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THE SECONDARY SPIKES OF CLIMBING FIBRE RESPONSES RECORDED FROM PURKINJE CELL SOMATA IN CAT CEREBELLUM

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SUMMARY

1. Extracellularly recorded climbing fibre responses in Purkinje cell somata in the cerebellar cortex were investigated in cats deeply anaesthetized with barbiturate. The effects on the amplitude of initial and secondary spikes of (a) preceding climbing fibre activation, (b) on-beam parallel fibre activation and (c) off-beam parallel fibre activation were studied.

2. When a climbing fibre response was preceded by climbing fibre activation there was a decrease in the amplitude of the initial spike of the second response at intervals up to 25 ms and little effect at longer intervals. Secondary spike amplitude was greatly increased at intervals up to 100 ms.

3. When a complex spike was preceded by on-beam parallel fibre activation there was a decrease in the initial spike amplitude at short intervals and an increase in the amplitude at long intervals. Secondary spike amplitude was increased up to 150 ms after an on-beam parallel fibre volley.

4. When a complex spike was preceded by off-beam parallel fibre stimulation there was an increase in initial spike amplitude at intervals up to about 200 ms and a decrease in secondary spike amplitude at intervals up to about 150 ms.

5. The results show that the amplitude of the secondary spikes can be modified by a preceding input to the Purkinje cell. The results also suggest that the secondary spikes are generated in the Purkinje cell dendrites and the initial spike in the soma.

INTRODUCTION

The classically described climbing fibre response (c.f.r.: Eccles, Ito & Szentágothai, 1967; also called 'complex spike': Thach, 1967) recorded from cerebellar Purkinje cells consists of a large initial spike followed by a series of one to five, or more, secondary spikes or wavelets at a frequency of 500–600 Hz. The duration of the response in extracellular recordings is 5–15 ms. In intracellular recordings, the secondary spikes are superimposed on an after-depolarization, which usually lasts for 12–25 ms. Many investigators (see Armstrong, 1974 for references) have noted that the form of the c.f.r. varies considerably from response to response with regard to the number and the amplitude of secondary spikes, even when experimental conditions are kept stable. Attempts to correlate the number of secondary spikes with

the spontaneous simple spike frequency (Mano, 1970) or with the number of impulses in the climbing fibre (Armstrong & Rawson, 1979) have not been successful.

In the Purkinje cell dendrites, climbing fibre stimulation gives rise to an initial spike followed by a plateau-like depolarization with a duration of up to several hundred milliseconds (Ekerot & Oscarsson, 1981; Llinás & Sugimori, 1982). Activation of the other excitatory input to the Purkinje cells, the parallel fibres, results in monosynaptic excitation of the dendritic tree followed by a disynaptic i.p.s.p. (Eccles *et al.* 1967). If the parallel fibre stimulation strength is sufficiently large, plateau potentials can also be elicited by this input (Campbell, Ekerot, Hesslow & Oscarsson, 1983).

The initial spike of the c.f.r. is probably generated in the Purkinje cell soma, whereas the generation site of the secondary spikes has not been clearly established. It was suggested by Granit & Phillips (1956) that the secondary spikes were also generated in the soma, and that their smaller amplitude was due to inactivation of the somatic membrane. Since the secondary spikes could not always be collided out by antidromic spikes, Martinez, Crill & Kennedy (1971) suggested that they are generated in the Purkinje cell dendrites and electrotonically conducted to the soma. If this is the case, then it is likely that the amplitude and the number of secondary spikes will be influenced by changes in ionic conductances in the dendrites. Indeed, Eccles, Llinás, Sasaki & Voorhoeve (1966*b*) have shown that the inhibition in Purkinje cells resulting from parallel fibre activation of interneurones can decrease the number of secondary spikes in a c.f.r. However, no systematic study of the factors affecting the secondary spikes has yet been conducted.

The aim of present study was to examine the effect on the c.f.r. of preceding climbing fibre or parallel fibre stimulation. The results reveal that the amplitude of the secondary spikes can be influenced by both excitatory and inhibitory inputs and suggest a dendritic origin for the secondary spikes.

METHODS

The experiments were performed on eighteen cats under deep pentobarbitone anaesthesia (initial dose 40 mg/kg I.P.; supplementary doses given as required). The animals were paralysed with gallamine triethiodide and artificially ventilated. In order to ensure an adequate level of anaesthesia (such that the pupils were maximally constricted) the paralysis was allowed to wear off periodically. The end-expiratory CO_2 concentration, arterial blood pressure and rectal temperature were continuously monitored and kept within physiological limits. A bilateral pneumothorax was performed in order to increase recording stability.

The left anterior lobe of the cerebellum was exposed and the cortex was covered with warm mineral oil. The left superficial radial nerve and the left sciatic nerve were dissected and mounted for stimulation. A monopolar stimulation electrode was inserted into the rostral part of the right dorsal accessory olive, which projects to the contralateral C3 zone of the cerebellar cortex (Groenewegen, Voogd & Freedman, 1979). An indifferent electrode was placed in the neck musculature. Except when stated otherwise, the inferior olive was stimulated at, or slightly above, the strength which evoked a maximal climbing fibre field potential from the cerebellar C3 zone, i.e. between 0·1 and 1·0 mA.

An opening was made in the occipital bone to permit insertion of a second monopolar stimulation electrode through the left posterior lobe of the cerebellum. The electrode was positioned in the white matter close to the interpositus nucleus such that a single shock could evoke both antidromic activation and direct and olivary reflex climbing fibre activation of the Purkinje cells (Armstrong & Harvey, 1966, Eccles, Llinás & Sasaki, 1966*a*). The threshold for antidromic activation was usually, but not always, lower than that for direct climbing fibre activation. The stimulus strengths used were always suprathreshold and usually gave rise to all three types of response.

Parallel fibre volleys were evoked by a concentric bipolar electrode which had an outer diameter of 1 mm. The central electrode was used as stimulation cathode. The threshold strength for evoking parallel fibre volleys was usually 0·2–0·5 mA. The stimulus strengths used were adjusted so that a single shock would produce clearly identifiable e.p.s.p.s and i.p.s.p.s in intracellular recordings, i.e. usually 0·3–1·5 mA. To increase recording stability, the concentric electrode was fitted to a small Perspex pressure foot which was gently placed on the cerebellar surface. The micro-electrode penetrations were made through a hole in the centre of the pressure foot. The minimum distance between the recording electrode and the centre of the stimulation electrode was 1·2 mm.

The stimuli to the nerves were square pulses with a duration of 100 μs, and to the inferior olive, white matter and cerebellar surface were square pulses with a duration of 200 μs. Intra- and extracellular recordings were made with capillary micro-electrodes filled with 3 M-potassium chloride or 4 M-potassium acetate solutions. The resistances were between 5 and 15 MΩ. All recordings were made with d.c. coupled amplifiers.

