SLOW SYNAPTIC TRANSMISSION IN FROG SYMPATHETIC GANGLIA

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SUMMARY

Bullfrog ganglia contain two classes of neurone, B and C cells, which receive different inputs and exhibit different slow synaptic potentials. B cells, to which most effort has been directed, possess slow and late slow EPSPs. The sEPSP reflects a muscarinic action of acetylcholine released from boutons on B cells, whereas the late sEPSP is caused by a peptide (similar to teleost LHRH) released from boutons on C cells. During either sEPSP there is a selective reduction in two slow potassium conductances, designated 'M' and 'AHP'. The M conductance is voltage dependent and the AHP conductance is calcium dependent. Normally they act synergistically to prevent repetitive firing of action potentials during maintained stimuli. Computer simulation of the interactions of these conductances with the other five voltage-dependent conductances present in the membrane allows a complete reconstruction of the effects of slow synaptic transmission on electrical behaviour.

INTRODUCTION

This chapter is concerned with slow synaptic transmission in amphibian sympathetic neurones. Although these forms of synaptic influence are indeed slow, slowness is not their most essential characteristic. Because the nature of the synaptic influence is more subtle than simple transmission of the nerve impulse from cell to cell the term 'neuromodulation' is often used. Although the forms of neuromodulation to be discussed are subtle, they can be quite striking under certain circumstances. Furthermore, their mechanisms are both complex and precise.

The frog lumbar sympathetic ganglia lie along two chains which are the most caudal extension of the sympathetic nervous system. Each ganglion is a collection of densely packed cells varying in colour from light yellow to deep orange. The nerve entering the ganglion (from higher up in the chain) contains axons that synapse on cells within the ganglion, as well as axons that pass through to synapse in more caudal ganglia. Most studies have been done on the largest, most caudal ganglia numbered IX and X (Kuba & Koketsu, 1978). Axons synapsing on cells in these ganglia all arise from preganglionic motoneurones in the spinal cord, but enter the chain at

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two different levels and exhibit different conduction velocities. Axons entering the chain rostral to the VI ganglion are moderately rapidly conducting \((2.4 \text{ ms}^{-1})\) and are called \(B\) axons. Axons entering the chain at more caudal locations conduct slowly \((0.4 \text{ ms}^{-1})\) and are \(C\) axons (Dodd & Horn, 1983a). These arrangements are sketched in Fig. 1.

These two classes of axon synapse selectively on two classes of cells found higgledy-piggledy within the ganglion. \(B\) axons synapse on cells of diameter \(30-60 \mu m\), which are called \(B\) cells, whereas \(C\) axons synapse on \(15-40 \mu m\) \(C\) cells. \(B\) cells typically receive only one suprathreshold axon input, whereas \(C\) cells may be multiply innervated. In both cases, the incoming axons spiral round the axon hillock, establishing synapses there and over the cell body (Weitsen & Weight, 1977). There are no dendrites, so all synapses are located close to recording electrodes on or in the cell body. There are typically 40 boutons per cell, though this probably varies with cell size, such that synaptic density is rather constant (Sargent, 1983).

**B CELLS**

The most important difference between \(B\) and \(C\) cells is the nature of the synaptic action exerted by stimulation of their incoming axons. Stimulation of the axon to a

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Fig. 1. Diagram of synapses on \(B\) and \(C\) cells. Synapses on \(B\) cells release only acetylcholine (ACh). Synapses on \(C\) cells release both ACh and T-LHRH. T-LHRH, unlike ACh, can diffuse within the ganglion. The righthand part of the diagram shows direct and orthodromic spikes in \(B\) and \(C\) cells. The direct spikes, triggered by brief current pulses monitored in the upper traces in each pair, precede and are larger than the orthodromic spikes. Note the two-component decay of the \(B\) cell direct spike afterhyperpolarization compared to the smooth decay of the \(C\) cell afterhyperpolarization. Also note the much slower orthodromic conduction velocity in \(C\) cells.
Modulation of ganglion cells

B cell results in two successive depolarizations, the fast and slow EPSPs. The fast EPSP reaches a peak in 1–2 ms and decays with a time constant roughly equal to the cell membrane time constant, about 15 ms. It is generated by a brief inward current, the fast EPSC, which lasts about 20 ms. The slow EPSP starts rising at about 50–100 ms after the end of the fEPSP, reaches a peak after 1–2 s, and lasts about 1 min. The underlying inward current, the slow EPSC, has a similar time course. The fast and slow EPSPs are both cholinergic but differ in every other respect. The fast EPSP is generated in the same way as the endplate potential at the nerve–muscle junction. It reflects the sum of numerous, almost synchronous mEPSCs peppered all over the cell. Because mEPSCs have very similar time courses to the fEPSP itself it is likely that each constituent mEPSC is independently generated, probably 1–2 per bouton. The exponential decay of the fEPSP reflects the random distribution of the individual channel openings. The channels have very similar conductances in B and C cells (approx. 30 pS) but the mean open time is about twice as long in C cells as in B cells (Marshall, 1985). Unlike the situation in the endplate, the channel open time shows very little voltage sensitivity (Kuba & Nishi, 1979; MacDermott, Connor, Dionne & Parsons, 1980). One curious feature of these ganglion cells is that the mEPSCs are very scattered in amplitude despite the apparently equivalent location of all the boutons. This could reflect variability in vesicle size or filling, effective receptor density (but see Land, Salpeter & Salpeter, 1981) or details of geometry. The postsynaptic receptors are nicotinic, and are blocked by curare in an apparently exclusively competitive manner (D. Lipscombe & H. P. Rang, personal communication). These receptors seem to be located almost exclusively under the boutons (Marshall, 1981).

The receptors mediating the slow EPSP are muscarinic, for the response is completely blocked by atropine in the 10–100 nmol l⁻¹ range. It is not yet known whether the receptors are principally of the M1 or M2 type (i.e. very sensitive or not to pirenzipine), nor is their anatomical distribution known. In cardiac muscle, Hartzell (1980) has shown by autoradiography that muscarinic ligand is found all over the cell surface but he was unable to decide if the receptors were located exclusively under terminals or at random on the surface, and similar ambiguities might attend on autoradiographic experiments in ganglion cells.

