Eur Biophys J (2004) 33: 211–226
DOI 10.1007/s00249-003-0373-0

REVIEW

Y. Pichon · L. Prime · P. Benquet · F. Tiaho

Some aspects of the physiological role of ion channels in the nervous system

Received: 28 August 2003 / Revised: 23 October 2003 / Accepted: 24 October 2003 / Published online: 14 January 2004

Abstract Recent analyses of the genomes of several animal species, including man, have revealed that a large number of ion channels are present in the nervous system. Our understanding of the physiological role of these channels in the nervous system has followed the evolution of biophysical techniques during the last century. The observation and the quantification of the electrical events associated with the operation of the ionic channels has been, and still is, one of the best tools to analyse the various aspects of their contribution to nerve function. For this reason, we have chosen to use electrophysiological recordings to illustrate some of the main functions of these channels. The properties and the roles of Na^+ and K^+ channels in neuronal resting and action potentials are illustrated in the case of the giant axons of the squid and the cockroach. The nature and role of the calcium currents in the bursting behaviour of the neurons are illustrated for *Aplysia* giant neurons. The relationship between presynaptic calcium currents and synaptic transmission is shown for the squid giant synapse. The involvement of calcium channels in survival and neurite outgrowth of cultured neurons is exemplified using embryonic cockroach brain neurons. This same neuronal preparation is used to illustrate ion channel noise and single-channel events associated with the binding of agonists to nicotinic receptors. Some features of the synaptic activity in the central nervous system are shown, with examples from the cercal nerve giant-axon preparation of the cockroach. The interplay of different ion conductances involved in the oscillatory behaviour of the *Xenopus* spinal motoneurons is illustrated and discussed. The last part of this review deals with ionic homeostasis in the brain and the function of glial cells, with examples from *Necturus* and squids.

Keywords Electrophysiology · Ion channels · Ionic homeostasis · Neurons · Synapses

Presented at the Biophysical Society Meeting on "Ion Channels: from Biophysics to Disorders", held in May 2003 in Rennes, France

Y. Pichon (✉) · L. Prime · P. Benquet · F. Tiaho
Equipe Canaux et Récepteurs Membranaires,
Université de Rennes 1, UMR 6026, Bâtiment 13,
Campus de Beaulieu, Avenue du Général Leclerc,
35042 Cedex Rennes, France
E-mail: yves.pichon@univ-rennes1.fr
Tel.: +33-2-23235061
Fax: +33-2-23235052

Introduction

In the following, we shall illustrate how ion channels participate in the main functions of the nervous system. This paper is aimed towards non-physiologist readers and corresponds to the main content of the presentation given at the Rennes meeting on ion channels. It is illustrated for its main part by original (partly unpublished) findings of our laboratory on three different nerve preparations: the squid giant axon, the cockroach nervous system and the *Xenopus* tadpole spinal cord. It is not intended to provide new and comprehensive information on the present status of this constantly changing field. More details will be found, for example, in Sakmann and Neher (1995) or Hille (2001). An interesting review of the functional role of ion channels in mammalian myelinated and unmyelinated nerve fibres has been written by Scholz and Reid (2002).

The first section will be devoted to a list of the different categories of ion channels and receptors found in the nervous system, followed by a comprehensive list of the main α-subunits of sodium, potassium and calcium channels that are present in the nervous system, together with their accepted nomenclature and distribution.

In the second section, we shall first summarize how the evolution of biophysical techniques has enabled the understanding of the role of ionic channels. We shall then illustrate the role of sodium and potassium channels in nerve conduction in the squid and cockroach preparations. The third subdivision will be devoted to

calcium channels and their role in neuronal bursting activity and synaptic transmission. It will be followed by a discussion of the suspected role of calcium channels in survival and growth of embryonic insect neurons. The properties of ligand gated ionotropic receptors will then be depicted in the case of the response of the insect neuronal membrane to ACh and carbachol. The complexity of the synaptic events in the nervous system will be illustrated in the following subdivision, with examples from the cockroach central nervous system (CNS). The interactions between various ion channels to induce oscillations will then be illustrated for motoneurons from the *Xenopus* tadpole in the presence of *N*-methyl-D-aspartate (NMDA), a glutamatergic agonist. In the last subdivision, the role of ion channels in ion homeostasis and the participation of glial cells in ionic regulation and signalling in the nervous system will be discussed, with examples from squid axons and *Necturus* ganglia.

Ion channel categories

The ion channels and receptors which are involved in the operation of the nervous system are shown in Table 1. They belong to six clearly different categories: voltage-gated channels, inward rectifiers, ligand-gated channels, ATP-gated channels, TRP-gated channels and gap-junction channels.

Voltage-gated channels correspond to a very homogeneous family of transmembrane proteins. They comprise six transmembrane domains and are assembled as four continuous subunits (Na^+ and Ca^{2+}) or a tetramer of four subunits (K^+). Each transmembrane domain has six membrane-spanning regions, one of which, the S4 segment, contains positively charged amino acids (lysine and arginine) and senses the voltage across the membrane and therefore regulates pore opening. Based on the bacterial K^+ channel model, the selectivity pore would be about 12 Å long and 3 Å wide and be lined by main-chain oxygen atoms. This pore is selective for Na^+, K^+ or Ca^{2+}.

Inward-rectifier channels, which are selective for K^+, possess only two transmembrane domains. The selectivity filter corresponds to the P domain and they form homo- or hetero-oligomers in the membrane.

Ligand-gated channels (LGIC), as their name indicates, are opened when the ligand molecules bind to a specific receptor. They comprise three clearly different families: Ionotropic nicotinoid, ionotropic glutamatergic and metabotropic receptors. The former are made of five homologous polypeptide subunits possessing four membrane-spanning regions. The part of the polypeptide that binds the ligand contains two cysteines which form the "*cis*-loop" disulfide bridges (*cis*-loop receptor subfamily). This subfamily is divided into two groups according to their ionic specificity: one containing the subunits forming cationic receptors (nicotinic and 5-HT_3 receptors) and one containing the subunits forming anionic (Cl^-) receptors ($GABA_A$ and $GABA_C$, glycine and GLUCl receptors).

The ionotropic glutamatergic LGICs possess four membrane-spanning regions and form three families of receptors selective to three different glutamate agonists, respectively NMDA, AMPA and kainate. Their ion channel is selective for cations.

Metabotropic receptors are activated indirectly by neurotransmitters, through second messenger systems, or directly by cAMP, cGMP, internal Ca^{2+}, G-proteins

Table 1 Different ion channel types

1. Voltage-gated channels (six transmembrane domains, four subunits)
   - Na^+, K^+, Ca^{2+}
2. Inward rectifiers (two transmembrane domains, K^+)
3. Ligand-gated channels (LGIC)
   - Ionotropic
     - Nicotinoid (four transmembrane domains, five subunits, cys-loop)
       - Nicotinic AChR (cations)
       - GABAA and GABAC (Cl^-)
       - Glycine (Cl^-)
       - 5-HT3 (cations)
       - Some glutamate-activated anionic channels
     - Glutamate-activated cationic channels (four transmembrane domains)
       - NMDA (NR1, NR2A–D, NR3A, cations)
       - AMPA (GluR1–4, cations)
       - Kainate (GluR5–7, Ka1,2, cations)
   - Metabotropic (seven transmembrane domains, cAMP, cGMP, Ca^{2+}, G-proteins, phosphorylation)
     - Muscarinic AchR (m1–5) (K^+ and Ca^{2+})
     - GABAB (K^+, slow IPSPs)
     - 5-HT (5HT1, 5HT2, 5HT4, 5HT5)
     - Adrenalin, dopamine
     - Metabotropic glutamate receptors (mGLUR1–7)
4. ATP-gated channels (three subunits, P2x)
5. TRP channels (six transmembrane domains, often calcium selective)
6. Gap-junction channels

or phosphorylation. They possess seven transmembrane domains and a large intracellular domain. They can be activated by Ach, GABA, 5-HT, adrenaline, dopamine and glutamate.

ATP-gated channels possess three subunits. Transient receptor potential channels (TRP) possess six membrane-spanning regions, are usually non-selective but can be selective for Ca^{2+}.

Human brain sodium, potassium and calcium channels

Human brain sodium channel α-subunits (Table 2) belong to eight genes (SCN1A to SCN11A) corresponding to the Nav1.1, 1.2, 1.3, 1.6, 1.7, 1.8 and 1.9 units of the present sodium channel nomenclature (Table 2). The last two channels are resistant to tetrodotoxin (TTX) and are associated with pain sensory fibres. The first four are particularly abundant in the brain. Sodium channels are clustered at the axon initial segment and at the nodes of Ranvier. Two β subunits (SCN1B and SCN2B) are also present in the CNS.

Human brain potassium channels belong to three distinct groups: voltage-gated potassium channels, inward rectifiers and calcium-activated potassium channels. Brain voltage-gated potassium channels subunits (Table 3) comprise 14 genes (KCNA1 to KCNG1). Two types of α-subunits are present in the brain; some are functional by themselves, but others modulate the activity of functional subunits. As for sodium channels, there is a correspondence between the KCN (human genome) terminology and the general Kv nomenclature.

