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

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SUMMARY

1. Responses evoked in Purkinje cells by climbing fibre activity were investigated by recording from Purkinje cell axons in the cerebellum of anaesthetized cats. Purkinje cell axons were identified by firing pattern and by latency of responses to stimulation of peripheral nerve and of the inferior olive.

2. Axonal climbing fibre responses usually consisted of one to two spikes, suggesting that normally only the initial spike or, at most, this and one of the secondary spikes are propagated down the Purkinje cell axon.

3. When two successive climbing fibre responses were evoked, the number of spikes in the second response was increased, usually up to three to five. This effect could be obtained at stimulation intervals of up to 100 ms.

4. In a few cases it was possible for a climbing fibre response to be preceded by a parallel fibre volley evoked by stimulation of the cerebellar surface. This increased the number of spikes in the axonal climbing fibre response.

5. The results suggest that the number of propagated spikes in the climbing fibre response can be modified by a preceding input to the Purkinje cell.

INTRODUCTION

In view of the very low discharge rate of olivary cells (1–2 Hz), it seems unlikely that the information carried by climbing fibres is simply transferred to the frequency code of the Purkinje cells, which usually have a background firing rate of 20–60 Hz. It has been speculated that the climbing fibre system is a ‘ teaching line ’ (Marr, 1969; Albus, 1971) which hetero-synaptically induces changes in the parallel fibre–Purkinje cell synapses. Indeed, in recent years, several investigators have shown that climbing fibre input can modify the responsiveness of the Purkinje cell (Colin, Manil & Desclin, 1980; Ito, Sakurai & Tongroach, 1982; Rawson & Tsilokskulchai, 1982; Montarolo, Palestini & Strata, 1982; Ebner & Bloedel, 1981).

However, this does not exclude the possibility that the Purkinje cell response to climbing fibre input is also important for the immediate output of these cells and for the information carried to the nuclear cells (Llinás & Hillman, 1969). A crucial question in this context is the extent to which the secondary spikes of the climbing fibre response (c.f.r.) are propagated down the Purkinje cell axon. If only the initial

spike is propagated, this extra action potential may be of little importance to the function of the nuclear cell, while a burst of impulses, due to propagation of secondary spikes, could have a marked effect. Previous investigations have not completely solved the problem. Thach (1967) suggested that only the initial spike of the climbing fibre response was propagated. Martinez, Crill & Kennedy (1971) showed that the largest secondary spikes could collide out an antidromic spike, suggesting that some secondary spikes are propagated. Ito & Simpson (1971) recorded from presumed Purkinje cell axon terminals and found that climbing fibre activation produced a burst of two to five impulses in about 50 % of the tested units. In the remaining cases only the initial spike of the c.f.r. was propagated.

Since the c.f.r. in the Purkinje cell soma often contains four to six spikes (Armstrong, 1974; Campbell & Hesslow, 1986), this would imply that many of the secondary spikes do not trigger that part of the Purkinje cell responsible for propagated action potentials. It has recently been shown, however, that the amplitude of the secondary spikes is strongly influenced by a preceding input to the Purkinje cell (Campbell & Hesslow, 1986). When two c.f.r.s occur at intervals up to 50–100 ms, the amplitudes of the secondary spikes of the second c.f.r. are usually greatly increased. On- or off-beam parallel fibre volleys preceding a c.f.r. can also increase and decrease respectively the amplitude of the secondary spikes. If only the larger secondary spikes are propagated down the axon, it would be expected that changes in secondary spike amplitude would change the number of spikes in the axonal c.f.r.

The present investigation was undertaken in order (a) to determine whether the secondary spikes are transmitted down the Purkinje cell axon and (b) to test the hypothesis that the number of spikes transmitted can be influenced by a preceding input to the Purkinje cell as suggested by Eccles, Ito & Szentágothai (1967) and Eccles, Llinás, Sasaki & Voorhoeve (1966).

