Neurotransmitters, Drugs and Brain Function.
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2 Control of Neuronal Activity

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The task of nerve cells is to communicate. They do this using a combination of electrical signals (action potentials) and chemical signals (transmission). However, even the chemical signal has to be transduced to an electrical signal (the synaptic potential) in order to continue the process of communication from one neuron to another. Information is then coded in the frequency and pattern of action potential discharges. This chapter considers the question of how these electrical signals are generated and how their frequency and discharge patterns can be regulated.

THE RESTING STATE: ION GRADIENTS, PUMPS AND POTENTIALS

The electrical signals are carried by the movement of charged ions across the cell membrane. This makes use of the potential energy stored across the cell membrane in the form of *ionic gradients*. Concentration gradients for the principal ions across a typical nerve cell membrane are indicated in Fig. 2.1(a). The cell interior has a high concentration of K^+ ions and a low concentration of Na^+, Cl^- and Ca^{2+} ions relative to the exterior.

The ionic gradients themselves are generated by ion 'pumps' (carriers) (Fig. 2.1(b)). Thus, the Na^+/K^+ exchange pump (Na^+/K^+ ATPase) in the outer membrane generates the primary Na^+ and K^+ gradients across the cell membrane. Other pumps (a Ca^{2+} ATPase and/or a Na^+/Ca^{2+} exchange pump) generate a high concentration gradient for Ca^{2+} ions. These pumps consume energy (in the form of ATP). It has been estimated that about 40% of the oxygen consumption of the brain is used to drive the Na^+/K^+ exchange pump.

There is also an *electrical gradient* across the membrane. At rest, the normal value of this potential (E_{rest}) in most nerve cells is around -70 mV (inside $-$ve). In general, the ion pumps themselves are not directly responsible for this (though they can contribute, since they are not electroneutral). Instead, it is due primarily to the passive diffusion of K^+ ions back out of the cell down the chemical concentration gradient previously set up by the Na^+/K^+ exchange pump, leaving a small +ve charge deficit on the inside of the membrane. However, if K^+ were the only ion involved, then, from the K^+ concentration gradient, the *Nernst equation* predicts that the membrane potential should be about -90 mV:

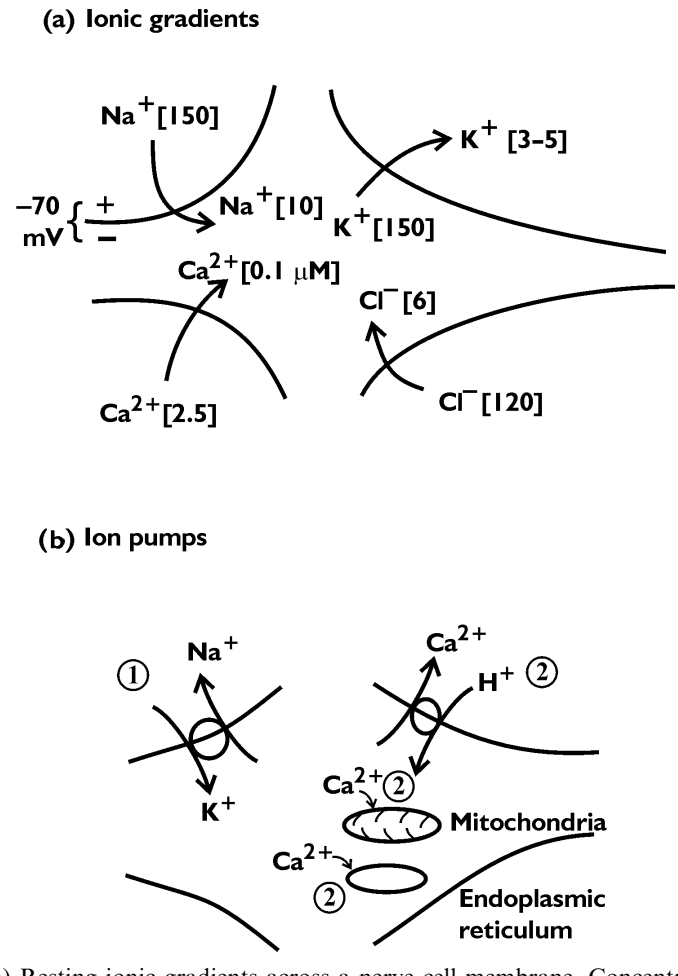


Figure 2.1 (a) Resting ionic gradients across a nerve cell membrane. Concentrations [ ] are in mM (except intracellular Ca^{2+}, in μM). Arrows show the direction of the electrochemical gradients for passive ionic movement. (b) Principal active ion pumps. (1): plasmalemmal Na^+/K^+ ATPase. (2) Ca^{2+} ATPases

$$E = RT/zF \ln([K^+]_{out}/[K^+]_{in})$$

This is the *equilibrium potential* for K^+ ions (E_K), i.e. the potential at which the electrical gradient pulling K^+ into the cell just balances the chemical concentration gradient forcing K^+ out of the cell. The 20 mV difference between E_{rest} and E_K is usually explained by assuming that the membrane is also slightly permeant to some other ion with a more positive equilibrium potential, such as Na^+. The membrane potential is then given by the *Goldman–Hodgkin–Katz (GHK)* or *constant-field equation*:

$$E = RT/zF \ln\{([K^+]_{out} + \alpha[Na^+]_{out})/([K^+]_{in} + \alpha[Na^+]_{in})\}$$

where α is the ratio of the permeability of Na^+ ions to that of K^+ ions (P_{Na}/P_K). The GHK equation then predicts a value of −70 mV for E_{rest} if the permeability of the membrane to Na^+ ions is about 4% of that to K^+ ions ($P_{Na}/P_K = 0.04$). However, it

should be noted that, at this potential, although the fluxes of total cations in and out of the cell are equal, the cell will gradually accumulate Na^+ and lose K^+, which will have to be corrected by the Na/K exchange pump; since this involves energy expenditure, it is not a true equilibrium state. The GHK equation can be expanded to include terms for other ions, such as Cl^- ions, which can have a profound effect on the membrane potential under certain circumstances (e.g. during the activation of Cl^- channels by inhibitory neurotransmitters).

ION CHANNELS

In fact, a nerve cell membrane is not really 'permeant' to ions at all, in the sense that ions cannot diffuse across the lipid bilayer, since they cannot dissolve in the membrane lipids. Passive diffusion occurs entirely through *ion channels* — pore-forming membrane proteins. The resting potential may then be generated either by two sets of channels, one set permeant to K^+ and the other to Na^+ (or some other ion with a more positive equilibrium potential), with the former in the majority or opening more often; or a set of channels primarily permeant to K^+ but with some weak permeability to Na^+. Recently, a special class of 'resting' K^+ channels, the 'twin-pore' TASK channels, has been identified in some neurons, thus supporting the former proposition. Another class of K^+ channels that can contribute to the resting potential of neurons are inwardly-rectifying K^+ channels (Kir channels) — so-called because they conduct K^+ ions more readily into the cell than outwards.

Both TASK and Kir can be inhibited by certain neurotransmitters which act on receptors that couple to phospholipase C-activating G-proteins, such as acetylcholine, substance P and TRH. As a result, these transmitters can induce a sustained depolarisation of the receptive neurons (e.g. cerebellar granule cells and motor neurons by TASK-inhibition, basal forebrain neurons by Kir-inhibition). Several transmitters, acting on receptors coupled to other G-proteins of the Gi/Go family, are capable of *activating* another class of Kir channels (G-protein-gated inward rectifiers or GIRK channels, also known as Kir3), thereby *hyperpolarising* the neuron (and inhibiting it).

Other ion channels are closed at rest, but may be opened by a change in membrane potential, by intracellular messengers such as Ca^{2+} ions, or by neurotransmitters. These are responsible for the active signalling properties of nerve cells and are discussed below (see Hille 1992, for a comprehensive account). A large number of ion channels have now been cloned. This chapter concerns function, rather than structure, and hence does not systematically follow the structural classification.

THE ACTION POTENTIAL (Fig. 2.2)

This is the basic unit ('bit') of information processing in the nervous system. It is a transient electrical signal generated by the opening of *voltage-gated Na^+ channels*. These are normally shut at rest (or largely so), but are opened when the nerve cell membrane is depolarised by (e.g.) an excitatory transmitter. Since the entry of Na^+ ions further depolarises the membrane, so opening more Na^+ channels, the process becomes regenerative once the threshold potential is exceeded: this is the potential at which the rate of Na^+ entry exceeds the rate of K^+ efflux (and/or Cl^- entry). The membrane

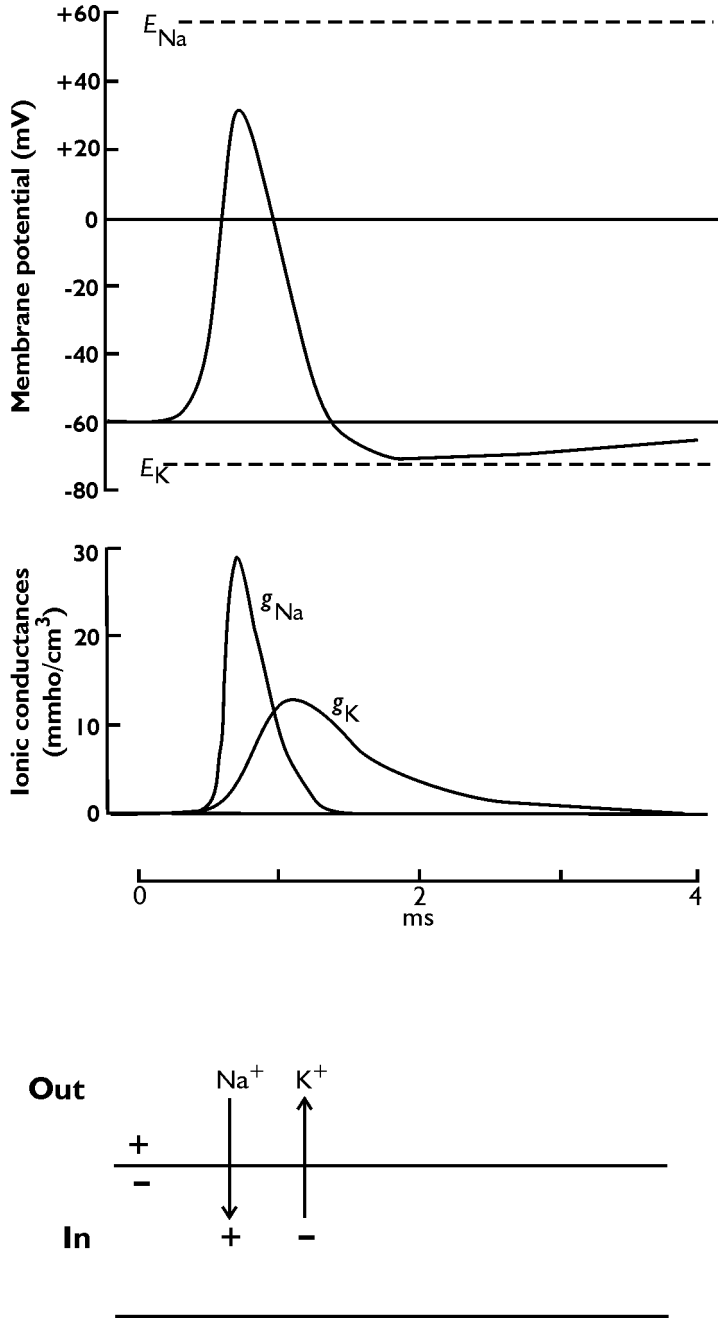


Figure 2.2 Ionic conductances underlying the action potential recorded from a squid axon. $g_{Na} = Na^+$ conductance; $g_K = K^+$ conductance. (Adapted from Hodgkin, AL and Huxley, AF (1952) *J. Physiol.* **117**: 500–544)