Nevertheless, the differences in behaviour of the amplitudes of the fast and slow EPSCs during repetitive stimulation may offer some clue as to localization. The first EPSC in a train is normally the largest, and even if the stimulation frequency is high enough to produce overlap, little summation occurs. In contrast, the slow EPSC evoked by a single stimulus is very small, and grows pari passu with the number of shocks in a train, until it reaches a limit governed by the available M-current (see below). The simplest interpretation of this is that the slow EPSP is generated by 'excess' acetylcholine (ACh), that comes off nicotinic receptors and escapes the gauntlet of acetylcholinesterase (AChE). Saturation of receptors and esterase (and hence availability of excess ACh) is more likely to occur with repetitive stimulation. This interpretation is supported by the observation that administration of anticholinesterases increases the amplitude of the slow EPSC much more than that of
the fast EPSP. It may be suggested, then, that the nicotinic receptors get 'first pick' of the released ACh, and that muscarinic receptors, possibly because of an extraboutonal location or because of a slow forward binding rate, get the crumbs from the table.

The fEPSC is normally large enough to depolarize the cell to the threshold, firing an action potential whose time course (itself greatly influenced by the underlying EPSC) largely obscures the subsequent sEPSP. In a tiny proportion of cells, this action potential can itself trigger a 'recurrent' EPSP. This most rare recurrent EPSP is probably cholinergic, because it is blocked by curare, and so it is probably due to an autapse.

If the fEPSP is suppressed (e.g. with curare) the sEPSP is found to be far too small to reach threshold, even when evoked by optimally effective tetani (e.g. 10–20 stimuli at 20 Hz). The slow EPSP is therefore useless in procuring straightforward transmission through the ganglion. It appears that the membrane depolarization occurring during the slow EPSP is not itself the important feature of this synaptic action, but instead it is a striking change in the 'electrical personality' of the nerve cell. This is shown in the experiment in Fig. 2. The first part shows the typical response of a B ganglion cell to a weak maintained depolarizing current pulse. The cell fires a couple of spikes and then falls silent. The same stimulus is then applied at the peak of the slow EPSP, and now the cell gives a vigorous repetitive discharge. One minute later the cell's behaviour has returned to normal. Merely depolarizing the cell (for example by passing current) to the level attained during the sEPSP produces little or no change in the firing pattern of the cell. Furthermore, the effect is not simply due to an increase in membrane resistance, since it cannot be mimicked by just increasing the depolarizing stimulus current strength (although there is an increase in input resistance during the slow EPSP or during muscarinic stimulation which can by itself make the cell more excitable; Schulman & Weight, 1976). Because the spiking behaviour of nerve cells reflects the participation of a range of voltage-dependent conductances, it seems intuitively likely that the major effect of the sEPSP must be to modulate one or more of the voltage-dependent conductances of these cells (Koketsu, 1984). Later in this chapter the details of this modulation will be described, and furthermore it will be shown that this modulation is indeed
adequate to explain the observed modification of repetitive firing behaviour during slow synaptic transmission.

C CELLS

Stimulation of the axon(s) innervating a C cell results in three successive potential waves: a fast EPSP closely resembling that seen in B cells, a fast IPSP reaching a peak in 100 ms and lasting 1 s or so, and a late slow EPSP peaking in 30 s and lasting for 5 min. The fast IPSP is muscarinically mediated and reflects a transient increase in permeability to potassium ions (Dodd & Horn, 1983b). Because it tends to hyperpolarize the cell towards E_K (approx. −100 mV) it is inhibitory in effect. Little is known about the channels involved, though they may resemble those responsible for a very similar muscarinic IPSP in heart muscle (Sakmann, Noma & Trautwein, 1983). In heart muscle the muscarinic K^+ channels are stimulated by ACh only within the recording patch electrode in the on-cell configuration, arguing against the participation of a second messenger. However, recent work has nevertheless implicated a GTP-binding protein in the receptor–channel linkage (Pfaffinger et al. 1985; Breitwieser & Szabo, 1985).

Kuffler and his colleagues (Jan & Jan, 1982; Jan, Jan & Kuffler, 1979, 1980; Kuffler & Sejnowski, 1983) have provided convincing evidence that the late slow EPSP is mediated by release of a peptide resembling mammalian LHRH. The evidence is as follows: (1) boutons on C cells, but not those on B cells, stain for M-LHRH; (2) an M-LHRH-like peptide is released by preganglionic nerve stimulation in a calcium-dependent manner; (3) the late sEPSP can be mimicked by application of M-LHRH to the cells; (4) M-LHRH antagonists block both the effects of applied M-LHRH and the late slow EPSP itself. Subsequently Eiden & Eskay (1980) and Sherwood et al. (1983) showed that the M-LHRH-like peptide in the ganglion is not M-LHRH, but closely resembles the LHRH of salmon brain, T-LHRH. Frog brain contains both M-LHRH- and T-LHRH-like peptides. Subsequently it was shown that T-LHRH closely, and very potently, mimics the late sEPSP, and that T-LHRH-based antagonists block it (Jones, Adams, Brownstein & Rivier, 1984; Jan & Jan, 1982). At present, therefore, T-LHRH or a very similar peptide is the most likely candidate for the transmitter of the late sEPSP.

Because boutons on C cells stain both for LHRH and for choline acetyltransferase, and stimulation of the same set of axons releases both LHRH-like and ACh-like material, it is suspected that both transmitters are co-released from the same boutons (which contain both small clear vesicles and large dense-core granules). Additional evidence for this comes from experiments in which fEPSPs and late sEPSPs could be fractionated by varying the stimulus strength (Jan & Jan, 1982). It was found that the different components of the fEPSPs and the late sEPSPs had identical stimulus thresholds, showing that the same axon generated both responses. It remains possible, however, that the two transmitters are not released precisely in parallel in both spatial and temporal coincidence. Certain boutons may release more or less of one transmitter, and peptide release may be slower than ACh release. Indeed, part of
the slow rise time of the late sEPSP could reflect slow diffusion or might reflect sluggish coupling of peptide release to calcium entry, such as would occur if hydrolytic processing were necessary prior to release.

Dodd & Horn (1983a,b) have shown that the excitability of C cells changes dramatically during the late sEPSP in a very similar manner to that discussed above for muscarinic modulation of B cells. Jones (1984) has shown that the biophysical mechanisms for LHRH action on C cells are identical, in at least one important regard, to those for the sEPSP in B cells.

**C-B interactions**

If axons innervating C cells are stimulated repetitively, a late sEPSP can also be recorded in B cells. Although this late EPSP is somewhat slower in B cells than in C cells, it is actually more reliably seen, corresponding to the greater variability of C cell responses to LHRH-like substances. L. Y. Jan & Y. N. Jan have shown that the late sEPSP in B cells reflects extracellular diffusion of peptide from boutons synapsing on C cells. This is an important demonstration that the anatomical and physiological 'polarity' of synapses need not coincide.