RESULTS

The afferent and efferent connexions of the Purkinje cell and the stimulating and recording arrangements used in this investigation are shown schematically in Fig. 1 *A*. Each Purkinje cell receives a single climbing fibre which makes extensive synaptic contacts with the proximal three-quarters of the dendritic tree (Palay & Chan-Palay, 1974). The climbing fibres were activated either by stimulating the inferior olive or by stimulation in the white matter in the vicinity of the anterior interpositus nucleus, which also resulted in antidromic activation of Purkinje cells. The parallel fibres were activated by means of a bipolar concentric electrode placed on the cerebellar surface. Such stimulation also elicited disynaptic inhibition in the Purkinje cell through cortical interneurones (only stellate cell shown). Forty cells were studied systematically with extracellular recordings and four cells with intracellular recordings. The units were identified as Purkinje cell somata according to well established criteria (Eccles *et al.* 1967). Most of them were recorded at depths of 300–400 μm, where one would expect Purkinje cell somata to be located. All units responded with typical c.f.r.s (complex spikes) and almost all of them with simple spikes. All spikes were biphasic and simple spikes and the initial spikes of c.f.r.s had large amplitudes, usually 20–30 mV.

Effect of preceding inferior olive stimulation on the secondary spikes of the climbing fibre response

Recordings from Purkinje cell somata revealed that when two shocks were applied to the inferior olive, within the interval range of 10–100 ms, the secondary spikes of the second (test) c.f.r. had a larger amplitude than those of the first (control) response. Examples of this phenomenon in extracellular recordings from three Purkinje cells can be seen in Fig. 1 *B–D*. Similar observations were also made in intracellular recordings (examples in Fig. 1 *E–G*). However, since it is difficult to record intracellularly for long periods it was necesary to confine the systematic study of the conditioning effect to extracellularly recorded responses. An increase in the amplitude of the secondary spikes was observed in each of the forty-four Purkinje cells recorded from. Other, less consistent, findings were that the number of secondary spikes decreased (Fig. 1 *C* and *F*) or that the wavelets, present in the control response, were absent in the test response (Fig. 1 *B* and *D*).

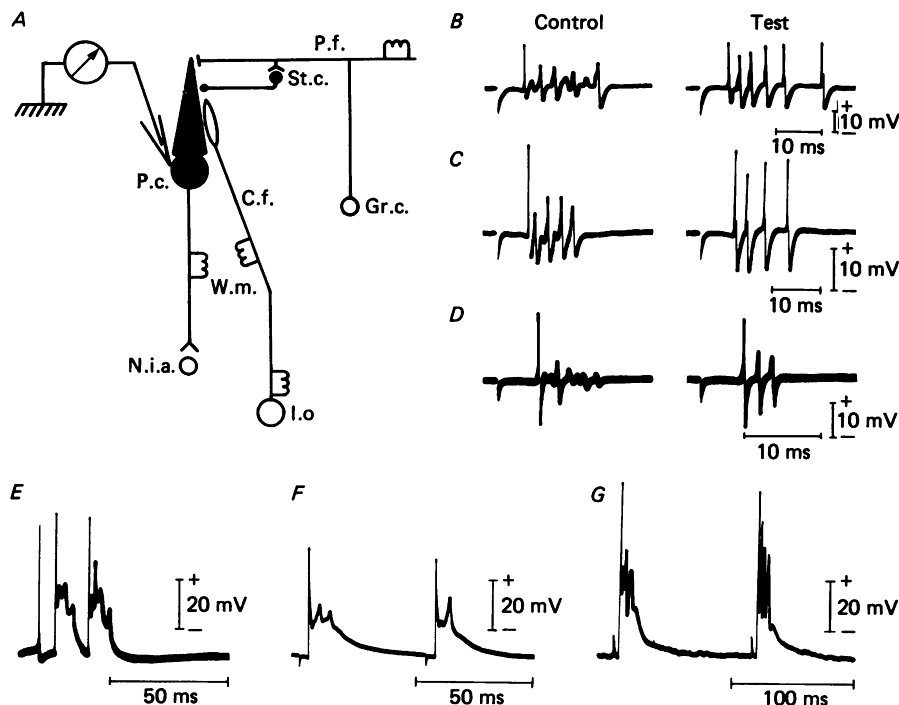

Fig. 1. *A*, stimulation and recording arrangement. Climbing fibres (c.f.) were stimulated by monopolar electrodes (cathodes) in contralateral inferior olive (i.o.) or in white matter (w.m.) close to the anterior interpositus nucleus (n.i.a.). W.m. stimulation resulted also in antidromic activation of the Purkinje cells (P.c.). Indifferent electrodes in temporal muscles. Parallel fibres (p.f.) stimulated by concentric electrode placed on cerebellar surface at least 1·2 mm from electrode recording extra- or intracellularly from soma of Purkinje cell. Connexions to Purkinje cell from i.o. and granule cell (gr.c.) shown schematically. St.c., stellate cell. Inhibitory neurones indicated in black. *B–D*, examples of extracellular recordings from three Purkinje cells showing c.f.r.s after single shocks to the i.o. (control) and after two shocks to the i.o. (test) at intervals of 22 ms (*B*), 26 ms (*C*) and 28 ms (*D*). In this, and in all following Figures, the control and test responses were recorded consecutively. *E–G*, examples of intracellular recordings from three Purkinje cells. In *E* a single shock to the superficial radial nerve produced two c.f.r.s. In *F* and *G* the c.f.r.s were evoked by two shocks to i.o. at intervals of 50 and 100 ms respectively.

Several experiments were performed in order to locate the source of the conditioning effect. The results of these experiments are shown in Fig. 2. The first test was designed to determine whether it was necessary that the first stimulus to the inferior olive elicited a c.f.r. in order to obtain the conditioning effect on the second response. Two single shocks were given to the inferior olive at an interval of 50 ms. The strength of the test stimulus was held constant (1 mA), while the strength of the conditioning stimulus was varied. Fig. 2*A* shows the effects of conditioning when the strength of the conditioning stimulus was just below threshold (sub *T*: 0·38 mA), at (*T*: 0·41 mA) and above threshold (supra *T*: 1 mA) for eliciting a c.f.r. The top row of traces shows control c.f.r.s recorded extracellularly. The lower rows of traces show conditioning or test regimes below, at and above threshold. The stimulus artifact of the

