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DISTRIBUTION OF CLIMBING FIBRES ON CEREBELLAR PURKINJE CELLS IN X-IRRADIATED RATS. AN ELECTROPHYSIOLOGICAL STUDY

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SUMMARY

1. The distribution of climbing fibres on cerebellar Purkinje cells has been studied with intracellular recordings in X-irradiated and normal rats.

2. In the treated rats, multiple steps in the post-synaptic potential were elicited in 57 % of the Purkinje cells by graded stimulation of the climbing fibres, the response was all-or-none in character in the other cells and in all Purkinje cells recorded in normal animals. In the neurones exhibiting the former type of response, no collision was seen along the afferent fibres during interaction experiments between just-threshold juxtafastigial and maximal olivary stimulations, whereas a collision always occurred when all-or-none responses were recorded.

3. These results show that in X-irradiated rats, the majority of Purkinje cells have a multiple innervation by two to four climbing fibres, instead of the one-to-one relationship seen normally.

4. Input resistances and total electrotonic lengths of Purkinje cells were measured in normal and treated rats. Mean values for these two parameters were higher than normal in multiply innervated cells.

5. Mean time course and mean current for reversal of the post-synaptic potential elicited in Purkinje cells by stimulation of the climbing fibres were nearly the same in mono- and in multiply innervated neurones. In multiply innervated cells, time courses and currents for reversal were independent of the size of the response or varied slightly with it, suggesting that the climbing fibres involved innervated territories whose electrotonic distance from the recording site were either the same or slightly different.

6. Interactions between two all-or-none steps of the graded post-synaptic potential evoked in multiply innervated cells by juxtafastigial and olivary stimulations revealed either a very weak or a very marked shunting effect between synapses of the two climbing fibres involved.

7. These results indicate that the over-all distribution of climbing fibre synapses on multiply innervated Purkinje cells is not grossly abnormal and that two fibres contacting a given cell can be either intermingled on the same dendrites, or segregated on distinct dendritic branches.

8. In general, the present study does not suggest the existence of a strong

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competition among climbing fibres innervating each Purkinje cell during development at least when granule cells are absent.

INTRODUCTION

Studies on the development of the neuromuscular junction and the rat submandibular ganglion, have shown that the establishment of the adult innervation implies the removal of already functional synapses (Redfern, 1970; Bagust, Lewis & Westerman, 1973; Bennett & Pettigrew, 1974, 1975; Lichtman, 1977). In the central nervous system, a very similar synaptic rearrangement has been disclosed in the rat cerebellum : the one-to-one relationship between climbing fibres and Purkinje cells in the adult is preceded at early developmental stages by multiple innervation (Delhaye-Bouchaud, Crepel & Mariani, 1975; Crepel, Mariani & Delhaye-Bouchaud, 1976*b*). The immature multiply innervated stage seems to persist in the adult when Purkinje cells develop in a cellular environment devoid of granule cells, as occurs in X-irradiated rats and in weaver and reeler mutant mice (Woodward, Hoffer & Altman, 1974; Crepel & Mariani, 1976; Crepel, Delhaye-Bouchaud & Legrand, 1976*a*; Mariani, Crepel, Mikoshiba, Changeux & Sotelo, 1977; Puro & Woodward, 1977). The experimental situation offers the possibility to study the synaptic distribution of climbing fibres on adult multiply innervated cells and especially to determine whether they are intermingled on the same dendrites or segregated on distinct territories, which would give some insights on the developmental processes leading to this distribution and on the mechanisms responsible for the regression of the multiple innervation during normal development.

The present experiments were carried out to study this distribution by determining time courses, reversal characteristics and summations of climbing fibre e.p.s.p.s. in multiply innervated Purkinje cells in X-irradiated rats and comparing them with controls, since these properties are related, in some favourable cases, to the distribution of synapses on the dendrites (Rall, 1967; Rall, Burke, Smith, Nelson & Frank, 1967; Rall, 1969; Calvin, 1969; Llinás & Nicholson, 1976). Since the accuracy of such correlations depends on electrotonic properties of the neurones, input resistances and total electrotonic lengths of Purkinje cells were also evaluated in control and treated rats. In a first series of experiments, additional evidence for a multiple innervation of Purkinje cells by climbing fibres in X-irradiated rats was obtained.

METHODS

Radiation procedure

The radiation procedure was the same as that previously used (Crepel *et al.* 1976*a*). Pups were gently placed in a plastic box containing soft polyether foam and their heads maintained in a plastic frame coated with the same foam. The beam of X-rays was collimated upon the head and an 8 mm thick lead shield with a small rectangular hole was positioned to protect the body except for the cerebellar area. Rats were irradiated one by one, at an exposure rate of 85 r/min and received seven successive doses on post-natal days 0, 3, 5, 7, 10, 12 and 14 (total dose: 1200 r). Histological controls revealed that, as in a previous study (Crepel *et al.* 1976*a*), this procedure prevented the formation of most of the granule cells.

Electrophysiological methods

Electrophysiological experiments were carried out on X-irradiated and untreated control Wistar rats.

Animals were studied 2–3 months after birth. They were anaesthetized with Na pentobarbitone (30 mg/kg) administered intraperitoneally, paralysed with gallamine triethiodide (Flaxedil) injected through the same route and were artificially respired. The level of anaesthesia was maintained by additional doses of 3 mg/kg every hour and the body temperature was maintained at 37 °C. The cerebellar vermis and the medulla oblongata were exposed. Electrical stimulations of the inferior olive were performed with a concentric bipolar electrode and juxtafastigial stimulations were done with fine bipolar electrodes insulated except at their tips and with a tip separation of 0·4 mm. Intracellular d.c. recordings of Purkinje cells located in Larsell's lobules V, VI and VII of the vermis were made with glass micro-electrodes filled with 3 M-KCl or 2 M-K citrate giving a d.c. resistance of 10–15 MΩ. Steady currents or rectangular current pulses were injected intracellularly through the recording micro-electrode using an active bridge circuit and were monitored on an oscilloscope. Usually, responses were filmed and were also stored on magnetic FM tape recorder (FM recording, bandwidth 0–5000 Hz) for subsequent averaging (see Results) on a minicomputer (Intertechnique Didac 4000).