potential then moves transiently toward (but does not usually quite reach) the Na^+ equilibrium potential ($E_{Na} \sim +50$ to $+70$ mV; Fig. 2.2)—i.e. the membrane potential is reversed to inside-positive. *Repolarisation* results (in the first instance) from the *inactivation* of the Na^+ channels—that is, as the depolarisation is maintained, the channels close again (though at a slower rate than that at which they open). Recovery then requires that they progress back from the inactivated state to the resting closed state: this takes a little time, so the action potential becomes smaller and eventually fails during high frequency stimulation or during sustained depolarisation—a process of *accommodation*.

Local anaesthetics and some anti-epileptic drugs such as phenytoin and carbemazepine act by blocking Na^+ channels. Many of these have a higher affinity for the inactivated state of the Na^+ channel than for the resting or open states. Hence, by promoting inactivation, they selectively reduce high-frequency nerve impulses ('use-dependence'). This provides a rationale for the use of phenytoin and carbemazepine in controlling epileptic discharges.

In unmyelinated fibres (including the squid axon, where the ionic currents responsible for the action potential were first elucidated, see Fig. 2.2), and in unmyelinated regions of neurons, such as dendrites, somata and axon terminals, action potential repolarisation is accelerated by the delayed opening of additional voltage-gated K^+ channels—so-called *delayed rectifier* K^+ channels. These may be sustained or transient (inactivating) in kinetic behaviour. Since they take a few milliseconds to close as the potential recovers, in addition to hastening repolarisation, current flow through these channels leads to a transient after-hyperpolarisation ('undershoot') following each action potential. Where the action potential leads to the opening of voltage-gated Ca^{2+} channels (as in nerve terminals and neuron somata or dendrites—see below), the entry of Ca^{2+} also induces the rapid opening of large (100–200 pS in symmetrical high $[K^+]$) conductance ('BK') Ca^{2+}-activated K^+ channels, which can also accelerate action potential repolarisation. However, K^+ channels are normally absent from nodes of Ranvier and action potential repolarisation in myelinated fibres results solely from Na^+ channel inactivation. Thus, blocking K^+ channels with drugs such as tetraethylammonium or 4-aminopyridine (Fig. 2.3) does not affect conduction along myelinated fibres (though they can increase transmitter release, by prolonging the action potential in unmyelinated nerve terminals). They can also improve conduction in myelinated fibres following *demyelination* (e.g. in multiple sclerosis). This is because the action potential now has to be conducted along the demyelinated segments of the fibres (continuous conduction), instead of 'jumping' from node to node (saltatory conduction). (This is assisted by the spread of Na^+ channels from the nodes along the internodes after demyelination.) Since K^+ channels are normally present along internodal segments of myelinated fibres and the internodal Na^+ channel density is relatively low (even after demyelination), current through the K^+ channels tends to 'shunt' the Na^+ current and block internodal action potential conduction. Cooling the nerve has a similar effect to blocking K^+ channels: hence MS patients are very sensitive to temperature.

CALCIUM CHANNELS: TRANSMITTER RELEASE

When an action potential arrives at the axon terminal, it induces the release of a chemical transmitter. Transmitter release is a Ca^{2+}-dependent process (see Chapter 4) and requires a charge of Ca^{2+}. This is provided through the action potential-induced

Table 2.1 Types of calcium channel

Type	T	L	N	P/Q	R
α-subunit(s)	1G,H,I	1C,D	1B	1A	1E
Threshold[1]	Low	High	High	High	High
Inactivation	Fast	Slow	Moderate	None (P) Moderate (Q)	Fast
Location[2]	s/d	s/d	t,s/d	t,s/d	t,s/d
Blockers	Ni^{2+}	DHP[3]	ω-CTX-GVIA[4]	ω-Aga IVA[5]	
Main functions	Pacemaker	Spike	Transmitter release	Transmitter release	Transmitter release

[1]Low threshold around −60 mV; high threshold around −40 mV.
[2]s = soma; d = dendrites; t = axon terminals.
[3]Dihydropyridines.
[4]ω-Conotoxin GVIA.
[5]ω-Agatoxin IVA.

opening of *voltage-gated Ca^{2+} channels*. A variety of Ca^{2+} channels have been described, characterised by their kinetics, single-channel properties, pharmacology (especially sensitivity to different toxins) and molecular structure (Table 2.1). Those primarily responsible for transmitter release belong to the N (α1B), P/Q (α1A) and R classes (α1E). So far, no pharmacological agents capable of uniquely modifying Ca^{2+} channels involved in transmitter release have been described (other than polypeptide toxins). These, and other (L-type, T-type), Ca^{2+} channels are also variably present in neurons somata and/or dendrites, where they contribute to the regulation of neural activity in other ways (see below).

REGULATION OF Ca^{2+} CHANNELS BY NEUROTRANSMITTERS

N and P/Q channels are susceptible to inhibition by many neurotransmitters and extracellular mediators that act on receptors coupling to *Pertussis* toxin-sensitive G-proteins (primarily G_o) — for example, noradrenaline (via α2 receptors), acetylcholine (via M_2 and M_4 muscarinic receptors), GABA (via GABA-B receptors), opioid peptides (via μ/δ receptors) and adenosine (via A2 receptors) (see Fig. 2.4). Inhibition results from the release of the $\beta\gamma$ subunits of the trimeric ($\alpha\beta\gamma$) G-protein following its activation by the receptor. The $\beta\gamma$ subunit then binds to the Ca^{2+} channel in such a way as to shift its voltage sensitivity to more positive potentials, so that the channels do not open as readily during a rapid membrane depolarisation. This effect is 'reversible' in the sense that it can be temporarily reversed by applying a brief, strong depolarisation but then returns on rehyperpolarisation in the continued presence of free $\beta\gamma$ subunits (Fig. 2.4(a)). One interpretation of this is that the binding of the $\beta\gamma$ subunits is itself voltage-dependent. It should be noted that no 'second messenger' is necessary for this form of inhibition: instead, the $\beta\gamma$ subunit reaches, and binds to, a neighbouring Ca^{2+} channel when released from the activated G_o protein. (This G-protein is very abundant in nerve cell membranes.) As a result, the process of inhibition can be quite fast — within 30–50 ms following receptor activation. This is thought to provide the principal mechanism responsible for *presynaptic inhibition*, whereby neurotransmitters inhibit their own release (autoinhibition) during high-frequency synaptic transmission. This process can be replicated by applying exogenous transmitters or their analogues (see Fig. 2.4(b))

