1

General neuropathology

In this chapter, we will introduce the basic tools for diagnostic neuropathology starting with practical neuroanatomy and neurohistology. In the following, we will describe the process of collecting and sampling tissues and subsequently the basic histological reaction patterns to injury of the different cell types of the nervous system. Based on this information, we then describe a number of basic lesion types or patterns of disease. We also show how neurological diseases are classified into different disease categories (e.g. inflammation, tumors, etc.) and which of the basic patterns can be expected to occur in each of these categories. Recognizing these patterns and histological responses, together with a basic understanding of the classification system, provides a critical diagnostic guide for classification of specific disease categories, each of which is covered in one of the subsequent chapters.

1.1 Principles of neuroanatomy for diagnostic neuropathologists

The nervous system is anatomically immensely complex with important structural and biochemical differences between its various regions. As a result these different regions have, to a certain extent, their own diseases. Therefore, some basic understanding of neuroanatomy is essential for diagnostic neuropathologists. This includes the recognition of the major anatomic regions of the central nervous system (CNS) and how they interact both topographically and functionally. Such information will help to interpret the clinical information, to examine the brain in a standardized way and serve as a basis for using a brain atlas. Excellent concise and schematic information in these topics can be found in current text books of veterinary neurology.

1.1.1 Anatomical orientation by using the ventricular system

An effective approach to learning neuroanatomy is to identify and correlate all of the CNS regions by their relationship to the ventricular system of the brain (Fig. 1.1). The CNS in the adult animal develops after closure of the neural tube. This tubular structure is still preserved in both the central canal of the spinal cord and the aqueduct in the midbrain. During further development of the brain the neural tube forms specific evaginations caudally to rostrally: the fourth ventricle, the third ventricle and, in the forebrain, bilateral ventricles originating from two vesicles bulging at the rostral end of the neural tube (Fig. 1.1A). This basic structure undergoes further bending and distortion during subsequent development but remains recognizable in the postnatal animal. All anatomical structures originate from the subependymal zone of the ventricular system. This development is depicted in Fig. 1.1A. The lateral wall of the lateral ventricle develops into the cortex and the basal nuclei. As a result of unequal growth the lateral ventricles assume a half-moon shape (Fig. 1.1B) and the forebrain expands to cover the thalamus and midbrain. The thalamus–hypothalamus develops around the third ventricle; the third ventricle becomes ring shaped because the two halves of the thalamus connect in the midline (*interthalamic adhesion*) forming the dorsal and ventral lumens of the third ventricle. The midbrain develops around the aqueduct, the medulla oblongata from the ventral part of the fourth ventricle. Dorsally it gives rise to both a thin layer of tissue (the *medullary velum*) and to the cerebellum, which forms above the fourth ventricle (Fig. 1.1C). The spinal cord develops from the central canal after closure of the caudal part

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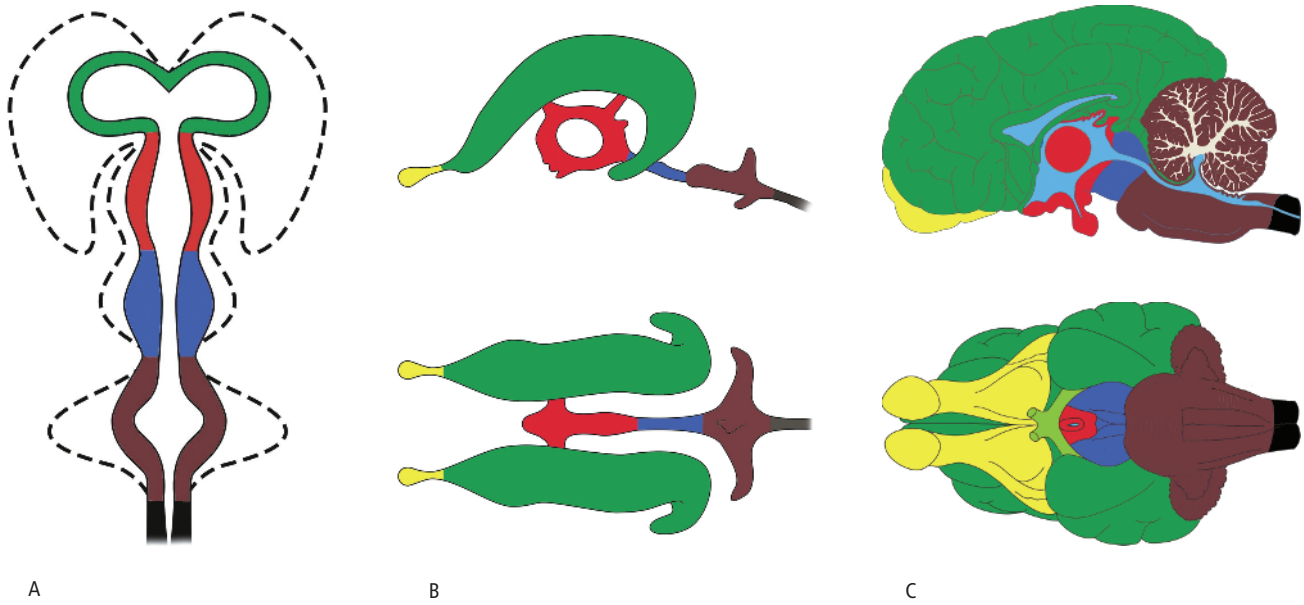

Fig. 1.1 Major divisions of the brain in relation to the ventricular system. A: Schematic drawing of the neural tube and its extensions (dorsal view). The dotted line indicates developmental growth of the periventricular tissues with the cerebral hemispheres overlapping the thalamus and midbrain. B: Schematic drawing of ventricular system dorsal and lateral view; different divisions of the ventricles are color coded. C: Medial and ventral view of an adult brain. The different colored areas arose from their respective color-coded sections of the ventricular wall. Yellow: olfactory bulb, tract and cortex; green: cerebral cortex; red: thalamus; dark blue: midbrain; brown: pons, medulla and cerebellum; black: spinal cord; light blue: cerebrospinal fluid. (Adapted from M. Stoffel: Funktionelle Neuroanatomie für die Tiermedizin, Enke, Stuttgart, 2011.)

of the neural tube. Additionally, there are several other extensions from within the ventricular system such as the olfactory canal extending from the lateral ventricles into the olfactory bulb, the *infundibular recess* extending ventrally from the third ventricle into the infundibulum, the lateral recesses of the fourth ventricle and the *suprapineal recess* dorsally from the third ventricle, which is best detected in sagittal magnetic resonance imaging (MRI) images. The choroid plexi in the walls of the lateral, III and IV ventricles develop from evaginations containing vessels and modified ependyma (*telea choroidea*) into the wall of the appropriate neural tube vesicles.

Thus when we transversely section the brain we can always identify some part of the ventricular system. Keeping in mind a three-dimensional concept of the ventricular system, as illustrated in Fig. 1.1, in each section we can thus correlate the shape of the ventricular system with the corresponding level of the CNS and also identify the relevant anatomical landmarks.

1.1.2 Major anatomical regions of interest

In this section we introduce the most diagnostically useful neuroanatomical sites of the CNS. The major regions of the CNS are the cerebral cortex and associated white matter, basal nuclei, thalamus/hypothalamus, midbrain, cerebellum, medulla oblongata and spinal cord. To perform a competent neuropathological evaluation, one should have at least a concept of how these major regions relate to each other topographically, preferably in all three dimensions, and be able to recognize the major landmarks.

This level of neuroanatomy is sufficient to start. Further information can be found in neuroanatomy textbooks and atlases, which should be consulted during the neuropathological examination to acquire a more detailed anatomical knowledge. This knowledge also needs to include the functional connections between certain structures, which are essential for the interpretation of secondary changes.

The CNS on external gross examination

External views of the brain are illustrated in Fig. 1.2.

Dorsally the cerebral cortex of the cerebral hemispheres is separated along the midline by the longitudinal cerebral fissure and divided into frontal, occipital, parietal and temporal lobes, the vermis of the cerebellum and the brainstem. Ventral and lateral views illustrate the olfactory bulb and tract extending into a bulbous structure, the piriform lobe representing the

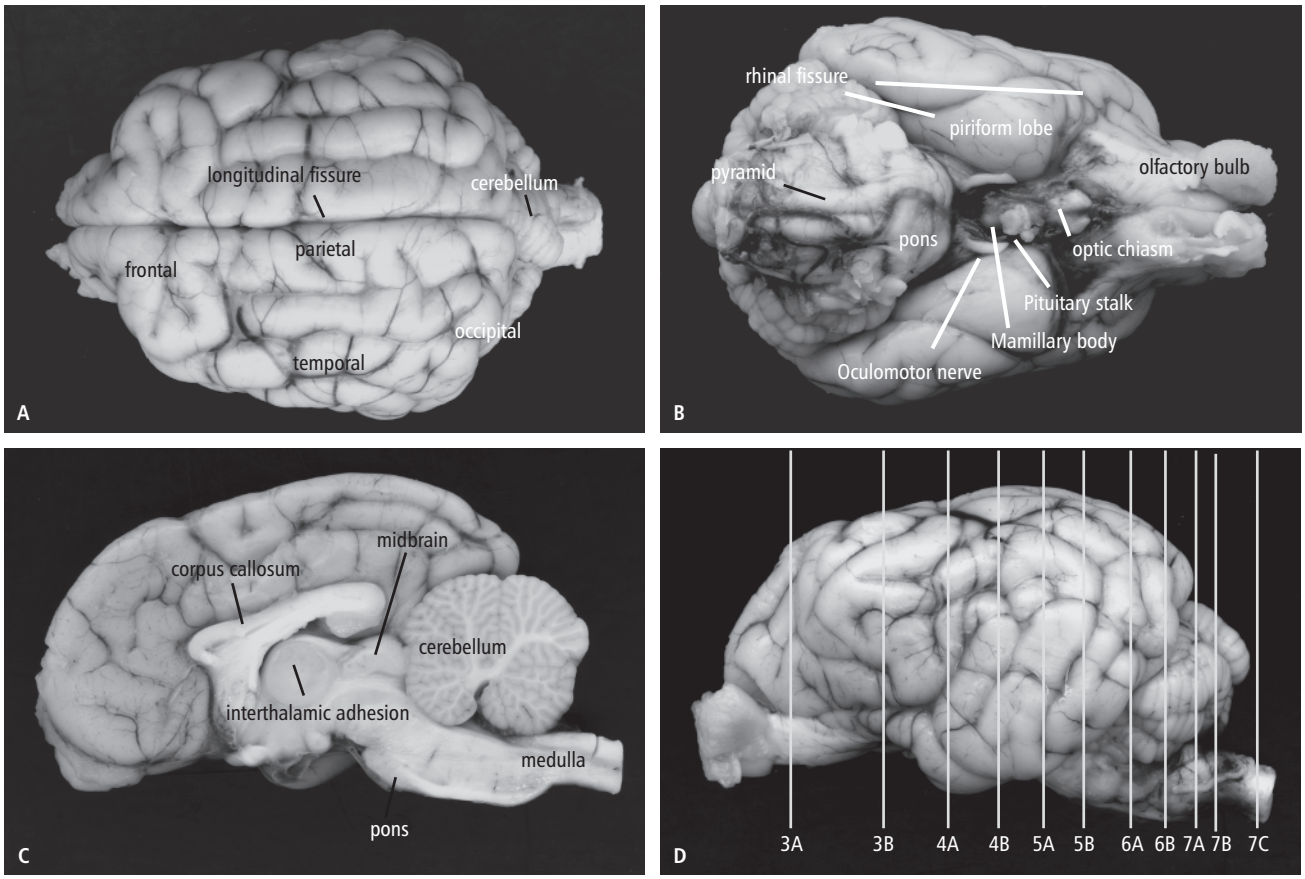

Fig. 1.2 Brain as seen externally. Dorsal (A), ventral (B), medial (C) and lateral (D) view indicating the transverse section levels shown in the subsequent figures (Figs.1.3–1.7).

most ancient part of the cortex (paleocortex) which is demarcated from the neocortex by the rhinal fissure. We need to recognize the optic chiasm, the pituitary stalk and the oculomotor nerves arising from the midbrain. The pons is the ventral bulge of white matter connecting the two cerebellar hemispheres, and also on the ventral aspect of the brainstem are the prominent pyramids, which are white matter tracts connecting the forebrain with the spinal cord. A medial view (Fig. 1.2C) following sagittal sectioning reveals the details of the ventricular system (as explained above), the corpus callosum, the interthalamic adhesion, the midbrain, brainstem and cerebellum. Fig. 1.2 D illustrates the levels at which the brain has been transversely sectioned to produce Fig. 1.3, Fig. 1.4, Fig. 1.5, Fig. 1.6 and Fig. 1.7.

The CNS in transverse sections

Serial transverse sections are illustrated in Fig. 1.3, Fig. 1.4, Fig. 1.5, Fig. 1.6 and Fig. 1.7. These brain slices have been stained to enhance the contrast between white and gray matter: the myelin content of the white matter is stained black. This is usually how brain sections are presented in a brain atlas and is somewhat reminiscent of T2W MRI images (see explanation below).

On transverse sections of the forebrain we can roughly discern three divisions according to the subcortical structures we can see: the frontal one-third containing the largest extent of the basal nuclei (Fig. 1.3), the middle one-third containing the thalamus/hypothalamus (Fig. 1.4) and the caudal one-third containing the midbrain (Fig. 1.5). Note that the caudal parts of the basal nuclei overlap with the thalamus and the caudal parts of the thalamus with the midbrain. Caudally to the forebrain we identify the brainstem, covered on its dorsal aspect by the cerebellum (Fig. 1.6 and Fig. 1.7). While studying the following transverse sections, keep the three-dimensional structure of the ventricular system in mind as the major feature for orientation to the major anatomical landmarks. In Fig. 1.3, Fig. 1.4, Fig. 1.5, Fig. 1.6 and Fig. 1.7 the colored drawing of the lateral view of the ventricular system (Fig. 1.1B) is shown indicating the level of sectioning.

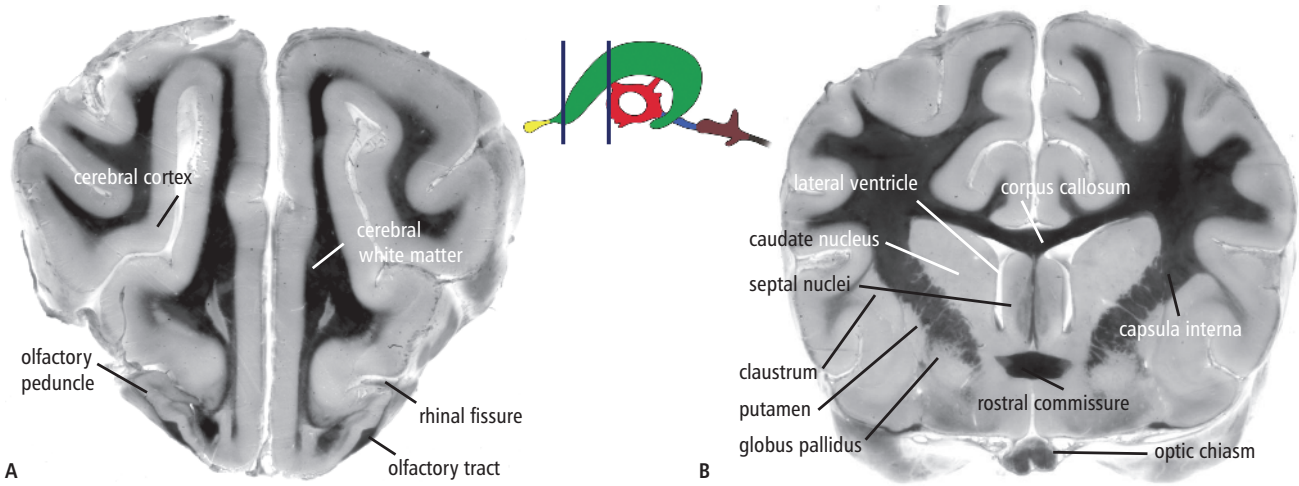

Fig. 1.3 A and B: Transverse sections frontal lobe and basal nuclei. Levels of sectioning shown in schematic drawing of the ventricles from Fig. 1.1.

Area of the basal nuclei (Fig. 1.3)

- Section A transversely slices the prefrontal area; the ventricles at this level consist of very narrow canals in the olfactory bulb (not visible). Section B transversely slices the rostral part of the lateral ventricles.
- Section A, ventral aspect, illustrates the olfactory bulb and associated tract (thin layer of white matter on the outside) extending caudally into the piriform lobe, a prominent bulbous structure best seen on ventral views (Fig 1.2B).
- The cerebral cortex is the gray matter on the surface of the hemispheres folded into gyri separated by sulci above the subcortical white matter. It has many functions associated with conscious perception of sensory input, voluntary control of movement and behavior.
- The basal nuclei consist of the caudate nucleus as a large convex structure protruding in the lateral ventricle and the putamen/pallidum/claustrum, distinct gray matter areas on the lateral side of the capsula interna. They all play a role in the control of motor function as part of the extrapyramidal system.
- Along the midline ventrally and bulging into the lateral ventricles are the septal nuclei, which belong to the limbic system and are involved in emotion.
- The corpus callosum is a large white matter tract connecting both hemispheres.
- The capsula interna, a wide white matter tract, bisects the deep gray matter nuclei of the hemispheres. It contains most connections from and to the cerebrum.
- The rostral commissure is a horseshoe-shaped band of white matter connecting both hemispheres ventrally.

Area of the thalamus (Fig. 1.4)

- Both sections show the lateral ventricles and the third ventricle. Section B slices through the lateral ventricles at the level where they curve back ventrally and rostrally; thus we see a dorsal and a ventral part. In addition to the lateral ventricles we see the third ventricle in the midline with – in section A slicing through the ring-shaped ventricle – a dorsal and a ventral portion.
- We can still see cortex, capsula interna and corpus callosum. In the wall of the lateral ventricle we see the caudal extension (the "tail") of the caudate nucleus; lateral to the capsula interna the caudal portions of the other basal nuclei. Section A shows the full extent of the piriform lobes which contain the amygdala, nuclear areas belonging to the limbic system.
- In section B the hippocampus appears, the particular shape of which results from inward folding of the cerebral cortex in the medial wall of the lateral ventricle. Envisage it as a sausage-shaped structure following the half moon of the lateral ventricle. At this level the hippocampus is exposed in its dorsal and ventral aspect. The hippocampus is part of the limbicsystem and plays an important role in memory.
- The fornix forms flattened bands of white matter attached to and containing the major connections of the hippocampus. They appear to be floating in the lateral ventricles.
- The gray matter in the centre is the thalamus, the major relay station for all sensory input, before it is projected in the cortex. The thalamus consists of many nuclear areas, some of which are anatomically quite distinct, notably the geniculate bodies (see below). Other prominent structures are the habenula

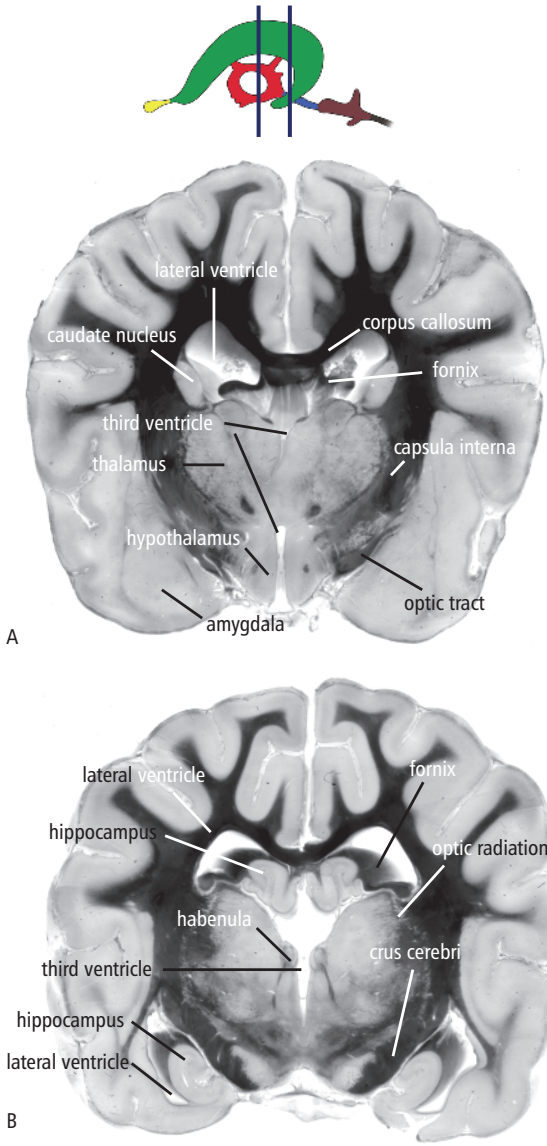

Fig. 1.4 A and B: Transverse sections at the level of the thalamus.

