Applied Neurophysiology

With particular reference to anaesthesia

J.A. Simpson MD, FRCP, FRCP (Ed), FRCP (Glas), FRS (Ed)
Professor of Neurology, University of Glasgow and Institute of Neurological Sciences, Glasgow

W. Fitch PhD, MB ChB, FFARCS
Reader in Anaesthesia, University of Glasgow and Royal Infirmary, Glasgow

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Preface

This book has not been entitled 'Clinical Neurophysiology' as that term has been adopted for the diagnostic specialties of electroencephalography, electromyography and related investigative procedures in neurology. No more is this a systematic textbook of vertebrate neurophysiology for neuroscientists. We were asked to write a book for anaesthetists primarily, to promote understanding of the structure and function of the human nervous system. For that reason we have presented concepts rather than critical discussions of experimental work. To give detailed references to original work would have required a different kind of book: we have instead recommended further reading where such information can be obtained.

We anticipated that the trainee practical anaesthetist would need to understand the neural mechanisms for consciousness and awareness, respiration, pain, muscle tone and the autonomic control of the cardiovascular system but that cellular physiology could be summarized as being readily available in other texts. It soon became apparent, however, that a selective approach had serious limitations. Although these topics are given special prominence, it was evident that a comprehensive though less detailed coverage was necessary for understanding of these important areas, and that the conventional arrangement of sensory and motor 'systems' etc. was detrimental to understanding the roles of the diencephalon and cerebral cortex.

The material has been organized to stress the distributed nature of functional systems and their integration, and attention is drawn to anatomical structures or important functional concepts by free use of italic type since their use in context usually makes it unnecessary to give formal definitions. In the main, the anatomical nomenclature and connections described are from *Human Neuroanatomy* by M. B. Carpenter. Unfortunately the elegant but simplified drawings of anatomical connections in other publications omit the important connections of the mid-brain and diencephalon and are often more relevant to laboratory animals than to man. Most of the illustrations in the present text attempt to show distributed systems more accurately. Unfortunately there is no substitute for a knowledge of the anatomy of the human nervous system but the already complicated drawings would be impossible to follow if they were burdened with lettering. As an alternative, the reader is 'talked through' the illustration by means of an extended legend, but at a second reading it is recommended that a good anatomical atlas should be compared.

Anaesthesiology is now probably the largest hospital specialty in collaboration with specialists in intensive care, ophthalmology, orthopaedics and many others. Certain chapters, such as those on vision, control of gaze, hearing and locomotion, are intended for such specialists but they have also been included to introduce concepts on brain stem automatisms and cortical function necessary to understand respiration and conscious awareness. It is hoped that the additional coverage of these chapters may interest other clinicians, psychologists, speech pathologists and even neurologists in training. The final section on the autonomic nervous system continues with an account of the regulation of the cerebral circulation. In view of the importance of this for the practising anaesthetist, we have provided quantitative data and references to original studies which were not thought necessary in other sections.

J.A.S.
W.F.

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PART 1 Cellular Organization of the Nervous System

Chapter 1
The excitable cell

The resting potential

Every living cell has a potential difference across its membrane due to the fact that charged particles are separated by a semi-permeable membrane which prevents the charged particles (ions) from redistributing themselves randomly. The special feature of the so-called 'excitable cells' (nerve, muscle) is that the permeability can be changed by processes which either increase (hyperpolarize) or decrease (depolarize) the potential difference.

The membrane is a double layer of phospholipid molecules with specialized protein molecules inserted into it, some of which are structured to form channels which allow passage of water and ions (*Fig.* 1.1). There are at least two types of *ion channels* which differ in channel diameter, so restricting the ion species which each will pass (in fact the ion and its shell of water). Thus, one type allows ready passage of potassium and chloride ions. The other will pass, less readily, sodium ions and the similarly dimensioned lithium ions. The channels also have a selectivity filter for certain ion species, apparently because of energy barriers which remove the shell of water molecules around the ions. In the sodium channel the sodium ions bind to fixed negatively charged sites (probably oxygen atoms) which force them into single file. Similar constraints probably exist in the potassium channel. Thus the resting membrane has little permeability to ions. There are some molecules which are capable of inactivating the passage of ions by blocking the channels. Tetrodotoxin (from the puffer fish and other poisonous animals) is a complex molecule which can bind to the outward-facing molecule of the sodium channels and so block passage of sodium ions. Similarly, tetraethylammonium ion applied internally blocks potassium conductance.

In addition to these specialized channels, there is an *active pumping mechanism* which transports sodium ions out of the cell and potassium ions into it. The structural basis for the pump is unknown but the action of certain poisons indicates that its carrier molecules are driven by energy derived from metabolic processes within the cell, probably from energy-rich ATP hydrolysed by Na-K-ATPase. The important part of this process is the removal of sodium from the cell cytoplasm as this permits the intracellular substance to have a sodium concentration only about 10 per cent of that of the extracellular fluid since the pump extrudes sodium at a rate that exactly balances the net passive inward membrane current. However, the membrane is slightly leaky to cations even when the channels are 'closed', although it is impermeable to anions other than Cl^- (eg glutamic and aspartic ions) and the imbalance of positive charges produced by the pump sets up an electric potential across the membrane (the *resting potential*). Potassium ions, which pass more readily than sodium, are retained within the cell in higher concentration than in the external fluid and chloride ions are extruded until the combined electrochemical gradient for potassium and chloride ions is about zero, while the imbalance of sodium ions makes the interior of the cell at a negative potential relative to the exterior.

The increased permeability to K^+ ions on depolarizing the membrane is vastly greater than the opposite effect of a hyperpolarizing current. This phenomenon is termed *delayed rectification.* Muscle membrane has an additional *anomalous rectification* in the opposite