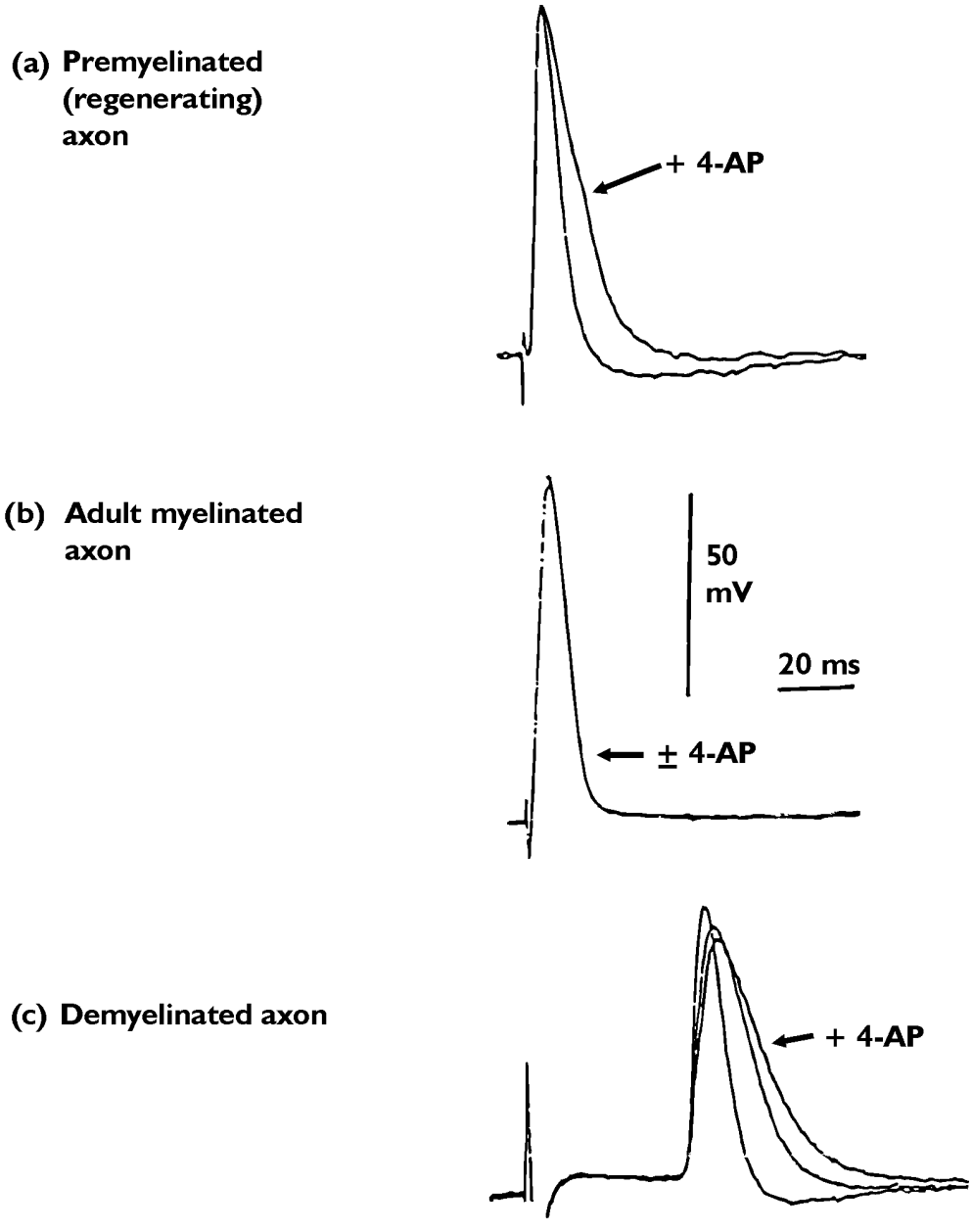


Figure 2.3 Role of K^+ channels in action potential repolarisation in mammalian axons, revealed using the K^+ channel blocking agent, 4-aminopyridine (4-AP, 0.5 mM). Records show intra-axonal recordings from (a) a regenerating sciatic nerve axon following nerve crush; (b) a normal sciatic nerve axon; and (c) a demyelinated ventral root axon after treatment with lysophosphatidylcholine. Note that 4-AP prolongs the action potential in (a) and (c) but not in (b). Thus, current through 4-AP-sensitive K^+ channels contributes to action potential repolarisation in premyelinated or demyelinated mammalian axons, whereas in normal myelinated axons repolarisation is entirely due to Na^+ channel inactivation. (Adapted from Fig. 2 in *Trends Neurosci* **13**: Black, JA *et al.* Ion Channel Organization of the Myelinated Fiber, p 48–54 (1990) with permission from Elsevier Science

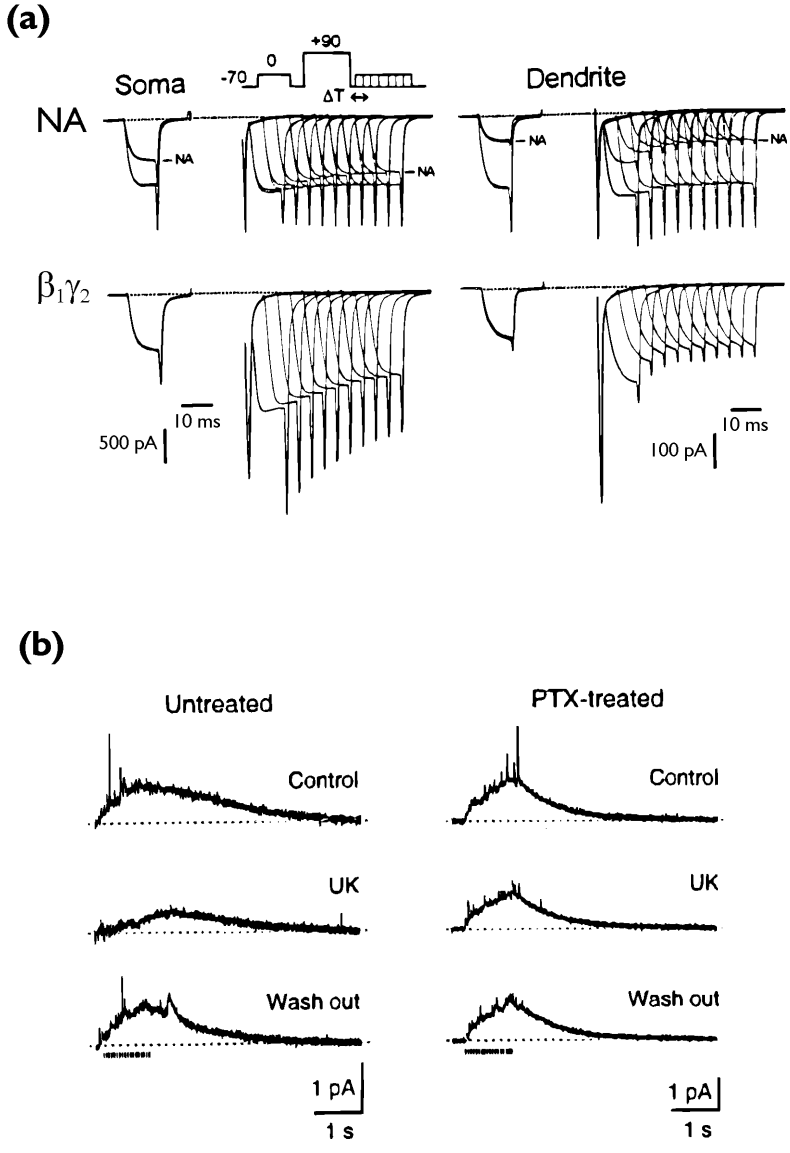


Figure 2.4 Noradrenergic inhibition of Ca^{2+} currents and transmitter release in sympathetic neurons and their processes. (a) Inhibition of currents through N-type Ca^{2+} channels by external application of noradrenaline (NA) or by over-expression of G-protein $\beta_1\gamma_2$ subunits, recorded from the soma and dendrite of a dissociated rat superior cervical sympathetic neuron. Currents were evoked by two successive 10 ms steps from -70 mV to 0 mV, separated by a prepulse to +90 mV. Note that the transient inhibition produced by NA (mediated by the G-protein G_o) and the tonic inhibition produced by the G-protein $\beta_1\gamma_2$ subunits were temporarily reversed by the +90 mV depolarisation. (Adapted from Fig. 4 in Delmas, P *et al.* (2000) *Nat. Neurosci.* **3**: 670–678. Reproduced with permission). (b) Inhibition of noradrenaline release from neurites of rat superior cervical sympathetic neurons by the α2-adrenoceptor stimulant UK-14,304, recorded amperometrically. Note that pretreatment with *Pertussis* toxin (PTX), which prevents coupling of the adrenoceptor to G_o, abolished inhibition. (Adapted from Fig. 3 in Koh, D-S and Hille, B (1997) *Proc. Natl. Acad. Sci. USA* 1506–1511. Reproduced with permission)

and suppressed by blocking the presynaptic receptors with antagonist drugs, which thereby selectively enhance the release of individual transmitters.

ION CHANNELS AFFECTING THE PATTERN AND FREQUENCY OF ACTION POTENTIAL DISCHARGES

The opening of Na^+ ion channels for the initiation of neuronal depolarisation and action potential generation, as described above, can be induced by excitatory neurotransmitters acting on receptors that are directly linked to cation channels. These include glutamate AMPA receptors (Chapters 3 and 10) and ACh nicotinic receptors (Chapter 6). The inhibitory neurotransmitter GABA has an opposing effect through receptors ($GABA_A$) that are directly linked to the opening of chloride channels, inducing an influx of Cl^- ions and subsequent hyperpolarisation (Chapters 1 and 11). There are, however, a number of other ion channels, generally for K^+ or Ca^{2+}, that have a more subtle controlling effect on neuronal activity. Their opening may be initiated by (or dependent on) preceding changes in membrane potential and ion flux, but they can be affected indirectly by various neurotransmitters, e.g. monoamines and peptides, acting on receptors linked to second messenger systems or more directly by various chemicals, some of which have clinical use. The role of these channels in controlling the overall activity of neurons is clearly important and needs to be considered.

'SLOW' K^+ CHANNELS AND ADAPTATION

The K^+ channels responsible for action potential repolarisation close fairly soon after repolarisation (usually within 5–10 ms). However, most nerve cells possess other K^+ channels which are opened during nerve cell discharges but which stay open much longer. These do not contribute much to the repolarisation of individual action potentials but instead affect the excitability of the neuron over periods of hundreds of milliseconds or even seconds.

Two principal types of channel having this effect have been identified and their properties are summarised in Table 2.2. The first type (*Ca^{2+}-activated K^+ channels* or K_{Ca} channels) are opened ('gated'), not by membrane voltage but by a rise in intracellular Ca^{2+} ion concentration. This means that they are activated by the Ca^{2+} influx through voltage-gated Ca^{2+} channels when these are opened during a somatic or dendritic action potential, or during trains of action potentials. They then close slowly as the intracellular Ca^{2+} concentration recovers, so producing a long-lasting after-hyperpolarisation (AHP) following an action potential or after trains of action potentials. The particular K_{Ca} channels thought to be responsible for the long AHP have a low conductance ($\sim$10 pS in symmetrical high [K^+] solution) so are called '*SK*' ('small-conductance K') channels: three variants of SK channel have now been cloned, SK1, 2 and 3. These are resistant to normal K^+ channel blocking agents such as tetraethylammonium or 4-aminopyridine, but can be selectively blocked (with varying affinities) by the bee-venom apamin or by certain quaternary ammonium compounds such as tubocurarine and derivatives therefrom.

The second type (*M-channels*) are voltage-gated, like delayed rectifier channels, but have a lower threshold (around -60 mV) and open 10–100 times more slowly when the

Table 2.2 'Slow' potassium channels

Type	Ca-activated	M-type
Descriptor	SK_{Ca}	K_M
Gene products	SK1–3	KCNQ2/3
Activated by	Intracellular Ca^{2+}	Voltage
Threshold	>100 nM $[Ca^{2+}]_{in}$	<-60 mV V_m
Blocked by	Apamin (SK2 > SK3 > SK1)	Linopirdine
Inhibited by	Acetylcholine[1]	Acetylcholine[1]
	Glutamate[2]	Glutamate[2]
	Noradrenaline[3]	Peptides[5]
	5-Hydroxytryptamine[4]	
Present in	Autonomic neurons	Sympathetic neurons
	Cortical pyramidal cells	Cortical pyramidal cells
	Hippocampal pyramidal cells	Hippocampal pyramidal cells
Function	Spike frequency adaptation	Spike frequency adaptation
		Membrane potential stabilisation

[1]Via m1, m3 muscarinic receptors.
[2]Via mGluR1,5 'metabotropic' glutamate receptors.
[3]Via β-adrenoceptors.
[4]Via 5-HT_2 receptors.
[5]Including bradykinin, angiotensin, substance P.

membrane is depolarised. They were originally called M-channels because they were inhibited by activating *M*uscarinic acetylcholine receptors. (This turns out not to be a very good definition since other channels can be inhibited by these receptors but the name has stuck.) It is now known that M-channels are composed of protein products of members of the KCNQ family of K^+ channel genes, mutations of which can give rise to certain forms of inherited epilepsy or deafness (depending where the proteins are expressed). M-channels, like SK channels, are generally resistant to common K^+ channel-blocking agents, but are selectively blocked by the 'cognition-enhancer' linopirdine and congeners thereof.