Table 2 Brain voltage-gated sodium channels α-subunits (six transmembrane domains)[a]

Nomenclature		Distribution	Main function	Pharmacology[b]	Diseases
SCN1A	Nav1.1	Brain	Action potential production	Sensitive to TTX	Seizures
SCN2A1	Nav1.2	Brain		Sensitive to TTX	Seizures
SCN2A2		Brain			
SCN3A	Nav1.3	Brain		Sensitive to TTX	
SCN8A	Nav1.6	Brain, spinal cord	Burst activity	?	Motor end plate (med) disease
SCN9A	Nav1.7	Dorsal root ganglia		Sensitive to TTX	
SCN10A	Nav1.8	Small sensory neurons	Pain	Resistant to TTX	Pain sensitization
SCN11A	Nav1.9	Sensory neurons	Pain	Resistant to TTX	Pain sensitization

[a]From Washington University database
[b]TTX: tetrodotoxin

Table 3 Brain voltage-gated potassium channels α-subunits (six transmembrane domains)[a]

Nomenclature		Distribution	Main function	Pharmacology[b]	Diseases
KCNA1	Shaker, Kv1.1	Presynaptic	Delayed rectifier	4-AP, α-dendrotoxin	Episodic ataxia 1
KCNA2	Shaker, Kv1.2	Presynaptic	Delayed rectifier	Tityus toxin Kα	
KCNA4	Shaker, Kv1.4	Presynaptic	A current	4-AP	
KCNA6	Shaker, Kv1.6	Brain	Delayed rectifier	α-Dendrotoxin	
KVNB	Shab, Kv2.1–2.2	Soma, dendrites		TEA	
KCNC1	Shaw, Kv3.1	Brain	Delayed rectifier	4-AP, TEA	
KCNC2	Shaw, Kv3.2	Brain	Delayed rectifier	TEA	
KCNC3	Shaw, Kv3.3	Brain	Delayed rectifier		
KCNC4	Shaw, Kv3.4	Brain	Delayed rectifier	BDS-I	
KCND1	Shal, Kv4.1	Brain	A-current	4-AP	
KCND2	Shal, Kv4.2	Brain	A-current	4-AP	
KCNEIL	Shal	Heart, muscle, brain			
KCNF1	Kv5	Heart, brain	Silent		
KCNG1	Kv6	Brain, muscle	Silent		
KCNQ2		Brain/sympathetic ganglia	M channels with KCNQ3 + calmodulin		Myokymia, benign neonatal epilepsy
KCNQ3		Brain	M channel with KCNQ2 + calmodulin		Benign neonatal epilepsy
KCNQ5		Brain/sympathetic ganglia	M current, aux. subunit KCNQ3		
KCNS1–3	Kv6	Brain	Silent		
KCNV2	Kv11.1	Brain	Silent		
KCN1–4	BCNG	Brain	Pacemaker H current	cAPM-gated; inhibited by cesium	

[a]From Washington University database
[b]4-AP: 4-aminopyridine; TEA: tetraethylammonium

Furthermore, KCNA1 to 6 (i.e. Kv1.1 to 1.6) correspond to the Shaker channel, KCNB (Kv2.1, 2.2) correspond to the Shab channel, KCNC1 to 4 (Kv3.1 to 3.4) to the Shaw channel and KCND1 and 2 (Kv4.1 and 4.2) to the Shal channel. Apart from KCNA4 and KCND2, which express A-current (rapidly inactivating) generating channels, the main function of the other genes is to express delayed rectifier channels. The voltage-gated potassium channels regulate the resting membrane potential and control the shape and the frequency of the action potentials (see below).

Brain inward rectifier channels belong to nine different genes (Table 4). Most of them belong to the KCNJ family [KCNJ2, 4, 6–7, 10, 12 and 13 (which correspond to Kir2.1, 2.3, 3.2, 1.2–4.1, 2.2 and 7.1)]. The three others (KCNH3 to 5 or HERG), which are related to cyclic nucleotide gated cation channels, are present in various parts of the brain. These channels play a role in the maintenance of the resting potential near the equilibrium potential for K^+ ions and the control of cell excitability. They are non-conducting at positive membrane potentials.

Table 4 Brain inward rectifying potassium channels (two transmembrane domains)[a]

Nomenclature		Distribution	Main function	Pharmacology	Diseases
KCNJ2	Kir2.1	Brain, heart, muscle	Shape of action potential, excitability	carbachol, polyamines, Ba^{2+}	ATS syndrome
KCNJ4	Kir2.3	Heart, muscle, brain			
KCNJ6–7	Kir3.2	Cerebellum			Weaver mice
KCNJ10	Kir1.2 – 4.1		Glial K^+ buffering		
KCNJ12	Kir2.2		Shape of action potential, excitability		
KCNJ13	Kir7.1		Membrane potential		
KCNH3	Kir7.1	Cortex			
KCNH4	Kir7.1	Brain			
KCNH5	Kir7.1	Brain			

[a]From Washington University database

Table 5 Brain calcium-activated potassium channels (four transmembrane domains)[a]

Nomenclature		Distribution	Main function	Pharmacology	Diseases
KCNK1	TWIK	CNS	Background K conductance	Apamin	
KCNK2	TREK	CNS			
KCNK4	TRAAK	Neural tissues			
KCNK7	TWIK-1-like	CNS			
KCNK12	THIK-2	Brain, kidney			
KCNK13	THIK-1	Ubiquitous			
KCNMB1	BK	Muscle, brain		Iberiotoxin	
KCNN1	SK1	Brain, heart			
KCNN2	SK2	Brain, adrenal gland			
KCNN3	SK3	Brain, heart, muscle			
KCNN4	SK4	T-lymphocytes, neurons		Charybdotoxin	
SUR2	ABCC9	Heart, brain			

[a]From Washington University database

Table 6 Brain voltage-gated calcium channels α-subunits (six transmembrane domains)[a]

Nomenclature		Distribution	Main function	Pharmacology/type[b]	Diseases
CACNA1A	α1A/Cav2.1	Brain/presynaptic	Transmitter release	P/Q type ω-AgaIVA/ω-conotoxin MVIIC	Episodic ataxia/ hemiplegic migraine
CACNA1B	α1B/Cav2.2	CNS	Transmitter release	N-type ω-conotoxin GVIA	
CACNA1D	α1D/Cav1.1	Brain, pancreas, neuroendocrine		L-type DHP, PAA, BZD	
CACNA1E	α1E/Cav2.3	Brain	Transmitter release	R-type resistant	
CACNA1G	α1G/Cav3.1	Brain	Pacemaker	T-type (low threshold)	

[a]From Washington University database
[b]DHP: dihydropyridines; PAA: phenylalkylamines; BZD: benzodiazepines

Brain calcium-activated potassium channels (Table 5) belong to 12 different genes. One half of the genes belong to the KCNK family (KCNK1, 2, 4, 7, 12 and 13 and correspond respectively to the TWIK, TREK, TRAAK, TWIK-1-like, THIK-2 and THIK-1 channels). The KCNMB1 gene corresponds to the BK (big K) large conductance channel. The four KCNN genes (KCNN1 to 4) correspond to intermediate or small conductance channels. The SUR channel is named after its high affinity for sulfonylurea. Calcium-activated channels play a role in oscillatory mechanisms (see below).

Human brain voltage-gated calcium channels are made of the combination of one α-subunit (α_1) and two subunits, one partly extracellular $\alpha_2\delta$ and one intracellular β. The different brain calcium α-subunits are listed Table 6. They belong to the five known calcium channel groups: four high-voltage activated channel types: P/Q-type channels (CACNA1A or Cav2.1), which are sensitive to ω-agatoxin IVA and ω-conotoxin MVIIC, N-type channels (CACNA1B or Cav2.2), which are sensitive to ω-conotoxin GVIA, L-type channels (CACNA1D or Cav1.1), which are sensitive to dihydropyridines (DHP), phenylalkylamines (PAA) and benzodiazepines (BZD), and R-type channels (CACNA1E or Cav2.3), which are resistant to all toxins, and one low-voltage-activated channel type: T-type channels (CACNA1G or Cav3.1). Three channels play a role in transmitter release (P/Q, N and R), whereas the low-voltage-activated T channels are involved in pacemaker activity (see below).

Evolution of the biophysical techniques and the understanding of the nature and function of ionic channels in the nervous system

Our understanding of the role of ion channels in the nervous system has followed the evolution of biophysical techniques. Portraits of six famous membrane biophysicists who contributed to this evolution are shown in Fig 1. At the beginning of the last century, the observation of the effects of changing the ionic concentration of the saline bathing a frog nerve-muscle preparation on its ability to respond to stimuli led Overton (1902) to the conclude that Na^+ or Li^+ ions were necessary for excitation to occur. The clear demonstration that sodium ions are involved in action potential generation has, however, awaited the advent of the voltage-clamp method which was initiated by Cole (1949) and Marmont (1949) in Chicago and further developed by Hodgkin, Huxley and Katz (Hodgkin et al. 1952) in Cambridge. The recordings of the ionic currents of the squid axon under these conditions were good enough to enable a mathematical description of the two main conductances with a set of three simple differential equations (Hodgkin and Huxley 1952), the so-called HH equations, which are still currently used.

The advent of fast amplifiers, sophisticated oscilloscopes and specialized oscilloscope cameras has boosted the development of electrophysiological studies on a variety of nervous preparations. Intracellular capillary glass microelectrodes and high-input impedance "cathode follower" amplifiers have been extensively used to record the electrical activity of large neurons such as those of *Aplysia* (Tauc and Gerschenfeld, 1961) or vertebrate spinal motoneurons (Brock et al. 1952). The same technique has been used for large axons such as those of the lobster (Dalton 1958), the crayfish (Dalton 1959), the

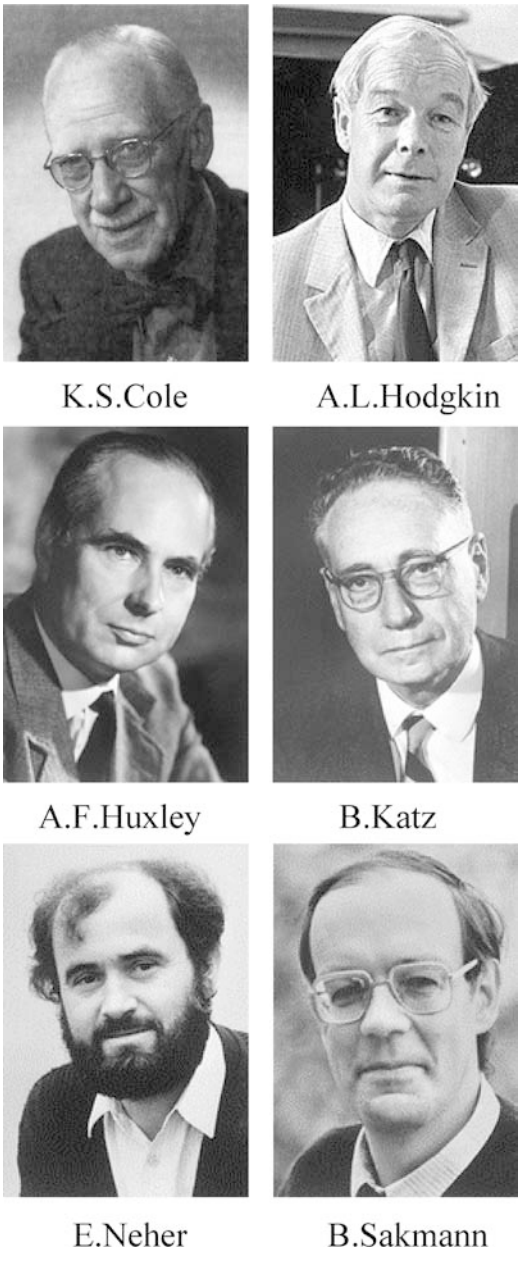
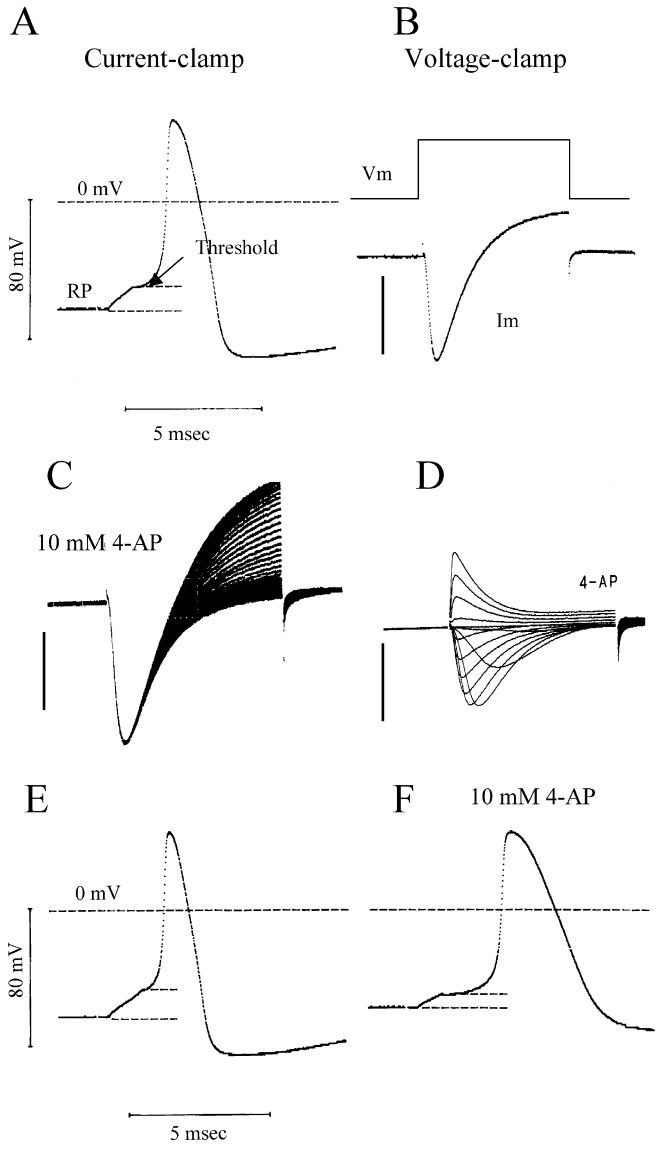