RESULTS

In control and treated rats, typical 'direct' and 'reflex' climbing fibre responses (Eccles, Llinás & Sasaki, 1966), were recorded before deterioration of the cells (Fig. 1*A*, *B*). They were often preceded by an antidromic response (Fig. 1A_2) when evoked by juxtafastigial stimulation, confirming the identification of impaled neurones as

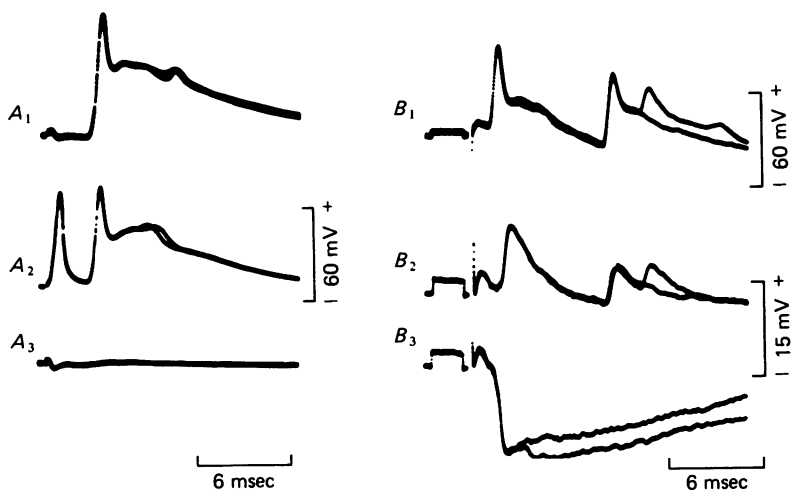

Fig. 1. Intracellular recordings of climbing fibre responses in X-irradiated rats. A_1–A_2: climbing fibre responses obtained by juxtafastigial stimulation. In A_2 the stimulation strength was higher than in A_1 and elicited an antidromic action potential. A_3: same cell as in A_1–A_2 after withdrawal of the micro-electrode. B_1–B_3: responses obtained in another cell by juxtafastigial stimulation. B_1: specimen records taken prior to deterioration of the cell and showing the 'direct' and 'reflex' climbing fibre responses. In B_2, the inactivation of the spike generating mechanism unmasked the underlying climbing fibre e.p.s.p. B_3: reversal of the direct climbing fibre e.p.s.p. shown in B_2 by a depolarizing current injected into the cell through the micro-electrode. A contamination by other synaptic activities is apparent (see text). Superimposed sweeps in all traces.

Purkinje cells (Eccles *et al.* 1966). Deterioration of the cell suppressed the spike generating mechanism and revealed the underlying climbing fibre e.p.s.p. (Fig. 1B_1, B_2), which reversed under depolarizing d.c. currents applied through the micro-electrode (Fig. 1B_3), in agreement with the chemical nature of the synapse involved (Eccles, 1964; Eccles *et al.* 1966; Llinás & Nicholson, 1976). In many instances, the inverted response was not the mirror of the climbing fibre e.p.s.p. before reversal (Fig. 1B_2, B_3), revealing contamination by other synaptic activities. Well impaled cells in both groups had resting potentials of up to – 60 mV. Purkinje cells with resting potentials lower than – 40 mV and exhibiting climbing fibre e.p.s.p.s. smaller than 10 mV in amplitude were discarded from the present study.

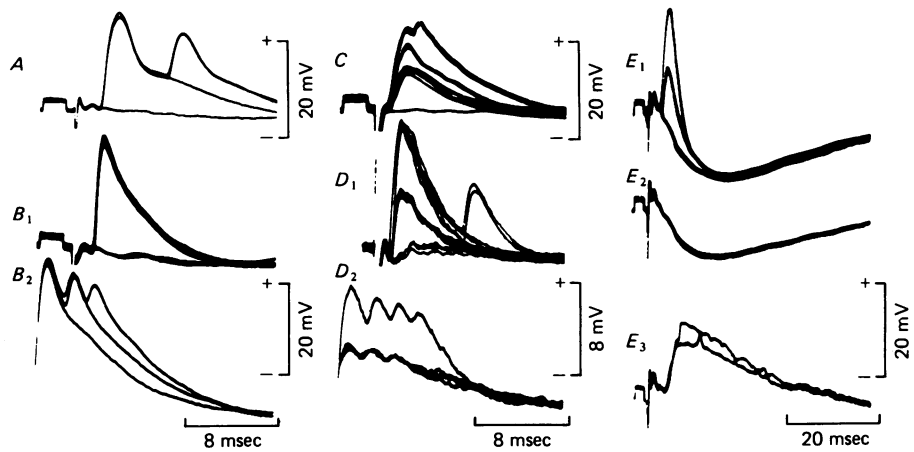

Fig. 2. All-or-none or stepwise graded amplitudes of the climbing fibre e.p.s.p.s. All responses were obtained by juxtafastigial stimulation, except in B_2 and D_2 which illustrate spontaneous climbing fibre e.p.s.p.s. *A–B*: examples of all-or-none e.p.s.p.s recorded in two monoinnervated Purkinje cells in X-irradiated animals. Spontaneous e.p.s.p.s in B_2 were recorded in the same cell as in B_1. *C–D*: examples of graded e.p.s.p.s recorded in two multiply innervated cells. The progressive increase of the intensity of stimulation revealed three steps in the response shown in *C* and only two steps in *D*. Spontaneous e.p.s.p.s. in D_2 were recorded in the same cell as in D_1. The two steps shown in D_1 were also present in these spontaneous responses. *E*: example of i.p.s.p. recorded in a multiply innervated Purkinje cell. In E_1, the stimulation strength slightly varied and elicited a graded e.p.s.p. with two steps, superimposed on a marked hyperpolarization of the cell; in E_2, the stimulation was sub-threshold for the e.p.s.p. and only elicited the i.p.s.p. E_3: reversal of this i.p.s.p. by hyperpolarizing current injected into the cell through the micro-electrode. A small contamination by a late e.p.s.p. is apparent (see text).