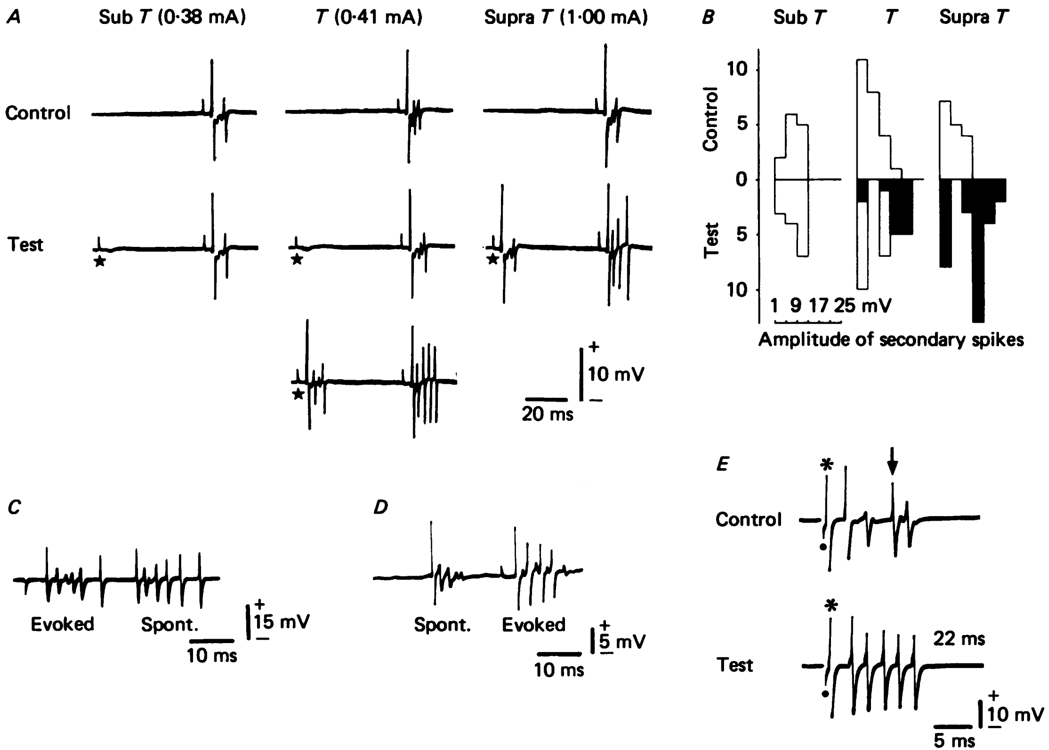

Fig. 2. Source of conditioning effect on secondary spikes. *A*, extracellular recordings from a Purkinje cell soma after inferior olive stimulation alone (control, 1 mA) or inferior olive stimulation (1 mA) preceded by a stimulus to the inferior olive below threshold (sub *T*; 0·38 mA), at (*T*; 0·41 mA) or above threshold (supra *T*; 1·00 mA) for eliciting a c.f.r. (test). Conditioning inferior olive stimulus artifact indicated by ★. *B*, quantitative analysis of secondary spike amplitude distribution. Each control or test histogram constructed from ten c.f.r.s. Amplitude distribution of secondary spikes (greater than 1 mV, see text) of control (upward) and test (downward) responses when conditioning stimulus was below threshold (sub *T*), at (*T*) or above threshold (supra *T*) for eliciting a c.f.r. Shaded bins indicate secondary spikes of c.f.r.s which were preceded by a conditioning stimulus which elicited a c.f.r. Bin width: 4 mV. *C*, extracellular recording from a Purkinje cell soma. C.f.r. evoked by stimulation of inferior olive followed by a spontaneous c.f.r. at an interval of 20 ms. *D*, spontaneous c.f.r. followed by inferior olive evoked response. *E*, extracellular recording from a Purkinje cell soma after white matter stimulation alone (control) or white matter stimulation preceded by a stimulus to the inferior olive at an interval of 22 ms (test). White matter stimulus artifact indicated by ●. Antidromic spike indicated by *. In control response, initial spike of c.f.r. generated by olivary reflex (see text) indicated by arrow.

conditioning stimulus is indicated by a star. With a subthreshold stimulus no effect was observed on the secondary spikes of the test response (Fig. 2*A*, left-hand column). At threshold, where the first stimulus to the inferior olive produced a c.f.r. in half the cases (middle column), an increase in the amplitude of the secondary spikes was observed in all cases where the first stimulus produced a c.f.r. and in no case where the first stimulus was ineffective. With suprathreshold stimulation (right-hand

column), there was always a clear increase in the amplitude of the secondary spikes of the test response.

The histograms in Fig. 2*B* show the amplitude distribution of the secondary spikes of ten pairs of control and test responses. When examining the conditioning effect on the secondary spikes in a quantitative manner, it was necessary to define the term 'secondary spike'. In the present study, to avoid possible uncertainties arising from the presence of wavelets, only biphasic spikes of amplitude greater than 1 mV were counted.

The histograms to the left in Fig. 2*B* show the effect of conditioning with a stimulus which was just below threshold for evoking a c.f.r. (0·38 mA). The amplitude distribution of secondary spikes was essentially similar in control (upwards) and test (downwards) responses. When the conditioning stimulus strength was increased to threshold (0·41 mA), half of the conditioning stimuli produced a c.f.r. It can be seen that those responses which were preceded by a conditioning c.f.r. (indicated by shading) had larger secondary spikes than those which were preceded by a stimulus which did not give rise to a c.f.r. When the conditioning stimulus was above threshold (1 mA) for eliciting a c.f.r. the test histogram shows a clear shift to the right compared with the control histogram, indicating the presence of larger amplitude secondary spikes in the test cases. An important fact, which is not revealed by the histograms, is that the augmentation of the secondary spikes occurred in practically every trial, although the magnitude of this augmentation varied. Furthermore, when an evoked c.f.r. was followed by (Fig. 2*C*) or preceded by (Fig. 2*D*) a spontaneous c.f.r., the secondary spikes of the second response were increased.

Thus, a c.f.r. must be elicited by the conditioning stimulus before the secondary spikes of the test response are affected. This suggests that the conditioning effect is generated either in the olivary cell or in the Purkinje cell.

In the unit illustrated in Fig. 2*E*, a single shock applied through the electrode in the white matter gave rise to three responses in the Purkinje cell (upper trace, control). The first response (latency 0·7 ms, *) is the antidromic spike generated by stimulation of the Purkinje cell axon. The second response (latency 3 ms) is the climbing fibre response generated by direct stimulation of the climbing fibre in the white matter. The third response is a c.f.r. (latency 9·5 ms, initial spike indicated with an arrow) resulting from a reflex discharge of the inferior olive cell following white matter stimulation. When the white matter stimulation was preceded by inferior olive stimulation (in this case, at an interval of 22 ms), the c.f.r. in the Purkinje cell was altered (Fig. 2*E*, lower trace, test). The antidromic spike (*) was unchanged. The secondary spikes of the direct c.f.r. were increased in amplitude and number. It is important to note that the effect on the secondary spikes occurs before a reflex response could occur and is therefore not due to an altered inferior olive output.

The time course of the conditioning effect was studied in twelve cells. The results from one cell are illustrated in Fig. 3. Two single shocks were given to the inferior olive at intervals of 10–150 ms. The amplitudes of the initial and secondary spikes of all responses were measured. Fig. 3 *A* shows examples of control (left-hand column) and test (right-hand column) responses at four intervals. It can be seen that there was a large increase in the amplitude of the secondary spikes at intervals up to 100 ms. At 150 ms there was no significant difference between control and test responses.

