Cellular and Subcellular Elements of the Nervous System

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A complete understanding of the structural and functional aspects to the brain at the cellular and subcellular level is needed to form links to clinical disease pathophysiology. For example, to understand the biology and pathophysiology of Parkinson's disease, it is important to understand the molecular aspects of the dopaminergic synapse. To understand Alzheimer's disease and related neurodegenerative illnesses such as ALS, an appreciation for the neuronal cytoskeleton is needed as well as to understand how disruption of axonal transport mechanisms can result in clinical disease, as shown on successive pages below. A central theme and approach is to therefore link the basic science of neuronal physiology and anatomy on a cellular level to help explain what is going on clinically.

Whereas glial cells are multipolar with diffusely radiating processes, vertebrate neurons are highly polarized into proximal dendritic trees, and distal axonal processes that give rise to synaptic contacts, as shown in Fig. 2.1 (invertebrate neurons are unipolar and lack dendrites).

Figure 2.1 emphasizes the central control aspect of genetic factors within DNA in the nucleus. This illustration as well as Fig. 2.3 also shows nuclear pores embedded within the dual membrane nuclear envelope and mediate the import and export of key signaling compounds as well as RNA and ribosomal proteins that shuttle across the nuclear pore complex. The average vertebrate cell has about 2000 nuclear pore complexes and can accomplish up to 1000 translocations every second. Cargo tagged by special nuclear localization signal (NLS) amino acid sequences have particularly efficient and selective transport into the nucleus (NLS example: PKKKRKV).

The most fundamental and central aspect that determines cellular functioning is DNA coiled within the nucleus. Minor defects in the sequence of DNA base pairs can give rise to tragic illnesses such as Duchenne muscular dystrophy or Huntington's disease (HD), as shown in the example summary (Fig. 2.2) with other genetically defined nervous system diseases listed including ALS (in certain cases only—most sporadic cases of ALS have no gene defect identified) and the very rare DRPLA.

Maintaining circulation is vital to neuronal health and involves not only circulation of oxygenated blood on a cellular capillary level, but also involves circulation of key proteins and organelles up and down the axon via the processes of slow and fast axonal transport. Exchange of glutamate versus glutamine occurs between neuron and glia as well, with neurons shuttling information in the form of neurotransmitter release mediated chemical activation at the synapse. A major form of circulation is in the form of cerebrospinal fluid that literally keeps the brain afloat within the cranial vault and is buoyant in a suspended form; this vital fluid is produced inside the ventricular cavities within choroid plexus and flows from one chamber into

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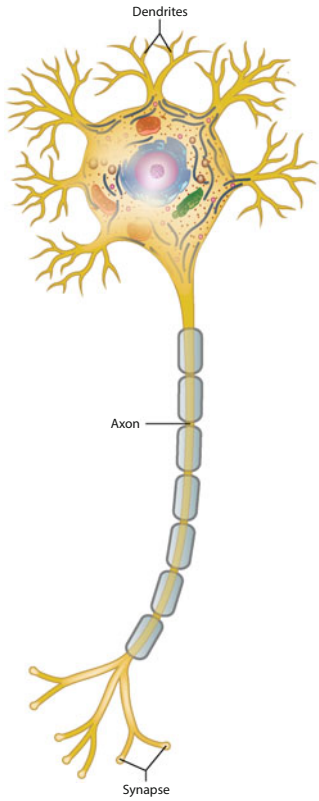

Fig. 2.1 The vertebrate neuron is highly polarized into proximal dendrites, with singular distal axonal processes that arborizes and give rise to synaptic contacts

the other (lateral ventricles into the central third ventricle and then into the midline central fourth ventricle).

With regard to this concept of intracellular circulation, a fundamental principle of neuronal function relates to the neuronal cytoskeleton shown in Fig. 2.3, which provides the framework for delivery of key proteins and organelles that shuttle back and forth along the microtubule system within the cell; disruption at any point can be deadly, with disruption of axonal transport being especially problematic as it isolates the cell body from its synaptic terminals.

Neurofilaments (NFs) are intermediate filaments within nerve cells that provide structural support for the neuronal cytoskeleton and can contain three types of subunits: neurofilament light chain (NF-L), medium chain (NF-M), and heavy chain (NF-H).

Neurofilaments assemble with neuronal cell bodies and then are shipped out down the axon via slow transport mechanisms along microtubules, at rates as fast as 2 cm per week. In actuality, this represents the net effect of an erratic stop and go pattern that at times is actually bidirectional, and sometimes rapid interspersed with periods of long pauses [1].

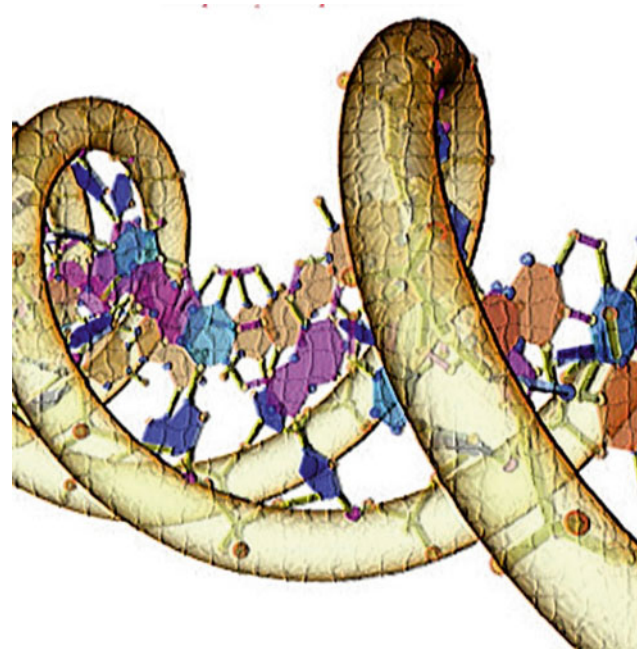

Fig. 2.2 Defects in the sequence of DNA base pairs can give rise to tragic neurologic illnesses such as Duchenne muscular dystrophy or Huntington's disease (HD). DNA encoding of neurological diseases: dystrophin gene deletions: Duchenne muscular dystrophy; SMN_1 gene deletion: spinal muscular atrophy; SOD_1 mutation: Familial ALS; Heavy neurofilament gene mutation: ALS; Huntington CAG expansion: HD; Atrophin CAG expansion: DRPLA

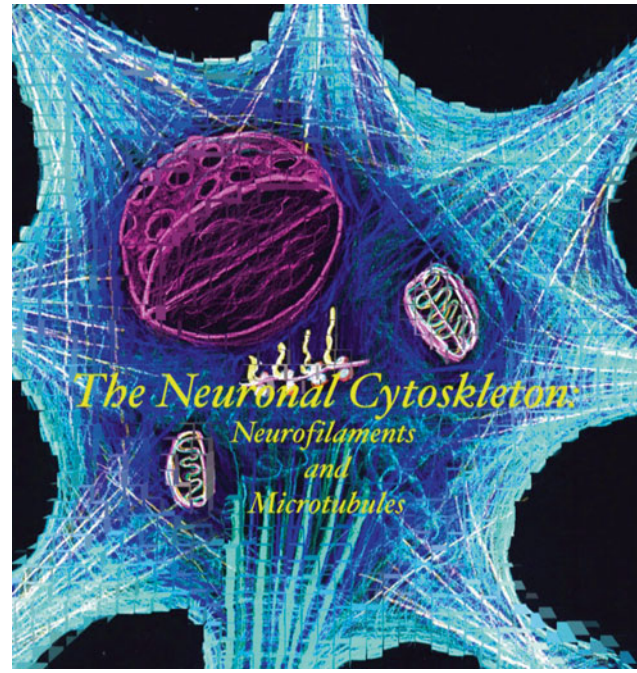

Fig. 2.3 The neuronal cytoskeleton provides the framework for delivery of key proteins and organelles that shuttle back and forth along the microtubule system within the cell

Defects in the transport mechanism and/or the genetic encoding of the neurofilament subunits may lead to pathologic neurofilament aggregations and induce neurodegenerative diseases, including ALS, where genetic defects in NF-H have been found.