Fig. 1 Six of the most famous membrane biophysicists who contributed to the understanding of the role of ion channels in the physiology of the nerve membrane. K.S. Cole introduced the squid axon and the voltage-clamp techniques. A.L. Hodgkin, A.F. Huxley and B. Katz unveiled the role of sodium and potassium ions in the genesis of the action potential and proposed a description of the ionic currents using a simple set of differential equations (Hodgkin and Huxley 1952). E. Neher and B. Sakmann invented the patch-clamp technique which enables the observation of the opening and closing of single ion channels



◀

Fig. 2A–D Action potential and membrane currents in a squid axon under normal conditions and during the application of 4-aminopyridine (4-AP), which blocks the potassium current. **A**, **B** Normal conditions. In **A**, under current-clamp conditions, the membrane potential (RP) was changed by the injection across the membrane of a square depolarizing current pulse (not shown). This resulted in a depolarization which reached the threshold (*dotted line*) and triggered an all-or-none action potential with its characteristic fast rising phase, overshoot and slower falling phase followed by a transient hyperpolarization. In **B**, under voltage-clamp conditions, the membrane potential (V m) was stepped from its original (resting) level to 0 mV (*upper trace*). This induced a net inward current followed by a steady-state outward current. Temperature: 2 °C. **B**, **D** Effects of 4-AP, a potassium channel blocker, on the ionic currents of a squid axon under voltage-clamp conditions. In **C**, the current induced by a step membrane depolarization from −60 mV to 0 mV is progressively modified by the application of 10 mM 4-AP in the bath: the peak current is not modified but the outward (potassium) current is reduced to nil. In **D** (modified from Pichon 1981), after the application of 10 mM 4-AP, the potassium current is suppressed and the ionic currents induced by 13 step membrane depolarizations of increasing voltages are carried by sodium ions. Temperature: 9 °C. The *vertical scale bar* in **B**, **C** and **D** represents a current density of 1 mA cm^{-2}. **E**, **F** Effects of potassium channel block by 10 mM 4-AP on the resting and action potential of a squid axon. **E** Normal action potential induced by a square depolarizing current pulse. In **F** (from Pichon et al. 1982), after the addition of 10 mM 4-AP to the bath, the resting potential is lower, the threshold for spike initiation is less negative and the falling phase of the action potential is prolonged. Temperature: 8 °C

cockroach (Boistel and Coraboeuf 1958; Yamasaki and Narahashi 1959) or of marine worms such as the fanworm, *Sabella penicillus* (Carlson et al. 1978). In several cases, however, owing to "space-clamp" problems, capillary microelectrodes were not adequate to voltage clamp the axons and to analyse the ionic currents. For these studies, single axons were voltage-clamped using external electrodes and "gap" techniques (sucrose-gap, air-gap, oil-gap). In all cases, the results were very similar to those obtained on the squid giant axon.

Another important technical development has been the purification of aequorin, a protein extracted from the marine jellyfish *Aequora*, which fluoresces when it binds calcium. When injected into a neuron, the corresponding light signal can be recorded and reflects the amount of free calcium in the cell. This technique is nearly as fast as electrophysiology and much more sensitive. The most convincing evidence that changes in membrane conductance correspond to the opening and closing of single ion channels was given by Katz and Miledi (1972) and Anderson and Stevens (1973) through their observations of membrane noise at the frog end plate. The next quantal leap in the evolution of the electrophysiological techniques applied to the nerve membrane has, however, been the advent of the "patch-clamp" technique initially developed by Neher and Sakmann (1976) to record single-channel currents from frog muscle fibres, and subsequently improved for high-resolution current recordings from cells and cell-free membrane patches (Hamill et al. 1981). Besides its interest in enabling the recording in excellent conditions of the opening and closing of ionic channels, the "patch-clamp" technique offered over the intracellular microelectrode technique the further advantage of enabling intracellular (whole cell) recordings from small cells and neuronal branches or growth cones from growing axons.

The missing link between the results of the sequencing of the membrane proteins and their function has now being fulfilled with the advent of single-cell RT-PCR measurements, allowing detection of mRNA molecules from individual cells which have been previously characterized with the patch-clamp technique, as shown for example by Lambolez et al. (1992) for single Purkinje cells. Another promising technique consists in using genetically modified animals in which specific ion channel genes have been modified, knocked out or overexpressed and comparing them with the original wild species, as illustrated by Sutherland et al. (1999) for the Shaker-type potassium channel. Finally, it is possible to exogenously express ion channels by injecting the corresponding cDNA into the neurons, as shown for example by Mochida et al. (2003) for calcium channels.

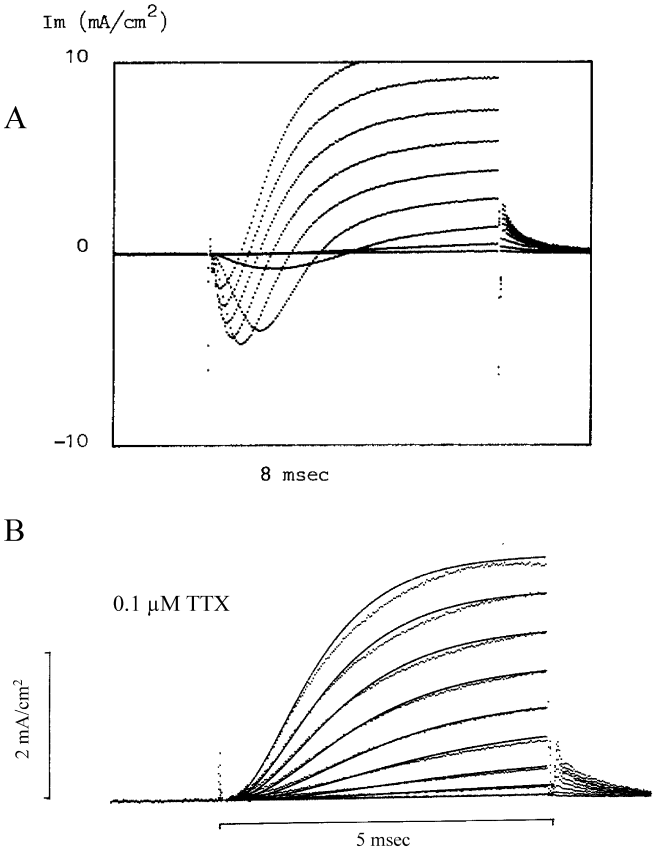


Fig. 3A, B Ion currents in a cockroach axon. **A** Family of membrane currents induced by nine 5-ms step depolarizations of a cockroach axon under voltage-clamp conditions. The current trace corresponding to the 90 mV depolarization is truncated. Leak current subtracted. **B** Potassium current induced by nine 5-ms step depolarizations of a cockroach axon under voltage-clamp conditions in the presence of 0.1 μM tetrodotoxin (TTX) to block the sodium current. Leak current subtracted. The *dotted lines* correspond to the digitized traces, the *continuous lines* to the fit of the current traces with the (modified) Hodgkin and Huxley (1952) equations. From Pichon et al. (1983)

Role of sodium and potassium channels in nerve conduction: examples from squid and cockroach preparations

The main functions of the two families of ion channels in excitation and conduction are illustrated Figs. 2, 3, 4. Under normal physiological conditions, the resting membrane potential (RP) of a squid giant axon in artificial sea water is about −60 mV. Injection of a suprathreshold depolarizing current pulse, under current-clamp conditions, induces a depolarization which gives rise to an action potential characterized by a fast rising phase to a positive value of about +45 mV, followed by a slower falling phase to a significantly hyperpolarized value of −85 mV (the after-hyperpolarization or negative after-potential) (Fig 2A).

In this same squid axon, under voltage-clamp conditions, the two ionic current components, the inward sodium current and the delayed outward potassium current, are clearly visible during a depolarizing pulse to 0 mV (Fig 2B). In Fig 2C, the potassium component of the current is progressively abolished by the addition of 10 mM 4-aminopyridine (4-AP) to the bath, leaving the sodium current alone, with its fast activation phase and its slow exponential inactivation decline. A sodium current "family" corresponding to square membrane depolarizations from the holding potential of −60 mV to +70 mV by 10 mV steps is shown in Fig 2D. In this example, the peak sodium current reverses between +30 and +40 mV. The effects of 4-AP on the resting and action potentials are illustrated in Fig 2E and Fig 2F. 4-AP depolarizes the membrane, reduces the threshold for action potential production, significantly lengthens the falling phase of the action potential and reduces the after-hyperpolarization.