In spite of their different structure and gating mechanisms, these channels have quite a lot in common in functional terms. First, they both open and close slowly. (The SK_{Ca} channels open slowly because of the time taken to build up the required concentration of Ca^{2+} in the cell, and close slowly because it takes hundreds of milliseconds or seconds for the Ca^{2+} to be extruded. M-channels open and close slowly because of their slow intrinsic gating.) Second, although there are differences in their distribution among different types of neuron (e.g. M-channels are abundant in sympathetic neurons whereas SK_{Ca} channels are more important in the enteric neurons in the intestine), they also co-exist in many neurons (such as hippocampal and cortical pyramidal cells — including *human* cells). Third, they have rather similar effects when they open (see below). Fourth, they are both closed by some important neurotransmitters. Thus, acetylcholine (acting via muscarinic receptors) and glutamate (acting via metabotropic glutamate receptors) close both types of channel, but noradrenaline (acting via β-adrenoceptors) closes only the K_{Ca} channels. This effect makes an important contribution to the postsynaptic action of these transmitters, and is discussed further below.

Figures 2.5 and 2.6 shows some experimental records illustrating the function of these channels. Figure 2.5 illustrates the function of SK_{Ca} channels in a hippocampal pyramidal neuron. In the record marked 'control' in Fig. 2.5(b) the neuron was depolarised by injecting a 1-s long depolarising current. This makes it fire action

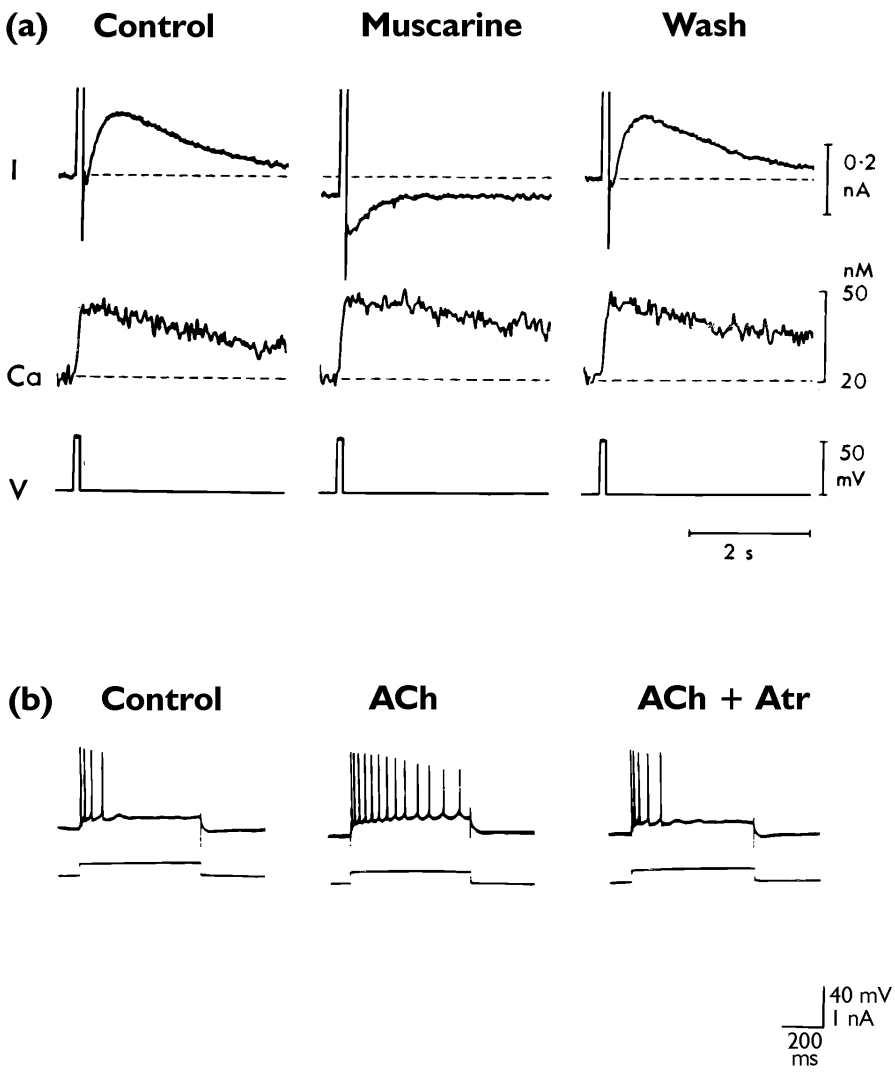


Figure 2.5 Effects of inhibiting SK_{Ca} Ca^{2+}-activated K^+ channels by stimulating muscarinic acetylcholine receptors (mAChRs) in rat hippocampal pyramidal neurons. (Micro-electrode recordings.) (a) Records showing SK_{Ca} current (I) and intracellular [Ca^{2+}] transient (Ca) following a 50 ms depolarisation (V). The depolarisation opens voltage-gated Ca^{2+} channels. The resultant Ca^{2+} influx leads to a rise in intracellular [Ca^{2+}] that (after a delay) activates the K_{Ca} current. The mAChR agonist muscarine (10 μM) does not affect the Ca^{2+} rise but inhibits the subsequent opening of the SK_{Ca} channels. (Adapted from Fig. 3 in Knopfel, T *et al.* (1990) *Proc. Natl. Acad. Sci. USA* **87**: 4083–4087. Reproduced with permission). (b) Records showing the effect of inhibiting the SK_{Ca} current on the firing properties of a hippocampal neuron. Under normal circumstances (control) the development of the SK_{Ca} current arrests action potential firing during tonic depolarisation induced by injecting 1-s depolarising current ('spike frequency-adaptation'). When the SK_{Ca} current is inhibited with acetylcholine (ACh, 200 μM) (see (a)) spike frequency-adaptation is reduced. This effect is reversed by adding 0.5 μM atropine, to block the mAChRs. Reprinted (adapted from Fig. 1) with permission, from *Acetylcholine Mediates a Slow Synaptic Potential in Hippocampal Pyramidal Cells*, Cole, AE and Nicoll, RA (1983) *Science* **221**: 1299–1301). American Association for the Advance of Science

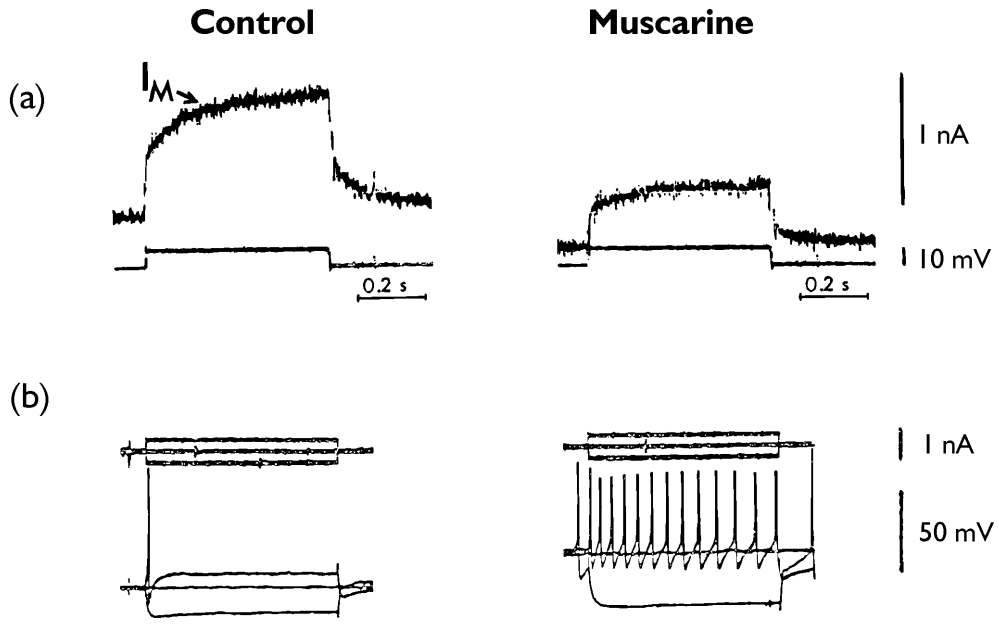


Figure 2.6 Effect of inhibiting M-type K^+ channels in rat superior cervical sympathetic neurons with the muscarinic acetylcholine-receptor (mAChR) stimulant, muscarine. Micro-electrode recordings from different neurons. (a). Current responses to +10 mV voltage steps from −50 mV holding potential. (b). Voltage responses to injecting depolarising and hyperpolarising currents from an initial resting potential of around −47 mV. Under control conditions, depolarisation produces a slow activation of the voltage-gated K^+ current, $I_{K(M)}$ ('I_M' in (a)); this raises the threshold for action potential generation so that the imposed depolarisation in (b) produces only a single action potential (i.e. this neuron, like that in Fig. 2.5, shows strong 'spike frequency-adaptation'). Muscarine strongly reduces $I_{K(M)}$; removal of this braking current now allows the neuron to fire a train of action potentials during the depolarising current injection. (Records in (a) A Constanti and DA Brown, unpublished; records in (b) adapted from Fig. 7 in *Intracellular Observations on the Effects of Muscarinic Agonists on Rat Sympathetic Neurones* by Brown DA and Constanti, A (1980) *Br. J. Pharmacol.* **70**: 593–608.) Reproduced by permission of Nature Publishing Group

potentials. However, the action potentials open Ca channels, so intracellular Ca^{2+} gradually rises as shown in Fig. 2.5(a), and this in turn opens SK_{Ca} channels to produce an outward (hyperpolarising) current. This current partly repolarises the cell and raises the threshold for action potential generation, so the action potential train in Fig. 2.5(b) dies out. The K_{Ca} channels were then inhibited with acetylcholine (or an analogue, muscarine). Now the SK_{Ca} channels cannot open, even though intracellular [Ca^{2+}] still rises (Fig. 2.5(a)). This allows the action potential discharge to continue throughout the length of the depolarising current injection (Fig. 2.5(b)). Thus, the SK_{Ca} channels induce an *adaptation* of the action potential discharge to a maintained stimulus: this adaptation is lost when the SK_{Ca} channels are prevented from opening.

