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MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL CHARACTERISTICS OF RAT CEREBELLAR SLICES MAINTAINED *IN VITRO*

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SUMMARY

1. The morphological and electrophysiological characteristics of sagittal cerebellar slices of adult rat cerebellum maintained *in vitro* were studied.

2. The ultrastructural preservation of the different neuronal cell types in many areas of these slices after 2–3 h incubation was very similar to that observed in material fixed *in situ*. A limited degree of glial swelling was observed in some regions.

3. The conduction velocity of parallel fibres was within the normal *in vivo* range and the fibres retained their ability to activate Purkinje cells and inhibitory interneurones.

4. Purkinje cells, recorded intrasomatically, responded to white matter stimulation with characteristic antidromic activation and climbing fibre responses, and typical parallel fibre responses were evoked following parallel fibre stimulation.

5. Climbing fibre excitatory post-synaptic potentials (e.p.s.p.s) were very similar whether recorded in the dendrites or somata of Purkinje cells. By contrast, marked differences in the associated spike potentials were evident, the initial fast, low-threshold somatic spike appearing in the dendrites as a slow, high-threshold spike. The secondary spikes, both in the soma and dendrites, were of the latter type.

6. The initial somatic spike was readily inactivated by cell depolarization but resisted moderate hyperpolarization, whereas the converse was true for the slow, high-threshold spikes recorded in the dendrites. These differences suggest that these responses are generated in the soma and in the dendrites respectively.

7. Climbing fibre and parallel fibre e.p.s.p.s recorded in Purkinje cell somata were reversed under depolarizing current injected through the recording micro-electrode. As *in vivo*, the parallel fibre e.p.s.p.s was more sensitive to injected current than the climbing fibre e.p.s.p. in several instances, despite the more proximal location of the synapses involved.

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INTRODUCTION

Previous electrophysiological studies on cerebellar slices maintained *in vitro* have concentrated chiefly on investigating the sensitivity of Purkinje cells to various putative neurotransmitters and the bioelectric properties of these cells (Okamoto & Quastel, 1973, 1976; Yamamoto, 1973, 1975; Chujo, Yamada & Yamamoto, 1975; Hounsgaard & Yamamoto, 1979; Llinás & Sugimori, 1979). There was little evidence to suggest that these slices would be suitable for the intracellular analysis of the synaptic potentials evoked in Purkinje cells by the activation of their excitatory and inhibitory afferents (Eccles, Ito & Szentágothai, 1967), probably because of either the horizontal plane of section of the slices or their thinness (80–100 μm).

Recent biochemical and morphological studies showing that thicker (400 μm) surface slices of rat cerebellum prepared using a bow cutter and guide were very well preserved *in vitro* (Garthwaite, Woodhams, Collins & Balázs, 1979, 1980) prompted us to examine the value of slices prepared in the same way, but cut in the sagittal plane to preserve the main afferent and efferent pathways (Palay & Chan Palay, 1974), for the *in vitro* analysis of synaptically driven activation of Purkinje cells.

This paper describes the anatomical and electrophysiological characteristics of these slices. Particular reference is made to the dendritic and somatic activation of Purkinje cells evoked by climbing fibres (climbing fibre responses) and to the reversal properties of the excitatory post-synaptic potentials (e.p.s.p.s) mediated in Purkinje cell somata via stimulation of both parallel fibres and climbing fibres.

Although the principal features of the climbing fibre responses are well established (Eccles *et al.* 1967), the site(s) of origin of the associated spike potentials remains uncertain. On the basis of experiments *in vivo*, Martinez, Crill & Kennedy (1971) proposed that they are generated in the dendrites, whereas computer modelling (Pellionisz & Llinás, 1977) indicated the initial segment to be the probable site of initiation. One reason for uncertainty is that, until now, climbing fibre responses in Purkinje cells have not been recorded intracellularly from an identified dendritic location, although intradendritic recordings of Purkinje cells *in vitro* and *in vivo* have been carried out in order to analyse the properties of dendritic spikes evoked by local stimulation (Llinás & Nicholson, 1971; Llinás & Hess, 1976; Llinás & Sugimori, 1979). The use of sagittal slices, where the position of the tip of the micro-electrode can be determined accurately under visual control, thus seemed a suitable way to approach this problem.

With respect to the reversal properties of the e.p.s.p.s evoked in Purkinje cells by parallel fibre and climbing fibre stimulation, when studied *in vivo*, these show differential sensitivity to injected current (Hackett, 1976; Crepel & Delhaye-Bouchaud, 1978; Dupont, Crepel & Delhaye-Bouchaud, 1979). This behaviour is contrary to predictions (Bennett, Freeman & Thaddeus, 1966; Rall, 1967; Calvin, 1969) based on the spatial distribution of these two classes of synapses on Purkinje cell dendrites (Sotelo, 1969; Palay & Chan Palay, 1974) and the cable properties of these cells (Crepel & Delhaye-Bouchaud, 1979). As reversal properties of these responses might depend on the current injection site (somatic or dendritic), a similar study using sagittal slices again seemed appropriate.

