characterized by specific loss of a given neuron type. Examples include loss of GABAergic medium spiny neurons of the striatum in Huntington's disease, reduced dopaminergic neurons of the substantia nigra in Parkinson's disease, decreased cerebellar Purkinje neurons in many spinocerebellar ataxias, and depletion of primary motor neurons of the spinal cord anterior horn in amyotrophic lateral sclerosis. Careful examination of potential murine models of human neurologic diseases is required to assess the relevance of the model to human pathophysiology.

Oligodendrocytes

Oligodendrocytes (oligodendroglia) form and maintain the myelin sheaths that surround processes of CNS neurons. Each oligodendrocyte sheathes multiple axons. Oligodendrocytes have round nuclei with condensed chromatin that stain darker than those of astrocytes and neurons, and they lack basal lamina. These cells are called "satellite cells" when they are found next to neuron cell bodies in gray matter. Immersion-fixed tissue commonly exhibits a clear "halo" artifact of the oligodendrocyte cytoplasm that gives the cells a "fried egg" appearance. This artifact does not occur when tissue is fixed by perfusion. Oligodendrocytes are often seen in linear rows between the nerve fibers of white matter tracts (Figure 11). Oligodendrocytes/myelin are the chief targets in autoimmune white matter disorders such as multiple sclerosis in humans and experimental autoimmune encephalomyelitis (EAE) in rodents. Destruction of myelin in the CNS is essentially permanent. Some common oligodendrocyte markers are carbonic anhydrase II, CNPase (2′,3′-cyclic nucleotide 3′-phosphohydrolase), MBP, and myelin oligodendrocyte glycoprotein (MOG).

In the PNS, axons are myelinated by Schwann cells. These cells have elongate, wavy nuclei

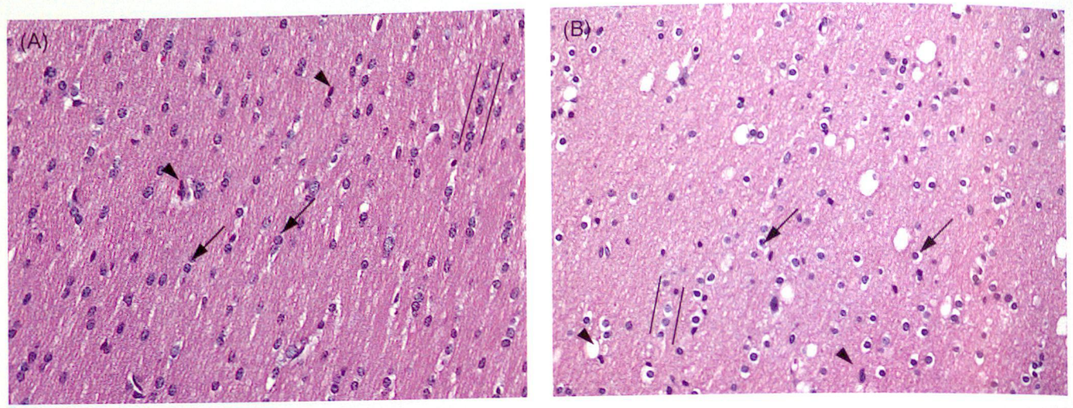


FIGURE 11 White matter in the adult mouse (A) and human (B). Oligodendrocytes (arrows) have round, dark nuclei and are arranged in long rows (bracketed by lines) oriented parallel to the axons that they myelinate. In H&E-stained sections, white matter is slightly more eosinophilic and less cellular than gray matter, and the neuropil has a more organized, streaming appearance. The human oligodendrocytes often have a slight perinuclear halo ("fried egg" appearance). This feature occurs as an artifact of immersion fixation and is not prominent in the mouse image because the animal was perfused with fixative. Occasional astrocytes (arrowheads) are evident as oblong, irregularly shaped nuclei. The cytoplasm of astrocytes (and microglia) normally blends with the neuropil and typically cannot be observed unless the cells are activated.

(see Peripheral Nerve section for further details) and form the myelin sheath for a single axon. Damaged myelin in the PNS may be repaired by Schwann cell proliferation, although in this event the length of each internode (the distance between Schwann cells) is reduced. Standard Schwann cell markers are CNPase, MBP, peripheral myelin protein 22, and S100β.

Astrocytes

Astrocytes are the most common glial cell, with the number of astrocytes equaling or exceeding that of neurons in most brain areas. These cells support neurons in many ways. Neurons rely on astrocyte-derived thiols to maintain stable glutathione concentrations; low glutathione renders neurons more susceptible to injury from oxidative stress. Astrocytes also take up and recycle neurotransmitters (glutamate and GABA), maintain the ionic composition of the extracellular milieu, and preserve the integrity of the blood–brain barrier. The cytoplasmic processes of astrocytes give them their starlike (stellate) shape. These processes extend outward and can make contact with any part of a neuron's surface. Astrocyte foot processes contact CNS capillaries and induce endothelial cells to form tight junctions during brain development *in utero*.

The cytoarchitecture of astrocytes is characteristic. Astrocyte nuclei are approximately the same size as many neuronal nuclei but are larger than oligodendrocyte nuclei. They are round to ovoid, have small or indistinct nucleoli, and have pale, vesicular euchromatin (Figure 11). Cytoplasmic processes of nonreactive astrocytes are inconspicuous in H&E-stained sections. However, when astrocytes react to injury, their cytoplasm and processes become distinctly eosinophilic and expand substantially due in part to the accumulation of intermediate filament proteins (e.g., GFAP) so that their cell borders become distinguishable. The culmination of these changes results in "gemistocytes," which are reactive astrocytes with blunt processes and markedly expanded and eosinophilic cytoplasm usually accompanied by nuclear eccentricity. The immunostain most frequently used to demonstrate astrocytes is GFAP.

Microglia

Microglia are the resident histiocyte-type cell and the key innate immune effector of the CNS. They are often described as either resting (i.e., ramified) or activated, but these terms fail to convey the dynamic remodeling of their fine processes and constitutive immunosurveillance activity. Their origin is highly debated. Whereas some microglia are derived from circulating

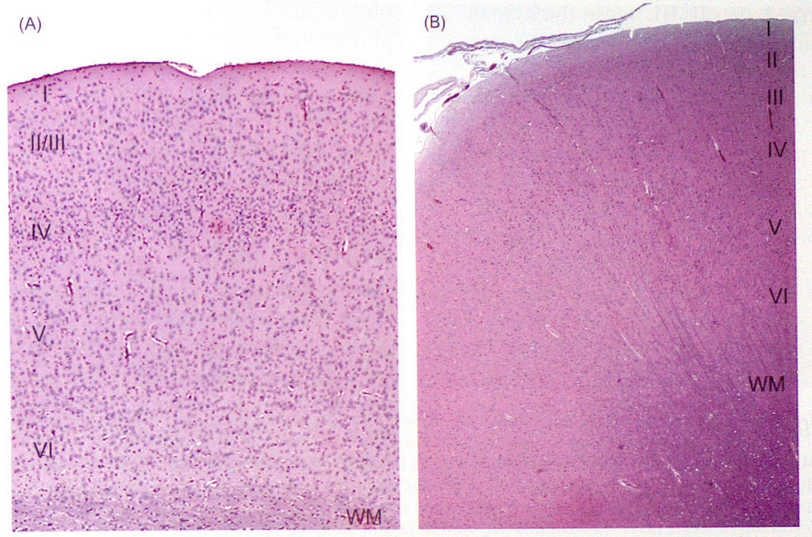


FIGURE 12 Neuronal organization in the cerebral cortex of an adult mouse (A) and human (B). The cortex contains six layers, although in the mouse, layers II and III are merged together as layer II/III. Layer I (molecular layer) lies beneath the meninges and contains neuropil and few neuron cell bodies. The remaining strata are layers II (external granular cell layer), III (external pyramidal cell layer, composed of small pyramidal cells), IV (internal granular cell layer), V (internal pyramidal cell layer, containing large pyramidal cells), and VI (multiforme layer, with elongate fusiform neurons). To highlight cellular detail of each species, the mouse image is presented at a higher magnification. WM, white matter.

bone marrow-derived monocytes, particularly in the setting of acute or chronic injury, evidence suggests that early microglia are derived from yolk sac progenitors. Thus, microglia in adult mice and humans are the result of a combination of proliferation of the resident population and migration into the CNS by myeloid progenitors. In H&E-stained sections of normal brain, microglia are relatively few in number. Such "resting" microglia have small, dark, rod-shaped nuclei with condensed chromatin (Figure 11); they are smaller than the nuclei of astrocytes. The cytoplasm of surveying (not activated) microglia is inconspicuous. In contrast, activated microglia that have become distended by phagocytosed material resemble foamy macrophages and are sometimes designated Gitter cells or foam cells. The markers frequently used to demonstrate microglia are CD68 in humans and CD11b or Iba-1 in mice.