Figure 2.6 shows the effect of the M-channels on the action potential discharges of a rat sympathetic neuron during an equivalent (1-s) injection of depolarising current. (Hyperpolarising currents were also injected in this experiment, giving the downward voltage response.) This cell shows even stronger adaptation under normal circumstances ('control'), because the depolarisation itself is sufficient to open extra M-channels,

even without the action potentials (Fig. 2.6(a)). When the opening of M-channels is inhibited by muscarine, this adaptation is again lost. Also note that muscarine has actually depolarised the cell — the level of membrane potential before injecting the current pulse has changed. This is because a few M-channels are open at the resting potential and actually contribute to the resting potential.

As mentioned above, M-channels and K_{Ca} channels co-exist in many neurons. This may seem odd, since Figs 2.5 and 2.6 suggest that they have the same effect. However, in practice, their effects are slightly different, depending on the pattern of stimulation, and in fact the two currents act synergistically — i.e. the effect of inhibiting both currents is far greater than the sum of inhibiting each individually. Their inhibition (separately or together) by neurotransmitters such as acetylcholine and noradrenaline removes a 'brake' on neural discharges and thereby induces a sustained increase in excitability. This is the prime mechanism underlying the arousal and attention-directing function of the ascending cholinergic and aminergic systems innervating the pyramidal cells of the cerebral cortex and hippocampus; the failure of this function, due to inadequate transmitter release, is thought to contribute to the cognitive deficits in such diseases as Alzheimer's disease.

SK_{Ca} and M channels are not the only K^+ channels regulated by transmitters. As noted above, transmitters can also close, or open, other K^+ channels that do not directly regulate excitability but instead determine the resting potential of the neuron, and hence depolarise or hyperpolarise the neuron.

Ca^{2+} CHANNELS: PLATEAU POTENTIALS AND PACEMAKING

As pointed out above, although the principal function of voltage-gated Ca^{2+} channels is to provide the charge of Ca^{2+} necessary for transmitter release, Ca^{2+} channels are also present on the somata and dendrites of most neurons. These include two classes of Ca^{2+} channel not involved in transmitter release — dihydropyridine-sensitive high-threshold L-type channels, homologous to the cardiac Ca^{2+} channels responsible for ventricular contraction and some pacemaking activity; and low-threshold, rapidly-inactivating T-type Ca^{2+} channels. These have multiple functions.

First, their opening during somato-dendritic action potentials provides the source of the increased intracellular $[Ca^{2+}]$ required to open Ca^{2+}-activated K^+ channels — BK channels, to accelerate spike repolarisation, and SK channels, to induce spike-train adaptation and limit repetitive firing. The BK channels are activated (primarily) following entry of Ca^{2+} through L-type channels; the source of Ca^{2+} for *SK* channel activation varies with different neurons, and may be either through L-type or N-type channels.

Second, as in the ventricular muscle fibres of the heart, opening of L-type channels can generate sustained plateau potentials following the initial Na^{2+}-mediated action potential — for example, in the rhythmically firing neurons of the inferior olive (Fig. 2.7).

The T-type channels have a special 'pacemaking' function. This is well illustrated in thalamic relay neurons (Fig. 2.8). At resting potentials $\leqslant -60\,\text{mV}$, these channels are inactivated and hence non-conducting (a voltage-sensitive closure process resembling Na^+ channel inactivation). Under these conditions, the relay neurons show sustained rhythmic firing when tonically depolarised. However, if the neurons are first hyperpolarised, T-channel inactivation is removed. Then, when the cells are depolarised, the T-channels open and generate a depolarising 'Ca^{2+} spike'. This in turn induces a rapid

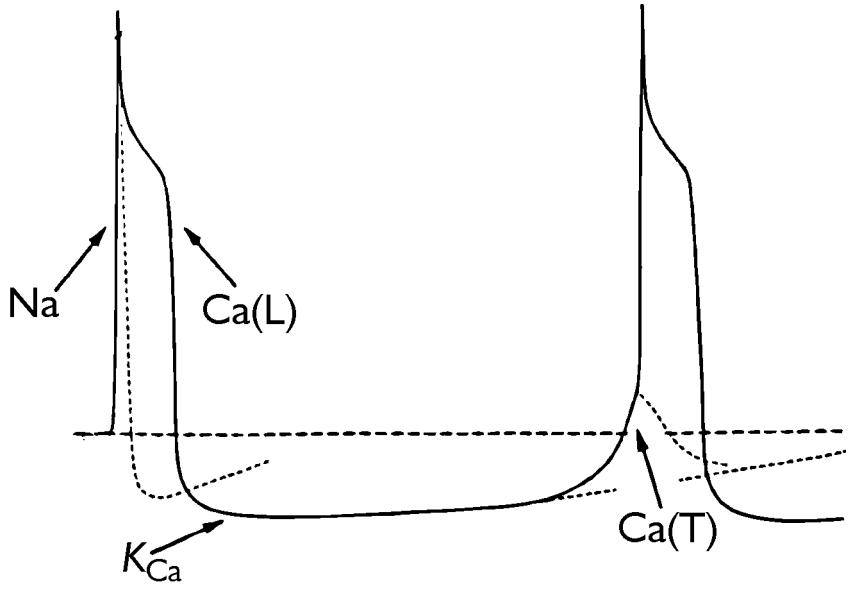


Figure 2.7 Oscillatory behaviour of guinea-pig inferior olivary neurons. The initial action potential, induced by the opening of conventional voltage-gated Na^+ channels, in turn opens voltage-gated L-type Ca^{2+} channels to produce a 'plateau potential'. The Ca^{2+} entry activates K_{Ca} channels, to produce a long-lasting (several hundred ms) after-hyperpolarisation. This de-inactivates the transient (T-type) Ca^{2+} channels (see Fig. 2.8). Hence, as the Ca^{2+} is extruded and the K_{Ca} current declines, the low-threshold T-type Ca^{2+} channels open, and the cell depolarises to reach the threshold for the Na^+ channel, giving a new action potential, and so on. The interval between the action potentials is 650 ms. (Adapted from Fig. 7 in Llinas, R and Yarom, Y (1981) *J. Physiol.* **315**: 569–584. Reproduced by permission of the Physiological Society)

'burst' of Na^+ action potentials. The burst is arrested first because the Na^+ channels inactivate, and then because the T-type Ca^{2+} channels inactivate. Both inactivation processes are removed when the cell hyperpolarises back again, so becoming available for another burst. As a result, the cells change their firing pattern from tonic firing to burst-firing simply dependent on membrane potential. This is thought to explain the switch between tonic firing in awake animals to burst-firing during slow-wave sleep. In the awake state, the neurons are maintained in a tonic state of depolarisation due to the release of neurotransmitters such as histamine and acetylcholine, which inhibit K^+ currents (see above), but hyperpolarise during slow-wave sleep when transmitter release diminishes — or when the receptors for the transmitters are blocked by anti-histamines or anti-cholinergic drugs. However, it should be emphasised that T-channels are quite widely distributed and their burst-inducing properties may also be important in some forms of epilepsy since they can be blocked by certain anti-epileptic drugs, such as ethosuximide.

Finally, entry of Ca^{2+} through somatic and dendritic Ca^{2+} channels activates calmodulin-dependent protein kinases to modulate transcription, and thereby plays a crucial role in certain components of neural development and plasticity.

Neither L nor T channels appear susceptible to the form of G-protein-mediated inhibition characteristic of N or P/Q channels. However, as in the heart cells, L-type Ca^{2+}

channels in the nervous system are susceptible to more indirect forms of modulation (both enhancement and inhibition) through receptor-mediated phosphorylation.

ANOTHER PACEMAKER CHANNEL: HYPERPOLARISATION-ACTIVATED CATION CHANNELS ('h-CHANNELS')

