

2. Nervous System

The nervous system is a network of cells specialized for the rapid transfer and integration of information. The fundamental unit of the nervous system is the neurone which with its processes transmits electrical signals. The cell bodies of neurones tend to be segregated into compact groups (nuclei, ganglia) or into sheets (laminae) that lie within the grey matter of the CNS or are located in specialized ganglia in the PNS. Groups of nerve fibres running in a common direction usually form a compact bundle (nerve, tract, peduncle, brachium, pathway). Many of these nerve fibres are surrounded by sheaths of lipid material called myelin which gives rise to the characteristic appearance of the white matter. In addition to neurones there are glial cells which play a supporting role. There are about five times more glial cells than neurones and they occupy approximately half the volume of the brain.

2.1 Cells of the Nervous System

Neurones

Neurones are more diverse in size and shape than cells in any other tissue of the body. They usually possess dendrites, a cell body, an axon and synaptic terminals.

Dendrites are branches which leave the cell body and share with it the function of receiving information from synaptic connections with adjoining neurones. They are usually tapered and possess various protuberances, such as dendritic spines which are sites of contact with other cells.

The *cell body*, also known as the soma or perikaryon, contains the nucleus. In the cytoplasm surrounding the nucleus is an extensive array of rough endoplasmic reticulum, the basophilic staining so-called Nissl substance which synthesizes proteins for the cell body and its processes. Large neurones replace as much as one-third of their protein content every day.

The *axon* is a slender process ranging in length from a few hundred microns to more than a metre. The diameter of axons usually varies from 0.1 to 20 microm although in some invertebrates it may be as much as a millimetre. The axon contains mitochondria, microtubules, neurofilaments and smooth endoplasmic reticulum. At its junction with the cell body, also known as the *axon hillock*, there is some rough endoplasmic reticulum but beyond the axon hillock there are no ribosomes. Axons are usually longer than dendrites and at their ends they branch to form terminals which make contact with other cells at *synapses*.

Synaptic terminals are the sites of release of chemicals (*neurotransmitters*) and are usually in close proximity to another neurone or effector cell. A synapse consists of a presynaptic element containing synaptic vesicles, a synaptic cleft and a postsynaptic element. The synaptic vesicles, which contain the neurotransmitter, vary in size in different cells from 25 to 250 nanom. The synaptic cleft between neurones is 20 to 30 nanom wide and at skeletal neuromuscular junctions it is 50 to 100 nanom wide. In electromicrographs electron-dense areas on the cytoplasmic faces of pre- and postsynaptic membranes indicate sites of specialization for exocytosis of vesicle contents and for localization of postsynaptic receptors respectively. While most neurotransmitters are released close to effector cells, some neurones

release substances which act at some distance from their site of release and thus are more like endocrine cells.

Thus neurones are specialized (i) to receive information from the internal and external environment; (ii) to transmit signals to other neurones and to effector organs; (iii) to process information (integration); and (iv) to determine or modulate the differentiation of sensory receptor cells and effector organs (trophic functions). Rapid processing of information by nerve cells relies on their electrical activity, coupled with neurosecretion at synapses; trophic functions may depend on electrical activity but also rely on axonal transport of substances to and from the cell body.

Neuroglia

There are three types of neuroglial or glial cells in the CNS - astrocytes, oligodendrocytes and microglia - and one type in the PNS - Schwann cells. Glial cells fill the spaces between neurones and accordingly have a role in maintaining nervous structure.

Astrocytes are star-shaped cells with processes extending into the surrounding tangled network of unmyelinated nerve fibres (neuropil). These processes contain bundles of fibrils and usually run at right-angles to the direction of the nerve fibres. Some processes expand into end-feet which are applied to the surfaces of blood vessels. They are also in contact with one another through gap junctions and have a high permeability to K^+ (and resting membrane potentials close to E_K), which help to dampen increases in extracellular K^+ concentration that might arise in local areas of the brain where there are high levels of activity.

Oligodendrocytes can be distinguished from astrocytes by having fewer and thinner processes. They do not possess gap junctions. They form myelin sheaths around axons in the CNS, as do *Schwann cells* in peripheral nerves, by enveloping them with concentric layers of plasma membrane. *Myelin* forms an insulating sheath around an axon with small areas of axonal membrane exposed between successive myelin segments known as the *nodes of Ranvier*. Schwann cells also encircle unmyelinated axons in the PNS without forming concentric layers of myelin.

Microglia are smaller glial-type cells of uncertain origin that appear to act as scavenger cells. Injury to the brain activates microglia which undergo proliferation and migrate to the point of injury where they turn into macrophages and remove the debris. Astrocytes then proliferate and wall off the damaged area forming a glial scar.

In summary, the functions of glial cells are (i) to maintain the structure of the nervous system; (ii) to form myelin sheaths around axons; (iii) to repair damage to nervous tissue; and (iv) to help maintain the surrounding ionic environment, particularly with respect to K^+ ions.

2.2 Electrical Signalling in the Nervous System

Neurones receive, conduct and transmit information and have regional specializations for these functions. For example, a sensory neurone has terminals at the periphery for processing information, an axon for conduction and terminals in the CNS for transmission of

signals to other neurones. Electrical signals are derived from changes in the resting membrane potential of the neurones and can be divided into two types - local potentials and action potentials. In general, *local potentials* occur at points of discontinuity (environment - sensory receptor, CNS synapses and nerve-muscle junctions) and they have their maximum amplitude at their point of origin; 0.2 to 2 mm away from this amplitude falls rapidly. In contrast, *action potentials* are transient changes in membrane potential that are conducted over longer distances, in some instances a metre or more, with little change in amplitude. The conducted action potentials can be likened to the transmission of Morse code along telegraph wires.

The role of these signals in the nervous system can be best illustrated by outlining a simple common reflex, the *withdrawal reflex*. This reflex results in the rapid withdrawal (latency about 30 ms) of a limb when a part of it is subjected to a noxious stimuli. For example, a hand placed on a hot object may be withdrawn initially without conscious direction. Underlying this reflex withdrawal is a specific sequence of neural events. In brief, the pathway comprises a sensory (afferent) neurone, an interneurone, a motor (efferent) neurone and skeletal muscle fibres. The noxious stimulus causes a local change of potential (receptor potential) in the sensory nerve terminal resulting in the generation of action potentials conducted along the sensory fibre to the CNS. Their arrival at the central terminal of the sensory fibre is followed by release of a neurotransmitter, which produces local potentials in the dendrites and cell body of the interneurone whereupon action potentials are again initiated. These action potentials are conducted along the axon of the interneurone to its point of synaptic contact with the motor neurone. Again, a transmitter substance is released causing a local excitatory potential followed by action potentials in the motor neurone. These action potentials are then conducted down the peripheral axon to the skeletal muscle fibres. At the nerve-muscle junctions a similar sequence of events occur, i.e., transmitter release, generation of a local excitatory potential in the muscle fibre followed by action potentials, conduction of the action potentials along the muscle fibre, and finally contraction. It should be noted that this is a very simple model and that withdrawal reflexes require the coordinated activity of many muscle groups, some of them excited, some of them inhibited.

Resting Membrane Potentials

Neurones, like other cells, are electrically polarized so that their interior is negatively charged with respect to the outside of the cell. Typical values for neurones are about - 7- mV, for glial cells - 90 mV, for skeletal muscle cells - 80 mV and for smooth muscle cells - 70 mV.

Local (Electrotonic) Potentials

A net movement of charge across the membrane changes the potential difference. A decrease in the magnitude of the potential difference with the inside becoming more positive is called a *depolarization*. An increase in magnitude is called a *hyperpolarization*. A depolarization is caused by the movement of positive charge into (or negative charge out of) the cell and a hyperpolarization is caused by movement of charge in the opposite direction. If the movement of charge (current) responsible for the change in potential is limited to a particular site on the membrane of an elongated cell, the change is greatest at that point and falls off exponentially with distance. As a result of the new potential gradients established along the cell and across the membrane, currents will flow between adjoining regions and

through the membrane. As the potential gradients inside the cell and across the membrane decline with distance, so do the currents. Such local potentials, sometimes referred to as *electrotonic potentials*, are typically restricted to within 1 or 2 mm of their point of origin. In addition to their being thus *decremental* such local potentials are *graded*, i.e., they increase in proportion to the increase in current. Because the membrane behaves as a capacitor (i.e., it stores electrical charge) the changes in potential are *distorted*, i.e., the rate of change in potential is slowed and lasts longer than the stimulus. Local potentials also *summate*.

These local changes in potential are important for the integration of electrical activity in dendrites and nerve cells and as receptor potentials in sensory receptors, and are the basis for the initiation and propagation of action potentials.

Action Potentials

Nerve and muscle plasma membranes have the capacity to generate action potentials. Though small currents produce the local potentials discussed above, large inward currents (such as the influx of Na⁺ at a sensory nerve terminal) depolarize the membrane to a point, termed '*threshold*', beyond which an all-or-nothing response, termed an action potential, ensues. During an action potential the polarity of the membrane potential temporarily reverses (i.e., the cell interior becomes positive with respect to the interstitial fluid) before returning to its original value. For example, the membrane potential of a neurone may move from a resting potential of -70 mV to a peak of +40 mV and back again in less than 2 ms. The magnitude of the action potential is not dependent on the magnitude of the signal initiating it. Moreover its duration is fixed and does not vary with the duration of the stimulus.

Immediately after each action potential there is a period called the *absolute refractory period* during which a second action potential cannot be initiated. Hence, no matter what the stimulus, summation of action potentials is impossible. The absolute refractory period is followed by the *relative refractory period* during which the cell membrane can only be brought to threshold with a larger than normal stimulus.