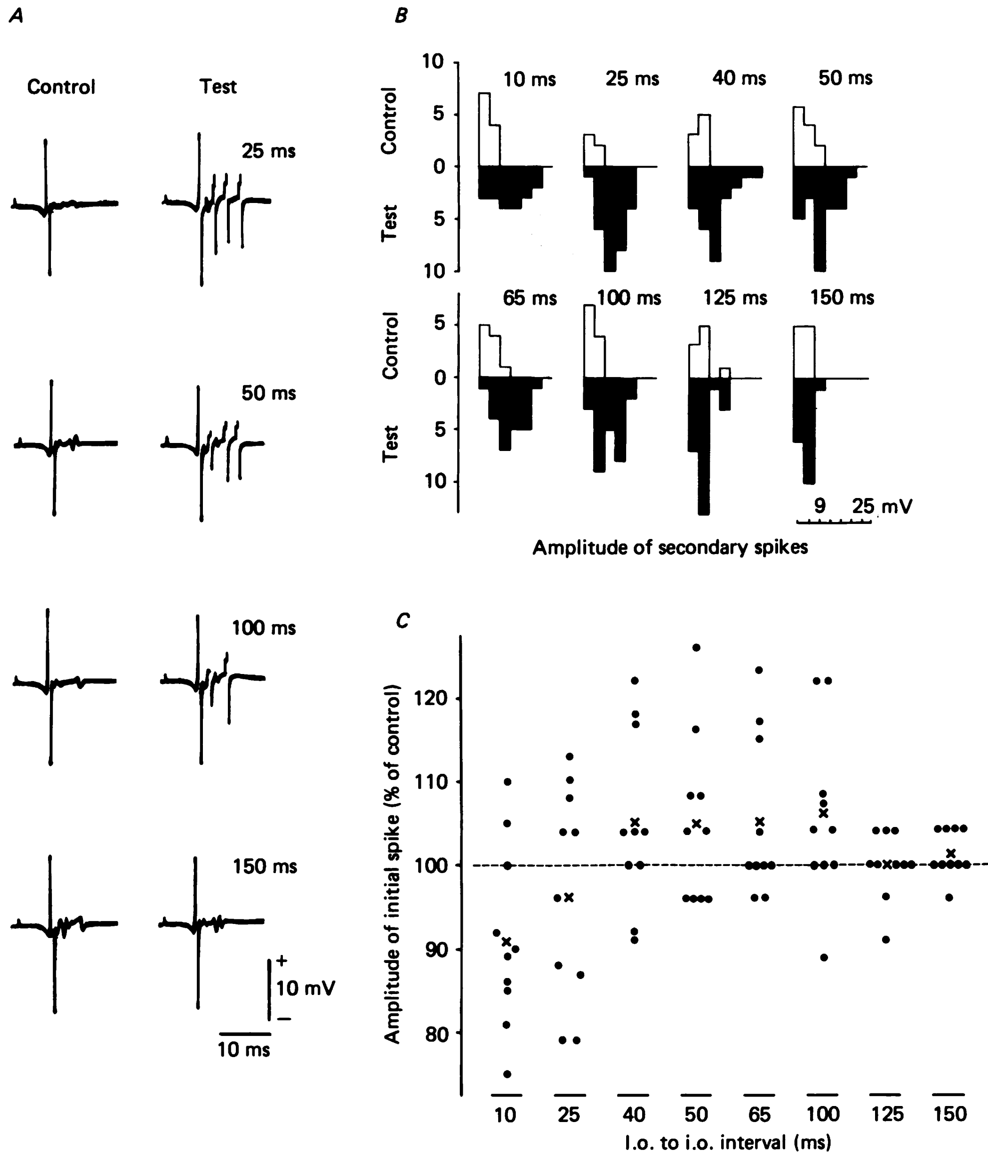

Fig. 3. Time course of inferior olive (i.o.) conditioning on initial and secondary spikes of test c.f.r.s. *A*, extracellular recordings from a Purkinje cell soma after i.o. stimulation alone (control column) or i.o. stimulation preceded by a stimulus to the i.o. at the intervals indicated in milliseconds (test column). *B*, quantitative analysis of secondary spike amplitude distribution at different i.o. to i.o. stimulus intervals. Each control or test histogram constructed from ten c.f.r.s. Control, upward. Test, downward, shaded. Bin width: 4 mV. *C*, amplitude of test initial spike (expressed as percentage of immediately preceding control response) *vs.* i.o. to i.o. stimulus interval (in milliseconds). ●, individual measurements; ×, mean values.

Fig. 3*B* is a quantitative illustration of the effect on the secondary spikes. The histograms were constructed, as described in the previous section, from ten pairs of control (upward) and test (downward) responses, and show that the amplitude of the secondary spikes increases after conditioning at intervals up to 100 ms. In this case, the number of secondary spikes apparently also increases, since the c.f.r. changes from an initial spike followed by wavelets (less than 1 mV in amplitude) to an initial spike followed by large biphasic spikes (Fig. 3*A*).

Fig. 3*C* shows the amplitudes of the initial spikes of test responses, expressed as a percentage of control responses, plotted against stimulus intervals. Mean amplitudes are indicated with crosses. At short intervals, presumably corresponding to the period of after-depolarization in the cell body following climbing fibre activation, the amplitude of the test initial spike was decreased. At longer intervals (up to 100 ms), there was a small (mean 5%) but consistent increase in the amplitude of the initial spikes. Thereafter, up to 150 ms, there was no difference between control and test responses.

Similar time courses were obtained in all twelve cells. The effect on the secondary spikes was always the same, although the magnitude of the amplitude increase varied (compare the examples in Figs. 1, 2 and 3) and the conditioning effect, in some cases, was difficult to observe at intervals longer than 50 ms. The effect of conditioning on initial spikes was always an initial reduction in amplitude lasting 12–25 ms. At longer intervals most cells showed no change in the amplitude of the initial spikes. In three cells there was a clear increase in initial spike amplitude, suggesting that a hyperpolarization followed the c.f.r. (e.g. Fig. 3*C*).

It is important to note that the effect of conditioning on the initial spikes is different from the effect on secondary spikes. At short intervals the amplitudes of the initial spikes are decreased while those of the secondary spikes are increased. At longer intervals the increase in the amplitude of the secondary spikes was much greater than that of the initial spikes. This suggests that the initial spike and the secondary spikes or wavelets are generated at different sites in the Purkinje cell.

Effect of preceding parallel fibre stimulation on the secondary spikes of the climbing fibre response

The effect on c.f.r.s of conditioning inferior olive stimulation at intervals of 10–150 ms may appear unphysiological since the normal discharge rate of the climbing fibres is of the order of 1 Hz. Nevertheless, the results suggest that the form of the c.f.r. can be affected by preceding events in the Purkinje cell. Thus, it was of interest to examine the effect of parallel fibre conditioning on the c.f.r.

Conditioning with parallel fibre stimulation was tested in Purkinje cells which lay both on and off the excited parallel fibre beam. A Purkinje cell was regarded as being 'on-beam' if parallel fibre stimulation evoked a simple spike in the cell body (eleven cases), or if a large e.p.s.p. could be recorded intracellularly in the same cell (one case). A cell was classified as 'off-beam' if a large i.p.s.p. and no e.p.s.p. could be recorded intracellularly on parallel fibre stimulation (four cells). The results from on- and off-beam stimulation were confirmed in nine additional cases in which either e.p.s.p.s or i.p.s.p.s were recorded, not from the cell under study, but from nearby Purkinje cells in the same track (five on-beam, four off-beam).