METHODS

Preparation of the slices

Adult male rats (Porton strain) were used in these experiments. The animals were stunned, decapitated and the cerebella rapidly excised. A portion of the vermis was isolated by two sagittal cuts and whole sagittal slices, nominally 500 μm thick, were prepared within 2 min of death using a convential bow cutter and glass guide as previously described for surface slices (Garthwaite *et al.* 1979, 1980). The slices were preincubated at room temperature (19–23 °C) for 20–30 min in a Krebs solution containing (mM): NaCl (124), KCl (5), KH_2PO_4 (1·15), $MgSO_4.7H_2O$ (1·15), $CaCl_2$ (2), $NaHCO_3$ (25) and glucose (10) continuously gassed with a mixture of O_2 (95%) and CO_2 (5%) before being transferred to the recording chamber.

Recording chamber

The design and operation of the constant-flow recording chamber was as described by Richards & Sercombe (1970) but the waste-outflow system was modified to allow recordings from partially submerged slices instead of surface slices. (The chamber was designed and made at the Neurophysiology Institute, University of Oslo.) This was done by positioning a hypodermic needle, which was connected to a vacuum line, above the level of the incubating fluid in a compartment interconnected with the main chamber. By means of a screw adjustment on the needle, it was possible to control the level of the fluid in relation to the position of the slice. Submerged preparations appeared macroscopically to remain better preserved and would be expected to be less sensitive to any fluctuations in the oxygen tension maintained above them. The composition of the incubating fluid was the same as that described for the pre-incubation period. The flow rate was normally maintained at 1–2 ml min^{-1}, and the chamber temperature kept at 36 ± 1 °C.

Electrophysiology

Two monopolar stimulating electrodes, each consisting of a thin platinum wire (20 μm in diameter) insulated except at the tip, were placed one in the cerebellar white matter and the other at the surface of the molecular layer, just below the pial surface. Extracellular field potentials and intracellular responses of Purkinje cells were recorded in Larsell's lobules IV to VIII with glass micro-electrodes filled with potassium acetate (20–80 MΩ d.c. resistance). In each case, the tip of the micro-electrode was positioned with the aid of a binocular dissecting microscope (magnification up to × 50), which allowed the different layers of the cortex to be readily identified.

Electron microscopy

Following 2–3 h incubation in the recording chamber, the slices were fixed for 30 min in 2·5% glutaraldehyde and 4% formaldehyde buffered with 0·1 M-phosphate buffer. After dehydration through ethanol and embedding in Spurr's resin, 0·5–1·0 μm sections were cut and stained with toluidine blue. Ultrathin sections were contrasted with uranyl acetate and lead citrate and viewed in a JEOL 100S electron microscope. A total of ten slices from three separate experiments were examined.

RESULTS

Morphology

Plates 1 and 2 show examples of the ultrastructure of the different anatomical layers of the cerebellar cortex in the slices after 2 h incubation under conditions identical to those used for the electrophysiological experiments described below. Although there were some areas showing obvious pathological changes, notably swelling of Purkinje cells and pyknosis of granule cells, the degree of preservation in most areas of the sagittal slices was broadly comparable with that documented previously for surface cerebellar slices (Garthwaite *et al.* 1979, 1980). Damage at the cut surfaces, evidenced by vacuolation of Purkinje cells and pronounced swelling, extended up to 50 μm. Below these margins, Purkinje cells (Pl. 1 *A*), granule cells (Pl. 2 *A*) and the inhibitory interneurones (basket cells, stellate cells and Golgi cells) were very similar in appearance to those fixed *in situ* by perfusion (cf. Palay & Chan Palay,

1974), as were the synaptic complexes (glomeruli) in the internal cell layer (Pl. 2*B*). In the molecular layer, numerous synapses characteristic of those of the parallel fibres and climbing fibres on Purkinje cell dendrites could be observed, the vast majority of which showed normal morphological preservation under the electron microscope (Pl. 1*B*). However, a degree of glial swelling was observed in some regions. Pl. 2*A* shows a slightly swollen glial cell in the internal granule cell layer and a limited expansion of glial processes in the Purkinje cell layer (Pl. 1*A*) and molecular layer (Pl. 1*B*) was evident.

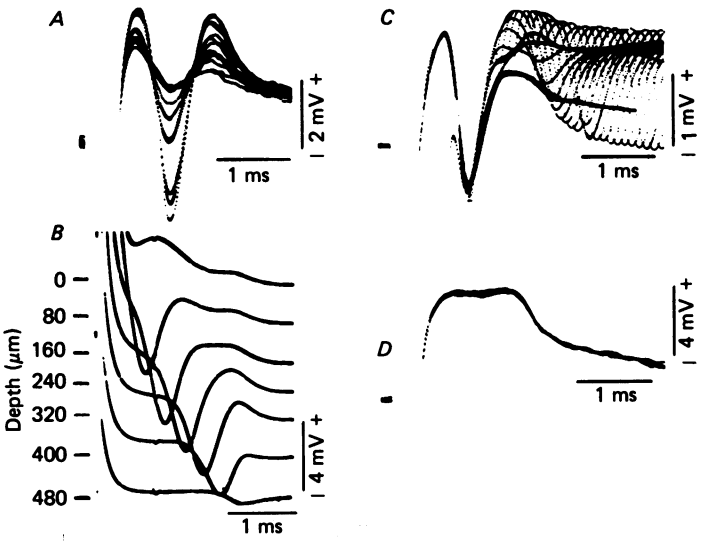

Fig. 1. Field potentials recorded in an 'on beam' position in the molecular layer (*A*, *B*, *C*) and in the Purkinje cell layer (*D*), following stimulation of the surface of the upper part of the molecular layer. In *A*, the stimulus strength was progressively increased to show the graded character of the parallel fibre volley. In *B*, the micro-electrode was advanced along the parallel fibre track in successive 80 μm steps from the upper surface of the slice at constant stimulus intensity. The depth of the electrode below the slice surface is indicated on the left of each trace. In *C*, the refractory period of parallel fibres was determined with paired supramaximal stimuli applied at various interstimulus intervals. *D*, response recorded in the Purkinje cell layer, 200 μm below the surface in the same experiment as in *B*. Superimposed sweeps in all records. Pulse duration, 0·2 ms in all traces.