Identification of mono- and multiply innervated cells

The mono- or multiple innervation of Purkinje cells by climbing fibres was determined in control and X-irradiated rats on the basis of criteria already used in previous studies (Eccles *et al.* 1966; Crepel *et al.* 1976*b*; Delhaye-Bouchaud, Mory & Crepel, 1978).

Fluctuation in size of the climbing fibre e.p.s.p.

Juxtafastigial or inferior olive stimulations evoked all-or-none climbing fibre e.p.s.p.s. in all Purkinje cells in control rats and in 46/108 tested cells (42·6 %) in X-irradiated animals. (Fig. 2*A*, B_1); in all these cells spontaneously occurring e.p.s.p.s. were all-or-none (Fig. 2B_2). Sixty-two (57·4 %) other Purkinje cells tested in treated rats displayed climbing fibre e.p.s.p.s. graded in size by steps (two to four, depending on cells) when the intensity of the stimulation was smoothly increased (Fig. 2*C*, D_1), or when they occurred spontaneously (Fig. 2D_2). A contribution of the mossy fibre pathway to these graded responses appears unlikely since (1) the latencies of graded climbing fibre e.p.s.p.s evoked by juxtafastigial or inferior olive stimulation were the same as those of direct or reflex climbing fibre responses recorded before cell deterioration, (2) the latencies of graded e.p.s.p.s directly elicited by juxtafastigial stimulation (1·3–5 msec, mean value 3·18 ± 0·43 msec, $n = 19$), were much longer than those of mossy fibre-mediated responses previously recorded from Purkinje cells in agranular cerebella (Llinás, Hillman & Precht, 1973; Crepel *et al.* 1976*a*), (3) the time courses of the graded responses were typical of climbing fibre e.p.s.p.s and did not change, or changed only slightly with the amplitude of the response for a given cell and (4) each component of the compound climbing fibre e.p.s.p. occurred randomly during the resting discharge, and its firing frequency (1–2/sec) was in the range reported for spontaneous climbing fibre responses (Thach, 1967).

In control and X-irradiated rats, juxtafastigial or inferior olive stimulations also frequently evoked prominent hyperpolarizations (Fig. 2E_1, E_2) which were readily converted into depolarizing potentials by hyperpolarization of the cell (Fig. 2E_3). The time course of these inverted i.p.s.p.s was much longer than those of the graded climbing fibre e.p.s.p.s. recorded in the same cells (Fig. 2E_1, E_3). In the case of Fig. 2E_3, the inverted i.p.s.p. was not exactly the mirror of the hyperpolarization seen in Fig. 2E_2, probably because of a small contamination by a late e.p.s.p., due to some remaining granule cells (see histological observations in Methods), and whose amplitude was enhanced by the cell hyperpolarization.

Interaction experiments between juxtafastigial and inferior olive stimulations

In the cells showing an all-or-none climbing fibre e.p.s.p., a collision always occurred along the afferent climbing fibre between impulses evoked by a conditioning juxtafastigial and a test inferior olive stimulation, until the interstimulus interval became greater than the sum of the refractory period of the climbing fibre plus the conduction time between the two stimulation sites. For instance, in the case of the cell illustrated in Fig. 3A_1–A_4, the all-or-none climbing fibre e.p.s.p. evoked by a test inferior olive stimulation (Fig. 3A_3) was cancelled in Fig. 3A_4 by a conditioning juxtafastigial stimulation, although the interstimulus interval was greater than the refractory period of the climbing fibre as revealed in Fig. 3A_2 where paired juxtafastigial stimulations with the same interval evoked two successive climbing fibre e.p.s.p.s.

In the cells showing graded e.p.s.p.s no collision occurred during juxtafastigial–inferior olive stimulations for any interstimulus interval if the juxtafastigial stimulation was at threshold and the inferior olive stimulation was strong enough to elicit the full response (Fig. 3*B*), or if the two stimulations evoked two all-or-none steps with distinct amplitudes (Fig. 3*C*). It was then always possible to summate these

climbing fibre e.p.s.p.s (Fig. $3B_4$, C_4). The correlation between the presence of graded e.p.s.p.s and the results of the collision experiments strongly suggests that in adult X-irradiated rats, slightly more than half of the Purkinje cells (57·4 %) each receive several (two to four) and probably independent climbing fibres, as already suggested by previous studies (Woodward *et al.* 1974; Crepel *et al.* 1976*a*).