Recordings from Purkinje cells lying on-beam revealed that parallel fibre conditioning resulted in an increase in the amplitude of the secondary spikes in fifteen of the seventeen cells tested (there was no effect in the remaining two cells). In some cases, there was a reduction in the number of secondary spikes, as suggested by Eccles *et al.* (1966*b*), but the remaining secondary spikes were increased in amplitude. Fig. 4*A* shows examples of control (left-hand column) and test (right-hand column) responses in one cell at five intervals. The uppermost trace in the test column (5 ms interval) shows a simple spike elicited by parallel fibre stimulation at 1·5 mA (*) followed by the c.f.r. elicited by inferior olive stimulation. The conditioning effect obtained by parallel fibre stimulation when the Purkinje cell lies on-beam is remarkably similar to that obtained by inferior olive conditioning. Although the effect in this unit is smaller, there is a clear increase in the amplitude of the secondary spikes and a decrease in the number of small spikes and wavelets at intervals up to 100 ms. The magnitude of this effect varied, presumably because of variations in the ratio of excitation to inhibition. In some units the effect of parallel fibre conditioning was comparable to that of climbing fibre conditioning.

The histograms in Fig. 4*B* were constructed in the same manner as those in previous Figures. They show that the parallel fibre conditioning produces an increase in the amplitude of secondary spikes at intervals up to 100 ms. In two of the remaining six cells in which time courses were examined, the effects of parallel fibre conditioning lasted up to about 50 ms, and in the other four cases the effects could be seen at intervals up to 100, 130, 150 and 155 ms.

The effect of on-beam parallel fibre stimulation on the initial spikes of the c.f.r.s (Fig. 4*C*) is similar to that of inferior olive conditioning, at least at short intervals (5 ms) where the initial spike amplitudes were reduced. However, at longer intervals (10–195 ms) the initial spike amplitudes were consistently increased.

It is noteworthy that the effect on the amplitude of the secondary spikes of on-beam parallel fibre stimulation is not dependent on the presence of an evoked simple spike. In the cell illustrated in Fig. 5, the parallel fibre stimulation strength was adjusted so that a simple spike was evoked in 50% of the trials (0·6 mA). Fig. 5*A* shows a control c.f.r. evoked by inferior olive stimulation (*). Fig. 5*B* shows a c.f.r preceded by parallel fibre stimulation (arrow) which evoked a simple spike. It can be seen that there is an increase in the amplitude of the secondary spike of the test c.f.r. A similar increase in present in Fig. 5*C*, where parallel fibre stimulation did not evoke a simple spike. This occurred even when the parallel fibre stimulation strength was further reduced to 0·1 mA. In Fig. 5*D*, however, where the c.f.r. is preceded by a spontaneous simple spike, there is no effect on the secondary spikes.

The effect of on-beam parallel fibre stimulation on the amplitude of the secondary spikes is shown quantitatively in a histogram in Fig. 5*E* which was constructed from twenty pairs of control and test c.f.r.s in the same unit. The test histogram indicates that c.f.r.s preceded by parallel fibre stimulation tend to have secondary spikes of larger amplitude than control c.f.r.s. That the effect is not due to presence of a simple spike can be seen from the shaded bins in the test histogram. These bins indicate secondary spikes of c.f.r.s ($n = 10$) preceded by parallel fibre stimulation which *did not* evoke a simple spike. The distribution for these secondary spikes is the same as the distribution for secondary spikes preceded by parallel fibre stimulation giving rise

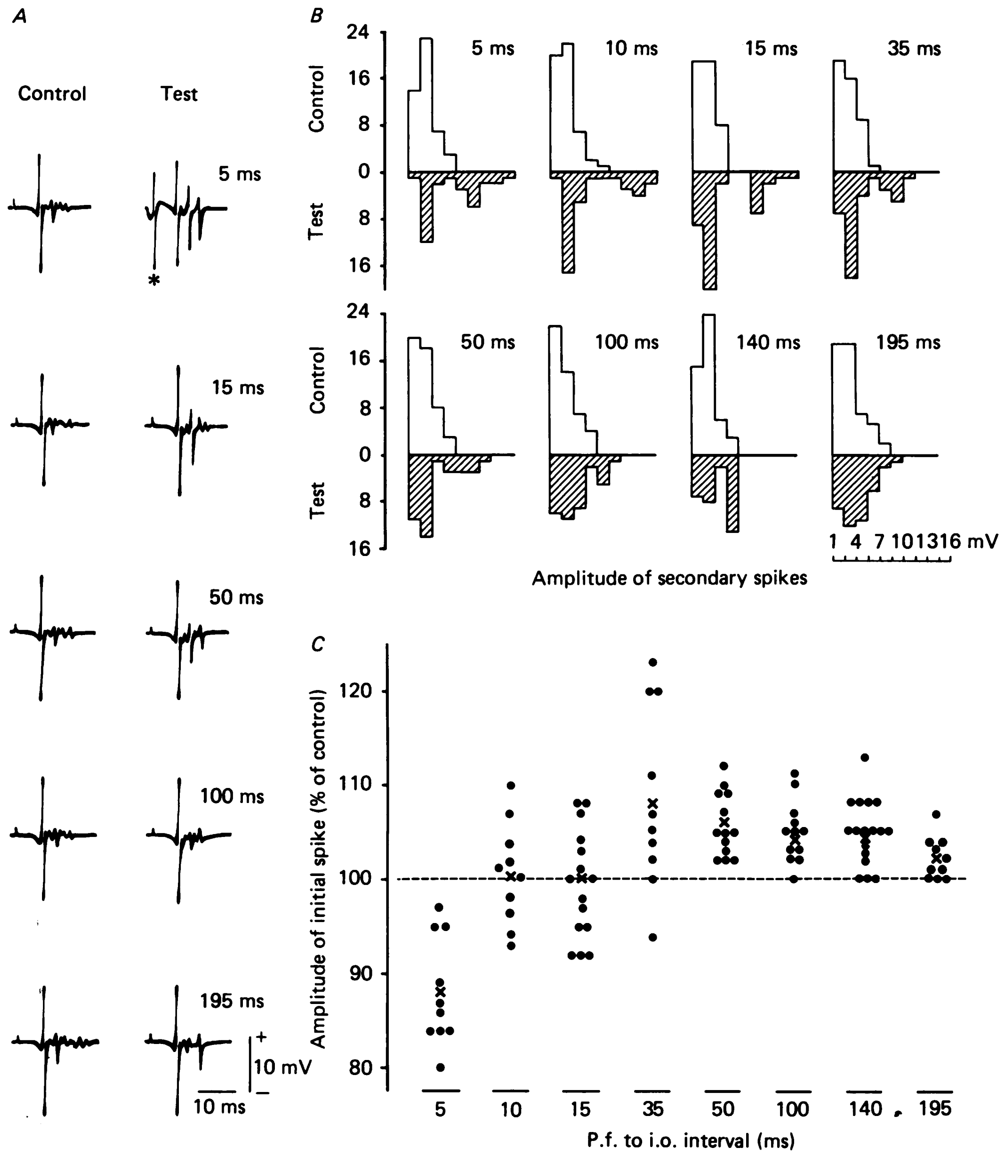

Fig. 4. Time course of parallel fibre (p.f.) conditioning (on-beam) on initial and secondary spikes of test c.f.r.s. *A*, extracellular recordings from a Purkinje cell soma after inferior olive (i.o.) stimulation alone (control column) or i.o. stimulation preceded by p.f. stimulation at the intervals indicated in milliseconds (test column). P.f. stimulation elicited a simple spike in the Purkinje cell (indicated by * at the shortest p.f. to i.o. interval: top trace, test column). *B*, quantitative analysis of secondary spike amplitude distribution at different p.f. to i.o. stimulus intervals. Control, upward. Test, downward, hatched. Bin width: 1·5 mV. *C*, amplitude of test initial spike (expressed as percentage of immediately preceding control response) *vs.* p.f. to i.o. stimulus interval (in milliseconds). ●, individual measurements; ×, mean values.

