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ELECTROPHYSIOLOGICAL PROPERTIES OF *IN VITRO* PURKINJE CELL SOMATA IN MAMMALIAN CEREBELLAR SLICES

BY R. LLINÁS AND M. SUGIMORI

From the Department of Physiology & Biophysics, New York University Medical Center, 550 First Avenue, New York, NY 10016, U.S.A.

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SUMMARY

1. The electrical activity of Purkinje cells was studied in guinea-pig cerebellar slices *in vitro*. Intracellular recordings from Purkinje cell somata were obtained under direct vision, and antidromic, synaptic and direct electroresponsiveness was demonstrated. Synaptic potentials produced by the activation of the climbing fibre afferent could be reversed by direct membrane depolarization.

2. Input resistance of impaled neurones ranged from 10 to 19 MΩ and demonstrated non-linearities in both hyperpolarizing and depolarizing directions.

3. Direct activation of a Purkinje cell indicated that repetitive firing of fast somatic spikes (s.s.) occurs, after a threshold, with a minimum spike frequency of about 30 spikes/sec, resembling the '2-class' response of crab nerve (Hodgkin, 1948).

4. As the amplitude of the stimulus was increased, a second form of electroresponsiveness characterized by depolarizing spike bursts (d.s.b.) was observed and was often accompanied by momentary inactivation of the s.s. potentials. Upon application of tetrodotoxin (TTX) or removal of Na^+ ions from the superfusion fluid, the s.s. potentials were abolished while the burst responses remained intact. However, Ca conductance blockers such as Co, Cd, Mn and D600, or the replacement of Ca by Mg, completely abolish d.s.b.s.

5. If Ca conductance was blocked, or Ca removed from the superfusion fluid without blockage of Na conductance, two types of Na-dependent electroresponsiveness were seen: (*a*) the s.s. potentials and (*b*) slow rising all-or-none responses which reached plateau at approximately −15 mV and could last for several seconds. These all-or-none Na-dependent plateau depolarizations outlasted the stimulus and were accompanied by a large increase in membrane conductance. Within certain limits the rate of rise and amplitude of the plateau were independent of stimulus strength. The latency, however, was shortened as stimulus amplitude was increased. These potentials were blocked by TTX or by Na-free solutions.

6. Substitution of extracellular Ca by Ba or intracellular injection of tetraethylammonium generated prolonged action potentials lasting for several seconds and showing a plateau more positive than those obtained in normal circumstances by either non-inactivating Na or Ca currents.

7. Spontaneous firing of the Purkinje cell was characterized by burst-like activity consisting of both s.s. and d.s.b. responses. Addition of TTX to the bath left the

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basic spontaneous activity and its frequency unaltered, indicating that Ca spiking and Ca-dependent K conductance changes are the main events underlying this oscillatory behaviour.

8. It is concluded that Purkinje cells demonstrate three types of voltage-dependent electroresponsiveness at the soma: (*a*) a fast Na-dependent spike, (*b*) a non-inactivating Na conductance which generates prolonged plateaus, where the amplitude of the plateau is produced by an equilibrium between the voltage-dependent Na and K conductances and (*c*) Ca-dependent spiking which is TTX-insensitive but responds to substances which block slow Ca conductances.

9. The Ca spikes are most prominent in the dendrites (see accompanying paper) and are followed by prolonged K conductance changes. This Ca and K activity is viewed as the most significant self-regulatory mechanism in long-term excitability of Purkinje cells. In the absence of Ca conductance, Purkinje cells behave in an erratic fashion, producing prolonged and unregulated periods of high frequency firing.

INTRODUCTION

Intracellular recording from cerebellar Purkinje cells has provided a significant body of information regarding the electrophysiological properties of these large neurones (Eccles, Llinás & Sasaki, 1966*a–d*; Martinez, Crill & Kennedy, 1971; Llinás, 1974). The strict geometrical organization of the cerebellar cortex itself (Ramón y Cajal, 1911; cf. Palay & Chan-Palay, 1974), in particular the laminar organization of the Purkinje cell elements, in addition to the localization obtained with intracellular markers (Llinás & Nicholson, 1971), has allowed the development of excellent criteria to establish unambiguously the site of micro-electrode impalement. This increased reliability in defining the location of the recording site has been important in demonstrating the membrane electroresponsiveness of these neurones (Llinás, Nicholson, Freeman & Hillman, 1968; Fujita, 1968; Llinás & Nicholson, 1971; Nicholson & Llinás, 1971; Llinás & Hess, 1976; Llinás, Sugimori & Walton, 1977; Llinás & Sugimori, 1978). Nevertheless, due to the difficulty in maintaining intracellular recordings and in modifying the extracellular ionic media in the *in vivo* condition, little is known about the ionic basis for these excitable properties. In order to investigate such a question and to define more accurately the difference between somatic and dendritic membrane characteristics, we have implemented an *in vitro* cerebellar slice preparation based on the techniques initially introduced by Yamamoto & McIlwain (1966) for the hippocampus and subsequently adapted to cerebellar research (Okamoto & Quastel, 1973; Yamamoto, 1974). This preparation has allowed long-term recording from Purkinje cells under direct visualization of the impalement site (at somatic or at different dendritic levels) and has also provided the means to control effectively the ionic composition of the extracellular milieu.

In this first paper we will describe the electrophysiological properties of the Purkinje cell as recorded *in vitro* and studied from an intrasomatic vantage point. The electrophysiological properties of Purkinje cell dendrites will be described in the accompanying paper (Llinás & Sugimori, 1980). Some of the present results have been published in preliminary communications (Llinás & Sugimori, 1978, 1979).

METHODS

Tissue preparation

Experiments were performed in adult guinea-pigs (400–600 g). The animals were decapitated with a small animal guillotine under ether anaesthesia. A rapid craniotomy which removed the squamous portion of the occipital bone allowed the total cerebellar mass, including the cerebellar nuclei, to be detached quickly with a metal spatula. The tissue was then immersed in 'standard' Krebs–Ringer solution (see Table 1) refrigerated at 6 °C. This procedure took, on the average, 30–40 sec. The cerebellar mass was then transected sagittally and a single slice about 2 mm thick was isolated from the vermis or one of the hemispheres. The slice was then affixed with cyanoacrylate to the bottom of a Plexiglass cutting chamber and side support given by surrounding the slice with agar blocks. Once secured, the tissue was immersed in standard Krebs–Ringer solution at 6 °C and further sectioned with an Oxford G501 vibrotome to yield about six 200-μm-thick cerebellar slices. These thin slices generally contained sagittal sections of all the cerebellar folia in the given rostrocaudal plane as well as the central white matter and cerebellar nuclear cells. Following this procedure, the slices were incubated in oxygenated standard Krebs–Ringer solution (37 °C) for about 1 hr.

Recording chamber and bathing solutions

After incubation, a slice was transferred to the recording dish (see Fig. 1*A*) where the tissue was continuously superfused with standard oxygenated Krebs–Ringer solution at 37 °C. The perfusion system was gravity-fed, allowing a routine flow of 0·5 ml./min with a maximum of 2 ml./min. This latter flow rate was used during solution exchange. The central chamber had a capacity of 2 ml. and the solution could be completely exchanged in approximately 10 min. The saline flowed into the central chamber by means of three small diameter channels which produced a close-to-laminar flow. The outflow of the chamber was accomplished via a cotton wick system which prevented turbulence by allowing continuous fluid movement (Fig. 1*B*).

The cerebellar slice was placed on a Sylgard plate at the bottom of the recording chamber and secured with a bipolar stimulating electrode pressing lightly on the white matter. The chamber was maintained at 37 °C by a surrounding water bath (Fig. 1*A*), which kept it at the same temperature as the perfusing solution (37 °C). The saline solution itself was temperature-regulated by passage through a heat exchanger at 37 °C (Fig. 1*A*).

Table 1 gives the solutions utilized in this set of experiments. The only drugs employed during the actual experiment were tetrodotoxin (TTX) and D600 (Knoll Pharmaceuticals) which were introduced into the solution at a concentration of 10^{-5} g/ml. and 0·1 mM respectively.

The standard' perfusion fluid was a Krebs-Ringer solution; this medium was utilized during the cutting, incubating and most of the recording time, and provided excellent pH buffering properties at different temperatures. This was especially significant during the sectioning and incubation periods. In order to prevent precipitation of the divalent cations (when replacing with Ca-free solutions), the standard medium was first exchanged with the control solution (see Table 1), and records were obtained in this control fluid before proceeding with the medium exchange. Neither the resting potential nor the Na- or Ca-dependent spikes showed any alterations after replacing the 'standard' with the 'control' solution. To increase oxygen availability and keep pH constant, 0·001 % H_2O_2 was added to all Tris-buffered solutions.

Recording and staining techniques

Purkinje cells were impaled with recording micropipettes under direct vision using Hoffman modulation microscopy (Hoffman, 1977) which allowed good visualization of the unstained cells (Pl. 1*A*). In order to prevent vapour condensation on the objective lens of the microscope, a small suction spigot was placed immediately to the side of its bottom surface (Fig. 1*A*). Intracellular recordings were obtained with micropipettes filled with 3 M-K acetate or with 1 M-tetraethylammonium chloride (TEA) having an average D.C. resistance of 60–80 mΩ. Antidromic and synaptic activation of the cells was obtained with a bipolar stimulation electrode located on the white matter immediately at the base of the folium studied. Direct stimulation of the Purkinje cells was implemented with a high-input impedance (10^{12} Ω) bridge amplifier. Capacity compensation allowed a frequency response of 10–15 kHz, depending on the microelectrode properties.