Ionic Basis of the Action Potential

This was first described by Hodgkin and Huxley in the early 1950s on the basis of *voltage-clamp* experiments. When an excitable cell is depolarized, Na⁺ conductance (G_{Na}) is increased, the Na equilibrium potential ($E_{Na} = +70$ mV) makes a greater contribution to the membrane potential and the cell is further depolarized. There is also a net movement of K⁺ ions out of the cell because of the shift of the membrane potential away from the K⁺ equilibrium potential ($E_K = -90$ mV). Likewise Cl⁻ ions will move into the cell. The movement of both these ions will tend to slow the change of the membrane potential. However, as the potential falls by about 15 mV towards threshold, the system becomes increasingly unstable as more and more voltage-dependent Na⁺ channels open. Once the membrane is depolarized beyond threshold there is an explosive increase in G_{Na} , the potential suddenly jumps to a potential approaching E_{Na} and an action potential ensues.

Threshold is the point at which the current generated by the movement of K⁺ and Cl⁻ ions is balanced by that generated by the influx of Na⁺ ions. Beyond threshold there is a net

influx of positive charge which continues to depolarize the membrane resulting in an all-or-nothing response.

The voltage-dependent G_{Na} mechanism can be activated only transiently. The automatic shutting off of the enhanced G_{Na} is called *sodium inactivation*. Thus an action potential is automatically terminated. Furthermore, the conductance cannot be reactivated until the membrane potential has returned to a value near the normal resting membrane potential. This results in the absolute refractory period following an action potential.

Repolarization following an action potential also depends on the outward movement of K^+ ions to bring the cell back to a potential near E_K . There is a voltage-dependent K^+ conductance (G_K) mechanism in the membrane such that the G_K is increased by depolarization but reaches a maximum some time after the change in G_{Na} . The increased G_K remains until the potential has returned to resting membrane potential. This process, called *delayed rectification*, is responsible for the rapid repolarization of the membrane during termination of the action potential and together with residual sodium inactivation is the cause of the relative refractory period.

During action potential the cell gains some Na^+ ions and loses some K^+ ions. It is calculated that only 1 in 3000 of the K^+ ions in a nerve fibre of 20 microm diameter is actually exchanged for Na^+ ions during the course of an action potential and this is insignificant. However, after several hundred impulses have passed, the total amount of K^+ ions lost becomes significant (particularly in small diameter fibres) and the ionic gradients must be recharged by the Na-K pump using metabolic energy.

The existence of specific membrane channels is supported by the action of specific pharmacological blocking agents. For example, *tetrodotoxin* blocks voltage-dependent Na^+ channels and hence the increase in G_{Na} . *Scorpion venom* prevents Na^+ inactivation and so after initiation of an action potential, the cell remains in a depolarized condition. *Tetraethylammonium* blocks K^+ channels and leads to a delayed recovery of the action potential. *Local anaesthetics* prevent the generation of the action potential by inhibiting the voltage-dependent opening of the Na^+ channels.

Propagation of the Action Potential

A propagated action potential can be considered as a wave of excitation passing down an axon. At any one time only a portion of the axon is depolarized. This local depolarization gives rise to a passive spread of current up and down the axon and this current in its turn depolarizes adjacent regions ahead of the action potential, bringing them to threshold and so generating an action potential in a new region. As regions which have just been excited are refractory, propagation of the action potential is *unidirectional*.

Because the action potential mechanism is relatively slow, the further local currents can spread the faster the action potential can travel. The *conduction velocity* can, in principle, be enhanced by increasing the *membrane space constant*, because current spreads further along the fibre, and by minimizing the *membrane time constant*, because the time taken to reach threshold decreases. The intracellular resistance is dependent on fibre diameter because

in larger fibres more ions are available to carry the current, whereas the extracellular resistance is not normally a limiting factor because of the large volume of the extracellular fluid. In considering biological adaptation that can increase conduction velocity, one solution is to increase fibre diameter (thus decreasing intracellular resistance and increasing the space constant), as for example in squid whose giant nerve fibres have diameters of up to 1 mm. The conduction velocity of such a squid axon is about 25 m/sec compared with about 1 m/sec for small (about 1 microm) unmyelinated fibres. An alternative development has been to increase the membrane resistance and to decrease the membrane capacitance by enveloping axons with myelin sheaths. Since resistances in series sum and capacitances in series sum inversely, by surrounding an axon with a spiral layer of myelin, the total membrane resistance is increased and the total membrane capacitance decreased. This greatly increases the space constant without increasing the time constant and the conduction velocity of small nerve fibres is enhanced. The conduction velocity of a myelinated nerve fibre of 20 microm diameter is about 100 m/sec at 37 °C. Most mammalian fibres greater than 1 microm diameter are myelinated.

In addition to the effects on membrane resistance and capacitance myelination restricts membrane current and the generation of action potentials to the nodes of Ranvier. The membrane in between is not excitable. The passing of an action potential from node to node is called *saltatory conduction*. However it should be realized that at any instant an action potential travelling along a myelinated axon will occupy a large number of nodes and not simply a single node. For example an action potential lasting 0.5 msec travelling at 100 m/sec will occupy a 50 mm length of the axon. In summary the benefits of myelination are (i) higher conduction velocities for rapid signalling, (ii) smaller diameter for conserving space and (iii) higher metabolic efficiency because of the reduced flux of ions and hence the reduced expenditure of energy required to restore ionic composition.

Extracellular Recording of Nervous Activity

Peripheral nerves contain both sensory and motor axons ranging from large-diameter, myelinated fibres with high conduction velocities to small, unmyelinated, slowly conducting fibres. During the conduction of an action potential along these axons, current is caused to flow in the tissue or medium surrounding the nerve. As the extracellular resistance is low (compared with the axonal membrane resistance and the intracellular longitudinal resistance), the field potentials generated in the surrounding medium will be small. However, if the whole nerve or an isolated axon is immersed in mineral oil, the increase in extracellular resistance by minimizing current dissipation results in the recording of greater potentials when two electrodes are placed on the surface of the nerve or axon.

Action potentials recorded by *extracellular electrodes* and a differential voltage amplifier are seen to be waves of *negativity* moving along the axon. Before an action potential reaches the recording electrodes there is no potential difference between them. When an action potential reaches the first electrode, the underlying membrane reverses its polarity and becomes negative with respect to the membrane under the second electrode. By convention this is shown graphically as an upward deflection. When the action potential is affecting the membrane under both electrodes simultaneously, the potential difference falls to zero between the electrodes. Later as the electrical activity moves away from the first electrode to affect

predominantly the area of the membrane under the second electrode, the potential difference is reversed. As this recording shows both negative and positive components it is referred to as a *di-* or *biphasic* action potential. This recording can be simplified if the action potential fails to reach the second electrode. This can be accomplished if a small segment of the axon between the electrodes is crushed or a local anaesthetic applied. Then the action potential is recorded as a single negative deflection - a *monophasic* action potential.

These mono- or biphasic action potentials, when recorded from a single axon, show the all-or-none and refractory characteristics of the intracellularly recorded action potential. In contrast, when the activity in a whole nerve is recorded, the record is now the summed activity of action potentials being conducted along all the fibres. This is called the *compound action potential*. It is not all-or-none because of the different thresholds of various fibres. It increases in amplitude as the stimulus is increased until all fibres are excited. Its absolute refractory period is the absolute refractory period of the largest axons.

The compound action potential may also display a number of peaks. The separation of the peaks will depend on the conduction velocities of the fibres in the nerve and on the distance of the recording electrodes from the point of stimulation. The greater this distance the greater the separation of the peaks. In addition, the amplitude of these peaks and their relationship to each other depend on the fibre composition of the nerve. As the contribution of each fibre to the compound action potential is proportional to its diameter, the large fast-conducting fibres, which also have the lowest threshold, dominate the record even though the small fibres are more numerous.

For convenience, Gasser and his colleagues in the 1930s divided the axons in peripheral nerves into three broad groups (A, B and C) according to their conduction velocities. The A fibres included all the peripheral myelinated fibres from 1 to 20 microm in diameter. This group was further subdivided into groups of decreasing size and conduction velocity, the *alpha*, *beta*, *gamma* and *delta fibres*. The B group comprised the small myelinated fibres in visceral nerves (preganglionic autonomic) and the C group all small unmyelinated, afferent and efferent fibres. A different grouping (I, II, III & IV) is often used for sensory fibres. As mentioned earlier, the conduction velocity of a myelinated fibre is proportional to its fibre diameter. However, since the conduction velocity is influenced by the cross-sectional area, the relationship is not linear. In the case of large fibres the conduction velocity (in m/sec) is approximately the diameter (in microm) multiplied by 6, for small myelinated fibres the factor is 4.5 and for unmyelinated fibres it is 1.7.

Conduction along fibres may be blocked by cold, anoxia, compression and drugs. The conduction velocity decreases by approximately 3% of the maximal velocity for each 1 °C of fall. This is important in limbs or superficial tissues where the temperature may be well below the core temperature. Conduction block occurs in the large myelinated fibres at about 7 °C and in the unmyelinated fibres at about 3 °C. Compression and anoxia preferentially block large myelinated fibres which have a higher metabolic rate. Thus painful stimuli, which are transmitted in small unmyelinated fibres, can still be felt when other modes of sensation are lost. In contrast, local anaesthetics tend preferentially to block small fibres, the surface area to volume ratio of which is high.

As changes in the conduction velocity of peripheral nerves can also occur as a result of accident or disease, studies of the compound action potential can be useful clinically. Thus neural degeneration and subsequent regrowth lead to the formation of thinner fibres with lower conduction velocities. Similarly, demyelinating disease, i.e. *multiple sclerosis* and *diphtheria*, may result in a reduction in thickness or a localized loss of myelin. Upon remyelination there is also a reduction in the distance between the nodes of Ranvier (internodal length). Under these conditions there is a decrease in the length constant and an increase in the membrane capacitance, both of which diminish the longitudinal spread of current and hence the conduction velocity. With localized loss of myelin there may also be a conduction block.

Spontaneous Action Potentials

Some neurones spontaneously undergo fluctuations in their resting membrane potentials. These may generate action potentials even in the absence of external excitation. Such fluctuations in membrane potential have recently been shown to be generated by specific changes in membrane conductances which may involve Na^+ , Ca^{++} , and Ca^{++} -dependent K^+ conductance pathways. Cells with these properties are important in the generation of rhythmic activity reflected in respiration and the electroencephalogram (EEG). They have also been shown to underlie the expression of some behavioural patterns in invertebrates.