Many illnesses have cytoskeletal disruptions at the microtubule level as a key aspect, including Alzheimer's disease where the microtubule framework falls apart due to alterations in the microtubule-associated protein Tau (see Fig. 2.4).

In general, one type of microtubule-associated protein is found only within the dendrites (MAP2) whereas the other type, associated with Alzheimer disease when phosphorylated (tau) is found only in the axon in healthy brain tissue (abnormally phosphorylated pathologic types of tau protein may be found in degenerative conditions within dendrites as well).

The microtubule motor proteins kinesin and dynein have polarity with respect to providing anterograde transport of kinesin bound proteins, filaments, and organelles from the cell body down the axon to the synapse, and up the reverse retrograde direction towards the cell body for dynein (see Fig. 2.5).

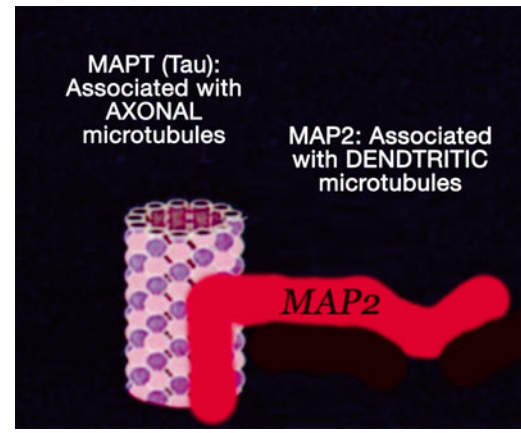

Fig. 2.4 In general, one type of microtubule-associated protein is found only within the dendrites (MAP2) whereas the other type, Tau, is found only in the axon in healthy brain tissue

Integrity of the microtubule system is critical for these transport processes to take place; the differences between slow and fast transport is summarized in Table 2.1.

In diseases such as Alzheimer's where the microtubule system has fallen apart due to mutations in the microtubule-associated protein Tau,

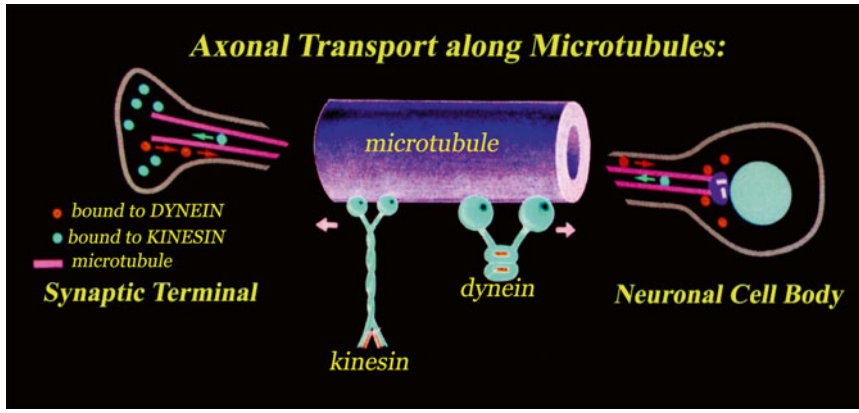

Fig. 2.5 Anterograde transport of kinesin bound proteins, filaments, and organelles travel from the cell body down the axon to the synapse

Table 2.1 Summary showing the differences between fast versus slow anterograde axonal transport

Fast transport rates are measured as high as 400 mm per day in sciatic nerve	Slow transport rates are usually found to be 1–5 mm per day
Fast transport is mainly for elements related to synaptic transmission and neurotransmitter vesicles	Slow transport is not significantly affected by colchicine
Microtubules mediate fast transport	Microtubule protein, enzymes, and mitochondria can travel slow route

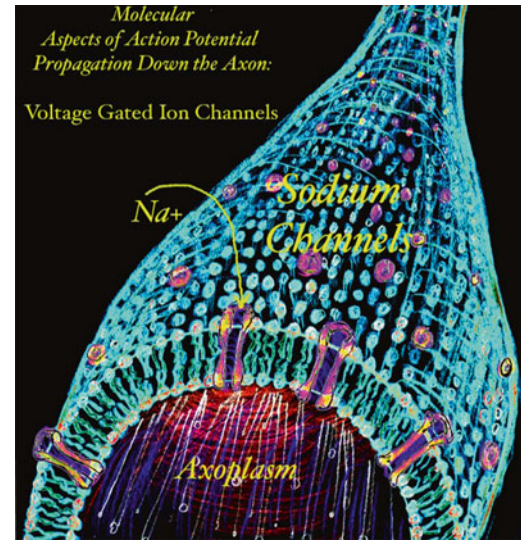

Fig. 2.6 Influx of extracellular sodium across voltage-gated channels is a key first step in propagating an electrical nerve impulse

the vital transport link between the nerve cell body and synaptic targets is lost.

With regard to the circulation of electrical impulses and flow of ions across channels embedded within axonal membranes, it is important to examine the key role played in this process by sodium channels in health and disease (see Fig. 2.6).

Using the squid giant axon for research on the basis of neural action potentials, it has been established since the 1950s that influx of extracellular sodium across voltage-gated channels is a key first step in propagating an electrical nerve impulse, followed by conductance changes for the efflux of intracellular potassium across separate and distinct channel protein complexes that are voltage sensitive; the process completes itself with local repolarization after sodium channel inactivation takes place. Much of the knowledge about sodium currents across nerve membranes can be attributed to the use of selective channel blockers in squid axon studies, such as the puffer fish poison tetrodotoxin that binds to the extracellular portions of the sodium channel, and thereby cause fatal blockade of neural impulse conduction.

Recent research has disclosed a key link between sodium channel defects and neurologic diseases with special reference to epilepsy [2].

Although 40% of seizure disorders have no obvious cause, most of these are likely due to genetic mutations, possibly within transmitter receptors or within axonal conductance channels.

Voltage-gated sodium channel gene mutations are now known to cause multiple types of seizure disorders including GEFS+ (genetic generalized epilepsy with febrile seizures Plus) and the Dravet syndrome (severe myoclonic epilepsy of infancy).

Whereas GEFS+ is linked to a missense mutation that alters sodium channel properties, the catastrophic Dravet syndrome is due to a loss of function, with status epilepticus occurring by about 6 months.

Most of these epileptic sodium channel gene mutations occur in the *SCN1A* gene, with patterns of dominant inheritance.

A key point of communication between one nerve cell and the other is the synaptic juncture, where the axonal impulse terminates to induce an influx of calcium on the presynaptic side, causing synaptic vesicles to fuse with presynaptic membrane leading to transmitter release across the synaptic cleft with activation of postsynaptic receptors. The drawing in Fig. 2.7 and electron micrograph in Fig. 2.8, both by the author, illustrate the structure of a chemical synapse with synaptic vesicles containing quanta of neurotransmitter chemical, to be distinguished by the less common but more immediate contact known as the electrical synapse (gap junction structure to permit further spread of a depolarizing impulse to the next cell).