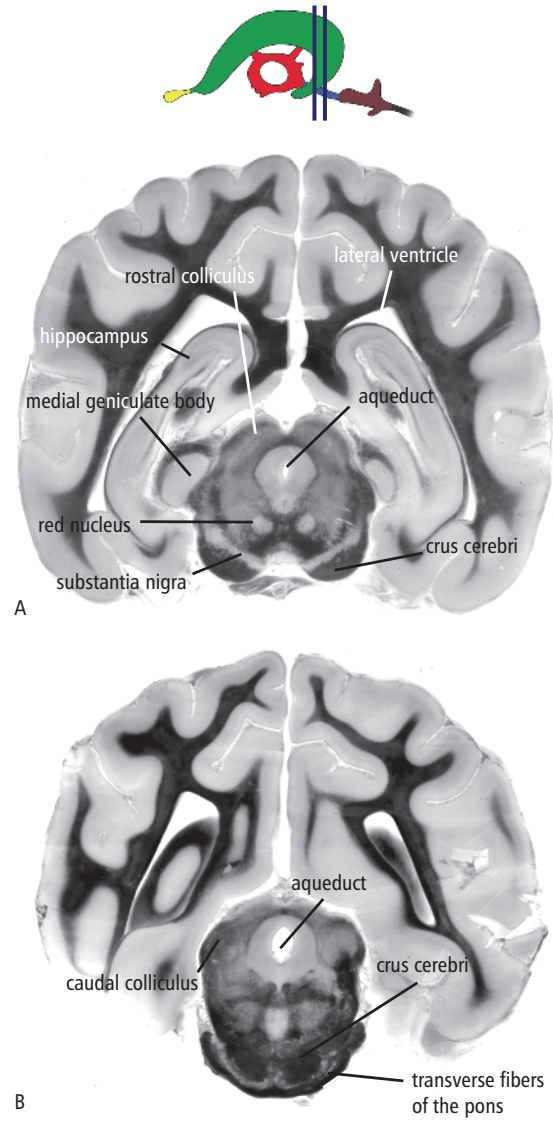

Fig. 1.5 A and B: Transverse sections at the lel of the midbrain.

protruding medially into the third ventricle; they play a role in control of circadian rhythms, emotional and social behavior and movement.

- The ventral extension of the gray matter on either side of the ventral portion of the third ventricle is the hypothalamus which regulates endocrine and vegetative functions. Ventrally is the pituitary gland (not present), attached to the hypothalamus via the infundibulum. When the latter is removed we can look directly into the third ventricle from the ventral surface.
- The optic tracts are the caudal and flattened extensions of the optic nerves and optic chiasm (easily seen on the ventral view), which can be recognized as distinct white matter structures; the optic tract eventually terminates at the lateral geniculate body, the primary visual centre in the thalamus.
- In section B of the thalamus we can see how the crura cerebri are starting to form from the internal capsule. The crura cerebri contain motor fibers, which continue into the spinal cord.

Area of the midbrain (Fig. 1.5)

- The ventricular system is limited here to the mesencephalic aqueduct, around which the midbrain developed. The lateral ventricles in the surrounding occipital lobes reach their maximal size at this level.
- This area contains the midbrain with, in its rostral part, the attached caudal extensions of the thalamus,

the lateral and medial geniculate bodies, which are involved in visual and acoustic function respectively. Section A shows the medial geniculate bodies. Note that the forebrain is no longer merged together with the subcortical structures: the midbrain is separated from the hemispheres by a meningeal space.

- In the lateral ventricle we can see the major extent of the hippocampus, which now appears as a continuous oval structure because it is sliced in its caudal part.
- The colliculi are four rounded protrusions on the roof of the midbrain and are associated with visual and acoustic orientation.
- The crura cerebri (corticospinal tract) at the base of the midbrain in the first section are the continuation of the internal capsule containing connections between forebrain and brainstem. In section B, these tracts traverse the pons.
- The red nucleus and the substantia nigra are prominent well demarcated nuclei in the ventral part of the midbrain, which play an important role in control of motor function (extrapyramidal system).
- In the caudal portion of the midbrain we discern the transverse fibers of the pons, a transverse protrusion at the base of the brainstem, and white matter connection between both cerebellar hemispheres. It also contains the large pontine nuclei, the relay station between forebrain and cerebellum.

Area of the pons, medulla and cerebellum (Fig. 1.6)

- The ventricular system expands into the fourth ventricle seen in sections A and B. In section B it has a lateral extension on either side (the lateral recesses).
- The cerebellar cortex is a strongly convoluted structure. It plays an important role in coordination of movement. The center of the cerebellum consists of white matter, and the embedded cerebellar nuclei.
- In the brainstem, white and gray matter are intimately mixed. The brainstem contains cranial nerve nuclei, which are responsible for motor and sensory function of the head, e.g. chewing, swallowing, movement of the lips. On either side of the midline is the reticular formation, which plays an important role in controlling the level of consciousness.
- Further useful white matter landmarks are the caudal cerebellar peduncle, the pyramids and the spinal tract of the trigeminal nerve. The pyramids are prominent triangular white matter tracts at the base on either side of the midline. They are the continuation of the crura cerebri containing motor connections between brain and spinal cord.

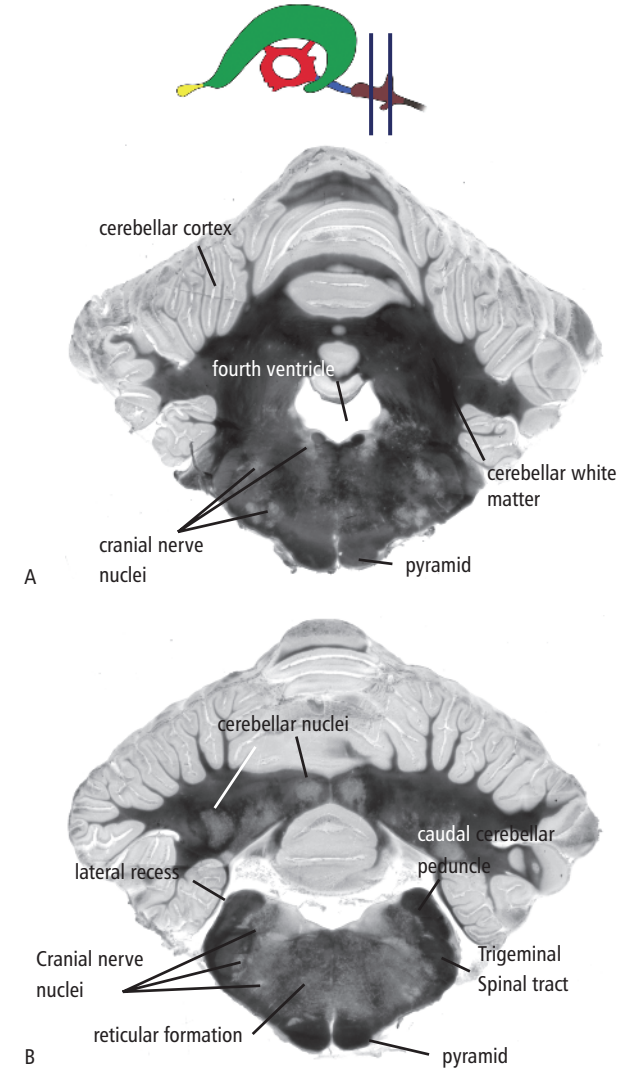

Fig. 1.6 A and B: Transverse sections through brainstem and cerebellum.

Area of medulla and spinal cord (Fig. 1.7)

- In section A we can see the thin roof of the fourth ventricle: the medullary velum. The ventricle becomes again surrounded by parenchyma in section B. At the level of the cord the ventricular system assumes a tubular configuration: the central canal.
- Further prominent gray matter structures in the medulla are the nuclei of the dorsal columns, the relay station for conscious proprioceptive impulses from the spinal cord, and the olivary nuclei, connecting the cerebellum with the extrapyramidal system, on either side of the midline just above the pyramids. The latter are quite large, triangular and can be easily recognized.

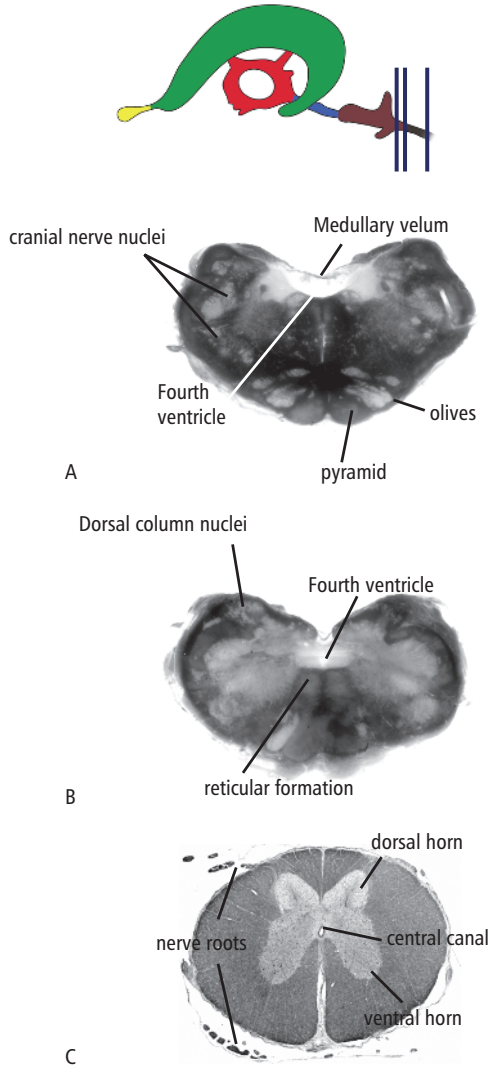

Fig. 1.7 A, B and C: Transverse sections through brainstem and spinal cord.

- In the cord, the gray matter is in the center with dorsal and ventral horns containing neurons responsible for movement of the limbs; especially important are the cervical and lumbar swellings associated with the fore and hind limbs.
- The white matter on the outside of the gray matter contains all connections between brain and spinal cord neurons.
- Note also the spinal nerve roots as the origin of the peripheral nerves; the dorsal nerve roots also contain dorsal root ganglia.

1.1.3 Histological neuroanatomy

Basic histological structure of the gray matter

There is a huge diversity in the histological appearance of the various anatomical areas of gray matter exemplified by the different sizes and shapes of neurons and their arrangement in layers and nuclei. The basic histological features of neurons as well as glial cells are, however, very similar throughout the CNS.

Neurons are generally the largest cells and are distinguished by their cytoplasmic content of clumps of chromatin, called *Nissl substance*, formed by aggregations of rough endoplasmic reticulum with ribosomes. In some neuron subtypes (e.g., pontine nuclei, inferior olivary nuclei), the Nissl substance is normally marginated (not to be confused with *chromatolyis*, discussed in Section 1.3). The *neuropil* is the tissue between neurons formed of countless neuronal cell processes (dendrites and axons) and synapses, which cannot be visualized on hematoxylin and eosin (HE)-stained formalin-fixed, paraffin-embedded (FF-PE) sections. In the neuropil are glial cells (oligodendrocytes, astrocytes and microglia), of which there are almost ten times the number of neurons. On routine HE stain, we usually only see their nuclei. Oligodendroglia have small, strictly round and hyperchromatic nuclei resembling nuclei of lymphocytes (Fig. 1.8A, small arrows), and their processes form myelinated internodal segments around axons (Fig. 1.9E,G). They are much more numerous in white matter. Astrocytes have round to oval nuclei that are larger, more irregular and paler than those of oligodendrocytes with less dense chromatin (Fig. 1.8A, thick arrows). The *astrocytes* and their processes basically occupy any remaining space in the neuropil, cover the surface of neurons and synapses, and form a continuous superficial layer (*glial limiting membrane*) of endfeet processes under the pia mater of the CNS. Either oligodendroglia and/or astrocytes can normally be located peripherally around neuronal cell bodies in the process of *neuronal satellitosis*. Microglia are small, thin, elongated cells without apparent cytoplasm in both white and gray matter and comprise up to 15% of all glial cells.

The gray matter is densely vascularized. The blood vessels in both the gray and white matter consist of an inner layer of endothelial cells connected by tight impermeable junctions, covered by a basement membrane and surrounded by pericytes and the *endfeet* of astrocytic processes. Together these structures form the *blood–brain barrier* (BBB). Large arteries penetrating the cortex have a perivascular space, called the *Virchow-Robin (VR) space*, formed by an extension of the arachnoid membrane, and which is continuous with the subarachnoid space. The VR space is no longer present at the level of capillaries and its function is unknown.

In the peripheral nervous system (PNS), the gray matter consists of ganglia (sensory and autonomic) and

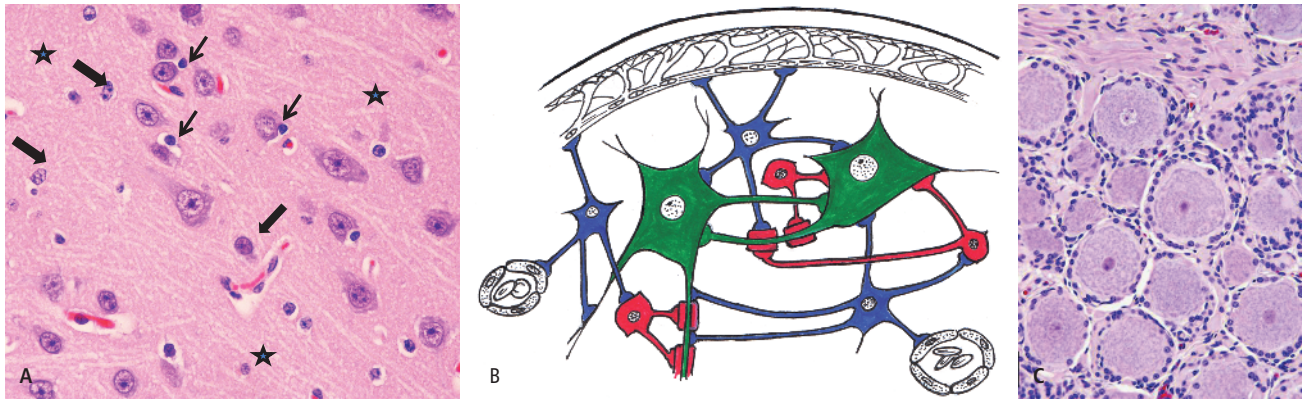

Fig. 1.8 Microanatomy of gray matter. A: Dog. Cerebral cortex with several neurons and glial cells, of which only the nuclei are visible. Small dark nuclei: oligodendrocytes (small arrows); the larger clear ones: astrocytes (large arrows). Most of the space between the neurons consists of neuropil (stars) and blood vessels. HE. B: Schematic drawing of gray matter structure with neurons (green), astrocytes (blue) making contact with neurons, blood vessels, oligodendrocytes and meninges. Oligodendrocytes (red) make contact with neuronal perikarya and particularly with the axons, where their processes form myelin sheaths. The surface is covered by meninges. C: Dog. Spinal ganglion. Neurons are surrounded by satellite cells. HE.

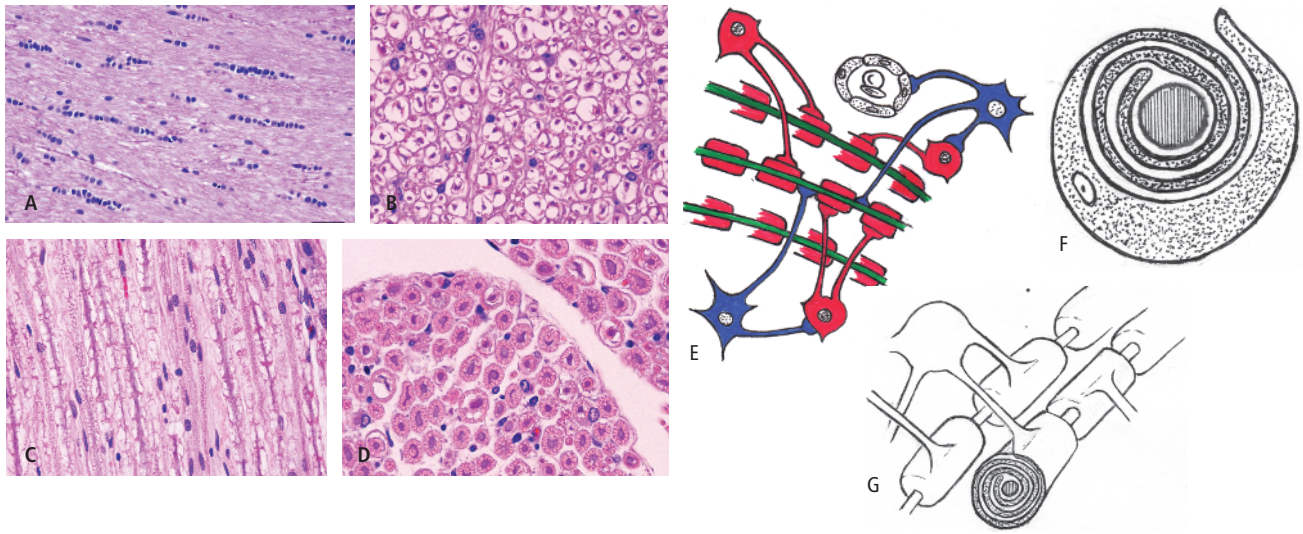

Fig. 1.9 Microanatomy of white matter. A: Dog. Longitudinal section of corpus callosum. HE. B: Dog. Transverse spinal cord section. The structure of the fibers of central white matter is discernible. Oligodendroglial nuclei in corpus callosum aligned in rows. HE. C: Dog. Longitudinal section of peripheral nerve. Note fishbone structure of myelin sheaths due to the Schmidt-Lantermann clefts. HE. D: Dog. Peripheral nerve cross-section showing individual axons surrounded by myelin sheath. HE. E: Schematic drawing of white matter structure with oligodendrocytes (red) covering axons (green) with myelin sheath segments separated by nodes of Ranvier, astrocytes (blue) and blood vessels. F: Schematic drawing of Schwann cell wrapping around an axon. G: More detailed drawing of CNS white matter showing oligodendroglial processes wrapping around axons to form myelin sheaths.

other less well demarcated accumulations of neurons (e.g. *Auerbach's and Meissner's myenteric plexus* in the gut). These ganglionic neurons are each surrounded by a layer of specialized Schwann cells called *satellite cells*.

Basic histological structure of the white matter

The white matter consists largely of tightly packed axons surrounded by myelin sheaths. On HE sections the myelin stains dark pink, although it is normally difficult to identify individual axons and their myelin sheaths. The sheaths are produced by oligodendrocytes, which wrap their processes around the axons in a spiral fashion creating segments of myelin called *internodes*, which are interrupted by the *nodes of Ranvier*. One oligodendrocyte can produce up to 60 internodes on regional axons. In the white matter, most oligodendrocytes are arranged in longitudinal rows along axonal tracts (Fig. 1.9). The white matter also contains many astrocytes, whose processes cover the axons at the nodes of Ranvier.

In the peripheral nerves, the myelin sheaths are produced by *Schwann cells*, with each cell contributing only one internode. Thinner non-myelinated axons are also wrapped by Schwann cell processes. The peripheral nerves also contain connective tissue with the *endoneurial fibroblasts* with their collagenous processes separating individual axons, the perineurium formed by modified Schwann cells isolating groups of axons as *fascicles* and fibroblast-derived *epineurium* wrapped around all the fascicles forming the peripheral nerve. In histological sections, the individual nerve fibers can be more easily identified than in the CNS. In longitudinal FF-PE sections the normal myelin sheaths often exhibit a "fish-bone" structure due to *Schmidt-Lanterman's clefts* within the myelin internodes (Fig. 1.9C).

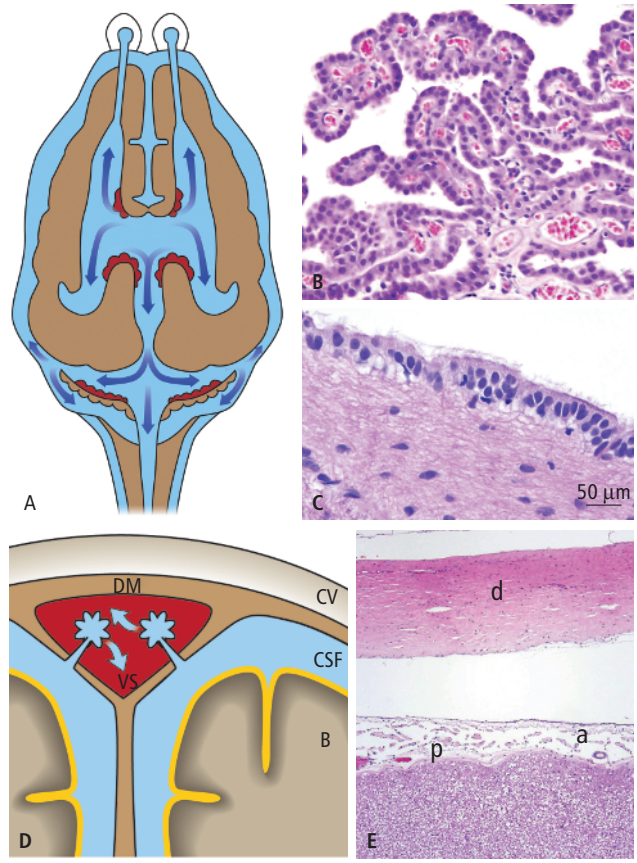

Fig. 1.10 CSF spaces. A: Schematic drawing of CSF flow (arrows). CSF produced by choroid plexus (red) flows caudally through the ventricles, and leaves the fourth ventricle into the arachnoidal space. B: Dog. Choroid plexus with vascular stroma covered by epithelial cells. HE. C: Dog. Fourth ventricle. Ciliated ependymal cells lining the ventricle. HE. D: Schematic drawing of CSF resorption via the arachnoidal granulations protruding into the venous sinuses (DM, dura mater; CV, bony cranial vault; CSF, cerebrospinal fluid in the subarachnoid space; B, brain lined by pia mater [yellow]). E: Dog. Meninges over the spinal cord d, dura mater or pachymeninges; a, arachnoid membrane with multiple trabecula; p, pia mater immediately overlying the neuropil. HE. The space between dura and arachnoidea is arteficial.