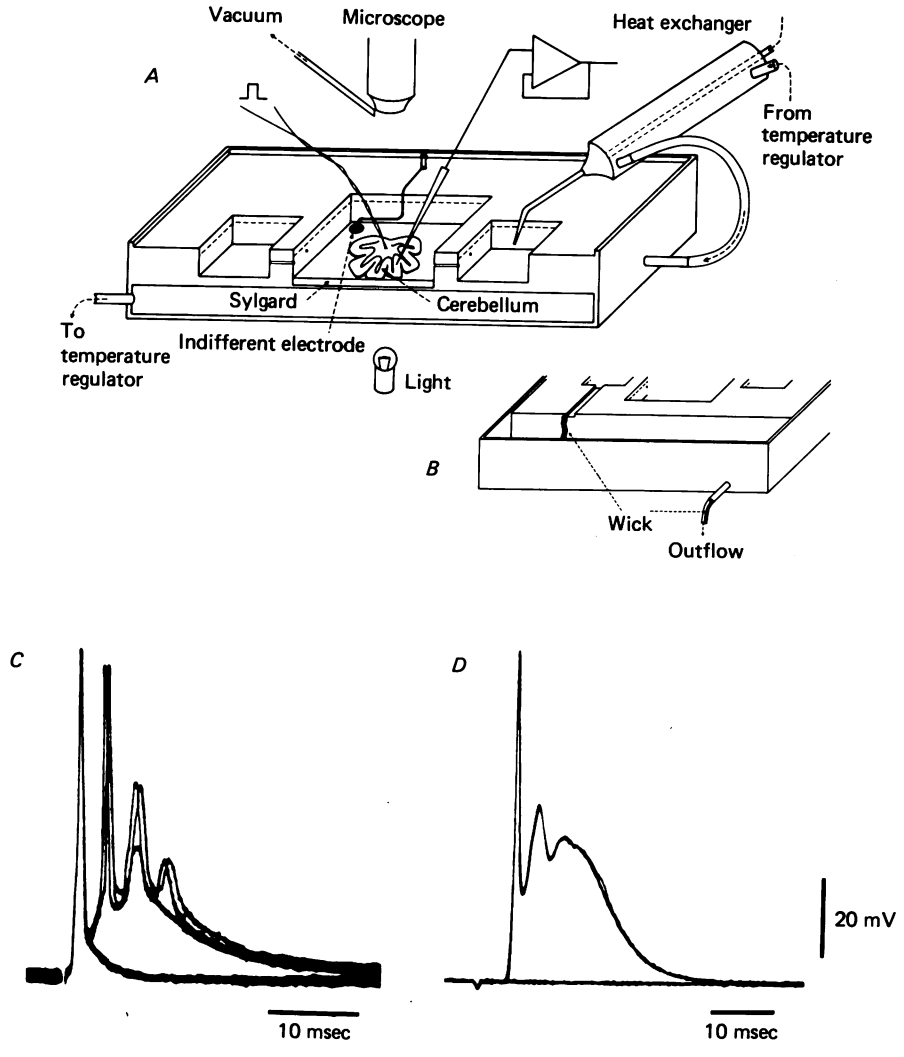

Fig. 1. Perfusion system and intrasomatic recordings from Purkinje cells following white matter stimulation. *A*, recording chamber consisting of three compartments. The cerebellar slice (central chamber) is immersed in a saline solution (broken line indicating fluid level). The saline is gravity-fed through a heat exchanger (to the right in *A*), and the fluid is removed through a wick (in *B*) into a reservoir. The temperature of the chamber is regulated by water flow maintained at 37 °C which passes from a temperature regulator through the heat exchanger, is circulated under the chambers and then returned to the temperature regulator. For further description, see text. *C* and *D*, intracellular recordings from Purkinje cell somata. In *C* white matter stimulation evoked an antidromic spike followed by all-or-none synaptic climbing fibre activation. *D*, all-or-none climbing fibre responses (from another neurone) superimposed in the absence of antidromic invasion.

Fast-green dye (Thomas & Wilson, 1965) or horseradish peroxidase (HRP) (Snow, Rose & Brown, 1976) were injected ionophoretically as neuronal markers. A cerebellar slice displaying several Purkinje and Bergmann glial cells stained by the HRP reaction product after intracellular recording is shown in Pl. 1*C*. The HRP staining technique utilized a micropipette electrolyte consisting of 4% HRP (Sigma type VI) in an 0·2 M-KCl solution buffered with 50 mM-Tris-HCl at pH 7·0. In these cases, pipettes were bevelled from a resistance of 120 MΩ to 60–70 MΩ with a Brown micro-electrode beveller (Kopf Instruments, California). The enzyme was injected using outward current pulses of 10–20 nA for somatic and 5–10 nA for dendritic staining. Injection was accomplished using 200 msec current pulses delivered at a rate of 2/sec for an average period of 10 sec. At the end of the experiment, the cerebellar slices were fixed with Karnovsky fixative (5 min), and the HRP reacted using the diaminobenzidine method (Kitai, Kocsis, Preston & Sugimori, 1976). The slices were then dehydrated and cleared following standard procedures and mounted for microscopic observation. This method permitted nearly 100% recovery of cells injected.

TABLE 1. Solutions

			Na-free		Ca-free				
	Standard (mM)	Control (mM)	Tris (mM)	Choline (mM)	Co (mM)	Cd (mM)	Ba (mM)	Mg (mM)	Mn (mM)
NaCl	124	130	0	0	130	130	130	130	130
KCl	5	6·2	6·2	6·2	6·2	6·2	6·2	6·2	6·2
KH_2PO_4	1·2	0	0	0	0	0	0	0	0
$CaCl_2$	2·4	2·4	2·4	2·4	0	0	0	0	0
$CoCl_2$	0	0	0	0	2·4	0	0	0	0
$CdCl_2$	0	0	0	0	0	1·0	0	0	0
$BaCl_2$	0	0	0	0	0	0	2·4	0	0
$MgCl_2$	0	1·3	1·3	1·3	1·3	1·3	1·3	3·7	1·3
$MgSO_4$	1·3	0	0	0	0	0	0	0	0
$MnCl_2$	0	0	0	0	0	0	0	0	2·4
Choline Chloride	0	0	0	130	0	0	0	0	0
Tris (pH 7·4 at 37 °C)	0	25	155	25	25	25	25	25	25
$NaHCO_3$	26	0	0	0	0	0	0	0	0
Glucose	10	10	10	10	10	10	10	10	10
O_2	95%	99·5%	99·5%	99·5%	99·5%	99·5%	99·5%	99·5%	99·5%
Co_2	5%	0·5%	0·5%	0·5%	0·5%	0·5%	0·5%	0·5%	0·5%

During the experiment itself the exact location of the recording pipette was determined by direct vision as seen in Pl. 1*A*. The modulation optics allowed unambiguous identification of the Purkinje cell soma and the more superficial main dendritic branches. Intrasomatic recordings were far easier to obtain than their dendritic counterpart since the optics allowed visual monitoring as the electrode touched the surface of the Purkinje cell membrane, causing a small but noticeable dimple. At this point a brief, single, outward current pulse produced a clear impalement of the cell without the application of further pressure. Pl. 1*B* shows a cell body and main dendrite stained with fast green after intrasomatic recording. Another cell filled from the soma with HRP and then prepared for observation following the procedure described above is shown in Pl. 1*D*.

RESULTS

Intrasomatic recording from Purkinje cells was obtained readily, and in healthy slices the recording could be maintained for 1 or 2 hr allowing repeated exchange of the bathing solution. On the average, intracellular recordings from a given cerebellar

slice could be performed for a period of up to 24 hr after isolation. However, in the present paper we shall restrict our results to recordings obtained within the first 8 hr following slice isolation.

Intracellular recording yielded a range of resting potentials which varied depending on the degree of damage induced by the impalement; when the initial resting potential was lower than 50 mV the recording was terminated. Generally, with initial resting levels of 50 mV and above, as the membrane 'sealed' around the recording pipette the resting potential stabilized at approximately 65 mV (see Table 2) and remained at this level for as long as the recording was continued, except for the membrane potential variations encountered in cells showing autorhythmicity. The resting potential was, on the average, more negative than those observed in the *in vivo* condition. This was probably due to removal, by slicing process, of the background synaptic input generated by the parallel fibre Purkinje cell synapses. The total data base for this and the accompanying paper (Llinás & Sugimori, 1980), was obtained from 157 intrasomatic recordings and 37 intradendritic recordings which fulfilled the above criterion in a total of fifty experiments.

TABLE 2. Values for resting potential, spike height and input resistance from ten Purkinje cell somata

Experiment no.	Resting potential (mV)	Spike* height (mV)	Input resistance (MΩ)†
21–4	65	75	10
21–5	69	85	18
21–6	65	75	13
26–1	66	72	14
26–2	67	73	18
26–3	64	70	15
26–4	63	66	18
27–2	68	80	19
27–3	70	70	15
28–2	70	83	11
Mean ± S.D.	66·7 ± 2·37	74·9 ± 5·77	15·1 ± 2·98

* Spike heights were measured from the resting potential to the peak of the spike

† Input resistances were determined with the hyperpolarizing current pulses (0·5 nA) in order to avoid delayed rectification.

Antidromic and synaptic activation

In addition to direct visualization, the Purkinje cells could be identified by their antidromic activation from the underlying white matter (Eccles *et al.* 1966*b*). As illustrated in Fig. 1 *C*, electrical stimulation of the white matter generated a typical antidromic invasion of this neurone, the action potential having an amplitude close to 80 mV (see Table 2) and a duration of 1 msec. The amplitude and time course of these action potentials were similar to those obtained in *in vivo* preparations. As previously described in other Purkinje cells (Eccles *et al.* 1966*a*), the spike consisted of an 'initial segment' (i.s.) and a 'soma-dendritic' (s.d.) component which could not be easily fragmented by repetitive stimulation. The latency of the invasion (0·2–0·3 msec) was in keeping with a conduction velocity of 15 to 20 m/sec, assuming a

utilization time of 50 μsec and was consistent with a Purkinje cell axonal diameter of 3–5 μm (Jakob, 1928).