The structure of the synaptic juncture is basically the same, whether it is sampled from a human patient as part of a diagnostic brain biopsy for encephalitis (Fig. 2.9) or from a mouse (Fig. 2.10); what is unique to humans is the vast

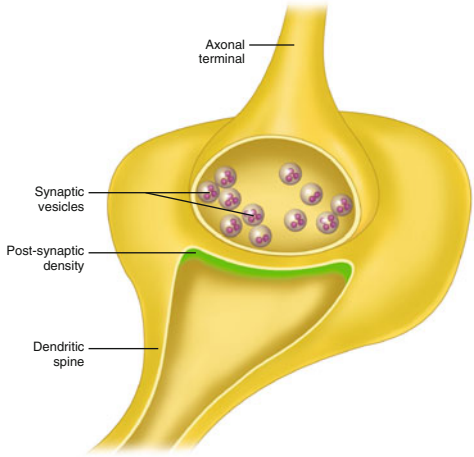

Fig. 2.7 The synaptic juncture, where the axonal impulse terminates to induce an influx of calcium on the presynaptic side, causing synaptic vesicles to fuse with presynaptic membrane

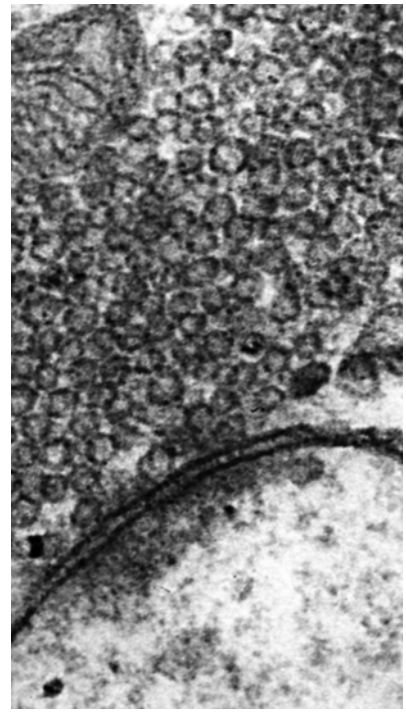

Fig. 2.8 Electron micrograph of a synapse within the mouse brain

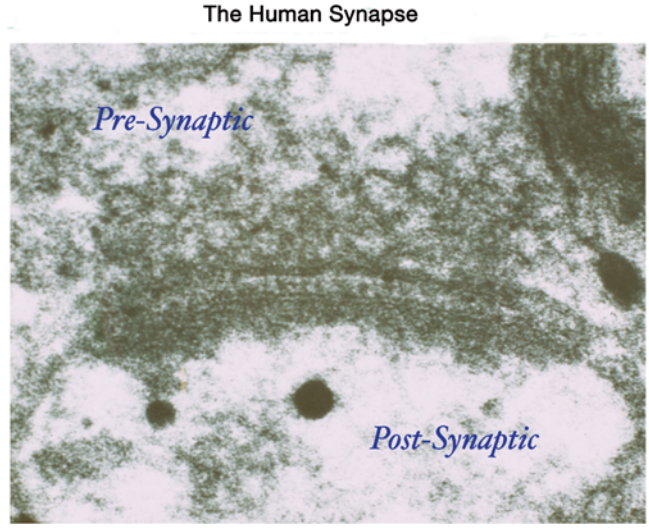

Fig. 2.9 Electron micrograph of a synaptic juncture sampled from a human patient as part of a diagnostic brain biopsy for encephalitis

number of synapses (150 trillion for the human neocortex alone) as well as the greater capability to form dendritic spines that may be linked to learning and memory through enhanced synaptic contacts. Interestingly, the density of synaptic contacts in the brains of rodents and humans is more or less constant at around 1100–1300 million/mm^3 [3].

Studies with animals show that stimulating environments and memory tasks produce greater densities of deep invaginating presynaptic contacts surrounded and encircled by the postsynaptic membrane in the form of well-developed dendritic spines (see Fig. 2.10, for example).

The electron micrograph by the author in Fig. 2.10 reveals the structure of a dendritic spine that forms a more direct contact with presynaptic structures that surround the invaginating postsynaptic finger-like projection. It is believed that neuronal plasticity of the synapse is linked to the growth of dendritic spines; spine formation and morphology are linked to the process of learning and memory.

Although multiple types of chemical synapses exist within the brain, and include transmitter

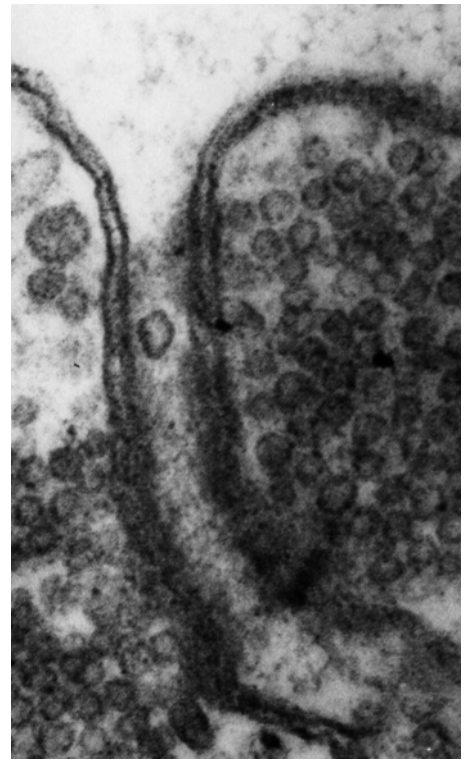

Fig. 2.10 Electron micrograph of a dendritic spine synapse

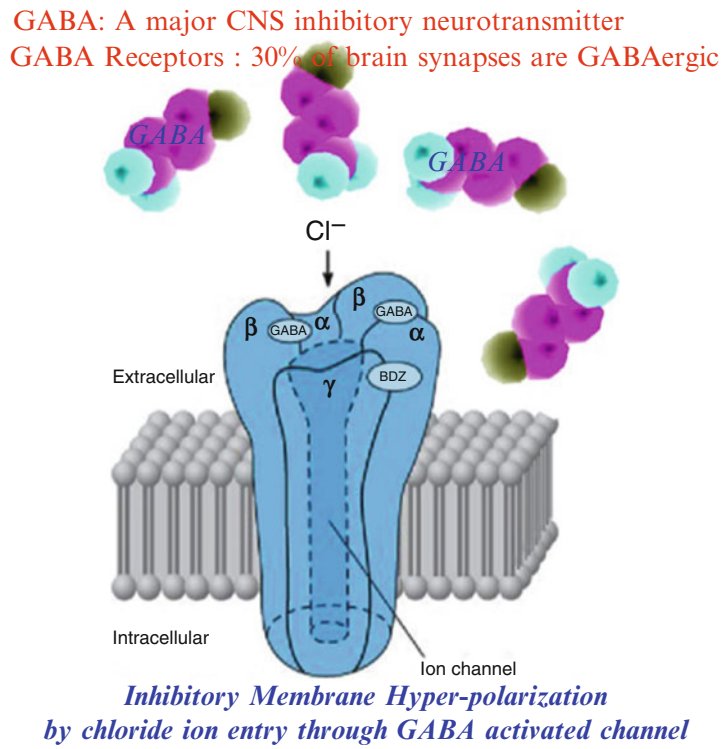

Fig. 2.11 Synapse for GABA (gamma amino butyric acid), which generates hyperpolarization of the postsynaptic terminal, thereby inducing inhibition

release specific for dopamine, or serotonin, or acetylcholine, for example, a particularly important synapse is for GABA (gamma amino butyric acid), which generates hyperpolarization of the postsynaptic terminal, thereby inducing inhibition (see Fig. 2.11).