Intra- and extraventricular space and cerebrospinal fluid

The leptomeninges form the outer (*arachnoid membrane*) and inner (*pia mater*) border of the cerebrospinal fluid (CSF)-filled *subarachnoid space* around the brain and spinal cord (Fig. 1.10). Surrounding the leptomeninges is the pachymeninges or *dura mater* separated from the arachnoid membrane by the sudural space. In the calvarium the inner periosteum is formed by the dura mater but in the spinal cord the dura mater is separated from the vertebral bodies.

The ventricular walls are generally lined by a single layer of ciliated *ependymal cells*. The *choroid plexus* consists of a vascular stroma covered by epithelial cells of ependymal origin evaginated into specific sites within the ventricular system. CSF produced by the choroid plexus through filtration from the blood flows caudally within the ventricular system and gains access to the extraventricular subarachnoid space through the lateral foramina within the fourth ventricle. CSF is reabsorbed into the blood through the arachnoidal villi protruding in the extracerebral veins and sinuses.

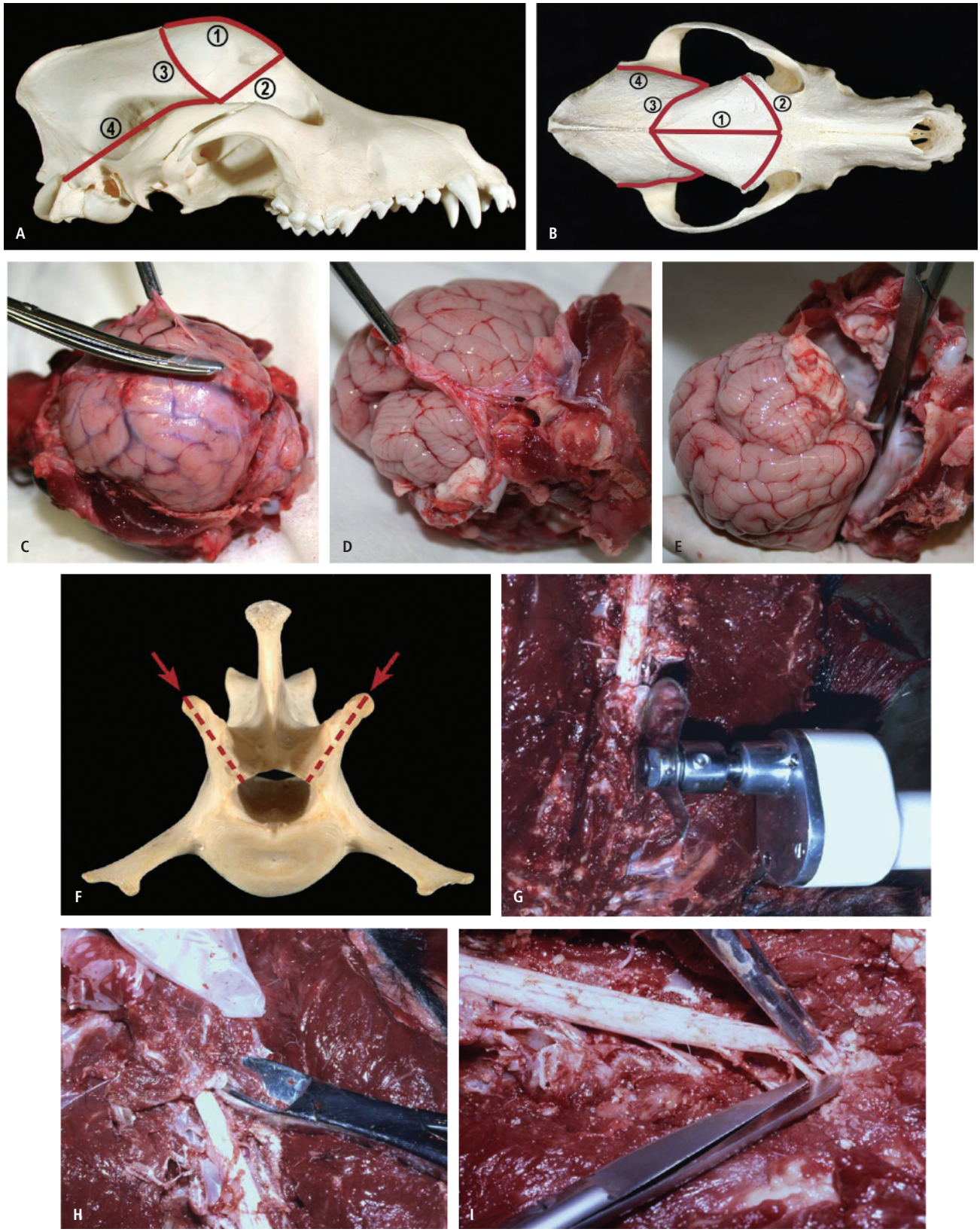

Fig. 1.11 Necropsy technique. Lateral (A) and dorsal (B) view of canine skull with lines marked in order (1, 2, 3 and then 4) for cuts using an autopsy saw, for partly removing the skull to easily access the brain : #1, 2 and 3 are to remove the frontal sinuses when present and #4 to remove the dorsal surface of the cranial vault. C: Removing the dura. D: Cutting the tentorium. E: Cutting cranial nerves with head upsidedown. F: Lumbar vertebral body indicating the site of the cut (using a Stryker saw) starting at the articular process (arrows) and extending down at an angle of about 30 degrees bilaterally resulting in a dorsal laminectomy and exposure of the spinal cord. G: Using the Stryker saw. H: Removing the roof of the vertebral column with rongeurs. I: Removing spinal cord by cutting spinal nerves.

1.2 Neuropathological techniques

1.2.1 Necropsy techniques

The CNS is protected by a solid bony calvarium and by the vertebral bodies. Thus, the skull and vertebral column have to be opened by considerable mechanical force to access the delicate CNS tissue. The latter is very soft and friable and should always be handled with care to avoid the many possible resultant artifactual changes. The brain therefore needs to be minimally touched, pressed or stretched during removal. Additionally, the spinal cord should not be folded or bent, nor should excessive pressure be placed on nerve roots during excision from the spinal canal. Post-mortem degeneration progresses rapidly within a few hours in nervous tissue. Thus, longer postmortem intervals considerably add to a range of artifactual changes.

Removal of the brain

Decapitate the animal by cutting ventrodorsally through the exposed soft tissues after extending the neck, opening the atlanto-occipital joint, separating the brain from the spinal cord before removing the head completely. Remove all skin and muscle from the head to expose the calvarium. Remove the dorsal cranial vault by using a saw (e.g. electric Stryker saw or Dremmel high-speed drill) cutting along the lines, as indicated in Fig. 1.11A and B, while avoiding contact with the underlying brain tissue. In very small animals, one can use a pair of rongeurs, starting at the medial side of one orbit until the dura mater is exposed; than remove the bone towards the foramen magnum. Always try to minimize touching the surface of the brain by cutting outwards from the brain. Then incise and remove the dura mater, falx and tentorium by using forceps and scissors or a scalpel (Figs 1.11C,D).

Then turn the head upsidedown, tilt to one side and shake gently to detach the brain from the skull and expose the cranial nerves; cut these cranial nerves transversely on the exposed side as close as possible to their exit foramina (Fig. 1.11E). Repeat for the other side.

Keeping the head upsidedown, hold the nose and shake gently; cut the remaining cranial nerves including the optic nerve and infundibulum and any other meningeal adhesions; detach the olfactory bulbs by arching the scissors or a wooden tongue depressor gently between bone and brain tissue; then shake gently to extract the brain completely.

Immediately after removal of the brain always examine the cranial vault, meninges, pituitary gland and fossa, the cranial nerves and their foramina to detect any relevant abnormalities. Sample the trigeminal (Gasserian) ganglia, and in ruminants the *rete mirabile caroticum*, which are both easily accessible at the base of the skull lateral to the pituitary fossa. Examine the whole brain for external gross lesions after removal and before fixation. Further detailed examination follows transverse sectioning of the brain.

Removal of the spinal cord

Expose the dorsal aspect of the spinal vertebrae by removing the paraspinal muscles. In small dogs and cats use rongeurs to remove the bone of the dorsal arch at the lumbosacral junction until you can see the cauda equina. Then, proceed cranially and remove the roof of each consecutive vertebra by cutting the lamina laterally on both sides without touching the cord (Fig. 1.11F–I). In large dogs, use an electric Stryker saw and perform a dorsal laminectomy by cutting through the lateral articular facets as an external guide, at approximately 30 degrees, to remove the dorsal arch and upper part of the vertebral arch. Once the cord is exposed, clamp the meninges over the cauda equina with a forceps and pull it gently horizontally, then, segment by segment in a cranial direction, cut the spinal nerve roots on each side with a scalpel blade or scissors, progressively lifting the cord (which remains confined within the dura mater) out of the spinal canal. When required for subsequent histological examination remove the dorsal root ganglia, which occur as tan, nodular thickenings of the nerve roots.

In large animals, suspend the eviscerated animal head down with the hind limbs maximally spread. Cut the vertebral column parasagittally with an electric saw to leave the cord intact and avoid damaging the nerve roots (at least on one side). Alternatively use a band saw after removal of the vertebrae from the carcass and again cut the vertebral bodies parasagittally. Remove the cord by cutting the spinal nerve roots on the remaining intact side of the canal. Avoid excessive bending of the labeled spinal cord segments for immersion fixation by placing labeled sections in a large rectangular flat container.

Depending on the neurological diagnosis, evaluate the vertebral canal and intervertebral foramina for any lesions that might cause stenosis; examine each intervertebral disc sagittally and the associated ligaments within the floor of the vertebral canal.

Evaluation of the neuromuscular system

In neurologically well documented cases in which neuromuscular disease is suspected, clinical biopsies of

muscle and nerve, or at postmortem, selected tissues are sampled and processed for appropriate evaluation (e.g. frozen sections for histochemistry, resin-embedding for semi-thin and subsequent thin transmission electron microscope (TEM) sections, teased fiber preparations for examining individual nerve fibers) in specialized neuromuscular laboratories.

For initial histological examination, small pieces of muscle and nerve can be immersion-fixed in formalin and embedded in paraffin. The orientation and quality of such nerve and muscle samples can be optimized by attaching them (e.g. suturing) outstretched on a solid (e.g. cardboard or a nerve biopsy apparatus) support while fixing. For most effective evaluation by any technique, it is most important to include longitudinal as well as transversely oriented sections from muscle and nerve samples.

Fixation procedures

For routine diagnostic neuropathological evaluation, immersion fixation of brain or spinal cord in 10:1 v/v of 10% buffered formalin solution to tissue is optimal. A single sheet of absorbent paper between the brain and the bottom of the container will prevent adherence of the brain and severe artifactual changes. Adequate immersion fixation of brains in 10% formalin takes between 5 and 10 days for small and large animal brains respectively. For specialized laboratory techniques other fixatives or procedures (e.g. freeze drying) may be used. Certain histological techniques require unfixed tissue, snap frozen and sectioned in a cryostat. Such frozen sections have an inferior morphological resolution as compared with FF-PE sections.

For TEM or scanning electron microscope (SEM) small pieces of fresh tissue can be immersion-fixed in buffered 3% glutaraldehyde, although more specialized ultrastructural studies require perfusion fixation for optimal preservation of detail.

1.2.2 Brain sectioning, macroscopic inspection and sampling for histology

Macroscopic inspection

Section the brain and cord only after an appropriate fixation time. Use a very sharp knife to avoid compression during sectioning. Specialized wide-blade brain knives are not necessary but do ensure a smoother cut surface which can be important for optimal photography. Always use the same standardized procedure: with the exception of very specific indications, make only transverse 3–4 mm thick slices starting at the frontal lobe and ending at the medulla so that one can always identify anatomical landmarks and reconstruct the brain for reexamination if necessary. Never make random cuts. Lay out the brain slices in their consecutive anatomical order for macroscopic inspection and for selection of areas for histological examination. Following autopsy and brain cutting after fixation, all regions must be examined (cerebral cortex, corpus striatum, thalamus, hippocampus, midbrain, cerebellum, brainstem and spinal cord). Pay attention to the following points, particularly when MRI is available:

- Check the ventricular system for stenosis, dilatation, compression and exudate. Examine the choroid plexi for swelling and congestion.
- During the entire examination look for alterations of all structures in size (e.g. aplasia, hypoplasia, atrophy, swelling), shape (e.g. cerebellar coning) and symmetry of both sides of the brain.
- Look for space-occupying changes (e.g. a tumor, abscess).

Other questions to consider are:

- Is there loss of substance (e.g. a cavity)?
- Is there a change of color (e.g. red indicates hemorrhage, white or yellow necrosis)?
- Is there a change of consistency (hardening or softening)?
- Are changes well demarcated from surrounding normal tissue?
- What is the pattern of distribution of the changes (single, multiple, bilateral or unilateral, anatomical localization)?

Often one may find very little change on macroscopic examination of the brain even when severe histological lesions are present.

Sampling for histological examination

When a definitive localization is suggested by neurological examination and confirmed by MRI, the examination can be concentrated on that specific anatomical area. However, representative sampling is the standard approach if no macroscopic lesions are present. Neurological disease is almost never the result of a small isolated single lesion except in the spinal cord. Small lesions are clinically often silent and even large lesions can remain unnoticed. When lesions of the CNS are the cause of neurological signs they are usually large or widespread. Still, often only specific regions are affected and in order to detect them a systematic approach with appropriate sampling is needed. Thus, where there are no grossly detectable lesions identified we aim to examine all the major divisions of the CNS histologically. These (depicted in Fig. 1.12) include: the area of

the basal nuclei (roughly rostral one third of the forebrain), the thalamus (roughly in the middle of the forebrain), the midbrain (roughly the caudal third), the cerebellum–pontine area and the medulla oblongata. The first two areas include cerebral cortex. Make sure to include some hippocampus in the section of thalamus. A somewhat more extensive survey additionally includes occipital and frontal cortex which would be included, e.g. with either blindness or behavioral or/and cognitive deficits respectively detected clinically.

The spinal cord should be always examined when there are relevant clinical deficits. A representative survey of the cord in the dog and cat includes at least one transverse and longitudinal section from each of the following segments: upper cervical segments C1 and C4, cervical intumescence (C7), upper thoracic (T4), lower thoracic (T12), lumbar intumescence (L5) and sacral segments (S1).

Depending on the size of the brain and the capabilities of the histology laboratory, several alternative approaches are possible. Nowadays few laboratories can process full transverse sections. Therefore, from the large brain slices, take alternating halves; e.g. basal nuclei left, thalamus right. Always routinely mark one side of the brain sections, either the left or right side, with an incision. These halves can be further divided in order to fit the size of the tissue cassettes; however, always standardize the system you use to cut the smaller sections so that you consistently recognize where you are anatomically. For documented cases of primary cerebellar disease we recommend sagittally sectioning the vermis prior to transverse sectioning of the cerebellar hemispheres.

Histological technique

The routine technique consists of paraffin embedding of the formalin-fixed tissues. Briefly, fixed tissue samples are dehydrated in graded ethanols, cleared in xylene and infiltrated with paraffin. Sections are cut 3–5 µm thick from the paraffin blocks and stained. The standard routine stain is HE, which allows the detection of lesions in nearly all cases. Special histochemical and immunocytochemical stains are used to define and characterize the detected lesions more precisely. Reliable methods are: Nissl stain (cresyl Echt violet) for neurons, luxol fast blue for myelin (best combined with HE), Bielschowsky silver-based stain for axons, trichrome (Gomorri) stain for connective tissue. However, special silver impregnations for neurons and glial cells are often difficult to reproduce and extremely cumbersome. Much more specific and reliable is the demonstration of cell-specific antigens with immunohistochemical labeling by relevant antibodies. The latter are referred to in the section on basic tissue reaction patterns. For special purposes, fresh unfixed tissues are snap-frozen but the

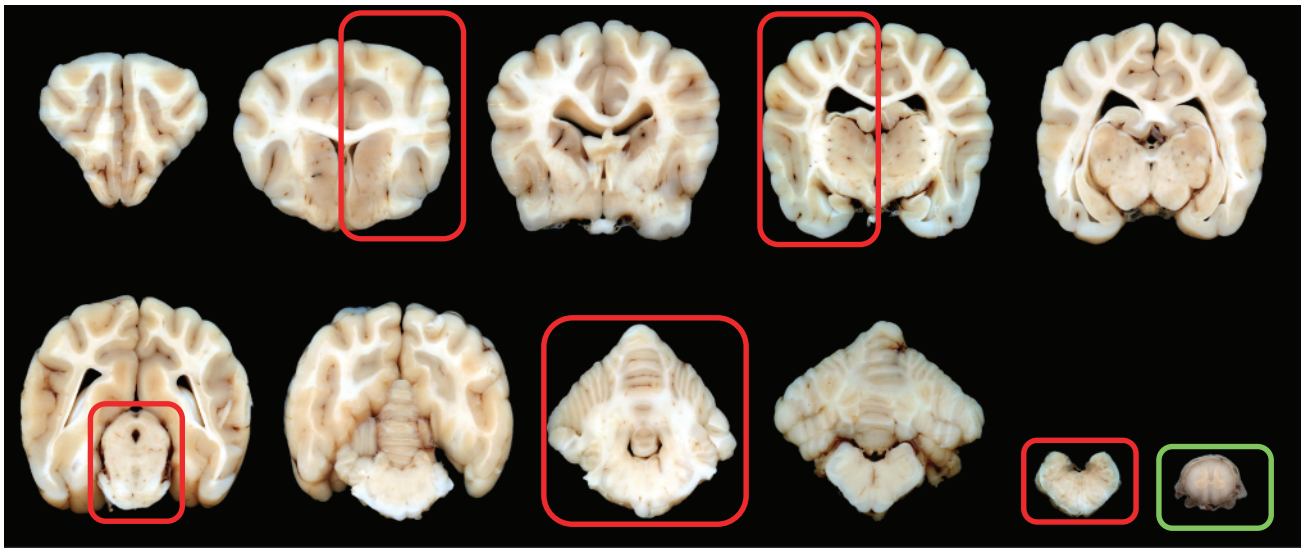

Fig. 1.12 Representative sampling. Serial sections of a fixed canine brain. The red boxes include the minimal areas of the brain which must be trimmed in for histological processing. Appropriate levels of the spinal cord (green box) should be included whenever possible.

morphological resolution of such frozen sections is of much lower quality than paraffin sections.

More precise structural resolution can be obtained by resin embedding (in which there is no fat extraction) for transmission electron microscopy. From such material so-called semi-thin sections can be cut and stained with toluidine blue for microscope study.

Examining microsopic slides histologically

At the microscopic level, scan all sections systematically, most effectively initially with low- and then medium-power magnification. In view of the great anatomical variations between the different regions, familiarity with the normal histological structure of all these regions helps to detect lesions. This familiarity comes only with experience, best started in a mentored one-on-one setting. When scanning slides it helps to consciously and constantly register microanatomical details. The morphology of reactions of the different cell types to injury is described in Section 1.3.

1.3 Basic tissue reaction patterns

The wide range of neuropathological entities in animals and man is due mainly to both the anatomical complexity and to inherent differences in vulnerability to injury in different areas of the nervous system. Cells in the CNS can mount a relatively limited number of reactions in response to injury: the same basic reactions can occur in different anatomical locations and combinations, thus giving the impression of a large variety of reaction patterns. As with any other organ system in the body, the CNS is subject to pathologic changes depending on genetic background (endogeneous causes: disease susceptibility, inborn degenerative diseases) of an individual, and external causes (exogeneous: e.g. trauma, viral, bacterial or protozoal infections, and metabolic–toxic agents). We will briefly discuss the reactive changes in the major CNS cell populations of neurons, oligodendrocytes, astrocytes, ependymal/choroid plexus cells, microglial cells and blood vessels to injury.

1.3.1 Reactions of neurons to injury

Microscopically the nervous system consists in part of neurons whose axons can extend over enormous distances. This creates the problem of neurons having to provide metabolic support in dendritic and axonal processes of the cell that are far removed from the perikaryon. Neurons are also highly differentiated and functionally specialized cells, which are not capable of regeneration to any significant extent. Another special feature of the CNS is the generation of action potentials and conduction of such signals along the axons. The efficiency of this process is greatly enhanced by the presence of segmented myelin sheath internodes allowing saltatory conduction across these segments.

A variety of molecular mechanisms have been unraveled, which can impair structural and functional integrity of neurons. Two important ones are *excitotoxicity* and *oxidative change*. Neuronal excitotoxicity depends on the excessive sustained release from neurons of certain excitatory neurotransmitters (e.g. glutamate, aspartate) and their decreased removal by astrocytes in the CNS in response to such factors as ischemia, anoxia or hypoglycemia. Subsequent binding of excessive glutamate to various types of ionotropic receptors (e.g. for N-methyl-d-aspartate, NMDA) on neurons results in transmembrane ionic fluxes with rising intracellular levels of calcium leading to activation of proteolytic enzymes, which then damage cell organelles. *Acidophilic neuronal necrosis* is considered to be the final common pathway resulting from neurotransmitter-induced neuronal excitotoxicity. Neurons are also particularly prone to oxidative damage, a final common pathway of cell pathology in many different diseases. During respiration mitochondria produce superoxide anions which, under normal circumstances, are reduced by *superoxide dismutases* (SOD) to H_2O_2. Under pathological circumstances H_2O_2 can be converted to hydroxyl (OH) radicals, which are highly reactive, particularly with lipids (in which the nervous system is very rich) inducing membrane damage and ultimately tissue destruction. Another class of reactive oxygen species includes the *nitric oxides* (NO) generated by *nitric oxide synthetases*. Reaction of NO with H_2O_2 can lead to the formation of the highly toxic peroxynitrite. Cells have developed defense systems such as SOD against such toxic events. Breakdown of the equilibrium between oxygen radicals and such defense mechanisms leads to cell pathology.