• Brain regions

Cerebral cortex

The cerebral cortex has an outer layer of gray matter and a central core of white matter. The cortex has two major types of neurons: granule (stellate) cells and pyramidal cells. Granule cells typically have small profiles with indented nuclei and no spines, and they function as intracortical interneurons. The larger pyramidal cells are triangular with variable amounts of cytoplasm, a central round nucleus with fine chromatin and prominent nucleolus, pronounced apical dendrites that extend toward the cortical surface, and many dendritic spines. Axons of pyramidal cells extend long distances as either corticocortical efferents or efferents to subcortical structures. Within a given region, mouse neurons are smaller and more densely packed than human neurons.

The mammalian cerebral cortex has a developmental period during which its elements are laid down in six parallel layers, each designated by a Roman numeral (Figure 12). The molecular layer (I) is the most superficial lamina. It is composed of neuronal processes (neuropil) and glia but has very few neuronal cell bodies. The next layers are the external granular cell layer (II) and external pyramidal cell layer (III). These levels cannot be readily distinguished in many regions of the mouse brain and are thus

usually referred to as II/III, but in the human brain they are fairly distinct. The deepest layers are termed the internal granular cell layer (IV), internal pyramidal cell layer (V), and multiforme layer (VI). Afferents to the cerebral cortex arise in the ipsilateral and contralateral cortex (terminating mostly in layers II and III) as well as in the thalamus (terminating in layers I, IV, and VI). Corticocortical efferents arise from layer III, corticostriatal efferents from layer V, and corticothalamic efferents from layer VI. Functional and evolutionary differences between the mouse and human cerebral cortices are described later.

Basal ganglia

The basal ganglia include the striatum (caudate nucleus and putamen), globus pallidus, subthalamic nucleus, and substantia nigra. These brain regions are deep gray matter masses of the telencephalon and mesencephalon (substantia nigra). In the human brain, the caudate nucleus and putamen are separate but semicontinuous structures in the striatum (sometimes referred to as the neostriatum) that are separated by the streaming fibers of the anterior internal capsule. The streaming of white matter tracts through the striatum gives it a striped or striate appearance, hence its name. In mice, the striatum is a single structure and is the most extensive of the basal nuclei. The rodent caudate/putamen is organized functionally along dorsoventral and mediolateral axes. The putamen and globus pallidus in the human brain are collectively also called the lenticular nucleus.

Histologically, the caudate nucleus and putamen are identical, and they have a common embryologic origin. Bundles of finely myelinated, small-diameter axons crossing the striatum toward the globus pallidus are called pencil fibers ("Wilson's pencils" or striatopallidal fibers) because of their appearance (Figure 13). The caudate nucleus and putamen are composed of semi-disorganized gray matter containing a mixture of projection neurons (principally the GABAergic medium spiny neurons) and cortical interneurons. The substantia nigra in humans is visible grossly by adolescence, as the neurons in this region accumulate neuromelanin—a coarse, dark cytoplasmic pigment that is a by-product of catecholamine (dopamine) synthesis. Histologically, the pigment is quite obvious in adult human tissue primarily as a result of years of intracellular accumulation. In mice, the cytoplasm of neurons has basophilic halos and bands visible on the cytoplasmic borders (Figure 14). Mice lack histologically visible neuromelanin, presumably because the short life span of this species prevents its accumulation.

Hippocampus

The hippocampus is part of the limbic system, the most primitive part of the cerebrum. The limbic system includes the amygdala, fornix, habenular nuclei, hippocampus, hypothalamus, septum, and anterior thalamic nucleus. Most limbic system nuclei are located in the diencephalon. The limbic system regulates emotions and behaviors that are important to socialization, survival (feeding, fighting, and fleeing), and memory. The limbic system is also known as the visceral brain and substantially influences autonomic function.

The hippocampus (from the Greek words for horse (*hippo*) and sea monster (*campus*)) has a distinctive C-shaped structural appearance resembling a seahorse. The parts of the formation include the Ammon's horn, dentate gyrus, and subiculum. Ammon's horn is a testament to the Greek god of shepherds, Ammon, who was depicted with large curved horns; the CA abbreviation for hippocampal fields derives from *cornu ammonis*, the Latin rendering of Ammon's horn. The hippocampus exhibits a rostrocaudal topographic orientation, both structurally and functionally. It is necessary for short-term memory function and is one of the first areas to show pathologic changes in Alzheimer's disease.

In mice, the hornlike shape of the hippocampus is readily apparent in coronal sections moving from cranial to caudal (Figure 15). The hippocampus in mice is oriented more dorsally within the cerebrum, whereas in the human brain it is oriented more ventrally (Figure 1). Ammon's horn is divided into regions CA1–CA4, with CA4 neurons located in the hilar area facing the dentate gyrus (Figures 15, 16A, and 16B). The pyramidal neurons of Ammon's horn are triangular with variable amounts of cytoplasm (more in human than in mouse cells) and dendritic spines (Figures 16E and 16F). As in the cerebral cortex, the hippocampal pyramidal

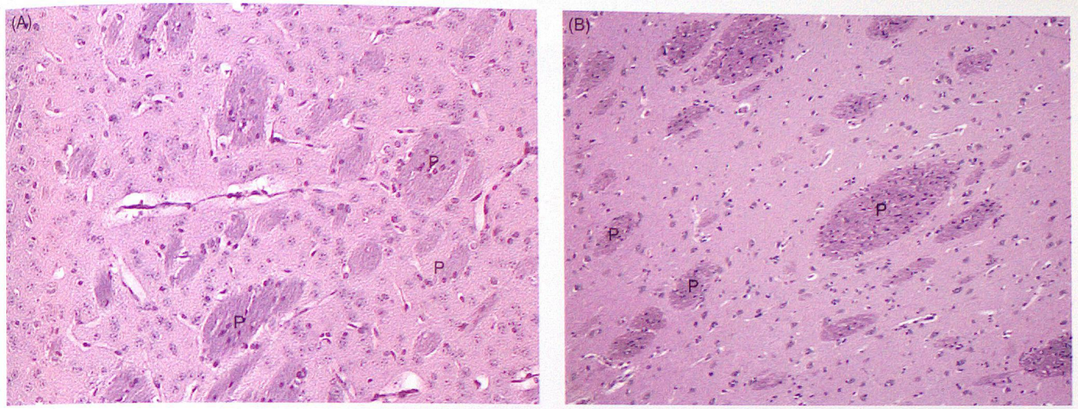


FIGURE 13 Caudate/putamen of an adult mouse (A) compared to the caudate nucleus of an adult human (B) (H&E with LFB). In humans, the caudate nucleus and putamen are separate but semicontinuous brain structures separated by streaming fibers of the anterior internal capsule. The alternating gray and white matter gives the tissue a striped appearance, so the two structures together are referred to as the "striatum" (meaning "striped"). In the mouse, the striatum is also referred to as the caudate/putamen. Bundles of finely myelinated, small-diameter axons (P) crossing the striatum toward the globus pallidus are called "pencil fibers" ("Wilson's pencils" or striatopallidal fibers) in humans; although the term is not frequently encountered in mouse neuroanatomy references, it aptly describes the appearance of white fiber tracts in mouse caudate/putamen. Histologically, the caudate and the putamen are nearly identical, and they have a common embryologic origin.