During the C-evoked late sEPSP in B cells there is a dramatic enhancement of directly evoked repetitive firing just as is seen with the sEPSP, and the biophysical mechanisms for these effects are identical (Adams & Brown, 1980; Jones et al. 1984). The C-evoked B cell late sEPSP therefore represents a clear-cut example of heterosynaptic modulation of orthodromic transmission. Unlike the heterosynaptic facilitation seen in *Aplysia* sensory neurones it is postsynaptic in origin.

THE VOLTAGE-DEPENDENT CONDUCTANCES OF B CELLS

To understand the modification of discharge pattern during the sEPSP it is essential to characterize all the major voltage-dependent conductances present in the somatic-axon-hillock membrane, partly because any of these might be targets for muscarinic action, and partly because during normal cell firing all of them may be brought into play and must therefore be quantitatively modelled in a final synthesis. This task has occupied the bulk of our energies over the last few years and is now nearing completion. The observed conductances fall into three groups: those for sodium, calcium and potassium.

The sodium conductances have been characterized by a whole-cell switch-clamp method in dissociated ganglion cells lacking visible processes. At first glance the sodium currents observed after suppression of K+ and Ca2+ currents look very like those seen in squid axon or vertebrate node of Ranvier. The most obvious difference is that the currents are somewhat slower at all potentials, and require rather larger (by 30 mV or so) depolarizations to produce equivalent degrees of activation or inactivation.

A closer scrutiny has revealed that there are probably two separate Na+ conductance systems. The major one (70–80% of the total current) is quite classical, showing rapid inactivation and block by tetrodotoxin (TTX) in the nanomolar
range. The minor component inactivates somewhat more slowly and is much less sensitive to TTX. Perhaps the most striking difference is that the minor, but not the major, component is sensitive to submillimolar amounts of cadmium (S. W. Jones, unpublished results). Similar distinctions are seen in vertebrate sensory cells (Bossu & Feltz, 1984).

So far only one calcium conductance has been described. It activates rapidly (within milliseconds) in the potential range \(-25\) to \(+50\) mV, and shows extremely slow current-dependent inactivation. Of the three types of calcium current described by Nowycky, Fox & Tsien (1983), it most closely resembles the L-form. Large prolonged hyperpolarizations do not reveal additional forms of calcium current and, with one possible exception, calcium entry via this pathway seems adequate to account for the observed calcium-dependent K\(^+\) currents. The calcium current is not very well blocked by simple calcium omission, because of residual calcium and shifts in activation voltage. However, it is well blocked by cadmium, manganese, cobalt or nickel. Because any one alone of these procedures may have independent side effects, a demonstration of calcium dependence is best achieved with a battery of tests.

Five different types of K\(^+\)-conductance have been described (Adams, Brown & Constanti, 1982a; Adams, Constanti, Brown & Clark, 1982c; Pennefather, Jones & Adams, 1985a; Pennefather, Lancaster, Adams & Nicoll, 1985b; Lancaster & Pennefather, 1986). One of these, which we call I\(_K\), is similar to the classical delayed rectifier current of squid axon, node of Ranvier and molluscan cell bodies. Its hallmarks are sigmoidal activation, very slow but complete voltage-dependent inactivation and sensitivity to millimolar external tetraethylammonium (TEA). It is rather slower than the nodal delayed rectifier (which, however, does exhibit both fast and slow components; Dubois, 1981). I\(_K\) is not reduced by omitting external calcium; indeed this procedure enhances it, possibly through a surface potential modification.

Two of the other K currents of B cells are also fairly classical. There is a rapid, transient, low-threshold K current strongly resembling the A current of invertebrate neurones (Connor & Stevens, 1971; Neher, 1971), and a rapid, voltage-dependent, calcium-activated current essentially identical to that of chromaffin or muscle cells (Marty, 1981; Adams et al. 1982c; Barrett, Magleby & Pallotta, 1982). We call the latter current I\(_C\) so as to avoid confusion with the other calcium-activated K\(^+\) current I\(_{AHP}\) (see below). The term I\(_K(Ca)\) will be used to refer to any type of Ca\(^{2+}\)-activated K\(^+\) current when the exact type is immaterial, or unknown.

Like I\(_K\), but unlike the other K\(^+\) currents, I\(_C\) is blocked by millimolar TEA. It can be separated from I\(_K\) by various tests. It activates much more rapidly, it is mostly blocked by calcium removal, metallic calcium antagonists or charybdotoxin (see Miller, Moczydlowski, Latorre & Phillips, 1985). Following depolarizations that activate both I\(_C\) and I\(_K\), the major rapid outward tail current that follows repolarization to rest potential is a mixture of I\(_C\) and I\(_K\), which, because of their somewhat similar kinetics and voltage dependence, can be quite difficult to separate. However, tail current following either very brief (1 ms) or very long (1 min) depolarizations consists almost exclusively of I\(_C\), because I\(_K\) either has not had time
to activate or has completely inactivated. It is, in fact, rather surprising that the tail currents in these two situations should be rather similar, because the intracellular calcium concentration and distribution must be very different. Nevertheless the kinetics of \( I_C \) are roughly compatible with a simplified version of the scheme proposed by Moczydlowski & Latorre (1983) from data obtained with reconstituted single channels:

\[
Ca + R \rightleftharpoons CaR \rightleftharpoons CaR^* \rightleftharpoons Ca_2R^*,
\]

where \( R^* \) is the open form of the channel and the calcium binding reactions are assumed to be very fast. This represents a reduced form of a more elaborate scheme developed by Magleby & Pallotta. In this scheme the voltage dependence of channel opening reflects voltage dependence of \( Ca^{2+} \) binding. For example, in bullfrog cells the steady-state conductance evoked by small increases in intracellular calcium grows e-fold for 11 mV membrane depolarization (Adams et al. 1982c).

Although inactivation of \( I_C \) has been reported, it is very slow (Pallotta, 1985), as is that of the calcium current itself. It is thus quite surprising that there are two reports that an \( I_C \)-like current can show extremely rapid inactivation under certain circumstances (MacDermott & Weight, 1982; Brown, Constanti & Adams, 1982). In both sets of experiments, depolarization to between \(-20\) and \(0\) mV from a rather negative holding potential elicited a large transient outward current that was blocked by \( Ca^{2+} \) omission, metallic \( Ca^{2+} \) antagonists or TEA. It is possible that this represents a distorted form of \( I_C \) caused by series resistance problems, poor space clamp or potassium accumulation, although \( Ca^{2+} \)-dependent transient outward currents have been described in other systems (Salkoff, 1983).