METHODS

The preparation of the nine cats used for these experiments was identical to that described by Campbell & Hesslow (1986). Methods of stimulation and recording were also similar. In the present investigation, parallel fibres were stimulated both with bipolar concentric electrodes and with monopolar silver ball electrodes (cathodal stimulation). Stimulus strength was 0·5–2·0 mA. In the present investigation, micro-electrode recordings were made from the white matter dorsal to the nucleus interpositus anterior. The electrode was inserted into the cerebellar cortex in the C3 zone just caudal to the inferior colliculus in lobulus IV and then driven towards the vicinity of the anterior interpositus nucleus (Fig. 1*A*). Care was taken to ensure that all recordings were from depths where no cell bodies are present.

RESULTS

Identification of Purkinje cell axons

The units studied had properties typical of axons. They all responded with monophasic spikes of relatively small amplitude, usually 1–10 mV, and they were easily lost when the recording electrode was moved. In order to identify Purkinje cell axons, the following criteria were employed. (a) Latency of c.f.r. In the C3 zone, the latency of c.f.r.s in Purkinje cell somata on direct stimulation of the inferior olive

is 3·5–4·5 ms (Campbell & Hesslow, 1986). The latency of the c.f.r.s in the axons would not be expected to differ much from the somatic latencies. Only units which consistently fired at least one spike after stimulation of the inferior olive at a latency of 4–5 ms were included. (b) Frequency of firing. Purkinje cells have a typical background firing frequency of 20–60 Hz. (c) Pattern of firing. Purkinje cells usually have long periods of quiescence, and pauses in firing after a c.f.r. A total of seventy-one units were classified as Purkinje cell axons according to these criteria. However, since the criteria are indirect, these units will be referred to as presumptive Purkinje cell axons.

Of those units which did not satisfy the aforementioned criteria, most could be classified as climbing fibres or mossy fibres. Presumed climbing fibres responded to inferior olive stimulation with a latency of 2–3 ms, had a spontaneous discharge rate of 1–2 Hz and fired in bursts of two to three spikes which, on conditioning with inferior olive stimulation, were reduced to a single spike. This effect of inferior olive conditioning is to be expected in the climbing fibres because of the recurrent inhibition demonstrated in olivary cells (Armstrong & Harvey, 1966; Crill, 1970). Only a few presumed mossy fibres were activated by inferior olive stimulation. These units responded at short latencies (*ca.* 2 ms) and had stable discharge frequencies of around 20 Hz. Since they also had higher thresholds than climbing fibres, their origin was presumably outside the inferior olive.

Effect of climbing fibre conditioning

Stimulation of the inferior olive generally resulted in a response consisting of one to two action potentials in presumed Purkinje cell axons.

Only rarely were more than two spikes evoked (see below for details), although bursts of up to five spikes were observed occasionally. As mentioned in the introduction, the amplitude of the secondary spikes of the somatic c.f.r. is increased by a preceding c.f.r. (Campbell & Hesslow, 1986). In order to test the possibility that this might be reflected in a greater number of spikes being propagated down the Purkinje cell axon, systematic conditioning experiments were carried out on seventy-one presumed Purkinje cell axons.

Some representative examples of recordings of axonal c.f.r.s from four different units are shown in Fig. 1*B*. These reveal that, when the axonal c.f.r. was conditioned by a preceding c.f.r., the number of spikes in the second response could be increased to up to five spikes. The magnitude of the effect for the unit shown in Fig. 1*B* is shown in Fig. 2*A*. The histogram is based on ten pairs of alternating test and control responses to inferior olive stimulation, and shows the distribution of responses with different numbers of spikes. The stimulation interval was 20 ms. Control responses are above, and test responses below, the abscissa. In determining the number of spikes in the c.f.r., only spikes occurring within 15 ms after the initial spike were counted. This value was chosen since it represents the normal duration of the somatic c.f.r.