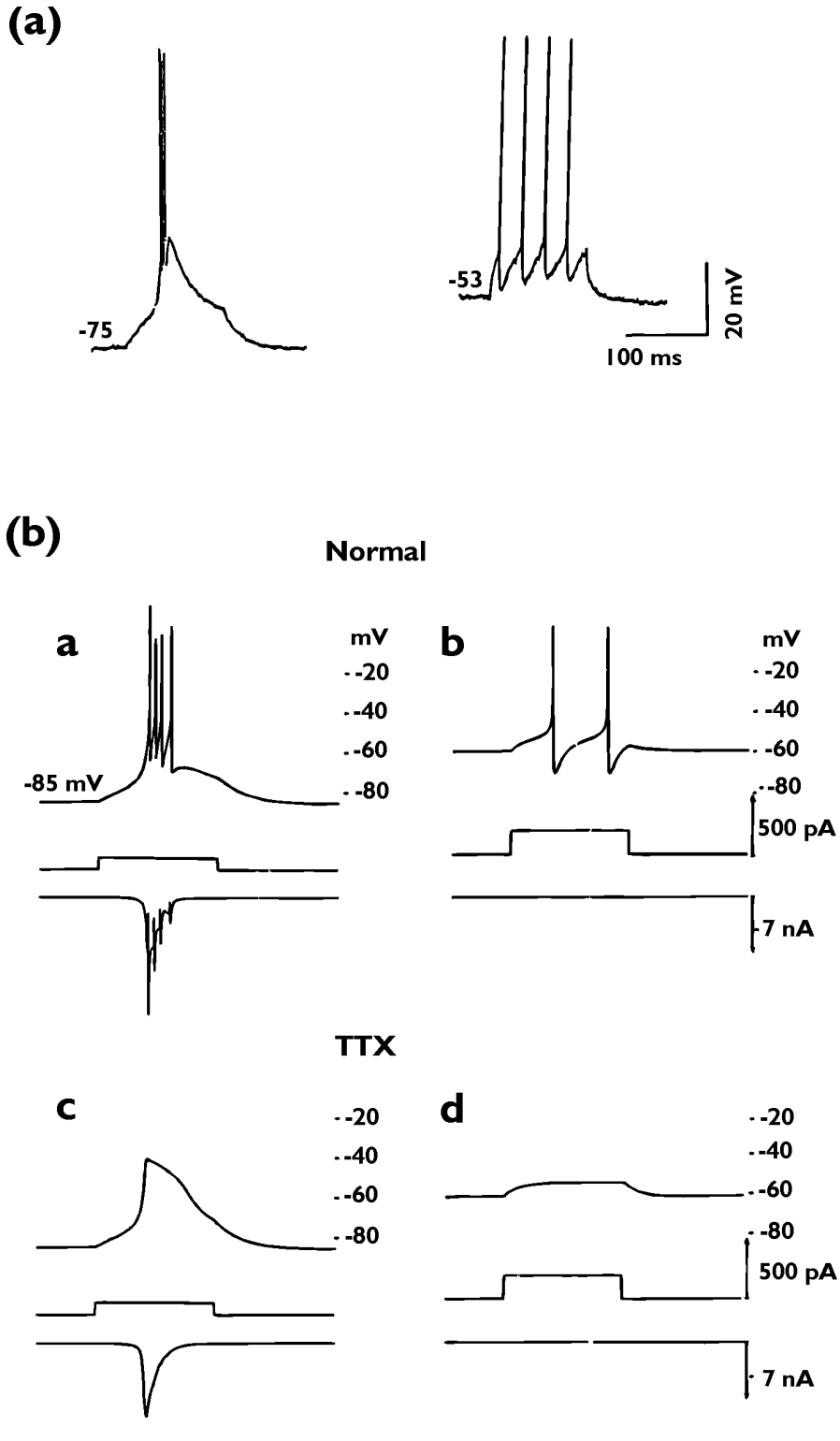
Another analogy with heart cells is the presence in many neurons of the cardiac pacemaker current I_h. (The neural current is sometimes dubbed I_Q.) This is a mixed cation current carried by channels that are permeant to both Na^+ and K^+ and which are opened by hyperpolarising the membrane — i.e. at potentials negative to the normal resting potential. It serves the same function as in the heart, to act as a pacemaker current. The way this works is illustrated in Fig. 2.9. As the membrane hyperpolarises (e.g. after an action potential, when K^+ currents are active), h-channels open to give an inward (depolarising) current (Fig. 2.9(a)). This leads to a slow depolarisation until the threshold for the T-type Ca^{2+} channels open, leading to a rapid depolarisation and spiking (Fig. 2.9(b)). The h-channels then switch off (because the cell is depolarised) and reopen during the subsequent hyperpolarisation. In this way sustained oscillations of membrane potential, leading to a steady rhythmic action potential discharge, can be maintained. The h-channels are blocked by low concentrations of Cs^+ ions, or by agents which block the cardiac current and slow the heart: such agents inhibit the neural membrane potential oscillations and discharges. Also like the cardiac pacemaker, the neural h-current is regulated by transmitters that activate adenylate cyclase, such as noradrenaline and 5-hydroxytryptamine: the cyclic AMP shifts the activation curve to more positive membrane potentials (by a direct action on the channels, not through phosphorylation), so accelerating the depolarisation and increasing the neural rhythm. Conversely, transmitters or mediators that inhibit adenylate cyclase, like enkephalins and adenosine, shift the activation curve to more negative potentials and slow rhythmic discharges.

RECORDING NEURAL ACTIVITY

RECORDING ION CHANNEL CURRENTS: THE PATCH-CLAMP

Currents through individual ion channels can be recorded using the *patch-clamp* technique (Fig. 2.10). A fine glass micro-electrode (tip diameter about 1 μm) filled with electrolyte solution is attached to the cell membrane by suction, forming a 'tight seal' (resistance 1 GΩ or more, i.e. 10^9 Ω), so that all current flowing through the channel enters the electrode. These currents are very small (a few picoamps, pA) so have to be amplified. The amplifier also incorporates a device for applying a potential to the pipette, so that the potential across the cell membrane at the tip of the pipette can be varied.

Figure 2.10(a) illustrates currents generated by K^+ ions flowing through an M-type K^+ channel in a ganglion cell membrane. By convention, the direction of current flow always refers to the direction in which +ve ions move. Thus, outward current is generated by +ve ions flowing out of the cell into the pipette (or −ve ions going the other way). Also by convention, outward current is depicted as an upward deflection in the recording. Note that the channel normally adopts one of two states — it is either


(a)
-75
-53
20 mV
100 ms
(b)
Normal
a
mV
-20
-40
-60
-80
-85 mV
b
mV
-20
-40
-60
-80
500 pA
7 nA
TTX
c
-20
-40
-60
-80
d
-20
-40
-60
-80
500 pA
7 nA

open or shut — but switches spontaneously between the two states. When it is shut, no current flows. When it is open, the current is fairly constant at any given potential. However, when the potential is changed, the amplitude of the channel current changes: this is because the current is given by Ohm's Law:

$$V = IR \text{ so } I = V/R, \text{ whence the single-channel current } i = g(V - E_K)$$

where g is the single-channel *conductance* (reciprocal resistance, units = Siemens, S), V is the membrane potential across the membrane patch and E_K is the equilibrium potential for K^+ ions. The conductance is normally constant, and is characteristic for the channel. Single-channel conductances are mostly within the range 2–100 picosiemens (pS): in this case, the conductance is about 8 pS with 2.5 mM $[K^+]$ in the pipette solution.

This channel is voltage-sensitive — that is, its activity is increased when the membrane is depolarised. Thus, the channel opens very infrequently and for very short periods at −50 mV, but opens more frequently and for longer times at −30 mV. This activity is expressed by the *open probability* (P_o), that is, the probability that, at any given time, the channel is open (or, in other words, the proportion of time the channel spends in the open state). In the example illustrated, P_o was 0.02 at −50 mV and 0.27 at −30 mV.

FROM SINGLE-CHANNEL CURRENTS TO WHOLE-CELL CURRENTS

Figure 2.10(a) shows the activity of a single channel. There are many hundreds or thousands of such channels in the entire membrane of a single ganglion cell. The currents through all of these channels add up to give the *whole-cell current*. This can be recorded using the patch pipette by filling the pipette with a solution of similar ionic composition to that of the cytoplasm (i.e. with high $[K^+]$ and low $[Na^+]$ and $[Ca^+]$), then rupturing the membrane under the pipette tip (with pressure) once a seal has been established, or by adding an antibiotic ionophore such as nystatin or amphotericin to the pipette solution and letting these diffuse into the membrane under the pipette tip. In the former case, the solution in the pipette is in direct contact with the cytoplasm, so substances in the cytoplasm diffuse into the pipette and vice versa; nystatin and amphotericin conduct small ions such as Na^+ and K^+ across the cell membrane under the pipette tip, so providing good electrical contact with the cytoplasm, but do not permit total mixing of the two solutions. An older, but still useful, method is to insert one or more fine *micro-electrodes* filled with a strong K^+ solution into the cell and then let them seal into the membrane.

Figure 2.8 *(opposite)* Effects of the T-type Ca^{2+} current on the firing behaviour of guinea-pig thalamic relay neurons. (a) Dependence of firing behaviour on membrane potential. At a hyperpolarised potential (−75 mV), a current injection produces a brief burst of action potentials superimposed whereas at −53 mV the cell responds with a sustained train of action potentials. (Adapted from Fig. 2 in Jahnsen, H and Llinas, R (1984) *J. Physiol.* **349**: 205–226. Published for the Physiological Society by Cambridge University Press.) (b) Interpretation of the records in (a). Each record show voltage-trace (top), injected current pulse (middle) and T-type Ca^{2+} current (bottom). At the hyperpolarised potential (record **a**), the T-type Ca^{2+} current is de-inactivated ('primed'), so a depolarising current pulse opens T-channels to produce a 'Ca^{2+} spike' with superimposed Na^+ spikes. The Na^+ spike can be blocked with tetrodotoxin (TTX: record **c**), leaving a 'pure' Ca^{2+} spike and T-current. The T-current is transient and inactivates, so terminating the burst. At a depolarised potential (**b** and **d**), the T-channels are fully inactivated so depolarisation does not initiate a T-current (record **d**) and now evokes a train of Na^+ spikes instead of a burst (record **b**). (Computer simulation, adapted from Fig. 24 in *Electrophysiology of the Neuron* by Hugenaard and McCormick (1994). Published by Oxford University Press, New York — see Further Study)