The remaining two potassium currents of B cells are less familiar, though they probably both have counterparts in many other types of nerve cell. They are both small, slow currents that can be overlooked in conventional voltage-clamp experiments. One, \( I_M \), is purely voltage dependent, and the other, \( I_{AHP} \), is purely \( Ca^{2+} \) dependent. In fact, strictly speaking, \( I_{AHP} \) is not a voltage-dependent current at all, since its 'voltage dependence' derives exclusively from that of calcium entry, but operationally it belongs to this family of currents.

\( I_M \) begins to activate at a rather negative potential, about \(-60\) mV, and is almost fully activated at the threshold for the sodium current. The activation–deactivation time constant is about \(150\) ms at \(-35\) mV, but becomes much faster at more negative potentials. The reversal potential for \( I_M \) is about \(-85\) mV, which is slightly more positive than the value estimated for \( I_{AHP} \), possibly due to \( K^+ \) accumulation in the narrow glia–neurone ('Frankenhauser–Hodgkin') space. \( I_M \) does not inactivate even during depolarizations lasting tens of minutes.

\( I_{AHP} \) has only been studied as a tail current following very brief depolarizations (such as action potentials or voltage-clamp steps) because it requires large depolarizations which elicit currents (particularly \( I_C \)) which dwarf \( I_{AHP} \). However, it is clear from envelope measurements that \( I_{AHP} \) can be maximally activated by pulses of 1–2 ms duration. The late tail current following such brief depolarizations or single
spikes decays approximately exponentially with a time constant of 250 ms. Following a burst of spikes, $I_{AHP}$ decays more slowly though its initial amplitude is much the same.

$I_{AHP}$ is abolished by blocking Ca$^{2+}$ entry (Fig. 3), suggesting that it is calcium dependent. If $I_{AHP}$ is defined as the slow current that is blocked by calcium antagonists, it may show a rising phase (Tokimasa, 1984). However, clear definition of the early part of the tail as a difference current is difficult because of the effects of Ca$^{2+}$ depletion on other very large early tail currents.

$I_{AHP}$ is partially blocked by the bee venom toxin apamin (see also Hugues et al. 1982; Romey & Lazdunski, 1984), which blocks Ca$^{2+}$-activated K$^+$ permeability in hepatocytes (Jenkinson, Haylett & Cook, 1983). Cook & Haylett (1985) have recently shown that a variety of bisquaternary compounds such as pancuronium, curare and hexamethonium not only block $G_{K(Ca)}$ in liver cells but also compete at the apamin binding site. These compounds also block $I_{AHP}$ in bullfrog neurones (Nohmi & Kuba, 1984; Pennefather et al. 1985a).

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**Fig. 3.** Effects of calcium influx blockade on spikes in a B ganglion cell. The upper row shows action potentials elicited by a brief current pulse. The chart recording on the left shows the block by cadmium of the spike afterhyperpolarizations (downward excursions) and the oscilloscope record on the right shows superimposed spikes in the absence and presence of cadmium. The middle traces show the effects of cadmium on the slower components of the spikes. In the bottom row the hybrid clamp protocol was used, so that the records are of the currents following a clamp to the resting potential imposed at the peak of the early afterhyperpolarization. This current is mostly $I_{AHP}$.
TRANSMITTER SENSITIVITY OF $I_M$ AND $I_{AHP}$

The B cells thus exhibit at least seven separate voltage-dependent conductances. Each is presumably mediated by distinct ionic channels (though so far only those for $I_C$ and $I_K$ have been clearly identified in single-channel recordings). However, of these seven only the two slow, small $K^+$ currents, $I_M$ and $I_{AHP}$, are clearly influenced by the activation of muscarinic receptors. An example of these effects is shown in Fig. 4. In Fig. 4A the cell was held at $-60\text{mV}$ and stepped at $20\text{mV}$ positive or negative. The positive step elicited a time-dependent outward current relaxation, $I_M$. This relaxation was reversibly almost abolished by addition of muscarine. The positive step does not elicit $I_{AHP}$ because it is not large enough to open calcium channels. Occasionally in this type of experiment the positive step does trigger an abortive poorly clamped action potential, in which case $I_M$ can be contaminated by $I_{AHP}$ and appear spuriously $Ca^{2+}$ dependent.

Fig. 4B shows what happens when a full spike is deliberately triggered. The bottom traces reveal that the spike is followed by a prolonged afterhyperpolarization (AHP), which is reduced and shortened in muscarine. This effect is partly due to an increase in membrane leak conductance, visible in Fig. 4A and discussed below. However, it is also partly due to a decrease in $I_{AHP}$ itself, as shown by the 'hybrid clamp' experiment in the top row of Fig. 4B. This shows the outward current tail that flows if the membrane potential is returned to rest at the peak of the AHP. The $I_{AHP}$ tail is reduced to about 70% of control amplitude by addition of muscarine, with little change in time course. Since muscarine has no effect on ganglion cells in which $Ca^{2+}$ current has been isolated by blocking or removing most $Na^+$ and $K^+$ current (P. R. Adams, unpublished observations; see also Tokimasa, 1985), the reduction in $I_{AHP}$ is probably not secondary to attenuation of Ca entry. This is not certain because the $I_C$ experiments involve the use of TEA which may block muscarinic receptors.

The magnitudes of the effects of muscarine on $I_M$ and $I_{AHP}$ seen in Fig. 4 are quite typical. Even high doses of muscarine ($100\text{µmol}\text{l}^{-1}$) do not block more than 80–90% of $I_M$ or more than 10–30% of $I_{AHP}$, even though the half blocking concentration for muscarine acting on $I_M$ is about $1\text{µmol}\text{l}^{-1}$. It is not known why this should be so, or whether the muscarine-resistant fraction of $I_{AHP}$ corresponds to the apamin-sensitive fraction.