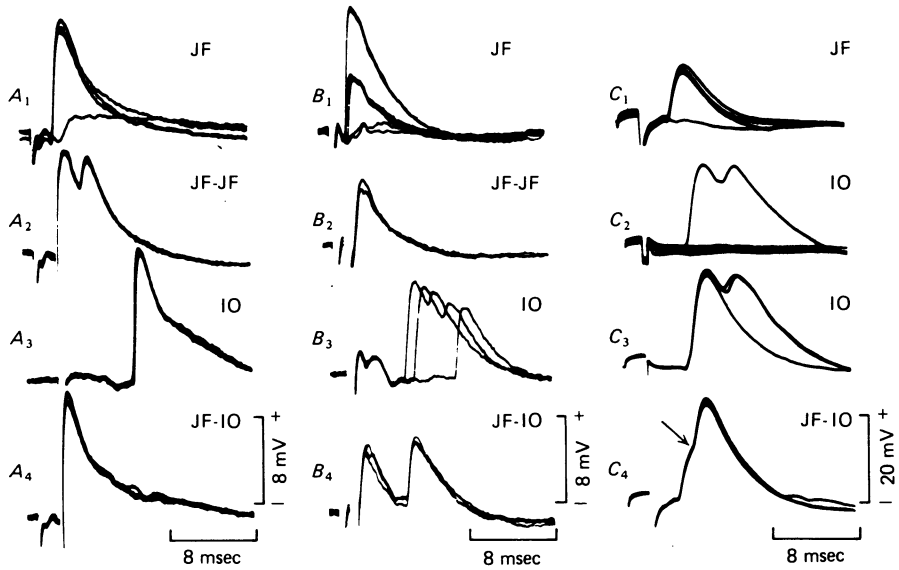

Fig. 3. Interaction experiments between climbing fibre e.p.s.p.s in mono- and multiply innervated Purkinje cells. A_1–A_4: example of collision in the afferent climbing fibre to a monoinnervated Purkinje cell. A_1: all-or-none climbing fibre e.p.s.p. evoked by juxtafastigial (JF) stimulation; A_2: responses obtained by paired JF stimulations with an interstimulus interval greater than the refractory period of the climbing fibre. A_3: the e.p.s.p. evoked by inferior olive (IO) stimulation; A_4: paired JF–IO stimulations with the same interval as in A_2. The response evoked by IO stimulation was cancelled by the conditioning JF stimulation. B_1–B_4: same type of experiments with another Purkinje cell where no collision occurred. In B_1 the JF stimulation elicited an e.p.s.p. with two steps obtained by slightly increasing the intensity of the stimulus. In B_2, two threshold JF stimulations were paired with an interstimulus interval shorter than the absolute refractory period of the climbing fibre (compare B_1 and B_2). B_3 and B_4 as in A_3 and A_4 respectively. In B_4, the climbing fibre e.p.s.p. evoked by the IO stimulation was not cancelled by the conditioning JF stimulation with the same stimulus interval as in B_2. C_1–C_4: example of an absence of collision in another multiply innervated cell. JF (C_1) and IO (C_2–C_3) stimulations evoked two different e.p.s.p. steps all-or-none in character but exhibiting different amplitudes. In C_4, JF and IO stimulations were applied simultaneously and a summation of the corresponding e.p.s.p.s occurred (arrow).

Passive electrical properties of Purkinje cells in control and X-irradiated rats

Input resistance

The input resistance of Purkinje cells was determined by measuring the voltage change produced by depolarizing and hyperpolarizing current pulses passed across the cell membrane (Fig. $4A_1$, A_2). Currents, usually ranging between -2 and $+2$nA,

were applied through the recording micro-electrode via a bridge circuit whose balance was tested before penetration and after withdrawal of the micro-electrode from the recorded cell (Fig. 4 A_3, A_4). For each Purkinje cell the transmembrane voltage change at the plateau of polarization was plotted against applied current (Fig. 4 B), the slope of the curve corresponding to the input resistance R_{in}. In both control and treated rats, some cells did not exhibit delayed rectification (Hodgkin & Huxley, 1952) with small depolarizing currents, but others did, as shown by the decrease of the slope of the curve with these positive currents (Fig. 4 B). A small anomalous rectification

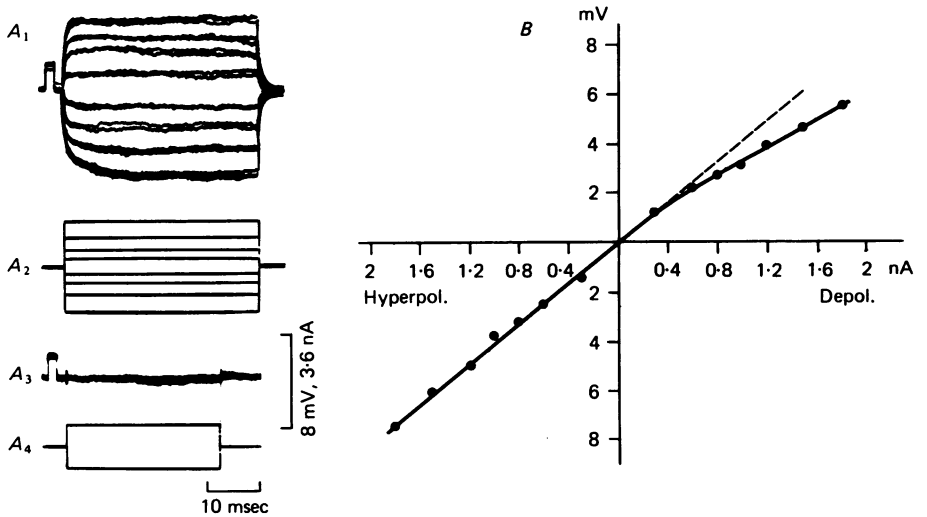

Fig. 4. Input resistance of Purkinje cells. A_1–A_2: transmembrane voltage changes (A_1) induced by various currents (A_2) injected into the cell through the recording micro-electrode. Note the local response with depolarizing currents. A_3–A_4: as in A_1–A_2 but with the electrode outside the cell. B: relationship between current and voltage for the cell illustrated in A.

(Nelson & Frank, 1967) also occurred in some cells when they were hyperpolarized (not illustrated). The mean input resistance of Purkinje cells determined around the resting potential was higher in multiply innervated cells than in controls ($8{\cdot}03 \pm 0{\cdot}70$ MΩ, $n = 15$, against $5{\cdot}90 \pm 0{\cdot}56$ MΩ, $n = 15$). However, individual values varied widely, probably partly because of differences in resting potentials of the tested cells (Ransom, Neale, Henkart, Bullock & Nelson, 1977) and partly because of their more or less severe deterioration.

