1

Brain Function, Physiology and the Blood–Brain Barrier

1.1 Introduction – An Overview of Brain Structure and Function

The brain is by far the most complex organ of the human body, and is responsible for our every thought, action, memory and feeling. Weighing only about 1.4 kg, protected by the bones of the skull and suspended in cerebrospinal fluid (CSF), this mass with the consistency similar to that of soft gelatine contains about 10^{11} specialized nerve cells, called neurons. Each neuron can form as many as 10^3–10^4 connections with other neurons via synapses. It also contains nearly 10 times more cells of a different type, called glial cells (the different classes of neurons and glial cells will be described in detail later in the chapter). Although it constitutes just 2% of the human body mass, it receives 15% of cardiac output, consumes 20% of our total O_2 consumption and accounts for 25% of total body glucose utilization.

The human brain is conventionally considered as being made up of three principal parts – the forebrain, midbrain and hindbrain (Figure 1.1a and b). The *forebrain* is made up of the *cerebral cortex* (or cerebrum), and, buried within the cerebral cortex beneath the *corpus callosum*, the *thalamus*, *hypothalamus*, *amygdala* and *hippocampus* (part of the limbic system, often referred to as the 'emotional brain'). The *midbrain* is the smallest region of the brain, functioning as a relay station for the transmission of auditory and visual information. Finally, the *hindbrain* is made up of the *cerebellum*, *pons* and *medulla*. Often the midbrain, pons and medulla are referred to as the *brainstem*.

1.1.1 The Forebrain

In the course of evolution, the mammalian *cerebral cortex* has expanded disproportionately compared to total brain volume. The human brain is distinctively larger than that of any other primate, mainly due to great expansion of the *cerebral cortex*,

Metal-based Neurodegeneration: From Molecular Mechanisms to Therapeutic Strategies,
Second Edition. Robert Crichton and Roberta Ward.

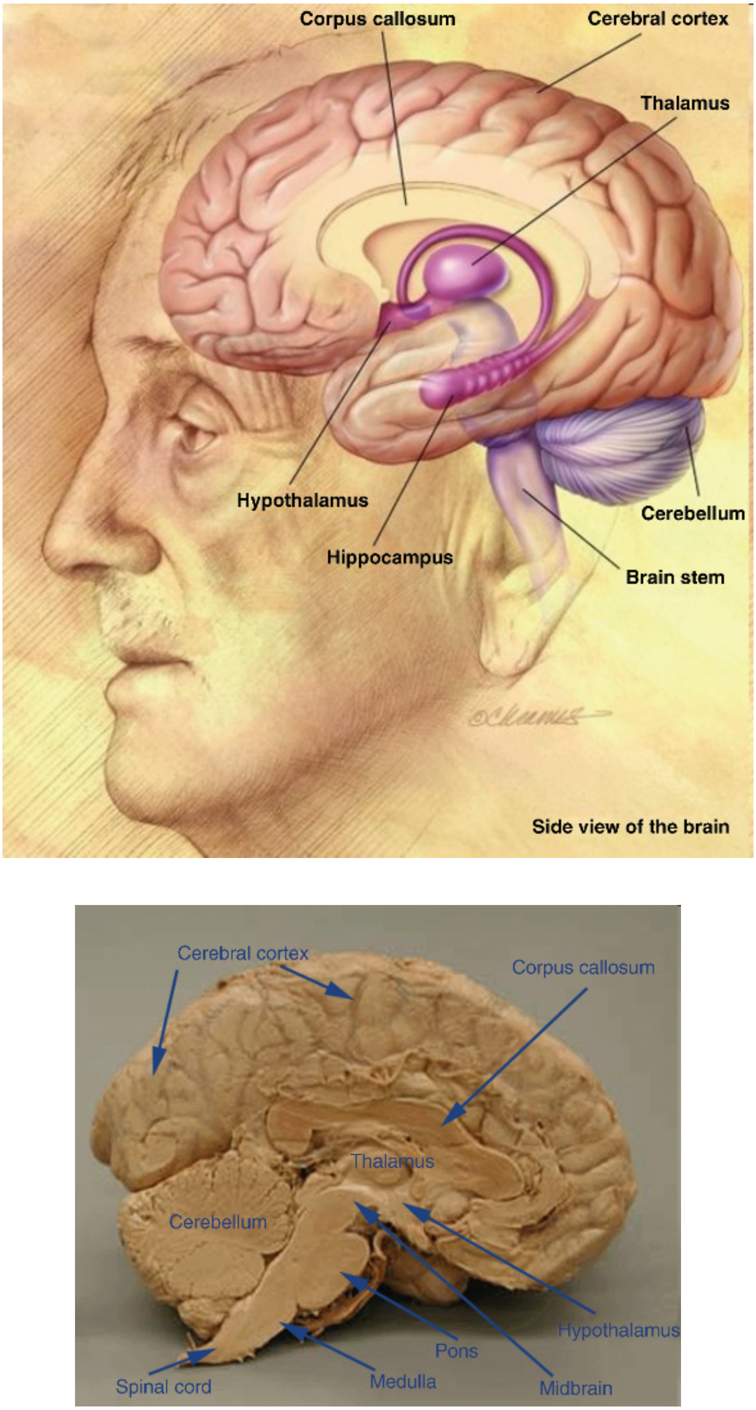

Figure 1.1 *(a) Drawing and (b) cross section of the human brain showing several important structures. (Reproduced from Wikipedia, "Alzheimer's disease: unraveling the mystery", available from the National Institute for Aging.)*

particularly the frontal lobes that are associated with executive functions such as reasoning, planning, abstract thought and self-control. The portion of the cerebral cortex devoted to vision is also greatly enlarged in human beings. The *cerebral cortex* is a huge sheet of neural tissue, profusely convoluted and folded to generate a vast surface area. It encompasses about two-thirds of the brain mass and lies over and around most of the other structures of the brain. It is the most highly developed part of the human brain and is also the most recent structure in the history of brain evolution. The *cerebral cortex* is made up of up to six horizontal layers, each with a different composition in terms of neurons and connectivity. This layer of the brain is often referred to as grey matter – in fact, the cortex is grey because nerves in this area lack the insulation that makes most other parts of the brain appear to be white. In contrast to the grey matter that is formed from neurons and their non-myelinated fibres, the white matter below them is formed predominantly by myelinated axons interconnecting neurons in different regions of the cerebral cortex with each other and also with neurons in other parts of the central nervous system (CNS). The phylogenetically most recent part of the cerebral cortex, the neocortex, is differentiated into six horizontal layers; whereas the more ancient part of the cerebral cortex, the hippocampus, has at most three cellular layers.

The *cerebral cortex* is divided into right and left *hemispheres*. Although their appearance is symmetrical, motor functions are directed by the opposite cerebral hemisphere (Figure 1.2). Thus, the right side of the brain controls muscles on the left side of the body and the left side of the brain controls muscles on the right side of the body. While the left hemisphere is associated with logic, the right hemisphere is associated with creativity.

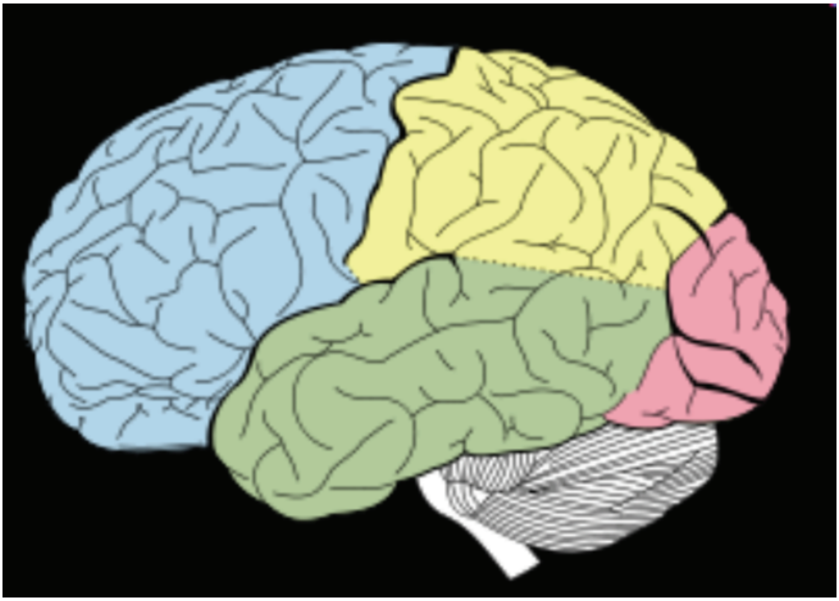

Figure 1.2 *The four topographically defined lobes of the cerebral cortex: frontal lobe (blue), parietal lobe (yellow), temporal lobe (green) and occipital lobe (pink). (Reproduced from Wikipedia, from Gray's Anatomy.)*

The *corpus callosum* is a wide, flat bundle of neural fibres beneath the cortex in the eutherian brain at the longitudinal fissure. It connects the left and right cerebral hemispheres and facilitates interhemispheric communication. It is the largest white matter structure in the brain, consisting of 200–250 million contralateral axonal projections.