With an increased stimulus amplitude, antidromic activation was often followed by an orthodromic firing (Fig. 1*C*), showing the well known spike burst response characteristic of climbing fibre activation of Purkinje cells (Eccles *et al.* 1966*a*).

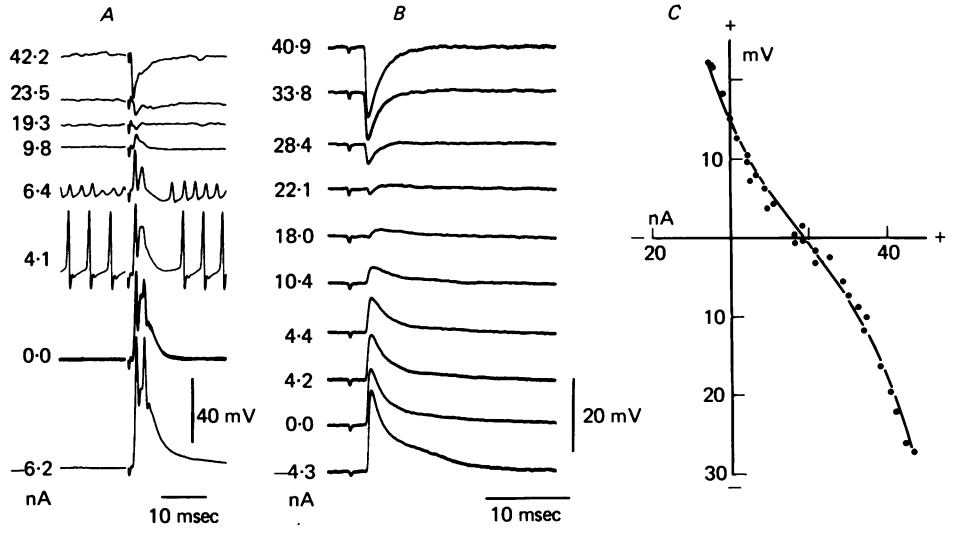

Fig. 2. Reversal of the climbing fibre-evoked synaptic potential. In *A* and *B*, D.C. current is injected across the Purkinje cell membrane at the magnitude shown to the left of each record. *A*, hyperpolarizing current increasing the amplitude of the synaptic activation. Depolarization by outward currents of 4–42 nA produce a reduction and finally a reversal (19.3 nA) of the synaptic potential. Note that with injections of 4·1 and 6·4 nA the cell fires repetitively. *B*, reversal of the e.p.s.p. in the absence of action potentials. The properties of the reversal are particularly clear at 18, 22 and 28 nA where the biphasic nature of the reversal is observed clearly. *C*, voltage–current relationship for the synaptic potential.

Although in some recordings (not shown) potentials generated by the disynaptic mossy fibre or parallel fibre system could be observed, in most cases only antidromic and climbing fibre activations of the Purkinje cells were seen. This is to be expected as the parallel fibres, due to their length (6 mm) (Brand, Dahl & Mugnaini, 1976), were transected at both ends and only a small percentage had the ascending portion of their axon in the slice itself.

The climbing fibre response was generally observed during the first 8 hr after cerebellar isolation. In some cases climbing fibre activation of visually identified Purkinje cells occurred following stimulation in the absence of the antidromic invasion, indicating that the Purkinje cell and olivary axons were probably severed at different distances from the stimulating electrodes. In some cases Purkinje cells could not be activated either antidromically or synaptically from the central white matter, and the stimulating electrode had to be moved closer to the recording site. The all-or-none nature of the climbing fibre response is clearly seen in records *C* and *D* of Fig. 1. The latency for this invasion indicates, in agreement with that

measured in *in vivo* preparations (Eccles *et al.* 1966*a*), a conductance velocity of 3–10 m/sec. (including the synaptic delay). The synaptic nature of this response was demonstrated by its reversal with artificial membrane polarization. Two such experiments are illustrated in Fig. 2. In Fig. 2*A* the amplitude of the climbing fibre response, consisting of a set of complex action potentials recorded at the resting

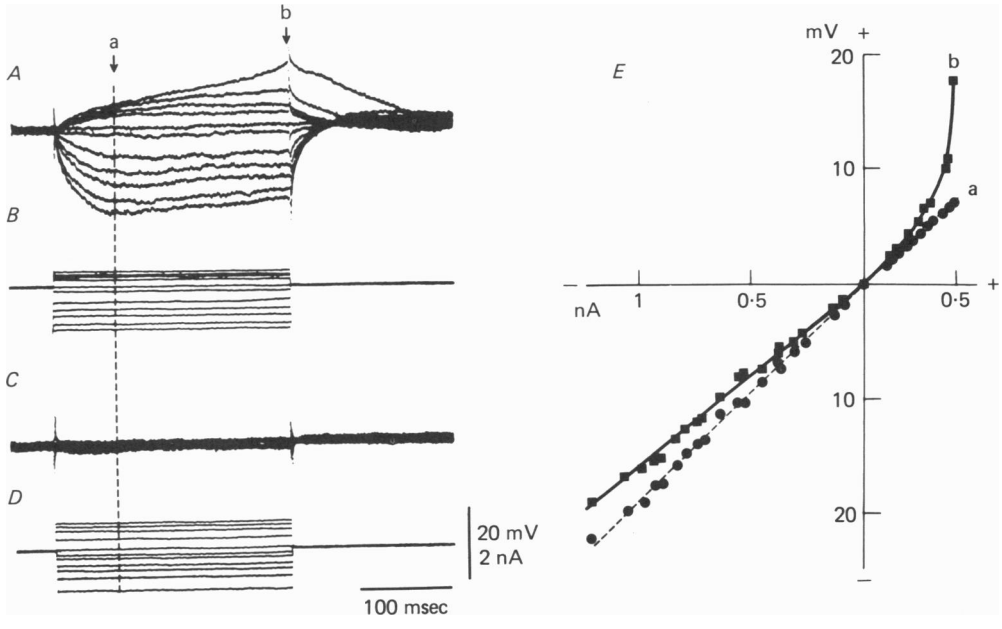

Fig. 3. Measurement of membrane resistance. *A*, membrane potentials produced by depolarizing and hyperpolarizing current steps (*B*). *C* and *D*, a similar set of records obtained with the electrode immediately outside the cell. *E*, plot of the current–voltage relationship at points a and b in *A*.

potential (0·0 nA), was increased by inward D.C. current injection (−6·2 nA) Outward DC currents produced a reduction and reversal of this all-or-none synaptic potential. At a DC current level of 4·1 nA, the cell responded with repetitive firing; at 6·4 nA these action potentials were decreased in amplitude; and a total inactivation was observed at 9·8 nA. An example of the reversal of a climbing-fibre-evoked excitatory post-synaptic potential (e.p.s.p.) in a cell with inactivated spike generation is shown in Fig. 2*B*. As in the *in vivo* preparation, the reversal properties of this e.p.s.p. were such that the early part of the response reversed first (22·1 nA) in accordance with the distributed nature of the climbing fibre Purkinje cell synapse (Llinás & Nicholson, 1976). The plot in Fig. 2*C* indicates the current levels required to reverse an *in vitro* climbing fibre e.p.s.p. and the actual relationship between e.p.s.p. amplitude and injected current. The current–amplitude relationship for this synaptic potential is in the same range as those found in the *in vivo* condition (Eccles *et al.* 1966*a*; Llinás & Nicholson, 1976; Hackett, 1976; Crepel & Delhaye-Buchaud, 1978).

Somatic input resistance

The input resistance at the Purkinje cell soma was determined using small amplitude square-current pulses injected through the recording pipette. The set of records in Fig. 3*A* shows the time course and amplitude of the membrane potential changes generated by the current pulses in *B*; the records in *C* and *D* show the potentials and current pulses after the electrode was withdrawn from the cell. The input resistance was calculated for six cells from the slope of the current–voltage relationship and is recorded in Table 2. As indicated by a in Fig. 3*E*, the current–voltage relationship in the hyperpolarizing direction is close to linear when measured at the time indicated by the first arrow in Fig. 3*A*. This point was chosen because it showed minimal anomalous rectification (cf. Grundfest, 1966). When measured at point b, for depolarizing pulses the response quickly became non-linear (see Fig. 3*E*, *b*) and a local response was observed even at low membrane depolarizations. With hyperpolarizing current, a time-dependent reduction in the potential change was often observed at this point. This anomalous rectification resembled that seen in other mammalian C.N.S. neurones (Nelson & Frank, 1967) and will be treated in detail elsewhere.

Repetitive firing

Short pulses

Short current injections on the order of 1 nA and 80 msec duration generated repetitive activation of Purkinje cells (Fig. 4*A–D*). In most cases this repetitive firing seemed to be superimposed on a small, rather slow rising, local response, as may be seen in record *B*. Here a current injection of 0·85 nA generated four action potentials followed by a prolonged after-depolarization (arrow). With larger current injections (*C* and *D*), the Purkinje cell fired at increasing frequencies and in *D* the after-depolarization outlasted the stimulus for several hundred milliseconds during which there was repetitive firing of the cell.