About one-third of all synapses within the brain rely on GABA as the neurotransmitter, which mediates inhibition through the intracellular influx of chloride ions. Focal lack of synaptic inhibition from loss of GABAergic nerve terminals can predispose the region to become epileptogenic, as seen in postinfarction seizures that arise at the borderzone rim of tissue that straddles healthy fully innervated brain tissue and chronic encephalomalacia comprising dead scar tissue from an old infarct. By emergent administration of benzodiazepines to such a patient experiencing a seizure, the excessive electrical firing can be reduced through this inhibitory mechanism, as the GABA channel is a multimeric complex with multiple subunits, one of which has a specific binding affinity for benzodiazepine compounds.

GABAergic synapses can be visualized in health and disease by PET imaging of 11C labeled flumazenil; whereas diminished receptor density has been found by PET imaging of epileptogenic foci, normal GABA receptor density has been found in Alzheimer's disease [4].

Glial cells play important nutritive and supportive roles for neuronal function, which is entirely dependent on intact perfusion at the capillary level as shown below in Fig. 2.12.

As illustrated in Fig. 2.12, Glial cells are anatomically interposed between capillaries and neurons, and play important supportive roles for neuronal functioning.

From an evolutionary perspective, glial cells are more highly developed within the human brain than other mammals. For example, with

regard to brain weights and glia–neuron ratios for layer II/III of prefrontal cortical area 9 L, the cotton-top tamarin (*Saguinus oedipus*) is a small New World monkey with a brain weight of only 10 g with a glia-to-neuron ratio of 0.446 versus 1.21 for the gorilla's 509 g brain; the 1373 g human brain is at the top of the evolutionary ladder with the highest glia-to-neuron ratio of 1.65 [5].

Human protoplasmic astrocytes have volumes that are 27 times greater than those found in the mouse brain, which enables the human protoplasmic astrocyte to contact and surround two million synapses versus only 100,000 synapses being contacted and covered by the same type of glial cell in mice [6].

One study on the cytologic aspects of the brain of Albert Einstein revealed higher glia-to-neuron ratios for cerebral cortex area 39 [7].

Recently, a unique type of astroglia has been found only in humans and certain primates known as the interlaminar astrocytes and polarized astrocytes; these specialized glia are absent from the brains of other species; intralaminar astrocytes are thought to be linked to information transfer between cortical layers [8].

With regard to information transfer between glia, it is important to note that glia show widespread expression for connexin proteins, which comprise gap junctions; 11 different connexin proteins have been found within the brain. Connexins have also been linked to the propagating waves of astrocytic intercellular calcium waves underlying the phenomenon of spreading depression, which moves across the cortex in both epileptiform and migrainous events [9].

Interconnection of glia via gap junctions allows for rapid and widespread sharing of information about the metabolic and ionic aspects of the extracellular environment.

Glia have specific biochemical specializations; for example, as shown in Fig. 2.13, the natural angiogenesis inhibitor thrombospondin 2 is selectively expressed within protoplasmic astrocytes; recent studies show that thrombos-

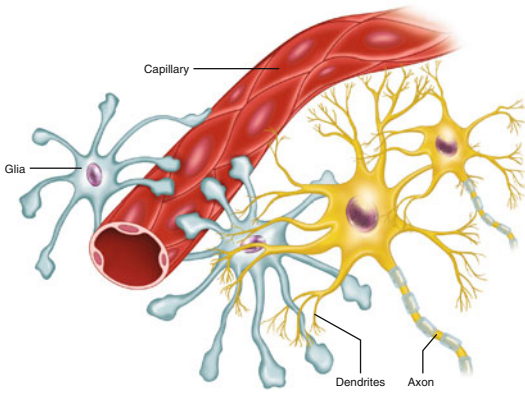

Fig. 2.12 Glial cells are anatomically interposed between capillaries and neurons, and play important supportive roles for neuronal functioning

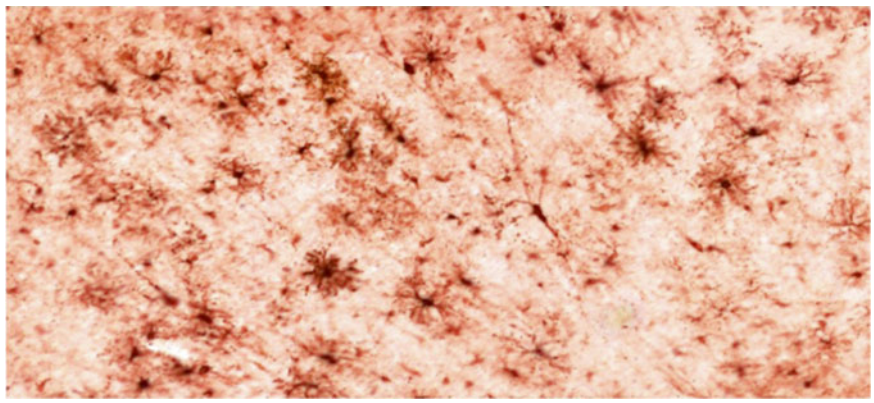

Fig. 2.13 Normal astrocyte morphology: Thrombospondin 2. Thrombospondin 2 is selectively expressed within protoplasmic astrocytes. Human gene map locus 6q27. THBS2 is a potent endogenous inhibitor of tumor growth and angiogenesis

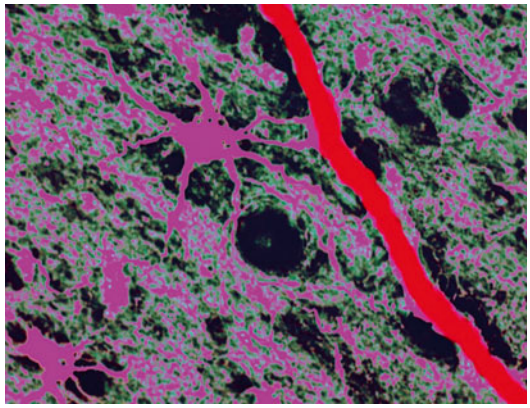

Fig. 2.14 The key nutrient role played protoplasmic astrocytes (*purple*) is illustrated in relation to end-feet processes that adhere to the walls of local capillaries (*red*)

pondins promote the formation of new synapses during development and in general act as key regulators of synaptogenesis in the central nervous system [10].

In Fig. 2.14, the key nutrient role played protoplasmic astrocytes (purple) is illustrated in relation to end-feet processes that adhere to the walls of local capillaries (red); initial absorption of nutrients needed the neuron takes place at the glia level initially, which passes this on to nearby neurons as needed; glucose is thought to shuttle across in this manner, with glia building limited supplies of glycogen in reserve as needed.

Whereas the cytoarchitecture of glial cell types remains constant throughout different regions of the brain, the shape and appearance of the neuron itself is characteristically and uniquely different for each region of the brain, as shown in the Fig. 2.15 composite.