Muscarinically mediated reductions of $I_M$ or $I_{AHP}$ can also occur physiologically (i.e. following presynaptic ACh release). After a single nerve shock, only a small fraction of the total $M$ conductance is turned off, but optimal trains can block nearly all of it. If the cell is held at a potential where there is standing $M$ current (i.e. $-60\text{mV}$ or more positive) the partial shut-down of $M$ conductance leads to a transient inward current which accounts for virtually all the sEPSC (Adams & Brown, 1982). This endows the sEPSC with its paradoxical voltage dependence, first noted by Weight & Votava (1970), such that it is enhanced by depolarizing the membrane. Furthermore, because $E_K$ is normally negative to the activation range for $G_M$, it is virtually impossible to reverse the sEPSC.
The effects of muscarine and related agonists on $I_M$ and $I_{AHP}$ are clearly mediated by muscarinic receptors for they are blocked by low concentrations of atropine. However, a variety of other receptors are also coupled in a similar manner to $I_M$ and $I_{AHP}$. These are LHRH receptors, substance P receptors and nucleotide receptors. The effect of T-LHRH is illustrated in Fig. 5, which is patterned after Fig. 4. T-LHRH, like muscarine, produces a large reduction in $I_M$ and a small reduction in $I_{AHP}$. T-LHRH is thought to be the natural transmitter for the late sEPSP, and
indeed a very similar reduction in $I_M$ occurs during this synaptic response. The effects of T-LHRH and related compounds, as well as those of the synaptically released transmitter, are blocked by antagonists based on the M-LHRH or T-LHRH.
structures, which do not affect the responses to muscarine. Therefore, a quite distinct LHRH receptor must exist in the cell membrane and be coupled to \( I_M \) and \( I_{AHP} \).

The description of the other receptor types is less complete, largely because of the lack of specific antagonists. Substance \( P \) effects on \( I_M \) are rather variable from cell to cell, some cells responding to concentrations as low as \( 1 \) \( \text{nmol} \cdot \text{l}^{-1} \) (being the most potent of any drug effects on this system), while other cells do not respond even at \( 10 \) \( \mu \text{mol} \cdot \text{l}^{-1} \) (Adams, Brown & Jones, 1983; Jones et al. 1984). This variability may reflect inconsistent desensitization or tissue penetration. Substance \( P \) attenuates the slow spike AHP (Akasu, Nishimura & Koketsu, 1983b) and thus probably reduces \( I_{AHP} \).

\( I_M \) is also reduced, to about 10% of normal, by high concentrations of UTP or ATP (Adams, Brown & Constanti, 1982b; Akasu, Hirai & Koketsu, 1983a; P. Pennefather, unpublished results), whereas UDP and ADP are almost ineffective. It is not known whether one or two nucleotide receptors are involved. ATP also produces a 30% reduction in \( I_{AHP} \) (P. Pennefather, unpublished results).

These data suggest two alternative extreme pictures of the coupling of the various receptor types to the voltage-dependent conductances (Fig. 6). In the first view, each receptor is separately coupled to each channel; either as a result of a permanent physical association of all five units (four receptors and one channel) or because each channel can transiently link with activated forms of any of the receptors. In the second view, there is never a physical association. Instead activation of any one of the four receptor types causes changes in the concentration of one or more second messengers ('\( X \)'), which in turn affects the number of channels available for activation. Attempts to identify \( X \) have so far proved inconclusive. Intracellular injections of cyclic nucleotides or calcium failed to affect the amplitude of \( I_M \). In the former case this could reflect rapid breakdown of the injected substances, although extracellular applications of stable or lipid-soluble analogues or of forskolin were equally ineffective. In the latter case there was evidence that \( \text{Ca}^{2+} \) did indeed reach the membrane, because activation of \( I_C \) was seen, but even here \( \text{Ca}^{2+} \) may not have reached all the membrane at adequate concentrations to inhibit \( I_M \). Tokimasa (1985) has recently suggested that massive calcium loading \textit{via} \( \text{Ca}^{2+} \) spikes may inhibit \( I_M \). If \( \text{Ca}^{2+} \) is indeed involved in control of \( I_M \), it is unlikely that calcium influx across the plasmalemma is itself the normal signal for \( I_M \) inhibition by muscarine because the effect of muscarine persists in \( \text{Ca}^{2+} \)-free solution or after addition of metallic calcium blockers. However, in several other cell types, including neurones, there is evidence that muscarinic or peptide receptor activation leads to enhanced phosphatidylinositol 4,5-bisphosphate (\( \text{PIP}_2 \)) breakdown, yielding inositol trisphosphate (\( \text{IP}_3 \)) and diacylglycerol (DAG) (Horwitz, Tsymbabov & Perlman, 1984). Conceivably \( \text{IP}_3 \) release also occurs in ganglion cells, resulting in \( \text{Ca}^{2+} \) efflux from endoplasmic reticulum (Streb, Irvine, Berridge & Schulz, 1983). Calcium release from such internal stores induced by caffeine or membrane depolarization is known to occur in these cells (Kuba, 1980; Adams \textit{et al.} 1984). It will be interesting to examine the effects of \( \text{IP}_3 \) injections on ganglion cells.
Fig. 6. Two extreme views of receptor–channel coupling in bullfrog neurones. Sub.P, substance P receptors; Nuc.TPS, nucleotide receptors; AHP, afterhyperpolarization; M, M current.

Extracellular application of DAGs to ganglion cells has so far not revealed consistent effects. However, it has been recently shown that active phorbol esters such as phorbol dibutyrate, at micromolar concentrations, affect the M system in two ways (Adams & Brown, 1986). First, they reduce the size of the available M current and, second, they block the effects of muscarine or LHRH on $I_M$. These effects are specific in that neither $I_{AHP}$ nor the action potential itself are affected.
In addition, phorbol esters do not block the 'leak' increase produced by muscarine (see below). It has been shown in a variety of other systems that phorbol esters also block biochemical responses to activation of various receptors which are linked to PIP$_2$ breakdown. For example, in chromaffin cells phorbol esters block the muscarinically evoked intracellular release of Ca$^{2+}$ and DAG (Vicentini et al. 1985). Fig. 7 sketches a way in which these various fragmentary observations might be interpreted. It suggests that receptor stimulation leads to phosphatidylinositol (PI) turnover via a coupling protein. C-kinase activation by phorbol ester might phosphorylate this coupling protein so as to impair the PI turnover response to receptor activation. Normally, however, release of Ca$^{2+}$ via IP$_3$ liberation and the release of synergistically acting DAG activates C-kinase which phosphorylates some component of the M channel system. The variable or unconvincing effects of phorbol ester or calcium acting separately on M conductance would be explained by a normal requirement for simultaneous [Ca$^{2+}$], and [DAG] elevation. This scheme is currently being tested in our laboratory.