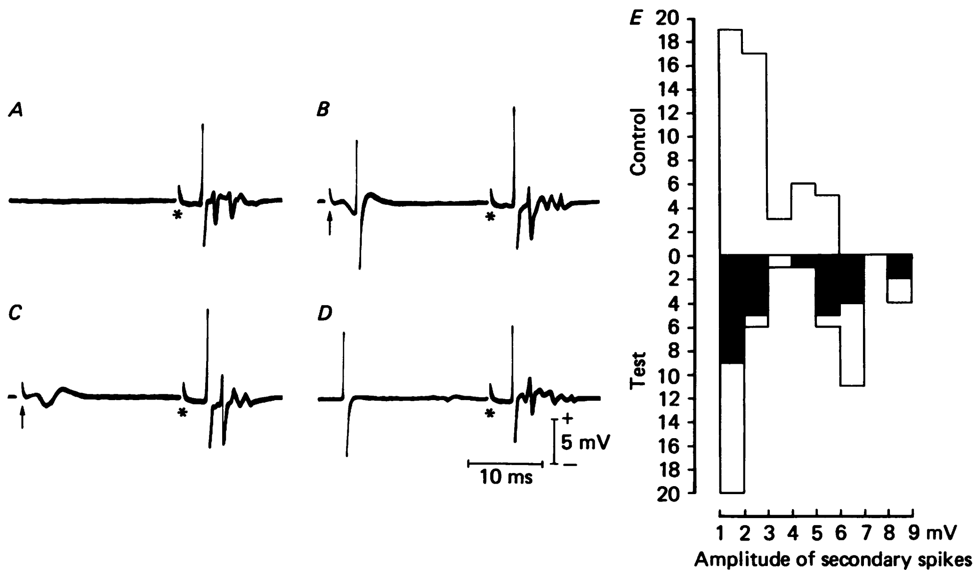

Fig. 5. Effect of parallel fibre (p.f.) conditioning on the secondary spikes of c.f.r.s in the presence or absence of a simple spike. *A*, extracellular recording from a Purkinje cell after inferior olive (i.o.) stimulation (*). *B*, recording from same cell after i.o. stimulation preceded by p.f. stimulation (arrow) which evoked a simple spike. *C*, recording from same cell after i.o. stimulation preceded by p.f. stimulation which did not evoke a simple spike. *D*, recording from same cell after i.o. stimulation preceded by spontaneous simple spike. P.f. stimulation strength 0·6 mA. P.f. to i.o. stimulus interval 22 ms. *E*, quantitative analysis of secondary spike amplitude distribution for Purkinje cell illustrated in *A–D*. Histograms constructed from twenty paired control (upward) and test (downward) c.f.r.s. Shaded bins represent secondary spikes from conditioned c.f.r.s ($n = 10$) which *were not* preceded by an evoked simple spike. Bin width 1 mV.

to a simple spike (unshaded bins). Thus, it appears that the increase in amplitude of the secondary spikes is generated by parallel fibre input to the Purkinje cell and not by the mechanisms responsible for spontaneous activity.

Recordings from Purkinje cells lying off-beam show that parallel fibre conditioning resulted in a decrease in the amplitude of the secondary spikes in all eight cells tested. The results from one cell are illustrated in Fig. 6. Examples of control (left-hand column) and test (right-hand column) responses at five intervals are shown in Fig. 6*A*. It was shown by Eccles *et al.* (1966*b*) that parallel fibre stimulation results in a reduction in the number of secondary spikes of a succeeding c.f.r. In the present investigation similar results were obtained, but they are described as decreases in the *amplitude* of the secondary spikes. This is a consequence of counting all potential deflexions larger than 1 mV as spikes.

At short intervals (< 10 ms), control and test response amplitudes are similar. At longer intervals (20–100 ms), there was a decrease in the number of secondary spikes with large amplitudes and an increase in the number of small spikes and wavelets (note that wavelets are not included in the histograms). This effect is shown quantitatively in Fig. 6*B*, where the decrease in amplitude of the secondary spikes is observed in the test histograms. This cell was not tested at intervals longer than