Long pulses

The repetitive firing properties of Purkinje cells became more evident with prolonged depolarizing pulses. As shown in Fig. 4*E–G*, a 1·3 sec pulse normally produced repetitive firing which, as with short pulses, reached threshold abruptly (arrow in *E*) and commenced with a frequency of approximately 30–40 spikes/sec. As the current pulse was further increased (Fig. 4*F*), a very regular firing occurred which was interrupted, near the end of the pulse, by a complex low-amplitude spike burst. Prior to the burst, the spikes showed a progressive decline in amplitude and were blocked (first arrow). Following the burst the membrane potential demonstrated a distinct hyperpolarization which reactivated spike firing (second arrow). This phenomenon is particularly clear in *G* where a larger depolarization generated high-frequency spike activation interrupted by bursts of spikes of smaller amplitude. As will be seen below, the regular bursting component of this response is generated by Ca-dependent dendritic spiking. These dendritic spike bursts (d.s.b.) were always followed by a large after-hyperpolarization at which time the Purkinje cell resumed firing somatic spike (s.s.) potentials. The type of oscillatory response illustrated in Fig. 4 suggests that beyond a certain level of depolarization, Na inactivation becomes

dominant and the s.s.-generating mechanism is blocked. This is indicated by the gradual decrease in action potential amplitude as the peak of the d.s.b. is approached. The larger after-hyperpolarization which followed each slow burst produced a momentary disappearance of the inactivation and fast firing was resumed until the level of Na inactivation was again reached.

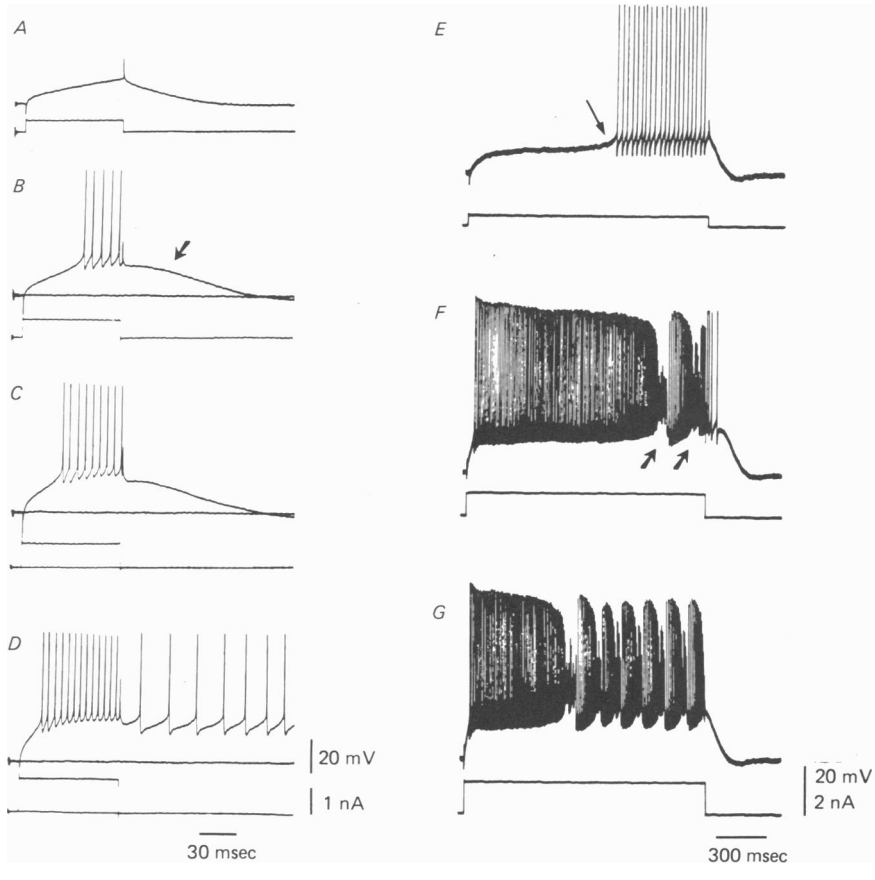

Fig. 4. Repetitive firing by direct stimulation. In *A–D*, square current pulses of increasing amplitudes are injected across the Purkinje cell soma. In *B –D* the break of the current pulse is followed by a prolonged after-depolarization (arrow) which in *D* becomes a plateau-response producing repetitive firing. *E–G*, repetitive firing obtained with prolonged current pulses. In *E* a 'threshold' current stimulus produces a repetitive activation of the Purkinje cell after an initial local response (arrow). In *F* and *G*, increases in current injection amplitude produce high frequency firing and an oscillatory behaviour marked with arrows in F.

Determination of firing frequency generated by square current pulses, was obtained (Fig. 4) and plotted as frequency–current (*f–I*) curves (see Fig. 5) These plots indicate that Purkinje cells have firing characteristics similar to those described by Hodgkin (1948) in the crab nerve as the 'class 2' response. As in other neurones, this repetitive s.s. firing showed no sign of a primary frequency range (Granit, Kernell & Smith, 1963; Calvin & Schwindt, 1972; Granit, 1972). Rather, as the depolarization

reached a certain level the cell fired repetitively. As in the case of the crab nerve, no single spikes could be generated with long pulses, and the frequency of firing could only be modulated by depolarization within a certain range. As will be seen below, the present results imply that the transition between resting and firing in this neurone is probably subserved by the abrupt onset of a slow inward current which shows little inactivation.

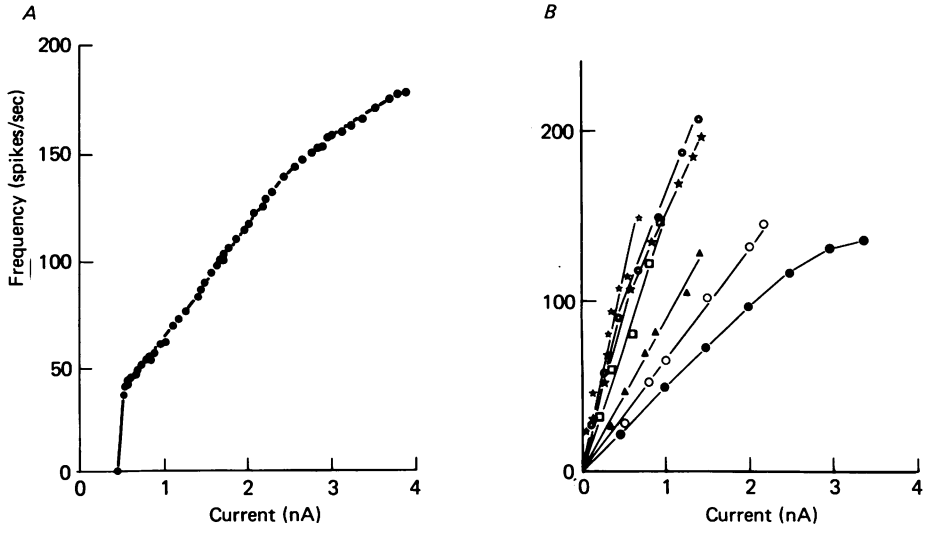

Fig. 5. Frequency–current (f–I) curves for Purkinje cell repetitive firing. *A*, typical f–I plot showing that repetitive firing commences at current injection level of approximately 30 nA. From this level a close-to-linear relationship between frequency and current is observed. In this cell the linearity is broken above 2·8 nA where a second slope is observed. *B*, f–I plots for seven other Purkinje cells. The current level at which the initial firing of the cell occurs is arbitrarily set to zero in order to compare the slopes of these firing rates. The cells were chosen because of their similar input impedances.

A second interesting finding relates to the plot in Fig. 5*B*. Here plots show that Purkinje cells with quite similar resistances often respond with different firing frequencies to current pulses of similar amplitude and duration, indicating different integrative properties. In order to facilitate the comparison of f–I plots obtained from these different cells, the immediate subthreshold current was normalized to zero.

Ionic basis of Purkinje cell firing

An analysis of the ionic species which serve as charge carriers for these different responses was accomplished by either modifying the ionic composition of the perfusing bath or by using drugs or ions known to block specific voltage-dependent conductances.

Purkinje cell firing in the absence of Na currents

Ca-dependent spikes. In order to investigate the electrophysiological properties of Purkinje cells in the absence of Na currents, two experimental paradigms were designed: (*a*) pharmacological block of Na conductance by bath application of TTX 10^{-5} g/ml. (Narahashi, Moore & Scott, 1964) and (*b*) replacement of Na with Tris or choline chloride (see Methods).

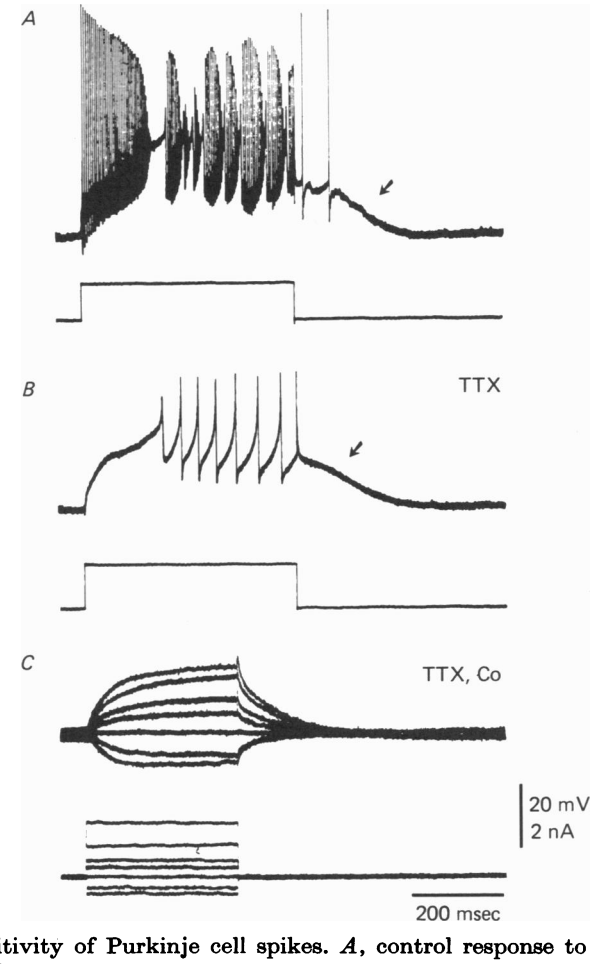

Fig. 6. TTX-sensitivity of Purkinje cell spikes. *A*, control response to square pulse depolarization. Note the after-depolarization (arrow). *B*, a similar pulse after the addition of TTX to the bath. Note that the fast (s.s.) spikes are blocked while the slower oscillations and the after-depolarization (arrow) remain. *C*, addition of cobalt chloride to the TTX saline removes all electroresponsiveness.