**Fig. 7.** Possible activation on targets of C-kinase in ganglion cells. PIP$_2$, phosphatidylinositol 4,5-bisphosphate; IP$_3$, inositol trisphosphate; DAG, diacylglycerol.
TRANSMITTER EFFECTS ON LEAKAGE

If a cell is voltage clamped at $-60\,\text{mV}$, virtually no time-dependent, voltage-sensitive current is present, and the behaviour is almost ohmic for moderate hyperpolarizations, until a slow creep current appears below $-100\,\text{mV}$. The slope of the $I/V$ relationship in this range thus reflects the membrane ‘leak’ resistance. This leakage is rather variable, and tends to be lowest when using high-resistance, sharp microelectrodes or after ‘sealing in’ of the electrode. Part of the leak conductance is thus probably due to the ‘hole’ caused by microelectrode impalement, either the physical discontinuity itself, or general membrane effects arising from influx of extracellular medium. However, part of the leakage conductance must reflect an intrinsic membrane property, because even the very best impalements never yield input resistances above $200\,\text{M\Omega}$. Whole-cell recording also usually yields input resistances in the negative potential region of several hundred megohms, even when the initial seal is many gigohms, is very stable, and membrane rupture appears to be clean and smooth. The intrinsic part of the leakage conductance is often increased by application of muscarinic or peptidergic agonists (e.g. Figs 4, 5). This effect is characteristically seen during prolonged application of high doses. Furthermore, it lags behind the onset and recovery of the effects of the same drug application on $I_M$ (Jones, 1985). This suggests that the mechanism of this effect may be quite different from that on $I_M$ or $I_{AHP}$, which is supported by the differential effects of phorbol esters on $I_M$ inhibition and leakage increase. The leakage conductance increase probably involves $\text{Na}^+$ and/or $\text{Cl}^-$ ions since the reversal potential is quite positive. It is thus capable of generating an inward current at potentials where $I_M$ is inoperative. However, it is not normally seen during slow synaptic transmission, unless drastic stimulation is used (Akasu, Gallagher, Koketsu & Shinnick-Gallagher, 1984).

C CELLS

Exploration of the voltage-dependent conductances of C cells has lagged behind that of B cells, largely because of their smaller size. The action potentials of these cells lacks the characteristic break in the afterhyperpolarization (see Fig. 1) which reflects the two-component ($I_C + I_{AHP}$) tail current in B cells. Correspondingly, hybrid clamp experiments reveal a tail current which decays much faster than $I_A$, but which is TEA and probably calcium insensitive (S. W. Jones, unpublished observation).

C cells possess a normal $M$ current, with almost identical kinetics and voltage sensitivity to that of B cells. However, $I_M$ in these cells is not reduced by muscarine. Thus although $I_M$ was first defined by sensitivity to muscarinic receptor activation, this is not a necessary characteristic. A muscarine-insensitive $I_M$ is also found in cultured sensory ganglion cells (Barry, Werz & Macdonald, 1983).

$I_M$ in C cells is sensitive to LHRH agonists, though less consistently so than in B cells. Application of LHRHs to cells held at $-60\,\text{mV}$ or more negative sometimes unexpectedly produces an outward current accompanied by a decrease in
leakage conductance, presumably through shutting of voltage-independent Na\textsuperscript{+} or Cl\textsuperscript{-} channels. Muscarine itself can produce similar decreased leakage outward currents in chloride-loaded rat sympathetic cells (Brown & Selyanko, 1985). In bullfrog C cells the decreased leakage may be followed by a period of increased leakage similar to that seen in B cells.

**EXCITABILITY EFFECTS OF NEUROTRANSMITTERS IN B CELLS**

We are now in a position to consider how inhibition of $I_M$, and to a lesser extent of $I_{AHP}$, might produce the dramatic changes in firing pattern that are seen during slow synaptic potentials or receptor stimulation. It is simplest to start by considering the subthreshold responses that occur during very weak direct current inputs or small fEPSPs. The normal resting potential of B cells is still somewhat uncertain, but is probably $-55$ to $-60$ mV, so that a small, standing M current is already present (but see Tosaka, Takasa, Miyazaki & Libet, 1983). An applied weak current step will cause the membrane potential to deviate from rest as a result of charging the membrane capacity. However, the fraction of M channels open is no longer appropriate to the new membrane potential, and this fraction will relax slowly, causing the potential to sag back towards its original value. To compute the exact time course of the potential trajectory one must solve the equations describing current flow in the circuit diagram shown in Fig. 8.

$G_M$ is a time- and voltage-dependent conductance, whereas the other values are fixed. The following equation applies ($\delta I$ is the applied current step):

$$\delta I = C \frac{dV}{dt} + G_L(V - E_L) + G_M(V - E_K).$$

(1)

$G_M$ is considered to be equal to the product of the maximum available M conductance (typically 85 nS) and the fraction of M channels that are open at any moment, $x_M$. Because opening or closing of M channels in voltage-clamp experiments follows an exponential time course, a simple, first-order differential equation adequately describes the time course of $x_M$:

$$\frac{dx_M}{dt} = \frac{(x_M,\infty - x_M)}{\tau_M},$$

(2)
where \( x_{M,\infty} \) is the steady-state fractional activation of the M conductance, and \( \tau_M \) is the time constant for activation/deactivation at any given voltage. Thus the key information which must be obtained in voltage-clamp experiments is the voltage dependence of \( x_{M,\infty} \) and \( \tau_M \). Fortunately these details are available in the range \(-100\) to \(-20\) mV (Adams et al. 1982a), and have been recently extended to more positive potentials (Lancaster & Pennefather, 1986).

The strong voltage dependence of \( \tau_M \) and \( x_{M,\infty} \) precludes general analytical solution of equations 1 and 2. However, if the injected current step \( I \) is very small, the membrane potential always stays very close to its original value, so that \( \tau_M \) is practically constant, and \( x_{M,\infty} \) is a roughly linear function of voltage. The equations can thus be linearized and solved to yield:

\[
\delta V = \delta I (Ae^{-t/\tau_1} + Be^{-t/\tau_2}),
\]

where \( A, B, \tau_1 \) and \( \tau_2 \) are algebraic functions of all the circuit parameters and the slope of the \( x_{M,\infty} \) curve at rest potential. For typical values of these parameters a double exponential (overdamped oscillation) or highly damped oscillation is expected, just as is observed in real cells. Intuitively one can see why this should be so. Over most of its activation range \( \tau_M \) is considerably larger than the effective cell membrane time constant \( C/(G_L + G_M x_{M,\infty}) \) so that the potential step produces an initial fast displacement which relaxes back to a steady-state value as M channels open or close.