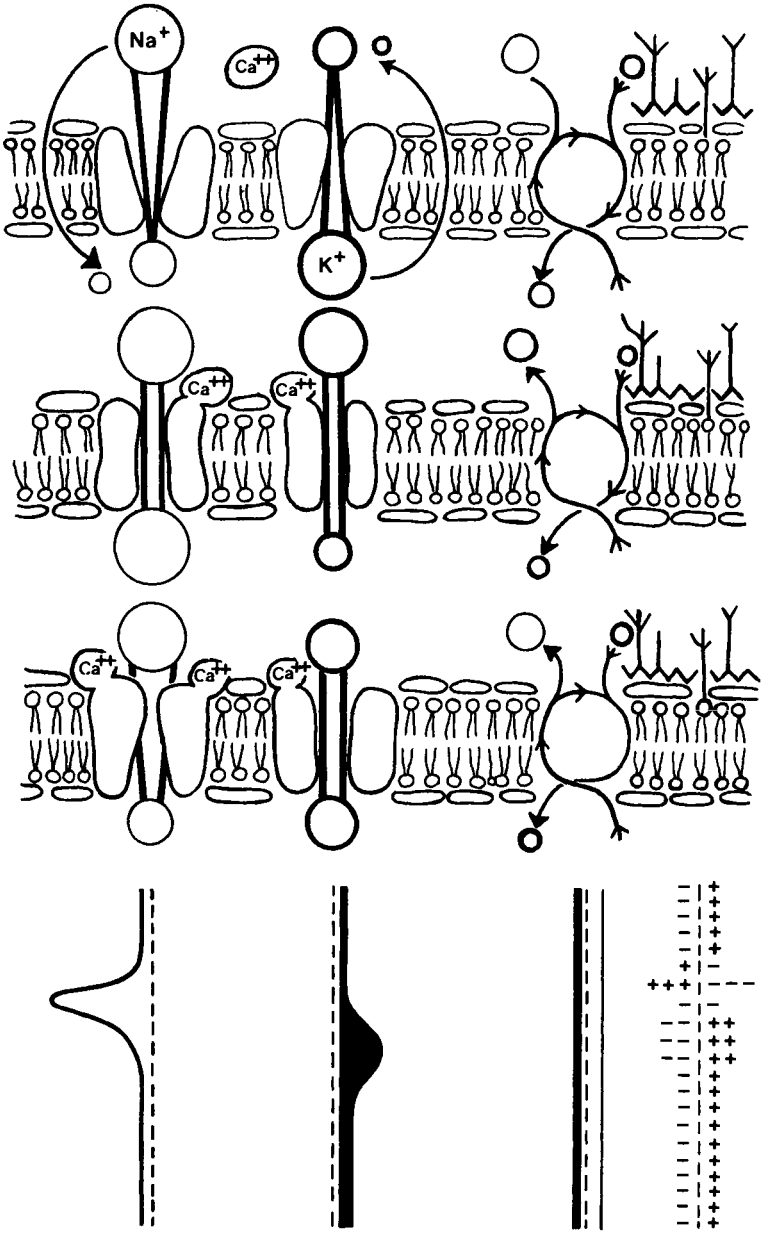

Fig. 1.1. Schematic diagram of the outer membrane of an excitable cell (extracellular space above, cytoplasm below). The structural protein bounding layers are separated by a double layer of orientated lipids. The outer surface has a glycoprotein 'backbone' into which are set a 'fuzz' of polysaccharides, glycoproteins and glycolipids (*right*) with fixed anions. The membrane is leaky (*upper*) but a metabolically driven pump extrudes Na^+ and introduces a smaller number of K^+ ions into the cell. The difference in charge is seen as a resting potential across the membrane (positive outside with respect to the cytoplasm). This sets up a gradient of both potential and cation concentrations across the membrane. This is abolished and temporarily reversed when an electric current activates the opening gates of specific channels allowing Na^+ ions to rush in and K^+ to emerge more slowly. Calcium ions (Ca^{++}) are necessary to open the gates. The sodium channel is soon closed by a voltage- and calcium-dependent inactivation gate but passive flux of K^+ continues until the balance is restored. Specific channels for transfer of calcium are not shown in the diagram which illustrates three states — resting (*upper*), activated (*middle*) and inactivated (*lower*).

Beneath each channel and the Na–K–ATPase pump are illustrated the fluxes of Na^+ and K^+ due to each. Intracellular shifts are to the left of the dashed line, extracellular extrusions to the right. The net charge distribution across the membrane is shown bottom right. This illustrates the change from resting to action potential (depolarization) with after-hyperpolarization which is slowly abolished by the sodium pump.

direction. Membrane permeability decreases during the outward flow of potassium current and increases with inward flow. Depolarizing drugs cause sufficient loss of potassium from muscles to raise the level of plasma potassium. In patients with severe burns or with extensive injuries to soft tissues, administration of succinylcholine may raise the potassium efflux to levels which may cause cardiac arrest. There is a similar risk in its administration to patients with widespread denervation (polyneuritis) or myotonic dystrophy.

The total body potassium is obviously significant for the excitable cell, but for its polarization and ability to produce action potentials, the external sodium level is more important than the level of potassium — a fact commonly overlooked in clinical medicine. Muscle is much more vulnerable than nerve to low potassium levels. Anomalous rectification seems to indicate a valve-like mechanism to resist a net outflow of potassium ions from the muscle cell, probably in the sarcoplasmic reticulum.

So long as the sodium–potassium pump operates, the intracellular cytoplasm is rich in potassium and organic anions but poor in sodium and chloride compared to the external fluid. This is a potentially unstable situation with *ion diffusion gradients* across the membrane. If the metabolic pump fails, there is a slow but continuous inward movement of Na^+ ions and compensatory loss of K^+ ions so that both concentration gradients gradually disappear. This is a comparatively slow process as the permeability to sodium is so low. It takes many hours for the resting potential to drop significantly and during this time action potentials can still be generated. The electrochemical gradients are the motive force; but eventually the gradients are dissipated. This demonstrates the importance of the metabolic process for 're-charging the battery', but also indicates that the ion shifts across the membrane are too slow to account for the action potential which is the hallmark of excitable cells, differentiating them from other cells which share the above mechanisms for polarizing the cell by electrochemical gradients.

The action potential

The time courses of passive diffusion of ions into and out of the cell described so far are insufficient to account for the generation of an action potential. If the membrane of an excitable cell is depolarized beyond a critical level (by passing an electric current through it, or by increasing the extracellular concentration of potassium) it develops a high permeability to Na^+ ions which then pass into the cell until the sodium equilibrium potential is reached. The influx of positive charges reverses the potential across the membrane. Activation of a sodium carrier has been postulated but it now seems that an adequate explanation is a voltage-regulated adjustment of a 'gate' mechanism at the external opening of the *sodium channels* (*Fig.* 1.2). At the normal resting potential, the channel appears to be occluded at its external opening by charged particles. When the membrane is depolarized (internal potential more positive) these gating particles are believed to change configuration by a series of steps to an 'open' position which allows sodium ions to pass freely through the channel. Passage of these positive ions into the cell further depolarizes it and therefore opens the gate still wider and the permeability increases explosively. The increase in conductance of sodium (with passage of sodium ions into the cell) is regenerative because of this gate effect, which is the characteristic feature of excitable cells. So far as nerve is concerned, the channel remains open for a very short time (about 1 ms) during which about 100 sodium ions pass through. Then the gate appears to be inactivated, even though the membrane remains depolarized. The method of *inactivation* of the gate is not clear, probably a further configurational change. Whatever it is, the gate is refractory to further opening for a short period.

Ion channels are believed to be protein polymers floating in the lipid membrane of the cell, with subunits of the complex arranged to form an obstructed pore through the membrane. Appropriate ions or an applied voltage alter the architecture of the channel and this *conformational change* opens or closes the channel to allow passage of ions, atoms or molecules if they are sufficiently small, appropriately charged and have a concentration gradient across the cell membrane. The conformational change may be induced by specific chemical substances (hormones or neurotransmitters) attached to receptor sites. The