Initially, as shown in Fig. 6*A*, depolarization of the Purkinje cell soma produced repetitive s.s. activation and oscillatory d.s.b. responses as described above and shown in Fig. 4*F* and *G*.

Following the addition of TTX (10^{-5} g/ml; Fig. 6*B*), total blockage of the s.s. potentials was observed; however, the d.s.b. potentials remained unmodified as did

the after-depolarization which follows pulse breaks (arrows in *A* and *B*). These broad action potentials could be distinguished clearly from the s.s. potentials by their slow rate of rise, high threshold and small amplitude. As with the d.s.b. in the absence of TTX, they were often followed by a prolonged after-hyperpolarization of rather sharp onset. In addition, following short depolarizations, prolonged all-or-

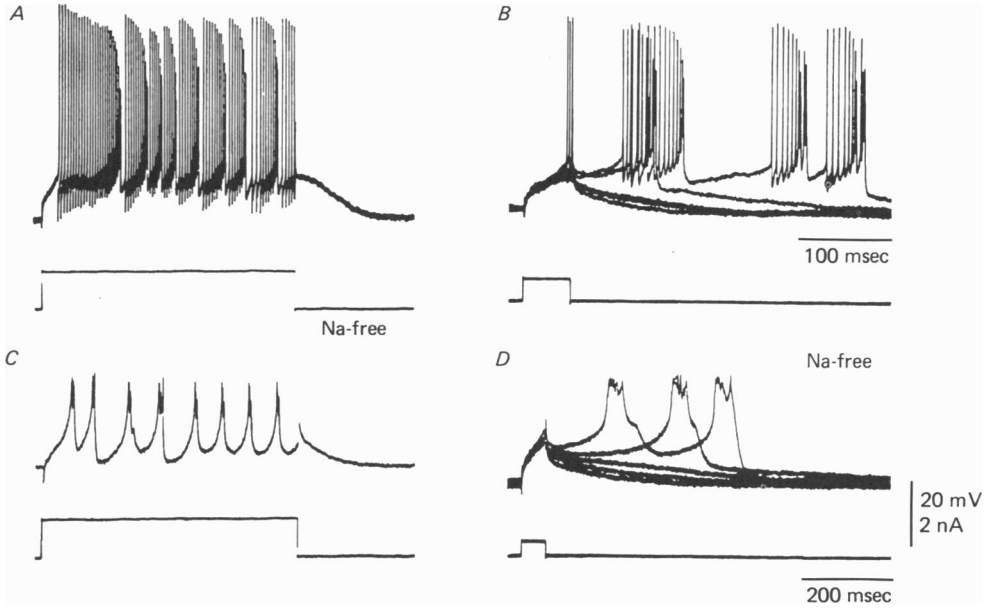

Fig. 7. Dependence of somatic spikes (s.s.) on extracellular Na. *A*, control record. *C*, same as *A* but 30 min after extracellular medium has been replaced by a Na-free solution. *B* and *D*, same as *A* and *C* but generated by short pulses. Note time calibrations.

none responses and plateau-type local responses lacking after-hyperpolarization, such as seen in Fig. 7*D*, were also seen after TTX administration. While the origin of these d.s.b.s will be discussed in detail in the following paper (Llinás & Sugimori, 1980), it is clear that they are calcium-dependent spikes. Indeed, replacement of Ca by 2·4 mM-cobalt chloride known to block Ca conductances (Baker, Hodgkin & Ridgway, 1971) completely blocked all active responses (Fig. 6*C*). While part of the after hyperpolarization is probably produced by a Ca-dependent K conductance, there is a significant voltage-dependent K conductance which is apparent only with larger depolarizations.

A second method used to investigate the role of calcium current in spike generation is to remove Na from the bath. In these Na-free preparations the ion was substituted by choline or tris (see Table 1). As shown in Fig. 7*A*, prior to Na substitution a prolonged current pulse initiated repetitive s.s. firing of the Purkinje cell and the subsequent d.s.b. oscillation. Thirty minutes after the replacement of Na by choline chloride (Fig. 7*C*) the same amplitude depolarization failed to elicit the s.s. potentials. At the same time the slow d.s.b.s described above were again present and reproduced the same membrane potential fluctuations which underlie the s.s. repetitive firing

in the presence of Na (Fig. 7*A*). In traces *B* and *D*, experiments similar to those in *A* and *C* are shown following shorter stimulus pulses. In trace *B* it can be seen that a short depolarization is capable of producing a rather prolonged all-or-none plateau depolarization upon which repetitive s.s. potentials are clearly seen. Following Na removal (in *D*), even shorter pulses can produce these long-lasting plateau potentials and the d.s.b. responses which ensue. As in the previous case, removal of Ca from the bath, the addition of Cd, Co, Mn or D600, or simply the substitution of Ca by Mg, abolished these TTX-insensitive responses.

Sodium-dependent action potentials in the absence of slow Ca conductance

Having determined the characteristics of the action potentials generated in the absence of Na currents, a set of experiments was designed to define the properties of the Na spikes when Ca currents are blocked. When Ca conductance was blocked by addition of Co, the typical Purkinje cell response to a prolonged pulse (Fig. 8*A*) was seen (Fig. 8*B*). Similar current pulses elicited a change in membrane potential which produced, after a delay, a rather slow rising response capped with s.s. potentials (Fig. 8*B*). These action potentials progressively decreased in amplitude as the response stabilized at a plateau-depolarization (at about -30 mV). At this point there was a total inactivation of the s.s.-generating mechanism. As the current amplitude was increased, the latency of this all-or-none slow response became shorter (arrows), but no significant change in the rate of rise was observed. Furthermore, the plateau-depolarization which followed the s.s. burst had the same amplitude, even though the injected current was doubled. The fact that the plateau which follows spike inactivation was insensitive to injected current strongly indicates that the membrane conductance is quite high during this time. This conjecture was tested as illustrated in Fig. 8*C*. The membrane potential change produced by a hyperpolarizing current pulse at rest was compared with that produced by the same current during the plateau. The difference between these two potentials indicates a five-fold decrease in input resistance during the prolonged plateau potential.

A similar set of findings was revealed by Ca current blockage with $CdCl_2$ (Fig. 9*B*), and when Ca was substituted by Mg (Fig. 9*C*). Depolarization in these preparations generated plateau potentials which, as shown in *B* and *C*, often outlasted the stimulus. If current injection was increased beyond the plateau level, as in the uppermost trace in *C*, a rapid fall of potential occurred at the end of the pulse. Also, in all cases shown in Figs. 8 and 9 the addition of TTX blocked this plateau response as well as the initial s.s. potentials (Fig. 9*D*).

In some cells, as shown in Fig. 9*E* and *F*, the prolonged depolarization after Cd blockage of Ca conductance generated a prolonged plateau-potential following a short stimulus. In this case the plateau, which lasted nearly 500 msec, was just short of s.s. inactivation. In record *F* another such prolonged plateau is shown with a duration of close to 10 sec. In these cases the frequency for s.s.firing was as high as 1500/sec, a frequency much higher than ever observed in any other condition.

The plateau potentials which are present after Ca current has been blocked or extracellular Ca removed, are blocked by TTX or by the absence of external Na (not shown). These findings indicate that the prolonged inward current which generates

the plateau is carried by Na through a slow or a non-inactivating TTX-sensitive channel. The observation that the plateau level may be shortened or abolished by a strong outward current injection (Fig. 9*C*), indicates that the plateau represents an unstable equilibrium state for at least two simultaneously occurring voltage-dependent phenomena: a non-inactivating Na and a non-inactivating K conductance.

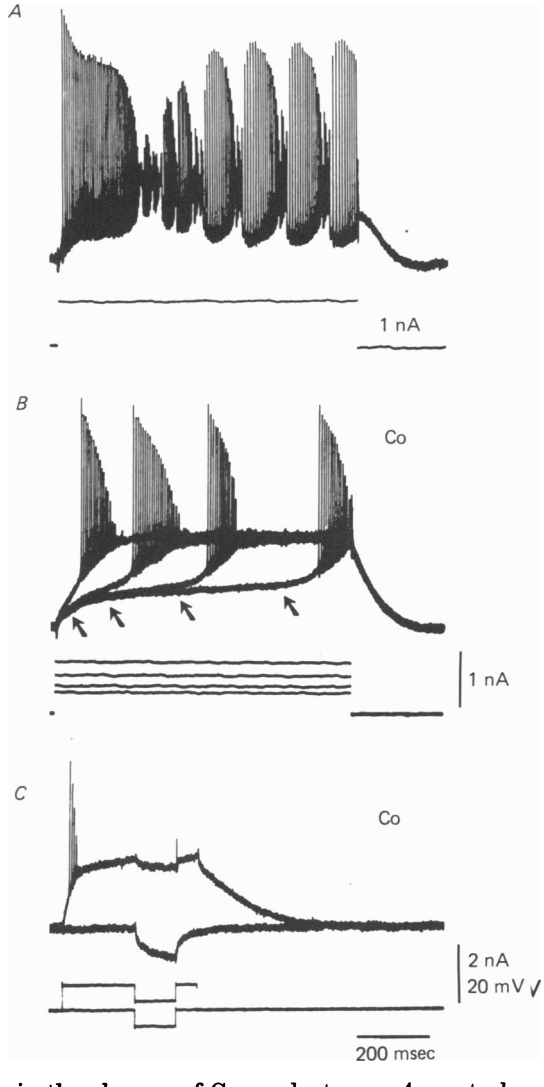

Fig. 8. Na spikes in the absence of Ca conductance. *A*, control response. *B*, response after Ca conductance was blocked by the addition of Co to the bath. Direct stimulation elicits slow all-or-none depolarizing responses which generate fast action potentials and reach a plateau level. As the stimulus is increased, the onset moves to the left but the rate of rise and the final amplitude of the plateau are independent of stimulus amplitude in the range illustrated. The slow onset of the all-or-none responses is indicated by arrows. *C*, increased conductance during the plateau of this response is illustrated by the reduction of the voltage produced by a test hyperpolarizing pulse.