Synaptic Transmission

Neurones communicate with one another at synapses. Transmission across the cleft separating the pre- and postsynaptic faces of opposing neurones is accomplished by *chemical means*. Rarely in the mammalian CNS but more commonly in invertebrates there are *electrical synapses* where the adjacent cells are joined by gap junctions allowing current to pass directly from one neurone to another.

A typical neurone in the CNS may receive inputs from many other neurones (*convergence*) and make synaptic contact with many other neurones (*divergence*). For example, a motor neurone in the ventral horn of the spinal cord may receive some 20000 to 50000 synaptic contacts. The so-called *synaptic boutons* are located mainly on the dendrites (axo-dendritic synapses) and cell body (axo-somatic synapses) of the neurone but in some cases they are found on the axon (axo-axonal synapses). Synaptic boutons release the contents of their synaptic vesicles into the intercellular space (*neurosecretion*). They contain mitochondria and the enzymes necessary to synthesize the neurotransmitter.

When an action potential invades a nerve terminal it causes an influx of Ca^{++} ions into the terminal which triggers the release by exocytosis of neurotransmitter. This neurotransmitter diffuses across the cleft and binds to receptor molecules linked to ion-selective channels in the postsynaptic membrane (*receptor-ionophore complex*). This usually produces a permeability change the nature of which is characteristic of the receptor-ionophore complex, not of the transmitter. In some cases, i.e., beta-adrenergic receptors, binding of the agonist to the receptor activates an enzyme instead of directly initiating a permeability change. The *synaptic delay*, which is the time between excitation of the nerve terminal and the permeability change in the postsynaptic membrane, is less than 0.5 ms. Diffusion across the

narrow cleft separating the pre- and postsynaptic membranes is very rapid and contributes only a small fraction to this delay. Most of the delay can be attributed to the time for presynaptic release of the transmitter. The action of the transmitter is terminated by diffusion away from the cleft, by enzymatic breakdown or by re-uptake into the terminal or surrounding cells.

The change in permeability may produce a small local change in membrane potential (*synaptic potential*) which is either a depolarization (*excitatory postsynaptic potential, EPSP*) or a hyperpolarization (*inhibitory postsynaptic potential, IPSP*). These small synaptic potentials last longer than an action potential and when they succeed each other by a sufficiently short interval they summate (*temporal summation*). Synaptic potentials elicited by impulses travelling along different pathways simultaneously can also summate (*spatial summation*). If the net result is a depolarization of sufficient magnitude an action potential is triggered. Inhibitory synaptic potentials tend to prevent the cell from reaching threshold.

One particular part of the neurone, the so-called *initial segment*, which is situated close to the axon hillock, has a lower threshold than that of the dendrites and cell body, and so the action potential is usually initiated in this region. Action potentials are not normally fired in the dendrites or the cell body. While synapses located close to the initial segment may be more effective than those synapses located further away, there is evidence to suggest that some of the more distal synapses of motor neurones may compensate by generating larger synaptic potentials. The coexistence of excitatory and inhibitory synaptic inputs and their capacity for temporal and spatial summation allows the neurone to *integrate* signals from a variety of other neurones.

Ionic Basis of Synaptic Potentials

By altering the relative conductances to Na^+ and K^+ ions, the membrane potential can be set to any value between E_{K} and E_{Na} . Synaptic transmission is mainly effected by changing one or both of these conductances, the relative contributions of which can be estimated from the *reversal potential*. An EPSP is a depolarization of a few mV resulting from an increased conductance to both Na^+ and K^+ ions. Na^+ ions move into the cell and K^+ ions move out but, as the movement of Na^+ ions predominates, the net effect is slight depolarization of the neurone. This brings the membrane closer to threshold and makes it more likely that an action potential will be triggered. Some inhibitory transmitters activate only G_{K} , so that a slight hyperpolarization, i.e. an IPSP, occurs moving the membrane further away from threshold. More commonly there is a simultaneous increase in both G_{K} and G_{Cl} . As E_{Cl} is close to the membrane potential, an increase in G_{Cl} alone would not change the membrane potential, but it means that more excitatory current is required to depolarize the cell as the membrane resistance is lowered and in addition any depolarization is opposed by Cl^- moving into the cell. The combined effect of increasing G_{K} and G_{Cl} is to move the membrane potential to a value between E_{K} and E_{Cl} . Cells already depolarized by excitatory activity will produce larger IPSPs but cells in a hyperpolarized state will generate smaller or no IPSPs while still being effectively inhibited.

Synaptic potentials are local potentials that spread passively along the membrane. Both EPSPs and IPSPs can arise in the same neurone because of the coexistence at separate sites

of different permeability mechanisms in the same cell. These permeability mechanisms in the membrane are ion-selective channels coupled to receptors which are activated by specific transmitters. The activation of these channels can be studied by the *patch-clamp* technique. Many possible transmitter substances have been discovered in brain and peripheral tissues, including acetylcholine, noradrenaline, dopamine, serotonin, glutamic acid, aspartic acid, gamma-aminobutyric acid, glycine and also many peptides, such as substance P and opioid peptides. Some are excitatory, some are inhibitory, and some have both actions, their effects being determined not by their receptors but rather by the particular ion-selective channels to which their receptors are linked. Receptors may be clustered in the postsynaptic membrane or in some cases scattered over the surface of the cell. Indeed many substances appear to exert slow modulatory influences on the excitability of groups of nerve cells. Such an action is more aptly called *neuromodulation*.

Presynaptic Inhibition

This is a different type of inhibition in which transmitter release is suppressed. It occurs where the terminal of one neurone makes synaptic contact with the synaptic ending of a second neurone which in turn excites third neurone. The first neurone controls the amount of transmitter released by the second neurone by depolarizing its terminal and causing partial inactivation of the voltage-dependent Na⁺ mechanism. This in turn reduces the amplitude of any action potential that arrives at the axon terminal and presumably the influx of Ca⁺⁺ ions which determine the amount of transmitter released.

2.3 Growth, Development and Regeneration of the Nervous System

Axonal Transport

The transport of substances along the axon from the cell body of the neurone to its terminals (i.e., anterograde or centrifugal transport) or in the opposite direction (i.e., retrograde or centripetal transport) is called axonal or axoplasmic transport. Since there are no ribosomes in axons and nerve terminals, proteins required for the functions of nerve cell processes must be synthesized in the cell body and transported to the cell periphery. Rates of axonal transport have been determined by measuring the rate of accumulation of enzymes and transmitters in occluded nerves or, more precisely, by tracing the movement of radioactively-labelled proteins after injection of labelled amino acids into regions containing cell bodies. This technique has revealed that there are several distinct rates of transport in axons - a fast rate of up to 400 mm per day, and two slower rates of 5 to 10 mm per day and 1 to 2 mm per day.

The slowest rate of transport appears to be that of the microtubules and neurofilaments. Other structural elements, notably actin, move somewhat faster. Fast axonal transport involves the movement of organelles such as vesicles along the axon. It is a local energy-dependent mechanism and is independent of the cell body and electrical impulses. Recent studies of the movement of vesicles in extruded axoplasm show that they travel along the microtubules.

A most elegant demonstration of retrograde transport comes from studies in which horseradish peroxidase is injected into the terminal regions. The intracellular localization of

this enzyme can be demonstrated by histochemical techniques. It is taken up into the nerve terminal by endocytosis and subsequently appears in the cell body. This technique has proved to be of considerable value for tracing neural pathways in the brain. Rates of such retrograde transport approach that of fast anterograde transport. Substances of physiological and pathological importance that are taken up at the nerve terminal and transported retrogradely include nerve growth factor, tetanus toxin and poliomyelitis virus.

Axonal Growth

The rate of growth of regenerating axons corresponds to the rate of slow axonal transport, i.e., about 1 to 2 mm per day. Structural components synthesized in the cell body and carried by axonal transport can be incorporated at the growing tip of the axon called the *growth cone*. The growth cone is motile and makes and breaks contact with its environment, apparently 'tasting' its surroundings to make sure its direction of growth is appropriate. For example, sympathetic and sensory fibres grow towards a source of nerve growth factor.

Aspects of Neural Development

Nerve Growth Factors

Substances derived from glial cells and target tissues can promote the development and survival of neurones. The best characterized of such substances is *nerve growth factor (NGF)*, a protein of MW 135000. Sympathetic and sensory neurones have an absolute requirement for NGF during the early stages of their development and disappear following the administration of antibodies to NGF at this time. Sympathetic neurones also require NGF for optimal maintenance at later stages. NGF is produced by target tissues and is specifically taken up into the nerve terminals of sympathetic and sensory neurones by adsorptive endocytosis and then transported to their cell bodies.

Cell Death

Many more nerve cells differentiate than actually survive into adult life. The amount of cell death is greatly increased if the targets for that particular group of cells are removed. Cells may also die if deprived of their afferent input. Once the initial development of the nervous system is complete, mammalian neurones do not divide.

Role of Activity

There are certain critical periods in the development of young animals during which particular groups of neurones must be used if their synaptic connections are to be functionally maintained. For example, if one eye of a young kitten is covered during the second to the fourth month of age the animal remains functionally blind in that eye for the rest of its life and there is a permanent deficit in the number of cortical synaptic connections relaying information from the deprived eye to the visual cortex. Strabismus (squint or 'lazy eye') in children has a similar result; the critical period here is from 2 to 5 years of age.

Neural Regeneration

Regeneration in the Peripheral Nervous System

If a peripheral nerve fibre is severed, the distal portion of the axon dies. Thereupon, Schwann cells surrounding the axon dedifferentiate and undergo mitosis, filling the nerve sheath distal to the cut. Cell bodies of cut neurones undergo chromatolysis, a process involving dispersion of Nissl substance and synthesis of new RNA. Thus RNA coding for proteins involved in synaptic functions is lost and replaced by newly synthesized RNA, which codes for proteins necessary for regrowth of the axon. The proximal ends of cut axons sprout and, provided that they can find the way to the distal portion of the nerve guided by Schwann cells, they grow within the old nerve sheath. This applies to both motor and sensory neurones. If, however, the more central portion (dorsal root) of a sensory nerve is damaged, regeneration into the spinal cord usually does not occur. There is little specificity in the regrowth of the peripheral axons, so that normal relations between particular motor neurones and muscles, and particular sensory neurones and their peripheral fields of innervation, are not accurately restored. The success of reinnervation of the original target area depends on the re-establishment of the original pathway. Hence in attempting to restore function, it is helpful to suture the cut ends of the corresponding bundles (fascicles) together again.