The ionic currents in a cockroach axon voltage-clamped using the "oil-gap" technique are very similar

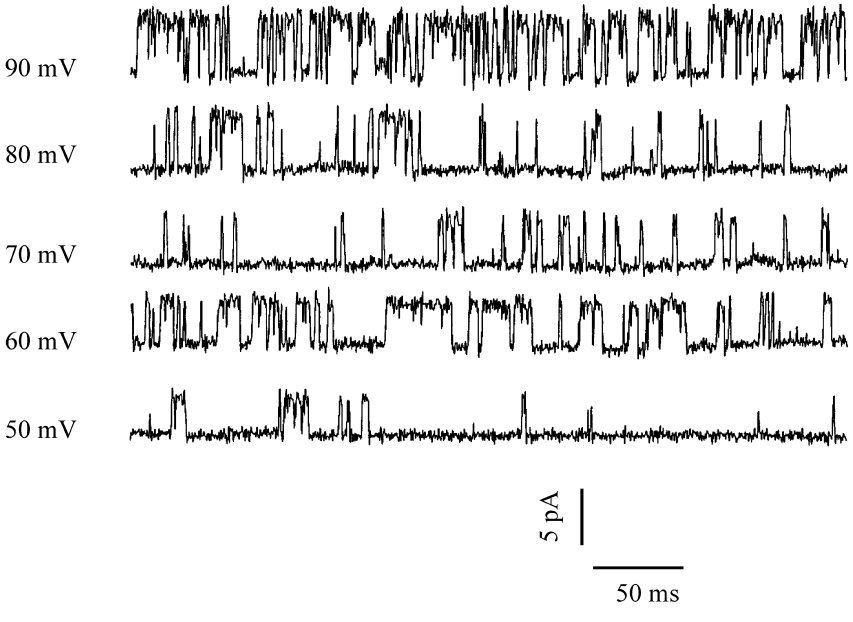


Fig. 4 Single potassium channel events induced by the depolarization (as indicated) of the membrane of a cultured cockroach neuron recorded in the "cell-attached" mode of the patch-clamp technique. The analysis of this current trace suggests that only one channel was present under the patch pipette. As expected, both amplitudes and frequencies increase with membrane depolarization. From Amar and Pichon (1993)

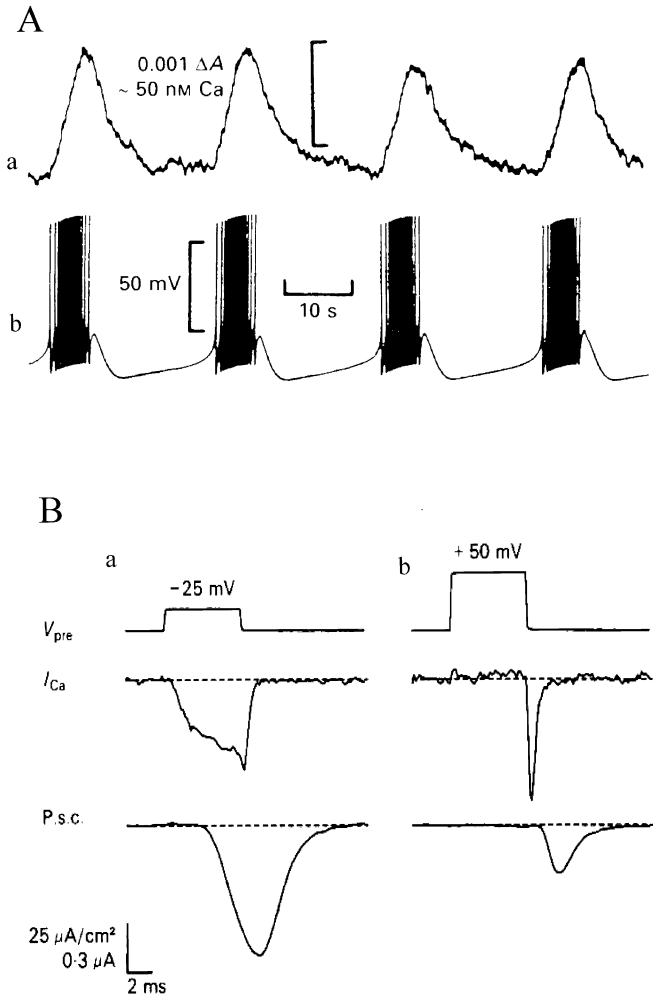


Fig. 5 Evidence and role of calcium channels in the bursting activity (**A**) and synaptic transmission (**B**). **A** Bursting activity in an *Aplysia* pacemaker neuron is associated with transient increases in intracellular calcium. The top trace (*a*) illustrates the changes in intracellular calcium concentration as detected using the calcium-sensitive dye arzenazo III, corresponding to the electrical activity of a giant neuron (R15 cell) recorded with an intracellular microelectrode (bottom trace *b*). The calcium concentration rises during the burst of action potential and falls during the quiescent period. Reproduced with permission from Gorman and Thomas (1978). **B** Relationship between presynaptic calcium currents (I_{Ca}) and postsynaptic current (P.s.c.) in the squid synapse. The presynaptic membrane was depolarized from its holding level (−70 mV) to −25 mV (*a*) and + 50 mV (*b*). The currents were corrected for linear leakage and residual currents. *Dotted lines* indicate the baseline current prior to voltage-clamp pulses. *Vertical bar*: the upper value (current density in μA cm^{-2}) corresponds to the calcium current, the lower value to the postsynaptic current. Temperature: 14 °C. Reproduced with permission from Augustine et al. (1985)

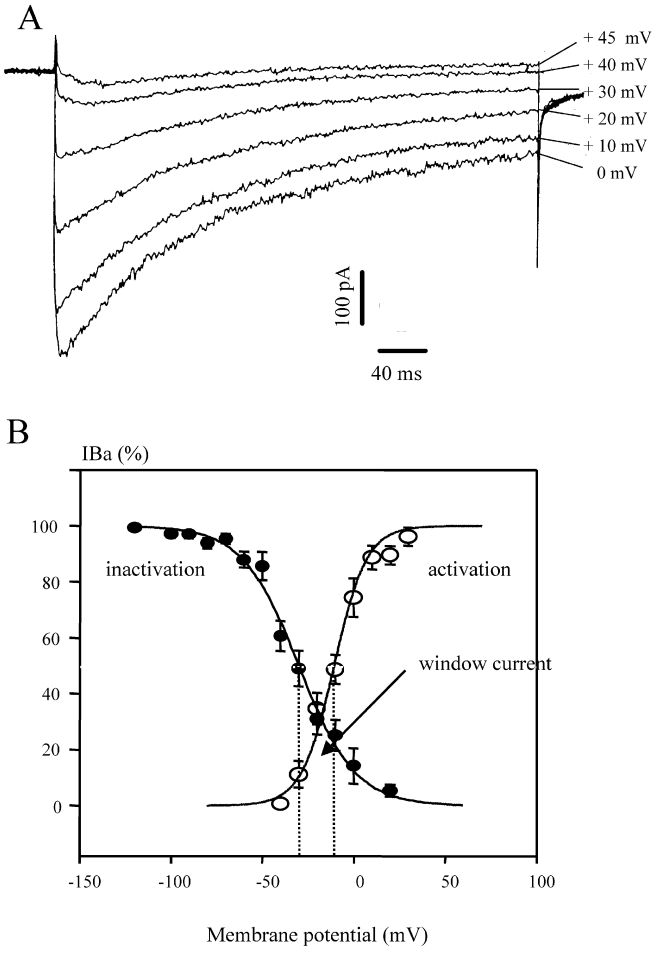


Fig. 6A, B Barium current through calcium channels in embryonic cockroach brain neurons under voltage-clamp conditions. **A** Family of inward barium currents in a cell from a 40-day-old culture. The membrane potential was stepped from its holding value of −50 mV to the values indicated on the *right* of each recording. The external solution contained 20 mM $BaCl_2$, 100 mM TEA and 0 mM NaCl. Reproduced with permission from Christensen et al. (1988). **B** Relationship between activation (*open circles*) and inactivation (*filled circles*) of the calcium channels and membrane potential for 10 (activation) and 20 (inactivation) neurons. An appreciable window current is present between −30 and −10 mV (*dotted lines*), enabling the flow of calcium ions at those membrane potential values which correspond to the resting potential in K^+-rich culture media. Reproduced with permission from Benquet et al. (1999)

to those observed in squid axons (Fig 3). In Fig 3A, one can clearly see the two ionic current components. The largest depolarizing pulse (+30 mV) is not sufficient to reverse the sodium current. In Fig 3B, 0.1 μM tetrodotoxin (TTX) had been added to the bath and the sodium currents are completely blocked. The time course of the potassium current (noisy discontinuous lines) can be fitted with Hodgkin–Huxley kinetics (continuous lines), with the potassium activation parameter *n* raised to the third power.

The single-channel events which underlie the potassium current have been analysed using the patch-clamp technique on cultured cockroach neurons. Recordings made in the cell-attached mode at five different depolarized levels from the holding potential are illustrated in Fig 4. Typical square-like openings together with bursting activity can be observed. As expected, both amplitudes (due to the increasing driving force) and the mean open times (due to the voltage

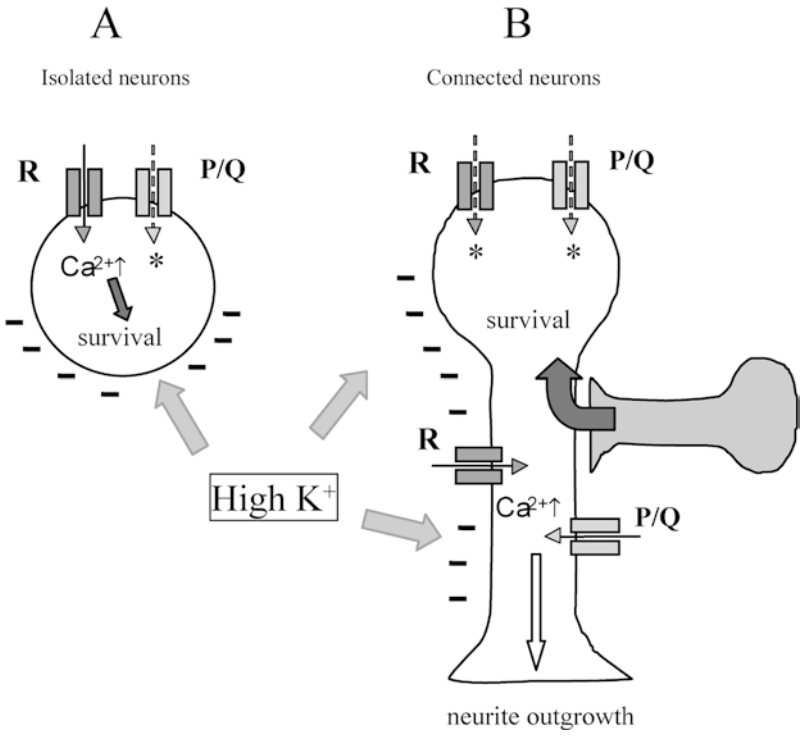


Fig. 7 Schematic diagram illustrating the hypothetical role of the voltage-gated calcium channels on survival and neuritic outgrowth of isolated (**A**) and connected (**B**) embryonic cockroach brain neurons in primary culture. In both cases, the neurons are bathed in a high potassium culture medium (high K^+), which depolarizes the membrane (as shown by the *minus signs*) and opens two categories of high-voltage-activated calcium channels: ω-AgaIVA-sensitive (P/Q) channels (*light grey pores*) and ω-AgaIVA-resistant (R) channels (*dark grey pores*), which are present in the neuronal membrane. In fresh cultures (**A**), survival (*dark grey arrow*) is associated with the R-like channels and not with the P/Q-like channels. In older cultures (**B**), none of the calcium channel blockers has any direct effect on survival but neurite outgrowth (*empty arrow*) is under the dependency of an entry of calcium through the two categories of channels, which are shown here on the elongated part of the neuron. *Interrupted arrows* and *asterisks* indicate that these channels are not involved. Reproduced with permission from Benquet et al. (2002)

dependency of the channels) increased with membrane depolarization.