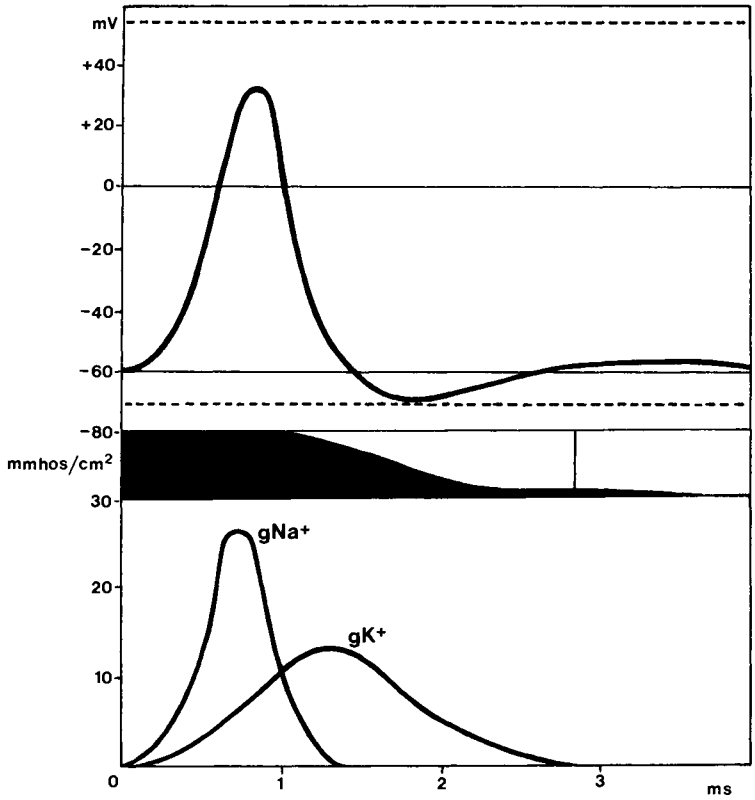

Fig. 1.2. *Upper*: Typical action potential recorded intracellularly. The dotted lines represent the sodium (*upper*) and potassium (*lower*) equilibrium potentials. The resting potential is −60 mV. *Lower*: The ionic conductances for sodium (gNa^+) and potassium (gK^+) across the cell membrane which generate the current responsible for the action potential. Between the graphs is an index of threshold to a second stimulus. During the first part of the action potential the membrane is completely unresponsive to a second stimulus (absolutely refractory). This is followed by a relatively refractory period. Under certain environmental conditions, a period of supernormality may follow the absolutely refractory period. Cyclical alternation of supernormality and subnormality leading to autorhythmicity occurs with low extracellular Ca^{++}.

architecture of each polymer is critical and may be distorted by changes in the fluidity of the lipid membrane in which it floats. Many lipid-soluble substances alter the fluidity and increase the rotational mobility of the protein structures, distort their architecture and permit increased passive diffusion of ions between the structures. Substances that do this act as local anaesthetics (*Fig.* 1.3).

Although the evidence is less satisfactory, it is likely that a similar voltage-dependent gate is opened at the internal end of the *potassium channel*. Depolarization of the membrane opens this a little later than the sodium channel and about ten times more slowly. Furthermore, the number of potassium channels per unit area of surface membrane is only about 10 per cent of the sodium channels (for nerve axon). For these reasons, the potassium conduction following depolarization is much slower than the sodium. Indeed it does not reach its peak until the sodium conductance has almost stopped, but it then continues for about three times the duration of the sodium conductance. While each gate is open the appropriate ion is transferred through its channel according to the concentration gradient, ie sodium passes rapidly into the cell and potassium flows out more slowly.

The sodium flux goes on until the sodium equilibrium potential is reached. As this takes place before significant K^+ loss has occurred, there is now a surplus of positive charges within the cell and the potential across the

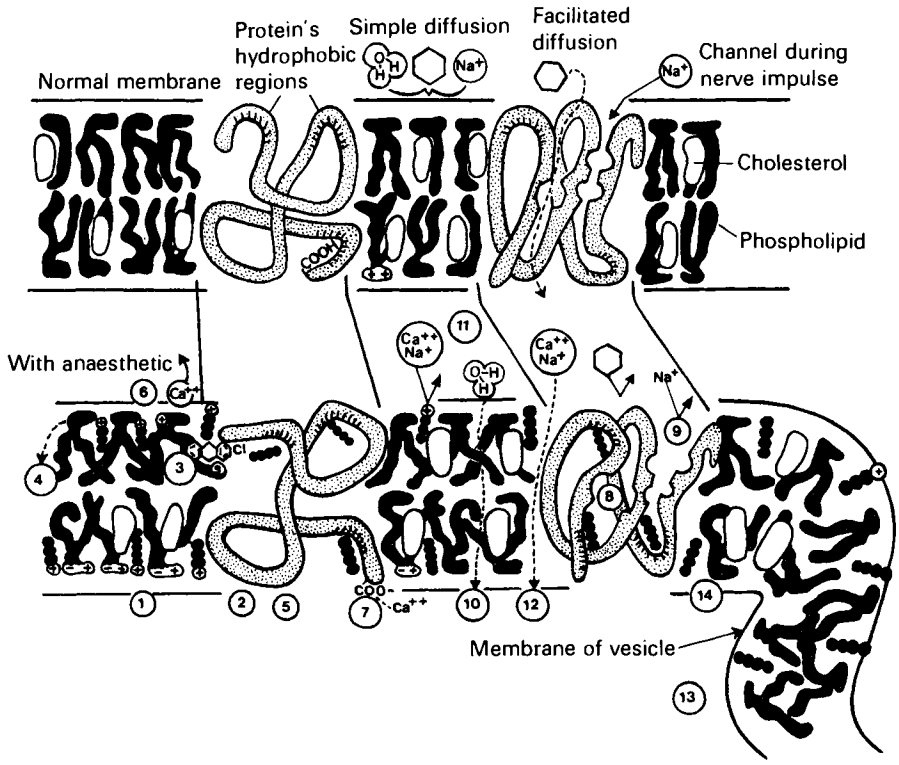

Fig. 1.3. Diagram of cell membrane showing some effects of drug occupation of membrane by anaesthetic agent. The lower diagram indicates: 1, drug occupation of membrane at low concentrations; 2, expansion of membrane with pressure alteration at higher concentrations; 3, increased rotational mobility of membrane units ('fluidization'); 4, decreased translocation of membrane lipids; 5, membrane enzymes activated or inhibited; 6, displacement of Ca^{++} by amine anaesthetics or; 7, increased membrane Ca^{++} by neutral anaesthetics; 8, diffusion of glucose and choline decreased; 9, ion exchange decreased during nerve impulse; 10, increased passive diffusion of water and urea; 11, decreased passive diffusion of ions by anaesthetic amines, or; 12, increased passive diffusion of ions by neutral anaesthetics; 13, increased exocytosis (neurosecretion); 14, dissociation of lipid-protein complexes at high anaesthetic concentrations.

membrane is reversed. Inactivation of the sodium channel while the potassium outflow is still increasing rapidly restores the potential to its resting value, but the potassium conductance continues beyond this stage so that the cell becomes hyperpolarized (*positive after potential*) until the resting state is restored by passive diffusion of potassium and active extrusion of sodium by the sodium pump. The relative proportion of potassium to sodium channels varies between tissues and species. The node of Ranvier of the mammalian myelinated nerve fibre is now believed to have many sodium but few potassium channels.

The other important cation is *calcium*. Although it plays a vitally important role in most physiological processes, the mechanisms involved are not well understood. At the excitable cell membrane, Ca^{2+} and (possibly Mg^{2+}) enters the cell by at least two channels. One of these is probably the Na^+ channel as Ca^{2+} entry is linked with Na^+. It occurs at the time of peak inward Na^+ current of the action potential and is blocked by tetrodotoxin. It is suggested that sodium–calcium exchange occurs and this channel is *voltage-dependent*. There is also at least one 'late' calcium channel (sometimes called the *slow channel*) which is not voltage-dependent and which may act as a second K^+ channel. These channels are still somewhat speculative and may not be represented in all excitable membranes. Evidence for their existence is mainly in invertebrate axons and in cardiac and smooth muscle, but they have been provisionally identified in the soma-dendritic area of vertebrate

motoneurones and autonomic neurones with α-adrenoceptors and it seems likely that they will also be found in axons, which show accommodation properties (*see below*). In muscle (striated and cardiac) the calcium channels are mainly in the transverse tubular system and sarcoplasmic reticulum.