The *thalamus* is a large mass of grey matter situated deep within the forebrain between the cerebral cortex and the midbrain. It has sensory and motor functions, relaying sensory and motor signals to the cerebral cortex. The *hypothalamus*, located below the thalamus and just above the brainstem, is involved in functions including homoeostasis, emotion, thirst, hunger, circadian rhythms and control of the autonomic nervous system. In addition, it controls the pituitary, linking the nervous system to the endocrine system via the pituitary gland, by the synthesis and secretion of a number of neurohormones (hypothalamic releasing hormones), which stimulate or inhibit the secretion of pituitary hormones. The *amygdala* located just beneath the surface of the front part of the temporal lobe is involved in memory, emotion and fear. The *hippocampus* is situated in the temporal lobe adjacent to the amygdala, and is important for learning and memory. In particular, it appears to be important in the consolidation of new memories, emotional responses, navigation and spatial orientation. The hippocampus is one of the first regions of the brain to suffer damage in mild cognitive impairment, with problems of memory initially, which, in some individuals, may develop to Alzheimer's disease, with enhanced memory loss and disorientation. It can also be easily damaged by hypoxia, and people with extensive damage to the hippocampus often suffer from anterograde amnesia, the inability to form or retain new memories.

1.1.2 The Midbrain

The *midbrain* is the smallest part of the brain located near the centre of the brain, between the pons and the thalamus and hypothalamus. It controls many important functions such as the visual and auditory systems as well as eye movement. Portions of the midbrain called the *red nucleus* and the *substantia nigra* are involved in the control of body movement. A large number of dopamine-producing neurons are located in the darkly pigmented substantia nigra. The degeneration of dopaminergic neurons in the substantia nigra is associated with Parkinson's disease.

1.1.3 The Hindbrain

The *cerebellum* or 'little brain' is located at the bottom of the brain, with the cerebral cortex above and the part of the brainstem known as the pons in front of it. Like the cerebral cortex, it is divided into two hemispheres. It consists of a continuous thin layer of grey matter, the cerebellar cortex, tightly folded rather like an accordion, representing a total surface area of some 500 cm^2 packed within a volume of just 300 cm^3. The morphological organization of the cerebellar cortex was first outlined by Camillo Golgi and Santiago Ramón y Cajal who together received the Nobel Prize for Medicine for their work in 1906. Within this grey matter are two important types of neurons, the Purkinje cells and the granule cells, and their representations in drawings by Golgi and Cajal are shown in Figure 1.3. Purkinje cells are among the largest cells of the brain and represent the sole output of motor coordination in the cerebellar cortex. There are so many of the tiny

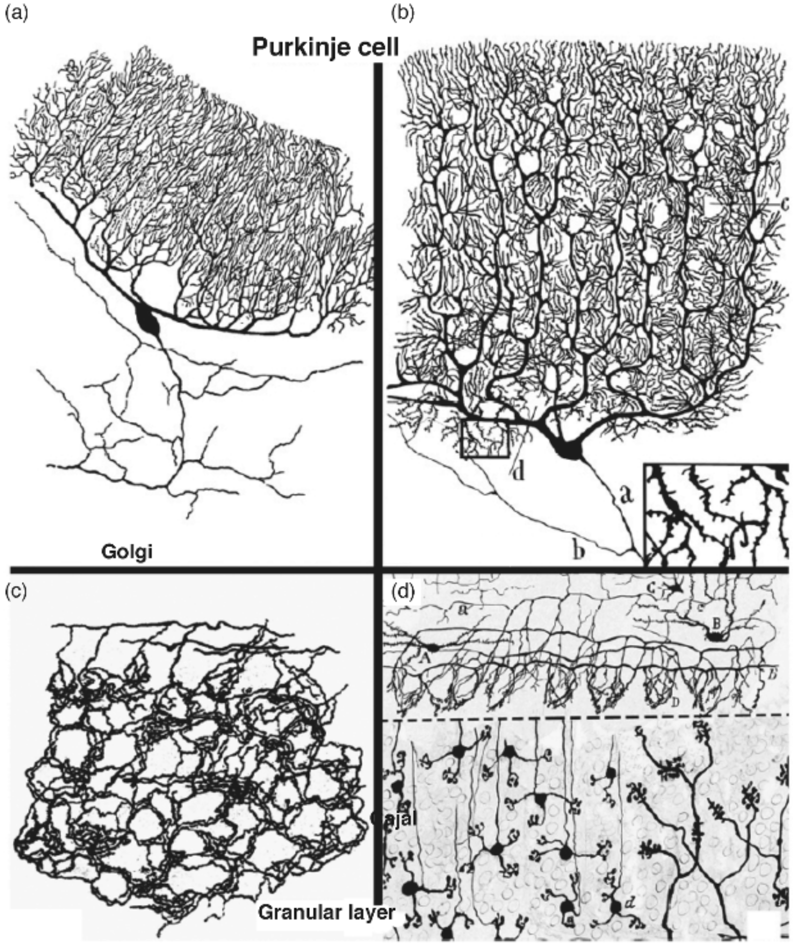

Figure 1.3 *Drawings by Golgi (a and c) and by Cajal (b and d) of a Purkinje cell and the nerve network in the granule cell layer. In part (d), a composite figure taken from two different drawings of Cajal is shown (the separation between them is marked by the broken line). The one at the upper third illustrates basket cells in the molecular layer and their descending axons in the Purkinje cell layer. Note the brush-like free terminals of these axons, the pinceau formations, which are completely independent of axons in the granule cell layer. The lower two-thirds represent on the right side the mossy fibres and on the left side the relay neurons, the granule cells with their characteristic claw-shaped dendritic endings, specialized to adapt to their presynaptic elements, the mossy fibres. [Reprinted from Sotelo (2010) with permission from Elsevier, © 2010.]*

granule cells that, although the cerebellum represents only 10% of the brain volume, it contains more neurons than the rest of the brain put together. Figure 1.4 presents a modern cellular and synaptic representation of the cerebellar cortex. In addition to the two principal types of neurons in the cerebellar cortex, there are stellate and basket cells in the molecular layer, and in addition to the granule cells, there are Golgi cells together with two new types

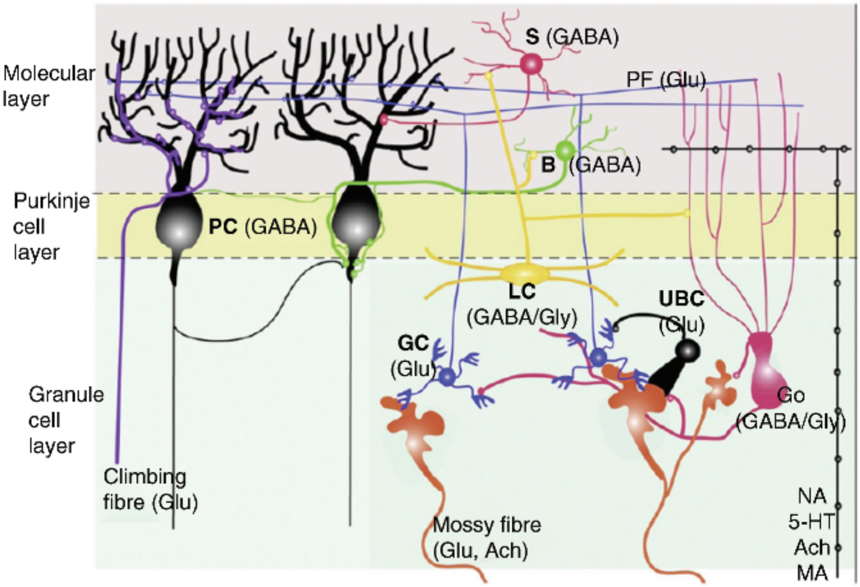

Figure 1.4 Cellular and synaptic organization of the cerebellar cortex. The schema, slightly modified from Chédotal (2010), not only includes the two new types of interneurons, Lugaro cells (LCs) and the unipolar brush cells (UBC), but also the neurotransmitter used by each class of neurons and the monoaminergic extracerebellar afferent fibres. [Reprinted from Sotelo (2010) with permission from Elsevier, © 2010.]

of interneurons, the Lugaro cells (discovered in 1894) and the unipolar brush cells in the granule cell layer. There are three types of axons: the mossy fibres and the climbing fibres, which enter the cerebellum from outside, and the parallel fibres, which are axons of the granule cells (Figure 1.4). The neurotransmitters used by each class of neurons and the monoaminergic extracerebellar afferent axonal fibres are indicated. Below the grey matter of the cortex are white myelinated nerve fibres running through the pons to and from the cerebellar cortex. Although it does not initiate movement, the cerebellum, which receives input from sensory systems in other parts of the brain and the spinal cord, plays an important role in the control of motor functions, integrating these inputs to fine-tune motor activity and thereby contributing to coordination, precision and timing of movement.