Electrophysiology

Parallel fibres

Stimulation of the surface of the molecular layer, just below the pia, elicited a parallel-fibre-like volley in the underlying tissue; the response was triphasic, positive–negative–positive and was smoothly graded with the intensity of the stimulus (Fig. 1*A*). Furthermore, as the micro-electrode was advanced through the molecular layer in the plane of the parallel fibres, the latency and amplitude of the response changed reciprocally (Fig. 1*B*), as expected for compound action potentials travelling along parallel fibres (Eccles *et al.* 1967). Corresponding conduction velocities, determined by plotting the latency of the response against distance along parallel fibres, ranged between 0·3 and 0·5 m s^{-1}. These are normal values for parallel fibres *in vivo* in this

species (Crepel, 1974). The refractory period of these fibres, measured with paired supramaximal stimuli applied at varying interstimulus intervals (Fig. 1*C*), ranged between 0·6 and 0·8 ms, which, once again roughly corresponds with the values found *in vivo* for parallel fibres (Eccles *et al.* 1967; Crepel, 1974).

Finally, three additional criteria were used to identify the responses recorded in the molecular layer as those of parallel fibres. First, the response disappeared when the recording micro-electrode was moved to an 'off beam' position (300 μm or more lateral to the stimulating electrode), whilst it was still prominent at such distances along the track of the parallel fibres. Secondly, when recorded in the Purkinje cell layer in an 'on beam' position, the response was greatly attenuated and appeared as a positive wave (Fig. 1*D*), as found *in vivo* (Eccles *et al.* 1967). Thirdly, typical parallel fibre responses and parallel fibre e.p.s.p.s were recorded from 'on beam' Purkinje cells and these responses always followed closely the parallel-fibre-like volley recorded in the molecular layer (see below).

Purkinje cells

Somatic recordings. When the micro-electrode track passed through the Purkinje cell layer, perpendicular to the surface of the slice, cells exhibiting spontaneous activity were routinely encountered and penetrated. Their resting membrane potentials at this somatic level ranged between -35 and -50 mV, and they exhibited spike potentials of up to 70 mV. The intracellular responses of thirty-four of these cells to white matter stimulation and to stimulation of the molecular layer were studied. The majority were recorded at depths no more than 200 μm below the surface, in order to ensure an exactly somatic location for the tip of the micro-electrode.

White matter stimulation applied at the base of the folia, or even near the cerebellar nuclei, routinely evoked an antidromic response in Purkinje cells, thus ensuring their identification. As *in vivo* (Eccles *et al.* 1967), the antidromic response was characterized by its short and fixed latency (less than 1 ms) and by the presence of an IS–SD inflexion on its rising phase (Fig. 2*A*). Usually, the antidromic response was followed by a typical all-or-none climbing fibre response or e.p.s.p. (cf. Eccles, Llinás & Sasaki, 1966), as illustrated individually in Fig. 2B_1 and 2B_2.

A local 'on beam' stimulation applied to the upper part of the molecular layer elicited, in many of the Purkinje cells studied, an e.p.s.p. which started 0·2–0·4 ms after the negative peak of the parallel fibre volley, and whose time-to-peak ranged between 1·0 and 2·2 ms (Fig. 2*C* and *D*). When the firing level was reached, one or several spike potentials were evoked, depending on the stimulus intensity. These responses clearly differed from the all-or-none climbing fibre responses and climbing fibres e.p.s.p.s recorded in the same cells and the firing level of the associated spikes was also different (Fig. 2*C* and *D*).

Finally, no inhibitory post-synaptic potentials (i.p.s.p.s) were recorded in the slices following white matter stimulation or local stimulation of the molecular layer, except possibly in two or three cases (see below). By contrast, a number of inhibitory interneurones were recorded extracellularly: these were identified by their location in the lower domain of the molecular layer, or granule cell layer, and by the fact that long trains of spikes were evoked in these cells by local and white matter stimulation (not illustrated).

Dendritic recordings. When the micro-electrode was advanced through the molecular layer in a plane perpendicular to the surface of the slice, Purkinje cell dendrites were routinely penetrated. These were identified (*a*) by their location in the molecular layer and (*b*) by the presence of climbing fibre responses (see below). The resting membrane potential of Purkinje cells at this dendritic location was up to −40 mV. Intracellular