The general shape of the response is much the same even for larger current steps which do not allow linearization. These potential trajectories have been obtained by numerical integration of the above equations 1 and 2 (Adams et al. 1982a,b,c).

The linearization approach, or alternatively numerical integration, can also be used to calculate the voltage trajectories generated by brief current inputs, such as small fEPSPs. These calculations predict that subthreshold EPSPs should be followed by a small hyperpolarizing bounce, if the initial membrane potential is within the M activation range. This bounce is quite different from the afterhyperpolarization that follows massive iontophoretic ACh applications, which is due to calcium entry through the nicotinic channels and activation of \( G_{K(Ca)} \) (Tokimasa & North, 1984). The absence of the fEPSP afterbounce in external records from whole ganglia may indicate that the normal resting potential of unimpaled cells is at least \(-60\) mV (Tosaka et al. 1983).

These calculations do not predict the membrane potential responses to larger depolarizing or hyperpolarizing current steps, or to normal strength synaptic inputs, since they do not incorporate the other six voltage-dependent currents. To extend the simulations it is necessary to have equations similar to 1 and 2 above for each conductance. Unfortunately all the other conductances show more complex kinetics than \( G_M \), and in no case does a complete description exist. However, in each case enough quantitative information is available to develop adequate approximate descriptions. The simplest are the purely voltage-dependent systems \( G_{Na} \), \( G_K \) and \( G_A \). These are modelled in a conventional Hodgkin–Huxley manner by including
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inactivation variables. Our equations for $G_K$ and $G_A$ are firmly based on our own measurements, while provisionally we have adapted the Frankenheuser–Huxley equations for nodal $G_{Na}$ to the overall time course of $I_{Na}$ in bullfrog neurones. Activation and inactivation kinetics were slowed by a factor of two and shifted in the depolarizing direction. Ultimately it should be possible to incorporate the more detailed analysis of inactivation referred to above.

$G_{Ca}$, $G_C$ and $G_{AHP}$ are more difficult to model because each depends, inter alia, on the subsurface calcium concentration. In general we have made the simplest possible assumptions consistent with our data, i.e. activation (or in the case of $G_{Ca}$, inactivation) by calcium binding:

$$Ca + R \rightleftharpoons CaR^\bullet.$$

In the case of $G_{AHP}$, it was necessary to assume that cooperative calcium binding was needed for the channel opening, to account for the almost complete activation produced by brief depolarizations. The kinetics of $G_C$ and $G_{AHP}$ activation are thus governed by the calcium binding/unbinding reactions, endowed in the former case with suitable voltage dependence. In the case of $G_{Ca}$, the calcium blocking reaction

$$Ca + (R^\bullet + R) \rightleftharpoons CaR$$

was assumed to be instantaneous.

The diffusion of calcium within the cell was represented by a simplified two-compartment system (Fig. 9). Both intracellular compartments were assumed to contain a calcium buffer of plausible amount and binding rate constants, and calcium was extruded across the cell membrane in a voltage-dependent manner.

Intact ganglion cells are surrounded by a tight glial capsule which greatly restricts the escape of potassium ions from the cell surface. This was represented as another well-mixed compartment around the plasmalemma (see Fig. 9). The thickness and permeability of this compartment were estimated experimentally by varying

![Fig. 9. Compartment model of ion diffusion in bullfrog ganglion cells.](image-url)
the length of 'loading' depolarizations and measuring the resulting change in $E_K$ (Lancaster & Pennefather, 1986).

Before considering the detailed behaviour of the computer model it would be well to consider in a qualitative manner how the various conductances might interact to generate complex trajectories such as action potentials, and how blocking specific channels might be expected to modify the observed responses. The response to prolonged hyperpolarizing inputs is fairly simple to understand. If the initial potential is positive to $-60\,\text{mV}$, i.e. within the activation range of $I_M$ and $I_A$, small steps will generate trajectories just like those described above for $I_M$ alone, whereas large steps, which remove $I_A$ inactivation, will be followed by a notch on the repolarizing phase (Connor & Stevens, 1971), just as is seen in real cells.

Brief suprathreshold depolarizing inputs generate spikes whose upswing (typically 100–500 $\text{V s}^{-1}$: Akasu et al. 1983a,b; MacDermott & Weight, 1982) is determined mainly by the cell capacity and the maximum sodium current (approx. 20 $\text{nA}$). The capacity of intact cells in whole isolated ganglia is typically 400 $\text{pF}$, larger than expected from the apparent cell diameter, and also larger than seen in acutely dissociated cells (approx. 100 $\text{pF}$: S. W. Jones & W. Gruner, unpublished observations). Presumably this reflects the contribution of initial segment membrane, although it is unclear quite how isopotential this compartment is with the soma (see Adams et al. 1982a,b,c). If it is not isopotential then the question of channel distribution would become important. In other cell types there is evidence for preferential localization of $\text{Na}^+$ channels in the initial segment (Matsumoto & Rosenbluth, 1985).

The action potential repolarizes at an almost comparable rate ($50\,\text{V s}^{-1}$), whereas sodium inactivation alone could only allow repolarization at a rate equal to the reciprocal of the cell time constant. Clearly a large outward current must activate during the spike itself, and there are only two possible candidates, $I_K$ and $I_C$. Of course in squid axon, $I_K$ (together with sodium inactivation) repolarizes the spike, but this does not seem to be the case in bullfrog neurones, because activation of $I_K$ takes several milliseconds. Brief spike-like depolarizations only activate $I_C$ and, correspondingly, application of calcium blockers reduces the repolarization rate as well as the maximum hyperpolarizing excursion (which now occurs somewhat later; Fig. 3, cf. Fig. 10). The fact that even after block of calcium entry, spike repolarization still occurs rapidly, albeit at a reduced rate, presumably reflects enhanced recruitment of $I_K$ by the broadened spike. TEA prolongs the spike to about the same extent as calcium deprivation. Since TEA is equally effective in blocking $I_C$ and $I_K$, this must mean that spike broadening enhances $\text{Ca}^{2+}$ entry enough to restore $I_C$ to at least its control level. If TEA is applied to a $\text{Ca}^{2+}$-deprived cell, further spike broadening is seen, and $\text{Na}^+$ inactivation, $I_M$ and even $I_{AHP}$ become the main repolarizing influences.