The sodium–calcium linkage promotes outward diffusion of sodium from the cell and the 'late' calcium channel increases K^+ conductance, both processes tending to restore the membrane potential after depolarization and indeed temporarily to hyperpolarize the membrane immediately after the action potential. These 'stabilizing' effects will be discussed after the action potential proper. Further important actions of calcium ions, including the release of neurotransmitters at nerve terminals, will be discussed in the appropriate section (p. 24). The calcium flux does not contribute materially to the action potential except in special experimental circumstances. Modulation of voltage-sensitive Ca^{2+} channels is a probable mechanism for the known effects of certain neurotransmitters and neuropeptides (p. 36) on the duration of neuronal action potentials. Despite the considerable amount of calcium entering the nerve cell, the concentration of calcium ions in axoplasm is low as most is sequestered in mitochondria and endoplasmic reticulum and some is bound to protein (calmodulin). Calcium in axoplasm is required for axoplasmic transport. In muscle fibre it binds reversibly with specific sites on myofibrils, causing them to contract.

In summary, the rapid change of potential (the 'action potential') is due to opening of voltage-dependent channels with different time courses, and the role of the energy-dependent pump mechanism is to restore the resting potential. The amplitude and time course of the action potential depends on the ionic gradients and channel sizes and hence the action potential is an 'all or none' response to adequate ('threshold') depolarization of the membrane. Calcium plays an important role in restoration of the membrane potential.

PROPAGATION OF ACTION POTENTIAL

The facility with which ions cross the membrane determines its electrical resistance. Flow of charge through a resistance constitutes electrical current but this can only take place when a circuit is completed. Ionic charge is drawn from an adjacent region of the excitable cell and the circuit is completed through the neighbouring membrane, most readily at those parts where current can easily flow across it — ie at the next potassium channels. This, of course, depolarizes the adjacent membrane setting up an action potential there, and so the action potential is propagated in all directions away from the initial site of depolarization (*Fig.* 1.4). What happens next depends on the structure of the excitable cell and differs for nerve cell soma, axon, dendrites or muscle. Further differences between these cells, the effective stimulus for depolarization, will depend on peculiarities of membrane structure, including the presence of specialized receptor sites which, like the ionic channels considered so far, are specialized protein molecules traversing the lipid membrane.

Chemical substances with affinity for each of these sites may depolarize the cell or block evocation of an action potential (and hence block conduction of the impulse). It will be convenient to postpone discussion of drugs

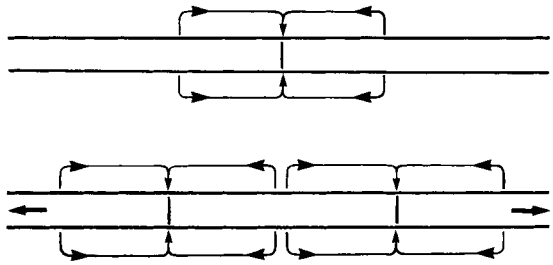

Fig. 1.4. *Orthodromic and antidromic propagation of a nerve impulse.* An area of depolarization of nerve membrane (*upper*) acts as a sink for current to flow into from neighbouring polarized membrane on each side. This creates depolarized areas which act as further current sinks and this change propagates in both directions (*lower*). The direction for normal transmission is termed 'dromic' and the other direction (e.g. towards the soma of a motoneurone) is 'antidromic'. An orthodromically conducted action potential spreads into collateral or terminal branches of an axon but an antidromic impulse starting in a branch tends to block at the confluence (T-junction effect). Action potentials will not spread retrogradely across a synapse, which conducts only in one direction, usually by release of a transmitter chemical by the pre-synaptic neurone which binds to receptors on the dendrites or soma of the post-synaptic neurone(s).

with affinity for receptors to the chapters on synapses and neuromuscular junction. Those that depolarize or block by action on the ion channels or the 'electrogenic' pump affect all excitable tissues to a greater or lesser degree. *Local anaesthetics* are believed to act by blocking activation of both sodium and potassium conductances; possibly ion channels are blocked only in the open state. The rate of rise, overshoot and rate of fall of the action potential are decreased and eventually depolarization is blocked. There are quantitative differences between nerve and muscle — the action being mainly on sodium conductance in nerve and on potassium conductance in muscle. The effects are transient.

A more complete block is produced by some naturally occurring toxins. *Tetrodotoxin* from the puffer fish (and certain other aquatic animals) has already been mentioned. Another is *saxitoxin*, a paralytic poison of equally high toxicity elaborated by a species of marine plankton (*Gonyaulax*) which may be so numerous in certain conditions as to cause a 'red tide'. Shellfish feeding in these waters concentrate the toxin and are highly poisonous. These biotoxins have a complex organic molecule. At one end of it there are one (tetrodotoxin) or two (saxitoxin) positively charged guanidium groups which can enter the sodium channel, but the main part of the molecule cannot do so and effectively plugs the sodium channel. (The effect is very specific and the affinity very high. Modern measurements of the number and distribution of sodium channels on an excitable membrane depend on the counting of isotopically labelled molecules of one of these paralytic poisons fixed to the surface of the cell.) A related molecule, guanidine hydrochloride, has its main effect on the cell membrane of cholinergic nerve terminals where it facilitates the release of acetylcholine (p. 19).

Anions

The most important anions within the cell are the comparatively large organic ions (eg glutamate, aspartate and organic phosphates), too large to move freely across the membrane and hence not contributing to the action potential but playing a role in the resting potential of the cell. On the other hand, the cell membrane is so freely permeable in both directions to chloride ions that their movements play no more than a negligible role during the action potential, the net flux being very small. Chloride shift out of the cell plays a greater part in the repolarization of the membrane. When the external sodium concentration is lowered (passively followed by the internal sodium) the action potential takes longer to subside to normal (*see* negative after potential, p. 4). During this time the membrane is more readily depolarized by a further stimulus and repetitive firing may occur, especially if external potassium is also low. A similar membrane hyperexcitability occurs if there is reduction in the permeability of the resting membrane to chloride. It appears that this abnormality is the cause of the membrane hyperexcitability of human myotonia congenita and that of goats with hereditary myotonia. The chloride permeability of cell membrane is also reduced by monocarboxylic aromatic acids and by certain sterols such as 20,25-diazocholesterol. Potassium and chloride conductances of mammalian muscle are both reduced by denervation. This reduces the critical membrane depolarization required to produce an action potential and reduces the repolarization electrolyte shifts, causing fibrillation potentials. No doubt similar spontaneous activity is generated in dying neurones.

Myotonic activity is reduced by procainamide, phenytoin or quinine as well as by reducing extracellular potassium.

Membrane stabilizers

The action of local anaesthetics such as procaine in blocking activation of the gate of the sodium channel (p. 3) has been described as 'membrane stabilization'. Phenytoin has a highly complex action, including stimulation of the sodium pump (driving Na^+ out of and K^+ into the cell) and increasing the availability of high-energy phosphates. The effects of quinine and quinidine are grossly similar to those of the local anaesthetics but their actions have not been identified at the molecular level. Cardiac glycosides (like ouabain) and aglycones (like strophanthidin) act specifically to inhibit Na^+

and K^+ transport. It is possible that this action is on the sodium–potassium pump ATPase which is sensitive to cardiac glycosides. This enzyme is also inhibited by calcium ions but only when applied intracellularly.