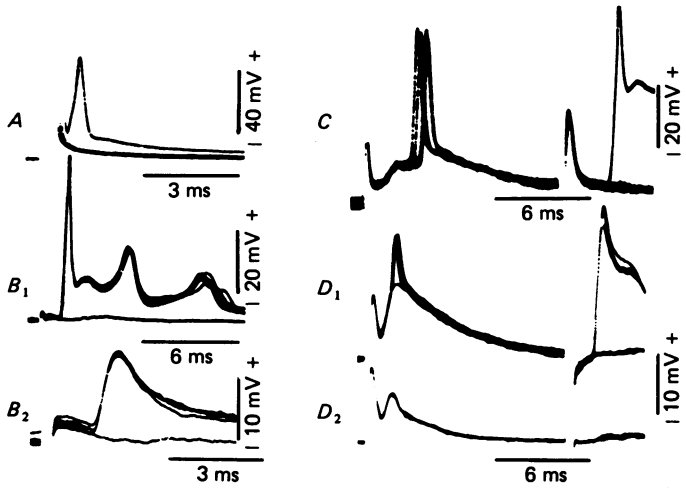

Fig. 2. Responses evoked in Purkinje cell somata by white matter stimulation (*A*, *B*) and by paired parallel fibre and white matter stimulation (*C*, *D*). Intracellular recordings in all traces except D_2. Superimposed sweeps in all records. *A*, antidromic spike potential recorded at a somatic level under hyperpolarizing current of 1·1 nA. Note the IS–SD inflexion on the rising phase of the response and the well-developed after-potential. B_1, all-or-none climbing fibre response recorded at a somatic level before cell deterioration. B_2, climbing fibre e.p.s.p. recorded in the same cell as B_1, but with a faster sweep speed, after inactivation of the spike-generating mechanism. *C*, parallel fibre and climbing fibre responses evoked at a somatic level in the same Purkinje cell, by paired parallel fibre and white matter stimulation. The parallel fibre e.p.s.p. gave rise to a spike potential whose firing level was clearly different to that of the initial spike of the climbing fibre response. D_1, as in *C* but in another Purkinje cell. Only a partial IS(?) spike was evoked at the peak of the e.p.s.p. D_2, extracellular field potential after withdrawal of the micro-electrode.

responses of thirty-one of these cells to white matter stimulation were studied. The dendrites were penetrated in the middle of the molecular layer (approximately half-way between the pia and the Purkinje cell layer) or in its lower half.

At this dendritic location, as for somatic responses, the climbing fibre response of Purkinje cells consisted of a series of spikes, up to 50 mV in amplitude, superimposed on a plateau of depolarization, and the response was all-or-none in character, as expected for the activation of Purkinje cells by climbing fibres (Fig. 3A_1 and *C*). However, striking differences between climbing fibre responses recorded in the soma and in the dendrites became apparent. In the former, the initial full spike had the duration (1 ms) of a fast somatic spike (Wong, Prince & Basbaum, 1979) and its threshold was apparently very low since it started near the foot of the climbing fibre e.p.s.p. (Figs 2B_1 and 3*E*), as usually observed in *in vivo* experiments (Eccles *et al.* 1967). When recorded in the dendrites, the duration of this initial spike was much longer (2–6·7 ms) and its apparent threshold was much higher, as it started during

the rising phase of the e.p.s.p. or near its peak (Fig. 3A_1, A_2 and C), i.e. later than the initial fast somatic spike. In addition, it often exhibited one or several inflexions on its rising phase (Fig. 3A_1 and C), thus resembling the slow, high-threshold dendritic spikes observed *in vivo* and *in vitro* (Llinás & Nicholson, 1971; Llinás & Sugimori, 1979; Wong *et al.* 1979). The secondary spikes of the response in intradendritic as, in most cases, in intrasomatic recordings, closely resembled the slow, high-threshold,

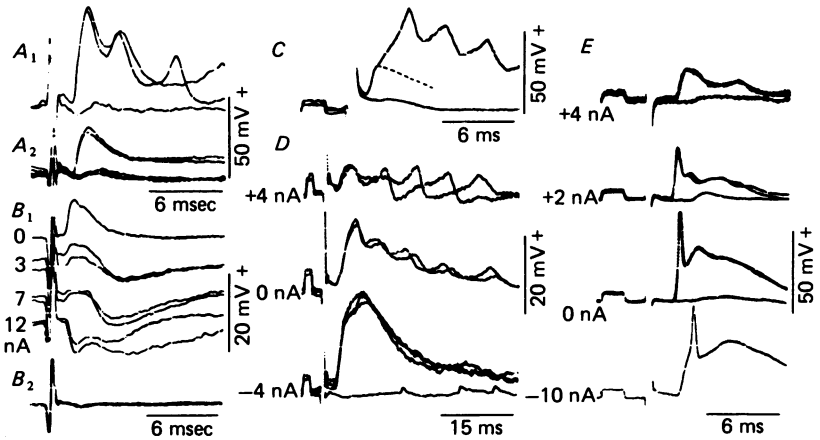

Fig. 3. Comparison of intradendritic (*A*, *B*, *C*, *D*) and intrasomatic (*E*) recordings of climbing fibre responses of Purkinje cells evoked by white matter stimulation. Superimposed sweeps in all records. A_1, climbing fibre response with full spikes recorded in the middle zone of the molecular layer. Note its all-or-none character. A_2, climbing fibre e.p.s.p. recorded in the same cell as A_1 after cell deterioration and inactivation of the spikes. B_1, reversal of a climbing fibre e.p.s.p. recorded in the lower third of the molecular layer under depolarizing current injected through the micro-electrode. Current values are shown on the left of each record. Bottom trace, extracellular field potential after withdrawal of the micro-electrode. *C*, as in A_1 in another Purkinje cell dendrite recorded in the middle zone of the molecular layer. Dashed line indicates the shape of the underlying e.p.s.p. *D*, effect of depolarizing or hyperpolarizing current on an intradendritic climbing fibre e.p.s.p. recorded in the middle zone of the molecular layer. Current values are given on the left of each record. *E*, effect of depolarizing or hyperpolarizing current on an intrasomatic climbing fibre e.p.s.p. in another Purkinje cell. Current values plotted as in *D*.

initial dendritic spike (Figs 2B_1, 3A_1, *C*, *D*), except in a few cases where the somatic spikes following the initial full spike were also of the fast type (not illustrated).