The above conclusion, i.e. that the plateau potential is produced by an equilibrium state between two conductances, was tested in cells where voltage-dependent K conductance was blocked by intracellular injection of TEA. Under these conditions the level of the Na-dependent plateau depolarization was shifted towards more positive values.

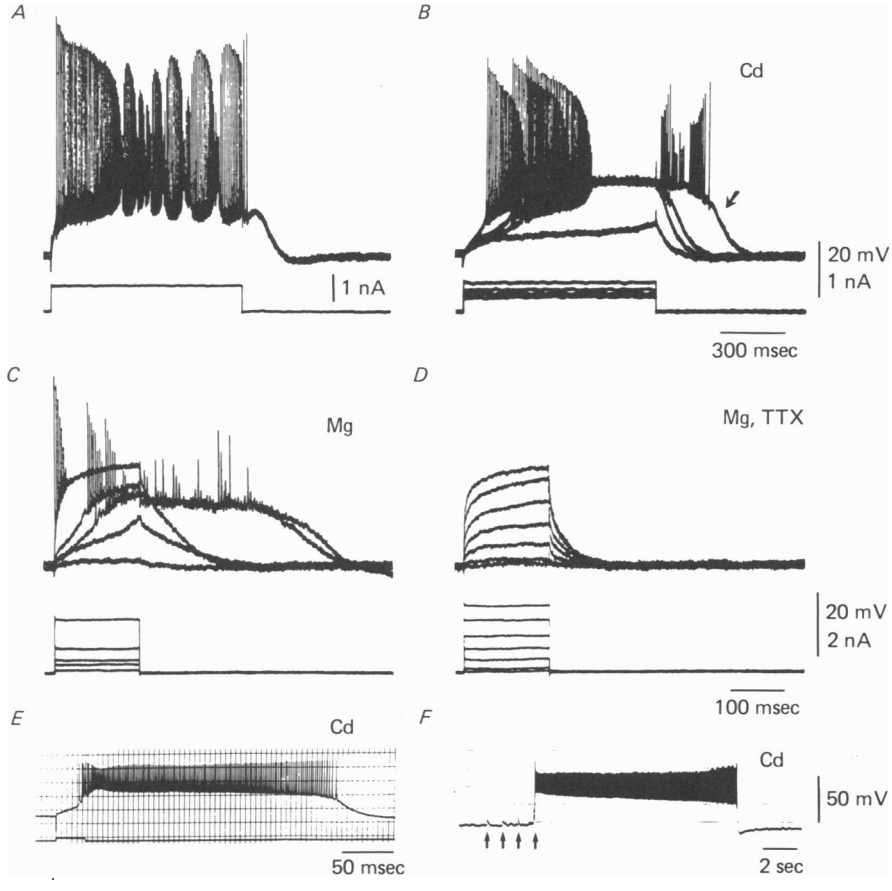

Fig. 9. TTX-sensitivity of the Na-dependent plateau. *A*, control response. *B*, initial firing and plateau all-or-none response following blockage of Ca conductance by $CdCl_2$. Note that the response outlasts the stimulus and shows firing at beginning and end of the plateau phase. *C*, a similar plateau phase which also generates fast action potentials is seen following extracellular Ca replacement by Mg. If the stimulus is increased beyond the plateau level (uppermost record), the plateau after-depolarization disappears. *D*, addition of TTX completely blocks the fast spikes and the plateau response. *E* and *F*, Ca conductance blockage by Cd produces Na-dependent plateau depolarizations which generate repetitive activation of Purkinje cells. These TTX-sensitive responses may last for several seconds. Time and voltage calibration for *A* given in *B*, and for *C* in *D*. Voltage calibration for *E* in *F*. Arrows in *D* represent subthreshold stimuli.

Purkinje cell spikes after extracellular Ca replacement by Ba ions and following intracellular TEA injection

Prolonged action potentials were produced in Purkinje cells at the somatic level (Fig. 10*A* following a short stimulus, up going arrows) when extracellular calcium had been replaced by $BaCl_2$ (see Table 1). These spikes, appearing 10–15 min after replacement, reached a plateau of about 55 mV from resting which could last as long

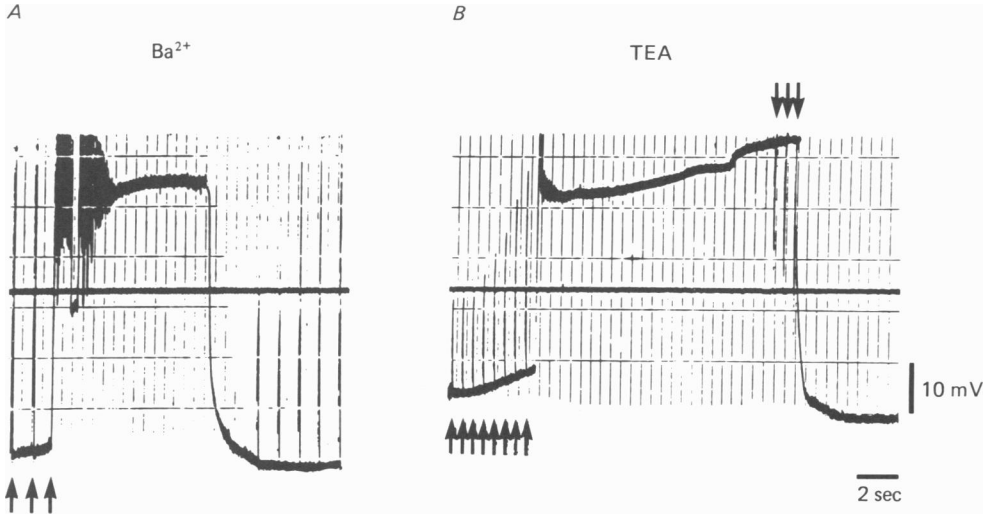

Fig. 10. Prolonged all-or-none response obtained by a short depolarization following extracellular replacement of Ca by Ba. *B*, action potential obtained following TEA injection into the Purkinje cell somata (arrows indicating stimuli).

as 10 sec. These spikes are probably related to the extracellular field potentials generated by local stimulation and to the large rises in extracellular K concentrations seen when Ba is added to the superfusing Ringer solution in the *in vivo* cat cerebellum (Nicholson *et al.* 1976). Because Ba is thought to move through the voltage-dependent Ca channel (cf. Hagiwara, 1973), the amplitude of this plateau potential suggests that in the normal Purkinje cell Ca current is probably counterbalanced by an outward Ca-dependent K conductance. In addition, since Ba probably moves more easily than Ca through the Ca channel and does not activate the Ca-dependent K conductance (cf. Meech, 1978), the amplitude and duration of this plateau-potential are larger than those observed in the Ca-containing medium.

Following intracellular injection of TEA (Fig. 10*B*), short depolarizing pulses (up-going arrows) generated a prolonged action potential having a slowly rising plateau phase which often reached close to the Na equilibrium potential (assumed to be +40 mV) and which could be terminated by applying a hyperpolarizing pulse (down-going arrows).

Two points may be made with respect to the TEA plateau. First, after TEA injection the plateau was larger than that obtained in the absence of TEA (see Fig. 6*B*), implying that a K conductance is indeed important in establishing the plateau

amplitude. Secondly, and more significant with respect to the genesis of the prolonged Na-dependent plateau, was the finding that TEA also increases the amplitude of the all-or-none response seen in the absence of Ca conductance. Because in the latter condition no inward current is carried by Ca, this voltage plateau must represent an equilibrium state between voltage-dependent Na and K conductances. Injection of TEA would then shift this equilibrium state to a more positive value by producing a partial blockage of the voltage-dependent K conductance (Armstrong & Binstock, 1965).

Spontaneous firing

So far we have examined the properties of Purkinje cells as revealed by short and long-duration current pulses applied through the recording electrode. In addition to this activity, Purkinje cells demonstrated in the absence of stimulation, an autorhythmicity which was characterized by prolonged periods of bursting followed by periods of electrical quiescence (Fig. 11). This may be seen at different times following preparation of the slices and appears to be present even after 24 hr. Because such electrical activity can be clearly seen extracellularly, we conclude that this autorhythmicity is not secondary to intracellular penetration. In addition, protracted recording from these cells indicates little variation in this behaviour for periods up to 3 hr. Basically this electrical activity is characterized by a fluctuation in membrane potential such that the firing level (i.e. the level at which the bursting would occur) is maintained for approximately 5–15 sec. The quiescent periods have approximately the same duration. Within 100 msec following a burst, the membrane potential reaches a peak hyperpolarization (beyond the resting level). Following this, the membrane potential slowly depolarizes at a rate of 1 mV/sec until the firing level is again reached.