The role of *calcium* as a membrane stabilizer is still controversial. One theory is that it occupies and neutralizes negative charges of adjacent molecules in the membrane. The external calcium ion concentration alters the dependence of the membrane permeability channels on the membrane potential, as described above. Decreasing the external calcium ion concentration facilitates depolarization and increasing it facilitates hyperpolarization. During the action potential there is an increased influx of calcium. Since almost all of the intracellular calcium is bound, a large influx during the action potential could cause a significant change in the amount of 'free' Ca^{2+} within the cell. It could be speculated that it might play a role in activating the K^+ channel gate. It has additional important actions in muscle cells.

It is perhaps misleading to use the vague term 'membrane stabilization' to cover all of the effects described in this section. They are certainly not identical. Procaine, for instance, reduces the maximum Na^+ and K^+ permeability without any great effect on the rate processes, whereas Ca^{2+} ions located on the cell surface reduce excitability by altering dependence of the rate processes on the membrane potential without affecting maximum Na^+ and K^+ permeabilities. To put it another way, high calcium ion concentration on the surface of the membrane opposes the increase in potassium conductance associated with small depolarizations and hence the explosive self-regenerative action potential mechanism is less likely to follow. Conversely, with low calcium at the cell surface the action potential mechanism is evoked by quite small depolarizations of the membrane — ie it is more excitable. Since at the time the action potential is prolonged and the negative after potential (p. 4) may still be significant after inactivation of the Na^+ channel gate has subsided, the slightly depolarized membrane again swings into the action potential phase, and so on repetitively. The normal process, termed *accommodation* (p. 10), is defective. This 'oscillation' of the membrane means that a single stimulus is followed by a train of action potentials and, if the calcium level at the cell surface is sufficiently low, by 'spontaneous' trains of action potentials. These are the basis of the sensory and motor phenomena of *hypocalcaemic tetany*. The same mechanism is invoked if the calcium ions are competitively displaced by certain other divalent cations, as in hypermagnesaemic tetany. The effect is most obvious on nerve axons. Muscle is also affected (eg the prolonged Q–T segment of the ECG), but the clinical manifestations of tetany are due to repetitive firing of peripheral nerve fibres. Latent tetany can be identified by measuring the time constant of accommodation (p. 10). Further lowering of the extracellular calcium is accompanied by failure to produce action potentials.

ANOXIA OF EXCITABLE CELLS

A similar decrease of accommodation, membrane instability and repetitive firing of action potentials going on to failure is caused by anoxia of excitable cells. This is the basis of paraesthesia or tetanic cramp when a sphygmomanometer cuff is applied, preceding loss of excitability and of conduction. Similar spontaneous phenomena are associated with hypoxia of the central nervous system. The sodium–potassium pump ATPase mechanism is oxygen-dependent. Its failure is preceded by an inability to restore membrane potential by extrusion of sodium ions. The effects of low calcium and hypoxia are so similar as to suggest a common site of action. They are additive in the Trousseau sphygmomanometer test used to diagnose latent tetany. The calcium effects are ionic, so there is no paradox in the fact that tetany may be provoked by hyperventilation, in which the respiratory alkalosis causes reduced ionization of extracellular calcium.

So far as we know, the sodium–potassium pump is the only 'electrogenic' mechanism that is oxygen-dependent. Calcium is also required for the resynthesis of ATP. There are other metabolic pumps which exchange cationic species across the membrane (no net gain in charge) and so are 'non-electrogenic'. They also require oxygen and calcium but their

malfunction is not recognizable as excitability changes.

There is an optimal level for extracellular calcium. Above this level, the accommodation of excitable tissues becomes so great that it becomes increasingly difficult to depolarize them. In human disease *hypercalcaemic states* cause hypotonia, muscular weakness, loss of reflexes and drowsiness. Calcium is less well tolerated after pretreatment with narcotics and rapid injection intravenously may cause falls in blood pressure and pulse rate. The action of cardiac glycosides on heart muscle is potentiated.

CALCIUM ANTAGONISTS

A number of substances block the entry of calcium into cells with slow calcium channels. In mammals these are mainly cardiac and smooth muscle cells and their effect is similar to that of reducing extracellular calcium — prolongation of the action potential and negative inotropic effect on heart muscle, and decrease of smooth muscle tone. These actions have some use in the treatment of angina pectoris, hypertension and supraventricular tachycardia. Examples are nifedipine, verapamil, lidoflazine and perhexiline. In therapeutic doses they have no significant effect on nerve membrane or skeletal muscle. Dantrolene sodium reduces the intracellular calcium in skeletal muscle, probably by an action on the membrane of the transverse tubules and possibly the triad junctions, so inhibiting the inward movement of '*triggering calcium*', which would normally release '*activator calcium*' from the sarcoplasmic reticulum. This is a large flux of calcium ions which diffuse to the adjacent myofibrils and bind to the troponin situated on the actin filament, a combination which is considered to cause a conformational change of the actin filament resulting in exposure of active sites on the actin molecule at which a reaction with cross bridges of myosin filaments occurs, leading to muscle contraction by a ratchet mechanism. This intracellular calcium flux is an essential part of the link between action potential and contraction of the muscle fibre (*excitation–contraction coupling*). The active sites are probably ADP molecules. The energy source for muscle contraction is ATP which is believed to combine with the head of each myosin cross bridge which then tilts and draws the actin filament which shortens the muscle fibre. The energy is provided by hydrolysis of the ATP (to ADP and phosphate ion) and so the head of the bridge returns to its normal position until further ATP is supplied (by creatine phosphokinase acting on ADP plus creatine phosphate) to initiate another cycle. The triggering calcium is reabsorbed into the sarcoplasmic fluid by an energy-dependent calcium pump in the walls of the longitudinal tubules and this ends the muscle contraction.

Failure of the reabsorption mechanism would cause persistent contraction of muscle fibre without further action potentials. This is termed *contracture*. It may be caused by potassium or caffeine and possibly by some anaesthetics (notably halothane), especially if there is a genetic defect of the sarcoplasmic regulation of calcium in muscle as in the rare muscular disease associated with malignant hyperpyrexia in man and pig. The hypermetabolism associated with the contracture causes oxygen desaturation despite increased oxygen uptake, respiratory acidosis followed by severe metabolic acidosis (as the hypoxic muscle changes to anaerobic metabolism) and leakage of creatine phosphokinase, potassium and myoglobin from the muscle cell into the bloodstream. The hypermetabolism rapidly raises body temperature to a dangerous level. The train of events can be arrested by inhibiting trigger calcium inflow into the skeletal muscle cell with dantrolene sodium, resulting in a decreased release of activator calcium in the terminal cisternae. Oral dantrolene may be of some prophylactic value before anaesthesia in known cases but treatment requires intravenous administration (10 mg/kg). There is a slight calcium-antagonist effect on smooth muscle (vascular and intestinal) but apparently only weak inotropic effect on heart muscle and no detectable effect on nervous tissues.

The train of events leading to contracture may be initiated by anything causing depolarization of muscle. This includes potassium, inhalational anaesthetics and depolarizing myoneural blocking drugs such as succinylcholine.

Membrane constants

The discussion on permeability of cell membranes to charged ions passing through narrow channels or exchanged by a metabolic pump has indicated that the membrane has 'resistance' in the electrical sense so that the basic laws of electricity flowing through chains of resistances may be applied. Additionally, the more extensive bilipid non-conducting part of the membrane has layers of ions on each side, ie space charges separated by an insulator. It therefore also has the properties of a condenser (capacitor). Since it has both capacity and resistance in parallel, any small potential change across the membrane (depolarization or hyperpolarization) will tend to decay exponentially to the normal resting potential. The time to decay to 1/e of the initial value is the electric *time constant* of the membrane.