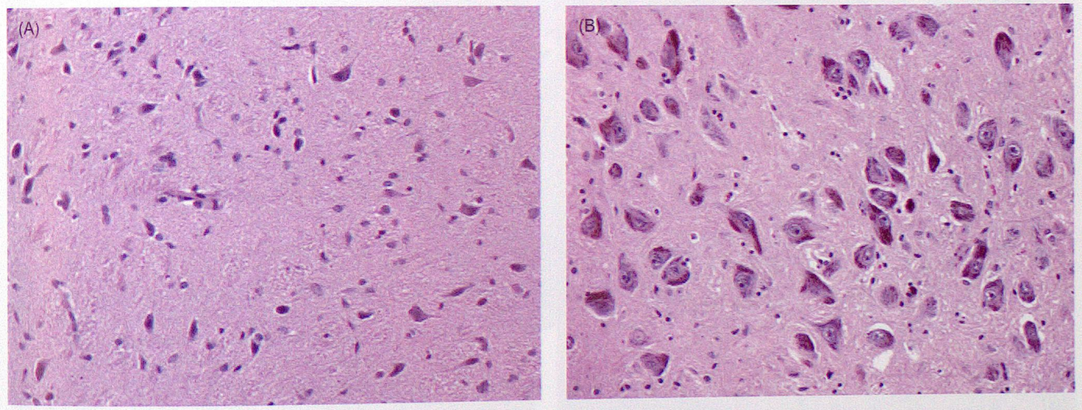
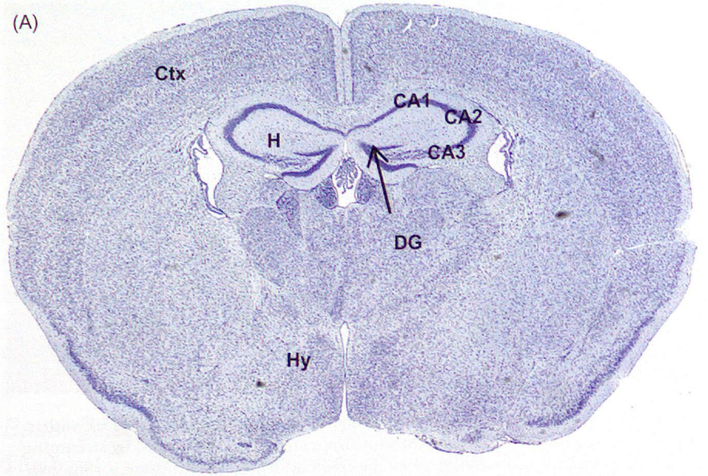
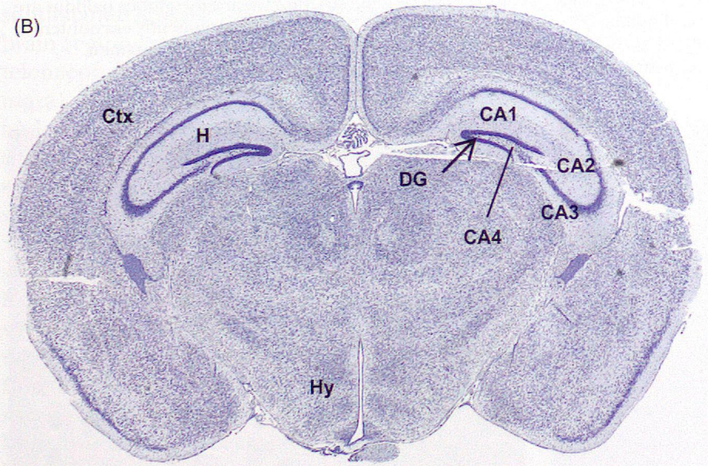


FIGURE 14 Substantia nigra in the adult mouse (A) and adult human (B). The region takes its name from the accumulation of neuromelanin (dark cytoplasmic pigment granules) in the large dopaminergic neurons in human substantia nigra. In the mouse, the same neuronal population is characterized by a narrow halo of cytoplasm bordered by a basophilic band. The pigment in the mouse is not visible, as it is in the human. It takes approximately 3 years of accumulation for pigment to become microscopically visible in humans.





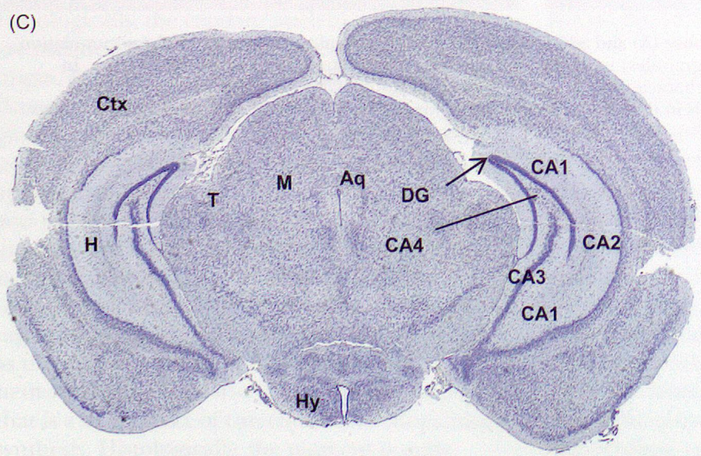
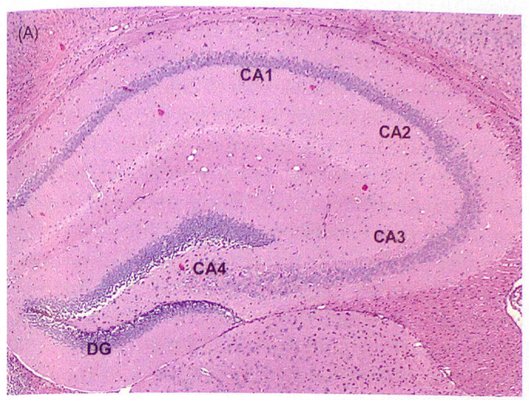
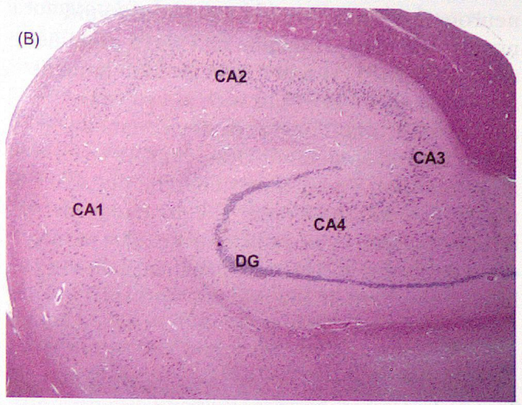
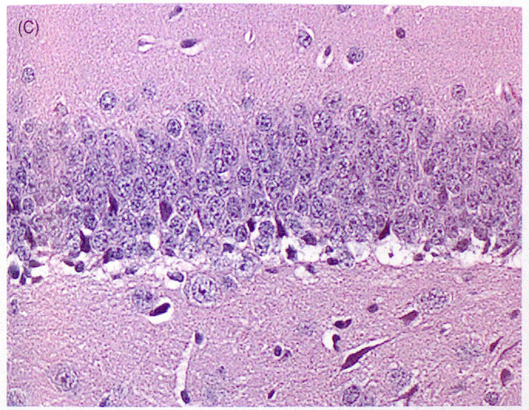
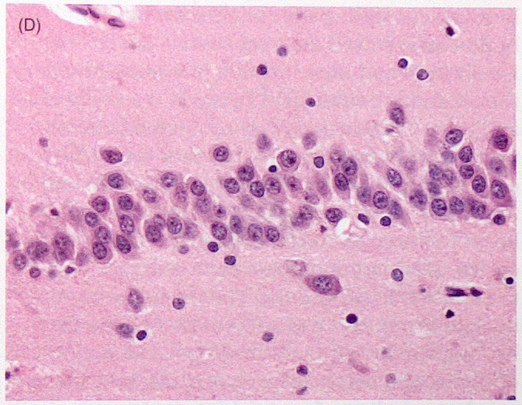