Regeneration in the Central Nervous System

Damaged neurones in the CNS of higher vertebrates have a very limited capacity for regeneration. Axotomy often results in the death of the injured neurone, possibly because cells must be nourished by their connections in order to be maintained. Cells surviving axotomy do produce axonal sprouts, but these do not grow into their proper pathway and form a tangled mass near the point of section. If cells in the CNS are denervated, intact synapses in their vicinity may sprout collaterals which form inappropriate synapses on the denervated cells.

2.4 Divisions of the Peripheral Nervous System

The peripheral nervous system (PNS) is that portion of the nervous system that lies outside the spinal cord and brain. It comprises both the somatic and the autonomic division.

Somatic Nervous System

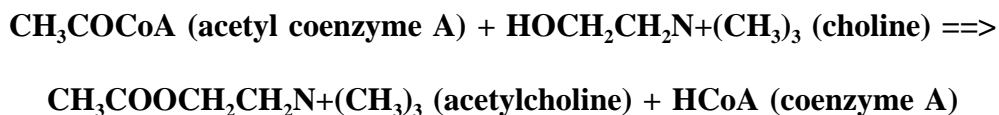
This division contains all the peripheral pathways responsible for direct communication with the environment. It includes all sensory (*primary afferent*) fibres from tissues such as eyes, ears, skin, joints and skeletal muscles and it includes the motor (*efferent*) fibres to skeletal muscles. The cell bodies of the sensory fibres are in the dorsal root ganglia or brain. The cell bodies of the motor nerves are in the spinal cord or brain. The sensory fibres and motor fibres are collected together to form *compound nerves* which are covered by a connective tissue sheath (*epineurium*). Within the nerve the fibres are arranged into bundles (*fascicles*). Each fascicle is surrounded by a *perineurium* and each fibre by an *endoneurium*. The somatic sensory fibres range in size from small unmyelinated to large myelinated axons. The motor nerves are all myelinated. The most distal portions of the somatic nerves are usually branched. The terminal regions of any one sensory fibre go to adjacent receptors of

the same type and the intramuscular portions of motor nerves branch to innervate a number of identical muscle fibres. Somatic nerves have only an excitatory effect on skeletal muscle. There are no peripheral inhibitory actions exerted on skeletal muscle.

Neuromuscular Transmission

Motor fibres give off unmyelinated terminal branches which form synapses (*neuromuscular junctions*) with skeletal muscle fibres. In adult mammalian skeletal muscles there is only one neuromuscular junction in the middle of each skeletal muscle fibre. At the neuromuscular junction the terminal branch of the axon lies in a shallow depression of the muscle fibre surface. The pre- and postjunctional membranes are separated by the junctional cleft which is about 100 nanom wide and contains a basement membrane (*basal lamina*). The postjunctional membrane is thrown into a series of folds, which together with the prejunctional membrane and junctional cleft, is called the *endplate*.

The neurotransmitter at the neuromuscular junction is *acetylcholine (ACh)*. It is synthesized in the cytoplasm in the reaction:



which is catalysed by the enzyme *choline acetyltransferase*. ACh is then packaged, together with ATP, into vesicles of about 50 nm diameter.

The mechanism of release of ACh is similar to that of other transmitters. Briefly, when a nerve fibre is stimulated an action potential invades the nerve terminal causing the influx of Ca^{++} . As a consequence ACh is released into the junctional cleft by exocytosis, together with ATP and, probably, soluble proteins contained within the vesicles. This step in the release of ACh is blocked by *botulinus toxin* produced by the bacterium *Clostridium botulinum* and by high Mg^{2+} (*in vitro*). The vesicular membrane which fused with the prejunctional membrane during exocytosis is subsequently removed by endocytosis and re-utilized. During transmission there is a synaptic delay of 0.2 to 0.3 msec. Much of this delay is attributed to the release of ACh, rather than to its subsequent diffusion across the cleft and the conductance changes that it provokes. Several hundred quanta of ACh, each quantum containing approximately 10000 molecules, are released by each nerve impulse. A quantum represents the number of ACh molecules contained within each vesicle. More than sufficient numbers of ACh molecules are released from the nerve terminals during each nerve impulse to ensure the generation of an action potential in the skeletal muscle fibre that it innervates. For this reason neuromuscular transmission is said to have a high safety margin.

The ACh diffuses across the cleft and binds to *nicotinic receptors*, so called because nicotine mimics the effects of ACh. These receptors are localized on the crests of the postjunctional membrane folds. Interaction of ACh with its receptors is blocked by *curare* and *alpha-neurotoxins*. These neurotoxins, i.e. alpha-bungarotoxin, alpha-cobrotoxin, are small proteins (M 8000) obtained from snake venoms. They bind irreversibly to ACh receptors and,

when labelled with radioactive iodine, have been used to estimate the number of ACh receptors (10^7) at the neuromuscular junction.

The binding of ACh to its receptor activates an ion-selective channel in the postjunctional membrane increasing Na^+ and K^+ conductance. It has been calculated from the reversal potential that the activation of ACh receptors increases Na^+ and K^+ conductances in the ratio 1.3:1. Recently it has been possible to estimate the opening time (about 1 msec) of individual channels by patch-clamp analysis. These conductance changes result in a localized depolarization at the endplate called the *endplate potential (EPP)*. Under normal circumstances the EPP exceeds threshold and an action potential is generated but in the presence of curare sub-threshold EPPs can be observed. This is either *non-quantal*, presumably the result of continuous leakage from the nerve terminal, or *quantal*, giving rise to *miniature endplate potentials (MEPPs)*.

The ACh released by each impulse is rapidly hydrolysed (within a few msec) to choline and acetate by the enzyme *acetylcholinesterase (AChE)* which is located in the basal lamina of the postjunctional membrane. This step is blocked by organophosphates, such as diisofluorophosphate, which bind to the active site of the AChE. Any that diffuses away from the endplate into the bloodstream is destroyed by pseudocholinesterase. The choline is taken up by a specific transport mechanism in the nerve terminal and re-utilized in the synthesis of ACh.

Myasthenia gravis. This disease affects about 0.01% of the population and is characterized by a failure in neuromuscular transmission with repetitive nerve stimulation. The symptoms are weakness and fatigue, particularly of extraocular muscles, difficulty in swallowing and speech, and, in advanced stages, respiratory failure. Hyperplasia of the thymus gland is frequently associated with this disease.

Biopsy samples of intercostal muscles of myasthenic patients show a reduction in the amplitude of the EPPs and MEPPs. Binding studies with iodine isotope-labelled alpha-neurotoxin have shown that there is a 90% decrease in endplate ACh receptors - a finding consistent with the neurophysiological studies. The symptoms of this disease therefore appear to reflect a deficiency in the number of ACh receptors at the neuromuscular junction.

Removal of the thymus gland often has a beneficial effect and it has long been suspected that myasthenia gravis is an autoimmune disease. This has recently been confirmed by the demonstration of antibodies to ACh receptors in these patients.

Autonomic Nervous System

The actions of the peripheral autonomic nervous system are normally involuntary and are directed to the control of individual organ function and to homeostasis. Classically the autonomic nervous system is regarded as being *solely motor* in function, its fibres going to cardiac muscle, to smooth muscle and to glands. However the terms 'autonomic afferents' and 'central autonomic processes' are also used. The peripheral autonomic nervous system is usually divided anatomically into the *sympathetic* and *parasympathetic* systems, and many tissues are innervated by both systems. When this occurs the two systems usually have

opposing effects. In addition to these nerves there is a network of nerves that can act independently of the CNS. This network is often considered as another division of the autonomic nervous system and is referred to as the *enteric system*. Both the sympathetic and parasympathetic systems modulate the activity of this system.

The organization of the autonomic nervous system differs from the somatic division as all final motor neurones lie completely outside the CNS. The cell bodies of the peripheral neurones are grouped together to form *ganglia*. The efferent fibres passing from the CNS to the ganglia, the *preganglionic fibres*, are slow conducting (class B and C) fibres which release the neurotransmitter ACh. The final motor neurones from the ganglia to the tissues, the *postganglionic fibres*, are largely slow-conducting, unmyelinated class C fibres. These fibres and fibres from visceral sensory receptors run together as *visceral nerves*.

Sympathetic System

All preganglionic sympathetic nerves have their cell bodies in the thoracic and upper lumbar segments ($T_1 - L_3$). The axons, along with somatic motor fibres, pass out of the spinal cord in the ventral roots. On leaving the spinal column, the preganglionic fibres separate from the somatic nerves to form the *white rami communicantes* and join a distinct group of sympathetic ganglia, the *vertebral (paravertebral or chain) ganglia*. These ganglia are segmentally arranged and lie along each side of the spinal column. The preganglionic fibres either:

- (a) synapse with postganglionic neurones in one or more of the vertebral ganglia, or
- (b) leave the vertebral ganglia in visceral nerves and pass to *prevertebral* ganglia in the abdomen or the *adrenal medullae*.

Many of the postganglionic fibres from vertebral ganglia leave the ganglia as the *grey rami communicantes* and join the spinal nerves that pass to peripheral tissue. Others, and also postganglionic fibres from prevertebral ganglia, join visceral nerves to particular organs. Most of these postganglionic fibres release the neurotransmitter *noradrenaline (norepinephrine)* and are referred to as adrenergic fibres. The adrenal medullae can be thought of as modified ganglia that release the hormone *adrenaline (epinephrine)*, and also some noradrenaline, into the blood stream rather than directly onto effector cells. Small groups of sympathetic nerves that do not release noradrenaline innervate the blood vessels of muscles, sweat glands and hair follicles in the skin, and release acetylcholine. The distribution of the postganglionic sympathetic nerves is not necessarily the same as the somatic motor nerves from the same segment and there is a lot of overlap between adjacent outflows. In brief, and very approximately, the T_1 outflow passes to the head, T_2 to the neck, T_3-T_6 to the thorax, T_7-T_{11} to the abdomen and $T_{12}-L_3$ to the legs.