Calcium channels: their role in neuronal bursting activity and synaptic transmission

These two important roles of the calcium channels are illustrated in Fig 5. In bursting neurons, such as *Aplysia* neurons (Fig 5A), the repetitive firing of the cell membrane induces a progressive increase in the intracellular calcium, as detected by the calcium-sensitive dye arzenazo III. This leads to a strong hyperpolarization of the membrane owing to the opening of calcium-sensitive potassium channels which stops the burst. After a few seconds, the free intracellular calcium decreases, the membrane potential of the cell drops and a new burst is elicited.

Recordings of the calcium channel activity associated with synaptic transmission have been made in the squid giant synapse preparation. In that preparation, owing to the large size of the pre- and postsynaptic elements, it is possible to make recordings in both pre- and postsynaptic axons. This is illustrated in Fig 5B. The presynaptic giant axon was voltage-clamped and the presynaptic membrane current recorded for two potential levels: −25 mV and +50 mV. At −25 mV, a typical calcium current with a slow activation phase is recorded. After a short delay, a large postsynaptic current is recorded in the postsynaptic axon. At +50 mV, there is no visible calcium current in the presynaptic axon since we are close to the calcium equilibrium potential and no postsynaptic current is seen. As expected, however, the fast presynaptic tail of the calcium current (fast transient), which is present at the end of the +50 mV pulse, induces a small postsynaptic current.

Role of calcium channels in survival and growth of embryonic cockroach brain neurons in primary culture

Embryonic cockroach brain neurons are known to survive in high potassium media, as are many other neurons in primary culture. The neurons have been shown to possess high-voltage-activated P/Q and R channels. Typical recordings of the barium[1] current induced by step membrane depolarizations are shown in Fig 6A. It

[1]Barium is currently used in these experiments to replace calcium to avoid calcium-induced inactivation of the channels. Furthermore, barium is more permeant than calcium in many calcium channels

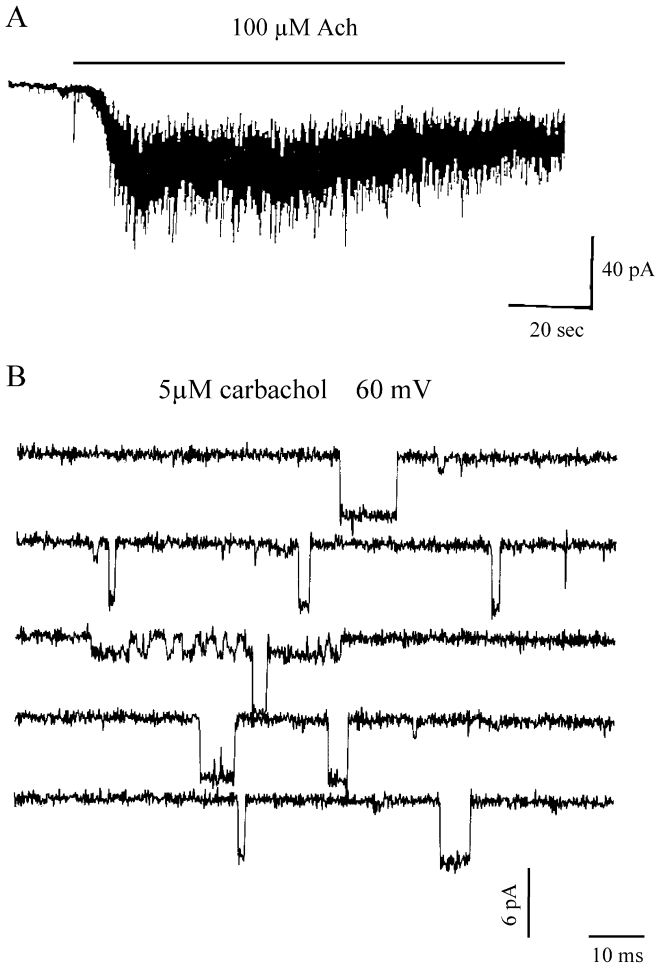


Fig. 8A, B Membrane currents associated with the application of cholinergic ligands. **A** Effects of bath application of 10^{-4} M Ach on the membrane current of an embryonic cockroach brain neuron. The agonist induces an inward current on which are superimposed large current fluctuations (noise), reflecting the opening and closing of individual Ach-gated channels. Whole-cell mode of the patch-clamp technique. **B** Single channel activity induced by 5 μM carbachol, a nicotinic cholinergic agonist, on a patch of embryonic cockroach brain neuron. The agonist induces the opening of two categories of single-channel events clearly different in size and corresponding to conductances of respectively 15 pS and 52 pS. Cell-attached mode of the patch-clamp technique. The membrane was hyperpolarized by 60 mV. From Beadle et al. (1989)

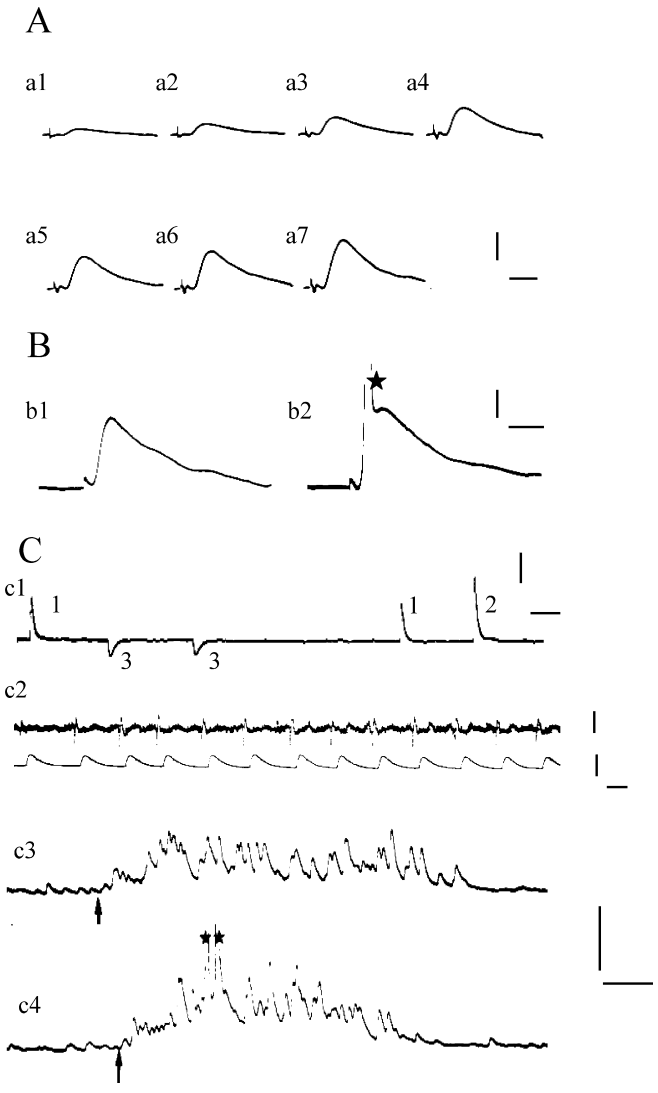


Fig. 9A–C Synaptic activity in a giant interneuron of the cockroach. **A**, **B** Graded electrical activity induced by the electrical stimulation of the presynaptic nerve. **A** The stimulus intensity was progressively increased from *a1* to *a7* as illustrated by the small diphasic deflection at the beginning of each trace, which reflects the number of active presynaptic fibres. The size of the postsynaptic potential increases with the stimulus intensity. **B** When the threshold is reached, a postsynaptic action potential is elicited (*b2*, *star*). **C** Synaptic activity under physiological conditions. *c1*: in the absence of any (known) stimulation, a random electrical activity is observed, consisting of EPSPs (1 and 2) and IPSPs (3). *c2*: stimulation of a presynaptic neuron (*top trace*) elicits small EPSPs in the postsynaptic axon (*bottom trace*). *c3* and *c4*: stimulation of several presynaptic neurons with an air puff (*arrows*) induces a complex EPSP which eventually gives rise in *c4* to two postsynaptic action potentials (*stars*). *Vertical scale bars*: 2 mV in **A**, 3 mV in **B**, 1 mV in *c1* and *c2*, 5 mV in *c3* and *c4*. *Horizontal scale bars*: 2 ms in **A**, 4 ms in **B**, 20 ms in *c1*, 10 ms in *c2* and 50 ms in *c3* and *c4*. Room temperature (20–25 °C). Note (1) that a single presynaptic action potential is not sufficient to trigger a spike, (2) that the same postsynaptic element receives multiple presynaptic inputs which may or may not give rise to postsynaptic action potentials. Spikes are truncated. Modified from Pichon and Callec (1970) and Callec et al. (1971)

exhibits a very fast activation phase and a much slower, exponential, falling phase. The voltage dependency of activation and inactivation of this current have been analysed and, as shown in Fig 6B, a clear window current is present between −30 and −10 mV, membrane potential values corresponding precisely to the resting potential of these neurons in high K^+ culture media. Survival of young (isolated) neurons was not modified by ω-Aga IV A, which blocks P/Q channels, but was abolished by mibefradil and verapamil in a dose-dependent manner. In older cultures, in which the different neurons are interconnected (connected neurons), survival was maintained despite the presence of calcium channel blockers in the culture medium, but neurite outgrowth was severely altered by both ω-Aga IV A and mibefredil. The proposed mode of action of high K^+