This conditioning effect was very reliable. In sixty-six of the seventy-one units tested, an increased spike number was observed in the second of two axonal c.f.r.s. Of the five units in which climbing fibre conditioning failed to increase the number of spikes, there was a decrease in two units and three units were unaffected. The histogram in Fig. 2*B* shows the over-all magnitude of the effect, using data from

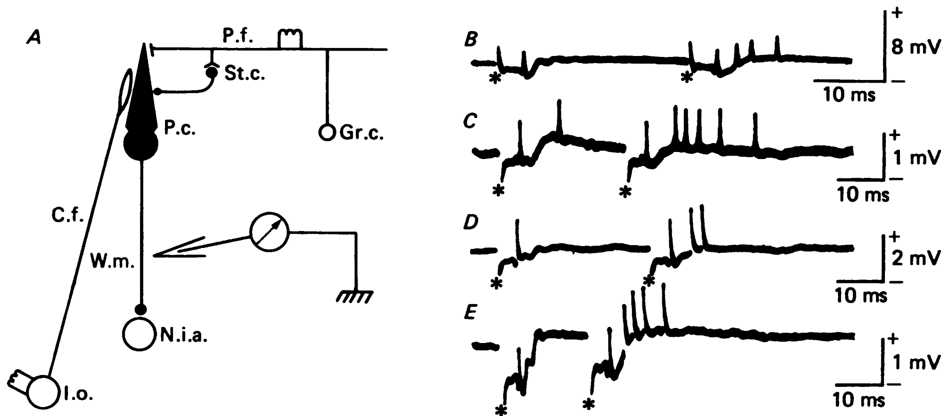

Fig. 1. *A*, stimulation and recording arrangement. Climbing fibres (c.f.s) were stimulated in the contralateral inferior olive (i.o.). Parallel fibres were stimulated with monopolar (cathode) or concentric bipolar electrodes on the cerebellar surface. Micro-electrode recordings were made from presumptive Purkinje cell (P.c.) axons in white matter (w.m.) dorsal to the anterior interpositus nucleus (n.i.a.). Gr.c., granule cell. St.c., stellate cell. *B–E*, typical recordings of responses in presumptive P.c. axons on stimulation of i.o. First shock to i.o. results in a response with one to two spikes, while response to second shock contains four to five spikes. Unit responses superimposed on background field potential. *, shock artifact of i.o. stimulation.

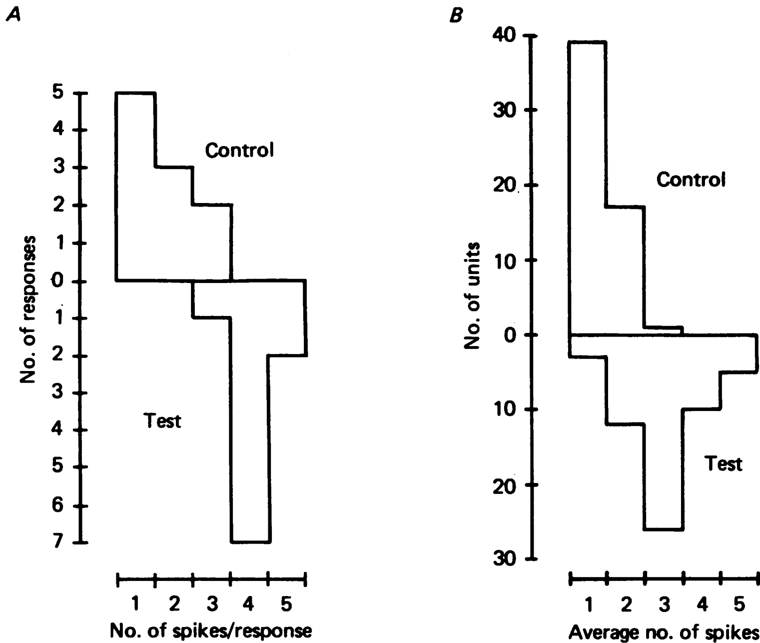

Fig. 2. Quantitative analysis of effect of climbing fibre conditioning. *A*, distribution of axonal c.f.r.s with different numbers of spikes for the unit in Fig. 1*B*. Each bar represents number of responses with the number of spikes indicated at the bottom. Histogram based on ten control (above) and ten test responses (below abscissa). Interval between shocks to inferior olive was 20 ms. *B*, distribution of fifty-seven units with different average numbers of spikes in control (above) and test responses (below abscissa). Each bar represents total number of units with the average number of spikes/response indicated at the bottom.