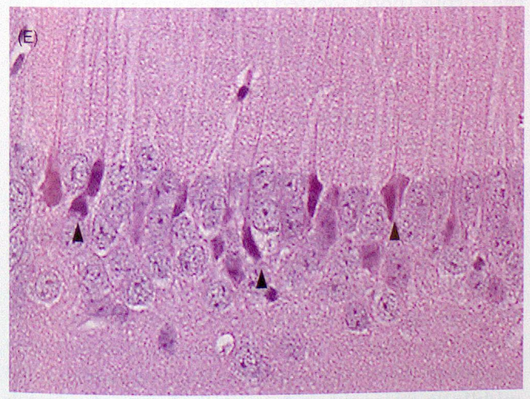
FIGURE 15 Coronal (cross) sections of adult mouse brain demonstrating the conformation of the hippocampus at its head (A), body (B), and near its tail (C) (Nissl stain). The hippocampus is a set of narrow zones that follow an S-curve starting at the dentate gyrus (DG; meaning "tooth-like"; the arrow points at its apex). The DG is a densely packed layer of granule cells. The hippocampus proper is divided into a series of cornu ammonis (meaning "ram's horn") subfields containing pyramidal neurons, which are designated CA1–CA4 regions. In the region of the mesencephalon (M), the CA3 field becomes quite narrow. The CA4 subregion does not have pyramidal morphology like other CA regions because it is actually the most superficial layer of the DG and contains mossy fibers. It is also referred to as the hilus, hilar region, or the polymorphic cell layer. Aq, mesencephalic aqueduct; Ctx, cerebral cortex (neocortex); H, hippocampus; Hy, hypothalamus; T, thalamus.











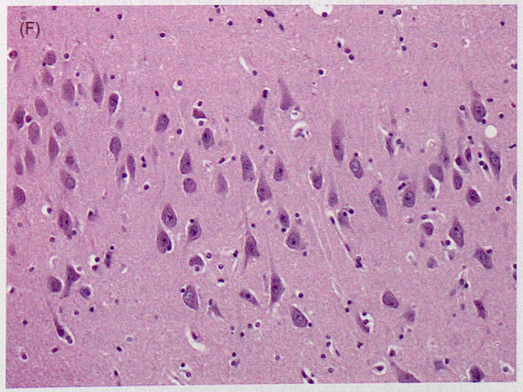


FIGURE 16 Organization of the hippocampus in the adult mouse and human (H&E). (A) Mouse dentate gyrus (DG) containing dense granule cells and CA1–CA4 regions containing pyramidal neurons. (B) Human DG and CA1–CA4 (hilus or superficial layer of DG) regions. The DG granule cells are more numerous and densely packed in the mouse (C) relative to the human (D). Granule cells project to the mossy fibers of CA4 and pyramidal cells of CA3. Mouse CA1 pyramidal neurons (E) are more densely packed, much smaller, and have less cytoplasm than their human CA1 counterparts (F). Note the presence of "dark neurons" (E; arrowheads)—a common histological artifact characterized by contracted, intensely basophilic neuron bodies—in the mouse. This artifact is thought to result from postmortem manipulation or trauma in ischemic brain tissue; it should not be interpreted as evidence of neuronal degeneration or death. The clear band of vacuolated cells (C) is another common processing artifact observed in the hippocampus of immersion-fixed mouse brains. Mouse and human micrographs are shown at different magnifications to better illustrate the cytoarchitectural features of each species.

neurons are characterized by a central round nucleus with fine chromatin and also a prominent nucleolus. The CA1 and CA3 regions in humans are very sensitive to insults such as hypoxia and seizures, and thus represent a common site of neurodegeneration, whereas CA2 is more resistant. These same vulnerabilities are recapitulated in the mouse. The dentate gyrus is a band populated by granule neurons. The mouse dentate gyrus is more densely cellular than the human counterpart (Figures 16C and 16D).

Thalamus/hypothalamus

The thalamus is a structure that underscores how understanding the connectivity between brain regions (and their functions) can facilitate localization and identification of lesions. Much of the anatomic connectivity between the thalamus and other CNS structures is conserved between rodents and humans. The thalamus forms the central part of the diencephalon and is the seat for multiple nuclei that relay sensory information between lower brain centers and the cerebral cortex. Distinct functions can be assigned to certain nuclear groups. The mediodorsal nuclear group receives afferent fibers from the hypothalamus and sends its axons to all regions of the frontal cortex. The central thalamic nuclei are the rostral terminus of the reticular activating system and thus act to modulate consciousness via projections throughout the cerebral cortex and basal ganglia. The lateral thalamic nuclei transmit information to cognitive regions throughout the cerebral cortex. Ventral thalamic nuclei receive afferent fibers carrying all sensory signals from the head and body before relaying them to the sensory cortex and the lateral thalamic nuclei. The connections of the ventral nuclear group, unlike the more diffuse associations of the remaining thalamic nuclei, occur in a specific somatotopic manner, with hind limb information transmitted on the lateral aspects, forelimb information carried more medially, and facial information (carried by the trigeminal nerve) near the midline. Connections between the thalamus and the cerebral cortex are reciprocal. In aged mice, mineralization of the lateral thalamus is often observed as an incidental finding.

The hypothalamus, which lies ventral to the thalamus within the diencephalon, is positioned near the junction of all major neural pathways as well as the pituitary stalk. It integrates the homeostatic functions of the autonomic, endocrine, and somatosensory systems. Numerous behaviors are regulated, including those involved in cardiovascular tone, ingestion (eating and drinking), parental care, self-preservation, sex, and thermoregulation. The brain nuclei in this region are poorly delineated due to the very complex partitioning of this region by numerous fiber tracts. In the sagittal orientation, the midline periaqueductal nuclei regulate hormonal secretion by the pituitary gland; the medial nuclei receive input from the limbic system and use it to modify feeding, fighting, fleeing, and sexual behaviors; and the most lateral nuclei coordinate multiple sensory inputs and behaviors such as eating and drinking. In general, parvocellular (small) neurons in the hypothalamus project to the median eminence, whereas magnocellular (large) neurons send fibers directly to the neurohypophysis (i.e., the caudal/posterior gland of the pituitary gland). As with the thalamus, the basic anatomic relationships and pathways in the hypothalamus are conserved between rodents and humans.

Cerebellum

The cerebellum has a gray matter cortical layer and a central white matter core. It can be divided into the corpus (body, located dorsally and laterally) and flocculonodular regions. The corpus is concerned with coordinated muscle movements and maintenance of muscular tone. The muscles in various body regions are controlled by specific lobules of the corpus in a stereotypical pattern based on the somatotopic projection of the body on the cerebellar surface. The flocculonodular lobes regulate equilibrium. The cerebellum is naturally divided into distinct lobes, the nomenclature of which varies with the species. The midline elements are referred to as the vermis, whereas the lateral wings are the hemispheres. The vermis consists of three lobes—the cranial or anterior, middle or posterior, and the flocculonodular—subdivided into nine lobules. Anatomical divisions of the cerebellum do not correlate exactly to the functional divisions. For example, the primary fissure is the anatomic divider between the cranial and middle lobes of the cerebellum, but the most craniolateral part of the middle lobe engages in the same tasks as the cranial lobe.

The cerebellum has very intricate neural circuitry, but its histologic appearance is simple throughout its structure and is remarkably conserved between rodents and humans. The cerebellar cortex contains three layers, which are, from deep white matter to cortical surface, the granular layer, the Purkinje cell layer, and the molecular layer (Figure 17). The microscopic pattern of the pale molecular layer and the darker granular layer creates an image of a tree and is thus referred to as the *arbor vitae*, which means "tree of life." Given this feature, the cerebellum is arguably one of the most beautiful anatomical structures in the body. The granular layer contains densely packed granule neurons, similar to the cells in the dentate gyrus of the hippocampus. These small neurons have axons that extend superficially into the molecular layer. The mouse granular layer is less densely populated than the human granular layer. The Purkinje cell layer in humans and mice is composed of a single row of large Purkinje neurons with a single axon that extends deep into the cerebellum and numerous dendrites that project into and widely arborize in the molecular layer. Mouse Purkinje cells are smaller and have less cytoplasm than human Purkinje cells. Thus, the molecular layer's abundant neuropil network consists mostly of the granule cell axons and Purkinje cell dendrites. The cell nuclei within the molecular layer are mostly glial cells.