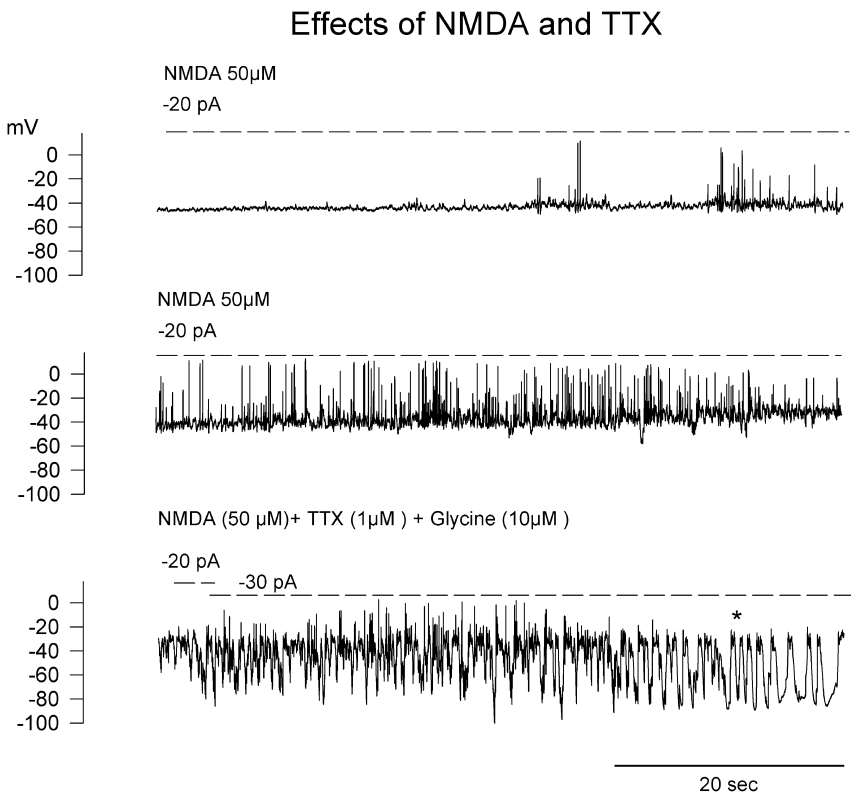


Fig. 10 Effects of bath application of 50 μM NMDA on the membrane potential of a (presumed) motoneuron of a stage 32 tadpole embryo. The agonist (50 μM) induces a depolarization which can be partly compensated for by injecting a repolarizing current (*dotted line*). The spike activity (*upward deflections on top of the continuous trace*) is blocked by TTX (1 μM) and replaced by low-frequency oscillations (*end of bottom trace*). Modified from Prime et al. (1999)

culture media on survival and neurite outgrowth is summarized in Fig 7 (from Benquet et al. 2002).

Properties of ligand-gated ionotropic receptors: response of the insect neuronal membrane to ACh and carbachol

Ach is the main excitatory neurotransmitter in the nervous system of insects. The effects on the membrane currents of a patch-clamped cockroach neuron to bath application of 100 μM ACh are illustrated in Fig 8A. ACh induces an inward current of a few picoamps in amplitude, on top of which are superimposed large current fluctuations which reflect the opening and closing of the ACh receptor channels. The effects of 5 μM carbachol, a nicotinic cholinergic agonist, on a membrane patch (cell-attached configuration of the patch clamp) are illustrated in Fig 8B. The agonist induces two clearly different categories of openings, corresponding to two different channels with conductances of respectively 15 pS and 52 pS.

Complexity of synaptic events: examples from the last abdominal ganglion of the cockroach

In the last (sixth) abdominal ganglion of the cockroach, a large number of presynaptic axons originating from the mechanoreceptors located on the cerci converge on a very small number of postsynaptic giant axons which, when active, trigger the escape behaviour of the insect. The synaptic events have been recorded from individual postsynaptic giant axons using the "oil-gap" technique. Various aspects of this highly integrative synapse are illustrated in Fig 9. Electrical stimulation of the cercal nerve, which contains the sensory fibres originating from the cercal mechanoreceptors, induces a monophasic response, the excitatory synaptic potential (EPSP) after a very short delay. The rising phase of this response approximates 1 ms and is followed by a slow exponential falling phase of about 5 ms (Fig 9A, B). The size of the response increases with the stimulus intensity (i.e. the number of excited presynaptic fibres) until the threshold for a postsynaptic spike is reached (b2). Individual synaptic events corresponding to the activity of different presynaptic elements are illustrated in Fig 9C. In c1, the postsynaptic giant axon receives the inputs from three different presynaptic fibres, two excitatory inputs which give rise to EPSPs 1 and 2, and one inhibitory input which gives rise to IPSP 3. In c2, repetitive activity in a single presynaptic fibre (diphasic action potentials) induces repetitive EPSPs but fails to induce a postsynaptic action potential. In c3 and c4, the cercus is stimulated with a puff of air (i.e. the physiological stimulus). Two action potentials are obtained in response to a strong puff (c4, arrows). The time course of the synaptic potential is complex and reflects the complexity of the synapse as well as the spatiotemporal characteristics of the presynaptic activity.

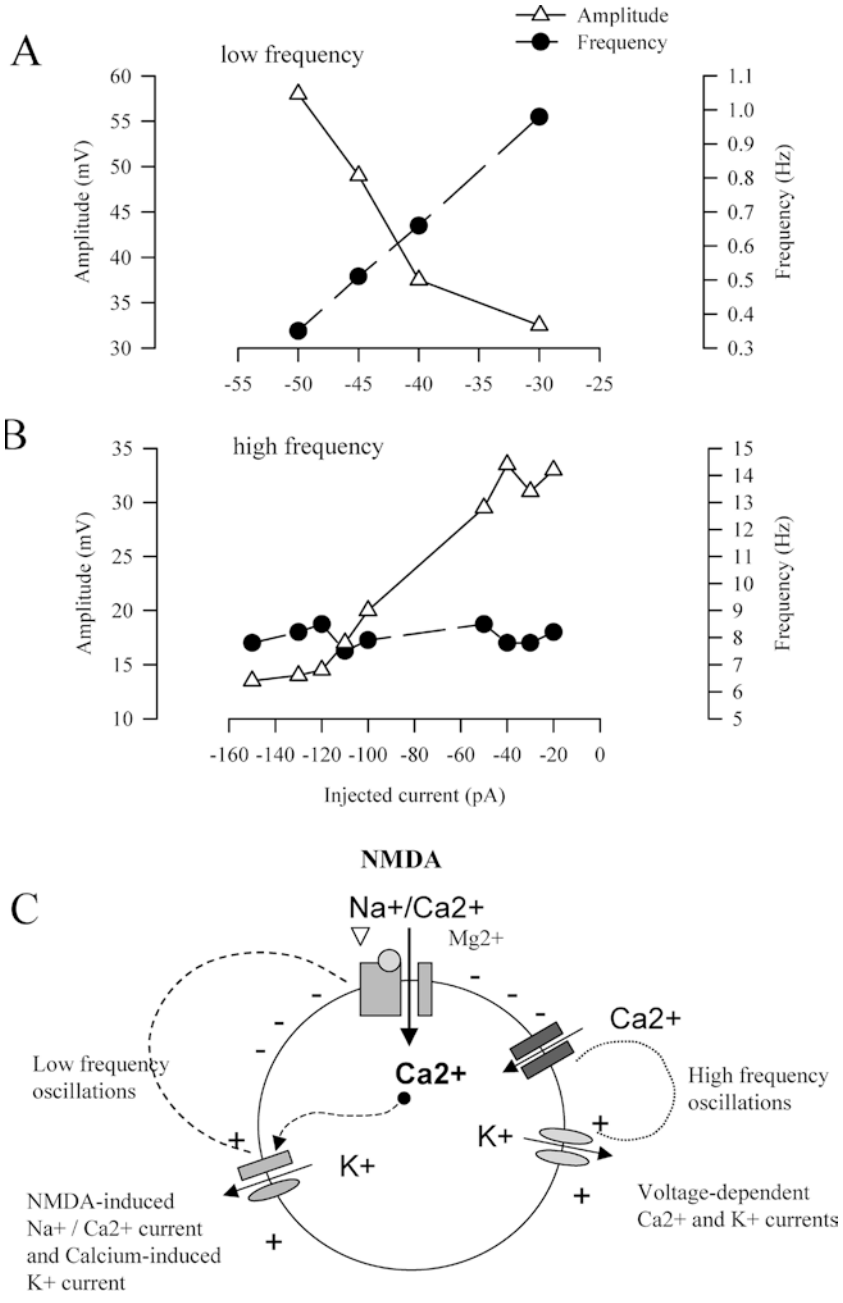


Fig. 11 Properties (**A** and **B**) and proposed interpretation (**C**) of NMDA-induced membrane potential oscillations of *Xenopus* motoneurons. **A** Low-frequency oscillations: the amplitude of the oscillations increases whereas their frequency decreases with membrane hyperpolarization induced by the injected current. **B** High-frequency oscillations: the amplitude of the oscillations decreases with membrane hyperpolarization whereas their frequency remains constant. **C** Schematic representation of the membrane mechanisms which might be responsible for the two categories of NMDA-induced oscillations. The slow oscillations would correspond to the opposite effects of NMDA (depolarization) and calcium-dependent potassium channels. The fast oscillations would correspond to the opposite effects of low-voltage activated calcium channels (T-channels) and of voltage-activated potassium channels. **A** and **B** modified from Prime et al. (1999)

Interactions between ionic channels and oscillations: oscillatory behaviour of *Xenopus* tadpole motoneurons

Motoneurons of the spinal cord of the *Xenopus* tadpole are responsible for the alternate contractions of the body wall during swimming. Under normal conditions, this activity is associated with the operation of a small neuronal circuit. Motoneurons are able under certain conditions, however, to produce oscillations in the absence of conducted synaptic activity (Prime et al. 1999). Figure 10 illustrates the effects of NMDA on the membrane potential of a motoneuron. The recordings were made using the ''whole-cell'' configuration of the patch-clamp technique. Bath application of 50 μM NMDA progressively depolarizes the neuronal membrane through the opening of the ion channels which are associated with the NMDA receptors. This depolarization induces a spike activity. Application of 1 μM TTX abolishes the spike activity and induces ''spontaneous'' membrane potential oscillations if the membrane is repolarized by passing current through the recording electrode (−20 pA then −30 pA) (Fig 10, bottom trace). Two categories of oscillations can be observed: low-frequency oscillations (Fig 11A) and high-frequency oscillations (Fig 11B). The relationships between the amplitude and the frequency of these oscillations and membrane potential (which is proportional to the injected current) are different. For low-frequency oscillations, the amplitude increases with membrane hyperpolarization whereas the