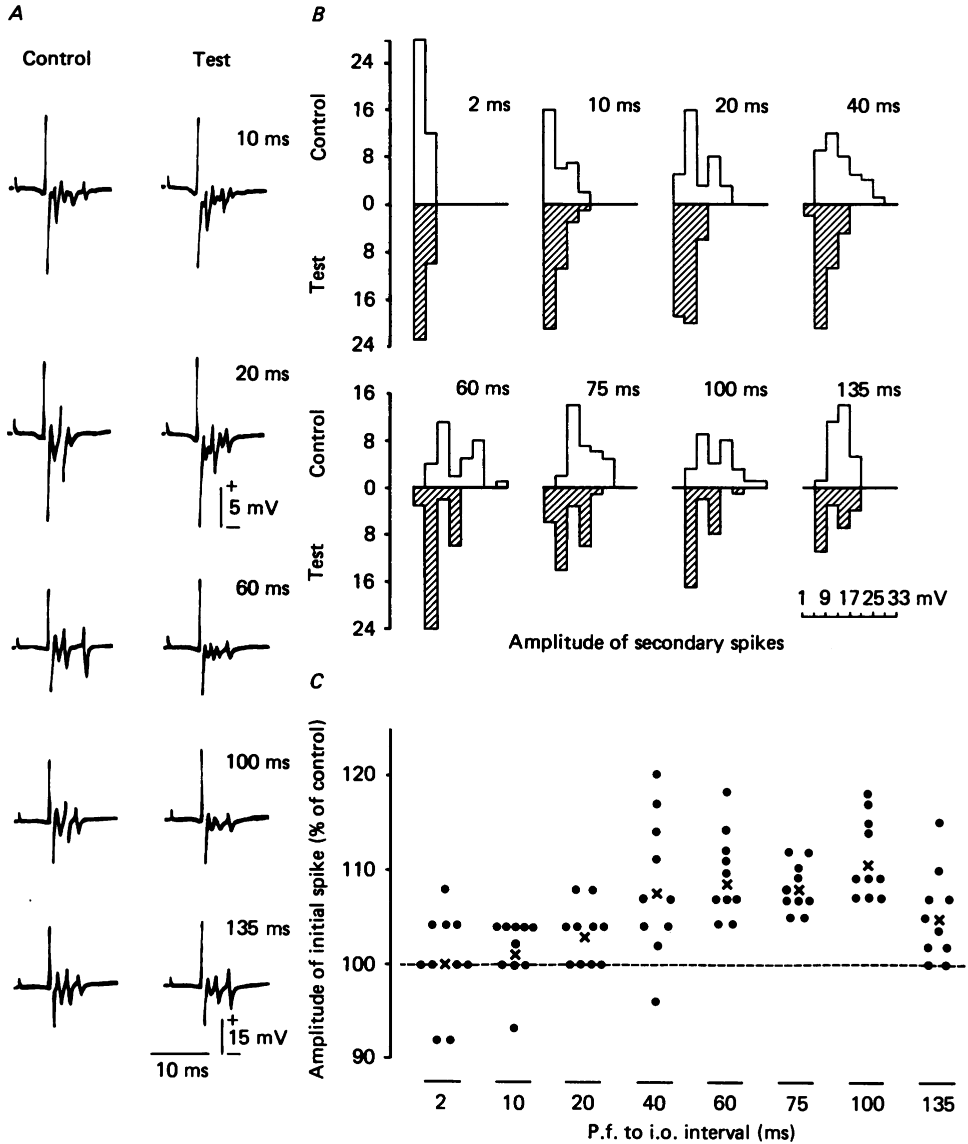

Fig. 6. Time course of parallel fibre (p.f.) conditioning (off-beam) on initial and secondary spikes of test c.f.r.s. *A*, extracellular recordings from a Purkinje cell soma after inferior olive (i.o.) stimulation alone (control column) or i.o. stimulation preceded by p.f. stimulation at the intervals indicated in milliseconds (test column). Note: upper two traces and lower three traces have different voltage calibrations. *B*, quantitative analysis of secondary spike amplitude distribution at different p.f. to i.o. stimulus intervals. Each control or test histogram constructed from ten c.f.r.s. Control, upward. Test, downward, hatched. Bin width: 4 mV. *C*, amplitude of test initial spike (expressed as percentage of immediately preceding control response) *vs.* p.f. to i.o. stimulus interval (in milliseconds). ●, individual measurements; ×, average values.

135 ms. After completion of the tests described above the cell was penetrated with the micro-electrode, and parallel fibre stimulation was found to generate an i.p.s.p., the duration of which was found to vary between 100 and 200 ms. In three other cells, conditioning effects were seen at intervals up to 150 ms, and in one cell up to 400 ms. Examination of the test c.f.r.s revealed that in a cell lying off-beam, there was no change in the amplitude of the initial spike at short intervals (< 10 ms) (Fig. 6*C*). At longer intervals, there was an increase in initial spike amplitude, reaching a maximum at 100 ms.

DISCUSSION

The main findings of the present study are summarized in a simplified and semiquantitative way in Fig. 7. The effects of climbing fibre and parallel fibre conditioning on the initial spike of a c.f.r. are shown in the upper half of the Figure. A preceding c.f.r. reduces the amplitude of the initial spike at short intervals and has no effect (in three units there was a small increase) at longer intervals. On-beam activation of parallel fibres results in a reduction of the initial spike amplitude at short intervals and an increase thereafter, while off-beam stimulation results only in an increase in initial spike amplitude. The effects of conditioning on the secondary spikes are depicted in the lower half of the Figure. Climbing fibre conditioning causes a large increase in the amplitude of the secondary spikes, as does on-beam parallel fibre stimulation, while off-beam p.f. stimulation reduces the amplitude of the secondary spikes. Although both the durations and the magnitudes of the effects were variable, the values used in Fig. 7 are representative of the investigated cells.

Effects on initial spike

The effects of conditioning on the initial spike can be satisfactorily explained by well known membrane properties of nerve cells. The after-depolarization which follows the initial spike of a c.f.r., and which lasts for 15–25 ms (Armstrong 1974), would be expected to inactivate the sodium channels responsible for the initial spike (Llinás & Sugimori, 1980*a*), and reduce the amplitude of the initial spike of a second c.f.r. occurring during this period. Such a depolarization will also reduce the difference between the membrane potential and the sodium equilibrium potential, which would also contribute to the reduction in the amplitude of the initial spike. The increase in the amplitude of the initial spikes which was observed at longer intervals in some units may be analogously explained by the hyperpolarization which sometimes follows the c.f.r. (Martinez *et al.* 1971).

Similar mechanisms can explain the effects of parallel fibre conditioning. Since on-beam parallel fibre stimulation results in an e.p.s.p. followed by an i.p.s.p. (Eccles *et al.* 1967), one would expect the initial spike of a subsequent c.f.r. to have a reduced amplitude during the e.p.s.p. and an increased amplitude thereafter. The duration of the conditioning effect (up to 200 ms) fits well with reports that the i.p.s.p. typically lasts for 150–200 ms (Eccles *et al.* 1967). It should be noted that such measurements are biased towards events occurring in the more proximal dendrites which are easier to record from, and where potential durations may be somewhat different from those occurring in the distal dendrites. The effect of conditioning on the initial spike of c.f.r.s

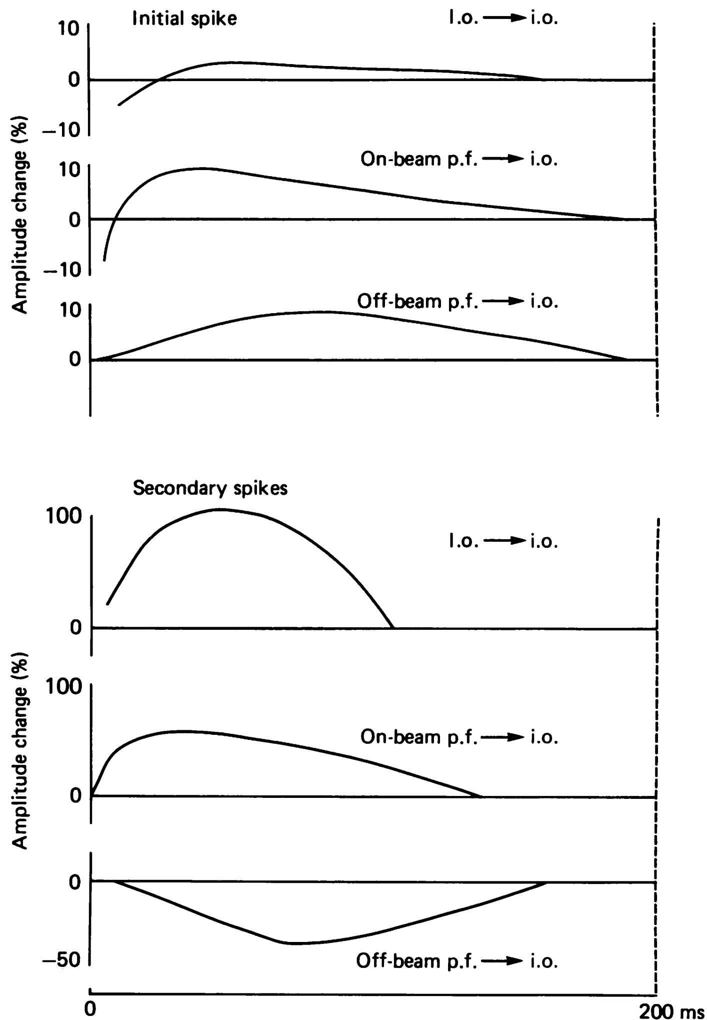

Fig. 7. Summary of conditioning effects. Schematic outline of the average effects of various conditioning procedures on c.f.r.s. Upper half of Figure shows amplitude, as percentage of control, of the initial spike of a c.f.r. when preceded at different intervals by inferior olive (i.o.) stimulation, parallel fibre (p.f.) stimulation on-beam and parallel fibre stimulation off-beam. Lower half of Figure shows corresponding changes in the amplitude of secondary spikes.

in Purkinje cells off the beam of excited parallel fibres was similar, except that at the shorter intervals the initial spike amplitude was unchanged. This is to be expected, since the corresponding intracellular event is a disynaptic i.p.s.p. with no preceding e.p.s.p.