The membrane resistance separating the central conducting core of the cell from the external conducting medium also gives it the properties of a cable, especially with elongated cell (fibres). If a steady potential change is produced at one transverse zone of the fibre (nerve or muscle) there is a distribution of current along its length. Steady current cannot easily pass through the membrane capacity so the through membrane current (and hence the membrane potential) will drop with increasing distance from the applied voltage. It decays exponentially according to a formula which contains a factor termed the *length constant*. The time constant and length constant of nerve and muscle fibre are such that they make inefficient cable conductors. The excitable cell tends to 'ignore' a continuous stimulus but responds maximally to an abrupt one. Less 'powerful' (eg lower voltage) stimuli have to be applied for a longer time to allow depolarization to reach the critical value (*threshold*). For this reason there is a *strength–duration curve* related to the membrane time constant, and the curve changes in degenerating neurones. Because there is a threshold for activation of the self-regenerative Na^+ conductance, even a stimulus current of infinite duration has a finite minimum strength (*rheobase*) which excites at the end of a finite maximum *utilization time*. For transmission of a signal over long distances some 'signal boosters' are required — the nodes of Ranvier and synapses at which the signal strength is renewed.

Refractoriness and accommodation

The voltage change which activates sodium and potassium conductances across the excitable membrane rapidly terminates the self-regenerative process by inactivating the sodium current, while potassium efflux continues (resulting in after-hyperpolarization). Both processes make the cell refractory to an immediately following depolarizing stimulus. During the sodium channel inactivation there is a *completely refractory period*, followed by a *relatively refractory period* of hyperpolarization.

Sodium inactivation occurs during the passage of an electric current, even if this is insufficient to cause the channel activation response. Thus a brief subthreshold stimulus increases excitability and lowers the threshold to a further stimulus following shortly, but a prolonged subthreshold stimulus raises the threshold to a following brief stimulus because the sodium channel inactivation and potassium efflux are dominant. In old terminology, the excitable cell is said to 'accommodate' to the passage of current through its membrane, regardless of whether it activates the sodium channel or not. If a stimulating current increases (linearly or exponentially) over a brief period but not instantaneously, there is a relation between the slope of current rise and its final exciting strength. There is a minimal current gradient. Currents rising at less than this critical rate will never excite. The rising threshold proportional to rate of rise of current is specified by a *time constant of accommodation*. Note that it is not the same as adaptation (p. 57) or habituation (p. 165). There is a limit to accommodation. It breaks down at a certain current level at which the excitable cell responds repetitively as long as the current is applied.

A consequence of accommodation is that — at threshold intensity — a constant current stimulates at the cathode when the circuit is completed (*'make' excitation*) but no repetitive firing occurs while current flows. When the current is stopped suddenly, a *'break'* excitation occurs at the anode. However, if there is

no accommodation, repetitive excitation occurs at the cathode as long as current continues to flow. When the external Ca^{2+} concentration is reduced or the excitable cell is made hypoxic, the accommodation is reduced and the breakdown of accommodation occurs at a lower level. Slowly rising stimuli excite at a threshold level little above that for instantaneously rising stimuli. At extreme, with accommodation abolished (as in peripheral nerve in severe tetany) the fibre continues to fire iteratively so long as the stimulus continues, or it even fires spontaneously. The rate of firing depends on the duration of refractoriness following each action potential. These factors of excitability, accommodation and recovery phenomena are fundamental to an understanding of the production of trains of action potentials ('spikes') by long-acting depolarizing stimuli, such as transmitter action at synapses or any other 'generator potential' as at sensory transducers.

Excitability, refractoriness and accommodation are not uniform throughout the cell. A typical *nerve cell* consists of a nucleated cell body (*soma*), with afferent fibres (*dendrites*) and an *axon hillock* leading into a more or less elongated axon which commonly branches at its distal end, each branch ending in a specialized knob-like structure containing vesicles of transmitter substance. The excitability factors just described vary considerably. The nerve cell dendrite–soma–axon hillock part is more excitable than the axon and terminal and its accommodation breaks down readily. Even the axon has a gradient of excitability and accommodation, with excitability and conduction velocity decreasing and accommodation increasing from proximal to distal. Finally, at the *nerve terminal* there is a specialized role for Ca^{2+} with respect to release of transmitter substance when the terminal is depolarized. Thus, spontaneous discharges are more likely to start in the proximal part of the neurone and to fire repetitively. Indeed, special inhibitory controls, such as the *Renshaw loop* (p. 244), are required to limit this tendency. When a neurone becomes metabolically deranged, this form of excitability tends to spread distally along the axon (a possible mechanism for the production of fasciculation in muscle). Long nerve fibres accommodate better than short ones, and motor neurones better than sensory neurones or muscle fibres. Clearly these regional variations must be correlated with the ultrastructure of the cell membrane, possibly the distribution of ion channels. For instance, in a myelinated nerve fibre the sodium channels are numerous at the nodes of Ranvier and relatively sparse in internodal segments.

Conduction velocity

We have already seen that regarded as a conductor of electricity, even a nerve fibre is a poor cable because its membrane is leaky and it has a space factor which makes the potential across the membrane drop in a rather short distance from the activated area. However, if there are ion channels within a suitable distance, the flow of current in the local circuits through adjacent membrane is sufficient to activate the voltage-dependent sodium channels and so induce the self-regenerative mechanism which starts off the action potential at full amplitude. The analogy of a spark passing along a line of gunpowder particles is a better analogy than an electric cable. The rate of conduction is, similarly, rather slow (about 10–15 m/s) and dependent on the diameter of the fibre (velocity is approximately proportional to fibre diameter). This is the situation in unmyelinated nerve fibres. Rapid conduction in unmyelinated fibres is only possible if the fibre diameter is very large (as in the giant axon of the squid).

In myelinated nerves the *myelin sheath* (*Fig.* 2.1) provides further insulation and local circuits can only be completed through the 'bare' *nodes of Ranvier* separating the myelin segments. Each segment is laid down by a single cell (the Schwann cells in peripheral nerve, the oligodendrocytes in the central nervous system). These nodes concentrate the electrical current density and, as just described, direct it to areas of membrane with high concentration of sodium channels. The next action potential is therefore generated at the next node (*Fig.* 1.5). The action potential jumps from node to node (*saltatory conduction*) (*Fig.* 1.5). This considerably increases the conduction velocity without requiring uneconomically large diameter axons. The fastest motor and sensory fibres in human peripheral