A wide spectrum of neuronal changes to injury has been described but here we will describe only a few common patterns.

Intraneuronal inclusions

Intracytoplasmic and intranuclear inclusion bodies, often with distinctive characteristic morphological, biochemical and ultrastructural features, can accumulate in neurons/glial cells as a result of certain degenerative, metabolic and viral diseases, and have often received the names of their discoverers (e.g. Negri and Lafora bodies).

Their usually distinctive morphology, intracellular localization (intranuclear versus intracytoplasmic or both) and biochemical and ultrastructural composition can be diagnostically important for specific diseases (Fig. 1.13A). Since most neurons are postmitotic, are

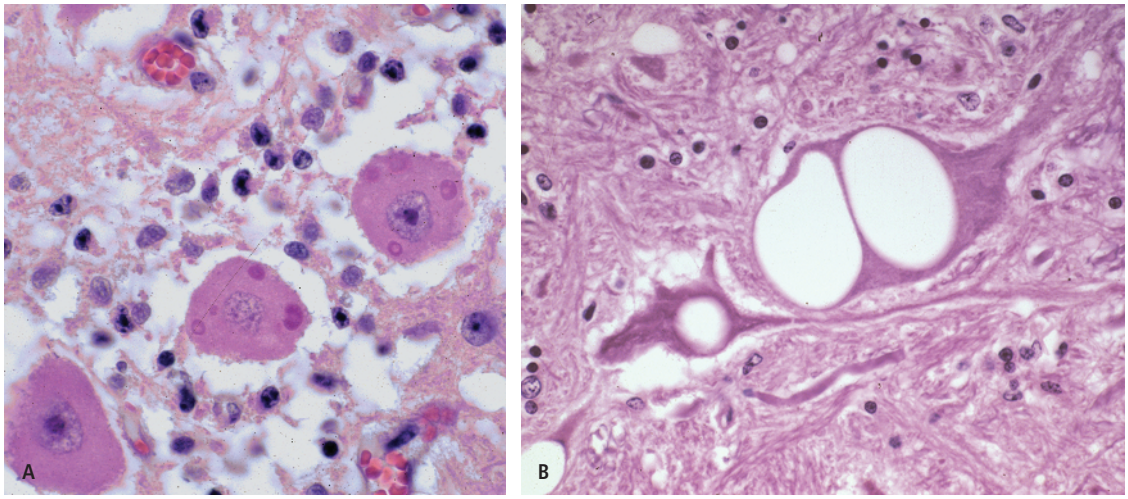

Fig. 1.13 A: Dog with rabies virus infection. Cerebellum. Multiple intraneuronal intracytoplasmic inclusion bodies (Negri bodies) in Purkinje cells. HE. B: Sheep with scrapie. Brainstem. Multiple intracytoplasmic intraneuronal vacuoles. HE.

usually not replaced and have no exocytic capability, neuronal or glial cytoplasmic storage of metabolites within hyperplastic lysosomes as a result of genetic or acquired lysosomal enzyme defects can be quite spectacular in the CNS (see Chapter 8). Empty cytoplasmic vacuoles in the neuronal cell body (Fig. 1.13B) and its processes are characteristic of prion-induced transmissible spongiform encephalopathies in animals, e.g. scrapie, bovine spongiform encephalopathy. However, they may occur in limited numbers also as incidental finding in normal cattle (e.g. in the red nucleus).

Eosinophilic inclusion bodies (pseudo-Negri bodies) of unknown significance are often found in neurons of the lateral geniculate body and hippocampus in cats and occasionally in other species. Similar small inclusions can occur in thalamic and cerebellar Purkinje cells in dogs. Widespread neuronal intranuclear inclusions were reported in a horse, resembling intranuclear neuronal inclusion body disease in humans.

Dark brown neuromelanin granules are normally found in the hypothalamus and sometimes in other neurons but rarely to the extent that it becomes grossly visible as in certain human neuroanatomical regions.

Chromatolysis

Central chromatolysis is a frequent reactive response in neurons. Histologically, there is an initial swelling of the cell body and processes, perinuclear dispersion of Nissl substance with loss of ribosomes from the rough endoplasmic reticulum (RER), a thin intact cytoplasmic border of Nissl substance and peripheral margination and flattening of the nucleus (Fig. 1.14). It can be commonly seen in lower motor neurons of the spinal cord in ruminants with postnatally acquired copper deficiency. It is also the result of a retrograde axonal reaction to nerve root injury, e.g. after brachial plexus avulsion. The histochemical stain, cresyl Echt violet, is very useful for visualization of the Nissl substance dispersion. This process can be either functionally and morphologically reversible with treatment or eventually lead to neuronal necrosis depending on the cause and severity of injury. The process of chromatolysis should not be confused with the normal morphology of cranial nerve nuclei (e.g. V, VII) which normally have only a peripheral rim of Nissl substance but a centrally placed nucleus.

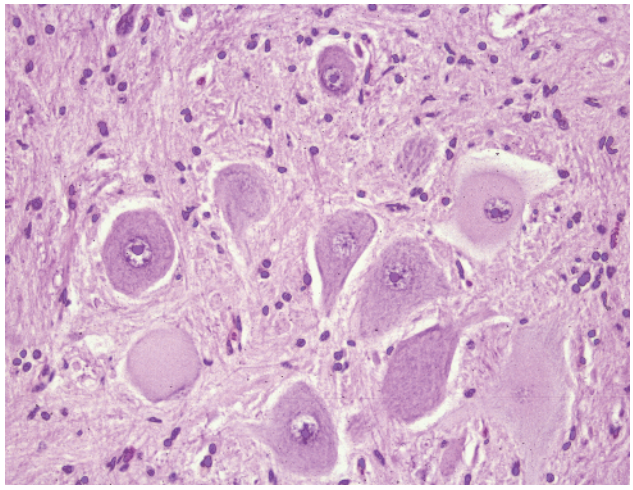

Fig. 1.14 Sheep with copper deficiency. Spinal cord. Various stages of chromatolysis in ventral horn motor neurons. HE.

Acidophilic neuronal necrosis

Cell death of neurons can be either necrotic or non-necrotic. *Necrosis* is solely due to external factors

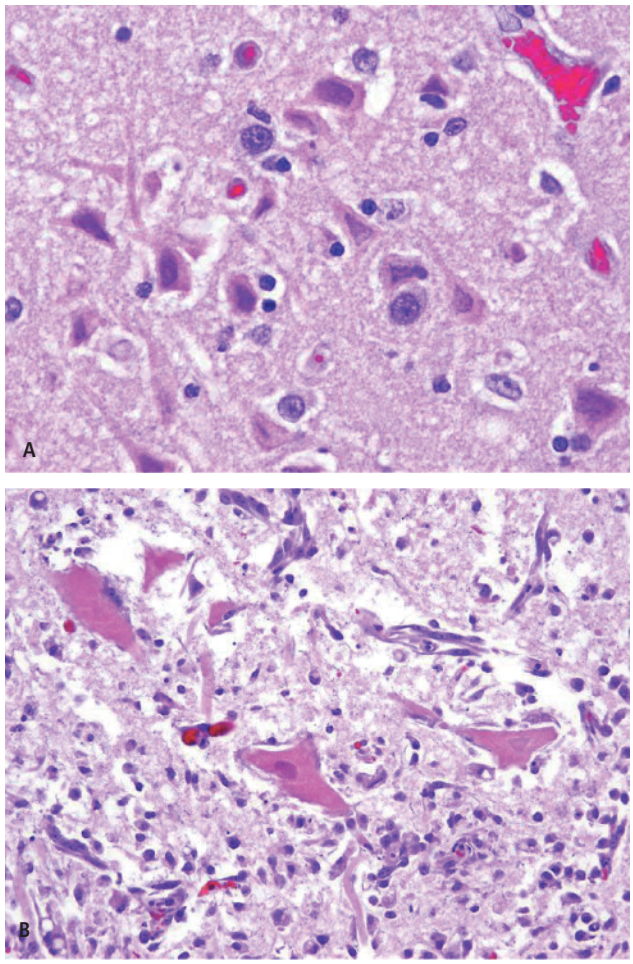

Fig. 1.15 A: Sheep with polioencephalomalacia. Cerebral cortex. Acidophilic (ischemic) neuronal necrosis with shrunken eosinophilic neurons. HE. B: Dog with spinal cord infarct. Myelomalacia with acidophilic neuronal necrosis. HE.

leading to membrane damage and cell swelling. Neurons, being primarily dependent on glycolysis for energy, are extremely sensitive to conditions interfering with glucose metabolism including ischemia, hypoglycemia or thiamin deficiency. Their morphologic reaction to anoxia/ischemia is acidophilic (ischemic) neuronal necrosis with marked granular change and eosinophilia of the cytoplasm in HE sections, acute swelling and later shrinkage of the cell body, as well as nuclear pyknosis of the centrally placed nucleus (Fig. 1.15). This is an irreversible lesion. Importantly from a diagnostic view, this change can only be detected after 6–8 hours following the triggering injury. The underlying mechanism (e.g. ischemia, hypoglycemia, anoxia, trauma, virus infection etc.) is thought to be mediated by excessive sustained release of various excitotoxic neurotransmitters (e.g. glutamate, aspartate), irrespective of the inciting event. Global ischemia results in acidophilic neuronal necrosis in specific neuroanatomical sites: cerebral cortex, hippocampus and Purkinje cells. Anatomically defined sites of selective neuronal susceptibility to ischemia as in the pyramidal neurons of the CA1 and CA2 sectors may be explained by their high concentration of dendritic glutamate receptors.

Apoptosis

In non-necrotic cell death such as *apoptosis*, components of the regulation of the cell cycle control the events leading to programmed cell death. Neuronal apoptosis is particularly common during fetal CNS development, in degenerative and some viral diseases. The two most important categories of molecules regulating apoptosis are the *Bcl2 family* and the *caspases*. Morphologically it is characterized by chromatin condensation, cytoplasmic blebbing, nuclear fragmentation and presence of so-called "apoptotic bodies". However, in HE-stained sections it might be difficult to definitely distinguish between necrosis and apoptosis and hence apoptosis is best confirmed with positive immunoreactivity to, for example, activated caspase 3.

Neuronal loss

Irrespective of etiology, individual necrotic neurons in the neuropil are removed by the process of *neuronophagia* mediated by activated phagocytic microglia, which accumulate around the neuron as microglial nodules (Fig. 1.16). Such focal microgliosis is also one characteristic histological feature of the triad of meningoencephalitis (perivascular cuffing, neuronal degeneration/necrosis, microglial nodules) that are hallmarks of neurotropic viral infections.

Neurons do not regenerate as a general rule. Thus typically in the CNS, neuronal damage frequently results in permanent loss of cells termed neuronal loss. There is a characteristic regional selective susceptibility of neurons or nuclei to different etiologic agents. A combination of irreplaceable loss of certain nerve cell populations and Wallerian degeneration of their axons results in neuropathological patterns which are highly typical of certain acquired or inherited degenerative diseases. Atrophy mainly occurs as a result of loss of transsynaptic afferent input in anterograde and retrograde degeneration (see below) or geriatric change.

Trans-synaptic degeneration

Neurons form interconnected networks, whereby axon terminals of one neuron make synapses with other

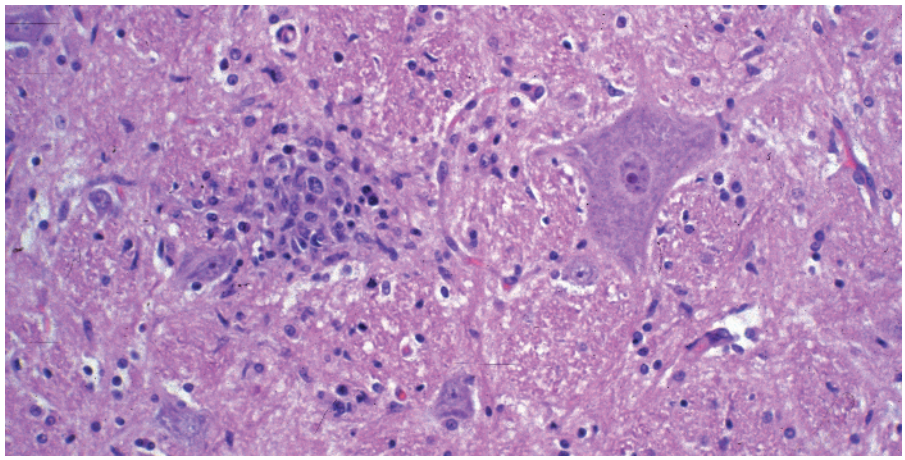

Fig. 1.16 Dog with neurotropic canine distemper virus encephalitis. Brainstem. Neuronophagia. Microglial nodule removing degenerated neuron. HE.

neurons. When a neuron and its processes degenerate, afferent and efferent synaptic contacts from neurons in both anterograde and retrograde locations are also lost (Fig. 1.17). This bidirectional process results in trans-synaptic neuronal degeneration, which maybe reversible depending on the primary injury. With a basic knowledge of neuroanatomical pathways between nuclear groups the concept of trans-synaptic degeneration can help to interpret certain lesion distribution patterns, e.g. Purkinje cell loss leads to anterograde trans-synaptic atrophy and eventually degeneration of the cerebellar nuclei and retrograde trans-synaptic atrophy of the cerebellar granule cells (see Chapter 8).

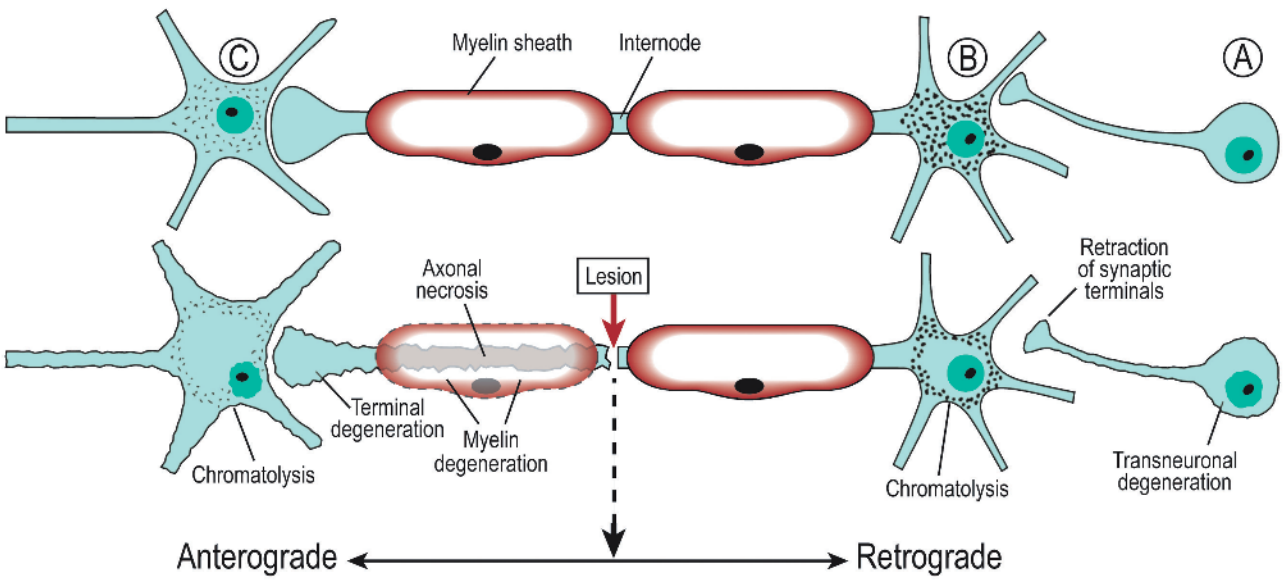

Fig. 1.17 Trans-synaptic neuronal degeneration following Wallerian degeneration. Diagram illustrates three contiguous interconnnected neurons A, B and C. Distal to the site of the transecting lesion is axonal necrosis and myelin degeneration with retraction of the synaptic junction and chromatolysis of the downstream neuron (C) in an *anterograde* direction. Upstream there is neuronal chromatolysis (B) and retraction of synaptic contacts from A in a *retrograde* direction.

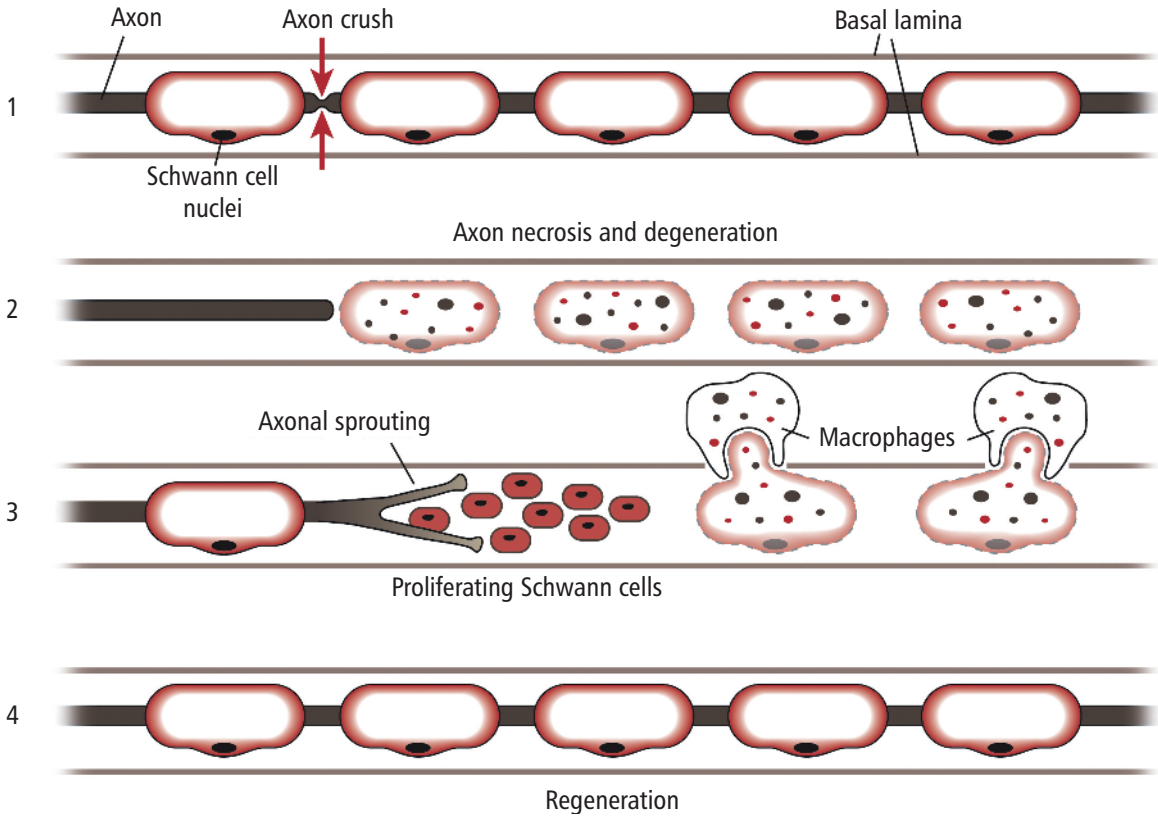

Fig. 1.18 A: Schematic drawing of Wallerian degeneration. 1. Peripheral axon covered by myelin sheath formed by Schwann cells. 2. Axon and myelin sheath distal to injury undergo degeneration. 3. Macrophages of blood monocyte origin ingest the axonal and myelin debris. Schwann cells proliferate within the persisting endoneurial tube forming densely packed cell chains, so-called Büngner's bands, providing support for axon sprouts arising from the damaged axon. 4. New myelin segments are formed around regenerating axon.