The *pons* is located on the brainstem, above the medulla oblongata and below the midbrain. Its white matter conducts signals from the cerebral cortex down to the cerebellum and medulla and carries sensory signals up into the thalamus. It is involved in motor control and sensory analysis. For example, information from the ear first enters the brain in the pons. It is also important for maintaining the level of consciousness and for sleep. Some structures within the pons are linked to the cerebellum, and are thus involved in movement and posture.

The *medulla oblongata* is the lowermost part of the brainstem, between the pons and spinal cord. It is responsible for maintaining vital autonomic body functions, such as breathing, heart rate and blood pressure.

1.2 The Cell Types of the Brain

The complexity of the CNS is staggering, regulating all aspects of the functions of our bodies, yet even today in our post-genomic era, we are still a long way from understanding it. The CNS, made up of the brain and the spinal cord, together with the peripheral nervous system (PNS) is made up of nerve cells, neurons. Each neuron makes connections with many other neurons at junctions known as synapses, and millions of new connections are formed and broken every second of our lives. We have already pointed out that in addition to *neurons*, the brain contains other types of cells known as *glial* cells. The brain also requires a blood supply, and so there are *endothelial cells* lining the capillaries that irrigate the brain. We discuss the characteristic features of neurons and glial cells here.

1.2.1 Neurons

Millions of neurons collect information about our environment (both external and internal), which they transmit to other neurons, where the data are either processed or stored. Millions more respond to this information to regulate the control of muscle contraction, hormone synthesis and so on. In spite of the overwhelming complexity of what we call the CNS, we understand the structure and function of neurons quite well. Most neurons contain four distinct regions – the cell body, the axon, the dendrites and the axon terminals. The cell body, which contains the nucleus, is the site of synthesis of all neuronal proteins and membranes. Most neurons have one single axon which conducts the electrical impulses, called action potentials, and which terminates in many axon terminals. The extensively branched dendrites receive signals at their interface with several hundred other cells. A single axon in the CNS can interact with many other neurons at synapses and induce responses in all of them simultaneously. The arrival of an action potential at the axon terminal of a presynaptic synapse results in the propagation of the electrical signal to postsynaptic neurons or other cell types.

We can distinguish three types of neurons. Multipolar neurons have profusely branched dendrites, which receive synaptic signals from several hundred other neurons and transmit them to many other neurons at the lateral branches of its terminals. Motor neurons transmit nerve impulses to muscle cells, and their single, often very long, axons extend from the cell body of the neuron to the effector muscle cell. Mammalian neurons have an insulating sheath of myelin covering all parts of the axon except for the nodes of Ranvier and the axon terminals at the neuromuscular synapse. Sensory neurons collect all sorts of information, concerning light, smell, sound, pressure, touch and so on, through specialized receptors and transform such information into electrical signals. In sensory neurons, the axon branches when it leaves the cell body. The peripheral branch carries the nerve impulse from the receptor cell to the cell body. The central branch then carries the impulse from the cell body, located in the dorsal root ganglion close to the spinal cord, either to the spinal cord or to the brain.

We will analyse in greater detail the action potential generated by successive cycles of hyperpolarization/depolarization, propagated by the opening and shutting of specific ion channels, when we consider the role of Na^+ and K^+ in neurotransmission (Chapter 2). An example of what is observed when a microelectrode is inserted into the axonal membrane of a presynaptic neuron is shown in Figure 1.5. The membrane potential observed shows that the neuron is ‘firing’ every 4 ms (i.e. the neuron is generating about 250 action

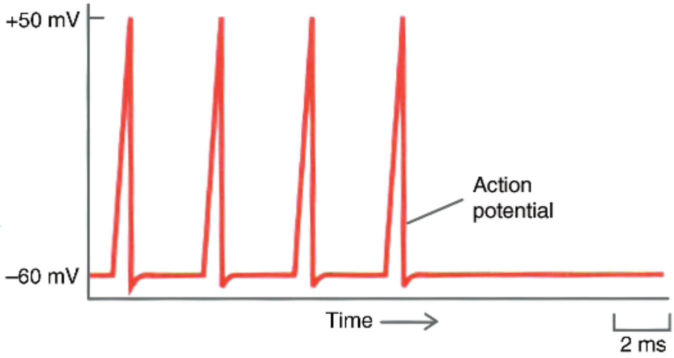

Figure 1.5 *The observed membrane potential across the plasma membrane of a presynaptic cell.*

potentials/s). The axons of larger vertebrate neurons, particularly motor neurons, are sheathed with myelin, a kind of biological insulating tape which allows them to propagate nerve impulses at velocities of up to 100 m/s. In contrast, in unmyelinated nerves, they travel no faster than 10 m/s.[1]

Neurons communicate information in one of two ways, either via chemical signalling or via electrical signalling. At a chemical synapse (Figure 1.6a), the axon terminal of the presynaptic cell contains vesicles which are filled with a neurotransmitter, such as adrenaline, glutamate or acetylcholine. When the action potential reaches the axon terminal, some of the vesicles fuse with the plasma membrane, releasing their contents into the synaptic

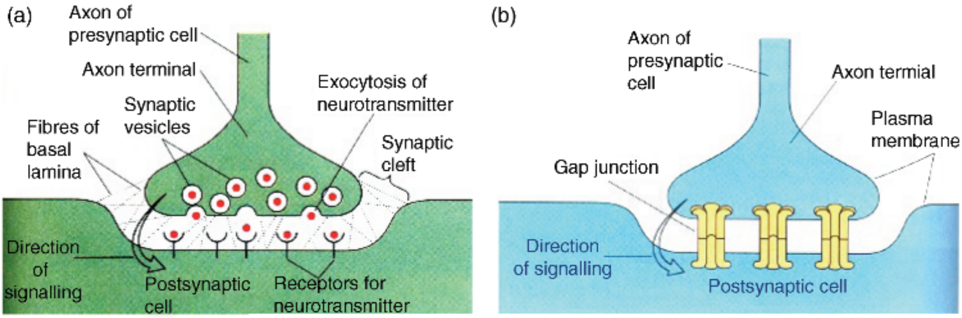

Figure 1.6 *(a) A chemical synapse. A narrow cleft, the synaptic cleft, separates the plasma membranes of the presynaptic and postsynaptic neurons. Transmission of electrical impulses requires release of a neurotransmitter by the presynaptic cell, its diffusion across the synaptic cleft and its binding to specific receptors on the plasma membrane of the postsynaptic cell. (b) An electric synapse. The plasma membranes of the presynaptic and postsynaptic cells are linked by gap junctions. The flow of ions through these channels allows electrical impulses to be transmitted from one cell to the other.*

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[1] One shudders to imagine the coordination problems of a giraffe if it had to rely on non-myelinated nerves!

cleft. The neurotransmitter diffuses across the synaptic cleft, binds to specific receptors of the postsynaptic cell and changes the membrane potential of its plasma membrane. If the postsynaptic cell is a neuron, it ultimately induces an action potential, resulting in transmission of the signal, whereas if the postsynaptic is a muscle cell, contraction results, and if it is a hormone-producing cell, it will release its hormone. Neurotransmitters must then be cleared out of the synapse efficiently so that the synapse can be ready to function again as soon as possible. Usually neurotransmission by chemical signalling results in amplification of the signal (referred to as 'gain'). In contrast, in an electrical synapse (Figure 1.6b), the presynaptic and postsynaptic cell membranes are connected by channels, known as gap junctions, that are capable of passing electrical current. Electrical synapses conduct nerve impulses faster, but unlike chemical synapses they do not have gain. Electrical synapses are often found in neural systems that require very rapid responses, such as defensive reflexes.

1.2.1.1 Axonal Transport

Neurons are the longest cells in the body, and whereas the cell body typically measures 6–120 μm in diameter in humans, the axonal process can run for up to a metre or more. The neuron must constantly supply new materials – proteins, membranes and metal ions – to the terminal to replenish those lost by exocytosis at the synapse. Ribosomes are present only in the cell body and dendrites of nerve cells, so that no protein synthesis can occur in the axons and synapses. The process by which axonal components are transported down the axons was first described in 1948 and is called axonal transport.