Not only does the neuronal cytoarchitecture vary, the 18 F-FDG PET scan also illustrates significant metabolic heterogeneity for gray matter structures, with the cerebral cortex being far more active in utilizing glucose to support the activities of densely arborizing large pyramidal neurons, versus the relatively inactive globus pallidus that contains simpler, spindly neurons that have less rich arborizations.

Artistic renderings of the cytoarchitecture unique to cerebral cortex pyramidal neurons versus those found within the hippocampus is portrayed in Figs. 2.16 and 2.17, respectively.

Out of the 130 billion neurons present in the normal human brain, about 31 million are lost annually as part of normal aging, representing a small fraction of 0.024 % annually [11].

However, consider the extreme situation if they were all lost overnight, as shown in the tragic case example within Fig. 2.18 where major head trauma led to fatal surges in intracranial pressure leading to cessation of intracranial circulation with herniation and brain death; the dynamic PET scan revealed no flow and metabolism within the brain.

The normal whole body appearance on 18F-FDG PET scan studies at extreme left highlights the marked dependence the brain has on glucose metabolism relative to the rest of the body; any transient interruption as in the case of cardiac arrest can also lead to brain death despite successful resuscitation and restoration of circulation.

Successful efforts to minimize the damage is shown in Fig. 2.19 through the use of mild therapeutic hypothermia; by maintaining core temperature at 33 °C for 24 h after resuscitation from cardiac arrest, postanoxic brain injury can be minimized with many patients showing excellent outcomes when the loss of circulation is brief; the mechanism presumably relates to hypothermic inhibition of the endogenous unfolding of programmed cell death, otherwise known as apoptosis, where caspase 9 is a key factor in the cascade.

Programmed cell death, otherwise known as apoptosis, is an event common to both the birth and development of the nervous system as well as brain death after global hypoxia with prolonged interruption of cerebral blood flow. Figure 2.20 outlines the final steps of apoptosis, where the final executioner pathway driven by caspase 3 arises through astrocytic generation of caspase 8 through TNF alpha, versus neuronal mitochondrial release of cytochromes to generate caspase 9.

As shown in Fig. 2.21, development of the brain and spinal cord is a complex event.

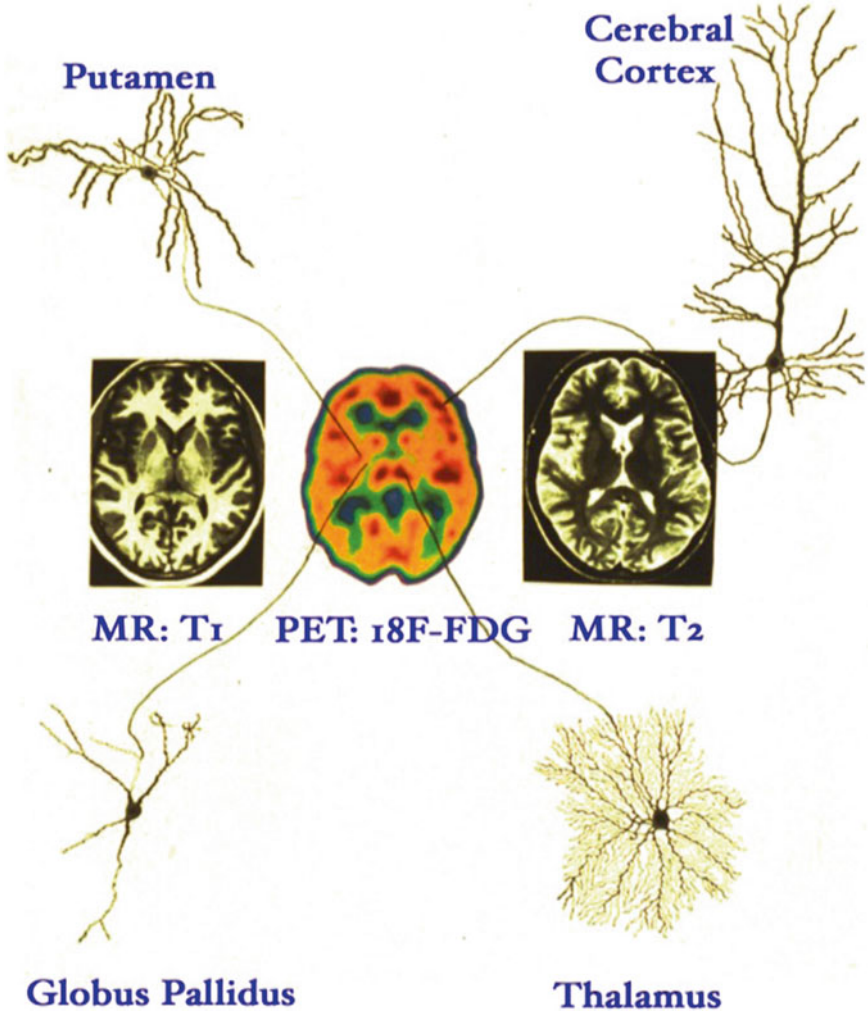

Fig. 2.15 The shape and appearance of the neuron itself is characteristically and uniquely different for each region of the brain

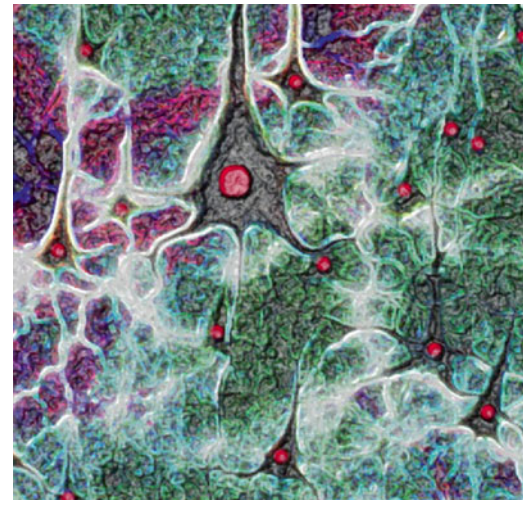

Fig. 2.16 Cerebral cortex neurons. Artistic rendering of the cytoarchitecture unique to cerebral cortex pyramidal neurons