Parasympathetic System

The preganglionic nerves of the parasympathetic system come from both the brain-stem and sacral spinal cord (S_2-S_4). The axons from the brain-stem leave in cranial nerves III, VII, IX and X and the sacral axons leave in the ventral roots. There are no vertebral or

paravertebral ganglia in this system. Instead all ganglia are found adjacent to or within the effector organ. The postganglionic fibres from parasympathetic ganglia are relatively short and nearly all release *acetylcholine* as a neurotransmitter. In most cases the distribution of the parasympathetic outflow is more restricted than that of the sympathetic system. Thus cranial nerve III supplies the smooth muscle of the eye (ciliary muscle and pupillary constrictor muscle), VII the lacrimal and submaxillary glands, and IX the parotid gland. The sacral parasympathetic outflow supplies the lower colon, rectum, bladder, the lower part of the ureters and the external genitalia. The major and most widely distributed parasympathetic outflow travels in cranial nerve X, the vagus. Approximately 70% of all parasympathetic preganglionic fibres leave the CNS in this nerve and supply all of the viscera in the thorax and most of the viscera in the abdomen. However, note that the vagus also carries many sensory fibres and a number of non-parasympathetic motor nerves, i.e., to laryngeal and pharyngeal muscle.

Enteric System

The autonomic nerves in the GIT differ from those in the other division as they form an extensive network in which many cells are not influenced by the CNS. The network is composed of ganglia and interconnecting bundles that lie in the wall of the intestinal tract (from the oesophagus to the rectum) and form the myenteric and submucosal plexuses. A number of different neuronal types have been identified in the plexuses but a detailed knowledge of the relationships between ganglia and between cells is lacking. However it is recognized that this system contains sensory neurones, interneurones (integrative) and excitatory and inhibitory motor neurones. Many of the excitatory neurones (interneurones and motor neurones) release acetylcholine as a neurotransmitter, but others, particularly interneurones, may release 5-hydroxytryptamine (5HT, serotonin) or other compounds. The inhibitory neurones do not release acetylcholine or noradrenaline as a transmitter and it has been suggested that they may be purinergic and release ATP, but more recent evidence suggests that a peptide (possibly vasoactive intestinal peptide, VIP) may be involved.

It should be noted that there is good evidence indicating that non-adrenergic, non-cholinergic nerves are also active in the regulation of other tissues, such as the smooth muscle of the respiratory tract and urinary system. The identity of the transmitter released by these fibres is not proven but it may be VIP.

Activity in Autonomic Neurones

The pattern of activity in the preganglionic autonomic nerves is controlled by the *hypothalamus*, and by centres in the *brain-stem* and *spinal cord*. The output to some tissues is phasic. To others it is continuous (tonic) and of low frequency (i.e., 1-2 Hz to blood vessels). The activity in all these pathways is either initiated or modified by sensory input from visceral or somatic receptors or by changes in emotional state. When the change in activity occurs as a result of visceral input it is often referred to as an *autonomic reflex*.

The activity in many postganglionic fibres is wholly dependent on preganglionic activity, and the ganglia act mainly as distribution centres for this activity. The extent of this distribution can be estimated from the ratio of preganglionic to postganglionic axons. It varies enormously but a ratio of 1:190 has been found in the human superior cervical ganglion and

1:30 in sympathetic-chain ganglia. However distribution is not the only function of autonomic ganglia. They can also show integrative activity, namely (i) postganglionic summation of preganglionic activity, (ii) complex, excitatory-inhibitory synaptic potentials, and (iii) inhibitory input from visceral receptors. The integrative capacity of ganglia appears to be most highly developed even when all connections to the CNS are severed. As local anaesthetics block this activity it is thought to be due entirely to peripheral reflex activity.

Synaptic Transmission in Autonomic Ganglia

Within autonomic ganglia there are both divergence of the presynaptic fibres and convergence on to postsynaptic neurones. In some cases synaptic transmission from individual fibres is always successful and the ganglion cells act as relays. In others the summed activity of converging neurones is required for successful transmission.

In each case the pre- and postganglionic transmission is *cholinergic*, acetylcholine being released from the preganglionic terminal to act on postganglionic *nicotinic* receptors. The resultant changes in permeability lead to a fast excitatory postsynaptic potential (EPSP). If it is large enough or if several smaller EPSPs sum to reach threshold, an action potential will be initiated and propagated to the effector tissue. Although this aspect of transmission is similar to that in skeletal muscle, ganglionic transmission does differ in that, first, the pharmacology of the nicotinic receptors is somewhat different and, secondly, the acetylcholinesterase in the synapses does not appear to be important in the termination of transmitter action. Rather the transmitter diffuses away.

In addition to the fast excitation, preganglionic activity can lead to slow inhibitory postsynaptic potentials (IPSPs) due to small catecholamine- or peptide-containing 'interneurones' in the ganglia, and to slow EPSPs due to a late cholinergic effect on *muscarinic* cholinergic receptors.

Neuroeffector Innervation

The axons of these postganglionic adrenergic neurones are long and unmyelinated, but the terminal regions branch extensively. Within effector tissues the extent and arrangement of the terminal branches vary. In some tissues they remain in bundles with only the occasional single terminal branch evident, whilst in others single terminals predominate. The neuroeffector junctions formed by the terminals also vary from narrow (25 nanom) to wide (100 nanom) junctional clefts. Irrespective of their structure the transmitting regions in these terminals are small swellings (approximately 1 microm in cross-section) called *varicosities*. Each neurone may have thousands of varicosities and each is responsible for the synthesis, storage and release of transmitter.

Neuroeffector Transmission

In parasympathetic nerves acetylcholine is synthesized as it is in somatic motor neurones. In adrenergic neurones noradrenaline is synthesized from tyrosine in reactions catalysed by the enzymes outlined. (Tyrosine $\xrightarrow{\text{tyrosine hydroxylase}}$ DOPA $\xrightarrow{\text{Dopa decarboxylase}}$ Dopamine $\xrightarrow{\text{Dopamine beta-oxidase}}$ Noradrenaline $\xrightarrow{\text{phenylethanolamine N-methyl transferase}}$ Adrenaline). All the reactions but the last take place in the cytoplasm.

The final step, from dopamine to noradrenaline, occurs in storage vesicles. In addition to containing one or other of the catecholamines and the enzyme dopamine beta-oxidase, vesicles also contain chromogranins (proteins) and ATP, the functions of which are unknown. Some of the transmitter leaks from the vesicles into the cytoplasm and regulates the activity of tyrosine hydroxylase through end-product inhibition. In addition to the transmitter noradrenaline, a number of peptides, i.e., somatostatin, vasoactive intestinal peptide and enkephalin, have been found in adrenergic neurones - the physiologic significance of these is not yet understood.

Like chemical transmission at other sites the release of transmitter from the *prejunctional* terminal follows invasion of the terminal by an action potential and an influx of Ca^{++} . The contents of the granules are then released by exocytosis from the varicosities into the junctional regions where they are free to interact with receptors. Unlike skeletal muscle where receptors are limited to the neuromuscular junctions, the postjunctional receptors are not all localized beneath the nerve and many are spread over the surface of the effector cell. In addition to these receptors on the effector cell there are receptors on the prejunctional membrane which are thought to control the release of transmitter.

The noradrenaline released from sympathetic nerves (and adrenaline from the adrenal medulla) produce their effects by interacting with alpha or beta adrenoreceptors as described by Ahlquist in 1948. These receptors are classified according to the effect of natural and synthetic catecholamines and pharmacological blocking compounds. The catecholamines have the following orders of efficacy on adrenoreceptors:

1. alpha 1 receptors, adrenaline > noradrenaline > isoprenaline
2. alpha 2 receptors, adrenaline > noradrenaline (isoprenaline is ineffective)
3. beta 1 receptors, isoprenaline > adrenaline = noradrenaline
4. beta 2 receptors, isoprenaline > adrenaline > noradrenaline.

In general, interaction of catecholamines with alpha 1 and beta 1 receptors leads to excitation and interaction with beta 2 receptors to inhibition. Thus stimulation of arteriolar smooth muscle occurs through alpha 2 receptors, stimulation of the heart through beta 1 receptors, and inhibition of bronchial smooth muscle through beta 2 receptors. However it must be remembered that some tissues have mixed populations of receptors and the response depends on both the agent, the receptors and their location. The alpha 2 receptors are thought to be mainly on the presynaptic membrane and their activation appears to inhibit transmitter release.

The acetylcholine released from the postganglionic parasympathetic fibres interacts with *muscarinic* cholinoreceptors on the postjunctional membrane. These receptors differ from the nicotine receptors, in that they are stimulated by muscarine, not nicotine, and are blocked by atropine and not tubocurarine.

In most tissues the excitatory effects of transmitters appear to arise through an increase in membrane conductance, mainly to Na^+ but in some also to Ca^{++} and K^+ . The subsequent

depolarization of the effector cell usually accompanied by action potentials initiates the response of the effector cell by causing an influx of Ca^{++} . Inhibitory actions of the transmitters can be accounted for in many tissues by an increase in conductance, mainly to K^+ , and subsequent hyperpolarization of the membrane. However, note that the catecholamine-induced increase in heart rate is a result of a decrease in K^+ conductance and that in some cases inhibition by catecholamines follows stimulation of adenylate cyclase and the production of adenosine 3',5'-monophosphate (cyclic AMP).