For purposes of analysis, each component of this phenomenon will be considered separately. Detailed investigation of this spontaneous activity (Fig. 11*C* and *E*) indicates that, as with prolonged pulses, the bursts are generated by Na and Ca-dependent action potentials. The prolonged Ca action potentials generally serve as both the electrical stimulus for the Na spike and as the mechanisms by which s.s. spikes are ultimately inactivated. This slow rising Ca spike is then followed by a precipitous hyperpolarization, probably due to a K conductance increase. As expected, introduction of TTX to the bath does not change the general character of the response other than to remove the fast action potentials (see Fig. 11*B*, *D* and *F*). The recordings reveal, on the other hand, that the underlying potential shift is related to the slow rising Ca current spike and that the subsequent sharp return towards the resting potential is related to the activation of K permeability. At the peak of these depolarizations, the Ca spikes themselves are followed by K activation which is countered by the next Ca spike such that at the peak of every burst there may be as many as three or four individual calcium spikes (see Discussion of Llinás & Sugimori, 1980). At this point then, a prolonged after-hyperpolarization becomes dominant and in most preparations has a duration close to 100 msec.

It must be noted that the membrane potential following this spontaneous Ca-dependent spike burst did not usually reach the initial resting potential level. However, at the end of each burst the membrane potential became more negative than

that seen at the maximum after-hyperpolarization during the burst and more negative than the initial membrane potential immediately prior to the spontaneous bursting. It is very probable that the bursting basically depends on a slow Ca-dependent depolarization which is terminated by a Ca-dependent K conductance. At low Ca current levels the membrane potential may actually reach firing level at

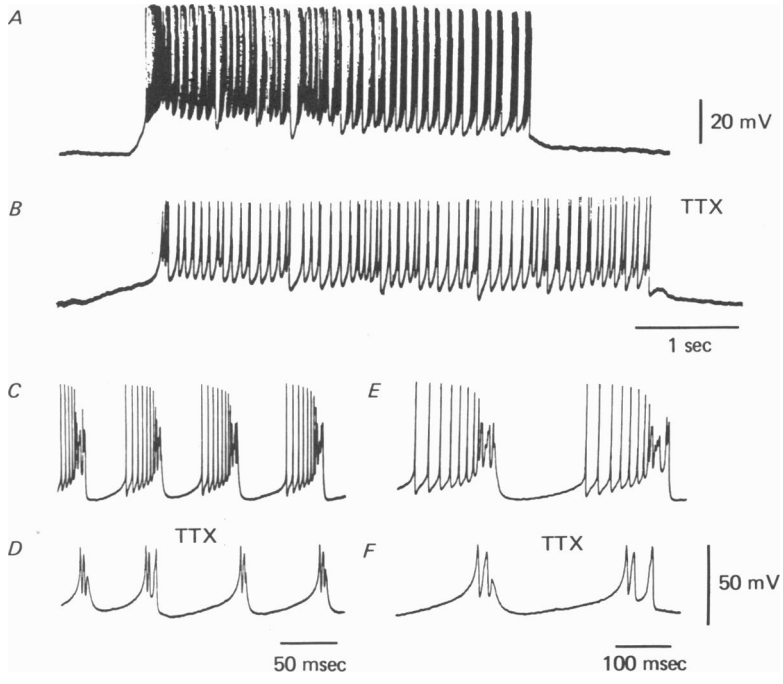

Fig. 11. Spontaneous repetitive firing of Purkinje cells. *A*, spontaneous depolarization lasting for 4 sec observed in the absence of any stimulus. *B*, addition of TTX producing a blockage of s.s. potentials as detailed in records *C–F*. In *C* and *E*, the spike bursting is shown to consist of fast spikes and dendritic spike bursts. In *D* and *F*, response after the addition of TTX to the bath. Note that the spontaneous bursting is unmodified by the TTX saline, while the s.s. potentials are blocked.

which time further Ca entry produces only a momentary K-dependent hyperpolarization. However, the slow fluctuations which regulate the over-all duration of the burst trains (10–15 sec.) are Ca-dependent and are probably modulated by metabolic factors (cf. Greengard, 1978). In addition, a 'local' response may itself be generating the membrane potential fluctuation underlying the autorhythmicity. This prolonged Ca current is probably different from that generating the Ca action potentials. However, it is also clear from our experiments that if Mn or any other Ca current blocker is added to the bath, or if Ca is removed, the autorhythmicity of these cells is blocked.

DISCUSSION

The present results indicate that the mammalian cerebellar cortex, and Purkinje cells in particular, can be kept *in vitro* for many hours after isolation. Furthermore, our results reveal many similarities between the electrical activity of the *in vitro*

and the *in vivo* Purkinke cell. Thus, the characteristics of simple and complex spikes which typify the firing ability of this cell throughout vertebrate phylogeny (Llinás & Hillman, 1969) seem to be maintained in this preparation. Furthermore, synaptic transmission (at least as far as the climbing fibre–Purkinke cell junction is concerned) can be maintained for a protracted time.

The *in vitro* preparation, in addition, provides new insights into the ionic basis for some of the electrophysiological properties of Purkinje cells. Also, it has revealed properties not previously observed in these cells or in any other vertebrate neurone. However, several points require discussion before the different firing modes are considered in detail.

Synaptic potentials

The results obtained in the *in vitro* preparation regarding the climbing fibre–Purkinje cell junction indicate that this synapse retains, *in vitro*, most of the properties described in the *in vivo* condition. The amplitudes and duration of the synaptic potentials are directly comparable to those seen in the intact preparation. This is perhaps best illustrated by the fact that reversal of the climbing fibre e.p.s.p. by currents injected at somatic level resembles very much that obtained in the *in vivo* condition. For example, the reversal of the e.p.s.p. near the equilibrium potential is biphasic, signalling that even after section of the climbing fibre the well known properties arising from the spatial distribution of the climbing fibre–Purkinje cell synapse are still present (Llinás & Nicholson, 1976). Equally interesting in this respect is the fact that the climbing fibre–Purkinje cell junction continues to function for at least 8 hr following slicing. This is in contrast with the recent experiment by Ito, Nisimaru & Shibuki (1979) who suggest that following lesion of the inferior olive, Purkinje cell inhibition of cerebellar nuclear cells is blocked; they propose that damage to the presynaptic cells (the inferior olivary nucleus) will produce a very rapid change of the climbing fibre–Purkinje cell synapse. However, no obvious change was observed in any of the properties of synaptic transmission between these two elements in the present set of experiments. In contrast, as is discussed in detail by Llinás & Sugimori (1980), climbing fibre activation generates sizeable Ca-dependent dendritic spikes and a prolonged K conductance. This K conductance may be quite important in modulating Purkinje cell excitability for a protracted period and, thus, may exercise a tonic regulatory effect on membrane potential capable of preventing artificially high levels of Purkinje cell activity in the presence of climbing fibre input.

Input resistance and repetitive firing

Little variation was found in the input resistance of Purkinje cells as tested at the somatic level. The range, as indicated in Table 2, was 10–19 MΩ with a mean of 15·1. Repetitive firing of these cells is characterized by a close to linear relationship between injected current and firing frequency. The action potentials show, therefore, no primary range as do, for instance, motoneurones (cf. Granit, 1972). On the other hand, the response to square pulses is characterized by high-frequency spike activity of sudden onset. On the whole, frequencies below 20/sec cannot be obtained with long pulses. Once the threshold level is obtained, the firing always occurs from a slow local response, as seen in Fig. 4*E*, very similar to that observed in the crab peripheral nerve by Hodgkin (1948). This so-called '2-class' response is characterized

by a non-smooth *f–I* plot as may be seen in Fig. 5. If Purkinje cells with similar input resistances are compared, the slope of the *f–I* plot can change significantly from one cell to the next, indicating that while Purkinje cells are morphologically a homogeneous population, even the simplest of the firing properties (i.e. the s.s. repetitive firing) shows distinct differences from one cell to the next.

Voltage-dependent action potentials and local responses

Experiments in which the voltage-dependent Na conductance was blocked by TTX or where extracellular Na was replaced by Tris or choline chloride (see Methods) indicate that membrane conductance to Na plays an important role in the generation of the fast action potentials (s.s.) recorded at somatic level. As in other neurones then, we must conclude that the action potentials evoked either by antidromic, orthodromic or direct stimulation, and characterized at the level of the soma by an amplitude of approximately 80 mV and a time course of approximately 1 msec, are indeed produced by a voltage-dependent Na conductance change.

The 'non-inactivating' Na-dependent spike

On the other hand, an unexpected finding was that of the prolonged Na-dependent potentials which may be seen in the soma following blockage of the voltage-dependent Ca conductance (Figs. 8 and 9). These rather prolonged potentials are characterized by a slow onset and plateau-like wave form. Their duration can vary from a few to several hundred milliseconds. Very characteristic is the flat plateau during which there is a large membrane conductance. Current pulses applied across the membrane during this time show that the input resistance is one fourth to one fifth of that at rest.

A second property of this response is its all-or-none nature. Two aspects of this all-or-none behaviour are worth noting: (*a*) its rather constant rate of rise which, over a wide range of current injection, is independent of the amplitude of the stimulating current and (*b*) its constant amplitude. Thus, as seen in Fig. 8, increased current injection produced a decrease in the latency of the response but changed neither the rate of rise nor the firing frequency of the spikes riding on this depolarization. The actual mechanism for the generation of this plateau is difficult to determine with any certainty since the properties of the current generating this response are not known. However, one obvious possibility is that since the plateau is blocked by TTX and by the removal of extracellular Na, it respresents a non-inactivating slow Na conductance. This voltage-dependent conductance is probably masked by the Ca–K conductance of the dendrites which modulate membrane potential; thus, it is seen clearly only under those conditions where the Ca current is blocked. Nevertheless, its slow onset and all-or-none character may be related to the special firing properties which these cells demonstrate. Thus, the abrupt onset of repetitive firing generated by prolonged depolarizations suggests that this non-inactivating Na current may be the mechanism for this '2-class' (Hodgkin, 1948) type of response.