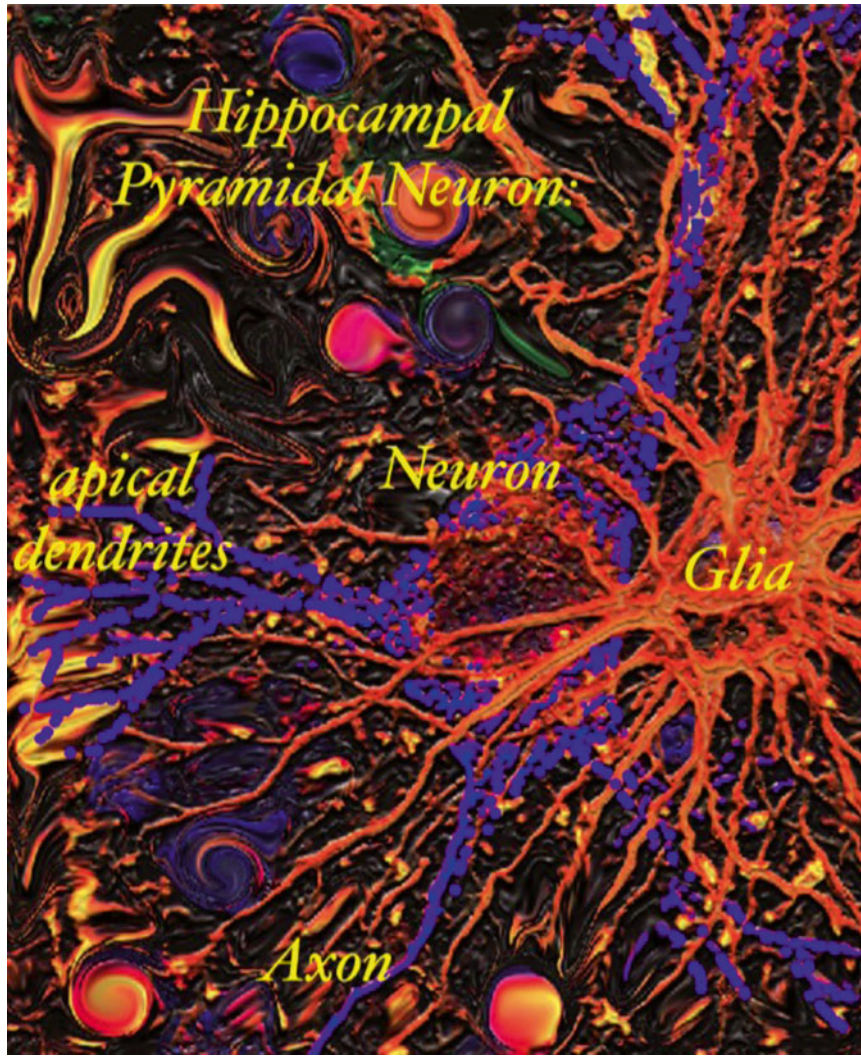

Fig. 2.17 Artistic rendering of the cytoarchitecture unique to hippocampal pyramidal neurons

Starting out as the primordial neural plate that fold upward to form a tubular structure, with central remnants persisting as the central canal of the spinal cord in adults which communicates with the ventricular system. Molecular analysis of neural crest migration indicates that a gradient of bone morphogenic protein (BMP) activity initially demarcates the neural plate borders, followed later by a transformation of this border of the neural plate into neural crest cells by a combination of Wnt signaling factors, fibroblast growth factors (FGFs), and retinoic acid (RA); connexin proteins also become upregulated during neural crest migration. The subsequent anterior–posterior patterning of cellular migration is controlled by semaphorins/neuropilins and Eph/ephrins [12].

Although there is great cellular proliferation during development, programmed cell death (Apoptosis) also takes place to refine and reduce the cell populations to the appropriate levels. Programmed cell death is actually an important

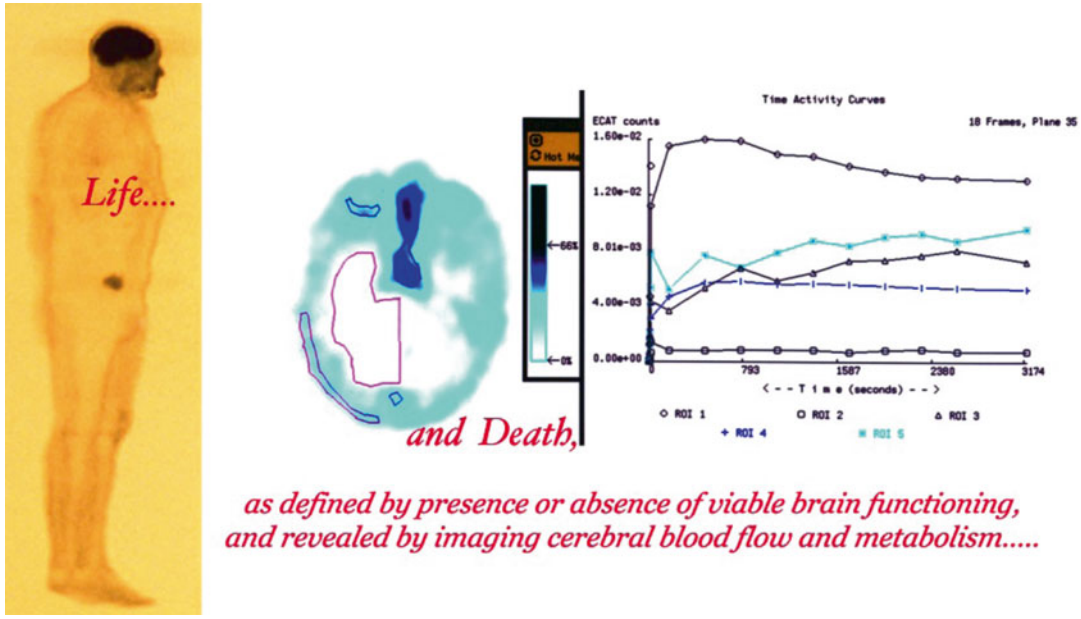

Fig. 2.18 The dynamic PET scan study at right revealed no flow and metabolism within the brain, making the diagnosis of brain death certain

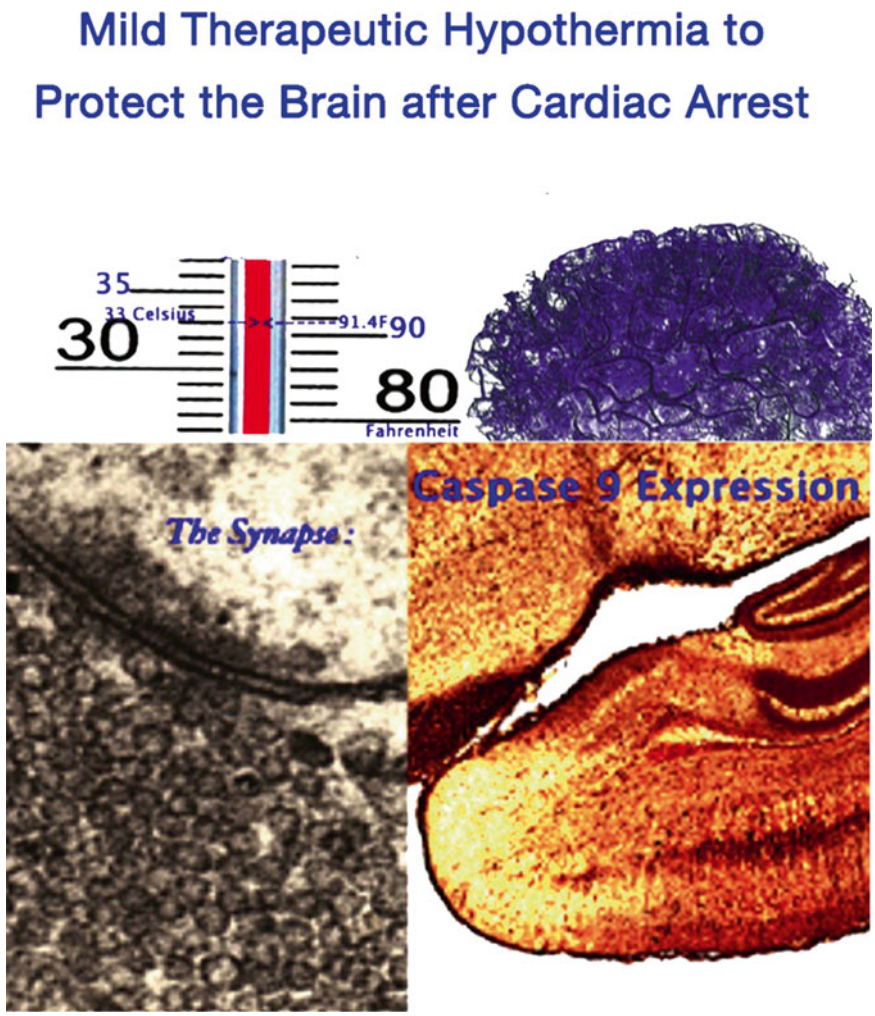

Fig. 2.19 Successful efforts to minimize postanoxic damage through the use of mild therapeutic hypothermia