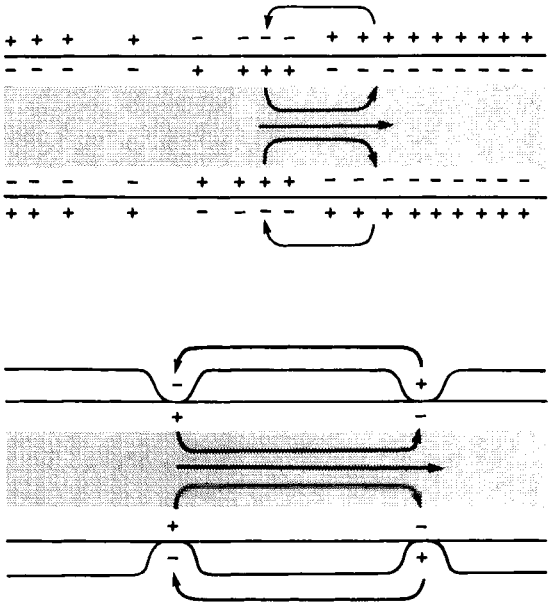

Fig. 1.5. Propagation of nerve impulse in a non-myelinated and myelinated nerve fibre (*lower*). The conduction velocity is increased in the myelinated fibre by saltatory conduction from one node of Ranvier to the next. A stimulus applied at a point on the fibre will propagate both ways (orthodromic and antidromic) but must then continue in that direction as it vacates a zone with inactivated sodium channels (absolutely refractory). Similarly, if two stimuli are applied at separate zones they will each evoke an action potential. These will eventually meet at a refractory zone and will not continue beyond it (occlusion).

nerve conduct at 50–85 m/s. The velocity depends mainly on (i) fibre diameter and (ii) internodal distance, and these factors are not constant throughout the length of a nerve. Conduction velocity is slower distally. Disease processes that decrease fibre diameter or, more importantly, decrease the internodal distance, will decrease the velocity of conduction.

Activation and inactivation of the ion channels vary with the ambient temperature. Raising the temperature increases conduction velocity until a temperature is reached at which impulse block occurs (about 46°C). Conversely, lowering the temperature slows conduction velocity but it also increases excitability and lowers accommodation (probably the mechanism for the tetany-like rigidity of muscle in hypothermia). These temperature effects are important in measuring conduction velocity of nerves for diagnostic purposes, and are also responsible for the clinical deterioration caused by overheating patients with multiple sclerosis. The initial beneficial effect on conduction velocity of raising the temperature is due to a more rapid increase of outward potassium current during the generation of the action potential, but inward sodium current at the node is also decreased and this ultimately blocks conduction.

Anaesthetics, general and local

A definition of an anaesthetic is 'a drug that directly blocks the membrane action potential without appreciably affecting the resting potential'. Many lipid-soluble substances conform to the definition and the potency of an anaesthetic is directly proportional to its oil/water partition coefficient (Meyer–Overton rule). The anaesthetic drugs electrically stabilize the membranes of excitable cells, partly by changing the fluidity of the membrane; it swells, becomes more fluid and its components are disordered, with modification of its enzyme activities. Membrane-bound Ca^{2+} may be increased or displaced and the transmembrane ionic fluxes may be increased or decreased according to the anaesthetic species. Neutral anaesthetics increase the passive fluxes of ions, amine anaesthetics decrease them. In some instances the effect is greatest in small fibres such as the pre-synaptic region so that transmission block occurs there additionally. Furthermore, certain drugs selectively block specific receptors. These factors, plus local morphological characteristics, cause regional or local predilection to blocking by some drugs.

Compound action potential

If all nerve fibres were identical, stimulation of a bundle of fibres would cause a summed action potential with amplitude proportional to the number of fibres stimulated, but with rise time and fall time, and conduction velocity identical with the action potential of one of the fibres. In practice, the fibres differ at least in diameter, internodal distance and myelin thickness. For these reasons alone the compound action potential is not a simple multiple of any single fibre potential and its components

propagate at different rates along the bundle of fibres, so increasing the discrepancy in recordings made at a distance (*Fig.* 1.6). The problem of interpretation is increased by the fact that neither of the recording electrodes can be intracellular for all fibres. Extracellular recording must be carried out with the fibres in an electrolytic medium (tissue fluid or an organ bath). Interpretation of differences of potential between two points in a conducting medium requires understanding of the properties of a *volume conductor*. In practice all biological potential measurements made in intact man have this limitation. Furthermore, measurement involves potential *difference* and this implies two electrodes. One of these can be made relatively 'indifferent' by siting it sufficiently far from the 'active' electrode (so called monopolar recording) or by placing it at a permanently depolarized area (injured cells). A discussion of the principles of volume conduction would be out of place in this book, but any reader intending to make a detailed study of action potentials must study this subject and in reading original papers it is essential to identify the type of recording used. For didactic presentation it is simplest to describe the compound nerve action potential as recorded monophasically (as nearly as possible 'monopolar').

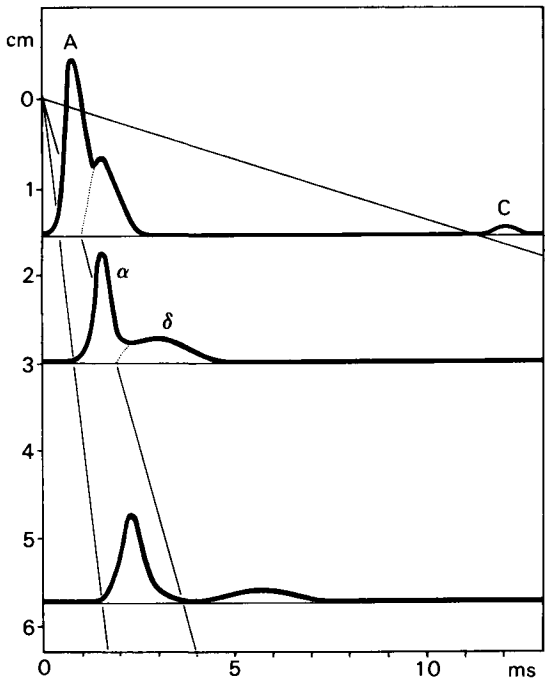

Fig. 1.6. Compound action potential of sural nerve. Diagram to illustrate dispersal with increasing distance from stimulating cathode. (In fact, C fibre potential would be at 30 ms or later in the top trace.) Progressive asynchrony, dependent on differing conduction velocities of fibre groups, causes each peak to widen and drop in amplitude. The diagrams are based on monopolar recording of a moist nerve in air. Each wave would be triphasic if recorded in a volume conductor. The compound wave is the summed potential at the site of the recording electrode. *In vivo*, the A_δ and later peaks can be identified by computer averaging of numerous responses.

The compound action potential recorded very near the site of stimulation has a single 'spike' with a hump on its falling phase. If the recording is made progressively further from the source, the 'spike' decreases in amplitude and the hump separates off into a separate wave, increasingly separated in time as conduction distance increases. Clearly the second wave is formed by action potentials from fibres conducting more slowly than those contributing to the initial 'spike'. With appropriate amplification and conduction distance it becomes clear that the 'compound action potential' is the algebraic summation of unit action potentials which differ in threshold, amplitude, duration and velocity of conduction (*Fig.* 1.6). These differences reflect the differences in diameter, myelination and internodal distances of the fibres and so it is possible to characterize the fibre type by the place where its action potential appears in the compound potential. There have been several nomenclature systems (requiring care in reading earlier literature). Unfortunately there is no universally agreed terminology related to fibre diameter and conduction velocity. The following terms in common usage depend on the type of nerve studied and so this must also be identified by the reader.