The degradation of acetylcholine appears to be essentially the same as in skeletal muscle. In contrast, once released from the neurone the action of noradrenaline is *not* terminated by degradation but is *inactivated* either by *uptake* or by *diffusion* away from the junctional region. The uptake processes transport the intact amine across plasma membranes either into the neurone (in which case it is called re-uptake, or *neuronal uptake*) or into the effector cell (*extraneuronal uptake*). Estimates of the relative activities of these two processes vary greatly but it would appear that in densely innervated tissues some 70% of the amine released is removed by neuronal uptake and 25% by extraneuronal uptake. Extraneuronal uptake and diffusion may be more important in diffusely innervated tissues. The major portion of the amine accumulated by the neurone is transported into vesicles for re-use as a transmitter, with obvious benefit to the neurone.

That portion of the transmitter taken up by the nerve and not recycled is degraded by *monoamine oxidase (MAO)* whilst the amine taken up extraneuronally is degraded largely by *catechol-O-methyltransferase (COMT)*. As the effects of catecholamines are not potentiated by inhibition of either or both of these enzymes it is believed that they are not involved in the termination of transmitter action. Rather, enhanced responses may be seen in the presence of inhibitors of neuronal uptake (i.e., cocaine and desipramine) or of inhibitors of extraneuronal uptake (i.e., corticosteroids).

Opposing Actions of the Parasympathetic and Sympathetic Systems

Many tissues receive a dual autonomic innervation and stimulation of one component usually results in effects opposite to those produced by stimulation of the other. Further, it is clear that the effect of stimulating any one component may vary from tissue to tissue, being excitatory in some tissues and inhibitory in others. The *parasympathetic* effects are largely directed towards *maintenance* and *conservation* of bodily function. Thus responses to parasympathetic stimulation include slowing of the heart and decreased atrial contractility, constriction of the pupils, bronchoconstriction, contraction of the bladder (detrusor muscle) and increased secretion and motility of the digestive tract.

In contrast, the effects of *sympathetic* stimulation are directed toward coping with stress and these tissues responses together comprise the '*fight or flight*' response described by Cannon in 1939. These changes include increased heart rate and contractility, bronchodilatation, pupillary dilatation, inhibition of intestinal motility, constriction of the splanchnic vascular bed, decreased muscle fatigue, and elevated blood glucose and free fatty acids. However this is not to say that the sympathetic system always acts *en masse* or that it is only active in stress. Many sympathetic effects are associated with normal activity and are

localized (i.e., pupillary dilatation and regional changes in blood flow), and these can occur without substantial changes in other tissues.

The activity of tissues having a dual innervation depends on the balance between parasympathetic and sympathetic discharge. Often one of the systems is dominant, for example the diameter of the pupils and the resting heart rate are largely determined by the level of activity (tone) in their parasympathetic nerve supplies. Changes in effector activity are usually the result of reciprocal changes in both parasympathetic and sympathetic activity. Mutual antagonism in the periphery can occur (i) as a result of dual reciprocal innervation of effector cells, such as occurs in heart, bronchial smooth muscle and detrusor smooth muscle, and (ii) from suppression of synaptic transmission in ganglia. The latter has been found in parasympathetic ganglia supplying detrusor muscle and in the ganglia that form the myenteric plexus of the intestinal tract. In fact most of the sympathetic supply to the intestinal muscle layer, excluding that accompanying blood vessels, ends in the myenteric plexus and appears to act by presynaptic inhibition of transmitter release.

Circulating Catecholamines

The activity of some autonomically innervated tissues is influenced by both noradrenaline released from nerves and by catecholamines released into the bloodstream from the adrenal medullae. In man adrenaline comprises some 80% of the catecholamines released from the gland, the remainder being noradrenaline. The adrenaline and noradrenaline are synthesized and stored in different cells. An additional step in the synthetic chain produces adrenaline by N-methylation of noradrenaline. The activity of the enzyme *phenylethanolamine-N-methyltransferase* responsible for this step is increased by steroids from the adrenal cortex.

The free plasma concentrations of these amines are low at rest, being 1 to 2 nmol/L for adrenaline and 0.2 to 0.8 nmol/L for noradrenaline. All of the adrenaline comes from the adrenal medullae and the noradrenaline from both the adrenal medullae and sympathetic nerves. Low levels are maintained at rest because the rate of release from the adrenal medullae is normally low and the half-life of the circulatory catecholamines is relatively short (less than 1 min in experimental animals). During periods of stress, physical or emotional, the rate of release of adrenaline can increase eight- to ten-fold.

Adrenaline has many actions similar to noradrenaline but, because it is a more potent beta agonist, it has more pronounced metabolic actions. These include elevation of blood glucose and free fatty acids and an increase in metabolic rate. Adrenaline also produces vasodilatation in those vascular beds in which the beta to alpha receptor ratio is high, i.e., in skeletal muscle and heart muscle.

2.5 Basic Design of the CNS

The CNS comprises the *brain* lying within the skull and the *spinal cord* lying within the vertebral column. During embryonic development the CNS initially develops as an infolding of the ectoderm, forming a hollow tube called the neural tube. On either side of this tube is found neural crest tissue which gives rise to dorsal root ganglia cells, autonomic ganglion cells and a wide variety of non-neural cells including Schwann cells and endocrine

cells. The inner surface of the neural tube is the principal site of generation of nerve cells which migrate in an orderly way to regions where they differentiate. Migrating nerve cells are closely associated with specialized glial cells which apparently act as guides for this migration.

As the neural tube grows it forms three longitudinal swellings at its anterior end. These swellings develop into the *fore-*, *mid-* and *hind-brain*. They retain their cavities (*ventricles*) which remain in communication with the canal in the *spinal cord* which is formed from the remainder of the tube. The forebrain enlarges to form bilateral swellings which grow first forward and the backward to cover most of the brain-stem, thus giving rise to the *cerebral hemispheres*. Further smaller raised swellings develop in the hind-brain to form the *cerebellum*. The cerebellum and the cerebral hemispheres have two unique features: (i) their surfaces are extensively folded, forming depressions called fissures or *sulci* and raised portions called *gyri* and (ii) unlike the brain-stem and spinal cord they have their grey matter on outside and white matter on the inside.

Spinal Cord

The spinal cord has a segmental structure with pairs of nerve roots, one pair on each side, arising at more or less regular intervals. Each pair comprises the *dorsal root* carrying information into the spinal cord from peripheral receptors and the *ventral root* carrying signals out which will make muscles contract. In cross-section each segment of the spinal cord shows a central butterfly wing-shaped area of grey matter and a peripheral zone of white matter. The grey matter contains cell bodies of neurones whose axons leave in the ventral roots to innervate muscles. In the dorsal horn of the grey matter lie cell bodies of interneurons concerned with the processing signals entering in the dorsal root axons.

The white matter consists of axons of cells in the brain descending to spinal cord cells and the axons of cells in the spinal cord ascending to synapse with cells in the brain. As like axons travel together, well-formed bundles (*tracts*) can be distinguished. Thus in the dorsal quadrant of the white matter we can distinguish the dorsal columns composed of axons ascending to the brain-stem with information from skin, joint and muscles. In the lateral quadrant there are both ascending tracts to the cerebellum and brain-stem and descending tracts from the cerebral cortex and brain-stem. The spinal cord segments perform the initial processing of afferent information and contain the neural circuits for many reflexes, some of which are the basis of movement and posture.

Brain

The brain consists of three major subdivisions - the *brain-stem*, the *cerebellum*, which is attached to the brain-stem, and the *cerebrum*.

Brain-Stem

This is an ancient part of the brain in an evolutionary sense. Although much smaller than the cerebellum, the brain-stem is vital to life while the cerebellum may, with considerable residual but not life-threatening disability, be dispensed with. The brain-stem

links spinal cord and cerebrum and is composed of four regions - the medulla oblongata, pons, mid-brain and diencephalon.

The *medulla* is continuous with the spinal cord and contains the same fibre tracts. The grey matter, however, is not organized in a continuous column but is broken into discrete nuclei, including motor and sensory nuclei for the throat, mouth and neck, and nuclei involved in the control of the respiratory and cardiovascular systems and of movement and posture.

The *pons* may be recognized on its ventral surface by the bulge of the brain-stem formed by axons descending from the cerebrum and turning up into the cerebellum which, in the intact brain, conceals the dorsal surface of the pons. The pons is continuous with the medulla and contains the same ascending and descending tracts in the reticular grey matter (*reticular formation*) that are involved in the control of the respiratory and cardiovascular systems. The pons also contains the nuclei of nerves for the motor and sensory functions of the face.

The *mid-brain*, the smallest part of the brain-stem, is continuous with the pons below and the diencephalon above. The ventral surface is characterized by the large *cerebral peduncles* lying laterally and carrying axons from the cerebrum to the brain-stem and to the cerebellum. The dorsal surface may be recognized by two pairs of protuberances, the *superior* and *inferior colliculi*. The superior collicular neurones are concerned with the processing of visual information and the inferior collicular neurones with auditory signals. The mid-brain also contains nuclei concerned with the state of wakefulness of the brain and ascending tracts from the spinal cord on their way to the diencephalon.

The *diencephalon*, nearest to the cerebrum, is covered by it in the intact brain and can only be seen if a brain is sectioned transversely or if one hemisphere is removed. Developmentally it represents that part of the forebrain from which the rudimentary cerebral hemispheres have budded off. Its walls have been thickened by the growth on either side of the mass of grey matter that constitutes the *thalamus*, and ventrally by the structures that make up the *hypothalamus*, so that the original cavity has become the narrow cleft of the *third ventricle*. The *thalamic nuclei* function in at least three ways. One group relays signals concerned with all types of afferent information, except olfaction, to the cerebral cortex on the same side. Another group relays signals to the motor cortex on the same side and receives information from the cerebellum and basal ganglia. A third group is concerned with sleep and wakefulness. The *hypothalamic nuclei* serve as regulating centres for autonomic functions (i.e. body temperature, heart rate, blood pressure), they control the release of hormones by the pituitary gland, and they are involved in the expression of the emotions and the regulation of food and water intake.