One of the puzzling aspects of this plateau-potential, on the other hand, is the rather constant amplitude which it displays throughout its time course. One likely explanation for this property is that, as in the case of other voltage-dependent spikes which show prolonged plateaus (Tasaki & Hagiwara, 1957), it is the product of two simul-

taneously-activated voltage-dependent conductances – in this case a Na and a K conductance. The amplitude of the plateau is close to – 20 mV which indicates that it is generated in approximately equal part by a K and a Na conductance change, assuming an E_K of – 80 to – 90 mV and an E_{Na} of + 40 to + 50 mV. Support for this suggestion is, in fact, furnished by the finding that if TEA is injected intracellularly in the absence of a Ca conductance, the amplitude of the Na plateau-response increases to + 2 mV, implying that the plateau was indeed the product of an equilibrium state between these two voltage-dependent conductances. However, in contrast to the prolonged Na-dependent spike demonstrated in squid by Tasaki & Hagiwara (1975), which can be reproduced by a modified Hodgkin-Huxley formulation (Fitzhugh, 1959), the coexistence in this preparation of normal spikes and of the slow plateau (see Figs. 8 and 9) does suggest that two different mechanisms are manifest. Thus, we propose that the fast action potential is generated by the usual voltage-dependent Na conductance (Hodgkin & Huxley, 1952) while the plateau is produced by a slow, non-inactivating, Na conductance.

Voltage-dependent Ca conductance changes and the oscillatory spontaneous activity of Purkinje cells

Following blockage of the fast and slow activating Na conductance changes, Purkinje cells continued to demonstrate electroresponsiveness. As illustrated in Fig. 6, Na conductance blockage uncovered at soma level a slow-rising and fast-falling Ca-dependent action potential which was followed by a hyperpolarization. In addition, as shown in Figs. 7 and 11, burst responses are produced in these cells by direct stimulation or spontaneously even in the absence of extracellular Na. Indeed the Ca conductance appears to be capable of generating, and to be the basis for, the oscillatory behaviour seen in Purkinje cells *in vivo* (Eccles *et al.* 1966*a*, *b*, *c*) and *in vitro*. On the whole, the frequency of these potentials is voltage-dependent and the amplitude of the after-hyperpolarization seems to be related to that of the spike burst. The mechanism underlying the generation of this response appears to be a voltage-dependent Ca conductance change followed by a Ca-dependent conductance as has been demonstrated in invertebrates (Meech, 1978; Lux & Heyer, 1979). Significant falls in extracellular Ca concentration and rises in extracellular K concentration are also seen in the cat cerebellum following repetitive local stimulation, both of which are diminished by application of Mn (Nicholson, Bruggencate, Stoeckle & Steinberg, 1978). The potentials seen in this study are characterized by a slow-rising multi-peaked onset, suggesting a spatially distributed electroresponsiveness. The after-hyperpolarization has a much faster rate of fall and is longer than the Ca burst itself.

Besides this bursting response, the Purkinje cell membrane seems to be capable of generating prolonged Ca-dependent responses which, as in the case of the non-inactivating Na response, can last for a protracted period and exhibit a plateau. The main difference between the Na and Ca plateau is that the latter may vary in amplitude and even develop into a Ca-dependent action potential This is particularly clear when short current pulses are injected into the soma as seen in Fig. 7 where the duration of the Ca response is seen to change dramatically from one pulse to the next. Of interest also is the fact that these plateau depolarizations occur when the stimulating pulse has a particular amplitude. If the stimulus is increased beyond this

level, the plateau discharge does not occur, probably because of the voltage-dependent K conductance change. Again, here equilibrium must be struck between the voltage-dependent Ca current and the voltage-dependent K current.

One intriguing difference between the Ca spike and the prolonged Ca-dependent plateau response is that during the plateau the Ca-dependent K conductance change appears to be absent or greatly reduced. While many hypotheses may be invoked for the lack of a powerful-Ca-dependent K current during these plateaus, such as a special location for the Ca–K exchange, a minimum Ca concentration increase, or the lack of modification of the Ca conductance into a K conductance (cf. Lux & Heyer, 1979), nevertheless it is clear that rate of fall of the Ca action potential seems to be strongly dependent on its amplitude. Whether this is partly due to summation of a Ca-dependent and voltage-dependent K conductance change must be determined. However, the effect of TEA in the presence of a Ca conductance (Fig. 10) strongly suggests that part of the after-hyperpolarization may be a voltage-dependent K conductance change.

In short, it appears that, besides the voltage-dependent Na conductance change and a slow or non-inactivating Na conductance, there is indeed a Ca dependent action potential generated in these neurones. As will be seen in the accompanying paper (Llinás & Sugimori, 1980), Ca spikes seem to be most prominent in dendrites. This suggests a specialization of the Purkinje cell membrane such that the soma and axon are particularly characterized by the presence of voltage-dependent Na conductance channels, while the dendrites have mainly a voltage-dependent Ca conductance change. The implications of this dichotomy in the membrane characteristics of the cell will be discussed in the accompanying paper.

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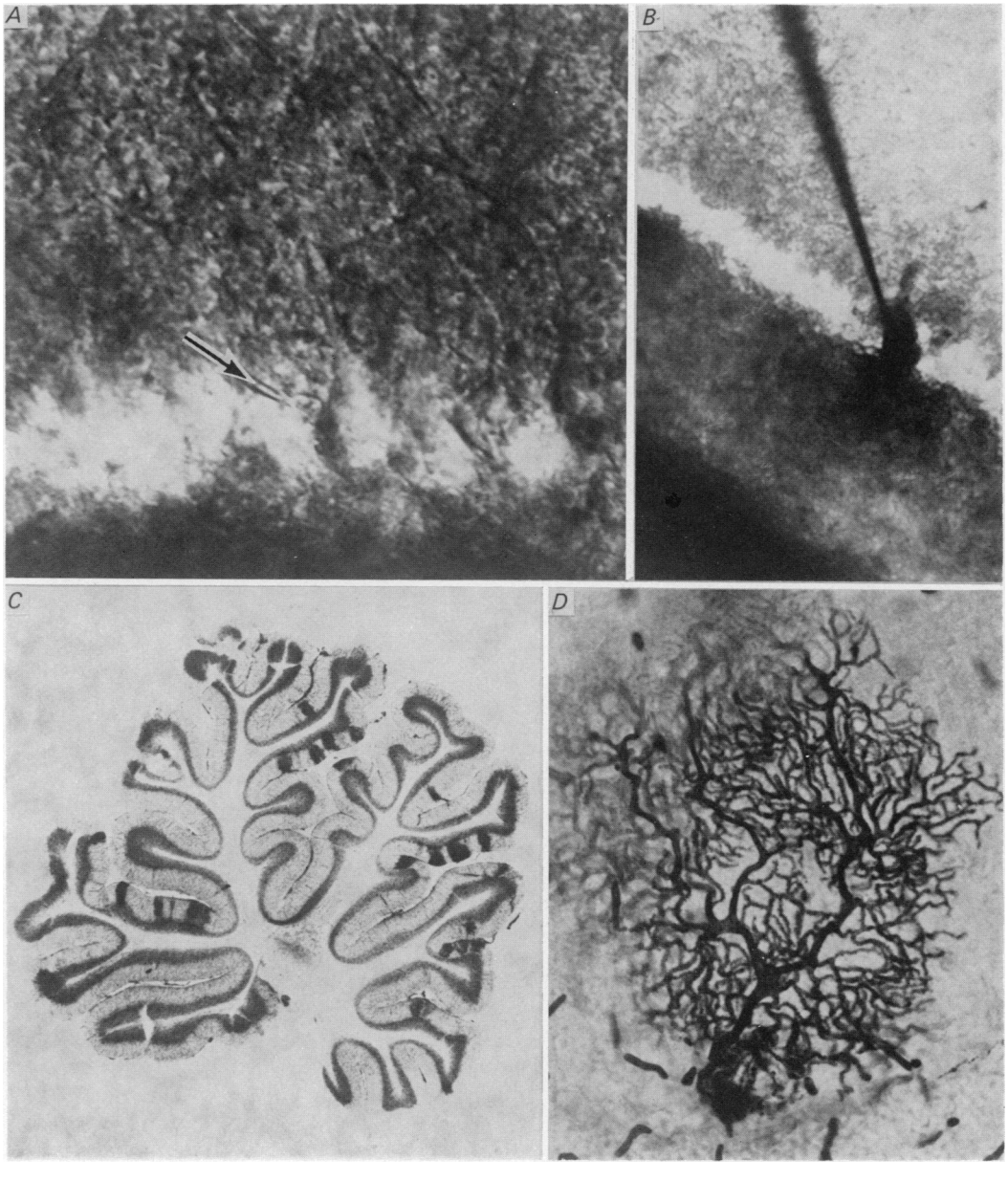

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EXPLANATION OF PLATE 1

Localization of intracellular recording. *A*, photomicrograph of Purkinje cell layer as seen through Hoffman modulation optics. The somata of Purkinje cells are seen as large spherical bodies. The shaft of the recording micro-electrode in the vicinity of the tip is indicated by an arrow. *B*, Purkinje cell body injected with fast green dye showing the initial part of the primary dendrite. *C*, a cerebellar slice seen after horseradish peroxidase injection of several Purkinje and Bergmann glial cells. *D*, photomicrograph of a Purkinje cell after horseradish peroxidase injection. demonstrating soma and dendritic arbour.