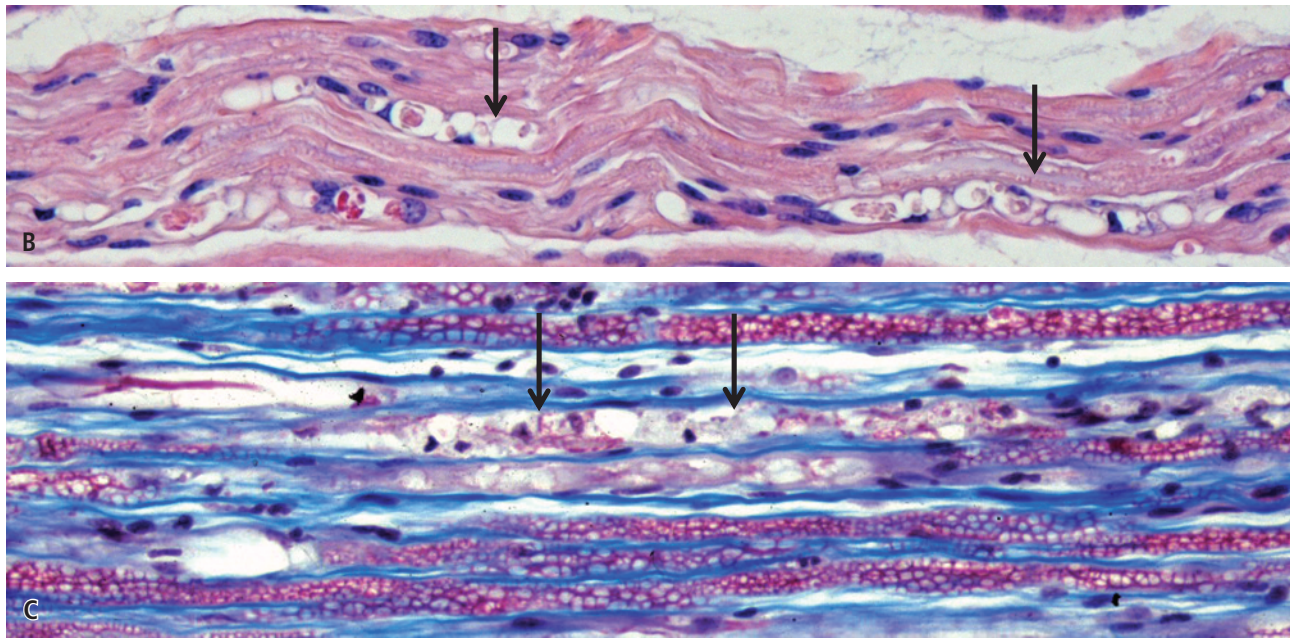

Fig. 1.18 B: Brown Swiss calf with motor neuron disease. Wallerian degeneration in peripheral nerve, longitudinal section: segments of fragmented axons in several fibers (arrows). HE. C: Dog with peripheral nerve injury. Longitudinal section. Wallerian degeneration, intact myelin sheaths are stained red. Degenerated fibers are replaced by macrophages containing myelin debris (arrows). Masson's trichrome stain.

Axonal and dendritic changes

The axonal and dendritic processes of neurons can undergo degeneration independent of the neuronal cell body. A typical reaction of the axon to a variety of insults is *Wallerian* (or *Wallerian-like*) *degeneration*. This process consists of a series of degenerative and reparative histological events which occur to the axon and myelin sheath classically following primary traumatic injury to the axon or dendrite (Wallerian degeneration) but may also occur following any other type of axonal damage, e.g. ischemia, degenerative axonal disorders (so called Wallerian-like degeneration). Though these changes occur also in the CNS, they are best studied in peripheral nerve injury. When a nerve fiber is focally damaged, the nerve process distal to the lesion undergoes anterograde degeneration due to interference with the vital mechanism of bidirectional axonal transport systems. In Wallerian degeneration, the axons undergo focal segmental swelling forming axonal spheroids due to interference with axonal transport and subsequent proximate metabolite accumulation, followed by distension of the myelin sheath, necrosis of both structures and their ingestion by macrophages of blood monocyte origin or of microglial cells in the CNS (Fig. 1.18). In peripheral nerves, Schwann cells proliferate within the persisting endoneurial tube and form densely packed cell chains, so-called *Büngner's bands* (Fig. 1.18). Complete functional regeneration following axonal sprouting and segmental remyelination is possible in the peripheral nervous system but rarely happens in the CNS. The most conspicuous feature in the neuron whose nerve fiber has been injured close to the cell body can be retrograde chromatolysis (see above).

The axon can swell segmentally many times its normal size and these axonal spheroids can be easily detected in a histological preparation (Fig. 1.19). Such swellings can also result from intrinsic metabolic hereditary disorders known as axonal dystrophies. Since there is little regeneration in the CNS, axonal damage usually ends as axonal loss, generally with a reactive astrogliosis, and can be best detected by using a combination of axonal and myelin stains.

Immunohistochemical identification of neurons and their processes

For routine diagnostic purposes, currently the most widely used antibodies for the immunocytochemical identification of neurons and their processes are those to synaptophysin; triple neurofilaments (NF-L, NF-M, NF-H molecular weights) either individually or in various cocktails, and also as phosphorylated or non-phosphorylated neurofilaments; and to Neu-N. There are, however, many more neuronal specific antigens, e.g. neural cell adhesion molecules (NCAM) and microtubule associated proteins (MAPs), often requiring strict fixation techniques; these are mainly for experimental use. Cells of the neuroendocrine system express synaptophysin, chromogranin A and neuron-specific enolase, which can all be visualized immunocytochemically.

Axons (both normal and undergoing pathological changes) in the CNS/PNS are best visualized immunocytochemically by antibodies to NF-200, and triple neurofilaments either in a phosphorylated or non-phosphorylated state. Amyloid precursor protein (APP) is a robust marker of early axonal injury.

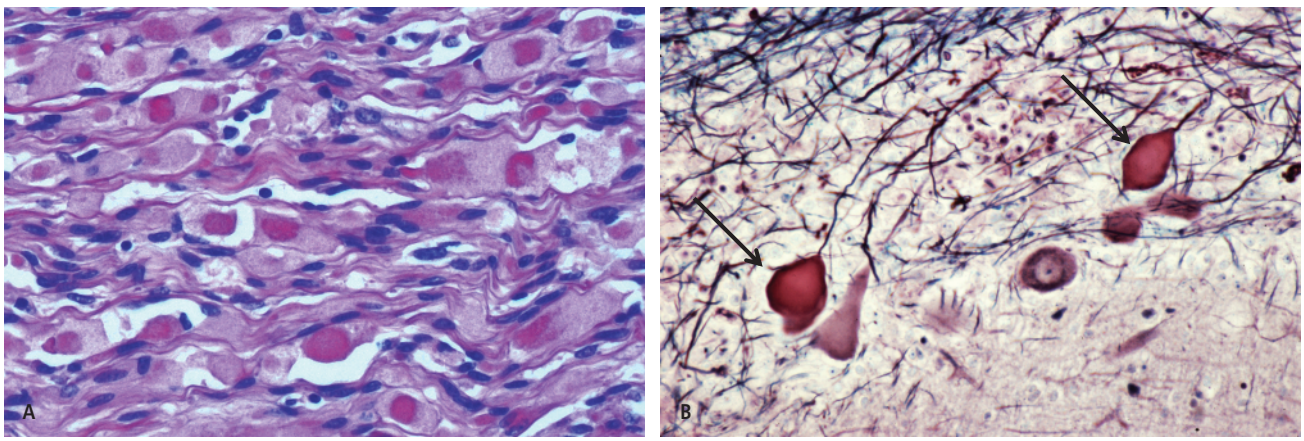

Fig. 1.19 A: Horse. Trigeminal nerve compressed by an abscess. Many swollen eosinophilic and fragmented axons. HE. B: Bovine with axonal dystrophy. Cerebellar cortex, axon swellings depicted by arrows. Holmes' silver stain.

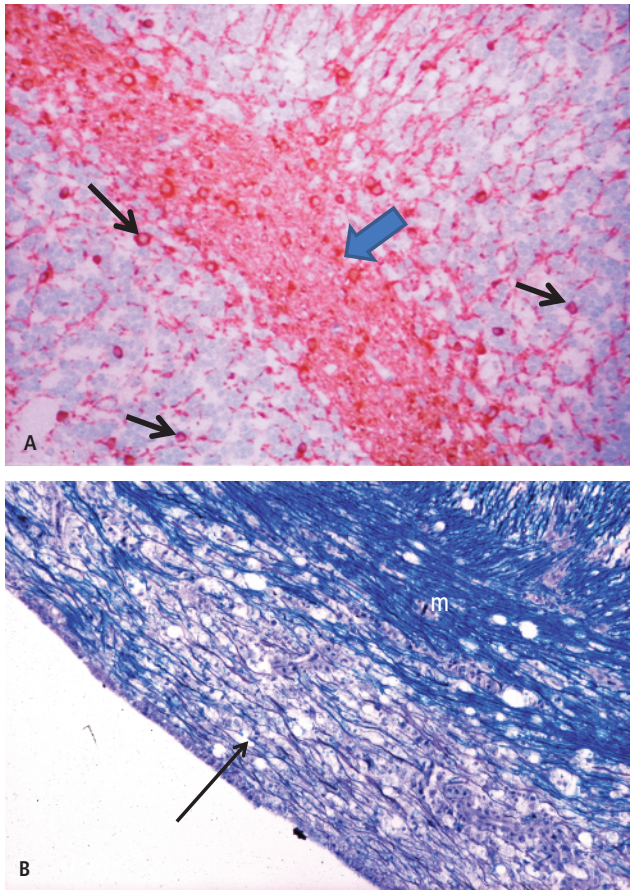

Fig. 1.20 A: Dog. Normal cerebellar white matter and adjacent granular layer. Oligodendrocytes (arrow) and myelin sheaths (big arrow) are labeled with an anti-CNP antibody. B: Dog. Brainstem. Demyelinating lesion in canine distemper infection (m, unaffected white matter with dark-staining axons covered by green/blue-stained intact myelin). In the demyelinating lesion there is focal loss of myelin staining but preservation of axons (arrow). Luxol fast blue–Holmes' silver stain.

1.3.2 Oligodendrocytes

These are highly specialized glial cells with small round dark basophilic nuclei and short processes, the distal ends of which flatten into wide membranous sheets that form myelin sheaths as explained above or clusters around neurons in the gray matter as normal satellitosis.

Demyelination – primary or secondary

When oligodendrocytes are damaged (e.g. by virus infection or ischemia), their complex membranous structures forming myelin internodes undergo degeneration and phagocytosis in the process of primary demyelination (Fig. 1.20B). In contrast to Wallerian degeneration, there is selective degeneration of the myelin sheath or oligodendrocyte in this primary demyelinating process with axons remaining intact for a long time. Secondary demyelination occurs after primary axonal necrosis with an obligatory loss of the myelin sheath. Functional remyelination by oligodendrocytes can occur in the CNS but not to the same degree as from their myelinating counterparts of Schwann cells in the PNS. To demonstrate demyelination histochemically the luxol fast blue/Holmes silver stain can distinguish between primary and secondary demyelination. In primary demyelination there is an absence of blue-staining myelin sheaths but the black silver-impregnated axons remain intact (Fig. 1.20B). In secondary demyelination there is a concommitant loss both of axons and then of their myelin sheaths. In the luxol fast blue/cresyl Echt violet/PAS stain (Klüver-Barrera) macrophages in the demyelinated area are demonstrated to contain myelin debris. Alternatively immunocytochemical procedures can be used to demonstrate specific antigenic markers for either axons (see above) or myelin.

Leukodystrophy is the term applied to an intrinsic dysfunction in oligodendrocytes with formation of unstable myelin in contrast to demyelination where there is an acquired lesion affecting myelin from normal oligodendrocytes.

Immunohistochemical staining

For routine diagnostic purposes (FF-PE tissue) there are many antigenic markers for oligodendrocytes/myelin sheath although no single specific unequivocal marker for oligodendrocytes. Some antigens expressed by oligodendrocytes include myelin basic protein (MBP), myelin associated glycoprotein (MAG), and proteolipid protein (PLP), galactocerebroside (GC), 2′-3′-cyclic nucleotide-3′-phosphatase (CNP) (Fig. 1.20A), transferrin and the transcription factor Olig2, as well as many antibodies that are useful for more specialized experimental purposes. Normal oligodendrocytes can be definitively labeled using *in situ hybridisation* for PLP mRNA.

1.3.3 Astrocytes

Astrocytes play a critical role in many normal functions including intercellular homeostasis of ions, glutamine and neurotransmitters and the detoxification of, for example, oxidants and ammonia. They insulate and isolate white matter tracts and are involved in inflammatory and immune responses, expressing cytokines, growth factors and adhesion molecules. Astrocytes provide intrinsic structural support and guidance for

fetal brain development. They form the glia limitans and perivascular foot processes, an integral component of the blood–brain barrier. They are morphologically described as protoplasmic or fibrillary in normal gray or white matter respectively. The distinction between the two forms requires special stains.

Gliosis

Both protoplasmic and fibrillary astrocytes react similarly and non-specifically to almost any kind of damage (e.g. edema, viral infections, malacia, degeneration) to the CNS by either hypertrophy (astrocytosis) or proliferation (astrogliosis). There are basically two reactive forms: in acute injury the *gemistocytic astrocyte*, in which there is substantial homogeneous eosinophilic enlargement of the perikaryon with multiple thick short processes on HE sections (Fig. 1.21D) and chronically a morphological transformation to the *fibrillary astrocyte* with large numbers of glial fibrillary acidic protein (GFAP)-containing thin elongate cell processes (Fig. 1.21C). Chronic fibrillary astrogliosis is also called *sclerosis*. On routine HE stains, astrocytosis is apparent as an increased number of cell nuclei in the affected area (Fig. 1.21B).

Fluid accumulation in the CNS, edema, is associated with a spongy vacuolated appearance of the tissue (see Section 1.3.7). In cytotoxic edema there is an accumulation of fluid within and marked swelling of astrocytic cell bodies and processes. A special kind of reactive astrocyte is the so-called Alzheimer type II cell found in clusters in gray matter in metabolic encephalopathy

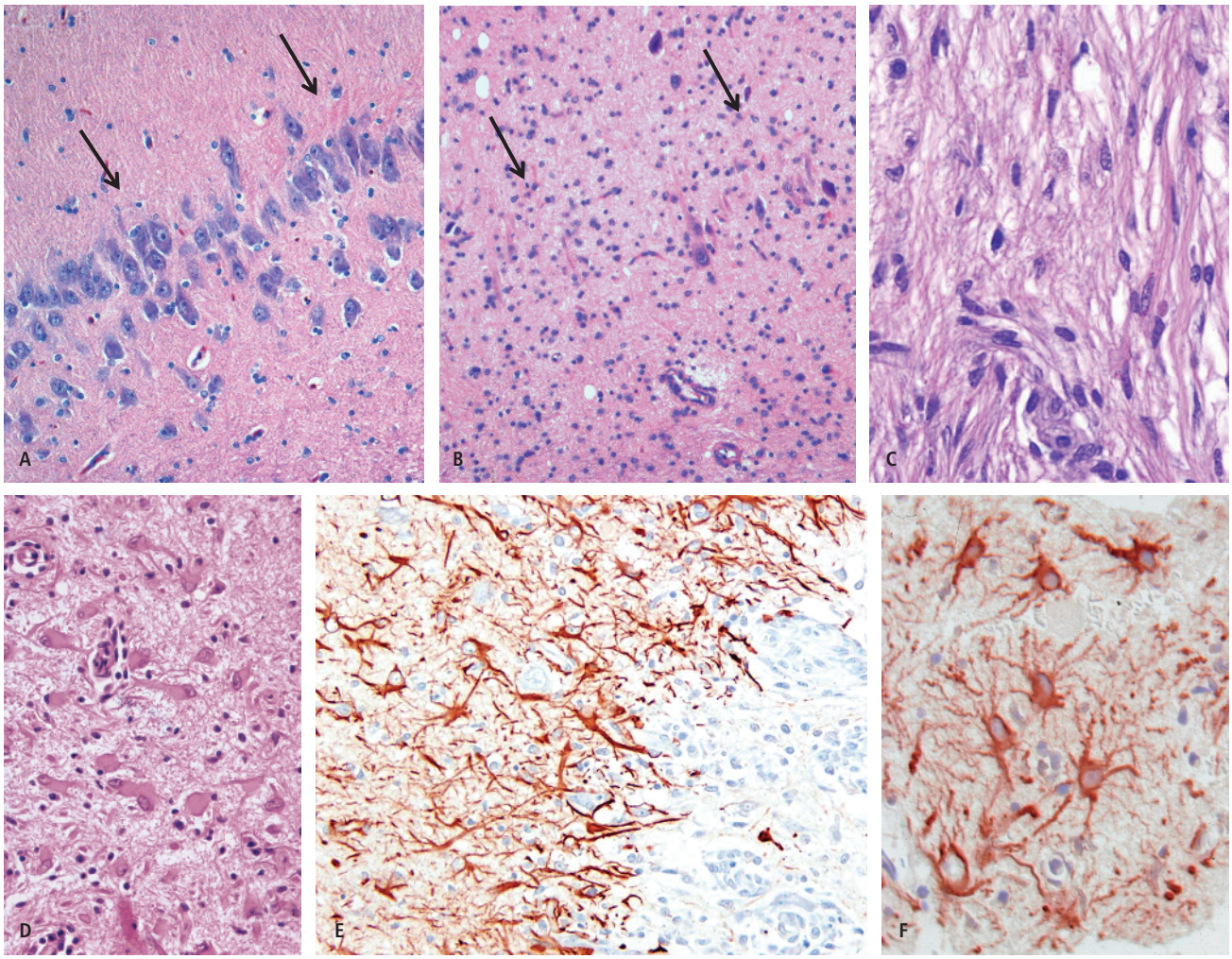

Fig. 1.21 Astrogliosis and astrocytosis. A: Normal dog. Hippocampus. Pyramidal cell layer. Relatively few glial cell nuclei. HE. B: Dog with hippocampal sclerosis. Same area as in A with nearly complete loss of neurons and massive increase in number of astrocytic nuclei. HE. C: Dog. Edge of old infarct. Fibrillary astrogliosis. HE. D: Dog with necrotizing encephalitis. Brainstem. Reactive gemistocytic astrocytes. HE. E: Dog. GFAP immunostaining of reactive fibrillary astrocytes around an oligodendroglioma. IHC. F: Dog. GFAP immunoreactive gemistocytic astrocytes with thickened processes. IHC.

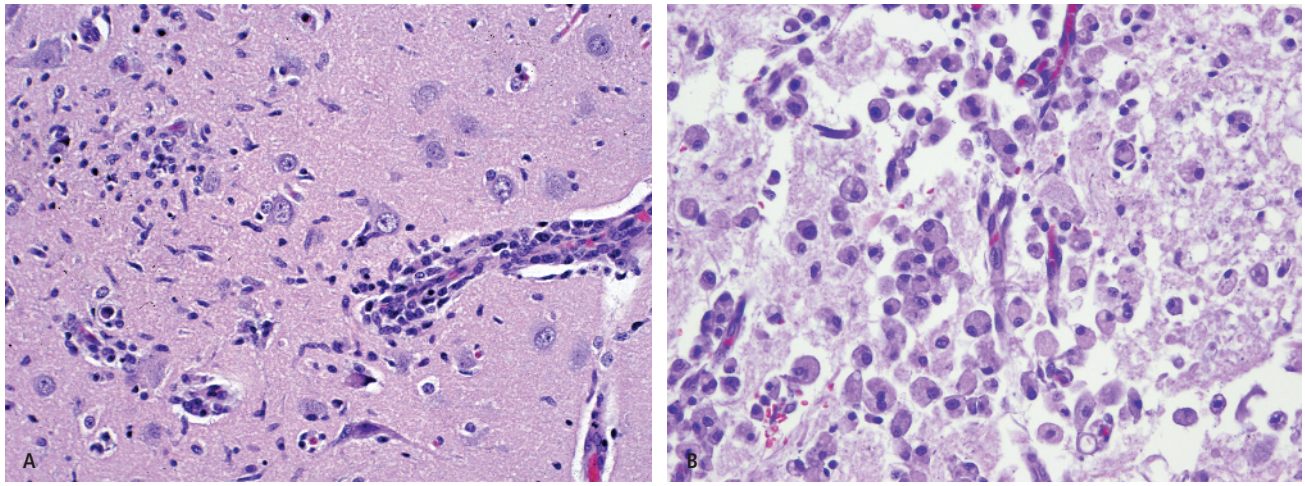

Fig. 1.22 A: Dog with viral encephalitis. Brainstem. Rod-shaped activated microglial cells. HE. B: Dog with cerebral infarct. Phagocytic gitter cells. HE.

associated with hepatic and less often renal disease (see Chapter 6). These cells have large pale nuclei with minimal cytoplasm. *Rosenthal fibers* are eosinophilic bodies found within processes of fibrillary astrocytes in tumors, sites of chronic injury and in **Alexander's disease** in the dog and sheep (see Chapter 8).

Immunohistochemical stains

The most widely used antigen detected immunocytochemically for normal, reactive and neoplastic astrocytes is glial fibrillary acidic protein (GFAP) (Fig. 1.21E,F). *Vimentin* and *nestin* can also be co-expressed with GFAP in reactive and neoplastic astrocytes. There is some cross-reactivity of GFAP with normal and neoplastic Schwann cells in the PNS.

1.3.4 Microglia/macrophages

Microglia and neuronophagia

The CNS contains a population of resident phagocytes, the so-called *resting microglia*, derived from monocytes of bone marrow origin entering the CNS during fetal development. These cells are capable of reactive proliferation in a variety of conditions. Activated reactive microglia, also called *rod cells*, appear as elongated, often twisted nuclei with very scant cytoplasm (Fig. 1.22A). This type of reaction occurs in more subtle lesions such as in retrograde neuronal degeneration and often in some protozoal and viral infections. These reactive microglia are mainly involved in removal of individual neurons in the process of neuronophagia mediated by chemokine-induced clusters of microglial cells forming microglial nodules.

Gitter cells and malacia

Acute necrosis of large areas of neuropil leads to almost complete loss of the original architecture of the affected area. This necrotic tissue is called *malacia*, which can be macroscopically visible when the lesion is large enough. The necrotic (malacic) tissue is initially infiltrated by densely packed actively phagocytic macrophages, also called gitter cells, which remove cell debris, axons and myelin (Fig. 1.22B). These cells are largely derived from blood-borne monocytes and to a much lesser extent from residential microglial cells.

Within weeks to months, a malacic area is completely cleared of all necrotic tissue and is replaced by a fluid-filled cystic cavity. Chronically reparative attempts with neovascularization and astrogliosis result in a fibrillary astroglial scar in and around the lesion and sometimes additional fibrous connective tissue formed by perivascular mesenchymal cells.