Studies on axonal transport have revealed that materials are transported in both directions. *Anterograde transport* proceeds from the cell body to the synaptic junctions, and is associated with axonal growth and renewal of synaptic vesicles [for a review, see Brown (2000, 2003)]. At the same time, a complementary mechanism translocates 'cargo', mainly consisting of 'old' membranes in the opposite direction, for their degradation in the lysosomes of the cell body (*retrograde transport*). Axonal transport can be further divided into three groups, according to their speed of migration. The fastest-moving material, consisting mostly of membrane-bound vesicles, has a velocity of 250–400 mm/day (3–4.5 μm/s) in the anterograde direction and 100–200 mm/day (1.5–2.5 μm/s) in the retrograde direction. The slowest-moving material, made up for the most part of polymerized cytoskeletal proteins, moves only a fraction of a mm/day. Organelles such as mitochondria move down the axon at an intermediate rate.

The mechanism of fast axonal transport has been understood for decades, but the mechanism of slow axonal transport has been discovered in 2005 (Roy *et al.*, 2005). Recent studies have revealed that the movement of individual 'slow' cargoes is actually rapid, but unlike fast cargoes, they pause frequently, making the overall transit rate much slower: this is the aptly named 'Stop and Go' model of slow axon transport (Brown, 2000, 2003; Roy *et al.*, 2007). A good analogy is the difference between the time taken by local and express trains to get to one's destination. Although both types of train travel at similar speeds between stations, the local trains take much longer to reach their destination because they stop at every station, whereas the express trains make only a few stops.

Generally speaking, to move any cargo, two components are required: 'motors' to move the cargo and 'rails' to transport them. As rails (Figure 1.7), the neurons, like most other cell types, use the complex network which constitutes the cytoskeleton made up mostly of the microfilaments, built from the protein actin, and the somewhat wider microtubules

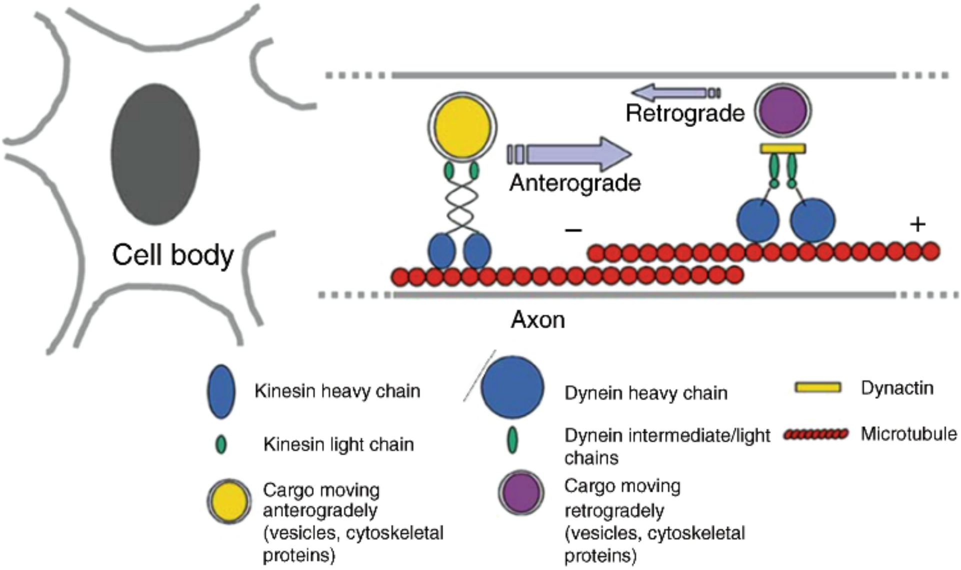

Figure 1.7 *Anterograde and retrograde axonal transport by motors kinesins and dyneins, respectively. Kinesins and dyneins move on microtubules and transport Golgi-derived vesicles, cytosolic protein complexes, cytoskeletal polymers and other cargoes such as ribosomes and messenger RNAs. [Adapted from Roy* et al. *(2005). With kind permission from Springer Science and Business Media.]*

composed of tubulin (Brown, 2003). The motors which drive the movement of cargoes in the anterograde or retrograde direction are the tiny molecular machines[2] constituted by the proteins kinesin and dynein, respectively (Figure 1.7). Kinesin is a dimer made up of two heavy chains complexed to two light chains (Figure 1.8) organized into three domains, each of which has a particular function. The globular head domain is responsible for the ATP hydrolysis-driven motor activity of kinesin and is the part of the molecule which binds to the microtubules. The central stalk region spaces the head from the surface of the membrane, while, in contrast to the head domain which binds microtubules and ATP, the tail domain is responsible for binding to the membrane of the vesicle which is transported (referred to above as the ‘cargo’). The movement of kinesin has polarity – it will walk only towards the (+) end of the microtubule, moving in 8 nm steps. In contrast, dynein, an extraordinarily large multimeric protein, walks along microtubules in the opposite direction, towards the (−) end, ensuring the transport of retrograde vesicles.

It is clear, as we will see in Chapter 2, that metal ions such as zinc, calcium, copper and probably also iron are transported to the synapses by axonal transport. Transport of material between extensive neuronal processes and the cell body is crucial for neuronal function and survival. There is growing evidence that deficits in axonal transport contribute to

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[2] The first such molecular machine for which an explanation for the mechanism was uncovered was the rotational motor, ATP synthase responsible for synthesis of ATP (the 1997 Nobel Prize in Chemistry, awarded to Paul Boyer, Jens Skou and John Walker).

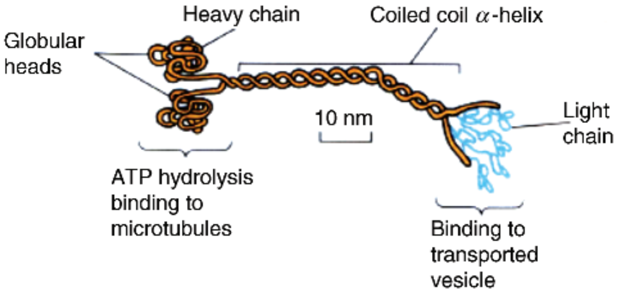

Figure 1.8 *Schematic model of kinesin, showing the arrangement of the two heavy chains and two light chains.*

the pathogenesis of multiple neurodegenerative diseases (Ström *et al.*, 2008), including amyotrophic lateral sclerosis (see Chapter 11). While most of the zinc in the CNS is tightly bound to macromolecules, a considerable amount, approximately 10–15% of the total zinc in the brain, exists as a pool of free or loosely bound zinc ions in synaptic vesicles with zinc transporter 3 (ZnT3) in their membranes which can be released into the synapse. Axonal transport of zinc transporter 3 and zinc containing organelles in the rodent adrenergic system has been reported (Wang and Dahmström, 2008).

1.2.2 Glial Cells

Glial cells were considered to perform poorly until quite recently compared to neurons. Over the past 20 years, it was believed that neurons performed the exciting functions of processing information storing memories and communicating with each other. In contrast, the glia, as their name indicates (Greek γλία 'glue'), were simply there to support neurons, feed them, clean up after them and look after them when they were sick. This was in large part due to the 'neuron doctrine' proposed by Santiago Ramon y Cajal, according to which the neuron is the basic unit of the nervous system (Agnati *et al.*, 2007; Sotelo, 2010). However, although Cajal and Golgi may have shared the 1906 Nobel Prize in Medicine, they never shared the same basic concepts. For Golgi, all axons fused into a diffuse nerve network, whereas for Cajal, they had free endings and communication between neurons was done by contiguity not by continuity.

An increasing body of work over the past two decades has revealed that the glia, which constitute 85% of the cells of the brain, are critical regulators of nervous system development, function and health, as we will see in what follows. There are three principal types of glial cells, namely astrocytes, oligodendrocytes and microglia, whose characteristics and functions are described here. For recent reviews, see the articles by Emery (2010), Fields (2010), Freeman (2010) and Graeber (2010).