An estimate of fibre diameter may be obtained by dividing the conduction velocity by a factor of 6 (large myelinated fibres) or 4·5 (small myelinated fibres), bearing in mind that the nerve fibre is not a true cylinder but has longitudinal grooves. The different conversion factors make it difficult to determine the spectrum of fibre diameters from a compound action potential. Conduction velocities shown in *Table* 1.1 are mainly derived from cat and

Table 1.1

Category	*Group*		*Fibre type*	*Conduction velocity (m/s)*	*Function*
Cutaneous afferent	Aα	II	Myelinated	20–100	Mechanoreceptors
Joint afferent	Aδ	III	Myelinated	4–30	Mechanoreceptors, cold, first pain
Visceral afferent	C	IV	Unmyelinated	2·5	Warm, second pain, post-ganglionic autonomic
Muscle afferent	A	Ia	Myelinated	50–125	From spindle primary
		Ib	Myelinated	50–120	From Golgi tendon organ
		II	Myelinated	24–71	From Golgi tendon organ
		II	Myelinated	20–70	Spindle secondary
		IV	Unmyelinated	2·5	Muscle nociceptors
		III	Unmyelinated	5–40	Muscle nociceptors
		IV	Unmyelinated	2·5	Muscle nociceptors
Efferent	Aα	I	Myelinated	41–120	Skeletomotor
	Aβ	II	Myelinated	50–85	Skeletofusimotor
	Aγ	III	Myelinated	10–40	Fusimotor
	B	III	Myelinated	3–15	Preganglionic autonomic

The efferent fibres are classified α–γ according to Leksell, muscle afferents I–IV according to Lloyd and other afferents (A–C) according to Erlanger and Gasser (their original A_{β} and $_{\gamma}$ groups, based on analysis of the compound action potential, were shown to be recording artefacts). Modern classification based on receptor of origin has not yet established a unified system of nomenclature but approximate equivalents are shown.

primate studies but are probably representative for human nerves.

The different types of nerve fibres have some important consequences for the anaesthetist as they have differing susceptibility to anoxia and to local anaesthetics. Conductivity of sensory fibres is blocked in the following order by compression with sphygmomanometer cuff B, Aα, Aδ (ie preganglionic autonomic, then large followed by progressively smaller myelinated somatic fibres). Similar sized motor fibres are blocked at the same time as afferent fibres (skeletomotor before fusimotor). Note the relative resistance of unmyelinated C fibres, important in conduction of pain impulses. (The literature on this should be consulted as various accounts of order of susceptibility are inconsistent.) With local anaesthetics the small myelinated sensory and (gamma) motor fibres fail first, followed by large myelinated fibres, as with compression block, but the order of unmyelinated C fibre blockade appears to depend on anatomical factors such as whether the nerve is sheathed (peripheral nerve) or unsheathed (posterior roots) and this factor confuses interpretation of many experimental studies on excised nerve. The susceptibility to cold block appears to be substantially the same as to cuff compression and local anaesthetics. (Note: the situation is rather different with tourniquet compression which damages the Schwann cell–myelin sheaths in the paranodal zones.)

Interaction between nerve cells and satellite cells

There is experimental evidence that glial cells are not necessary for production of action potentials by neurones but it has been suggested that they act as a buffering mechanism to limit local concentrations of K^+ outside nerve membranes producing action potentials and at the same time they accumulate potassium which would otherwise diffuse away from the nerve cell. This is a controversial subject. The glial cells, especially astroglia, probably

regulate the extracellular fluid of the central nervous system to a constitution typified by cerebrospinal fluid rather than blood plasma. The blood–brain barrier formed by capillary endothelial cells (Chapter 27) buffers the nerve cells and glia from changes in the chemistry of the blood involving large molecules, but small molecules can penetrate the CNS rapidly from the blood. Nevertheless the CSF varies little in its pH, P_{CO_2} and HCO_3 and this regulation may be effected by glial cells. This is an active metabolic regulatory process rather than a barrier function.

There is also controversy regarding the suggestion, based on ultramicroscopic evidence of small vesicles in some glial cells, that they mediate exchange between the nerve cells and intercellular space by pumping material from one surface of the glial cell to the other. It is also suggested that glial cells prepare substrates for the nerve cells, acting as energy donors to them.

There is little doubt that astrocytes are not merely structural scaffolding for the central nervous system. Microglia are the histiocytes of the central nervous system, constituting a non-haemic source for macrophages and for production of antibodies. The main satellite cells are the oligodendrocytes in the CNS and the Schwann cells in the peripheral nervous system. These are intimately wrapped around all axons. Around the larger axons they wrap multiple layers of myelin, one satellite cell to each node. The insulating effect and contribution to saltatory conduction of the nerve impulse have been described above. Small fibres without myelin coverage are also infolded into oligodendrocytes or Schwann cells, often several nerve fibres to one satellite cell. The thickness of the myelin sheath is not proportional to the diameter of the axon and the ratio varies from one nerve to another. In statements about the relation between diameter and conduction velocity of myelinated nerve fibres the diameter refers to total diameter (including myelin).

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Chapter 2
Neuromuscular junction

STRUCTURE

During development, or on reinnervation, a number of motor nerve terminals may form junctions on each skeletal muscle fibre but soon all disintegrate except one. Polyneuronal innervation is exceptional in human muscle. Within a depression on the surface of the muscle fibre, termed the 'soleplate area', each terminal axon divides into several terminal branches which lose the myelin sheath but remain covered by a continuation of the Schwann cell sheath except on the surfaces facing the receptor surface ('subneural region') on the muscle from which it is separated by a *primary cleft* of about 50 nm (500 Å) width (*Fig.* 2.1). In mammals the terminal branches end in knobs which are normally grouped closely together in a *'plaque'* applied to a discrete region of a muscle fibre. *Terminations 'en grappe'* do not occur in man, with the possible exception of extraocular muscle.

The subneural apparatus does not follow the contours of the nerve terminals but is thrown into folds (*secondary clefts*) radiating out from the sides and deep surfaces of the synaptic gutter or *primary synaptic cleft.* This is not a structure for providing an extensive receptor surface, as formerly thought. *Acetylcholine receptors*, identified by isotope or other labelling of alpha-bungarotoxin, which has a marked binding affinity for these receptors, are restricted to the crests of the sub-synaptic folds (*Fig.* 2.2) and only the mouths of the secondary clefts. There is considerable evidence for cholinergic receptors on the pre-synaptic membrane (nerve terminals) with a modulatory role in neuromuscular transmission. The receptors are conceived as integral membrane proteins with binding sites for acetylcholine which regulate the opening and closing of channels which traverse the membrane, the whole having a structure shaped like a doughnut. The ligand-activated channel allows passage of ions through the otherwise impermeable post-synaptic membrane.

The molecular structure of the depths of the secondary clefts remains unknown. They contain an amorphous surface material which continues into the primary synaptic cleft. Its nature and function is unknown. It may derive from vesicles described by some authors in the sarcoplasm of the muscle soleplate. The subneural apparatus and the basal lamina in the primary and secondary clefts contain *acetylcholinesterase*, an enzyme which hydrolyses the neurotransmitter acetylcholine. It is not confined to the motor endplate and its exact site of production is unsettled.

FUNCTION

The neurotransmitter acetylcholine is synthesized from choline and acetyl coenzyme A and stored in the distal part of motor nerves. The reaction is catalysed by the enzyme choline acetyltransferase which originates in the soma of the cell and travels down to the nerve terminal by axoplasmic flow. The flow rate is greatly exceeded by the rate of release of transmitter at the nerve terminal so some storage mechanism is required to provide a reserve of transmitter near the release sites.

Coenzyme A (CoA) is acetylated with energy which is usually supplied by glucose and adenosine triphosphate. The enzyme acetyltransferase transfers the acetyl groups to choline to form *acetylcholine* (ACh) which is then stored until required. The mechanism is probably the same as in sympathetic ganglia in