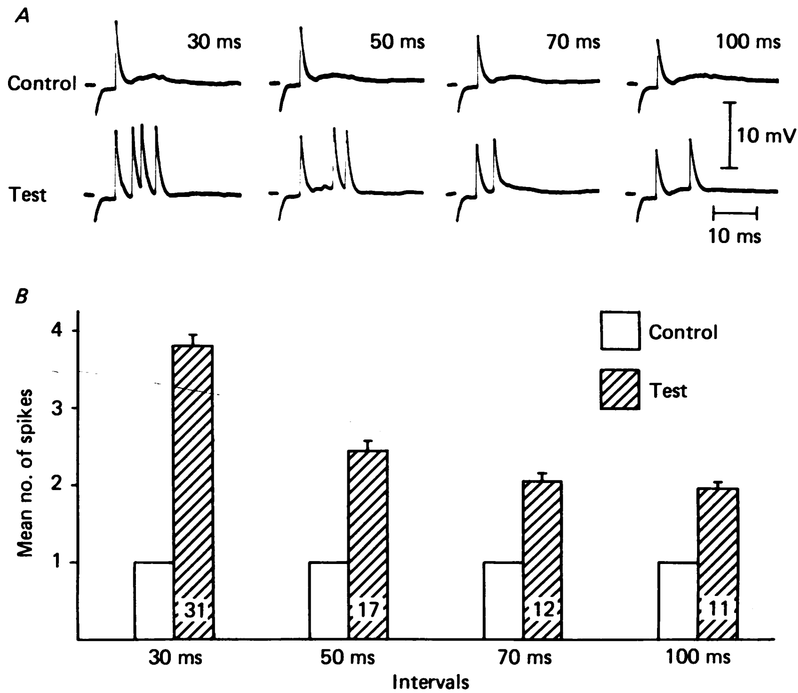

Fig. 3. Time course of effect of climbing fibre conditioning. *A*, records of control (upper traces) and test (lower traces) responses at different intervals. *B*, histogram showing mean spike number (+S.E. of mean) for the same cell in control (unshaded) and test (shaded) responses. Number of response pairs from which means were calculated are shown within the shaded areas.

fifty-seven units in which climbing fibre conditioning was tested at least one interval between 15 and 25 ms, and for which there were at least ten pairs of responses. The average number of spikes was calculated for each unit both with and without preceding climbing fibre conditioning. Control responses are depicted above and test responses below the abscissa.

The effectiveness of climbing fibre conditioning in increasing the number of spikes in the axonal c.f.r. was tested at different intervals in fourteen units. An example is shown in Fig. 3*A*. The bar graph in Fig. 3*B* is based on the same unit. Each bar shows the average number of spikes in control responses (unshaded) and conditioned responses (shaded). S.E. of mean is indicated in the test bars. There was no variation in the number of spikes in the control responses. The number of response pairs is indicated within the shaded bars. A clear effect of conditioning is present up to 100 ms. This unit was not tested at longer intervals. In the other units, an effect was always present at intervals up to 50 ms. Thereafter, the effect gradually disappeared. In six units the effect lasted about 100 ms, but could not be seen at longer intervals.

Effect of parallel fibre conditioning

With the techniques used in the present investigation, the effects of parallel fibre