The connections within the cerebellum are quite complex. Fibers travel to and from the cerebellum via the cerebellar peduncles. The rostral (superior) peduncle contains afferent fibers from the ventral spinocerebellar tract but consists mainly of efferent fibers from the deep cerebellar nuclei to higher brain regions. The middle peduncle is composed entirely of axons from the contralateral pontine nuclei (which serve as the relay center for impulses from the ipsilateral (with respect to the nuclei) cerebral cortex). The caudal (inferior) peduncle contains afferent fibers from the olivary and vestibular nuclei as well as the dorsal spinocerebellar tract, and it relays efferent fibers to the vestibular nuclei and reticular formation. Afferent fibers to the cerebellum are of two types. Climbing fibers originate exclusively in the contralateral olivary nucleus and pass through the granular and Purkinje cell layers to enter the molecular layer, where they synapse with the abundant dendrites of the Purkinje cells in a somatotopic manner. Mossy fibers, the largest input path, originate in neurons of the brain stem nuclei and synapse with neurons in the granular layer. Axons from granule cells within the molecular layer relay impulses to many types of neurons. Axons of the Purkinje cells provide the primary efferent fibers from the cerebellar cortex; their axons descend to the deep cerebellar nuclei, after which projections from these nuclei extend to the inferior olivary nucleus, red nucleus, and thalamus. The cerebellum has no known primary motor nuclei or direct fiber connections to the lower motor neurons of the spinal cord.

Although remarkably conserved, several differences in cerebellar anatomy distinguish the brains of mice and humans. The cranial lobe spreads laterally to a greater extent in humans than it does in rodents. The paraflocular lobule is relatively large in rodents, but it is relatively insignificant in humans. The lobules of the middle lobe are uniformly small in rodents, but their lateral portions are much larger in humans due to an increase in the length and number of the folia. A generalization among species is that mammals with well-developed limbs capable of extensive independent movement (especially primates, including humans) have expanded lateral cerebellar hemispheres. At the tissue level, the corticopontocerebellar pathway that integrates higher brain centers with the cerebellum is most highly developed in humans. In addition, the cerebellar nuclei of rodents are not as discrete as those found in humans and primates. In general, these anatomic differences are associated with quantitative distinctions in cerebellar function, but the quality of the basic functions is comparable for humans and mice.

Brain stem

The brain stem includes the midbrain and pons rostrally and the medulla oblongata caudally. Neurons in this region are arranged in longitudinal columns of nuclei that represent rostral extensions of the functional zones found in the spinal cord gray matter. In general, the location of each nucleus is predictive of its function. For example, afferent fibers for somatic sensations from the head synapse in the sensory nucleus of the trigeminal nerve (cranial nerve V). This cranial nerve nucleus is located in the

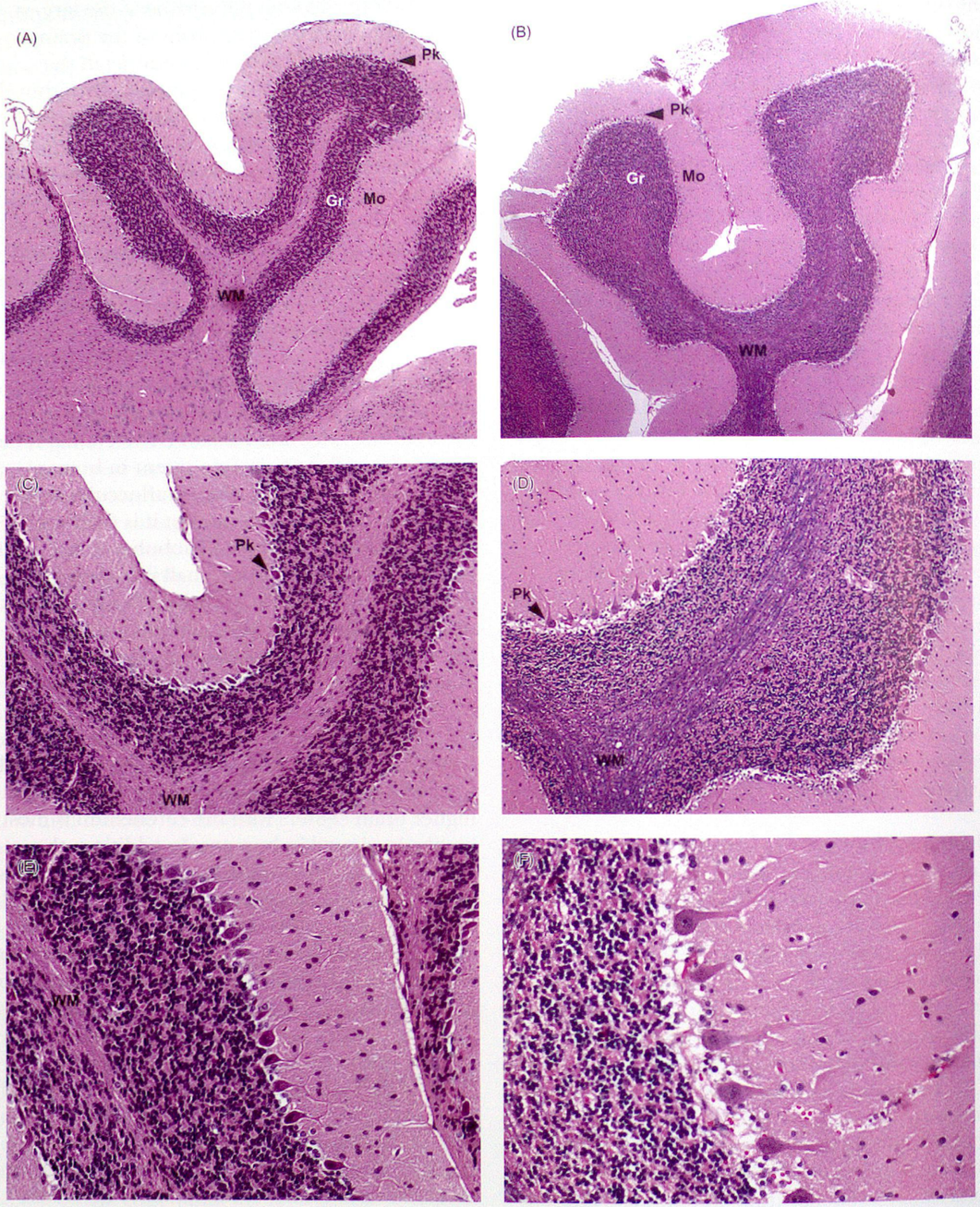


FIGURE 17 Cerebellum of the adult mouse (A, C, and E; H&E) and human (B, D, and F; H&E with LFB). The region is uniformly organized in three layers, which are (from outside to in) the molecular layer (Mo), Purkinje cell layer (Pk, with arrowheads), and the granular layer (Gr). The molecular layer is a broad expanse of densely packed neuronal processes with few neuronal bodies. The Purkinje cell layer is a single layer of large, torpedo-shaped cells with prominent apical processes extending into the molecular layer; the cells in the mouse are smaller and have less cytoplasm (E) relative to human cells (F). The granular layer is packed with small, dark, round granule cells, and it is more cellular in the human than in the mouse. The white matter (WM) core of each cerebellar folium (meaning "leaf") is visible in both species. Mouse and human micrographs are shown at different magnifications to better illustrate the cytoarchitectural features of each species.

same gray matter column as those that relay somatic sensations from the spinal nerves serving the forelimb (nucleus cuneatus) and hind limb (nucleus gracilis). The remaining tissue in the brain stem consists of various white matter tracts and the scattered neurons that form the reticular formation. On transverse sections, the reticular formation is divided into radiating, wedge-shaped zones, oriented with their apices near the floor of the fourth ventricle (Figures 7F and 7G). From medial to lateral, these wedges include the raphe nuclei on the midline, the gigantocellular (large cell) zone, the intermediate (medium to large cell) area, and the parvicellular (small cell) region. The lack of readily defined divisions between elements of the brain stem makes histological analysis of this region a formidable task.