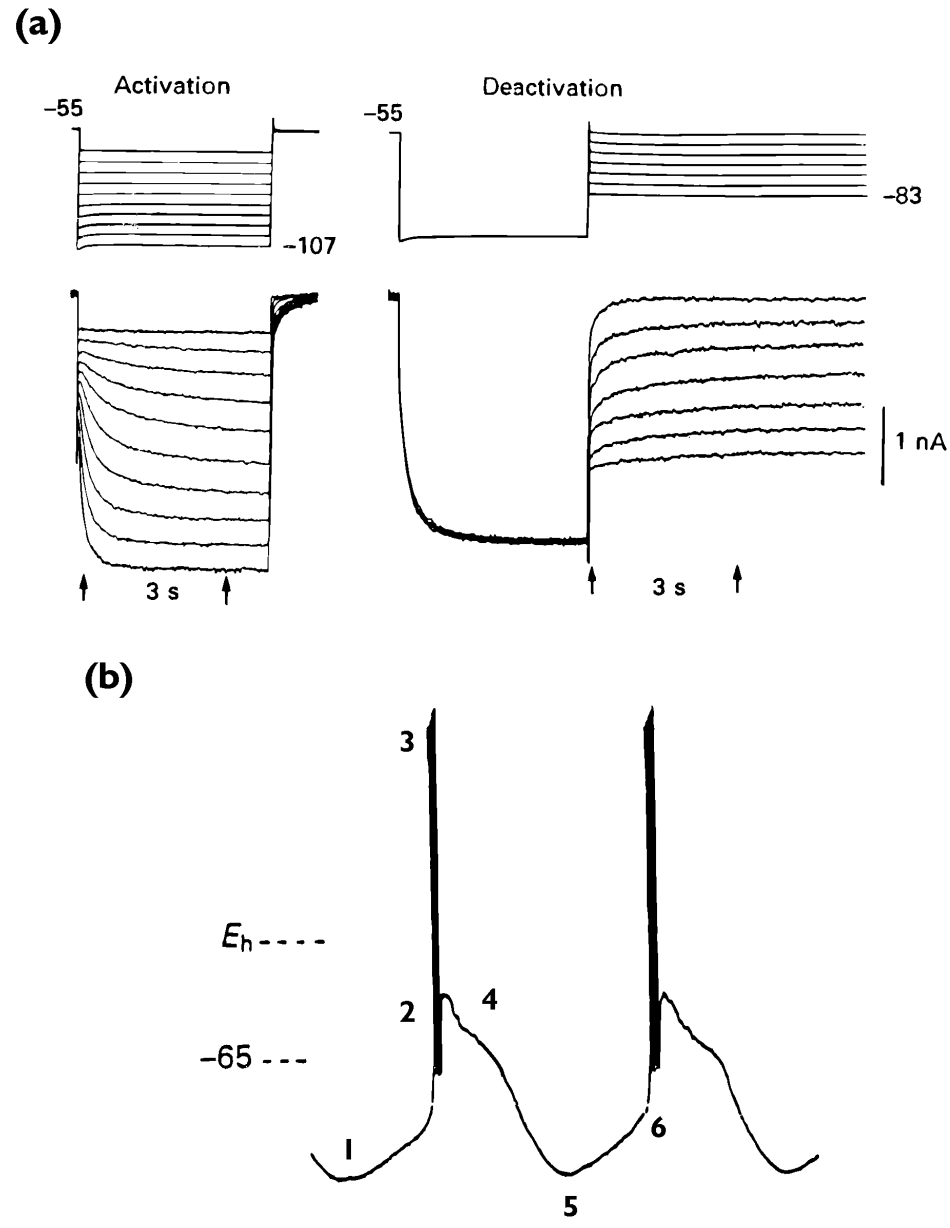


Figure 2.9 Hyperpolarisation-activated cation current I_h and its role in pacemaking in a guinea-pig thalamic relay neuron. (Adapted from Figs 2 and 14 in McCormick, DA and Pape, H-C (1990) *J. Physiol.* **431**: 291–318. Reproduced by permission of the Physiological Society.) (a) Records showing the time-dependent activation of the h-current by hyperpolarisation and its deactivation on repolarising. (b) Interpretation of rhythmic activity in a thalamic relay neuron. (1) The inter-spike hyperpolarisation activates I_h to produce a slowly rising 'pacemaker' depolarisation. (2) This opens T-type Ca^{2+} channels to give a more rapid depolarisation, leading to (3) a burst of Na^+ spikes (see Fig. 2.8). At (4) the depolarisation has closed (deactivated) the h-channels and has inactivated the T-channels. The membrane now hyperpolarises, assisted by outward K^+ current (5). This hyperpolarisation now removes T-channel in-activation and activates I_h (6), to produce another pacemaker potential

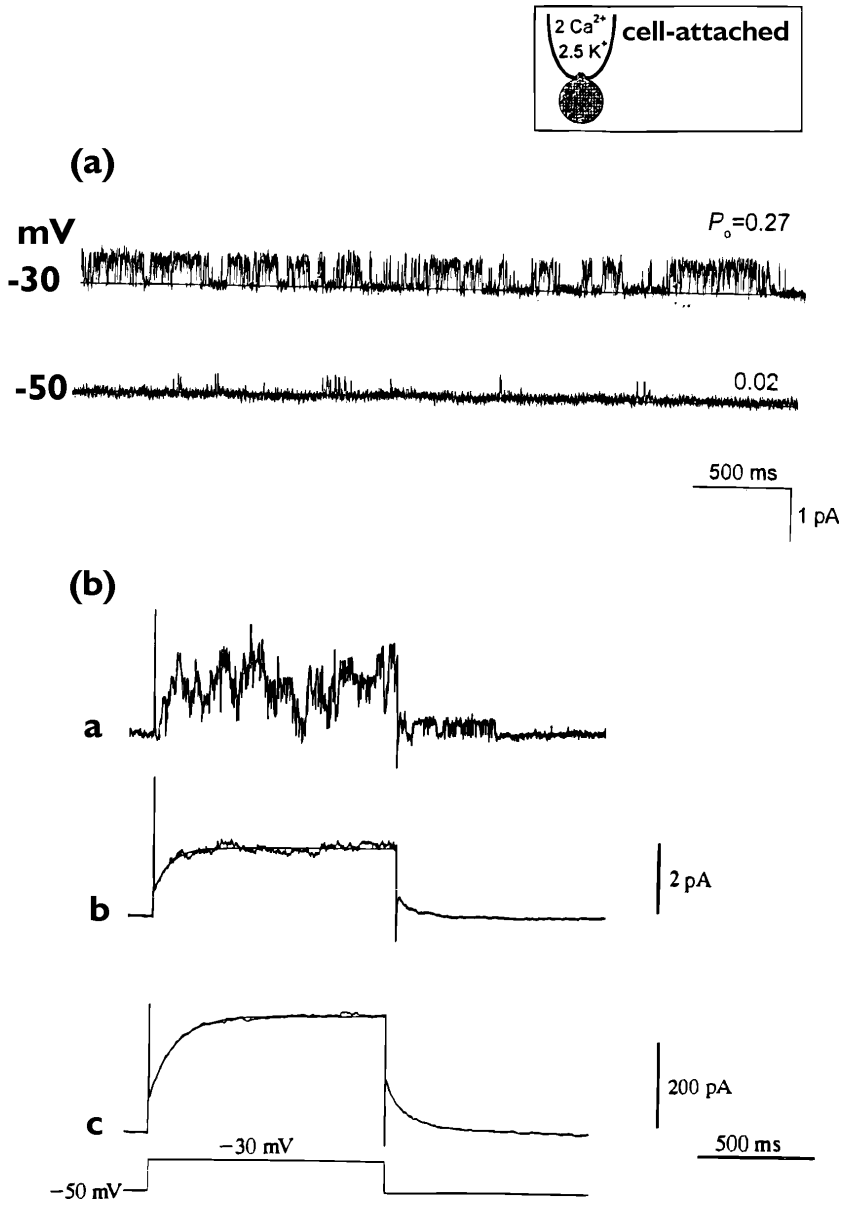


Figure 2.10 M-type K^+ channels: from single-channel currents to whole-cell currents. (a) Single-channel currents recorded from a dissociated rat sympathetic neuron using a cell-attached patch pipette held at estimated membrane potentials of −30 and −50 mV. (Adapted from Fig. 3 in Selyanko, AA and Brown, DA (1999) *Biophys. J.* **77**: 701–713. Reproduced with permission of the Biophysical Society.) (b) **a**: a cluster of single-channel openings recorded from a dissociated ganglion cell with a cell-attached patch pipette on stepping for 1 s from an estimated membrane potential of −50 mV to −30 mV. **b**: an averaged 'ensemble' current obtained on averaging the currents generated by 45 steps like that shown in **a**. **c**: mean whole-cell current recorded with a nystatin-perforated patch pipette during four steps from −50 to −30 mV. (Adapted from Fig. 3 in Selyanko, AA *et al.* (1992) *Proc. Roy. Soc. Lond. Ser. B* **250**: 119–125. Reproduced by permission of The Royal Society)

Figure 2.10(b) shows the relation between the activity of a small cluster of perhaps five individual M-channels recorded from a small patch of membrane with a cell-attached patch pipette (records **a** and **b**) and the whole-cell M-current recorded when the membrane patch under the electrode is permeabilised with nystatin B (record **c**), as seen when the membrane patch or the whole-cell membrane potential is suddenly stepped from −50 to −30 mV and back again. As predicted from Fig. 2.10(a), this depolarisation greatly increases the activity of the channels. However, they do not open instantly but instead take many milliseconds to open — that is, their voltage-gating is relatively slow compared to that of (say) a Na^+ channel. The time taken by any individual channel to assume its new level of open probability varies stochastically about a mean. This mean value is given by the time constant τ ($= 1/(1 - e)$). This can be estimated for a single channel, or for the small cluster of channels seen in Fig. 2.10(b), by repeating the depolarising step many times, then averaging the currents to give an ensemble current (record **b** in Fig. 2.10(b)). In this example, the average time-constant after 45 steps was 86 ms. The whole-cell current (record **c**) gives the current through all the channels in the cell membrane, so, since there are several hundred of them, the current is much larger (note that the current scale is 100 times larger) and one now sees an 'averaged' time-course after a single step (though in this experiment four steps were applied and averaged, to obtain a smoother trace). As one might expect, the time-course of the whole-cell current is quite similar to that of the ensemble of the currents through the small cluster of channels. (They may not be exactly the same, since individual channels in different parts of the cell may vary somewhat in behaviour, depending on their local environment.)

FROM CURRENT TO VOLTAGE

Currents through single channels and across the whole cell membrane are recorded under *voltage-clamp* — that is, the membrane potential is fixed. In a normal cell, however, the voltage is not fixed: the effect of the current is to change the voltage, and signals are normally seen as voltage signals. Figure 2.11 shows how the current through M-channels affects the membrane voltage. When the cell (a frog ganglion cell) was artificially hyperpolarised to −90 mV (left column) so that all of the M-channels were shut, very little current flowed when the voltage was changed (i.e. the membrane conductance was very low or its resistance was very high) (Fig. 2.11(a)). As a result, when a current was injected across the membrane (Fig. 2.11(b)), there was a large voltage change. (The time-course of this voltage change is dependent on the product of the membrane resistance and capacitance. Membrane capacitance is determined by the lipid composition of the membrane and is relatively constant at around 1 $\mu F/cm^2$ membrane.) However, when the cell was left to depolarise to its 'natural' level of (in this case) −46 mV (right-hand column), many M-channels were now open. A hyperpolarising step closes some of the channels, giving a slow decline in current, whereas depolarisation opened more, giving a slow increase in current — the gating of M-channels being characteristically slow, as shown in Fig. 2.10. So now when depolarising current is injected into the cell (bottom record), the membrane begins to depolarise as before but the depolarisation opens more M-channels, and the K^+ current through these extra M-channels hyperpolarises the membrane nearly back to where it started. Conversely, if one tries to hyperpolarise the membrane by injecting hyperpolarising current, the outward flux of K^+ ions diminishes as M-channels close, so the membrane