Total electronic length of Purkinje cells

According to Rall (1969), the transient response of the membrane of a neurone with dendrites to a current step intracellularly applied at one location comprises several time constants, namely the passive membrane time constant τ_m ($= \tau_0$) and smaller equalizing time constants $\tau_{1\cdots n}$ that govern rapid equalization of the membrane potential over the length of the processes. These parameters allow to calculate the 'total electrotonic length' L for a theoretical equivalent cylinder ($L = l/\lambda$, where

l = the anatomical length of the cylinder, i.e. that of neuronal soma and dendrites, and λ = the corresponding characteristic length) with the following equation:

$$L = \pi\left(\frac{\tau_m}{\tau_1} - 1\right)^{-\frac{1}{2}}.$$

In spinal motoneurones, in keeping with Rall's model, the membrane transient response produced by a current step injected in the soma is the sum of a slow and a fast exponential whose individual slopes give respectively τ_m and τ_1 (Lux, Schubert &

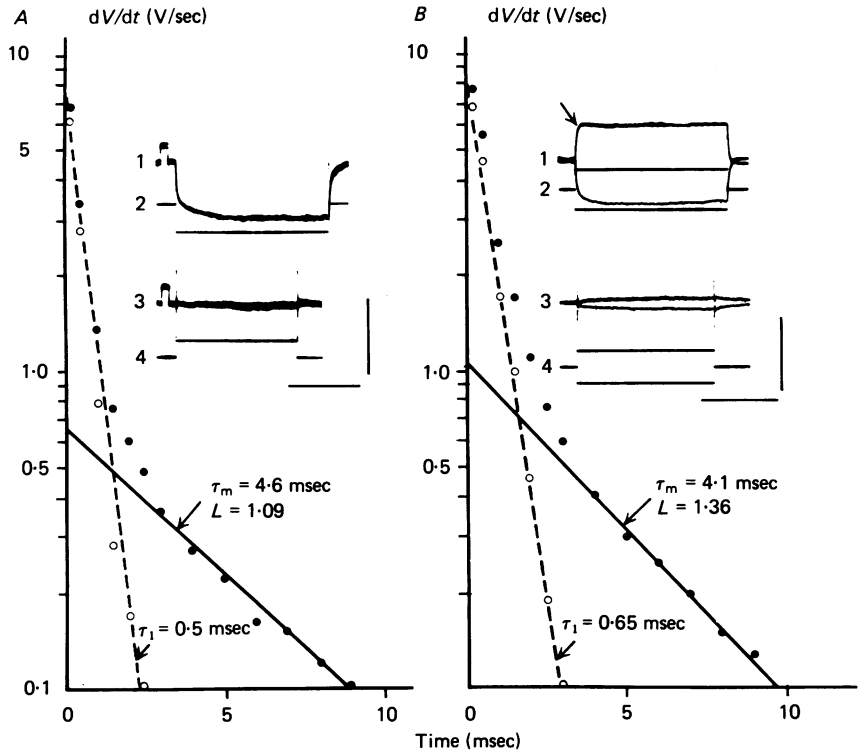

Fig. 5. Total electrotonic length of Purkinje cells. *A*: experiments on a monoinnervated Purkinje cell in a normal animal. Same cell as in Fig. 4 A_1–A_2. Inset 1–2 shows the membrane voltage response (1) to a hyperpolarizing current (2). Inset 3–4 shows voltage records after withdrawal of the micro-electrode (3) with a depolarizing current pulse (4). Calibration bars represent 8 mV and 20 msec. The rate of change of potential with respect to time as a function of time after the onset of the current pulse is plotted on a semilogarithmic scale. The negative slope of the continuous line is related to the membrane time constant τ_m, and that of the dashed line to the equalizing time constant τ_1 (see explanations in text). *B*: same experiments as in *A* in a multiply innervated Purkinje cell. Note the local response (arrow) of the membrane in inset 1 with a depolarizing current pulse (2); calibration bars represent 16 mV and 20 msec. In *A* and *B*, filled circles represent data points and open circles the difference between the extrapolated continuous line and the data points.

Kreutzberg, 1970; Nelson & Lux 1970; Burke & Ten Bruggencate, 1971). Since a reasonable estimate of these parameters can be obtained by using a single micro-electrode in a bridge circuit (Ransom *et al.*, 1977), this method was applied to mono-innervated Purkinje cells in control rats and to multiply-innervated Purkinje cells in

X-irradiated animals. Specimen records in Fig. $5A_{1-2}$ and B_{1-2} illustrate transient electrical responses elicited in these cells by hyperpolarizing current. The bridge balance was tested as previously (Fig. $5A_{3-4}$, B_{3-4}). In both mono- and multiply-innervated Purkinje cells, the rate of change of the transient response varied linearly on semilogarithmic co-ordinates for time intervals greater than 3–4 msec. At earlier times, a deviation occurred; subtraction of this extrapolated slow exponential from the experimental curve revealed another much faster exponential (Fig. 5*A*, *B*). These data, very similar to those obtained with spinal motoneurones, allowed the calculation for each cell of τ_m, τ_1 and L (Fig. 5*A*, *B*). Mean values were respectively $5{\cdot}57 \pm 0{\cdot}89$ msec, $0{\cdot}40 \pm 0{\cdot}25$ msec and $0{\cdot}96 \pm 0{\cdot}12$, $n = 11$ in monoinnervated Purkinje cells and $4{\cdot}36 \pm 0{\cdot}58$ msec, $0{\cdot}50 \pm 0{\cdot}22$ msec and $1{\cdot}25 \pm 0{\cdot}10$, $n = 12$ in multiply innervated cells, the difference for L values being significant ($P < 0{\cdot}05$).

Properties of climbing fibre e.p.s.p.s in control and in X-irradiated rats

Time course of the climbing fibre e.p.s.p.

The time-to-peak and half-decay time of the climbing fibre e.p.s.p.s. were measured in fifteen mono- and twenty-seven multiply innervated Purkinje cells recorded in X-irradiated rats and their values were compared to those found in normal animals. For multiply innervated Purkinje cells these parameters were determined for both the minimal and the maximal amplitude of the response. On the whole, the mean times to peak and half-decay times of all-or-none e.p.s.p.s were nearly the same in control and in X-irradiated animals ($0.99 \pm 0{\cdot}09$ and $1{\cdot}89 \pm 0{\cdot}16$ msec against $1{\cdot}02 \pm 0.06$ and $1{\cdot}81 \pm 0{\cdot}12$ msec). The mean time-to-peak and half-decay time of graded climbing fibre e.p.s.p.s were $0{\cdot}93 \pm 0{\cdot}04$ and $1{\cdot}88 \pm 0{\cdot}10$ msec and did not vary significantly with the amplitude of the response.