Three major anatomic differences distinguish the functionally equivalent zones of the brain stem and the spinal cord. The first feature is the shape and position of the central canal. In the brain stem, the canal forms the fourth ventricle, is wide, and is displaced dorsally. The gray matter of the brain stem forms a "V" lining the floor of the ventricle rather than assuming the "X" conformation that surrounds the central canal of the spinal cord. Second, the gray matter columns in the brain stem form distinct nuclei, whereas those of the spinal cord are continuous. When the brain stem is viewed in cross section, both dorsal (sensory) and ventral (efferent) columns are separated by white matter tracts (Figures 7F–7H). The efferent columns are also divided into discrete nuclei in the longitudinal plane. Finally, the brain stem contains an additional gray matter column that is lacking in the spinal cord. Nuclei in this column supply special visceral efferent fibers to innervate the striated muscles originating from the pharyngeal arches.

The brain stem has three major functions. The first task is to integrate and modulate the activities of other brain centers. To this end, neurons of the brain stem nuclei and the reticular formation receive inputs from many ascending and descending pathways. The second purpose, coordination of autonomic sensory and motor activities, is performed by the various nuclei that contribute to cranial nerves V and VII–XII. Some nuclei have two functional components (sensory and motor), whereas others contribute fibers to more than one cranial nerve. Finally, many elements in the brain stem have prominent roles in regulating basal homeostatic functions. For example, the reticular formation contains major centers that regulate the involuntary reflexes that sustain cardiovascular and respiratory functions.

Like the cerebellum, brain stem anatomy is remarkably conserved between humans and rodents, although there are some species differences. The medial portion of the inferior olivary nucleus, which relays afferent fibers from higher and lower brain centers to the cerebellum, is present in all mammals. However, the lateral expansion of the cerebellar hemispheres in humans is accompanied by enlargement of the lateral portion of the inferior olivary nucleus (Figure 7G), with the enhanced development of the nucleus occurring in a craniocaudal direction. The increase in size corresponds with the increase in connections between this nucleus and the cerebellum. Two direct routes from the spinal cord to the thalamus are enlarged in humans because the size and specificity of the thalamic and cortical connections are greater than in rodents. These fiber bundles are the medial lemniscus, which carries the sensory information from the contralateral nuclei gracilis and cuneatus in the medulla oblongata, and the spinal (or lateral) lemniscus, which represents the conjoined sensory fibers of the spinothalamic and spinotectal tracts.

Functional and Structural Organization

Functional domains of the nervous system correlate with particular anatomic sites in the CNS, and neurons with comparable functions are clustered in regionally stereotypical patterns. Therefore, although mouse and human brain vary somewhat in the organization and size of various brain regions, they are comparable with respect to specific anatomic pathways, functions, and relationships therein. Several gross morphologic and tissue differences are readily apparent (as discussed previously). For example, humans have a considerably greater amount of white matter (Figure 18) due in large part to their markedly elevated encephalization quotient (the measure of actual brain size compared with that predicted

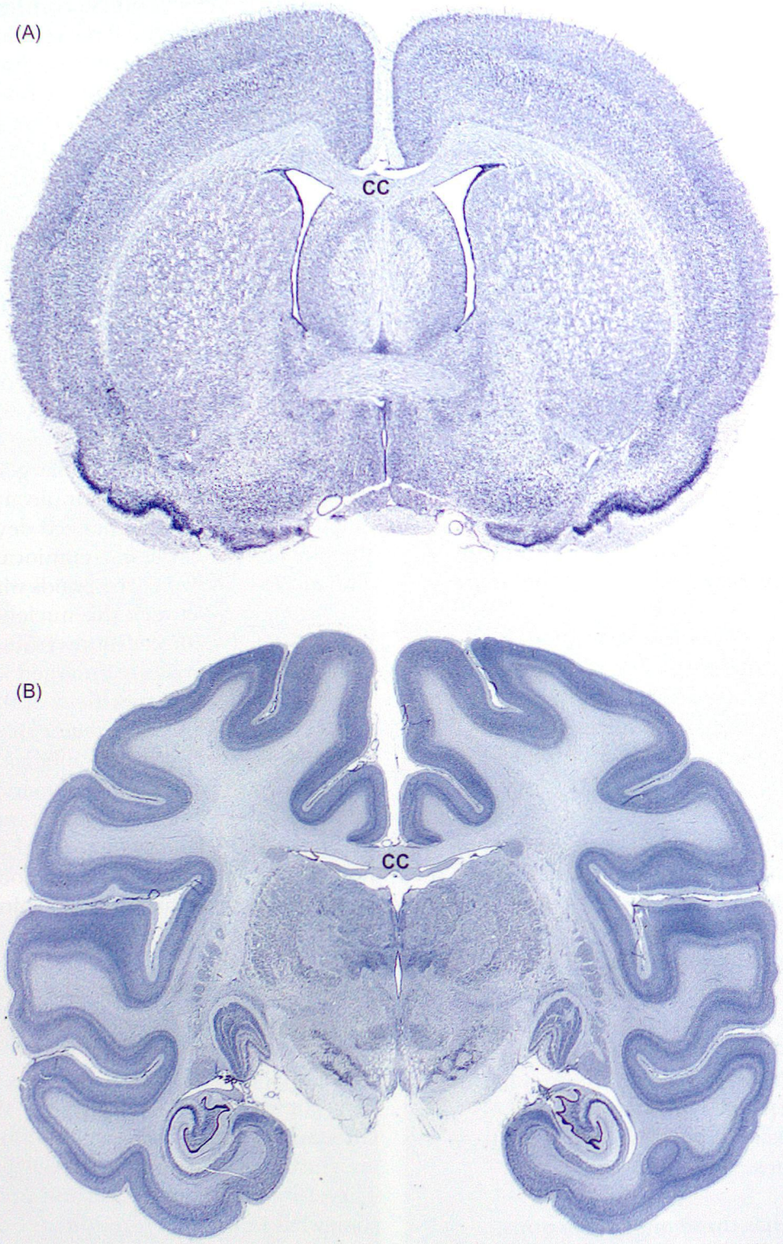


FIGURE 18 Nissl stain showing that the proportion of gray (dark blue) to white (pale blue) matter is much higher in the adult rodent (rat) brain (A) relative to its primate (cynomolgus monkey) counterpart (B). The primate brain is shown at a markedly reduced size to facilitate this comparison. cc, corpus callosum. Source: Images provided by Dr. Robert Switzer III of Neuroscience Associates (Knoxville, TN).

- Need-to-know
  - Humans have much more white matter than mice because they have more neurons and, therefore, more neuronal processes.

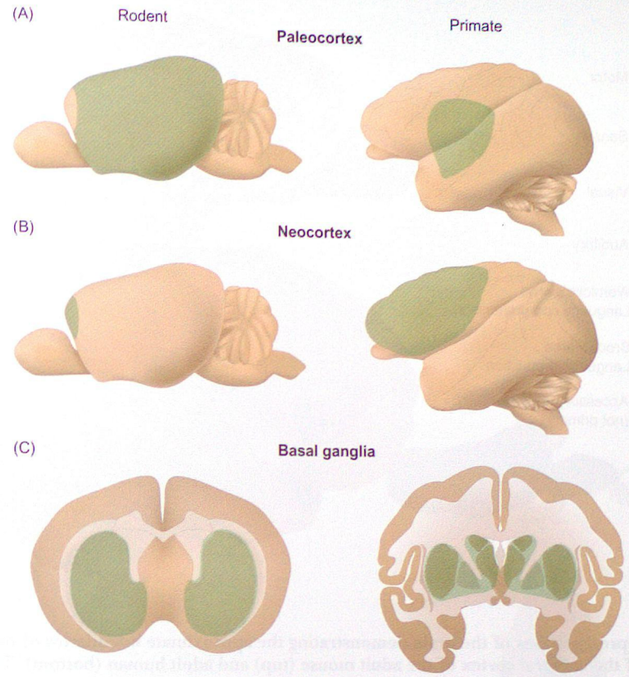


FIGURE 19 Schematic representations demonstrating the approximate boundaries of the paleocortex (A), neocortex (B), and the basal ganglia (striatum) (C) in rodents (left) and primates (right). Source: Figure by Sara Samuelson, S. S. Illustrations.