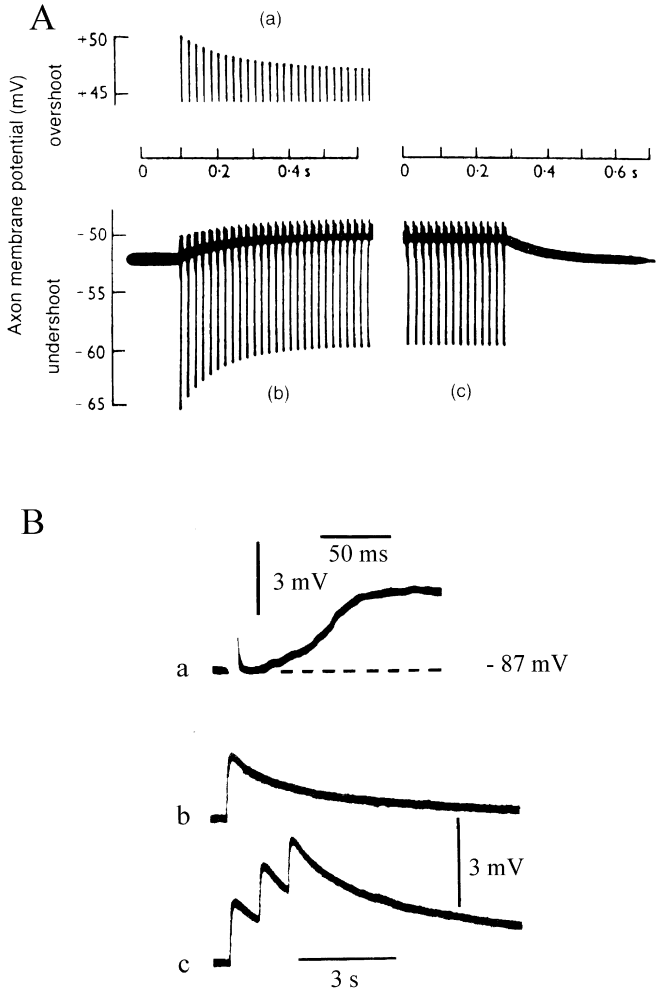


Fig. 12A, B Effects of the flow of potassium ions through the voltage-gated potassium channels of the axonal membrane on the extra-axonal potassium concentration. **A** Evolution of the overshoot (*a*) and the undershoot (*b* and *c*) of the action potential of a giant axon of *Loligo forbesi* during repetitive stimulation at 50 Hz at 19.8 °C. K concentration: 10 mM. Trace *c* illustrates the end of the train of action potentials which lasted about 1 s. Reproduced from Frankenhaueuser and Hodgkin (1956) after inversion of the membrane potential polarity (depolarization upwards). Potassium accumulation in the vicinity of the axonal membrane is reflected in the decrease of the undershoot. **B** Depolarization of glial cells induced by nerve activity in *Necturus* optic nerve. Modified from Orkand et al. (1966). Note the high value (−87 mV) of the resting potential of the glial cell, which reflects its high potassium permeability. Nerve stimulation is indicated by the artefact at the beginning of the top record (*a*). The two other traces (*b* and *c*) are shown on a much slower time base. In *c*, the stimulus was repeated three times at 1 s intervals and the glial depolarizations summed

frequency decreases. For high-frequency oscillations, the amplitude decreases with membrane hyperpolarization and the frequency remains constant. These differences suggest that the origin of the two categories of oscillations is different. Based on the observations of Nowak et al. (1984) it is proposed, as for the lamprey (Wallen and Grillner 1987), that the low-frequency oscillation corresponds to the opposite effects of NMDA (depolarization) and calcium-activated potassium channels (hyperpolarization) since it is known that NMDA channels let Ca^{2+} enter into the cell (as indicated by the arrow). The model for the high-frequency oscillations is based on the observations of Dale (1985) and Gu and Spitzer (1993). These oscillations would correspond to the opposite effects of voltage-gated calcium channels (T-type) and voltage-gated K^+ channels at depolarized potentials (Fig 11C).

Ionic homeostasis and participation of glial cells in ionic regulation and signalling in the nervous system

Besides their effects on the membrane potential, the ion flux which flows through the channels can produce indirect effects such as ion accumulation or depletion. An early example of potassium accumulation was given by Frankenhaueuser and Hodgkin (1956), who observed that repetitive stimulation of a squid giant axon induced a marked decrease of the after-potential which closely reflects the extracellular potassium concentration (Fig 12A).

Depolarizations are also induced in adjacent glial cells, as illustrated for the optic nerve of *Necturus* by Orkand et al. (1966) (Fig 12B). Many, but not all, glial cells have a high resting potential (as in *Necturus*) since their membrane is highly potassium permeable. As illustrated in Fig 12Ba, the time course of the glial cell depolarization is very slow (several hundred milliseconds) compared to the duration of the nerve stimulation. This indicates that it takes some time for potassium to return to its resting value. Potassium accumulation can reach disastrous levels in the case of ischemia [up to 80 mM in the rat (Hansen 1978)]. Calcium ions have been found to accumulate intracellularly and be responsible for the so-called "glutamate toxicity". Ionic homeostasis, which is necessary for the normal function of the nervous system, is achieved through a multiplicity of mechanisms and includes passive diffusion, carrier-mediated effects and spacial buffering (see Pichon et al. 1995). This last mechanism is based on the high K^+ permeability of the glial cells.

Another important and yet little documented observation is that glial cells probably play a role in signalling in the nervous system. Thus, as illustrated in Fig 13, repetitive stimulation of a squid giant axon induces a clear hyperpolarizing response in the adjacent glial cell. This response could correspond to the opening of potassium channels caused by a rise of intracellular calcium resulting from the binding of glutamate released by the axon during the stimulation on the Schwann cell metabotropic glutamate receptors (Abbott et al. 1995).

Present and future

As mentioned above, the fact that the ion channel genes have been sequenced on several species, and the development of combined electrophysiological and single-cell

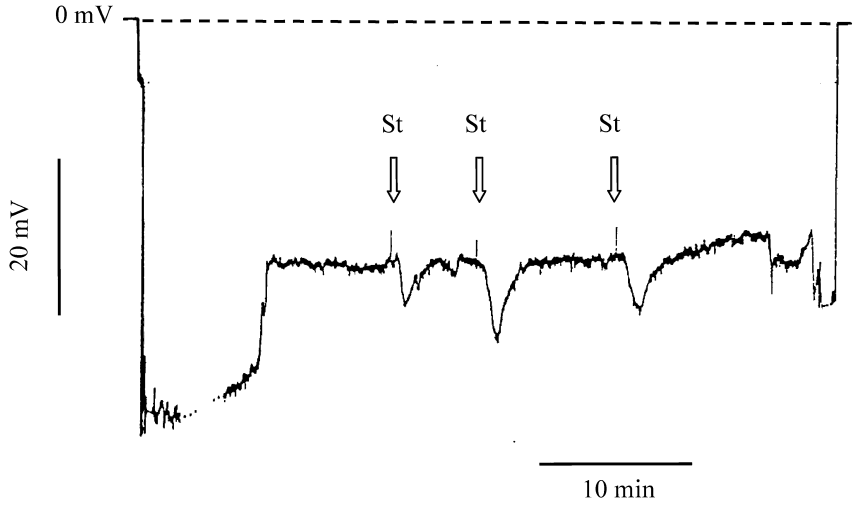


Fig. 13 Effects of repetitive stimulation of a squid axon on the resting potential of an adjacent glial cell. The glial cell was impaled with an intracellular microelectrode. The resting potential dropped from its initial value of −52 mV to a plateau value of about −30 mV. Repetitive stimulation of the giant axon (*St*, *arrows*) at 50 Hz induced transient hyperpolarizations of the glial membrane after a short delay. These hyperpolarizations are supposed to reflect a transient increase of the resting membrane permeability to potassium, mediated through the effects of glutamate on non-NMDA glutamate receptors located on the glial membrane, and an entry of calcium into the glial cytoplasm. From Pichon and Abbott (unpublished)

RT-PCR approaches, enable detailed analyses of the role of the ion channels in various parts of the nervous system. This combination of techniques has been used, for example, to estimate the respective contribution of the Shaker-like K^+ channels (Kv1.1, 1.2 and 1.3) to the membrane current in mouse rod bipolar cells (Klumpp et al. 1995). Similarly, Baranauskas et al. (1999) have used this approach to understand the role of the Kv2.1 and Kv3.1/3.2 K^+ channels in the delayed rectifier currents in rat *Globus pallidus* neurons and suggested that burst activity in these neurons, which is observed in Parkinson disease, could arise from an alteration of the balance between the Kv2 and Kv3 channel types. The same combination of techniques has enabled Alessandri-Haber et al. (2002) to analyse the molecular determinants of emerging excitability in rat embryonic motoneurons.

Besides this approach, the use of transfection techniques enables investigations of the role of the different components of the ionic currents. Thus, in a recent study of transfected mammalian cortical neurons in culture, Pal et al. (2003) have been able to show, through the analysis of the current density, that Kv2.1-encoded K^+ channels are responsible for the expression of apoptosis.

In another area, the use of sophisticated imaging techniques together with electrophysiology enable precise investigations of the correspondence between electrical activity in different parts of a neuron and ionic currents, such as calcium "spikes". This has recently been demonstrated by Debarbieux et al. (2003) who showed, using two-photon microscopy of calcium transients and intracellular microelectrode recordings, that the entire dendritic tree of rat mitral cells has an "axon-like behaviour".

Finally, the last, but not necessarily the least, sophisticated and easy to use computer programs such as NEURON (Hines and Carnevale 2001) enable fast and reliable tests of different working hypotheses which take into account most of the biophysical properties of the neurons (see Prime and Pichon 2003).

The multiple facets of the physiological significance of the large diversity of ion channels which are present in the nervous system and of their (dynamic) distribution are in the process of being identified with this combination of techniques which is now accessible to neurophysiologists and biophysicists. Two examples borrowed from the literature can illustrate this aspect of modern research.

The first one is the recent review of Mathie et al. (2002) on the cerebellum. In this paper, in which the authors concentrate on cerebellar granule neurons and on potassium channels, they show that at least 26 potassium channel α-subunits are expressed, whereas a good empirical model of the function of these neurons can be obtained with just six distinct K conductances. This apparent redundancy, together with the observation that the expression of many of these subunits is highly regulated, suggests that the resulting electrical properties of the cerebellar granule neurons are highly dynamic and subject to constant fine-tuning.