Astrocytes are the star-shaped glial cells (Figure 1.9) found in both the brain and the spinal cord, and are the most abundant cell type in the mammalian brain. They outnumber neurons by over fivefold (Sofroniew and Vinters, 2010) and exert many complex functions in the healthy CNS. They are closely associated with neurons, and most synapses are intimately ensheathed by astrocytes processes (Figure 1.10). Interestingly, as one

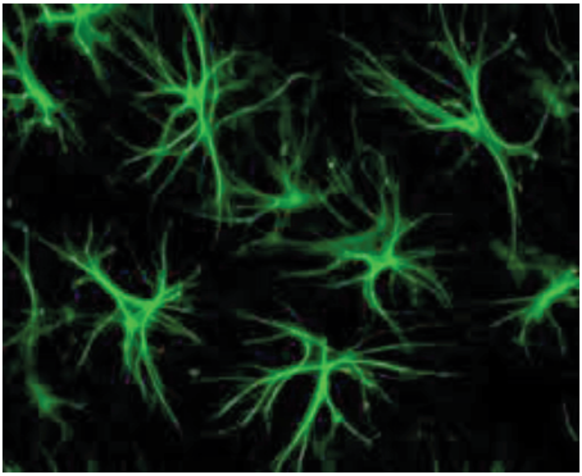

Figure 1.9 *Astrocytes. Immunocytochemical stained astrocytes with an antibody against glial fibrillary acidic protein. (Reproduced from Wikipedia.)*

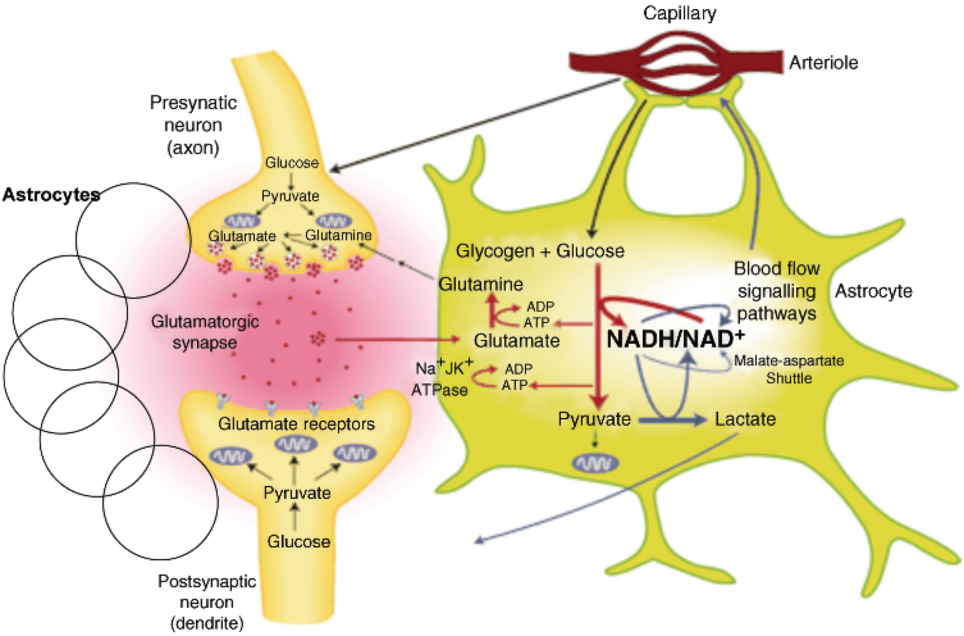

Figure 1.10 *An astrocyte surrounding the synapse of a glutamatergic neuron. On the left-hand side, astrocytes (represented schematically as circles) are shown surrounding the synapse). On the right-hand side, the functioning of a single astrocyte is shown.*

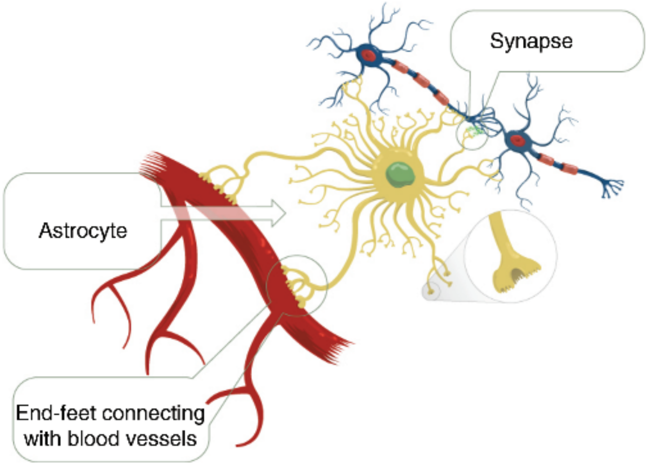

Figure 1.11 *Astrocytes are in close contact with neurons and blood vessels. (Reproduced with permission from Genetic Science Learning Center, learn.genetics.utah.edu.)*

moves up the evolutionary scale, astrocytes in the brain cortex increase in both number and size, with humans having not only the most astrocytes but also the biggest (Kobb, 2009). Astrocytes have hundreds of ‘end-feet’ spreading out from their cell body, embracing all penetrating arterioles, and capillaries enwrap multiple neuronal somata (Figure 1.11) in addition to thousands of individual synapses (Gourine and Kasparov, 2011) as well as other astrocytes and neurons. Among the functions which have classically been attributed to them for some time in addition to their role of ‘gap fillers’ are the biochemical support of endothelial cells involved in the blood–brain barrier (BBB), supply of nutrients such as lactate to neurons, maintenance of ion balance by clearing K^+ released by neurons when they are stimulated, thereby preventing its accumulation in the extracellular space, and their involvement in the repair process after neuronal injury, filling the space and replacing the CNS cells that cannot regenerate. However, in recent years, new roles for astrocytes in synapse formation, maturation, efficacy and plasticity have been identified. This includes their function as a gliotransmitter.

Since the mid-1990s it has become apparent that in response to stimulation, astrocytes are able to release Ca^{2+} from their internal stores, for example endoplasmic reticulum, which propagates intracellular Ca^{2+} waves across long distances from one astrocyte to another. Perisynaptic processes of astrocytes express active G-protein-coupled receptors that sense neurotransmitters released from the synapse during synaptic activity and increase intracellular cytosolic levels of calcium (via endoplasmic reticulum, inositol 1,4,5-triphosphate and ryanodine receptors), providing the conduit for the release of Ca^{2+} into the cytosol (Parpura, Grubišić and Verkhratsky, 2011) to stimulate the release of various transmitters, for example ATP, glutamate, D-serine and GABA, in the vicinity of the synapses. For example, glutamate released from the presynaptic neuron during neuronal firing is taken up by astrocytes, converted to glutamine and released back to the neuron. This has led in turn to the conclusion that astrocytes not only modulate neuronal behaviour but may also be involved in thought processing. Astrocytes can communicate with other astrocytes,

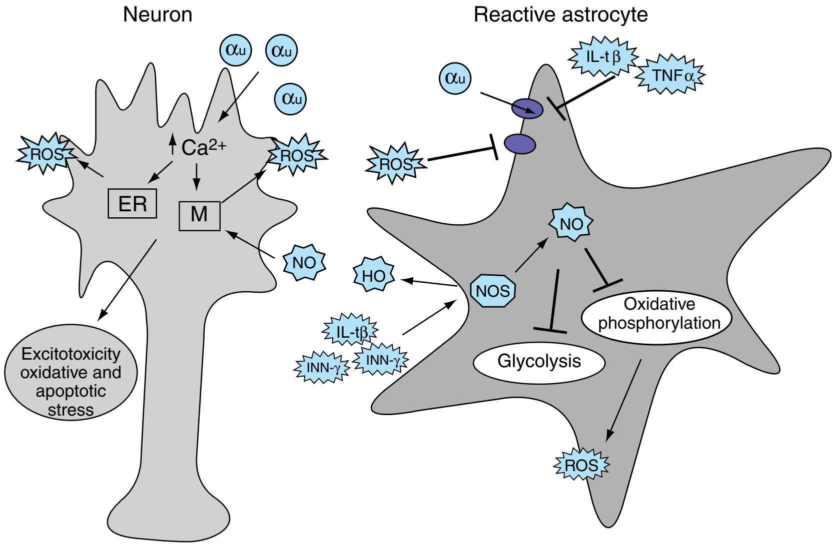

Figure 1.12 *Activated astrocytes. Excessive glutamate accumulates in the synaptic cleft due to decreased uptake (caused by ROS and cytokines), increasing influx of calcium into neurons which will damage ER and mitochondria. This is turn will increase oxidative stress and metabolism. Cytokines stimulate iNOS in astrocytes producing NO which will inhibit the mitochondrial respiratory chain components. Enzymes involved in glycolysis and oxidative phosphorylation are vulnerable to inhibition by NO. Neurons and astrocytes will become compromised. The exchanges of metabolic precursors will be disrupted such that there may be synaptic loss and even cell death. ER, endoplasmic reticulum; M, mitochondria; iNOS, inducible nitric oxide synthase; NO, nitric oxide; ROS, reactive oxygen species; Glu, glutamate; IL-1, interleukin-1; TNF-α, tumour necrosis factor-α. [Reprinted from Stipursky* et al. *(2011) with permission from Elsevier, © 2011.]*

and are able to release, uptake and degrade neurotransmitters from distant synapses to control the transmission of information between neurons, perhaps signalling to neurons via Ca^{2+}-modulated glutamate release, thereby causing the neurons to fire. As we will see below, the astrocytes serve as a relay between neurons and oligodendrocytes to stimulate myelin formation. They are also thought to suppress synaptic transmission in the hippocampus by releasing ATP which is hydrolysed to adenosine; this in turn binds to neuronal adenosine receptors, inhibiting synaptic transmission, and may therefore contribute to long-term potentiation in the hippocampus. If the normal role of astrocytes is to control neurotransmitter levels at synapses, this implicates them directly in psychiatric disorders, since all drugs for the treatment of mental illnesses act by controlling the synaptic levels of different neurotransmitters. Astrocytes can be either neuroprotective or neurotoxic. On activation (astrogliosis) (Figure 1.12), a variety of mediators are released which include proinflammatory cytokines, glutamate, ATP and free radicals, each of which may decrease neuronal survival and increase their susceptibility to neurotoxins.