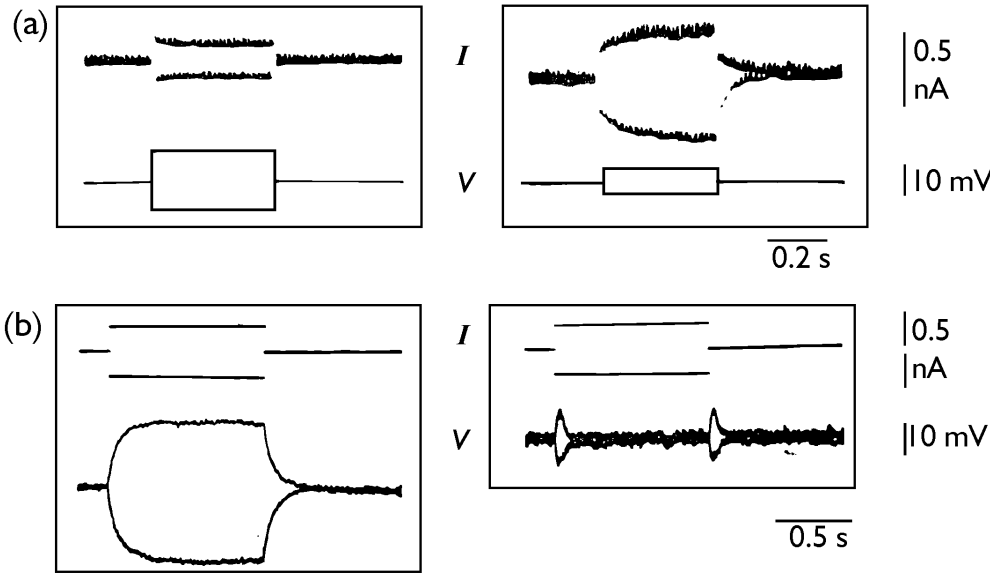


Figure 2.11 M-type K^+ currents: from voltage-clamp to 'current-clamp'. Recordings from frog sympathetic neurons. Upper traces in each record show currents (I), lower traces show voltage (V). (a) Voltage-clamp records showing membrane currents evoked by 0.5-s voltage steps from holding potentials of −90 mV (left), where K_M channels are shut, and −46 mV (right), where K_M channels are open. (b) Voltage responses to 1-s current injections at the same two potentials observed when the voltage-clamp circuit is switched off. Note that the effect of activating the current is to severely reduce the voltage response to current injection. (Adapted from Fig. 6 in Brown, DA (1988) *Ion Channels*, Vol. 1 (Ed. Narahashi, T), Published by Plenum Press, New York, pp. 55–99)

re-depolarises back to where it started. Hence, because M-channels are voltage-sensitive, changes in voltage affect current through M-channels and changes in current through M-channels in turn affect voltage, in such a manner as to stabilise the membrane potential — a negative feedback effect. This is exactly the opposite effect to current through voltage-gated Na^+ channels: current through Na^+ channels depolarises the membrane and this increases the number of open Na^+ channels, so generating more depolarisation, to give positive feedback and hence generating the 'all-or-nothing' action potential.

Figure 2.12 shows another example of how current is converted into voltage — this time a synaptic current. The bottom trace shows a synaptic current recorded under voltage clamp at a preset voltage of −60 mV from a ganglion cell on giving a single shock to the preganglionic fibres. The synaptic current is generated by acetylcholine released from the preganglionic fibres, which opens nicotinic cation channels in the ganglion cell membrane to produce an inward cation current. The top trace shows what happens when the voltage-clamp circuit is switched off, to allow the membrane potential to change. The inward synaptic current now generates a depolarisation (the synaptic potential), which in turn initiates an action potential. This is exactly what synaptic potentials should do, of course, but no Na^+ current is seen under voltage clamp because the membrane potential is held below the threshold for Na^+ channel opening. This threshold is readily exceeded when the clamp circuit is turned off.

EXTRACELLULAR RECORDING

The action potential shown in Fig. 2.12 was recorded from inside the neuron with a micro-electrode. In many instances (particularly in humans!) it is neither convenient nor practicable to use intracellular recording. However, action potentials can still be recorded with extracellular electrodes, by placing the electrode near to the cell (Fig. 2.13). In this case, the electrode tip picks up the local voltage-drop induced by current passing into or out of the cell. Note that (1) the signal is much smaller than the full (intracellularly recorded) action potential and (2) it is essentially a differential of the action potential (because it reflects the underlying current flow, not the voltage change). Nevertheless, since neural discharges are coded in terms of frequency and pattern of

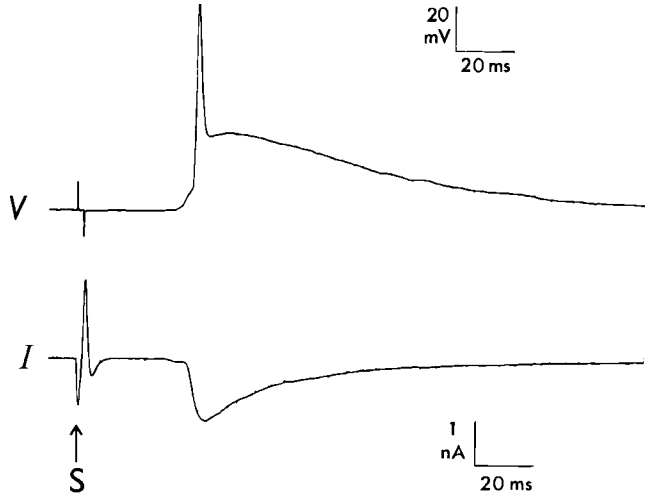


Figure 2.12 From voltage-clamp to 'current-clamp': micro-electrode recordings of synaptic current (*I*, lower trace) and synaptic potential with superimposed action potential (*V*, upper trace) from a neuron in an isolated rat superior cervical sympathetic ganglion following a single stimulus (S) applied to the preganglionic nerve trunk. The interval between the stimulus and the postsynaptic response includes the conduction time along the unmyelinated axons of the preganglionic nerve trunk. (SJ Marsh and DA Brown, unpublished)

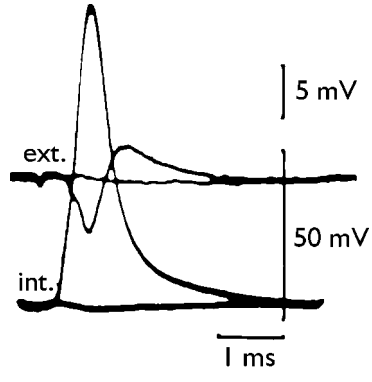


Figure 2.13 Relation between the action potential recorded intracellularly from a cat spinal motoneuron following antidromic stimulation (int.) and the local field potential recorded with an extracellular electrode (ext.). (Adapted from Terzuolo, AC and Araki, T (1961) *Ann. NY Acad. Sci.* **94**: 547–558). Published by NYAS

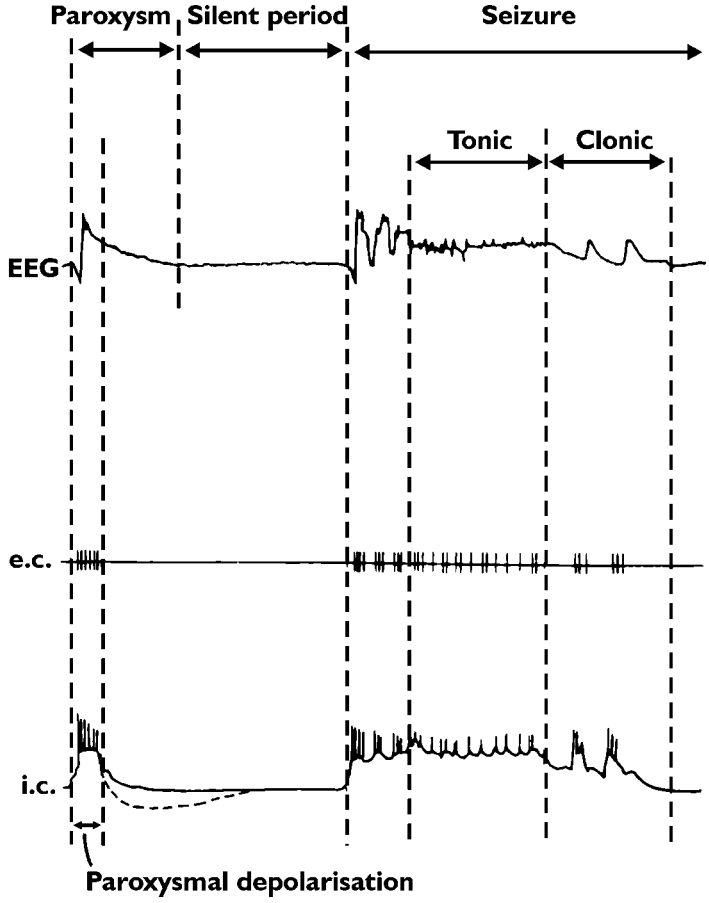


Figure 2.14 Relation between the EEG recorded from an epileptic focus on the surface of the cerebral cortex (EEG) and the activity of a single cortical neuron recorded extracellularly (e.c.) and intracellularly (i.c.) during an experimental epilepsy induced by topical application of penicillin. Note that the large EEG excursions correspond to the large (synchronised) depolarisations of the neuron, not to action potential discharges. (Adapted from *Brain Res.* **52**: Ayala, GF *et al.* Genesis of Epileptic Interictal Spikes. New Knowledge of Cortical Feedback systems suggests a Neurophysiological Explanation of Brief Paroxysms, 1–17 (1973) with permission from Elsevier Science)

action potential firing, such 'unit recording' provides the most convenient and useful method of studying neural activity in the intact nervous system.

Problems arise when the electrode is in contact with lots of cells. If these are firing asynchronously, the signals may cancel out so that individual action potentials become lost in the noise. This problem becomes less when the cells are made to discharge synchronously, by (for example) electrical stimulation. This is made use of to record *evoked potentials* with surface electrodes — for example, to measure conduction velocities along peripheral nerve trunks. Evoked potentials can also be recorded from the brain, via the scalp, along with the EEG (see below). However, the signals are very small (not surprisingly) so have to be averaged by computer. These are used to assess function of sensory systems or in evaluating the progress of demyelinating diseases.

THE EEG

This is a record of fluctuations in activity of large ensembles of neurons in the brain — primarily of the cortical pyramidal cells underneath the recording electrode. Unlike evoked responses, the EEG itself does not represent action potential activity: instead, it originates principally from summed synaptic potentials in pyramidal cell dendrites which (being longer-lasting) summate. However, as with extracellular recording in general, the strongest signal arises when activity of many neurons is synchronised. This happens (for example) in sleep, when large slow-wave activity is recorded: when the subject is woken, the EEG becomes *desynchronised*. Another instance of synchronised activity occurs in epilepsy (Figure 2.14) in which large numbers of neurons show a simultaneous depolarisation (the paroxysmal depolarising shift), again reflecting large underlying synaptic potentials.

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Hugenaard, J and McCormick, DA (1994) *Electrophysiology of the Neuron: an Interactive Tutorial* (Oxford University Press, New York). (This book is accompanied by a disk that allows the properties and functions of the different ion currents to be explored using a PC or Mac. Versions are also available through the web at *http://tonto.stanford.edu/eotn/*)