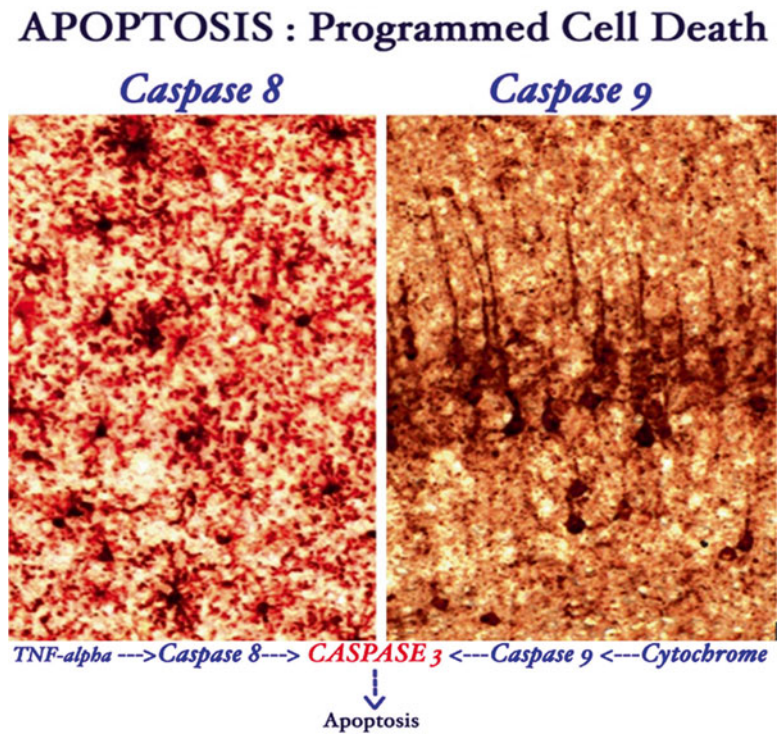

Fig. 2.20 The final steps of apoptosis

process in neuronal development that acts to eliminate certain areas and remove excess neurons that had proliferated. As suggested by Fig. 2.21, pyramidal neurons within the cerebral cortex proliferate during development—through the process of programmed cell death, not all of these neurons and associated glial cells survive. Recent research indicates that ephrin molecules play a role in this brain region-specific apoptosis process in regions where erythropoietin-producing hepatocellular (Eph) receptor tyrosine kinases (RTKs) cluster with their ephrin (Eph receptor interacting proteins) through direct cell to cell contact [13].

The final product is a masterpiece of the developmental process: containing 130 billion neurons and 150 trillion synapses in the human neocortex alone, each brain is uniquely different with regard to fine details of interconnections and patterns of cortical gyration. As shown in Fig. 2.22, the MRI study by the author showed a surprising variability to the patterns of cortical infoldings for the primary central fissure and neighboring cortical sulci.

The effects of age on these infoldings producing mild global cortical atrophy is shown in Fig. 2.23 for the case example of a 68-year-old male with new onset of mild to moderate cognitive impairment, who also displayed mild prominence to the ventricular system thought to be less likely of a congenital nature.

The development of the ventricular system of the brain is influenced by many genes and proteins; recent research indicates that one of these influencing factors is SOCS7, which is a member of the suppressor of cytokine signaling (SOCS) family of proteins. Lack of this protein in the developing mouse brain was found to be linked to hydrocephalus [14].

Whereas the intrasulcal cerebrospinal fluid spaces are readily visible on 3D surface renderings of MR brain images, the 3D morphology of the

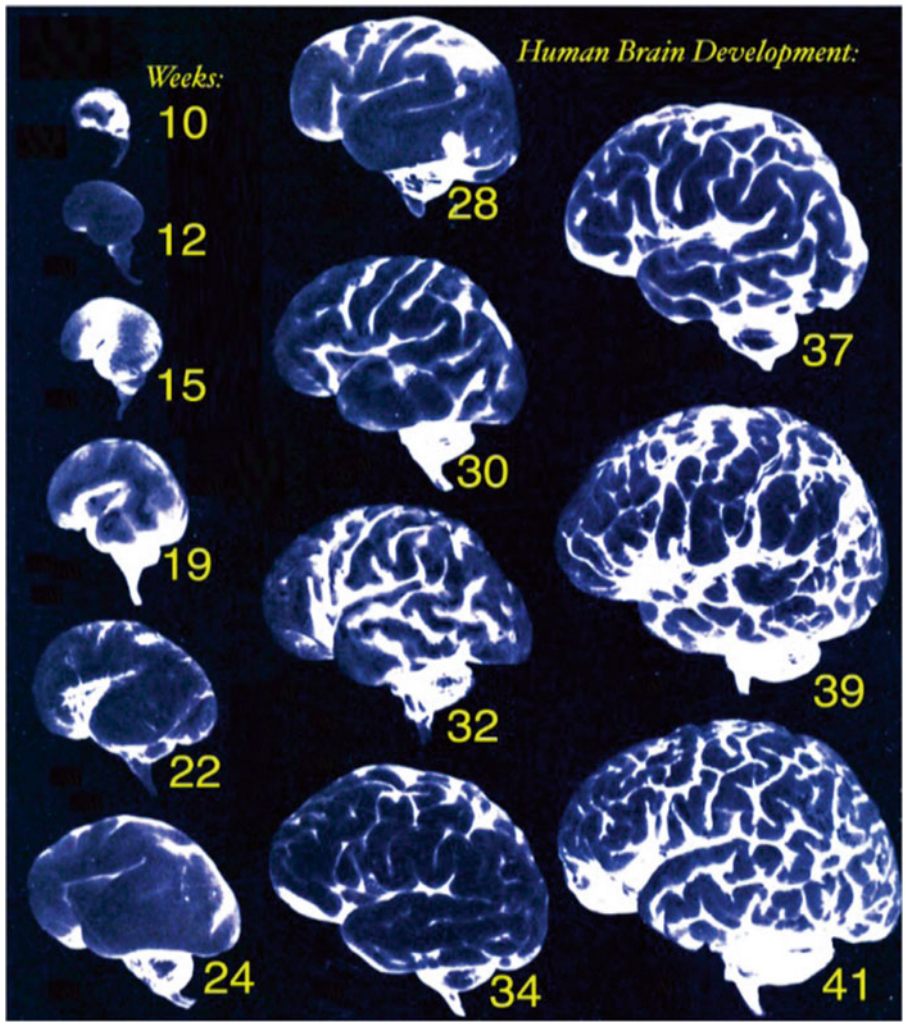

Fig. 2.21 In utero development of the brain

internal ventricular spaces are not readily apparent. As shown in Fig. 2.24, the drawings by Leonardo da Vinci on his studies of the ventricular system were reasonably accurate and also included making a wax corrosion cast of the ventricular system post-mortem using molten hot liquid wax.

Lower panels reveal a MRI-derived 3D computer reconstruction dating to 1991 that selectively outlined the morphology of the ventricles at various viewing angles (overlying cerebellar and cortical structures are shown at right).