Oligodendrocytes (Figure 1.13a) are involved in the electrical insulation of nerve fibres (axons). These cells morphologically resemble cellular octopuses, wrapping up to 150 layers of myelin sheath approximately 1 μm thick around the axons of neurons, rather

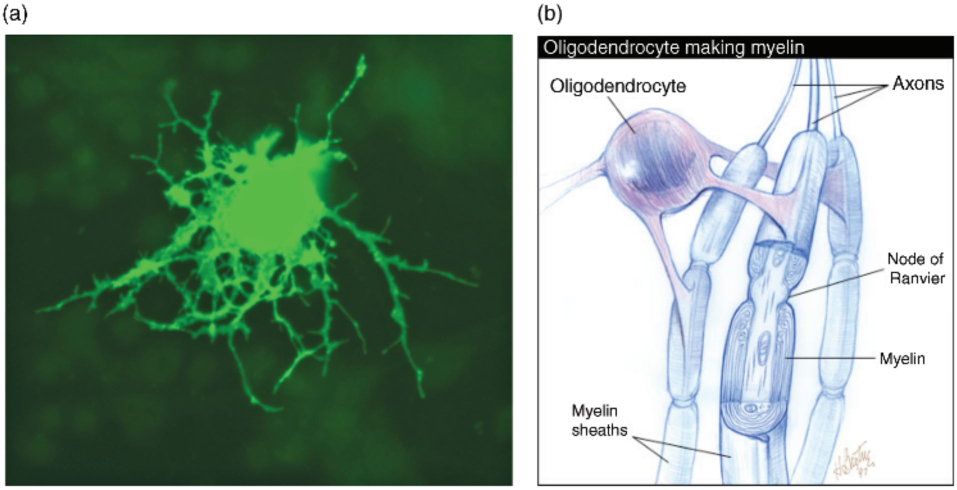

Figure 1.13 Oligodendrocytes. (a) Fluorescent micrograph [Reproduced from Wikimedia Commons.] (b). Oligodendrocytes form the myelin sheaf around neurons, thereby allowing faster transmission of electrical impulses. One oligodendrocyte may be responsible for the myelination of up to 50 nerve fibres. [Reproduced from blustein.tripod.com.]

like electrical insulating tape (Figure 1.13b). One oligodendrocyte can extend its processes up to 50 neuronal axons. In the PNS, the function of the oligodendrocytes is replaced by Schwann cells, which, however, can wrap around only one axon. Mature oligodendrocytes with highly ramified processes will form membrane sheets following the generation of oligodendrocytes–axon contact. Compression of these primary membrane wraps will result in the generation of multi-lamellar insulating myelin sheaths around axons. The myelin sheaths form internodes which are separated by nodes of Ranvier where action potentials are generated (Figure 1.14). The internodes are flanked by

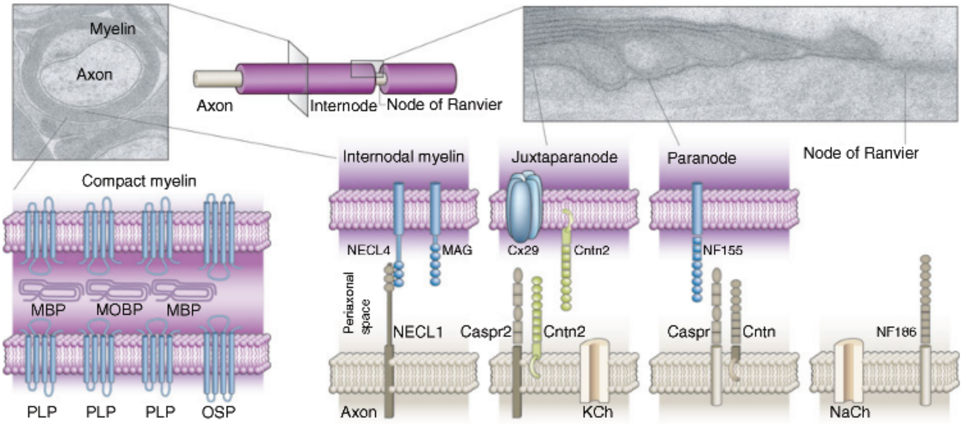

Figure 1.14 Oligodendrocytes and the axon myelin unit. [Reprinted from Nave (2010) by permission from Macmillan Publishers Ltd.]

paranodes, beside which are clusters of potassium channels named the juxtaparanode (Figure 1.14). High numbers of proliferating oligodendroglial progenitor cells are present to ensure remyelination can occur when necessary. New studies of human brain using magnetic resonance imaging (MRI) have demonstrated that myelinating oligodendrocytes sense electrical activity in axons, revealing that white matter changes after learning complex tasks. Studies on effects of long-term training, in particular, in professional musicians, show strong relations between training and white matter structure (Ullén, 2009). Adult subjects learning to juggle show increased white matter organization in a brain region important for visuomotor control (Scholz *et al.*, 2009), while adults learning to read showed increased volume, anatomical organization and functional connectivity of white matter tracts linking cortical regions important for reading (Carreiras *et al.*, 2009). This implies that white matter changes during learning, and that myelin formation can be controlled by electrical impulse activity. Myelination of a non-myelinated axon will increase the speed of transmission of nerve impulses through the nerve fibre by at least 50-fold. In addition, neurons benefit from the myelination of their axons by a reduction in the amount of ATP utilized. Both chemical signals from the environment and the internal controls regulate the development of oligodendrocytes from immature progenitor cells. Recent studies have shown that these oligodendrocyte progenitor cells (OPCs) can transform not only into mature oligodendrocytes but also into astrocytes and neurons under appropriate conditions. Such cells are being transplanted into patients with spinal cord injuries in experimental studies to cure paralysis. One intriguing aspect of these OPCs is that for reasons that we do not understand, neurons form synapses with them. One exciting challenge is that these OPCs could perhaps be programmed to form myelin, opening the possibility of treating currently debilitating diseases such as multiple sclerosis.

The proliferation and differentiation of oligodendrocyte lineage cells are controlled by growth factors, cytokines, such as platelet-derived growth factor, and brain-derived neurotrophic factors. Myelination depends on precise interaction of axonal and oligodendroglial signals. These include neural cell adhesion molecule (NCAM), which plays an important role in axonal pathfinding, nerve branching, cell migration and synaptic plasticity. LIMGO-1, a transmembrane protein, is important in neurogenesis, axon guidance and myelination during development. Female sex hormones are beneficial for re-myelination, while ageing has a detrimental effect. Oligodendrocytes express the proteoglycan protein NG2 as well as glutamate receptors and receive transient synaptic input from unmyelinated axons, that is neuronal activity will modulate myelination.