From the brain-stem at different levels the various *cranial nerves* have their superficial origins. There are twelve pairs of these and they innervate the skin and muscles of the face, structures in the head and neck and, in the case of the vagus (X) nerves, the thoracic and many of the abdominal viscera. Strictly speaking the olfactory (I) nerves are connected to the forebrain, and not the brain-stem. The optic (II) nerves are joined to the diencephalon via the optic chiasma. Cranial nerve pairs III and IV emerge from the mid-brain, V, VI, VII and VIII from the pons and the remaining four pairs from the medulla.

The nuclei from which these cranial nerves have their deep origins in some instances extend through a considerable length of the brain-stem, and many of the nerves contain a mixture of fibres, motor and sensory, somatic and autonomic.

Cerebellum

This is a large midline organ overhanging the dorsal aspect of the brain-stem to which it is attached by three large bundles of white matter on each side - the superior, middle and inferior *cerebellar peduncles* which carry neural signals in and out of the cerebellum. Three cerebellar lobes can be recognized. On the superior aspect the anterior lobe is separated from the larger middle lobe by the primary fissure. The anterior and middle lobes are further divided into a narrow median strip - the *vermis* - and two lateral *cerebellar hemispheres*. Rostrally, and seen only on the inferior aspect, are the *flocculus* and *nodule* together forming the *posterior lobe*. The anterior and middle lobes are transversely folded, the folds being termed *folia*. This folding vastly increases the surface area of the cerebellum.

All parts of the cerebellum have the same structure - a thin superficial layer of grey matter, the *cerebellar cortex*, covering a much larger expanse of white matter formed by axons entering and leaving the cortex. Deep in the white matter, close to the brain-stem, lie the *intracerebellar nuclei*. A section at right angles to the long axis of a folium shows that it is much wider laterally than medially and that subsidiary foldings of the folium, each with cortical grey matter and white matter, give the section the appearance of a tree, hence the name *arbor vitae*. The cerebellum has its major role in the control of the rate, range and direction of movement. The small, phylogenetically old, posterior lobe is connected to the organ of balance (labyrinth) and is concerned with the reflexes which ensure an upright posture.

Cerebrum

The cerebrum consists of the right and left *cerebral hemispheres*. Connecting them in the midline is a large thick band of white matter known as the *corpus callosum* and made up of axons passing between the hemispheres. Each hemisphere comprises an outer layer, about 1 cm thick, of grey matter which is the *cerebral cortex*, covering a dense thick inner layer of white matter. The grey matter has a rich blood supply. The white matter is less well endowed. The white matter is composed of axons connecting different regions of the cortex with each other and connecting the cortex with the rest of the brain. Within the white matter of each hemisphere is a collection of neuronal cell bodies lying between the thalamus and the cerebral cortex. These nuclei, known as the *basal ganglia*, are concerned with the initiation and control of movement.

The cerebral cortex is folded into gyri and sulci, thereby increasing the surface area. The deepest sulci are called *fissures*. On the lateral surface of each hemisphere the *lateral fissure* partially separates off the temporal lobe from the rest. This lobe contains the *primary auditory cortex* which receives signals from the auditory receptors in the inner ear.

The *central sulcus* runs from the medial surface to the lateral fissure. It is not as prominent as the lateral fissure but there are usually well-formed and continuous gyri on

either side of the sulcus. The central sulcus forms the posterior boundary and the anterior wall of the central sulcus is the *precentral gyrus*. This is the *primary motor cortex* containing neurones whose axons run down through the brain-stem and spinal cord and synapse with motor neurones.

The *parietal lobe* lies behind the central sulcus. The posterior border of the sulcus is the postcentral gyrus of the parietal lobe - the *primary sensory cortex* which receives information from receptors in skin, joint and muscle.

The *occipital lobe* is most posterior and little of it can be seen on the lateral surface. Examination of the medial surface, however, shows the *calcarine fissure* of the occipital lobe where the *primary visual cortex* is located. The occipital lobe is entirely concerned with the processing of visual information.

The *corpus callosum* connects the two hemispheres and is surrounded by the *cingulate gyrus* which is involved in the control of emotional behaviour.

Meninges, Ventricles and Cerebrospinal Fluid

The CNS is covered by three membranes or *meninges* comprising a tough outer layer, the *dura mater*, a middle layer, the *arachnoid mater*, in which lie the blood vessels, and an inner layer, the *pia mater*. Between the arachnoid mater and the pia mater is the *subarachnoid space* which is communication with the ventricles and contains *cerebrospinal fluid (CSF)*, a specialized extracellular fluid. At the base of the brain the subarachnoid space becomes enlarged to form cisterns, the largest being the *cisterna magna*.

The ventricles comprise the *lateral ventricles* in the cerebral hemispheres, the *third ventricle* in the diencephalon and the *fourth ventricle* in the hind-brain. The third and fourth ventricles are connected in the mid-brain by a canal called the *cerebral aqueduct*.

Blood-Brain Barrier

Slow diffusion of many substances of low M between the blood and CSF and between the blood and brain suggests the existence of a *blood-CSF barrier* and a *blood-brain barrier*. These barriers are permeable to respiratory gases, to glucose and to fat-soluble drugs like volatile anaesthetics. The endothelial cells of the capillaries within the CNS are held together by 'tight' junctions and it is these which limit diffusional exchanges of water and water-soluble solutes. However, these cells are involved in transport of solutes between blood and brain interstitial fluid. Note that the blood-brain barrier is absent in certain structures called *circumventricular organs* which abut on the third and fourth ventricles i.e., the subfornical and pineal organs, the area postrema and the median eminence.

Cerebrospinal Fluid

The CSF is formed predominantly by the *choroid plexus* - rich networks of blood vessels covered with epithelial cells (*ependyma*) projecting into the ventricles. Fluid formed in the lateral ventricles passes to the third and fourth ventricles and to the central canal of the

spinal cord. This movement is aided by the cilia on ependymal cells. The fluid escapes into the subarachnoid space through foramina in the ependymal lining of the fourth ventricle and circulates around the brain and spinal cord. Finally it is reabsorbed through the arachnoid villi into the sinuses of the venous system. In most regions of the brain, substances are free to diffuse between the ependymal cells and so there is a ready exchange of solutes between the CSF and the extracellular spaces of brain and spinal cord. The local environment of the neurones is further controlled by the activity of glial cells which can adjust both potassium and hydrogen ion concentrations. Of the 700 ml of CSF formed per day about 70% is derived from choroid plexuses and the other 30% comes from endothelial cells lining the brain capillaries. The volume of CSF in adult man is about 140 mL (compared with about 250 mL for brain interstitial fluid) and in the horizontal position its pressure is about 10 mmHg, i.e., a little less than local venous pressure.

The composition of CSF differs from what one would expect if it were simply an ultrafiltrate of plasma, indicating that it is actively secreted. One model of the formation of CSF by the choroid plexus is illustrated. Note the unusual feature that the Na-K-ATPase is located in the apical plasma membrane. Note also that though CO₂ crosses the blood-brain barrier readily, HCO₃⁻ does not. The HCO₃⁻ in CSF is synthesized within the epithelial cells, in the reaction



The initial reaction is catalysed by carbonic anhydrase which is present in the epithelial cells. The primary secretion has more HCO₃⁻ than plasma (about 45 mmol/L) and it buffers H⁺ ions produced by neuronal and glial metabolism, reducing the concentration to about 23 mmol/L. This decrease in bicarbonate ion may also reflect the mixing of choroidal CSF with fluid secreted by the capillary endothelial cells which is thought to have a higher Cl⁻:HCO₃⁻ ratio.

The functions of the CSF are:

1. *Conferring of buoyancy.* The brain has little mechanical strength or rigidity. It weighs 50 g in CSF compared with 1500 g in air and so flotation in CSF protects it against deformation and damage from the innumerable accelerations imposed by movements of the head. Counter-pressure of CSF surrounding the blood vessels within the cranium and the spinal cord compensates for gravitational effects of alterations in posture or external acceleration. Also, variations in the distribution of CSF with the cardiac cycle maintain fluid pressures constant within the cranium.

2. *Maintenance of a constant ionic environment in brain.* This is important because the activity of neurones is highly sensitive to ionic changes. The CSF also provides another route for removal of substances which have limited lipid solubility or are too large to move easily across capillary walls. In other tissues the lymphatic system, which is not found in the CNS, serves this function.

3. *Control of respiration and pH.*

4. *Control of water balance.* The concentration of NaCl in CSF may be a factor mediating thirst and the release of antidiuretic hormone.

2.6 Appendix

Basic Electrical Properties of Biological Membranes

Current, Voltage, Conductance and Capacitance

Small ions can flow across biological membranes, thereby carrying current and separating electrical charge. Some of the properties of such membranes can be expressed in electrical terms. The potential difference (V) across biological membranes is normally some 60 to 100 mV, the cytoplasm being negative with respect to the interstitial fluid. The potential difference is a measure of energy separated across the membrane per unit charge (joules/coulomb) and is therefore independent of area. In contrast, current (coulomb/second), which is directly proportional to the potential difference, increases with the available area. For any V, the actual current flow (I) is determined by the electrical conductance (G) of the membrane. Conductance expresses the ease with which current flows. Its reciprocal, the membrane resistance (R), is more familiar from elementary physics. Ohm's law states that

$$I = V / R \text{ or } I = G * V.$$

Conductance (siemens) and resistance (ohms) are also determined by the area involved. When area is taken into account, the resistance is often referred to as the *specific membrane resistance* and has the units ohms * area (since $V/I = \text{ohms}$, $V/I/\text{area} = \text{ohms} * \text{area}$).

In practice it is sometimes possible to determine the resistance of a plasma membrane by inserting micro-electrodes into a cell, passing a known current across the membrane, and measuring the change in potential difference which results. This is permissible since the cytoplasm offers comparatively little resistance to current flow. In some cells, membrane resistance so measured is found to be influenced by the direction of the induced change in potential, i.e. there is not a linear relationship between current and voltage - Ohm's law being obeyed strictly over only a narrow range. Thus the resistance calculated from the change in potential following a hyperpolarizing current (one which makes the interior of the cell more negative) may differ from that calculated when potential is decreased by the same amount following a depolarizing current. This asymmetrical behaviour is referred to as *rectification*. It indicates that current flows more easily across the membrane in one direction than in the other.