Minimum and maximum responses were also compared for a given multiply innervated cell. The time course of the response was independent of the size of the e.p.s.p. in eleven of the twenty-five Purkinje cells tested. In the other fourteen, it varied: in eight the minimum response had a shorter time course than the maximum one, whilst the inverse was true in the remainder. The mean difference in time-to-peak was $0{\cdot}12 \pm 0{\cdot}03$ msec, $n = 25$ (range 0–0·4 msec). These results suggest that the overall distribution of climbing fibre endings on multiply innervated Purkinje cell dendrites is not grossly abnormal and that the different climbing fibres contacting each cell innervate territories whose electrotonic distance from the recording site is either the same or is slightly different.

Reversal of the climbing fibre e.p.s.p

The reversal of climbing fibre e.p.s.p.s by depolarization was studied in control and in X-irradiated rats. Direct current injections were done through the recording micro-electrode when the spike generating mechanism of the cell was completely abolished. Climbing fibre e.p.s.p.s were produced by either juxtafastigial or inferior olive stimulations. For all-or-none e.p.s.p.s in control and treated rats, either supra-threshold stimulations were applied for each current step and the size and polarity of the responses determined by comparison with the extracellular field potential, or responses to supra- and sub-threshold stimulation were compared: the latter procedure was especially important when the stimulation also evoked other synaptic

activities in the tested cell. In control rats, an averaging technique was used also and results have been published elsewhere (Crepel & Delhaye-Bouchaud, 1978). For graded e.p.s.p.s the study was done both for responses elicited by threshold and maximal stimulation (Fig. 6). As in control rats, these e.p.s.p.s were often contaminated by other responses (compare Fig. 6*A* and *B*) and in this case, cells were discarded when threshold and maximal stimulation strengths were too different, since contamination of the maximal response was likely to be greater than that of those

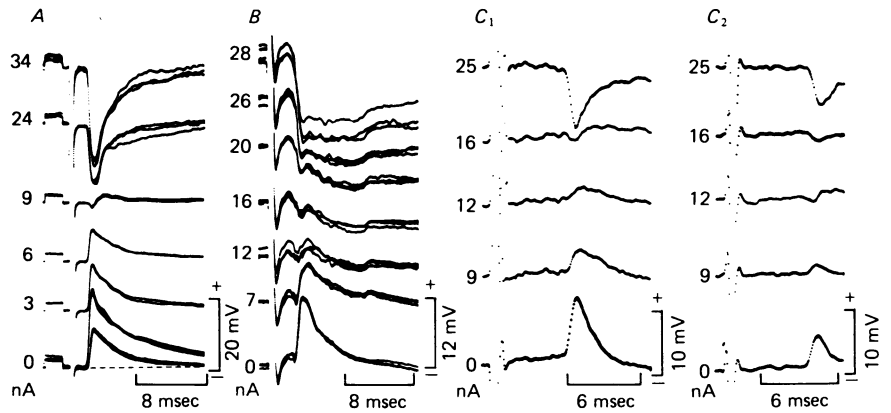

Fig. 6. Reversal properties of graded climbing fibre e.p.s.p.s evoked by juxtafastigial stimulation in X-irradiated rats. *A*: example of reversal of a graded e.p.s.p. by depolarizing direct current. The intensity of the stimulation was adjusted to evoke the maximum response except in the bottom trace (O nA) where it was slightly decreased to show that the response consisted of two steps. As in controls, the rising phase of the e.p.s.p. reversed with lower current values than the peak of the response. Current intensities are indicated on the left of each record. *B*: same experiments as in *A* in another multiply innervated cell: reversal of the maximum response. An important contamination of this response by other synaptic activities is apparent in the upper trace. Two to three superimposed sweeps in all traces in *A* and *B*. C_1–C_2: example of reversal of averaged e.p.s.p.s (50 μsec/bin) under depolarizing direct currents in another multiply innervated cell (full explanations in text). C_1: reversal of the maximum averaged response. C_2: reversal of the threshold one. Note that it occurred with lower current values than that of the maximum response. In C_1 and C_2, reversal of the initial part of averaged climbing fibre e.p.s.p.s occurred before that of the peak of the response, and contamination by other synaptic activities was no longer detectable.

elicited by threshold and sub-threshold stimulations. Averaged responses were derived from responses to eight sub-threshold, eight threshold and eight maximum stimulations for each level of current; the averaged response to the sub-threshold stimuli was then subtracted from the threshold and from the maximum ones to get climbing fibre e.p.s.p.s without underlying synaptic activities (Fig. 6C_1, C_2). Finally, the current was frequently turned off during current injections and the size of the e.p.s.p. was measured at resting potential level to test the stability of the recording.

The reversal properties of these e.p.s.p.s in control rats will be summarized only very briefly here, since they have been already reported elsewhere (Crepel & Delhaye-Bouchaud, 1978). The reversal was biphasic and the initial part of the response generally reversed first as previously found in the cat (Llinás & Nicholson, 1976).

The mean current value to reverse the peak of the climbing fibre e.p.s.p. was 13·4 ± 1·2 nA, $n = 32$. In X-irradiated rats, the reversal of all-or-none e.p.s.p.s occurred as in controls (not illustrated) and needed very similar current values, i.e. 15·25 ± 1·04 nA, $n = 16$. The reversal of graded responses was also biphasic and the initial part of the response reversed first in all Purkinje cells tested (Fig. 6*A*, *B*, C_1, C_2). Mean current values for reversal of the responses to threshold and maximum stimulations were respectively 14·24 ± 1·03 and 15·45 ± 1.35 nA, $n = 20$, these values being not significantly different from those in monoinnervated cells.