Microglia are regarded as the resident immunocompetent effector cells for the innate immunity in the brain macrophages of the CNS (Figure 1.15). They can communicate with the astrocytes and neurons and with cells of the immune system by a large number of signalling pathways. In normal circumstances, they have two important roles: (a) as surveillance cells, to regulate and supervise the removal of cell debris after neuronal death, after which the microglia will return to their quiescent state, and (b) controlling apoptosis. The microglia are as common as the nerve cells and are characterized by an extremely plastic, chameleon-like phenotype (Figure 1.15). They are the most susceptible sensors of brain pathology, and when they detect any signs of brain lesions or nervous system dysfunction, they undergo a complex, multi-stage activation process that converts them into ‘activated microglia’. This was convincingly demonstrated with the advent of lectin and antibody markers which label all of the stages of microglial activation. In the healthy adult

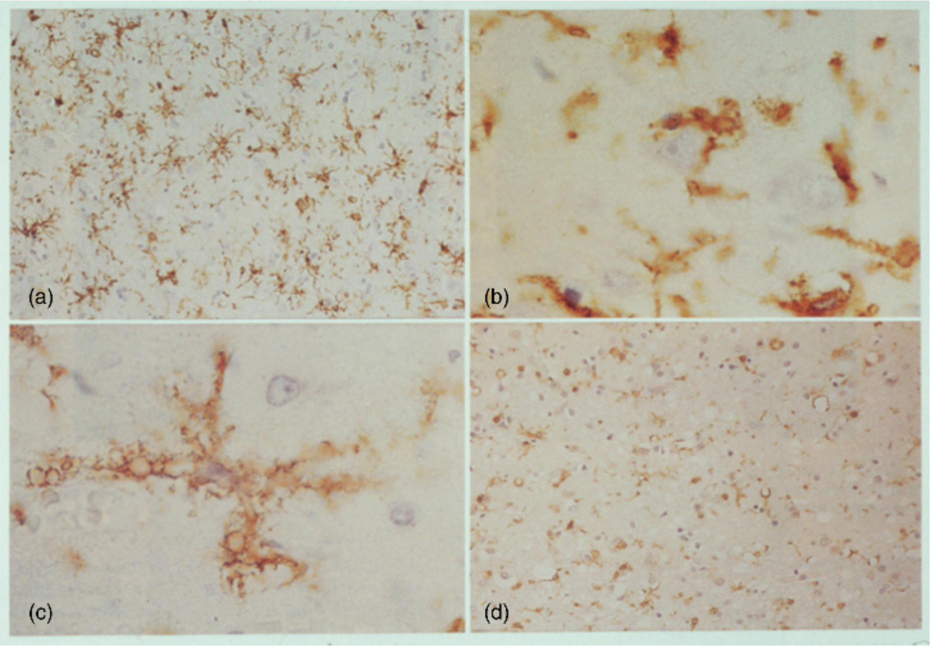

Figure 1.15 *Microglia. Microglia showing highly branched processes in the ramified state. [Reproduced from Ward, Crichton and Dexter (2012).]*

brain, they exist in a non-activated state, equipped with receptors for neurotransmitters, neuropeptides, hormones and immune signals. When activated (Figure 1.16), microglia show a phenotypical repertoire which includes the synthesis of MHC Class 1 and Class II antigen-presenting proteins, the release of proinflammatory cytokines such as IL-1, IL-2, IL-6, TGF-α1, CREB, the synthesis of complement components and their receptors, glutamate ROS, RNS, together with the mitogens M-CSF, GM-CSF and IL-3 (Table 1.1). In addition, microglia are the major source of IL-18 which enhances microglia caspase-1 expression as well as matrix metalloproteins. These can act detrimentally or beneficially upon surrounding cells, since microglia move to the site of injury, proliferate and phagocytose cells and cellular compartments. Their activity in immunological disease states, such as multiple sclerosis, to remove myelin debris is in line with their pathological role as macrophages and antigen-presenting cells. However, expression of immune proteins by microglia is not synonymous with inflammation, since these molecules can have CNS-specific roles. Microglia can have functionally adapted phenotypes, like their involvement in neurogenesis, neuropathic pain and post-lesional 'synaptic stripping'. Experimental studies support the view that microglia have a role in the maintenance of synaptic integrity and are capable of removing defunct axon terminals, thereby helping neuronal contacts to remain intact. In the healthy CNS, microglia do not present as macrophages, indicating that their day-to-day function is different. These specific non-macrophagic microglial functions are now beginning to be explored. That one and the same cell type can have opposite biological effects is well illustrated by the contradictory role of macrophages in cancer

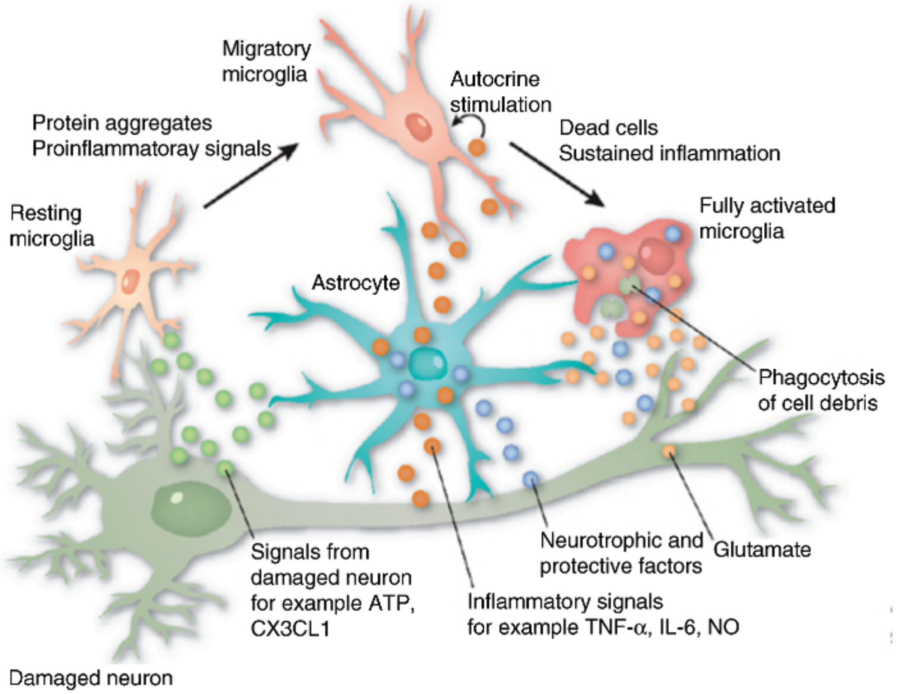

Figure 1.16 *Microglia respond to several immunological signals, for example cytokines and aggregated proteins, and are activated. Such activation may be toxic to the surrounding glial cells and neurons or protective. [Reprinted from Monk and Shaw (2006) by permission from Macmillan Publishers Ltd.]*

(Sica *et al.*, 2008). While they express activities that can prevent the establishment and spread of tumour cells, they may simultaneously elicit functions which support tumour growth and dissemination (Figure 1.17). This paradoxical ambivalent relationship between macrophages and cancer reflects the functional plasticity of these cells, which can express different functional programmes in response to different microenvironmental signals.

Table 1.1 *Products generated by the activation of microglia.*

Cytokines	Chemokines	Receptors	Additional factors
IL-1	IP-10	CCR2, 3, 5	MHCI, II
IL-6	MIP1a	IL-10R	CD80, 86
IL-10	MIP1b	IL-12R	CD95, 178
IL-12	MCP1	IL-18R	Complements
IL-18	IL-8	IFN-γR	COX2
TNF-α	RANTES	TNF-αP	NO
TNF-β		TGF-βR	Superoxide
		FCγRI-III	Hydroxyl radical
		CRI-3,4	PGE2
		Prostaglandin receptors	PGD2
			NGF, BDNF

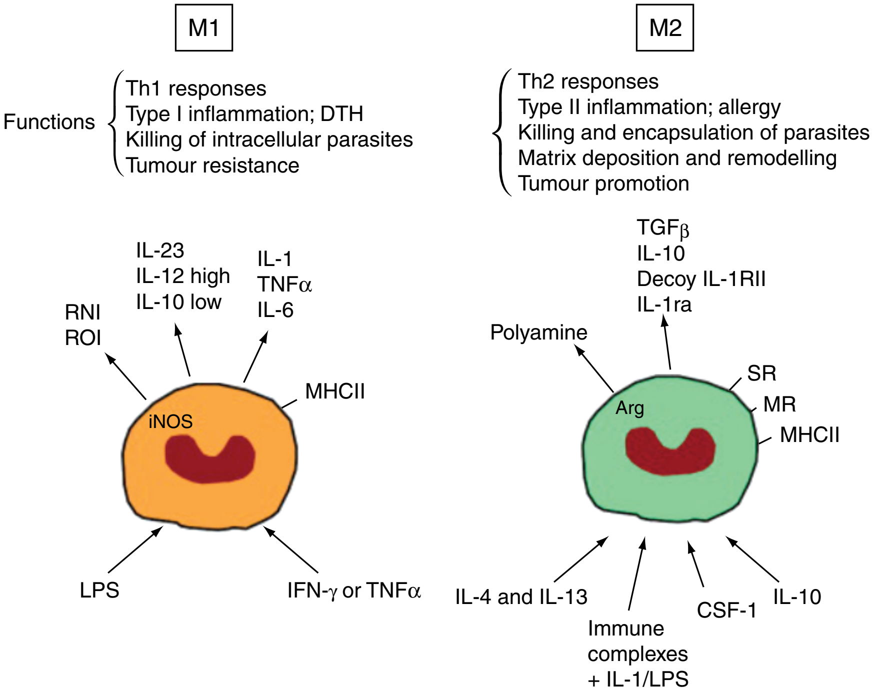

Figure 1.17 *In the presence of interferon (IFN)-γ, lipopolysaccharide (LPS) and other microbial products, monocytes differentiate into M1 macrophages. In the presence of macrophage colony-stimulating factor (CSF-1), interleukin (IL)-4, IL-13, IL-10 and immunocomplexes in association with either IL-1R or TLR ligands, monocytes differentiate into M2 macrophages. M1 and M2 subsets differ in terms of phenotype and functions. M1 cells have high microbicidal activity, immunostimulatory functions and tumour cytotoxicity. M2 cells have high scavenging ability, promote tissue repair and angiogenesis and favour tumour progression. [Reprinted from Sica* et al. *(2008) with permission from Elsevier, © 2008.]*