The conductance (reciprocal of the resistance) of a membrane can be thought of as reflecting the number and selectivity of water-filled ion-conduction pathways spanning the lipid bilayer. For example, K⁺ ions generally pass more readily across plasma membranes through these pathways than do Na⁺ ions. Therefore, replacement of Na⁺ by K⁺ in the medium produces a large increase in measured membrane conductance, though the number of available pathways may be unaltered. Conductance, measured by electrical techniques, is not to be confused with *permeability*, measured by isotope exchange or other chemically-based methods. For example, the electrically 'neutral' one-to-one coupled movement of Na⁺ and Cl⁻ across a membrane (co-transport) can be detected using Na⁺ isotopes. In such a case

the membrane is permeable to Na⁺ but does not have an electrically measurable conductance. However, if an ion moves only by simple passive diffusion across a membrane, then it is possible to relate measured conductance and measured ion permeabilities by using appropriate conversion factors and transference numbers which express the fraction of total current carried across the membrane by the ionic species of interest.

Plasma membranes do not respond to current passed across them with an instantaneous change to a new steady potential difference. Initially, some of the current passed changes the amount of charge (Q) stored on the membrane itself. The membrane, in separating charge across it, acts like a capacitor and the charge separated per unit driving force (coulombs/volt) is the *capacitance*, C, equal to Q/V, and its unit is the farad (F). The capacitance per unit area of *specific membrane capacitance* is similar form most biological membranes, about 1 microF/cm².

Because use is made of electrical analogues in understanding ion movements across single membranes and epithelia and because such concepts as resistance and capacitance are employed in discussing circulatory and respiratory physiology as well, the following should be noted:

(a) The total resistance (R) in a circuit containing resistors in series is $R_1 + R_2 + R_3$. Since $G = 1/R$, the total conductance (G) for this circuit is given by $1/G = 1/G_1 + 1/G_2 + 1/G_3$ or $G = G_1 G_2 G_3 / (G_1 + G_2 + G_3)$.

(b) The total resistance (R) in a circuit containing resistors in parallel is given by $1/R = 1/R_1 + 1/R_2 + 1/R_3$ or $R = R_1 R_2 R_3 / (R_1 + R_2 + R_3)$. Here the total conductance (G) is given by $G = G_1 + G_2 + G_3$.

(c) The total capacitance (C) in a circuit with capacitors in series is given by $1/C = 1/C_1 + 1/C_2 + 1/C_3$ and with capacitors in parallel it is $C_1 + C_2 + C_3$.

Membrane Space and Time Constants

Nerve and muscle fibres are elongated, like a submarine cable, and are endowed with so-called 'cable' electrical properties. Because the membrane is not a perfect insulator, when current is injected into a fibre it flows longitudinally in both directions and progressively leaks out across the membrane. By inserting glass micro-electrodes into large fibres at points of increasing distance from the site of current injection it is possible to record electrotonic potentials along the fibres. A simple electrical model for the passive properties of an axon is shown. In such experiments it can be seen that the amplitude of the electrotonic potential declines exponentially with distance from the current electrode. This exponential process is expressed by the *membrane space constant (lambda)* which is defined as the *distance* in which voltage falls to 1/e (about 37%) of its original value. It is independent of the size of the event. It can be shown that

$$\lambda = \sqrt{r R_m / 2 R_i}$$

where r is the radius (cm) of the fibre, R_m is the specific membrane resistance (ohm cm²) and R_i is the specific intracellular resistance (ohm/cm).

Moreover when current is passed across the membrane of a cell the membrane capacitance will slow the time course of the consequent voltage change. Therefore the electrotonic potential will rise exponentially before it reaches a plateau. This exponential rise is expressed in the *membrane time constant (tau)* which is defined as the *time* required for the voltage to reach 1/e of its final value. For cells in which the applied current is distributed homogeneously, as in the case of a spherical cell, it can be shown that tau equals the product $R_m C_m$ where C_m is the specific membrane capacitance (microF/cm²). Thus C_m can be calculated, if resistance is known, from the time course of the change in the voltage following the passage of current across the membrane. This relationship is valid only when current is applied uniformly across the whole surface of a cell or fibre. If, however, current is applied focally by injection, as in the experiments considered above, then the apparent time constant becomes longer the further away from the point of current injection the measurement is made and the response appears after a delay.

Number of Ions Crossing the Membranes

The number of ions that must be separated to give rise to the membrane potential can be calculated from the relationship $C = Q/V$. With a membrane potential of 100 mV and a specific capacitance of 1 microF, the charge separated across each cm² of membrane is therefore 10⁻⁷ coulombs. Since 1 mol of univalent ion carries 96500 coulombs, or approximately 10⁵ coulombs, 10⁻⁷ coulombs represents about 10⁻¹² mol of univalent ion. Since the number of ions in a mol (Avogadro's number) is 6.02 * 10²³ this represents 6 * 10¹¹ ions. Therefore the number of ions separated across the membrane to give a membrane potential of the magnitude measured across the plasma membrane is infinitesimal when compared with the molar concentrations of ions in biological fluids, and the principle of bulk electroneutrality is not violated.

During the course of an action potential Na⁺ ions move into the nerve fibre during the rising phase and K⁺ ions move out to repolarize the membrane during the falling phase. The net gain of Na⁺ ions and net loss of K⁺ ions have been determined in squid giant axons using radioactive isotopes of Na⁺ and K⁺ as tracers. These experiments show that 3 to 4 * 10⁻¹² mol of each ion actually crosses each cm² of membrane during the passage of one impulse.

The number of K⁺ ions crossing the membrane can be calculated in relation to their intracellular concentration by equating a nerve fibre to a hollow cylinder. Its surface area is given by 2πrL and its volume by πr²L, where r is the radius and L the length. Therefore the volume enclosed by a cylinder of 1 cm² surface area is

$$\pi r^2 \quad 1/2\pi r = r/2.$$

In the case of a 20 microm axon this equals 5 * 10⁻⁴ cm³. Since the axon contains about 140 mmol/L K⁺, there are 7 * 10⁻⁸ mol of K⁺ in each segment enclosed by 1 cm² of membrane. Therefore, in comparison, the 3 to 4 * 10⁻¹² mol of K⁺ ions per cm² of membrane that actually leave the interior of the nerve fibre during the passage of one impulse represent only about 1/3000 of the internal K⁺ pool and in the short term this is insignificant. In the smallest axons, however, it may become significant and such fibres can conduct only a few action potentials before their gradients must be recharged by the Na-K pump.

Voltage Clamping

The ionic basis of the action potential was described by Hodgkin and Huxley in the early 1950s. Their interpretation was based on voltage-clamp experiments on squid giant axons. In these experiments a feedback apparatus was used to deliver a current across the membrane in order to clamp membrane potential at any selected voltage. The current passed in order to clamp this voltage was monitored.

If the command voltage was set to the resting potential, no current flowed. When the potential was changed rapidly to more positive values and an action potential invoked, there was a brief inward current followed by a strong prolonged outward current. Replacing external Na⁺ with the impermeant cation choline abolished the inward current, indicating that it was carried by Na⁺ ions. This was confirmed by clamping the cell to a voltage more positive than E_{Na} . This reversed the inward current, so there were now two outward currents, with different time courses, the first carried by Na⁺, the second by K⁺. From such experiments it was possible to derive the ionic conductance changes underlying the action potential as shown.

Reverse Potential

Synaptic potentials result from current flow through ion-selective channels. This flow tends to change the membrane potential towards some particular value. For example, at the neuromuscular junction, activation of acetylcholine receptors increases Na⁺ and K⁺ conductances in the ratio 1.3 : 1. Assuming $E_{Na} = +60$ mV and $E_K = -90$ mV, the potential value the cell tends towards is thus -5 mV.

As the current flow is local and is opposed by current flow drawn from neighbouring regions of the cell, this value is not actually reached. If, however, an experimenter injects current into the cell and changes the value of the resting potential so that the cell is made positive inside, the synaptic potential will still tend towards -5 mV. Thus, instead of being a depolarizing potential, it will now be a hyperpolarizing one. This technique is used experimentally to determine the reversal of the synaptic potential and so to measure the relative conductances of Na⁺ and K⁺.

Patch Clamping

Passive ion movement across membranes is through water-filled pores or channels. These channels may always be open, or they may fluctuate between open and closed states. The opening of some channels is voltage dependent, i.e., sodium channels of the nerve cell membrane. The opening of others depends on the presence of chemical transmitters, i.e., the acetylcholine-sensitive channels at the muscle endplate.

If channels fluctuate between open and closed states, the number of ions crossing an area of membrane in a given time will also fluctuate. In macroscopic measurements of ionic conductance we are not aware of this since the high density of channels smoothes out these oscillations to give an average or steady state. There are, however, two ways in which the behaviour of individual channels may be studied. If ionic currents passing across a given area of membrane are subjected to very high amplification, small variations in current can be

recorded, particularly if the density of channels is low or if the number open at any given time is reduced by experimental means. These variations in current are referred to as membrane 'noise' and their study as 'fluctuation analysis' or 'noise analysis'. In essence this involves the separation of 'noise' due to ion flow through the membrane from other 'noise' sources in the system and the investigation of the properties of the former by statistical analysis. By these means the average density of the channels and their individual properties can be determined.

A more elegant technique involves isolating a small piece of membrane containing only a few channels in which current flow through individual channels can be recorded directly. This technique, developed by Neher in the mid-1970s and known as 'patch-clamping', requires that specially prepared micro-electrodes be brought up to a membrane surface and a tight seal formed. Isolation of a small piece of membrane affords the opportunity of studying the channels uninfluenced by other regions of the cell, either by manipulating the external environment (the outside-out patch-clamp) or the inside environment (the inside-out patch-clamp). Experiments of this type have shown that the primary mechanism by which many specific external stimuli affect cellular activity is through combination with an external receptor on the cellular surface and opening or closing ionic channels. Some unexpected results of this research include the surprisingly large number of different channels that coexist within the surface membrane and that many channels, even those activated by ligands, spontaneously open and close.