When reversal of these graded climbing fibre e.p.s.p.s was considered in each multiply innervated cell, currents for reversal of the threshold and of the maximum response were either the same (six out of fourteen cells), or were slightly different (eight out of fourteen cells). In the latter case, the threshold response reversed with either lower (Fig. 6C_1, C_2) or higher current values than did the maximum one. Differences in current for reversal were 2·21 ± 0·87 nA on the average and ranged between 0 and 6 nA. Finally, half of the Purkinje cells tested behaved as predicted as regards correlations between time courses and current for reversal of their e.p.s.p.s (Rall, 1967; Calvin, 1969; Llinás & Nicholson, 1976). For the other cells, currents for reversal changed with the amplitude of the response, whereas time courses remained constant. The absence of correlation conflicts with data gained on *Aplysia* neurones (Graubard, 1975) and it could be due either to a change in the relative position of the micro-electrode and of the recorded cell during current injection, or in some cases, to a segregation of the climbing fibres involved on separate dendritic branches. In the latter case, the propagation into these dendrites of the currents injected at the soma level would depend on their branching pattern.

Therefore, with the exception of this last discrepancy, these results corroborate conclusions concerning the distribution of climbing fibre synapses on multiply innervated Purkinje cells (see previous section).

Additive properties of components of the graded climbing fibre e.p.s.p.

If the different climbing fibres innervating each multiply innervated Purkinje cell are intermingled on the same dendrites, one may expect that a very marked shunting effect will occur between the corresponding synapses, whereas the shunt will be minimal if the synapses are borne by different dendritic territories. Therefore, interactions between two all-or-none steps of graded e.p.s.p.s evoked respectively by juxtafastigial and inferior olive stimulations, were studied in multiply innervated cells. This was done by measuring the size of the climbing fibre e.p.s.p.s evoked by inferior olive stimulations timed to occur at successive points along a climbing fibre e.p.s.p. elicited by juxtafastigial stimulation. The cells used for these experiments were those in which difference in amplitude of the two all-or-none responses (Fig. 7A_1, A_2), and the absence of collision during juxtafastigial–inferior olive stimulation (Fig. 7A_3, *B*) ruled out the possibility that they were mediated through the same climbing fibre and confirmed the multiple innervation of the Purkinje cells. When, as occurred in some multiply innervated cells, the threshold juxtafastigial and inferior olive stimulations activated the same climbing fibre, these neurones were discarded. In the twelve cells successfully studied, the shunting effect developed during the rising phase of the conditioning e.p.s.p. and was maximal near its peak. Thereafter,

the size of the test response rapidly returned to normal (Fig. 7*C*). These interaction experiments revealed the presence of two very different populations of Purkinje cells as regards the magnitude of the shunt. In five cells, the minimal size of the remaining test climbing fibre e.p.s.p. (as per cent of the control value) ranged between 11 and 26 %; in the seven other cells, it ranged between 66 and 94 % (Fig. 7*C*). These shunting values did not overlap with those previously found in normal rats during experiments on interactions between parallel fibre and climbing fibre e.p.s.p.s (Crepel & Delhaye-

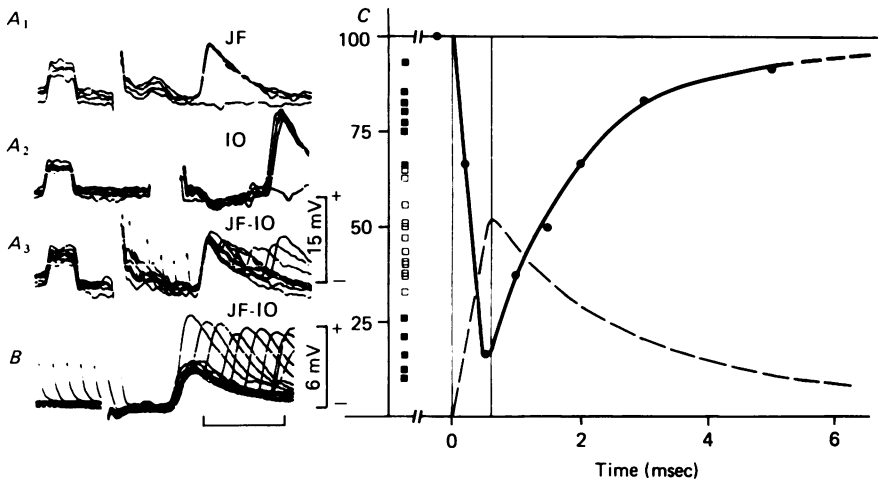

Fig. 7. Shunting effect among components of graded climbing fibre e.p.s.p.s in multiply innervated cells. Superimposed sweeps in *A*, *B*. A_1: all-or-none step of the e.p.s.p. evoked by juxtafastigial (JF) stimulation. A_2: other all-or-none step of the e.p.s.p. evoked in the same cell by inferior olive (IO) stimulation. A_3: paired JF and IO stimulations at various interstimulus intervals to show the shunting effect of the former on the latter (see text). *B*: same interaction experiments in another cell. The shunting effect was prominent in *A* and weak in *B*. *C*: same cell as in *A*, plotting of the size (ordinate) of the test e.p.s.p. evoked by IO stimulation in per cent of the control value (filled circles and continuous line) against time intervals (abscissa) from the beginning of the conditioning response obtained by JF stimulation. The time course of the conditioning response has been redrawn (dashed line). Minimum values of the size of the test e.p.s.p. during the rising phase of the conditioning response are plotted on the left of the graph (filled squares). The shunting effect of the parallel fibres e.p.s.p. on the climbing fibre e.p.s.p. in control rats has been included also in the same plot (open squares). Full explanations in the text.

Bouchaud, 1978) as illustrated on the left side of Fig. 7*C*. It is probable therefore that the two climbing fibres activated by juxtafastigial and inferior olive stimulations contacted different dendritic branches in the second group of cells, whereas they innervated the same dendrites in the first group. In this latter case, it is likely that the synaptic distribution of the two climbing fibres was either identical or slightly different, on the basis of results concerning time courses and reversal properties of these graded e.p.s.p.s (see previous sections).

DISCUSSION

Two main conclusions can be drawn from the present study. Firstly, the majority of Purkinje cells are multiply innervated by climbing fibres in the adult X-irradiated rat. Secondly, the over-all distribution of climbing fibre synapses on these cells is not grossly abnormal and two different climbing fibres contacting a given cell can be either totally or partially intermingled on the same dendrites, or segregated on distinct dendritic branches. Before considering implications of these data for mechanisms responsible for the final refinement of these connexions during development, several points have to be discussed.