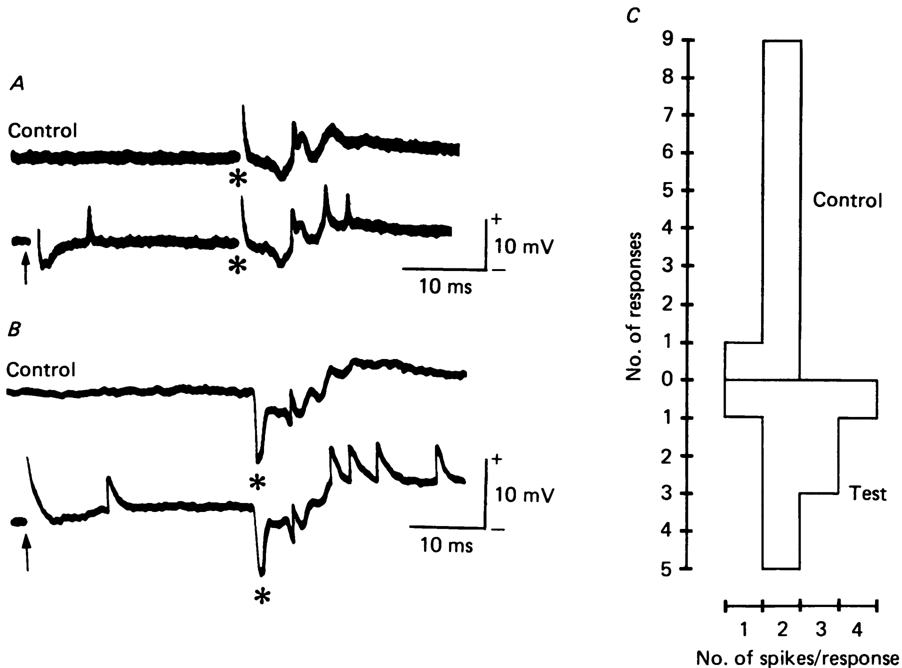

Fig. 4. Effect of parallel fibre conditioning on number of spikes in an axonal c.f.r. *A* and *B*, typical recordings from two Purkinje cell axons which were activated by parallel fibre stimulation. Upper traces (controls) show responses to inferior olive stimulation. Lower traces show c.f.r.s preceded by parallel fibre stimulation. Unit responses to inferior olive stimulation superimposed on a background field potential. Inferior olive shock artifact indicated by *; parallel fibre shock artifact indicated by arrow. *C*, quantitative analysis of effect of parallel fibre conditioning for the unit in *A*. Histogram shows distribution of axonal c.f.r.s with different numbers of spikes per response. Number of responses with a certain number of spikes when inferior olive stimulation was preceded by parallel fibre stimulation (test) are shown downwards, and control responses upwards.

conditioning are much more difficult to study than the effects of climbing fibre conditioning. All recordings were made from axons in the white matter in the vicinity of nucleus interpositus anterior and parallel fibres were stimulated with a concentric electrode or with a silver ball electrode on the cerebellar surface. Since only a small proportion of all Purkinje cells are located in the superficial parts of the folia and surface stimulation only activates the superficial parallel fibres, the number of Purkinje cell axons which could be activated by surface stimulation was rather small ($n = 7$). It was also impossible to determine the relation of the corresponding Purkinje cell to the beam of excited parallel fibres. Although all the units studied were excited by stimulation of the cerebellar surface, it is likely that many of them also received a substantial inhibitory input from interneurones activated by parallel fibres.

In order to exclude the possibility that the spike evoked by the surface stimulation was due to an inadvertent climbing fibre activation or stimulation of the Purkinje cells directly, three different tests were employed. In two cases it was possible to move the surface electrode along the folium and observe a corresponding gradual change

in the latency of the spike evoked by surface stimulation. This suggests that the response resulted from stimulation of parallel fibres. In five cases the order of application of the two shocks was reversed, i.e. the inferior olive stimulation preceded surface stimulation. Thus, if the surface electrode had been activating a climbing fibre, then surface stimulation preceded by inferior olive stimulation should produce an increase in the number of the spikes in the surface response, as shown above. Thirdly, it was possible in three units to observe an effect of conditioning when the stimulation strength was below threshold for evoking a spike. This excludes the possibility of climbing fibre activation. All seven units included in this study satisfied at least one of these criteria. The possibility, not excluded by these tests, that the surface stimulation activated Purkinje cells directly, can be rejected because the latencies of the spikes produced by the surface stimulation were too long, sometimes up to about 10 ms (cf. Fig. 4*B*).

It was thus possible to test the effect of parallel fibre conditioning in seven presumed Purkinje cell axons. The results show that parallel fibre stimulation preceding olivary stimulation increases the number of spikes as compared with control responses (five out of seven tested units). This can be seen in the records from two units in Fig. 4*A* and *B*. The upper trace for each unit shows the response in the axon to olivary stimulation alone (*) while the lower trace shows the response to parallel fibre stimulation (arrow) followed by olivary stimulation (*) at an interval of about 20 ms. The histogram in Fig. 4*C* shows the distribution of the number of spikes in the unit in Fig. 4*A* for ten pairs of control (above abscissa) and test (below abscissa) responses elicited by olivary stimulation. The stimulation interval was 20 ms. The average number of spikes is clearly greater for those responses which were preceded by parallel fibre stimulation.