- Need-to-know
  - Rodent paleocortex does not fully recapitulate the human neocortical circuitry and functions.

by body weight). The human cerebral cortex can be organized by reference to surface topography (sulci and gyri), but the lissencephalic mouse brain does not enable comparable division. Such differences clearly demonstrate that functional areas of the brain are not bound to gross anatomical landmarks. Function is based on connected pathways; thus, the two species can be compared on the basis of internal nuclei (gray matter) and tracts (white matter).

The cerebral cortex can be divided into three regions based on phylogeny. The archicortex is the most primitive domain and consists of the olfactory bulbs, olfactory tracts, and olfactory cortex (the piriform lobe of mammals). The archicortex is quite large in proportion to the brain in rodents relative to humans. The paleocortex, the next oldest region, consists of the various derivatives of the limbic system: hippocampus, parahippocampus, and cingulate gyrus (in humans). Neocortex is the most evolutionarily recent form of cortical development, and it is functionally distinct from paleocortex and archicortex. Neocortex consists of the frontal (rostral), parietal (dorsolateral), temporal (ventrolateral), and occipital (caudal) lobes. Interspecies differences in cortical function hinge on the ratio of paleocortex (limbic system functions) to that of neocortex (associative and cognitive function). The cerebral cortex of rodents consists almost entirely of paleocortex with almost no neocortex (Figure 19), and the amount of neocortex increases in a graduated manner with ascent up the phylogenetic tree. The neocortex is extensive in primates (Figure 19), including humans.

Functional studies have shown that practically all regions of the cerebral cortex are connected with underlying subcortical centers (e.g., via the thalamus and basal ganglia). Nevertheless, particular cortical areas consistently relate to major functional modalities (Figure 20) and are referred to as projection areas for sensation, motor control, or sense integration (vision,

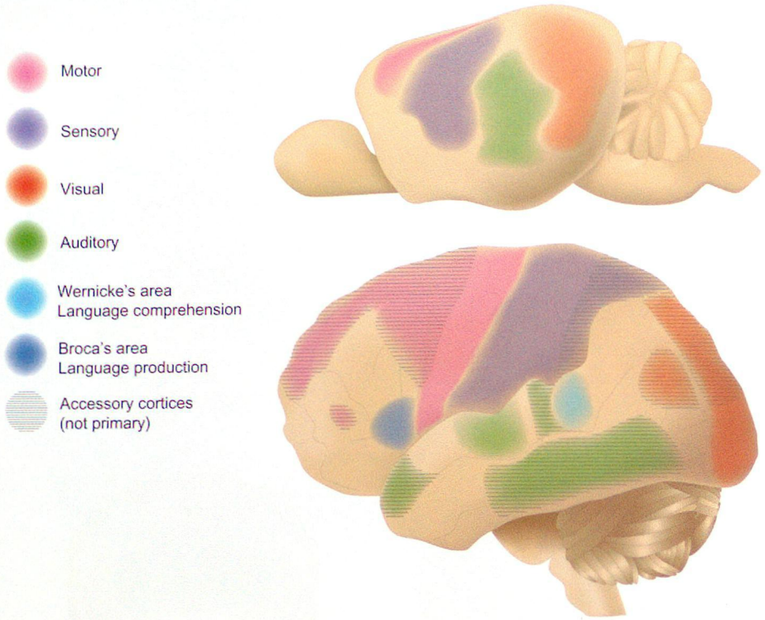


FIGURE 20 Schematic representations of the brain demonstrating the approximate boundaries of major functional zones as projected on the surface of the cerebral cortex of the adult mouse (top) and adult human (bottom). The mouse lacks accessory cortical areas. Source: Figure by Sara Samuelson, S. S. Illustrations.

olfaction, and audition). For example, in the forebrain, the primary somatic centers reside in the dorsolateral cerebral cortex, with the motor area located rostral to the sensory area. Topographical arrangements of these functional areas differ between mouse and human but are stable within each species. In rodents, the visual cortex is located more laterally than in humans. In the human but not the mouse, the sense integration areas also have accessory or associative cortices (Figure 20). These associated cortices do not have direct connections to the senses and are thought to be involved in higher mental processes.

Within the somatosensory and motor functional zones, body regions are projected somatotopically. In humans, the somatotopic maps of somatosensory and motor cortices are represented on opposite sides of the central sulcus (with motor rostral to it and sensory caudal to it). The maps begin on the medial surface and extend laterally, with caudal body parts represented most medially and rostral parts represented progressively more ventrally and laterally. Mammals vary considerably in the degree of separation and location of the somatosensory and motor cortices. Generally, body parts with the greatest sensitivity have the largest representation in the somatosensory cortex, and body parts with the most dexterity have the largest representation in the cortical motor map. Large representations on sensory cortex do not necessarily translate to large representations of motor cortex. For example, in the case of human hands, the index finger occupies the most sensory cortex, whereas the thumb occupies the most motor cortex.

Mice and humans make different use of their sensory and motor modalities. Thus, the proportional representation of various body parts on these cortices as a homunculus ("little man") or "musunculus" ("little mouse") differs markedly (Figure 21). Mice use olfaction and vibrissae (facial whiskers) in contexts that humans do not (or cannot). Each whisker has its own dedicated cortical area; these areas are called barrels because of their cylindrical shape, and they extend through the depth of cortical layer IV. The barrel fields are the face areas of the somatosensory and motor cortices of mammals that make extensive use of vibrissae (whiskers) for exploration of the world; they are a tactile early warning system—indicating

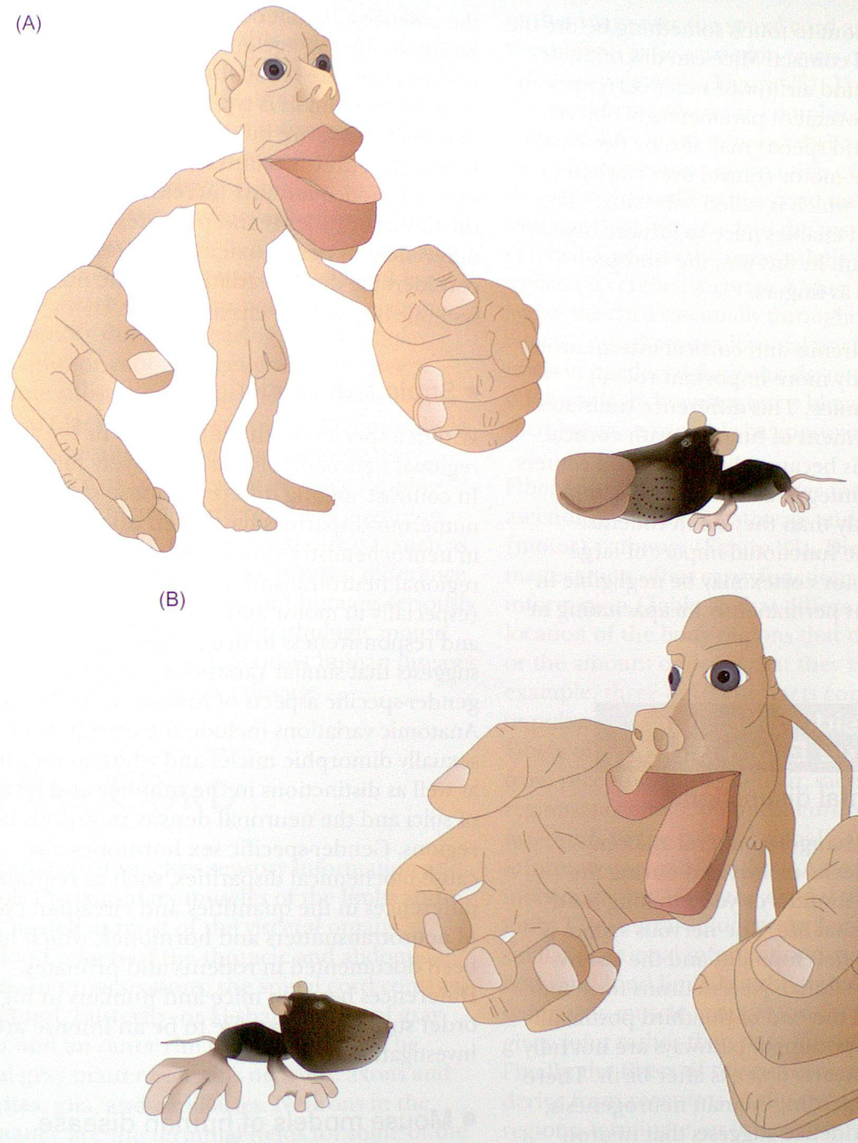


FIGURE 21 Sensory (A) and motor (B) homunculi ("little man") and "musunculi" ("little mouse"), where the sizes of the figures' body parts are proportional to the volume of somatosensory cortex (A) and motor cortex (B) devoted to their control. For somatosensory input, the hands and face are dominant in the human, whereas the nose (olfaction) and whisker barrels predominate in the mouse. For motor output, hands are the dominant structure in humans, whereas in mice motor tasks are divided nearly evenly between the limbs and such facial features as the upper lip, lower jaw, vibrissae (facial whiskers), and eye muscles. The homunculi are based on the models at the Natural History Museum in London. The somatosensory "musunculus" is derived from Vanderhaeghen et al. (2000), whereas the motor maps were defined using data from Pronichev and Lenkov (1998). Source: Figures by Sara Samuelson, S. S. Illustrations.