After cell deterioration, typical climbing fibre e.p.s.p.s were revealed; these had times-to-peak and half-decay times which were very similar to those recorded in the soma (compare Fig. 3A_2 and B_1 with Figs. 3E and 2B_2). As with climbing fibre e.p.s.p.s recorded in a somatic position, these dendritic e.p.s.p.s were readily inverted by current injected through the recording electrode (Fig. 3B_1). Currents for reversal were similar to or even smaller than those required at the somatic level (see below) or those previously reported in experiments *in vivo* (Llinás & Nicholson, 1976; Hackett, 1976; Crepel & Delhaye-Bouchaud, 1978). In the example shown in Fig. 3B_1 the falling phase of the response reversed much earlier than the initial part, probably because of contamination of the e.p.s.p. by a subsequent i.p.s.p., as already reported *in vivo* (Crepel & Delhaye-Bouchaud, 1978). However, given its sensitivity to current,

the i.p.s.p. in this case was probably generated in the dendrites, near the recording site.

Finally, in Purkinje cells in which the spike-generating mechanism was maintained after impalement, the effect of small depolarizing or hyperpolarizing currents passed through the tip of the recording electrode on the spike potentials of the climbing fibre response was investigated. Here again, a marked difference between the behaviour

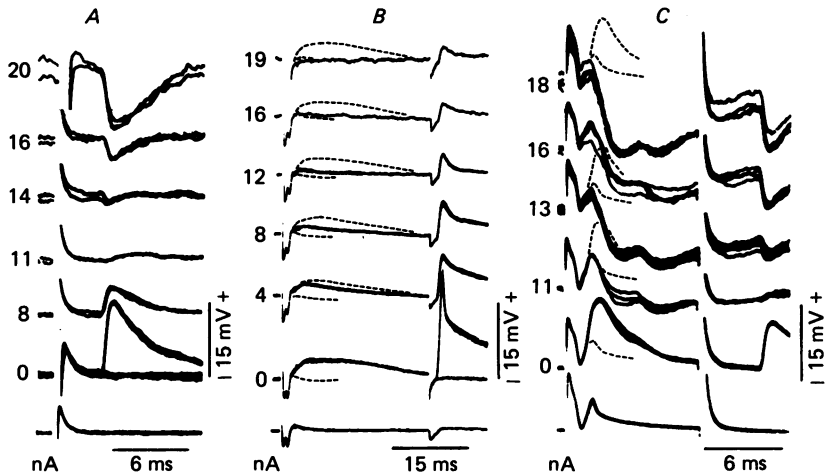

Fig. 4. Reversal properties of climbing fibre and parallel fibre e.p.s.p.s recorded at a somatic level in Purkinje cells. Superimposed sweeps in all records. *A*, reversal of a climbing fibre e.p.s.p., evoked by white matter stimulation, under depolarizing current injected through the micro-electrode. Current applied is shown on the left of each record. Bottom trace: extracellular field potential after withdrawal of the electrode. *B*, sensitivity to current of parallel fibre and climbing fibre e.p.s.p.s evoked in the same cell at a somatic level by paired parallel fibre and white matter stimulation. The d.c. current values are shown on the left of each record. Bottom trace as in *A*. At each current level, two dashed lines are superimposed on the trace, the upper showing the shape of the parallel fibre e.p.s.p. at 0 nA and the lower that of the underlying extracellular field potential. Note that the parallel fibre e.p.s.p. was more attenuated than the climbing fibre e.p.s.p. by depolarizing current. *C*, as in *B* in another cell. In this case the peak of the parallel fibre e.p.s.p. and of the climbing fibre e.p.s.p. reversed at the same current value.

of these spikes was noticed depending on their somatic or dendritic location. In the dendrites, besides the effect of current on the climbing fibre e.p.s.p. previously described (Fig. 3*D*), moderate depolarization either had no effect or even enhanced the successive slow, high-threshold spikes of the climbing fibre response, whereas small hyperpolarization always abolished them (Fig. 3*D*). By contrast, in the soma, the initial fast, low-threshold spike resisted much more severe hyperpolarization, with a shift in the rising phase of the e.p.s.p. (fig. 3*E*), whereas it was always strongly inactivated by small depolarizations of the same magnitude as those which had no effect on or enhanced the size of the spikes recorded in the dendrites (Fig. 3*E*).

Reversal properties of climbing fibre and parallel fibre e.p.s.p.s. In the present experiments, only climbing fibre and parallel fibre e.p.s.p.s recorded at a somatic level, as determined by the placement of the micro-electrode in the Purkinje cell layer under visual control, were used for the comparison of their reversal properties. Depolarizing

current applied to the cells through the recording micro-electrode led to a progressive decrease in the size of the climbing fibre e.p.s.p. until it completely reversed (Fig. 4*A*). In the eleven cells studied, the reversal was biphasic, the initial part of the response reversing first (Fig. 4*A*), as expected from the anatomical distribution of the climbing fibre/Purkinje cell synapses (Palay & Chan Palay, 1974; Llinás & Nicholson, 1976). Currents for reversal were approximately the same as those found *in vivo* (Llinás & Nicholson, 1976; Crepel & Delhaye-Bouchaud, 1978), ranging from 8 to 15 nA for well-impaled neurones.