Effects on secondary spikes

A striking feature of Fig. 7 is that the effects of conditioning on the secondary spikes differ from the effects on the initial spike. These differences are difficult to reconcile

with the traditional view that the secondary spikes are abortive somatic spikes (Granit & Phillips, 1956) which are smaller than the initial spike because the large c.f.r. after-depolarization inactivates the somatic membrane. First, there is a large increase in the amplitude of the secondary spikes of a c.f.r. after climbing fibre conditioning and a smaller but consistent increase after on-beam parallel fibre stimulation. The effect of climbing fibre conditioning was also observed by Martinez *et al.* (1971), who suggested that it was due to the hyperpolarization which sometimes follows the c.f.r. If this was correct, however, one would expect an even more marked amplitude increase with on-beam parallel fibre conditioning, since the hyperpolarization following parallel fibre stimulation is much greater than that which follows the c.f.r. Instead, there is a smaller increase.

Secondly, the effects of on- and off-beam parallel fibre conditioning on the initial spike of a c.f.r. are similar at longer intervals, whereas the effects of on- and off-beam parallel fibre stimulation on the secondary spikes are opposite. Again, if the secondary spikes are abortive spikes generated in the Purkinje cell soma, on- and off-beam stimulation should have similar effects.

These observations suggest that the secondary spikes are generated by a different mechanism from that responsible for the initial spike of the c.f.r. A plausible hypothesis is that they are generated in the dendritic tree and electrotonically conducted to the soma. This fits well with the observation of Martinez *et al.* (1971) that secondary spikes cannot always be collided out by antidromic spikes in the Purkinje cell axons, suggesting that secondary spikes need not involve the soma-hillock region of the Purkinje cell. Furthermore, Llinás & Sugimori (1980*b*) have observed spike-like potentials in Purkinje cell dendrites. This does not exclude the possibility that the secondary spikes may, under some conditions, trigger action potentials in the soma-hillock region of the Purkinje cell. Indeed, there is good evidence that this does occur (Ito & Simpson 1971; Campbell & Hesslow, 1986).

In view of this, some other explanation must be proposed to account for the effects on the secondary spikes. A plausible cause for the effect of climbing fibre conditioning is the long-lasting plateau-like depolarization which is triggered in the Purkinje cell dendrites by climbing fibre activation (Ekerot & Oscarsson, 1981). Such a depolarization might facilitate triggering and/or conduction of dendritic spikes evoked by a second climbing fibre response. The duration of this potential has been estimated to be 50–100 ms in the proximal dendrites, which is consistent with the duration of the conditioning effect.

It is possible that this long-lasting depolarization can explain the effects of on-beam parallel fibre conditioning as well. It has recently been shown (Campbell *et al.* 1983) that activation of parallel fibres by stimulation of the cerebellar surface can evoke similar plateau-like potentials. A difficulty with this explanation is that the stimulation strengths required to evoke plateau potentials by parallel fibre volleys (at least 0·5–1 mA) were higher than those necessary to obtain the conditioning effect reported here (sometimes down to 0·1 mA). More importantly, secondary spike amplitude was often increased with stimulation strengths well below the threshold for evoking simple spikes, and thus, presumably, weaker than the natural input to the cells. It cannot be excluded, however, that a weaker stimulation can evoke plateau potentials in finer distal dendrites. Surface stimulation activates mainly superficial parallel fibres and

distal dendrites in the Purkinje cells, in which plateau potentials are more difficult to observe. A second difficulty is that the duration of the conditioning effect was greater for on-beam parallel fibre stimulation (usually 100–150 ms) than for climbing fibre activation (50–100 ms), although the effectiveness of parallel fibre stimulation in evoking plateau potentials was less than that of climbing fibre activation (Campbell *et al.* 1983). Again, this might reflect different properties of proximal and distal dendrites.

The reduction in the amplitude of the secondary spikes after off-beam parallel fibre stimulation may be explained by the i.p.s.p.s produced by such stimulation. A hyperpolarization could prevent triggering of dendritic spikes, and reduced membrane resistance during the i.p.s.p. would impede electrotonic conduction of such spikes.

Functional considerations

An important question raised by the present study is whether the conditioning effects observed occur under normal physiological conditions. With regard to preceding climbing fibre stimulation, it should be noted that the firing frequency of the inferior olive is very low, usually below 2 Hz (Armstrong, 1974), and it might be thought that c.f.r.s do not occur at intervals below 100 ms. However, c.f.r. intervals short enough to permit a conditioning effect have been reported in the literature (see Campbell & Hesslow, 1986 for references).

The parallel fibre volleys evoked by stimulating the surface of the cerebellar cortex are also artificial, and such synchronous excitation of the Purkinje cells probably never occurs *in vivo*. However, recent findings suggest that the vertically oriented axons of the granule cells make repeated synaptic contacts with the dendrites of a single Purkinje cell, and that a single impulse in such an axon is sufficient to evoke a simple spike in the Purkinje cell (Llinás, 1982; Bower & Woolston, 1983). Such activation may produce a sufficient depolarization in the Purkinje cell to affect the secondary spikes. Indeed, since in the present study a conditioning effect was sometimes seen even in the absence of a simple spike, it may be suggested that relatively small inputs from the granule cells can affect the form of the c.f.r.

A possible objection to the proposed interaction between granule cell (parallel fibre) input and the secondary spikes of c.f.r.s is that previous investigations have not found any relationship between the secondary spikes and the spontaneous simple spike frequency (Mano, 1970). If the simple spikes are produced solely by parallel fibre input, one would expect that the secondary spikes of a c.f.r. would be increased in amplitude following spontaneous simple spike firing. This is not the case. However, there is now considerable evidence that there is an intrinsic pace-maker activity in the Purkinje cell. Thus, the spontaneous firing rate is not a reliable measure of the excitatory input to the cell (see Gruol, 1983 for references). It has been suggested (Eccles *et al.* 1967) that the number of secondary spikes in a c.f.r. signals the over-all excitability level of the Purkinje cell. The present study suggests that the form of the c.f.r. may be influenced by the *input* to the cell and not by the background firing level.

Since the function of the secondary spikes is unknown, it is difficult to speculate on the functional significance of modifying them. However, there is evidence that the secondary spikes of c.f.r.s can be transmitted down the Purkinje cell axon (Ito &

Simpson, 1971) and therefore, since the amplitudes of the secondary spikes can be altered, the number of spikes transmitted down the axons may also vary, depending on the input to the Purkinje cell. This problem will be addressed in the accompanying paper (Campbell & Hesslow, 1986).

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