Need-to-know

- Shapes and proportions of body parts encoded in the somatosensory and motor cortices differ markedly.
- Mice have whisker barrels to innervate the vibrissae (facial whiskers), which serve as *de facto* fingers.

that the face is about to touch something before the skin makes actual contact. Mice can discriminate textures, shapes, and airflow or water currents with their vibrissae; movement parameters of objects (e.g., trajectory and speed) may also be detectable. Rodents also have motor control over vibrissae, the movement of which is called "whisking." The whisking function enables mice to localize objects and orient to them. In this way, the vibrissae function de facto as fingers.

Corticocortical circuits and cortical interneurons have a functionally more important role in humans than in mice. This difference translates to greater impairment of function with cortical injury for humans because the subcortical centers are more highly integrated and thus function less independently than they do in rodents. Consequently, the functional impact of large lesions in the motor cortex may be negligible in lower animals but permanently incapacitating in primates.

Other Considerations in Comparative Brain Biology

Developmental distinctions

The most rapid changes in neural anatomy and physiology occur *in utero* and during the first few days (or months or years in long-lived species) of postnatal life. The nervous system is the first to begin development and the last to become fully functional. New neurons form in young mice until the end of the third postnatal week. Mouse hippocampal pathways are not fully functional until nearly 6 weeks after birth. There are fewer data regarding human neurogenesis, but increasing evidence suggests that neuron numbers increase markedly during the first 2 years of life and continue to be produced into adolescence, and that full maturation of neuronal populations in certain areas of frontal cortex may take decades. Specific neural changes often occur in relation to the life span in mammalian species. Many age-related anatomic differences have been documented, including the extent of cerebral fissuration, regional differences in the rate of brain and spinal cord myelination, and neuronal densities in various brain regions. Another example is the reduction in the number of glial cell nests in the subependymal layer of the paleocortex that occurs with age. Functional variations among age groups may be considerable. For instance, the blood–brain barrier in developing animals is typically more porous than that of mature but not senescent adults. Levels of neurotransmitters, their receptors, and their synthetic pathways increase with age in the brains of rodents and primates. Age-related differences in neurotoxicity of xenobiotics may be considerable due to variations in the efficiency of systemic detoxifying enzymes.

Strain and gender

Within a species, strain differences in regional neuroanatomy are relatively minor. In contrast, among different mouse strains, numerous disparities have been documented in neurochemistry (hormone production and regional neurotransmitter levels), behavior (especially in motor and stereotyped actions), and responsiveness to drugs. Mounting evidence suggests that similar variations occur for some gender-specific aspects of human neurobiology. Anatomic variations include the existence of sexually dimorphic nuclei and white matter tracts as well as distinctions in the number and location of sulci and the neuronal density in various brain regions. Gender-specific sex hormones also cause biochemical disparities, such as regional differences in the quantities and circadian cycling of neurotransmitters and hormones, which have been documented in rodents and primates. Differences between mice and humans in higher order structures continue to be an intense area of investigation.

Mouse models of human disease

Mouse models—genetically engineered, induced, and spontaneous—are used to study a variety of human diseases. However, there are some limitations to these models based on species differences in neural structure and function. Mouse models rarely provide a faithful recapitulation of human disease. For example, several research groups have made different transgenic mice that express distinct elements of the human β-amyloid precursor protein, which is thought to play a role in the development of Alzheimer's disease (AD), but only one of these models demonstrates both functional and morphologic alterations representative of this disease in humans. Even in this example, neuronal loss is markedly reduced in mice compared with human AD. In another case, the first transgenic mouse model of Huntington's disease (HD) was created using an enormous expansion of genetic material (~150 CAG repeats) relative to the human disease entity, but nevertheless this model has been used extensively as a model of human HD. Researchers making use of behavioral tests should be aware that a number of mouse strains spontaneously develop hearing and vestibular defects and/or retinal degeneration as they age. Some substrains of mice (129 substrains, in particular) lack a corpus callosum. Behaviorally, a number of strains are predisposed to stereotypic behavior, such as bar chewing or "cage flipping." In addition to intrinsic differences in cognition that make modeling neurological diseases such as dementia difficult in mice, the marked difference in life span between rodents and humans seriously complicates interpretation of pathologic mouse models that recapitulate late-onset human diseases such as AD and Parkinson's disease.

Spinal Cord

The spinal cord receives sensory information and controls the voluntary muscles of the limbs and trunk, as well as most of the visceral organs and blood vessels of the thoracic and abdominal cavities. In cross sections, the spinal cord consists of a central, butterfly- or H-shaped zone of gray matter and an outer rim of white matter. The central gray matter contains neurons, axons and dendrites, glia, and capillaries. Neurons in the gray matter are the terminal fields for some of the white matter tracts (Figure 22). Mouse and human spinal cords are anatomically similar, but there are significant differences that are discussed next.

The spinal cord white matter contains multiple, bilaterally symmetrical fiber tracts segregated among the dorsal, lateral, and ventral columns (funiculi). Tracts are named according to the direction in which impulses are conveyed, with sensory (ascending) tracts beginning with the prefix "spino-" and motor (descending) tracts ending with the suffix "-spinal." Fibers may be carried in tracts on the same side as their brain center (ipsilateral), or they may enter the spinal cord and then cross the midline to be carried in tracts on the opposite side (contralateral) (Figure 23). The pattern of decussation may be quite complex. For example, fibers of the ventral spinocerebellar tract represent axons from neurons on one side of the spinal cord that cross the midline to ascend on the contralateral side and then cross back in the rostral (superior) cerebellar peduncle to reach their destination in the ipsilateral cerebellar cortex. Other tracts send axons across the cord essentially throughout their entire course (e.g., the reticulospinal tract). The courses of tracts in the dorsal funiculus do not overlap. In the other funiculi, however, tracts blend together so that their locations can only be approximated.

Fiber tracts in the white matter include both ascending (sensory) pathways and descending (motor) pathways (Figure 23). Distinct white matter tracts often carry functionally comparable information (Table 2) that differs only in the location of the body regions that they innervate or the amount of detail that they transmit. For example, three different tracts convey sensory impulses about muscle tone to the cerebellum. Fibers of the dorsal spinocerebellar tract originate from receptors in the lumbar, sacral, and caudal regions; terminate on interneurons in the caudal two-thirds of the cervical spinal cord; and transmit information regarding the position and tone of individual muscles in the caudal trunk and hind limb. The ventral spinocerebellar tract carries similar positional information from the caudal body and hind limb, but the signals deal with synergistic muscle groups positioned around a given joint rather than data for single muscles. Finally, the fibers of the cuneocerebellar tract derive from receptors in the cervical and thoracic regions, terminate on interneurons located in the most rostral region of the cervical spinal cord, and convey information regarding the position and tone of individual muscles in the forelimb.

The descending spinal cord tracts transmit motor information to the periphery. The corticospinal tract is the best known fiber tract. In humans, this huge bundle has undergone an evolutionary expansion that parallels the enhancement of dexterity of distal muscular and the development of "skilled" motor capacities; it is located in the lateral column. Unlike humans, the corticospinal tract in rodents is much smaller and located in the