Identification by immunohistochemically detected markers

Both resting and activated microglia and macrophages derived from blood monocytes in the canine CNS can be broadly identified immunocytochemically by antibodies to both CD18 and CD11d in conventional FF-PE tissue. A fraction of microglia and macrophages may stain with antibodies to CD68, lysozyme and MAC (*myeloid/histiocyte antigen*). However antibodies capable of discriminating between these functional cell types in the CNS (resting or activated microglia, macrophages) are not yet available in the dog. Both activated microglia

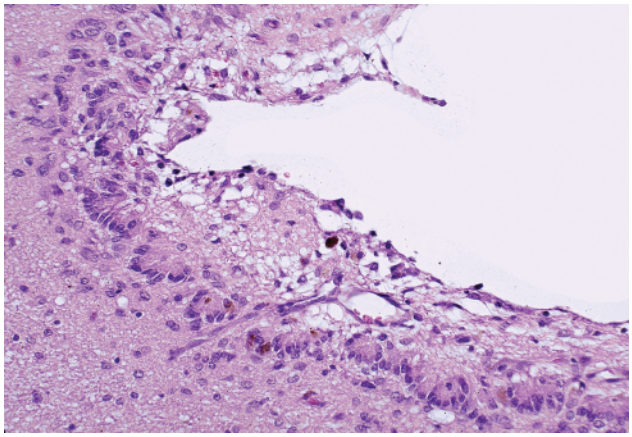

Fig. 1.23 Dog. Fourth ventricle. Traumatic damage to the ependyma leads to rosette formation, supra-ependymal astrocytosis and partial occlusion of mesencephalic aqueduct. HE.

and blood-borne macrophages express MHC class I and II antigens, various adhesion molecules, chemokines and cytokines and can act as antigen-presenting cells in modulating both inflammatory and immune responses.

1.3.5 CSF spaces

Ependymal cells

A single layer of usually ciliated ependymal cells lines the ventricles, mesencephalic aqueduct and central canal of the spinal cord, and allows for the regulated bidirectional flow of proteins and fluid between the ventricles and the interstitial space of the brain. Viral cytolysis, inflammation (*ependymitis*) or traumatic loss of these cells can lead to attempts at repair with rosette formation, sub- and supra-ependymal astrogliosis (Fig. 1.23) and occlusion with partial or complete obstruction of CSF flow at critical stricture points and subsequent non-communicating hydrocephalus. Atrophy with loss of cilia can occur with increased and sustained hydrostatic pressure of hydrocephalus. Normal and neoplastic ependymal cells are variably immunoreactive for GFAP and more consistently positive for vimentin. The subependymal zone around the lateral ventricles is a niche environment for neural stem cell populations.

Choroid plexus

The choroid plexus develops from evaginations of blood vessels covered by modified ependymal cells into specific sites within the lateral, third and fourth ventricles. The modified ependymal epithelial cells secrete CSF that fills both the ventricular system and subarachnoid space. The CSF delivers nutrients to and removes waste metabolites from the CNS. There is a tight-junction-mediated barrier between epithelial cells of the choroid plexus and the CSF (blood-cerebrospinal fluid-barrier). Bacteria, protozoa and viruses commonly invade the CNS by infecting the choroid plexus and disseminating within the ventricular system once the integrity of tight junctions between the epithelial cells is compromised. Canine distemper virus gains access to the CSF after productive viral infection of the plexus epithelium and subsequent dispersion of high titers of infectious viral particles into the CSF. Normal and neoplastic choroid plexus epithelium is usually immunohistochemically reactive for both low and high molecular weight *cytokeratins* while the lamina is immunoreactive for collagen IV. Neoplastic choroid plexus epithelium variably expresses GFAP.

Meninges

Leptomeninges (pia mater and arachnoid membrane) and the pachymeninges (dura mater) can be involved mainly as meningitis in bacterial or viral infections of the CNS associated with infection of the subarachnoid space. The major reactive change of the leptomeninges consists of fibroblastic proliferation and fibrous collagenous thickening. In chronic inflammatory disease, the latter may become very extensive and potentially occlusive.

Osteomyelitis, trauma and skull fractures may impinge on the contiguous dura mater and focal fibroblastic proliferation of the inner surface of the dura can occur in response to chronic dural irritation (tumors, vertebral subluxation, meningitis etc.). *Dural metaplastic ossification* can occur incidentally and mainly in large-breed dogs particularly in cervical and lumbar segments with formation of elongated bony plaques, often containing hematopoietic bone marrow.

Meningothelial cells express vimentin and variably cytokeratin.

1.3.6 Blood vessels

Blood–brain barrier

The CNS is extremely well vascularized with a very dense capillary network.

In contrast to other organs, the endothelial cells of the blood vessels in the nervous system are connected by *tight junctions* creating an effective protective mechanical and biochemical barrier between the blood and the nervous tissue. Lipid-mediated passive diffusion of small molecules is possible but most traffic across the BBB is by rate-limited, receptor- and carrier-mediated transport of nutrients, proteins and electrolytes. Also involved in the BBB as a functional system are the pericytes, which – together with the endothelial cells – form a *basement membrane*, and the astrocytic foot processes, which cover most of the external surface of the vessels.

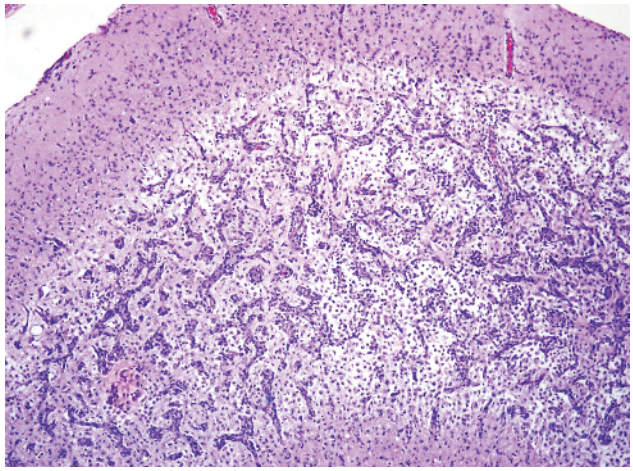

Fig. 1.24 Dog with chronic band-like (pseudolaminar) polioencephalomalacia. Cerebral cortex. Marked neovascular proliferation. HE.

In the normal state, little protein and a few inflammatory cells cross the BBB. Because of this blood–brain barrier the nervous system has been considered to be an *immunologically privileged site*. However, as we will see, this barrier can be breached rendering the CNS vulnerable to many immunopathologically mediated events. In infectious/inflammatory diseases, the BBB plays a very important role, regulating access of immune cells to the CNS. Endothelial cells are very active when homeostasis is lost: they react by upregulating MHC antigens and adhesion molecules, and can express various cytokines such as IL-1, IL-6 and IL-8, hence mediating inflammation in the CNS as explained in depth in Chapter 3.

Reactive blood vessels

The endothelial cells of the blood vessels of the CNS react in a variety of conditions by hypertrophy and proliferation (Fig. 1.24) initiated by hypoxia and resulting in upregulation of expression of vascular growth factors such as *vascular endothelial growth factor* (VEGF). Such growth factors are important in development and homeostasis and they can be affected by or participate in tissue damage and repair. Proliferation of endothelial cells results in neovascularization, which is easily recognized in routinely stained HE sections and immunohistochemically by antibodies to either CD31 or von Willebrand factor VIII related antigen.

1.3.7 Disturbance of water balance: edema

Homeostasis of exchange of water and electrolytes between the blood and the CNS depends on complex mechanisms in which the BBB plays an important regulatory role. Edema is the result of excess fluid accumulation in the CNS parenchyma and is frequently associated with most disease categories described in subsequent chapters. Classically, three different types of edema are recognized depending on their pathogenesis, but frequently these types co-exist.

Vasogenic edema

Vasogenic edema is the most common form of edema and is seen in many types of injuries such as trauma, focal and diffuse inflammatory processes, tumors and infarcts. The physical breakdown of the tight junctions between endothelial cells of the BBB causes increased vascular permeability with leakage of serum proteins into the intercellular space of the neuropil (Fig. 1.25A). The resulting increased osmotic pressure draws more water into the intercellular space. There is a preferential focal or global accumulation of fluid in the white matter since it has more extracellular space than gray matter. Such edema is readily detected by MRI and gross examination. Microscopically with HE staining, edema appears as pale areas with widespread separation of myelinated axons by clear vacuolated spaces (Fig. 1.25B). In chronic edema there is a reactive gemistocytic astrogliosis.

Cytotoxic (cellular) edema

Cytotoxic edema occurs when the energy-dependent sodium and potassium pumps in endothelial and glial cells are impaired. Intracellular accumulation of sodium ions leads to fluid retention resulting in intracellular swelling (Fig. 1.25A). This effect is first apparent in endothelial cells, then in astrocytes and subsequently in neurons and oligodendrocytes. The physical blood–brain barrier remains intact and hence cytotoxic edema has low protein content. This type of edema is seen in various toxic and metabolic disorders (discussed in Chapter 6). Furthermore, it occurs in combination with vasogenic edema in ischemia. Macroscopically, brain swelling appears generally less severe in cytotoxic edema than in vasogenic edema. Histologically, cytotoxic edema causes a *spongy state (status spongiosus)* with sharply defined vacuoles in the white matter due to intramyelinic edema (Fig. 1.25C). Cell swelling can also be seen as vacuoles within processes of perineuronal and perivascular astrocytes. Initially the status spongiosus is devoid of reactive changes; in chronic edema diffuse reactive astrogliosis becomes apparent.

Interstitial edema

Interstitial edema results from increased permeability of the desmosomal junctions between lining ependymal cells of the ventricular system. With increased intraventricular pressure the ependymal lining ruptures and CSF accumulates interstitially in the periventricular white matter. In contrast to vasogenic edema, interstitial

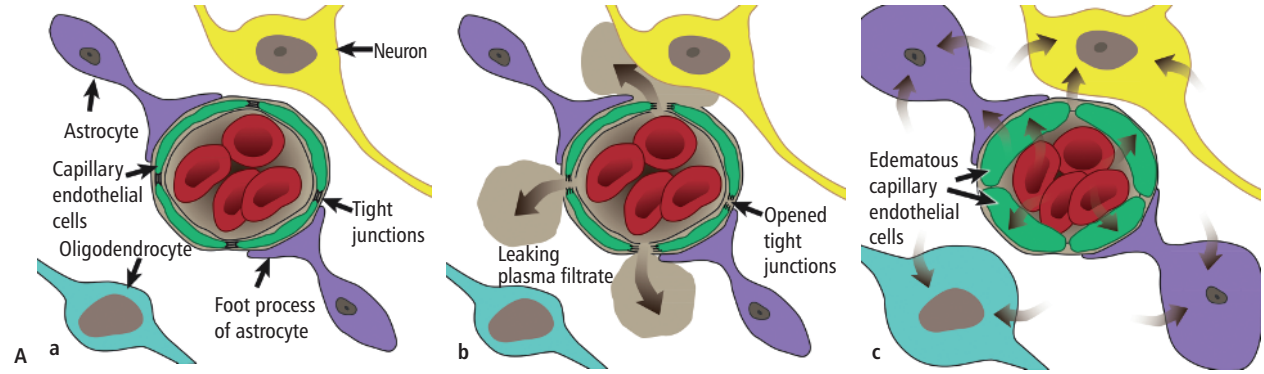

Fig. 1.25 Edema. A: Schematic drawing; a: normal capillary with endothelial cells connected by tight junctions creating the blood–brain barrier (BBB), supported by astrocytic endfeet processes; b: opening of the BBB with leakage of plasma into the interstitium (vasogenic edema); c: failure of ion/water pumps, water from blood enters the intracellular compartment (endothelial cells, astrocytes, neurons) with swelling of cells resulting in cytotoxic edema.

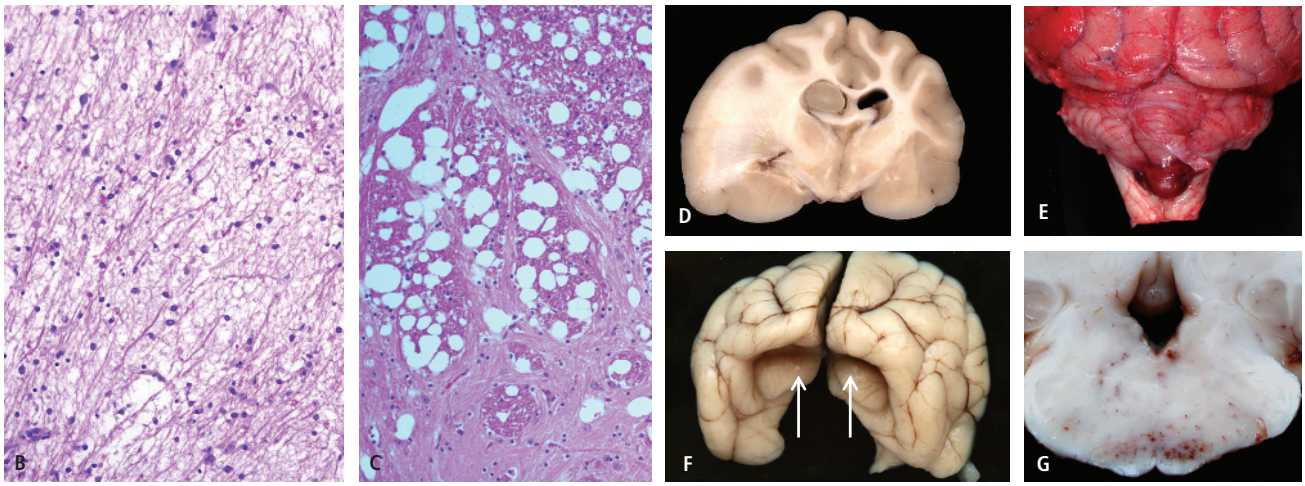

Fig. 1.25 B: Dog with brain tumor. Vasogenic edema in peritumoral white matter, separation of axonal fibers by clear spaces. HE. C: Calf with liver failure. Internal capsule. Spongy state with sharply delineated vacuoles. HE. D: Cat with brain tumor (meningioma extending in lateral ventricle). Massive edema particularly in white matter with swelling of the left hemisphere associated with the tumor. E: Dog with cranial trauma. Cerebellar coning with hemorrhagic necrosis of the herniated vermis and brainstem compression resulting from severe brain edema. F: Dog with brain tumor. Caudal view of occipital lobes, herniated cerebral cortex (arrows) underneath tentorium (tentorium has been removed). G: Dog with brain tumor and high intracranial pressure. Medulla oblongata. Multiple small hemorrhages (*Duret* hemorrhages).

edema has low protein content. Small pools of interstitial edema may coalesce leading to fluid-filled cavities of various sizes eventually leading to macroscopically visible cystic structures. In the spinal cord or brainstem such cavitations are called *syringomyelia* or *syringobulbia* respectively. In the brain, mostly connected to the lateral ventricles, they are called diverticula.

Effects of brain edema

Edema may cause focal or global CNS swelling usually associated with space-occupying masses such as abcesses, tumors or hemorrhages. Because the brain and spinal cord are closely confined by meninges and the rigid calvarium and vertebrae, brain or spinal cord swelling causes increased intracranial or intraspinal pressure, respectively, with life-threatening secondary complications as described in Chapter 4. The brain is compressed against the skull resulting in flattening of gyri and narrowing of sulci. In transverse sections marked mechanical distension, particularly of the white matter, and variable compression of the ventricular system can be observed (Fig. 1.25D). Global swelling of the cerebral hemispheres causes compression of the brainstem, which is flattened and distorted with close apposition of rostral colliculi. The mesencephalic aqueduct is narrowed, and in the brainstem and cerebellum small target hemorrhages (*Duret hemorrhages*) due to stretching and necrosis of blood vessels may be present

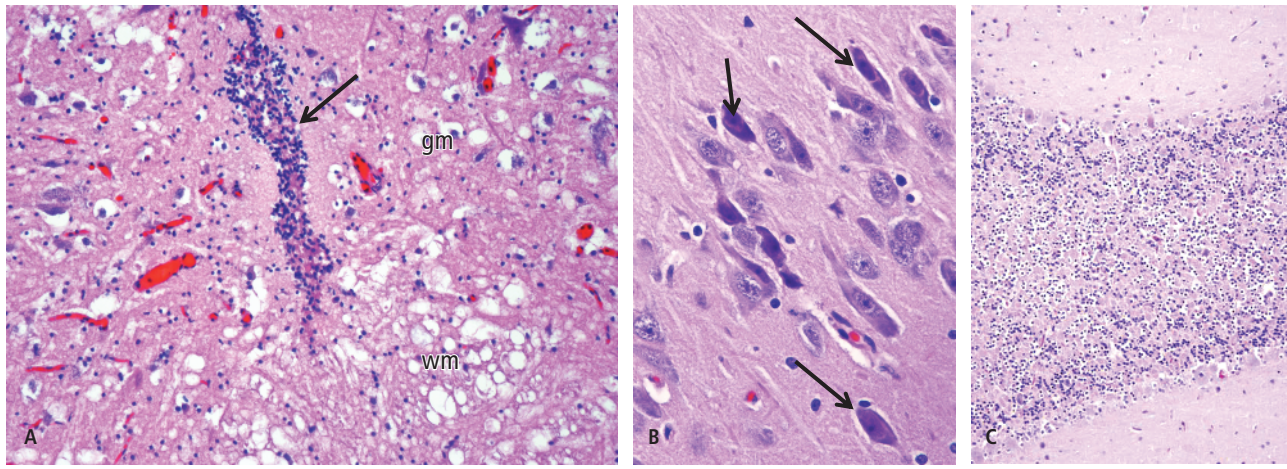

Fig. 1.26 Artifacts. A: Dog. Spinal cord. Advanced postmortem autolysis, marked vacuolation of gray (gm) and white (wm) matter, the structure of the central canal (large arrow) is largely disorganized with only the nuclei of the ependymal and subependymal cells indicating its presence. HE. B: Dog. cerebral cortex. Dark neurons (arrows) next to morphologically normal neurons. HE. C: Bovine. Cerebellar cortex. Extensive postmortem lysis of cerebellar granule cell layer, a common artifact in large animals. HE.

(Fig. 1.25G). The brain parenchyma expands caudally resulting in *cerebellar vermal herniation* (Fig. 1.25E) and herniation of the cerebral cortex underneath rigid structures (e.g. tentorium cerebelli: *transtentorial or subtentorial herniation* (Fig. 1.25F); falx cerebri: subfalcine herniation). Such herniation severely increases pressure in focal areas leading to small hemorrhages and ischemic necrosis following disruption of blood supply with a usually fatal outcome.

1.3.8 Artifacts, postmortem degeneration, pseudolesions and old age

Artifacts

Postmortem autolysis progresses quickly in nervous tissues leading to artifactual changes that may significantly interfere with detection and correct interpretation of lesions (especially for the untrained eye). In advanced autolysis, the CNS becomes mushy, macroscopic structures may become ill defined and cavities are caused by gas-forming bacteria.

The most common artifact of autolysis at the histological level is vacuolation (Fig. 1.26A). The latter can be especially prominent in the white matter. These vacuoles are irregular in shape and size and often not well delineated. Also rough handling of the tissues causes mechanical tearing and disruption of the tissue architecture with increased intercellular space, separation of neuronal layers and vacuolation. Inadequate processing (e.g. prolonged contact with 70% ethanol) causes significant vacuolation principally of the white matter. In the gray matter, there are excessively large clear spaces around neurons, blood vessels and glial nuclei. Artifactual vacuolation has to be distinguished from pathological spongy state in the CNS as discussed in Chapter 6.

Purkinje cells undergo rapid perineuronal vacuolation, and granule cell depletion is also common after a prolonged interval before fixation (Fig. 1.26C). With advancing autolysis, neurons become pale and pink with a slightly foamy cytoplasm. Sometimes, oligodendroglial nuclei are uniformly surrounded by clear halos or opaque material, rendering the impression of a swollen cytoplasm. In advanced autolysis, the choroid plexus and ependyma become denuded of their epithelial-derived lining cells (Fig. 1.26A). A reliable sign of good tissue preservation is the presence of cilia on ependymal cells.

Inadequate fixation either in time or in fixative volume leads to blurring of structural detail in histological sections. Pale-basophilic staining, round structures (mucocytes, Buscaino bodies) may appear in significant numbers in the parenchyma as an artefact of formalin fixation, probably by reaction of myelin components with formalin.

A very common finding even in fresh, well fixed tissue are *dark neurons*. Their name derives from single or clusters of neurons anywhere in the CNS, which appear shrunken and strongly basophilic with corkscrew dendrites (Fig. 1.26B). Their significance has been hotly debated but they are most probably artifact due to tissue handling. Others interpret the uptake of the dye *fluorojade* as an early degenerative change in such neurons.