The second example is the ion channel redistribution and function during the early development of myelinated axons. Myelination isolates electrically most of the axolemma whereas the Na^+ channels are highly concentrated at the (unmyelinated) node of Ranvier (see Vabnick and Shrager 1998). Recent experiments have revealed a surprising level of complexity within this zone, with several components, including ion channels, being sequestered with a high precision and sharply demarcated borders (Kazarinova-Noyes and Shrager

2002). Targeting of specific channel stubtypes to distinct membrane domains of axons is regulated by local signals from the myelinating glial cells (Rasband and Trimmer 2001; Salzer 2002).

References

Abbott NJ, Brown ER, Pichon Y, Kukita F (1995) Electrophysiology of squid Schwann cells. In: Abbott NJ, Williamson R, Maddock L (eds) Cephalopod neurobiology, vol 14. Oxford University Press, Oxford, pp 197–212

Alessandri-Haber N, Alcaraz G, Deleuze C, Jullien F, Manrique C, Couraud F, Crest M, Giraud P (2002) Molecular determinants of emerging excitability in rat embryonic motoneurons. J Physiol (Lond) 541:25–39

Amar M, Pichon Y (1993) Potassium conductance and potassium channels in a primitive insect: the cockroach *Periplaneta americana*. In: Pichon Y (ed) Comparative molecular neurobiology. Birkhaüser, Basel, pp 352–365

Anderson CR, Stevens CF (1973) Voltage-clamp analysis of acetylcholine produced end plate current fluctuations at frog neuromuscular junction. J Physiol (Lond) 235:655–691

Augustine GJ, Charlton MP, Smith SJ (1985) Calcium entry and transmitter release at voltage-clamped nerve terminals of squid. J Physiol (Lond) 369:163–181

Baranauskas G, Tkatch T, Surmeier DJ (1999) Delayed rectifier currents in rat globus pallidus neurons are attributable to Kv2.1 and Kv3.1/3.2 K^+ channels. J Neurosci 19:6394–6404

Beadle DJ, Horseman G, Pichon Y, Amar M, Shimahara T (1989) Acetylcholine-activated ion channels in embryonic cockroach neurones growing in culture. J Exp Biol 142:337–355

Benquet P, Le Guen J, Dayanithi G, Pichon Y, Tiaho F (1999) ω-Aga IVA-sensitive (P/Q-type) and -resistant (R type) high-voltage-activated Ba^{2+} currents in embryonic cockroach brain neurons in primary culture. J Neurophysiol 82:2284–2293

Benquet P, Le Guen J, Pichon Y, Tiaho F (2002) Differential involvement of Ca^{2+} channels in survival and neurite outgrowth of cultured embryonic cockroach brain neurons. J Neurophysiol 88:1475–1490

Boistel J, Coraboeuf E (1958) Rôle joué par les ions sodium dans la genèse de l'activité électrique du tissu nerveux d'inserte. CR Acad Sci Paris 247:1781–1783

Brock LG, Coombs JS, Eccles JC (1952) The recording of potentials from motoneurons with an intracellular electrode. J Physiol (Lond) 117:431–460

Callec JJ, Guillet J C, Pichon Y, Boistel J (1971) Further studies on synaptic transmission in insects. II. Relations between sensory information and its synaptic integration at the level of a single giant axon in the cockroach. J Exp Biol 55:123–149

Carlson AD, Pichon Y, Treherne JE (1978) Effects of osmotic stress on the electrical activity of a the giant axon of a marine species, *Sabella penicillus* L. J Exp Biol 75:237–251

Christensen BN, Larmet Y, Shimahara T, Beadle D, Pichon Y (1988) Ionic currents in neurones cultured from embryonic cockroach (*Periplaneta americana*) brains. J Exp Biol 135:193–214

Cole KS (1949) Dynamic electrical characteristics of the squid axon membrane. Arch Sci Physiol 3:253–258

Dale N (1985) Kinetic characterisation of the voltage-gated currents possessed by *Xenopus* embryo spinal neurons. J Physiol (Lond) 489:473–488

Dalton JC (1958) Effects of external ions on the membrane potential of a lobster giant axon. J Gen Phyiol 41:529–542

Dalton JC (1959) Effects of external ions on the membrane potential of a crayfish giant axon. J Gen Phyiol 42:971–982

Debarbieux F, Audinat E, Charpak S (2003) Action potential propagation in dendrites of rat mitral cells *in vivo*. J Neurosci 23:5553–5560

Frankenhaueuser B, Hodgkin AL (1956) The after-effects of impulses in the giant nerve fibres of *Loligo*. J Physiol (Lond) 131:341–376

Gorman AL, Thomas MV (1978) Changes in the intracellular concentration of free calcium ions in a pacemaker neurone, measured with the metallochromic indicator dye arzenazo. III. J Physiol (Lond) 275:357–376

Gu X, Spitzer NC (1993) Low-threshold Ca^{2+} current and its rôle in spontaneous elevations of extracellular Ca^{2+} in developing *Xenopus* neurons. J Neurosci 13:4936–4948

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 391:85–100

Hansen AJ (1978) The extracellular potassium concentration in brain cortex following ischemia in hypo- and hyperglycemic rats. Acta Physiol Scand 102:324–329

Hille B (2001) Ion channels of excitable membranes, 3rd edn. Sinauer, Sunderland, Mass., USA

Hines ML, Carnevale NT (2001) Neuron: a tool for neuroscientists. Neuroscientist 7:123–135

Hodgkin AL, Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol (Lond) 117:125–134

Hodgkin AL, Huxley AF, Katz B (1952) Measurement of current-voltage relations in the membrane of the giant axons of *Loligo*. J Physiol (Lond) 116:424–448

Kazarinova-Noyes K, Shrager P (2002) Molecular constituents of the node of Ranvier. Mol Neurobiol 26:167–182

Katz B, Miledi R (1972) Statistical nature of the acetycholine potential and its molecular components. J Physiol (Lond) 224:665–699

Klumpp DJ, Song EJ, Ito S, Sheng MH, Yan LY, Pinto LH (1995) The Shaker-like potassium channels in the mouse rod bipolar cell and their contributions to the membrane current. J Neurosci 15:5004–5013

Lambolez B, Audinat E, Bochet P, Crepel F, Rossier J (1992) AMPA receptor subunits expressed by single Purkinje cells. Neuron 9:247–258

Marmont G (1949) Studies on the axon membrane. 1. A new method. J Cell Comp Physiol 34:351–382

Mathie A, Clarke CE, Ranatunga KM, Veale EL (2003) What are the roles of the many different types of potassium channel expressed in cerebellar ganule cells? Cerebellum 2:11–25

Mochida S, Westenbroek RE, Yokoyama CT, Itoh K, Catterall WA (2003) Subtype-selective reconstitution of synaptic transmission in sympathetic ganglion neurons by expression of exogenous calcium channels. Proc Natl Acad Sci USA 100:2813–2818

Neher E, Sakmann B (1976) Single channel currents recorded from denervated frog muscle fibres. Nature 260:799–802

Nowak L, Bregetowski P, Ascher P, Herbert A, Prochiantz A (1984) Magnesium gates glutamate-activated channels in mouse central neurones. Nature 307:462–465

Orkand RK, Nicholls JG, Kuffler SW (1966) Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. J Neurophysiol 29:788–806

Overton E (1902) Beiträge zur allgemeinen Muskel-und-Nervenphysiologie. Pflügers Arch 92:346–386

Pal S, Hartnett KA, Nerbonne JM, Levitan ES, Aizenman E (2003) Mediation of neuronal apoptosis by Kv2.1-encoded potassium channels. J Neurosci 23:4798–4802

Pichon Y (1981) Pharmacological characterisation of ionic channels in unmyelinated axons. J Physiol (Paris) 77:1119–1127

Pichon Y, Callec JJ (1970) Further studies on synaptic transmission in insects. I. External recording of synaptic potentials in a single giant axon of the cockroach, *Periplaneta americana* L. J Exp Biol 52:257–265

Pichon Y, Meves H, Pelhate M (1982) Effects of aminopyridines on ionic channels and ionic channel noise in unmyelinated axons. In: Lechat P, Thesleff S, Baumann WC (eds) Aminopyridines and similarly acting drugs. Effects on nerves, muscles and synapses. Pergamon, Oxford, pp 53–58

Pichon Y, Poussart D, Lees G V (1983) Membrane ionic current, current noise and admittance in isolated cockroach axons. In: Chang DC, Tasaki I, Adelman WJ, Leuchtag HR (eds) Stucture and function in excitable cells. Plenum, New York, pp 211–226

Pichon Y, Abbott NJ, Brown ER, Inoue I, Revest P (1995) Periaxonal ion regulation in the squid. In: Abbott NJ, Williamson R, Maddock L (eds) Cephalopod neurobiology, vol 16. Oxford University Press, Oxford, pp 229–251

Prime L, Pichon Y (2003) Role of ligand-gated ion channels in the swimming behaviour of *Xenopus* tadpoles: experimental data and modelling experiments. Eur Biophys J (this issue)

Prime L, Pichon Y, Moore LE (1999) *N*-Methyl-D-aspartate-induced oscillations in whole-cell clamped neurones from the isolated spinal cord of *Xenopus laevis* embryos. J Neurophysiol 82:1069–1073

Rasband MN, Trimmer JS (2001) Developmental clustering of ion channels at and near the node of Ranvier. Dev Biol 236:5–16

Sakmann B, Neher E (1995) Single channel recording. Plenum, New York

Salzer JL (2002) Nodes of Ranvier come of age. Trends Neurosci 25:2–5

Scholz A, Reid G (2002) Properties and localisation of ionic channels in myelinated and unmyelinated nerve fibres. In: Rouzaire-Dubois B, Benoit E, Dubois JM (eds) Ion channels and physiopathologies of nerve conduction and cell proliferation. Research Signpost, Kerala, pp 7–47

Sutherland ML, Williams SH, Abedi R, Overbeek PA, Pfaffinger PJ, Noebels JL (1999) Overexpression of Shaker-type potassium channel in mammalian central nervous system dysregulates native potassium channel gene expression. Proc Natl Acad Sci USA 96:2451–2455

Tauc L, Gerschenfeld HM (1961) Cholinergic transmission mechanisms for both excitation and inhibition in molluscan central synapses. Nature 192:366–367

Vabnick I, Shrager P (1998) Ion channel redistribution und function during development of the myelinated axon. J Neurobiol 37:80–96

Wallen P, Grillner S (1987) *N*-Methyl-D-aspartate receptor-induced, inherent oscillatory activity in neurons active during fictive locomotion in the lamprey. J Neurosci 7:2745–2755

Yamasaki T, Narahashi T (1959) The effects of potassium and sodium ions on the resting and action potentials of the cockroach giant axon. J Insect Physiol 3:146–158