Microglia also show a strong respiratory burst capacity, via NADPH oxidase, possess cytotoxic cytokines such as TNF-α and can produce both reactive nitrogen and oxygen species. In normal circumstances, this inflammatory response would be rapid and decisive and would then decline. However, chronic exposure to proinflammatory signals from systemic infection during an individual's lifetime might promote an exaggerated microglial response that could contribute to neuronal deterioration instead of facilitating a protective homoeostatic response.

1.3 The Blood–Brain Barrier

The brain is unique among all organs of the body, hidden behind a relatively poorly permeable vascular barrier, which limits its access to plasma nutrients, such as metal ions. There are three principal barrier sites between blood and brain (Abbott *et al.*, 2009): the

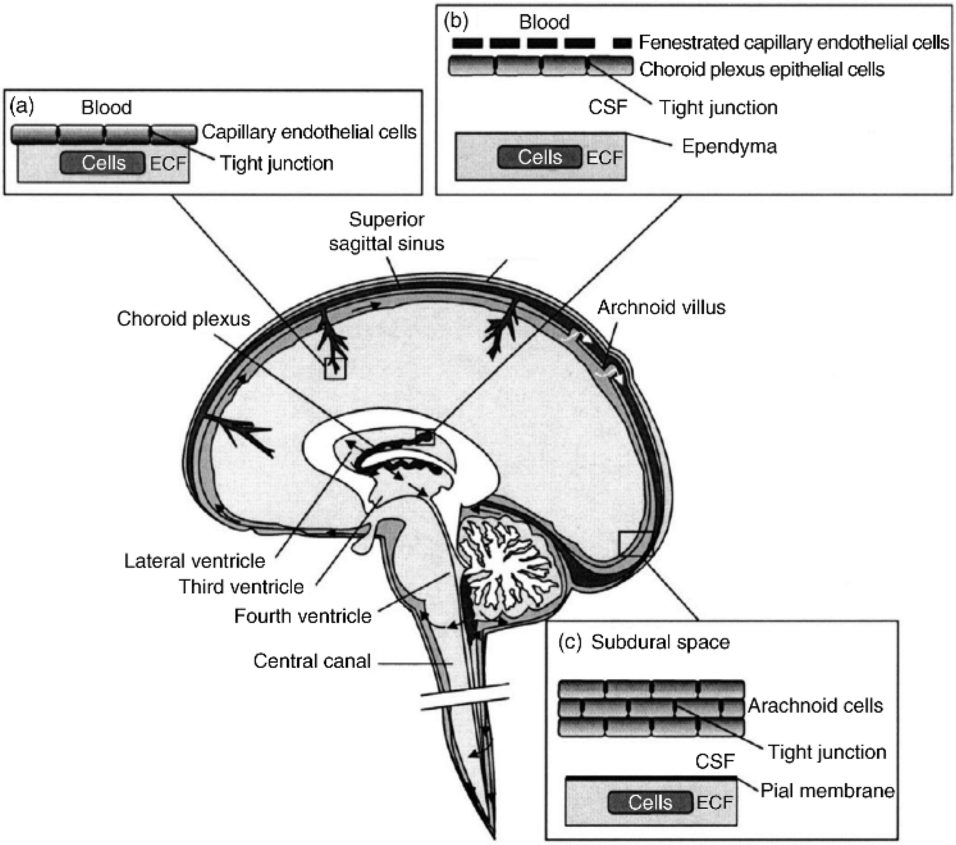

Figure 1.18 *Barriers of the brain. (a) The BBB proper. (b) The blood–CSF barrier. (c) The arachnoid barrier. [Reprinted from Abbott* et al. *(2009) with permission from Elsevier, © 2009.]*

BBB proper, the blood–CSF barrier (BCSFB) and the arachnoid barrier (Figure 1.18). The BBB is created at the level of the cerebral capillary endothelial cells which form the walls of the capillaries by tight junction formation. The BCSFB is located at the choroid plexus in the lateral, third and fourth ventricles of the brain. Tight junctions are formed between the epithelial cells at the CSF-facing surface of the epithelium. The CSF is secreted into the ventricular space of the brain, whereas the rest of the brain extracellular fluid (ESF) is, at least in part, secreted across the capillary endothelium of the BBB. The third barrier is provided by the arachnoid epithelium, lying underneath the dura and completely enclosing the CNS.

In an adult human being, the BBB has a surface area of 12–18 m^2 and is essentially composed of the cerebral capillary endothelial cells, joined by tight junctions, a basal lamina, pericytes and astrocyte end-foot processes, as illustrated in Figure 1.19. The endothelial cells form tight junctions which seal the paracellular pathway between the cells, such that substances which enter the brain must use dedicated endothelial cell

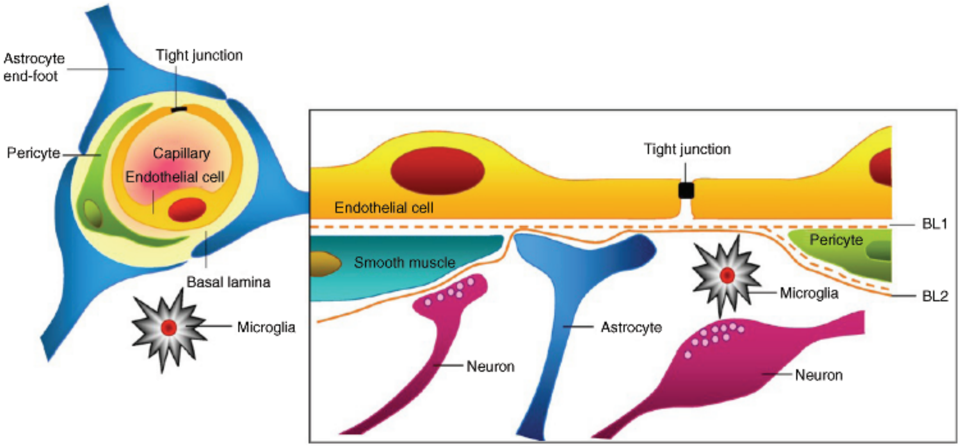

Figure 1.19 *Cell associations at the BBB. [Reprinted from Abbott* et al. *(2009) with permission from Elsevier, © 2009.]*

transport systems. The movement of solutes across the BBB may be facilitated by passive or active transporters in the endothelial cell membranes. Since no brain cell is further than about 25 μm from a capillary, once the BBB is crossed, diffusion distances for solutes to neurons and glial cells are short. Unlike other blood vessel epithelia, the BBB epithelia express different receptors at the luminal membrane (facing the circulation) compared to the abluminal membrane, surrounded by astrocyte end-feet, neuronal processes and interstitial fluid. Pericytes, the connective tissue cells which occur around small blood vessels, are distributed along the length of the cerebral capillaries, partially surrounding the endothelium. Both the cerebral endothelial cells and the pericytes are enclosed by the local basement membrane, forming a distinct perivascular extracellular matrix [basal lamina 1 (BL1)], different from the extracellular matrix of the astroglial end-feet bounding the brain parenchyma (BL2). Foot processes from astrocytes form a complex network surrounding the capillaries. The network of astrocytic processes forms the infrastructure on which all other CNS cells and vessels are anchored. They have a multitude of functions including regulation of the ionic milieu in the intercellular space, uptake and/or breakdown of some neurotransmitters, supplying nutrients to the neurons, and formation of the BBB. Microglia are also found in the vicinity of the BBB.

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