Multiple innervation of Purkinje cells by climbing fibres in the X-irradiated rat

Until now, the existence of a multiple innervation of Purkinje cells by climbing fibres in the X-irradiated rat was suggested mainly by the graded character of climbing fibre responses extracellularly recorded (Crepel *et al.* 1976*a*) and by the unusually large fluctuations in the size of the spontaneous climbing fibre e.p.s.p.s (Woodward *et al.* 1974). The two criteria used in the present study, i.e. the difference between the all-or-none and the stepwise graded e.p.s.p.s and the presence or absence of collision along afferent climbing fibres during juxtafastigial-inferior olive interaction experiments strongly support this interpretation. In particular, they rule out the possibility of an innervation of these multiply innervated Purkinje cells by several collaterals belonging to the same climbing fibre, except if the branching occurred within the inferior olive and if a conduction failure arose at this level. Conduction failure has been demonstrated for other axons (Parnas, Spira, Werman & Bergman, 1969; Waxman, 1971; Van Essen, 1973; Westerfield, Joyner & Morre, 1978) but is unlikely here. To account for data obtained during such interaction experiments and those concerning the spontaneous firing of components of the graded climbing fibre e.p.s.p. (see Results, and also Woodward *et al.* 1974), the conduction failure would have to be total in the antidromic direction and only intermittent and at random in the orthodromic one.

Electrotonic properties of Purkinje cells

The validity of the criteria used in the present study to define the synaptic distribution of climbing fibres on to multiply innervated Purkinje cells as compared to controls depends on electrotonic properties of these neurones (Rall, 1967, 1969; Rall *et al.* 1967; Lux *et al.* 1970; Nelson & Lux, 1970; Llinás & Nicholson, 1976). The mean values given in the results have to be considered as very crude approximations of these parameters (Hubbard, Llinás & Quastel, 1969; Ransom *et al.* 1977). However, all classes of Purkinje cells were studied in the same experimental conditions and therefore it seems possible to infer some conclusions from comparisons of the means of their input resistances and total electrotonic lengths. The higher value of R_{in} in multiply innervated cells as compared to controls could be the consequence of an almost complete absence of excitatory inputs via parallel fibres leading to a reduced shunt; but it could also result from a shrinkage of these neurones. It is known that in X-irradiated rats, the peripheral limits of the dendritic trees of Purkinje cells are at the same distance from the soma as in normal animals (Berry & Bradley, 1976). The

higher value of L in multiply innervated cells might therefore be due to a smaller characteristic length of the neurones. In any case, these L values are of the same order of magnitude as those of spinal motoneurones (Nelson & Lux, 1970; Ransom *et al.* 1977) and according to Rall (1967, 1969) and Calvin (1969), it is possible to correlate time course and sensitivity to injected currents with the distribution of the synapses involved on Purkinje cell dendrites. This conclusion is supported by the biphasic reversal of the climbing fibre e.p.s.p. in mono- and multiply innervated cells, given the interpretation of this type of reversal previously put forward in the cat (Llinás & Nicholson, 1976).

Mechanisms responsible for the regression of the multiple innervation of Purkinje cells by climbing fibres during normal development

In the case of the peripheral nervous system, removal of supernumerary synapses during development has been explained mainly by an intrinsic tendency for presynaptic fibres to retract (Brown *et al.* 1976), by an influence of the post-synaptic cell on the presynaptic endings (Benoit & Changeux, 1975; Changeux & Danchin, 1976) and by a competitive interaction among synapses innervating each target cell (see for instance Lichtman, 1977; Brown & Ironton, 1978). At the neuromuscular junction, this competition would be achieved through a continuous reorganization of synaptic contacts, which in turn would lead to a withdrawal of redundant terminals, rather than to their degeneration (Barker & Ip, 1966; Korneliussen & Jansen, 1976; Kuffler, Thompson & Jansen, 1977; Brown & Ironton, 1978).

In the case of the cerebellum, the presence of a direct competition between climbing fibres during development can be questioned. On the one hand, the fact that in adult X-irradiated rats, in some multiply innervated Purkinje cells, the same dendritic segments can bear at least two different climbing fibres indicates that in these animals, competitive interactions among these fibres are not very strong. The segregation in other neurones of the two climbing fibres tested on distinct dendritic branches does not necessarily invalidate this conclusion since, at least for Purkinje cells supplied by three or four fibres, the remaining untested fibre(s) might be intermingled with the tested ones. On the other hand, the fact that a sizeable proportion (42·6 %) of Purkinje cells are monoinnervated by climbing fibres in these adult agranular cerebella, whereas multiple innervation seems to be the rule at earlier stages of development in these treated rats (Crepel *et al.* 1976*b*) and in very immature (4–6 day old) normal rats (Crepel & Delhaye-Bouchaud, unpublished data) suggests that the competition nevertheless exists (but the establishment of the monoinnervation in these cells might also be the result of an intrinsic tendency of climbing fibres to retract, as suggested for peripheral nerves (Brown *et al.* 1976)).

Mechanisms underlying competition among climbing fibres might be similar to those previously considered in the case of the neuromuscular junction despite a wider synaptic distribution on the target cell, since Kuffler *et al.* (1977) have shown that competition among synaptic terminals belonging to different fibres can occur even in the absence of intimate contact between them. Also, the establishment of the monoinnervation of Purkinje cells during normal development seems to involve interactions with post-natally formed cerebellar cortical neurones (Crepel, 1977; Delhaye-Bouchaud *et al.* 1978).

In conclusion it seems reasonable to postulate that during normal development, climbing fibres have primarily a tendency to fill all available synaptic space on dendrites of the Purkinje cell and only a weak intrinsic ability to retract and/or to compete for the control of each post-synaptic cell. Therefore, the role of granule cells would be to strengthen the direct competitive interaction among climbing fibres and/or their tendency to retract, possibly by decreasing the supply of some trophic factor emitted by the post-synaptic cell (Purves, 1975), which would ultimately lead to the withdrawal of redundant climbing fibre terminals.

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