After the normal spike has reached its maximum hyperpolarizing excursion, $G_C$ must return rapidly to zero, even though the internal calcium concentration remains transiently elevated (e.g. Smith, MacDermott & Weight, 1983). At $-80\,\text{mV}$ the $I_C$ tail current has a time constant of only 1 ms, and were it not for $I_{AHP}$ the spike AHP...
would decay passively back to rest. Indeed, in our early work this was usually the case, because unsuspected damage had eliminated or obscured the effect of $I_{AHP}$. Even here, however, the decay is not completely passive, because the AHP itself is long and large enough to turn off some of the standing M conductance, resulting in a brief depolarizing afterbounce.

Lightly damaged cells, possessing a normal $I_{AHP}$, show a slow component in the AHP decay. The exact shape of the AHP following its initial excursion is rather variable and this variability correlates with the degree of damage the cell has sustained. Rather leaky cells show an initially fast decaying AHP with a slow tail. The slow tail becomes proportionately larger as the leak decreases, until no fast decay is present. Even less leaky cells show an additional rising phase to the AHP (i.e. arising out of the initial hyperpolarizing excursion) followed by a slow decay phase. These various complex waveforms arise purely from variations in the passive leak resistance, and not the underlying ionic conductances, as shown by a passive model of the cell membrane (Adams, Pennefather & Lancaster, 1985; Adams & Galvan, 1986).

We are now in a position to consider the pattern of discharge expected during a maintained depolarizing stimulus. The first spike occurs as described above, and is followed by a brief hyperpolarization, which brings the potential below rest. If the cell has little or no $I_{AHP}$, this is followed by a short, overshooting depolarization, which could reflect partly the normal sag that occurs in a subthreshold depolarizing electrotonic potential, and partly turning off of M channels during the early spike afterhyperpolarization. The overshooting depolarizing 'bounce' then settles down to a maintained level of depolarization which is largely determined by the steady-state, current/voltage relationship. This level is normally subthreshold, because development of outward M current subtracts from the applied stimulus current.

If AHP current is present this will be triggered by the initial spike, and will mask the afterdepolarizing bounce and prevent further spiking. Both $I_M$ and $I_{AHP}$ act by subtracting from the stimulus. $I_{AHP}$ acts early on because it is activated by calcium entry. Adaptation by this mechanism has the drawback that it wears off since the calcium load is rapidly dissipated. $I_M$ takes time to develop but then continues to act. These two currents thus play complementary roles in adaptation. Alone neither could produce complete adaptation but together they effectively limit firing.

**COMPUTER SIMULATIONS**

The qualitative analyses and predictions in the preceding section have been tested and refined by the detailed computer simulations. The interpretation of the repolarization and AHP of the action potential given above is completely borne out by these calculations. Both $I_C$ and $I_K$ appear as brief outward currents coincident with repolarization, $I_C$ being normally much the larger (20 nA vs 5 nA). However, inhibition of $\text{Ca}^{2+}$ entry considerably reduces $I_C$, with a compensatory increase in $I_K$ due to slight spike broadening (Fig. 10). $I_C$ by no means disappears because at positive potentials resting internal calcium is adequate to allow some activation.
Similarly, in voltage-clamp experiments (Lancaster & Pennefather, 1986) or simulations, considerable $I_C$ can be triggered by large depolarizations in the presence of cadmium.

The only other outward current significantly activated by single spikes according to this model is $I_{AHP}$. As observed experimentally this shows: (1) only a very brief rising phase, (2) near maximal activation by single spikes and (3) exponential decay which gets slower following a burst of spikes. As expected, the form of the AHP following single spikes depends critically on input resistance.

The calculations also confirm the synergistic actions of $I_M$ and $I_{AHP}$ in producing spike frequency adaptation during long current pulses. Inhibition of $I_M$ allows the firing of isolated extra spikes late in the depolarizing pulse, while inhibition of $I_{AHP}$ produces rather little increase in firing. But inhibition of $I_{AHP}$ does allow the observation of a depolarizing 'bounce' following the initial spike. This bounce is most prominent when the empirically determined voltage dependence of M activation at positive potentials (Lancaster & Pennefather, 1986) is used, as opposed to the original extrapolation of data in the negative potential range (Adams et al. 1982a). This is because, in the original formulation, considerable M activation can occur during the spike itself. Ideally (and in fact experimentally) one would want little net change in the M-activation variable during single spikes, with activation (during the upswing) just balancing deactivation (during the afterhyperpolarization). This would allow a gradual and sustained increase in $I_M$ during the depolarizing pulse.

As expected, inhibition of both $I_{AHP}$ and $I_M$ allows sustained repetitive firing during the pulses (Fig. 11). In real cells, firing does cease after 1 or 2 s, presumably because of slow sodium inactivation processes not yet incorporated in the computer program. These predicted effects of separate or combined inhibition of $I_M$ and $I_{AHP}$
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have been experimentally verified using curare and/or muscarine as relatively selective blockers (Pennefather, Jones & Adams, 1985a). It should be noted that in our early work (Brown & Adams, 1980; Adams et al. 1982b) inhibition of $I_M$ alone was enough to allow repetitive firing, because these cells were somewhat damaged and lacked $I_{AHP}$.

CONCLUSIONS

Our knowledge of synaptic transmission and excitability characteristics in frog sympathetic ganglia is probably now more complete than in any other comparable miniature nervous system. The way that the fast and slow synaptic potentials generate electrical activity, and the interactions between them, is now reasonably well understood. Nevertheless, our ignorance remains daunting. Many of the suspected channels have not yet been identified in individual recordings. The biochemical coupling mechanisms are only beginning to be explored. Perhaps most acutely, the physiological relevance of these elaborate phenomena to normal impulse traffic across the ganglion and to target tissues such as blood vessels and slime glands is quite obscure. Even if, in these ganglia, the phenomena discussed in this chapter have little functional importance, it is now clear that very similar processes occur in mammalian central neurones such as rodent hippocampal pyramidal cells (Halliwell & Adams, 1982; Cole & Nicoll, 1984; Madison & Nicoll, 1984; Lancaster & Adams, 1986; Gähwiler & Brown, 1985) and human neocortical neurones (Halliwell, 1986).

![Control](image1.png) ![Im and I_{AHP} inhibited](image2.png)

Fig. 11. Computer simulation of repetitive firing in a B cell. Manual clamp to $-60 \text{ mV}$ resting potential. The control model fires only two spikes to a $3 \text{ nA}$ current pulse, but maintained firing is seen after inhibition of $I_M$ and $I_{AHP}$. Compare with Fig. 2.
Slow synaptic modulation of voltage-dependent conductances is likely to become an increasingly attractive theme for investigation.

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