Parallel fibre e.p.s.p.s were also annulled or even reversed by depolarizing current injected at a somatic level (Fig. 4*B* and *C*) and their reversal properties were compared with those of the climbing fibre e.p.s.p.s in a small number (7) of cells. In four of them, parallel fibre e.p.s.p.s were more sensitive than climbing fibre e.p.s.p.s (Fig. 4*B*), whereas in the remainder the currents for reversal were identical (Fig. 4*C*), or the climbing fibre e.p.s.p.s reversed first. In the case of the cell illustrated in Fig. 4*C*, the falling phase of the parallel fibre e.p.s.p. reversed before the peak of the response. This could indicate that the parallel fibre e.p.s.p. was contaminated in this case by a small i.p.s.p.

DISCUSSION

The present study demonstrates for the first time that, in 500 μm thick sagittal cerebellar slices, well preserved at the ultrastructural level, the parallel fibre volley, the antidromic activation of Purkinje cells and their two classes of excitatory responses can be obtained. This allowed, as a first application, an intracellular analysis of climbing fibre responses in the somata and dendrites of Purkinje cells and of the reversal properties of parallel fibre and climbing fibre e.p.s.p.s *in vitro*. In previous studies where thinner slices were used, only the antidromic activation of Purkinje cells and their climbing fibre responses were obtained, mainly with extracellular recording (Yamamoto, 1973, 1975; Chujo *et al.*, 1975; Hounsgaard & Yamamoto, 1979).

Parallel morphological examination of the preservation of cellular and subcellular structures in incubated brain slices has only rarely been presented (Yamamoto, Bak & Kurokawa, 1970). In the present study, the different classes of neurones of the cerebellar cortex all showed good preservation in most areas and synaptic elements appeared normal. Apart from the few badly damaged regions, presumably resulting from mechanical trauma during cutting, the principal deviation in comparison with material fixed *in situ* (Palay & Chan Palay, 1974) and with slices cut from the surface of the cerebellum (Garthwaite *et al.* 1979, 1980) was the slight swelling of some glial elements, notably in the Purkinje cell layer and molecular layer. Reasons for this are at present unclear.

Electrophysiology

Parallel fibres in the slices exhibited properties comparable to those recorded *in vivo* (Eccles *et al.* 1967; Crepel, 1974). In particular, their conduction velocity fell within the normal range: this supports the usefulness of the sagittal slices for physiological studies since, for such unmyelinated fibres, this parameter is very sensitive to modifications of the milieu (Gasser, 1955). The physiology of parallel

fibres, hitherto, has been mainly studied *in vivo* (Eccles *et al.* 1967; Crepel, 1974) and to much lesser extent *in vitro* (Gardner-Medwin, 1972). Neither of these preparations would readily permit activation of parallel fibres at different depths within the molecular layer in order to gain insight into their properties in relation to their anatomical position. The present results open up the possibility of performing this analysis in the future.

The good preservation of the tissue is probably also responsible for the fact that many of the recorded Purkinje cells had resting membrane potentials of up to −50 mV, full spikes of up to 70 mV and exhibited e.p.s.p.s which apparently shared all the main features of those recorded *in vitro* (Eccles *et al.* 1967), including differential reversal under depolarizing current applied to the cell through the recording micro-electrode (Hackett, 1976; Crepel & Delhaye-Bouchaud, 1978). However, the absence in most Purkinje cells in the slices of i.p.s.p.s following white matter or parallel fibre stimulation (Eccles *et al.* 1967) is puzzling, since inhibitory interneurones, whose location in the lower domain of the molecular layer corresponds to that of the basket cells (Palay & Chan Palay, 1974), were morphologically intact and responsive and since the sagittal orientation of the slice should preserve the axons of these neurones (Palay & Chan Palay, 1974). Further studies will be required to determine the reason(s) for this failure.

The comparison of dendritic and somatic climbing fibre responses of Purkinje cells revealed no clear differences with respect to the e.p.s.p. According to previous interpretation (Eccles *et al.* 1967), this probably means that this e.p.s.p. is generated in the dendrites and electrotonically conducted to the soma. By contrast, marked differences regarding the spike potentials of the response were observed depending on the recording site. The fact that the duration and latency of the initial full spike of the response was longer in the dendrites than in the soma would suggest that, as claimed by Pellionisz & Llinás (1977), it is generated in the initial segment, or in the soma, and electrotonically propagated to the dendrites. However, the initial spike recorded in the dendrites might also be generated locally by a high-threshold, slow regenerative process, therefore having an additional delay with respect to the underlying e.p.s.p. This would explain both the timing of the response and the resemblance between the initial spike of the dendritic climbing fibre response and active slow dendritic spikes previously recorded *in vivo* and *in vitro* (Llinás & Nicholson, 1971; Llinás & Hess, 1976; Llinás & Sugimori, 1979; Wong *et al.* 1979). In keeping with this interpretation and with experiments *in vivo* by Martinez *et al.* (1971), the long duration of the subsequent partial spikes of the burst response in dendritic as, in most cases, in somatic climbing fibre responses, suggests that they might also be actively generated in the dendrites, rather than in the initial segment as proposed by Pellionisz & Llinás (1977).