When such abnormalities are not associated with reactive changes (e.g. invasion of macrophages, gliosis,

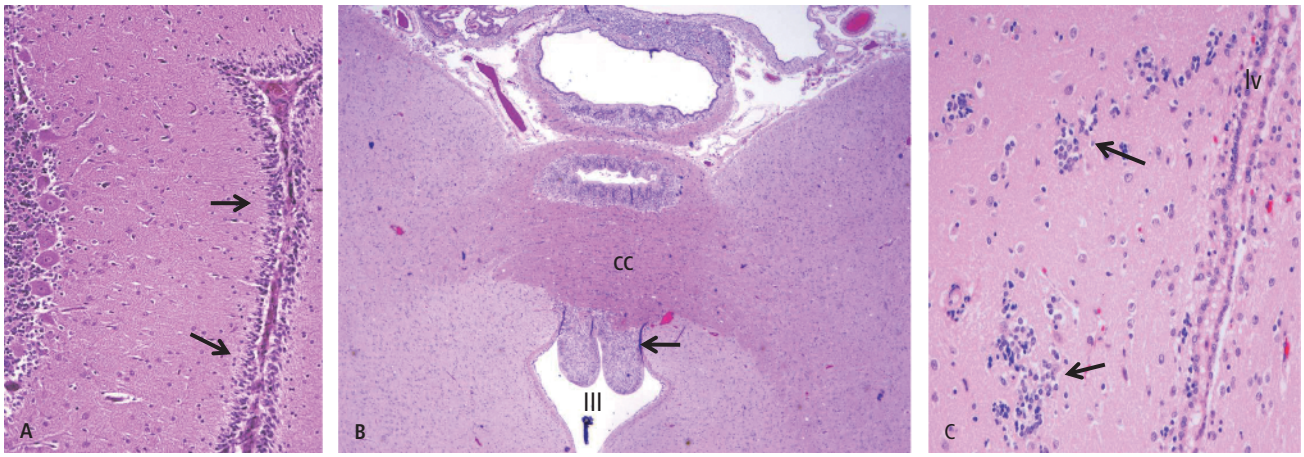

Fig. 1.27 Pseudolesions. A: Young dog. Cerebellum with an external granule cell layer (arrows). HE. B: Dog. Subcommissural organ (arrow) (cc, caudal commissure; III, third ventricle). C: Dog. Caudate nucleus. Clusters of primitive neuroectodermal cells (arrows) in the caudate nucleus in vicinity of lateral ventricle (lv). HE.

invasion of inflammatory cells, capillary proliferation) it is prudent to assume that they are artifacts unless they are found in optimally preserved and processed tissues.

Pseudolesions

Be aware of some normal microanatomical features suggesting a lesion:

- Very young animals may still have germinal cell layers notably subpially in the cerebellar cortex (*external granule cell layer*) (Fig. 1.27A). The density of glial cells in immature white matter is also much higher than in adult brains.
- The *circumventricular organs* (CVO). These small, highly specialized structures in the wall of the third and fourth ventricles contain numerous capillaries without a blood–brain barrier. Ependymal and neural cell processes contact the CVOs, which play a role in the coordination between neural and hormonal functions. The CVOs include the organum vasculosum, the subfornical organ, the subcommisural organ (Fig. 1.27B) and the area postrema in the fourth ventricle.
- Focal accumulations of *primitive undifferentiated glial and neuronal cell precursor cells* occur in the forebrain in subependymal and perivascular sites around the lateral ventricles (Fig. 1.27C). They are very prominent and dense in fetal and neonatal animals, also occurring in the thalamus and midbrain; they may again increase in numbers with old age.
- In the brainstem, neurons of certain nuclei such as the olivary and pontine nuclei uniformly lack the perinuclear Nissl substance; this is not to be confused with chromatolysis.
- In certain mammalian species normal secretory neurons of the hypothalamus and locus coeruleus contain cytoplasmic melanin.
- Eosinophilic intracytoplasmatic inclusion bodies occur occasionally in neurons (e.g. in the lateral geniculate body) in different species, notably cats (pseudo-Negri bodies).

Old age

Tissue changes associated with old age are quite consistent, and it is a quantitative problem whether to call them pathologic or simply physiologic attrition with age.

Old dogs often show a marked hydrocephalus and apparent shrinkage of the cerebrum (Fig. 1.28B) without ever having suffered from neurological signs during their lives. Although there is gliosis and increased numbers of glial cells around neurons (satellitosis) in the gray matter in old dogs with brain atrophy, it is not clear whether there is significant loss of neurons. Another conspicuous feature in old brains is a diffuse status spongiosus of the white matter (Fig. 1.28D), sometimes with fibrillary gliosis ("scar" tissue). This appears to be due to degeneration and loss of myelin associated with deposits of non-degraded ubiquitin–protein conjugates and complex galactolipids. A consistent finding in all species is the appearance of degenerated neurons and large spheroids in the accessory cuneate nucleus. The number of so-called subependymal and cortical glial nests in old brains seems to be increased compared with younger animals (in the rhinencephalic cortex, ventricular angles, caudate nucleus). A typical sign of old age is an accumulation of lipofuscin granules in the cytoplasm of neurons, in particular in the brainstem nuclei (Fig. 1.28C). Around blood vessels one often sees macrophages containing lipopigment. Senile plaques and congophilic angiopathy (Fig. 1.28F,G) have also been described in very old animals as discussed in Chapter 8 on degenerative disease. There is also a fibrotic thickening of the meninges and of the stroma of the choroid plexus.

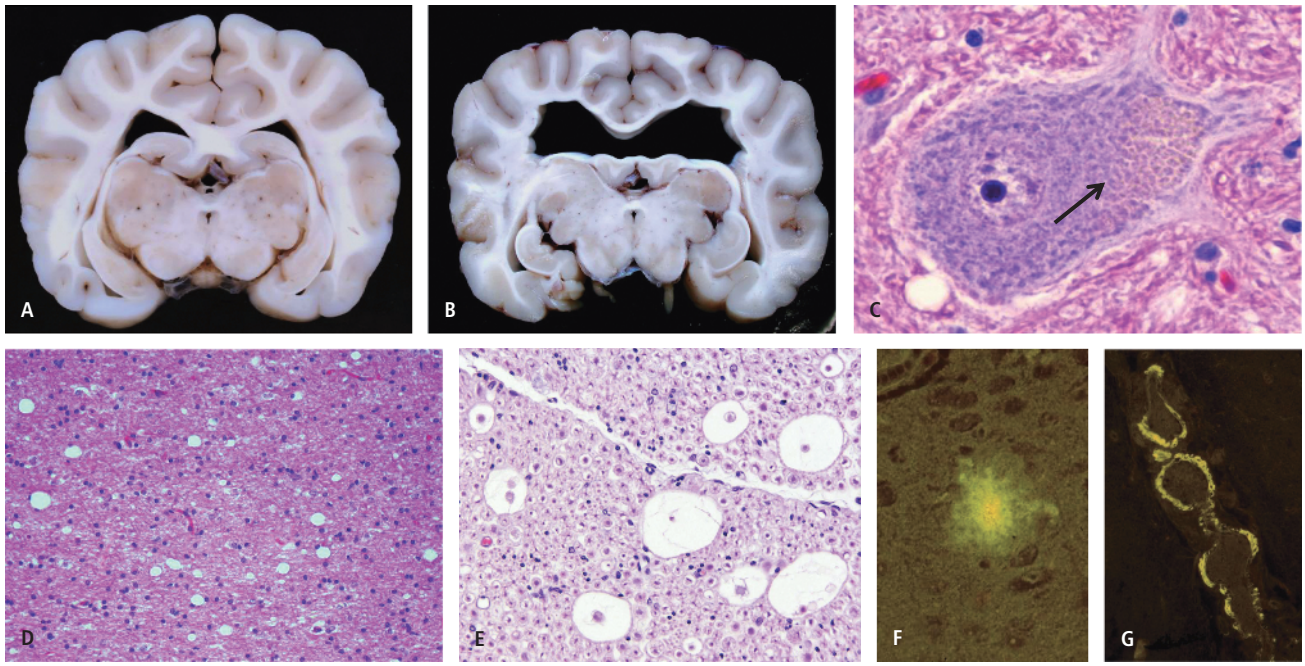

Fig. 1.28 Geriatric changes. A: Normal 2-year-old dog. Cross-section of forebrain. B: Normal 14-year-old dog. Section at the same level, marked thinning of the cerebral cortex and subcortical white matter with compensatory enlargement of the ventricles C: Old dog. Brainstem. Lipofuscin pigment accumulation (arrow) in a neuron. HE. D: Old dog. Cerebrum. Vacuolation and gliosis in the white matter. HE. E: Old dog. Spinal nerve roots. Ballooning of myelin sheaths. HE. F: Old dog. Cerebral cortex. Senile plaque (accumulation of beta amyloid). Thioflavine stain, UV fluorescence. G: Vascular amyloidosis. Thioflavine stain, UV fluorescence.

In old cats, we often see focal meningothelial proliferation/hyperplasia. In some species such as horses, there is mineralization and iron deposition in the walls of blood vessel walls mainly in the basal nuclei. In the PNS, old age is frequently characterized by extensive vacuolation of myelin sheaths particularly in nerve roots (Fig. 1.28E).

1.4 Recognizing major lesion patterns

Diagnostic neuropathology is based on the detection of lesions and their subsequent interpretation. In addition to being familiar with the basic reaction patterns as described in Section 1.3, two further essential requirements for a successful start in diagnostic neuropathology are: (a) being able to recognize the major gross and histological lesion patterns and (b) a working knowledge of the classification of neurological diseases including the major morphological hallmarks (lesion patterns) of each disease category.

1.4.1 The major lesion patterns

As we have seen in Section 1.3, the nervous system can only mount a relatively limited number of reactions to injury. It follows therefore that there are also only a limited number of *basic* lesion types. Of course there are myriads of morphological variations of these basic lesion types but it is possible and necessary to recognize their most essential common features or patterns. At the macroscopic level we can recognize the following patterns: abnormal anatomy, space-occupying mass lesion, hemorrhage, malacia/necrosis, pallor/softening of the white matter. While even small hemorrhages can be seen with the naked eye, small foci of incipient malacia may require microscopic detection. Major microscopic patterns include, apart from malacia: spongy change, intracellular accumulation of abnormal material, hypercellularity, selective loss of neurons, axons or myelin. All these lesion patterns are briefly described below and illustrated in Fig. 1.29.

Deviation of normal anatomy

With some basic knowledge of neuroanatomy, abnormal anatomic disturbances are readily identified macroscopically as, for example, hydrocephalus or cerebellar hypoplasia. Deviation of the normal anatomy is most often a congenital malformation but can also be the result of some other acquired pathological process leading to atrophy of a certain region. Occasionally, part of the brain or cord, or sometimes the whole brain, is swollen

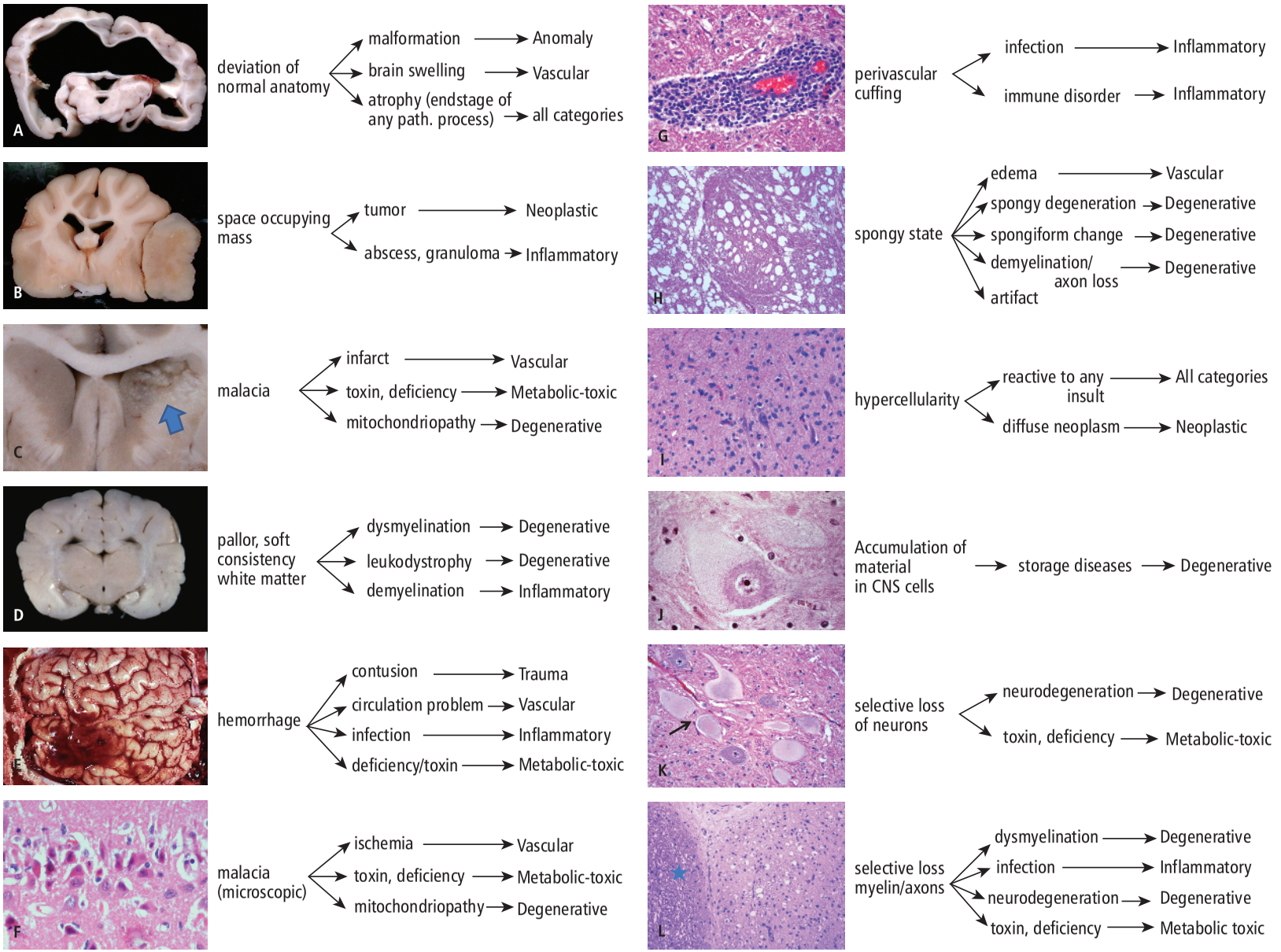

Fig. 1.29 Major lesion patterns and their interpretation. A: Major gross lesion patterns and their interpretation. B: Major histological lesion patterns and their interpretation.

due to mass lesions with cerebral edema. The resultant increased intracranial pressure can force areas of the brain underneath the tentorium cerebelli (*subtentorial herniation*) or the cerebellar vermis caudally through the foramen magnum (*cerebellar vermal herniation*). Also the cerebrocortical gyri are flattened and widened and the sulci compressed.

Space-occupying mass

Such a mass is usually easy to detect. Space-occupying mass lesions in small animals are generally due to primary or secondary tumors but can also be associated with excessive focal accumulation of inflammatory cells (abscess, granuloma) and result in a midline shift.

Malacia

Malacia is defined as grossly or microscopically detected softening and necrosis of the tissue resulting from destruction of most cells in the affected area leading to complete loss of the original architecture. Complete destruction with cavitation is easy to detect grossly, while acute lesions display a gelatinous consistency often with a change of color to gray or yellow. Histologically, malacic lesions are demarcated from normal tissue and appear paler or darker than their surroundings. The original architecture is lost, and the acutely affected tissue appears less compact, vacuolated and with widespread cell necrosis.

Pallor/softening of the white matter

Extensive lesions of the white matter are visible to the naked eye. The white matter is normally brilliantly white and in many areas is clearly demarcated from the gray matter. Following paucity/absence/loss of myelin the white matter becomes grayish, less distinguishable from the gray matter and may appear softened. Histologically on HE, loss of myelin is characterized by pale staining. Specific myelin stains are very useful to detect and define this lesion.

Hemorrhage

Accumulation of erythrocytes outside the blood vessels is usually grossly visible as red to dark brown in acute hemorrhage while orange/yellowish discoloration of the tissue is more indicative of chronicity. Hemorrhage results from damage to the blood vessels and is usually associated with malacia.

Perivascular cuffing

Accumulation of inflammatory cells around the blood vessels in the parenchyma is probably the easiest lesion to detect at the histological level. Such perivascular cell accumulations or cuffing can often be detected at low-power magnification and are highly characteristic for an inflammatory/infectious disorder but may also occur in a very limited extent in other disorders (e.g. hippocampal necrosis in cats).

Spongy state (status spongiosus)

This is a very common pattern resulting from many different types of injury and is also a common artifact. The tissue looks moth-eaten, there is an excessive number and size of clear spaces around neurons, glial cells and capillaries, and diffuse or distinct vacuoles in the neuropil and or specifically in gray and/or white matter. Such vacuolar change can be caused by postmortem artifact, (intra-myelinic) edema, loss of axons and/or myelin, or vacuolation of neurons, glial cells or their processes. A guide to the interpretation of this spongy state is extensively covered in Fig. 6.14.

Hypercellularity

This is apparent as focal or extensive areas with increased numbers or density of cells. Usually, in HE sections the cell nuclei are most prominently stained, thus we detect too many nuclei. This change is very common and there are several variations of this change.

- Infiltration and accumulation of inflammatory cells. Accumulation of large numbers of inflammatory cells in the leptomeninges and subarachnoid space can sometimes be detected on gross examination as a diffuse whitish or yellowish clouding of the meninges.
- Astrogliosis or microgliosis (proliferation of astroglial or microglial cells), a reactive change particularly of astrocytes or microglial cells, which can occur as a result of any kind of injury. Hypercellularity can thus be a feature of many disease categories.
- Proliferation of endothelial cells and increased density of microvessels or neovascularization in the affected area is a common reactive change in different types of injury.
- Diffuse neoplasia. While most tumors consist of compact expansile masses, in certain tumor types (e.g. astrocytomas), neoplastic cells may diffusely invade the tissue, leaving the original architecture of the tissue more or less undisturbed.

Accumulation of abnormal intracellular material or bodies

Such abnormal material can consist of inclusions, granules, foamy material or clear vacuoles and is relatively

easy to detect in neurons because of their size. Such change can also be associated with swelling of cells and/or displacement of other cell components such as the nucleus.

Selective changes in neurons/axons/dendrites

Detection of this pattern requires careful histological examination and may be difficult without experience. The architecture of the tissue is changed, regularity is disrupted, the tissue appears less compact and there is loss of certain tissue elements. Recognition of these changes is greatly facilitated by knowing the normal microanatomy of the various areas of the CNS. This knowledge is acquired by making it routine to consciously register anatomical features while reading histological slides. Special histochemical or immunohistochemical stains for myelin and axons are very helpful to recognize selective change in the white matter. For example, such stains are required to distinguish between primary or secondary demyelinating lesions.

1.4.2 Lesion distribution pattern

As explained in later chapters, the anatomical distribution pattern of the lesions is of paramount importance in the differential diagnosis. Lesions which are either single (focal) or multiple (multifocal–disseminated) can already suggest different disease categories. Generally, bilateral involvement of specific anatomic areas is typical for metabolic–toxic and degenerative diseases. Particularly in the latter the lesions are mostly, but not always, symmetrical. Last but not least lesions may have a predilection for either gray or white matter. As we will see, the specific anatomical location of lesions may be highly diagnostic in many diseases.

1.4.3 Classification of neurological diseases

Recognition of the major patterns is a first important step in neuropathological diagnosis. The recognized patterns must then be placed in the appropriate disease category against a background of historical and clinical information. For diagnostic purposes, it makes sense to categorize diseases according to their common characteristics. To classify lesions, it is obviously best to consider disease mechanisms that are associated with specific morphological changes, and thus with certain patterns of lesions as described above. This is true to a certain extent, at least at the level of the large disease categories. However we prefer to use a classification system based not only on morphological criteria but which also includes assessment of clinical data, including that obtained from MRI images. With this approach we can distinguish the following groups of diseases: vascular, inflammatory, traumatic, anomalous (malformation), metabolic–toxic, idiopathic, neoplastic, degenerative. The acronym **VITAMIN D** may be helpful as an initial memory aid for the neurologist as well as the pathologist.

While diseases in this scheme are classified according to traditional pathologic criteria, these categories not only have common morphological features (thus exhibiting one or more of the above lesion patterns) but also include some common clinical neurological denominators or at least trends with respect to breed, age, onset/course, involvement of other organ systems, focal or multifocal localization and CSF evaluation changes.

Vascular diseases

The main lesions of the CNS vasculature include vasculitis, hemorrhage and infarction. The latter results from focal vascular obstruction (e.g. septic or bland fibrin thrombi and emboli, fibrocartilage, atherosclerosis, intravascular lymphoma) or less commonly from global ischemia and consist of sharply demarcated areas of neuropil destruction termed malacia. Vascular diseases can occur in all age groups and provoke peracute, severe, often lateralizing neurological signs which remain stationary for some time and may gradually regress. Extraneural signs are found when the primary problem is located in the cardiovascular system, e.g. bacterial endocarditis. Cerebrospinal fluid is usually altered with protein increase, excess red blood cells (RBCs) and sometimes pleocytosis with macrophages.

Inflammatory diseases

The hallmark of this group is parenchymal invasion of blood-derived leucocytes around blood vessels (perivascular cuffing) and infiltrating into the parenchyma, as well as proliferation of endogenous microglia (hypercellularity), resulting in encephalitis, myelitis, meningitis, ependymitis, choroid plexitis and neuritis. Inflammatory lesions are most frequently associated with infections but also with immune-mediated disorders. Infectious diseases of the CNS can occur in any age group but are generally more frequent in young animals. They are usually of acute onset with rapid progression but a subacute–chronic course may occur. Infections can be restricted to the CNS but may also be associated with disseminated lesions in other organ systems. Inflammatory lesions are generally *multifocal*, *disseminated* or *diffuse*. Focal lesions may become space-

occupying masses. Neurological signs may suggest multifocal involvement but one single localizing sign may predominate. Pleocytosis and protein increase in the CSF is typical of CNS inflammatory diseases.