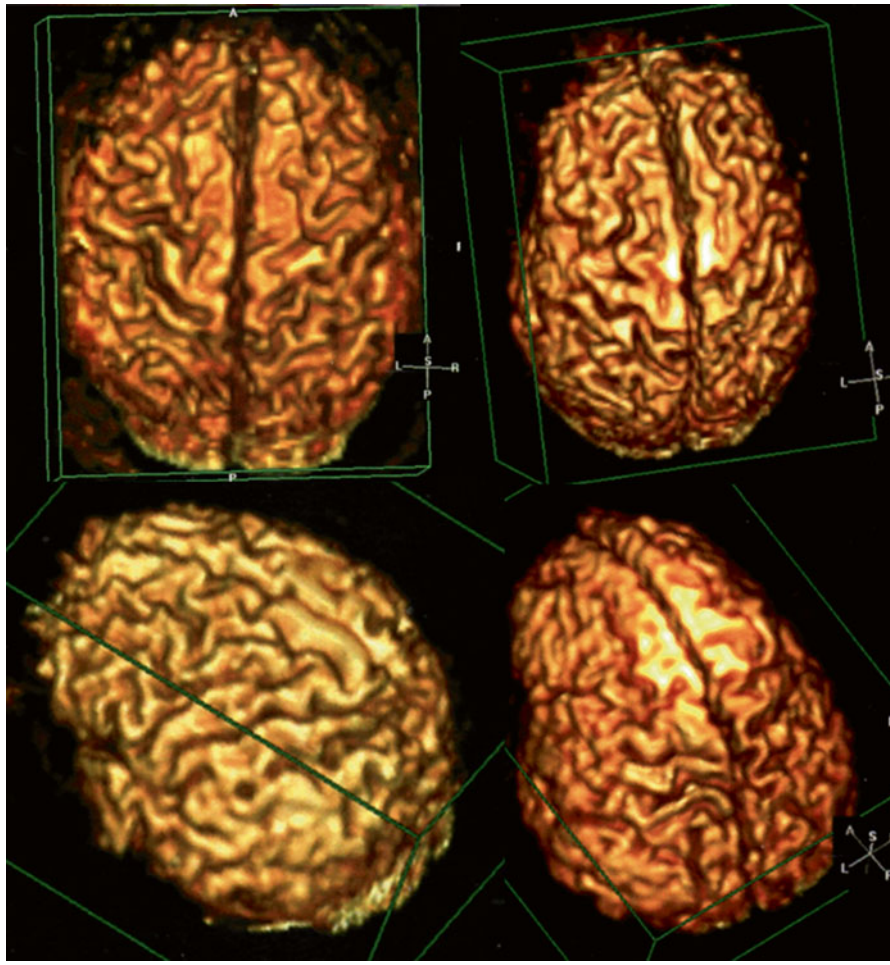

Fig. 2.22 3D reconstructions of brain MR data for four patients show a surprising variability to the patterns of cortical infoldings for the primary central fissure and neighboring cortical sulci

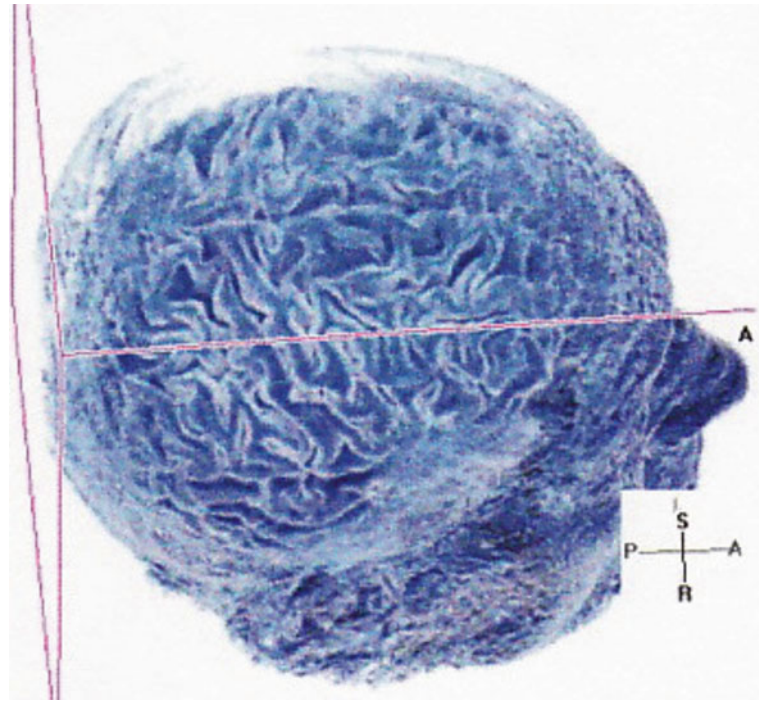

Fig. 2.23 Mild global cortical atrophy for the case example of a 68-year-old male

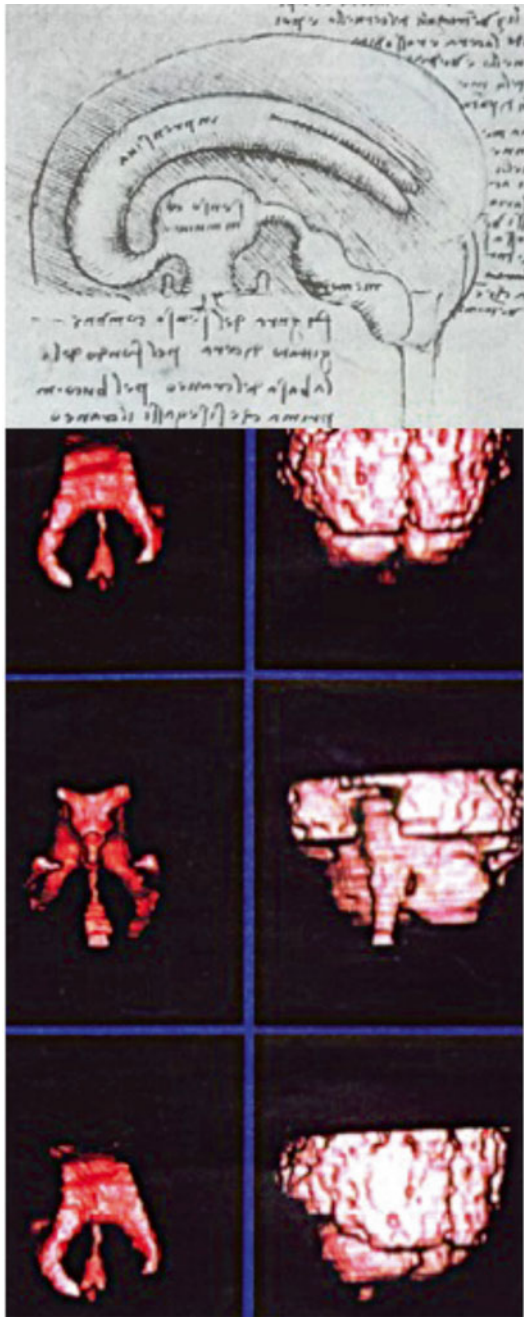

Fig. 2.24 Drawings by Leonardo da Vinci (*above*) on his studies of the ventricular system were reasonably accurate; compare with a MRI-derived 3D computer reconstruction (*below*) that selectively outlined the morphology of the ventricles at various viewing angles

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