The differential effects of polarizing current on the spikes of the response depending on the recording site also support this view. The rapid inactivation of the initial somatic spike with depolarizing current and its resistance to hyperpolarization accord with properties expected from sodium-mediated, fast, low-threshold spikes (Hodgkin & Huxley, 1952; Llinás & Hess, 1976; Wong *et al.* 1979), whereas in the dendrites, the suppression of the successive slow spikes of the response by small hyperpolarizing currents confirms their high threshold and, therefore, their probable different generating mechanism. The unusual increase in amplitude of these active dendritic

and perhaps calcium-dependent (Llinás & Sugimori, 1979) spikes in some neurones by small depolarizing currents might reflect the presence of an anomalous rectification (Nelson & Franck, 1967) of dendritic membranes in these cells. This was not tested because of changes in electrode resistance during application of d.c. current which, by unbalancing the bridge, biased measurements of cell input resistance.

Finally, the fact that parallel fibre e.p.s.p.s were more sensitive to injected current than climbing fibre e.p.s.p.s in several cells unambiguously recorded (and injected) at a somatic level, and in the absence of any visible contamination of these responses by an i.p.s.p., can hardly be reconciled with the assumption that the equilibrium potentials for the two classes of e.p.s.p.s are the same. As previously discussed (Dupont *et al.* 1979), the lower apparent sensitivity of parallel fibre e.p.s.p.s to depolarizing current in other Purkinje cells might be due to the presence of some active components in these responses.

In general, the present experiments demonstrate that the sagittal slices of cerebellum represent a suitable and convenient experimental model for the analysis of the excitatory synaptic potentials in Purkinje cells and are likely to prove useful in, for example, detailed pharmacological studies of these responses.

Note added in proof. Intrasomatic and intradendritic recordings of climbing fibre responses of Purkinje cells in cerebellar slices maintained *in vitro* have been recently presented by Llinás & Sugimori (*J. Physiol.* **305**, 171–195 and 197–212), with similar conclusions as in the present work as regards the local origin of the spikes of the dendritic response.

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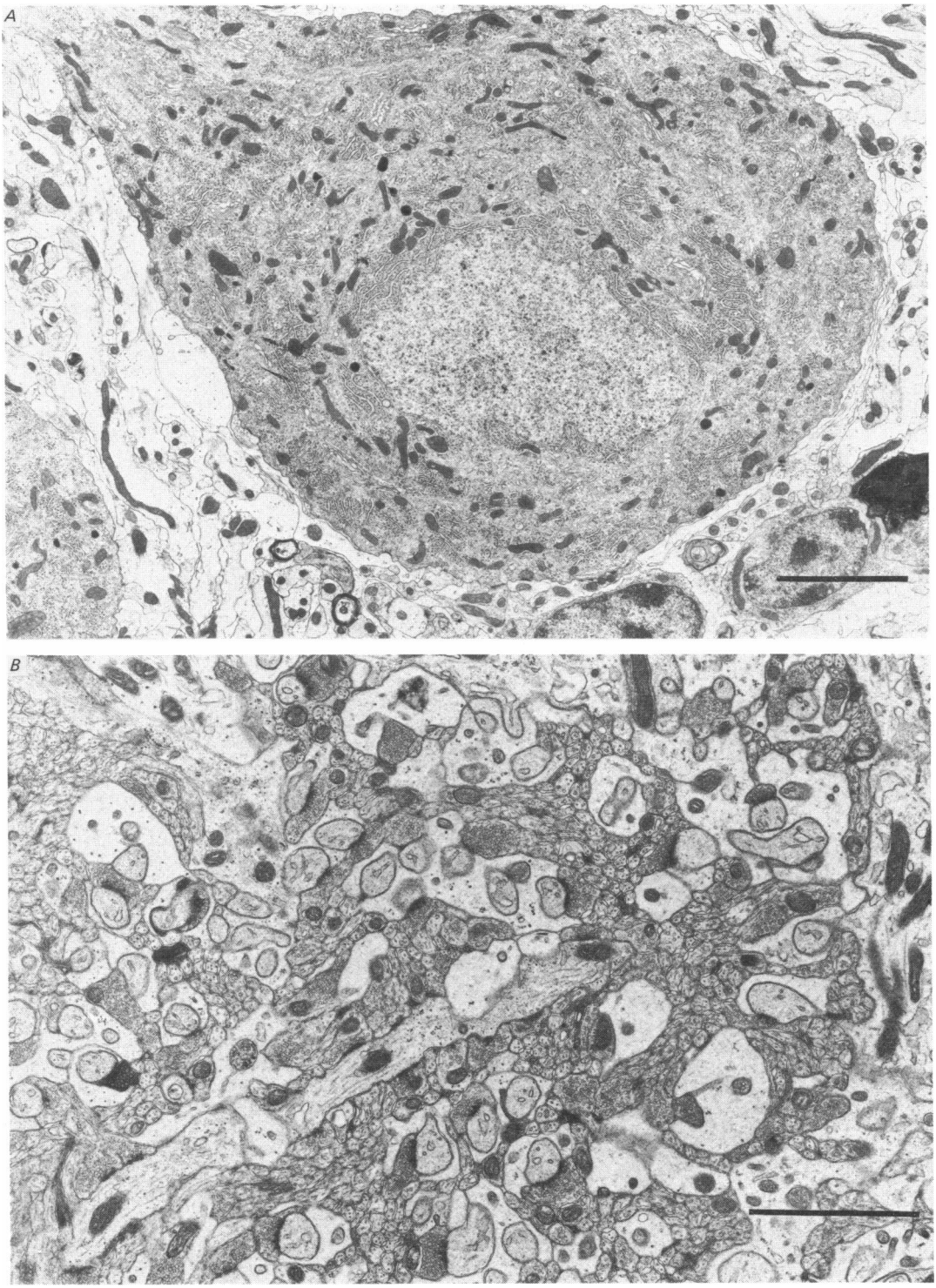
EXPLANATION OF PLATES