Trauma

Cranial or spinal trauma results in mechanical disruption of tissue compounded by traumatic injury to blood vessels resulting in hemorrhage and usually malacia. Trauma leads to peracute neurological signs, which in the case of hemorrhage, may rapidly worsen. The neurological signs may improve in the days and weeks following trauma. In the case of endogenous spinal cord trauma (e.g. intervertrebral disc disease) a subacute to chronic intermittent course is frequently seen. Neurological signs are mostly focal. CSF sampling is contraindicated in cranial trauma due to concurrent edema and raised intracranial pressure. In spinal cord trauma various spectra of CSF changes may be found.

Anomalies or malformations

Abnormal development of the CNS *in utero* may lead to gross or more subtle malformations or anomalies of the normal anatomy, e.g. hydrocephalus, cyclopia, cerebellar hypoplasia or spina bifida. Most malformations occur as single point genetic mutations, and much less commonly as a result of intrauterine transplacental infections or intoxications. Malformations are usually clinically apparent at birth or within the first months of life. Usually, neurological signs are focal and remain stationary, although some compensation or progression may develop in time. Routine CSF evaluation fluid is unremarkable.

Metabolic–toxic diseases

Deficiencies and toxins can lead to acute destruction of nervous tissue in certain anatomically restricted sites in a *bilateral, usually symmetrical distribution pattern*. Tissue destruction is frequently severe with malacia, cavitation and hemorrhage. An additional common microscopic lesion is a spongy vacuolar change. In some conditions lesions are more discrete and therefore resemble degenerative diseases. Metabolic–toxic diseases are usually in groups of animals, and of rapid onset and progression reaching maximal clinical intensity within a short time. Surviving animals may gradually recover. Neurological signs reflect a particular localization but without lateralization. There are usually non-specific but marked changes in the CSF in both protein level and cell content. Metabolic encephalopathies resulting from primary extraneural organ failure (e.g. renal or hepatic) exhibit a fluctuating course and diffuse localization.

Idiopathic diseases

Idiopathic means of obscure or unknown cause. Because this designation is still applicable for many animal CNS diseases, idiopathic disorders comprise the single largest group of diseases, which in our opinion is no longer practical. Therefore we use the term idiopathic for a group of diseases with abnormal *functional* neurological signs but without morphologically detectable changes of the nervous tissue. Such syndromes or diseases are not covered in this book. Examples are epilepsy, myasthenia, narcolepsy and the scores of clinically ill defined "movement disorders". Such diseases often demonstrate clinically a paroxysmal (e.g. seizures) or fluctuating course. Routine CSF evaluation is unremarkable. It is important to note that in such cases on neuropathological examination no lesions are detected even though the animal may demonstrate severe neurological signs.

Neoplasia

Primary CNS tumors, pituitary tumors and metastatic tumors originating from surrounding tissues (e.g. nasal cavity) or from other organs all compromise the CNS by either destructive invasion or compression by tumor cell proliferation (hypercellularity). Such focal space-occupying lesions can result in a rise in intracranial pressure and secondary peritumoral edema. Tumors can also obstruct CSF flow with secondary distension of the ventricular system. Tumors mostly affect older animals. The clinical course is usually subacute to chronic but a sudden increase in intracranial pressure may lead to rapid progression. Neurological signs are focal and lateralizing. In metastatic brain tumors, signs may be multifocal and clinical evidence of the primary tumor may be found. CSF changes include mild to severe protein elevation, sometimes with exfoliation of tumor cells.

Degenerative diseases

These diseases are characterized by progressive degeneration of specific cell types in the nervous system in a *bilaterally symmetrical* and restricted anatomical localization. Common patterns are: selective change of neurons/myelin/axons with gliosis (hypercellularity), spongy state, pallor or loss of white matter and abnormal accumulation of material in neurons. Such lesions are attributed to specific gene defects. Most degenerative diseases occur in young animals in certain breeds. Sometimes they are of late onset. The course of the disease is slowly progressive. Most degenerative diseases are restricted to the CNS; however in lysosomal storage diseases there is also selective involvement of extraneural tissues. Selective involvement leads to focal neuro-

logical signs without lateralization. The CSF is usually normal.

1.4.4 General strategy

Gross lesions

Recognition of the major lesion patterns and the anatomically based lesion distribution is the important first step and will direct the neuropathological assessment towards specific disease categories. The latter are covered in the subsequent chapters of this book. It is important to use this systematic stepwise approach before assessing details. Fig. 1.29 shows examples of major lesion patterns. The first five images relate to recognizable changes at the macroscopic level: deviation from normal anatomy, space-occupying mass, hemorrhage, malacia and selective white matter changes. When such changes are seen, various diagnostic options are listed with their respective VITAMIN D category.

At the next level, microscopic lesions are generally more difficult to interpret for beginners with one exception: it is easy to recognize perivascular cuffing, the hallmark of inflammatory diseases. With further experience additional patterns can be recognized including, in increasing order of difficulty, microscopic malacic lesions, spongy change, intracellular accumulation of abnormal material, hypercellularity and selective lesions/loss of neurons, axons or myelin.

The major patterns may often occur in combinations, e.g. hemorrhage together with malacia or hypercellularity together with pallor of the white matter. It is important to realize that lesions are dynamic processes and their morphology can be modified with time.

Detecting changes and recognizing the major patterns are both major steps towards diagnosing neuropathological problems. In the following chapters these basic patterns will be further subdivided and detailed and their underlying mechanisms will be discussed. In approach, pattern recognition and understanding disease mechanisms is most effective in achieving a neuropathological diagnosis.

1.5 Neuropathology in the clinics: magnetic resonance imaging (MRI)

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In recent years, MRI has become an essential non-invasive diagnostic method in veterinary neurology. This technique allows visualization of the nervous system in sections, very much like in the macroscopic examination of brain slices. The interpretation of MRI images requires not only physical and neuroanatomical but also neuropathological knowledge. Thus this is an area where diagnostic imaging and diagnostic pathology increasingly overlap. In fact, neuropathologists are often consulted to help in the interpretation of images and, conversely, the MRI findings have become an integral part of the documentation of neurological cases submitted to necropsy. Therefore neuropathologists should become familiar with the principles of MR image interpretation.

1.5.1 Basic MRI physics

MRI generates sectional images of the body by exploiting the nuclear magnetic resonance of atomic nuclei in the body. The most commonly used (and most abundant) atom in this respect is hydrogen.

According to the quantum theory, hydrogen nuclei act like rotating gyroscopes, which have a dipole moment about their rotational axis, the so-called nuclear spin. In MRI, the rotational axis of the nuclear spin, which has a random and disorganized orientation at rest, is polarized by a strong magnetic field and consequently hydrogen nuclei of the body align along this field. This may occur in two directions: either parallel or anti-parallel to the direction of the magnetic field, where they rotate along their axis in a frequency that is defined by the strength of the magnetic field and the proton in question (precession). Then, a short radio frequency pulse is emitted by a coil, which is placed around the body part of interest. The hydrogen nuclei with the same frequency as the emitted radiopulse will absorb the energy (resonance frequency or "Larmor frequency") and as result the nuclear spin changes its orientation in the magnetic field (resonance). When the radio wave pulse is turned off resonating protons return to their original low-energy (equilibrium) state, a process called relaxation, and the released energy can be registered by a receiver coil. An image can be produced because relaxation rates of hydrogen protons vary depending on their chemical binding in a given tissue and the water content of the latter. Therefore, with the additional help of paramagnetic contrast media, MRI is able to detect subtle differences between soft tissues. In the nervous system, this method allows the direct and anatomically exact reproduction of gray and white matter, the ventricular system with the CSF, the spinal cord, the spinal nerves, the intervertebral discs and the surrounding fat. Only the resolution of bony structures and bone/air interfaces is poor.

Two relaxation components are differentiated: the so-called T1 (or longitudinal) and the T2 (or transverse) relaxations. The length of T1 relaxation (T1 relaxation

time) lies in the range of seconds for fluids but is much shorter for soft tissues (tenths of seconds). The T2 relaxation time, like the T1 relaxation time, has a great influence on the signal intensity of a given tissue and image contrast. In T2-weighted (T2W) images, brain tissue has a short T2 relaxation time, whereas CSF has a long T2 relaxation time which is displayed as high signal intensity structure (white).

1.5.2 Principles of interpretation

Practical examples are shown in Fig. 1.30, Fig. 1.31 and Fig. 1.32. Please note that radiologists use the perspective of the examiner standing in front of the animal. The right side of the image corresponds with the left side of the animal.

The standard image planes in an MRI examination are oriented in a transverse, sagittal and dorsal direction. However, any other sections are also possible. As these slices have a certain thickness, MRI renders a two-dimensional image containing all the information within these tissue slabs. By using a variety of so called sequences, the different components of the tissue can be seen in different ways. In the brain, the basic examination usually includes T1W (read as T1-weighted) images, T2W images and FLAIR (fluid attenuated inversion recovery) images for the suppression of the signal from free fluid such as the CSF.

T1W images are basically used for differentiating fat from water and carry excellent anatomical information. Tissues with short T1 relaxation times such as fat or tissues that accumulate contrast agents have high signal intensity and appear bright, substances with a long T1 such as CSF have low signal intensity. As the white matter in the brain contains more fat than the cortex (due to its myelin content) and has therefore a shorter T1 relaxation time, the T1W image provides good gray–white matter contrast and hence an excellent anatomical picture of the brain. Due to the usually increased water content causing prolongation of the T1 relaxation time, most pathological changes appear as hypointense structures compared to normal tissue.

In T2W images, brain tissue has a comparatively shorter T2 relaxation time and lower signal intensity than CSF, which has high signal intensity in this sequence, resulting in good portrayal of the ventricular system. The contrast of the white to gray matter is reversed compared to T1W sequences: the gray matter is more hyperintense than the white matter on T2W images. T2W are sensitive for pathologies since they emphasize change of water content as it occurs in most pathologies including tumors, edema and inflammation. Increased water content causes a prolongation of the T2 relaxation times and therefore lesions are more hyperintense on T2W images than normal tissues. Furthermore, iron-containing structures such as the basal nuclei and nuclei in the brainstem and cerebellum are hyperintense in T2W images compared to the surrounding brain tissue.

Special sequences and images using contrast agents can selectively increase the contrast between different tissue components and diseased versus normal areas. In the brain, the single most important sequence is the FLAIR (fluid attenuated inversion recovery) sequence which selectively suppresses the signal from free fluid such as the CSF but not of brain edema facilitating the detection of lesions in the subarachnoid space and in the brain parenchyma close to the ventricular system.

The diagnostic yield of a MRI investigation can be enhanced with the injection of paramagnetic contrast agents such as gadolinium. Paramagnetic substances accelerate energy exchange of spins in the vicinity of the contrast agent thus shortening the T1 relaxation time of the hydrogen protons and leading to increased signal intensity. Since the contrast agent does not penetrate the intact blood–brain barrier (BBB) of normal brain, only structures without BBB will be hyperintense after application of contrast media. Examples are the pituitary gland or the highly vascularized choroid plexus. In pathological tissue with absence or disturbance of the BBB, lesions will show high signal intensity and increase the contrast to normal tissue thus enhancing the sensitivity of an MR examination. The degree and pattern of contrast uptake also varies between different types of abnormalities therefore increasing also the specificity of the examination. See MRI Atlas.

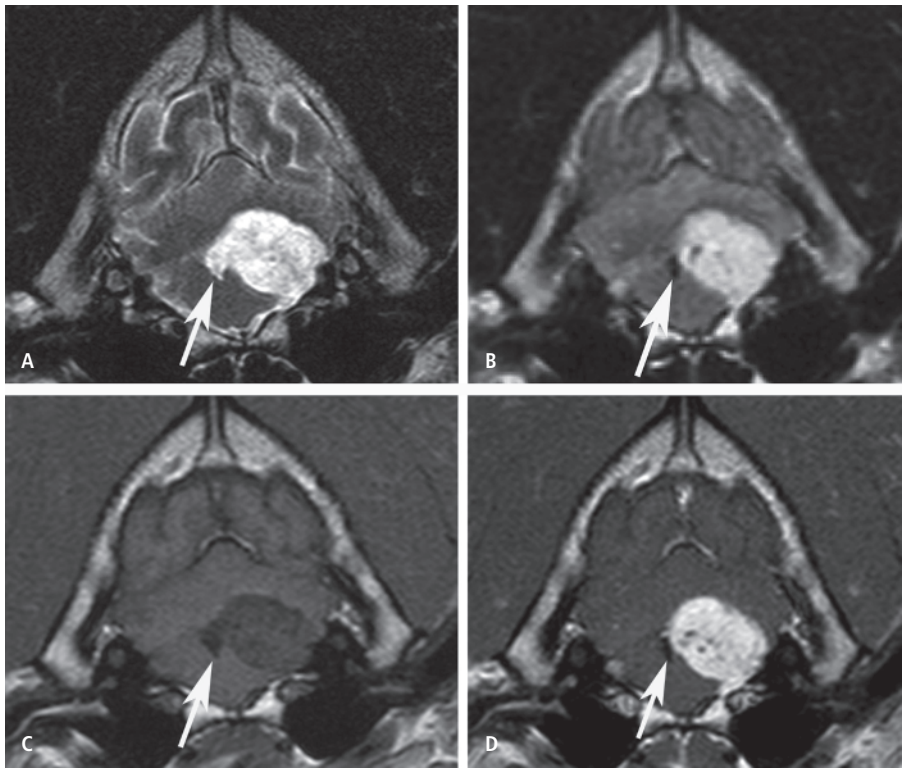

Fig. 1.30 Male, 8-year-old crossbreed dog with clinical signs of central vestibular disease. MRI (High Field Open Magnet from Philips, 1 Tesla) including transverse T2W (A), FLAIR (B), T1W pre- (C) and post-contrast (D) images of the posterior fossa. Diagnosis was choroid plexus papilloma. There is a 1.5 × 2 cm extra-axial lesion in the left cerebellopontine angle causing mild right-sided deviation of the medulla. The lesion has broad-based contact with the left petrosal bone, which is clearly delineated and has normal signal intensity (SI). The lesion shows high SI in FLAIR and T2W images, and low SI in the pre-contrast T1 sequence, and there is severe contrast uptake of the lesion. Note the small triangular area dorsal to the medulla and continuous with the lesion (arrows) representing the compressed fourth ventricle. It displays the SI of fluid with T2W hyper-, T1W hypo-, FLAIR hypointensity, and there is no contrast enhancement. In the post-contrast series, fine hypointense and sharply delineated tubular structures can be seen coursing through the lesion. The meninges surrounding this lesion appear normal.

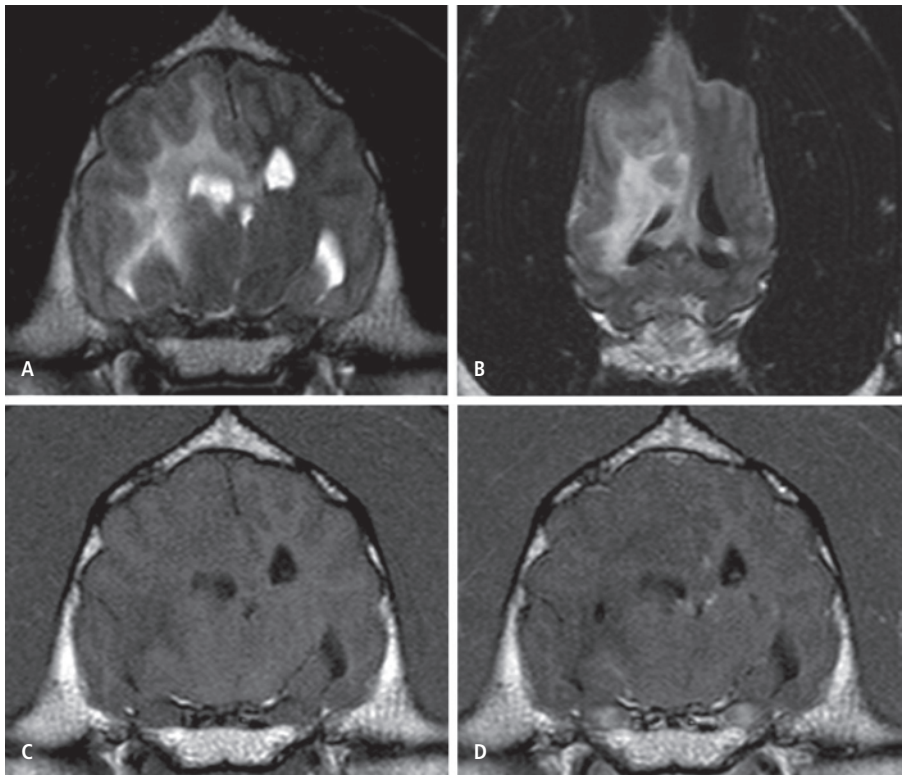

Fig. 1.31 Male, 7-year-old Flatcoated Retriever with reduced consciousness. MRI (High Field Open Magnet from Philips, 1 Tesla) including transverse T2W (A), dorsal FLAIR (B), transverse T1W pre- (C) and post-contrast (D) images of a severe vasogenic brain edema, located in the white matter. It is hyperintense in T2W and FLAIR images with high contrast to the dark ventricles in the FLAIR studies. Typically, edema is hypointense in T1W images and shows no contrast uptake. Space occupying mass in right frontal lobe visible in dorsal FLAIR image.

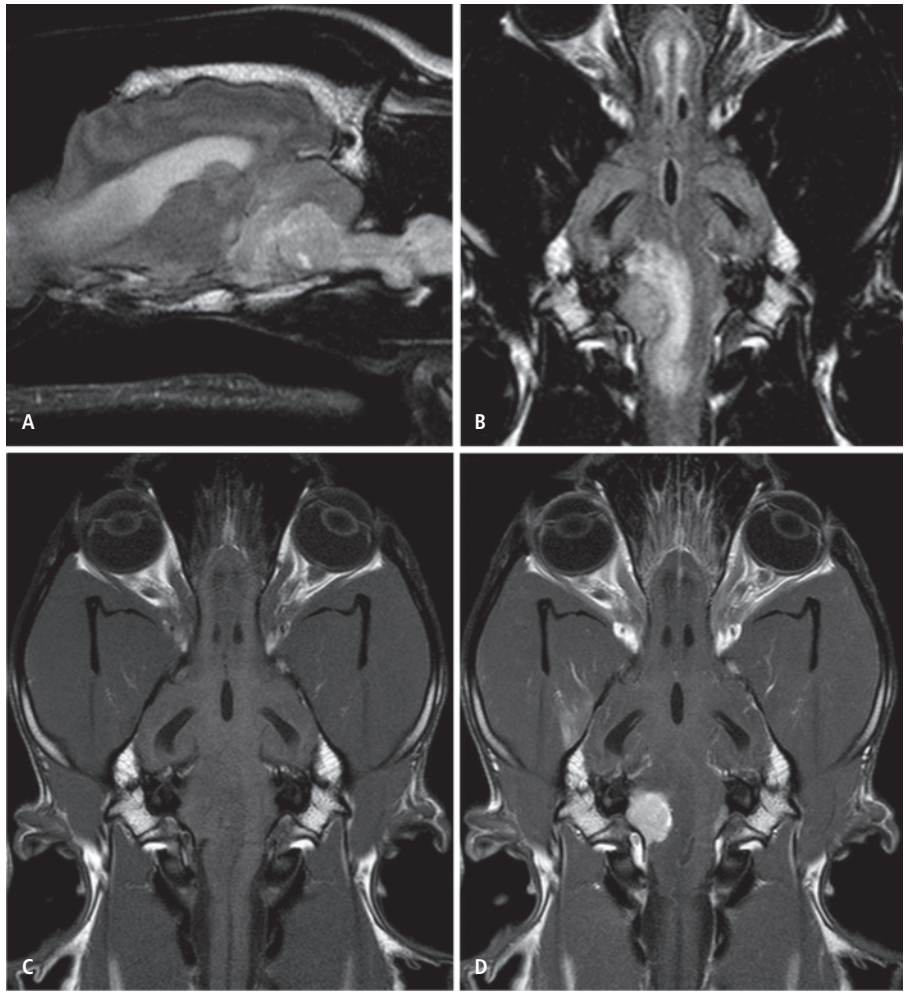

Fig. 1.32 Male, 11-year-old Magyar Vizsla dog with suspected right-sided brainstem lesion. MRI (High Field Open Magnet from Philips, 1 Tesla) including sagittal T2W (A) , dorsal FLAIR (B), dorsal T1W pre- (C) and post-contrast (D) images of the brain. Diagnosis was meningioma. The paramedian sagittal T2 sequence reveals dilated right lateral ventricle with high SI, and also ill defined high SI in the brainstem, cerebellum and spinal cord. In the center, there is arounded focal lesion delineated by a hyperintense rim. In the dorsal FLAIR, the CSF in the ventricular system is suppressed, the perifocal edema presents hyperintense, and the well delineated extra-axial lesion slightly hypointense compared to the edema. There is also hyperintense periventricular edema in the olfactory bulb bilaterally. In the pre-contrast T1 image, lesion and edema present mildly hypointense compared to the brain, and there is massive homogeneous contrast enhancement of the broad-based tumor.

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This book is accompanied by a companion website which is maintained by the Division of Diagnostic Imaging, Dept of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Switzerland:
www.wiley.com/go/vandevelde/veterinaryneuropathology