PLATE 1

Electron micrographs showing *A* Purkinje cells and *B* the molecular layer in a sagittal cerebellar slice incubated in the recording chamber for 2 h. Although a degree of glial expansion is evident, Purkinje cells, nerve fibres and synaptic terminals are well preserved. Scale bars represent 5 μm in *A* and 2·5 μm in *B*.

PLATE 2

Electron micrograph showing *A* cells in the internal granule cell layer and *B* a detail of synaptic glomeruli. The astrocyte cell body in *A* is possibly slightly swollen but the granule cells appear normal. As in Pl. 1, synaptic elements retain their normal ultrastructure. Scale bars represent 5 μm in *A* and 2·5 μm in *B*.



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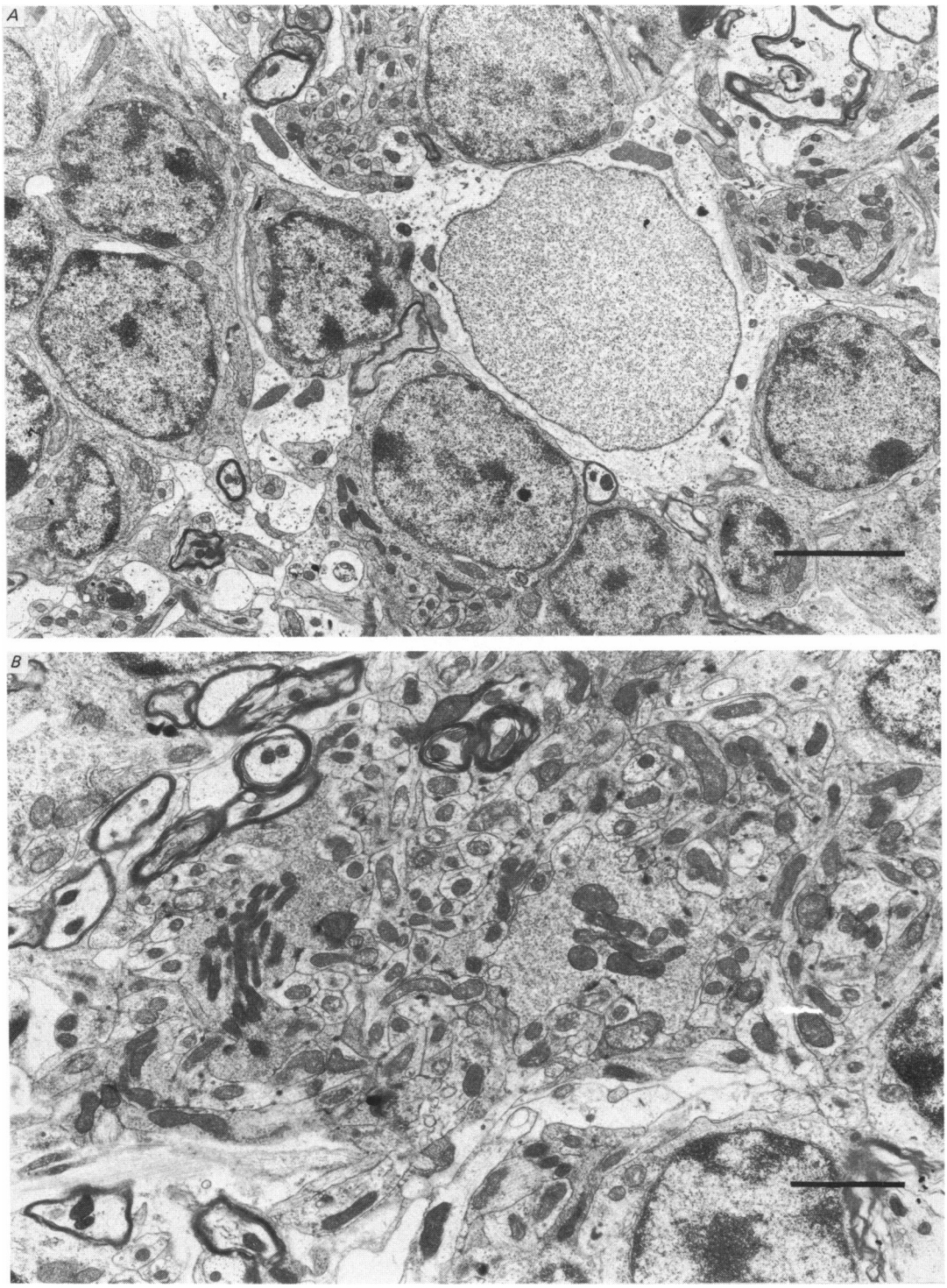

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