In general, the effect of parallel fibre conditioning was weaker than that of climbing fibre conditioning. On average, in those cells where an effect was present there was a 1·5-fold increase in the number of spikes. In one unit there was a 4-fold increase in spike number. For each of the seven units, χ^2 was calculated. The number of spikes in the test responses was significantly greater than that of the control responses ($P < 0{\cdot}01$) in five units. In the remaining two units there was no difference.

DISCUSSION

Identification of Purkinje cell axons

Identification of Purkinje cell axons was based on the latency of the response to inferior olive stimulation (4–5 ms), the spontaneous discharge rate (20–60 Hz) and the firing pattern of the unit (high frequency discharge with intermittent periods of quiescence). Climbing fibres could not satisfy any of these criteria. The latency of a response in a climbing fibre after inferior olive stimulation must be about 1–2 ms less than the latency of c.f.r.s recorded from the cerebellar surface (4–5 ms) and the discharge rate of olivary neurones is only 1–2 Hz (Armstrong, 1974). Furthermore, olivary cells usually fire in bursts of up to five spikes. Because of the strong recurrent inhibition of these cells, a second stimulus to the inferior olive would be expected to produce a discharge with fewer spikes (Armstrong & Harvey, 1966; Crill, 1970). Unpublished observations (N. C. Campbell & G. Hesslow) suggest that this is indeed

the case. In the presumptive Purkinje cell axons in this study, however, the effect observed was an increase in the number of spikes.

It is also unlikely that the above criteria could be satisfied by mossy fibres. It is possible that stimulation of the inferior olive could activate mossy fibres, for example by current spread to the lateral reticular nucleus. However, the conduction velocity of mossy fibres is usually faster than that of climbing fibres, and the latency of a response to inferior olive stimulation would usually be expected to be shorter than 4–5 ms (Eccles *et al.* 1967).

It is also important to note that seven of the units classified as Purkinje cell axons, using the aforementioned criteria, could be activated by surface stimulation at latencies up to 10 ms. This makes it extremely unlikely that they were mossy fibres and provides support for their classification as Purkinje cell axons. Since these units responded to olivary conditioning in a manner identical to that seen in the other presumed Purkinje cell axons, it would seem safe to assume that a sufficiently large portion, presumably all, of the tested units were indeed Purkinje cell axons.

Propagation of secondary spikes

The present results show that, with single shocks to the inferior olive, usually only one or two of the spikes in the resultant c.f.r. are propagated down the Purkinje cell axon, although it was sometimes observed, without prior conditioning, that axonal responses to inferior olive stimulation contained up to five spikes. It is possible that these multiple spike responses can be explained partly by the occurrence of spontaneous c.f.r.s in the Purkinje cell before the evoked response. When a spontaneous axonal c.f.r. contains only one spike, it cannot be distinguished from a simple spike. However, such responses must occur and it is likely that axonal c.f.r.s with more than one or two spikes have been preceded by a spontaneous c.f.r. This could also explain the large number of axonal c.f.r.s with many spikes reported by Ito & Simpson (1971). It should be noted, however, that these authors recorded from presumed axon terminals in Deiters nucleus and it cannot be excluded that the difference in the proportion of responses with multiple spikes between their study and the present one reflects differences between Purkinje cells projecting to Deiters nucleus and to the anterior nucleus interpositus.

The results also show that the effects of inferior olive conditioning on the axonal discharges mirror the effects seen in the soma. When a somatic c.f.r. is preceded by a c.f.r., the secondary spikes of the second response have an increased amplitude (Campbell & Hesslow, 1986), an effect which is often very marked. The present study demonstrates that there is a corresponding increase in the number of spikes in the axonal c.f.r. The time courses of the somatic and axonal conditioning effects are also in good agreement. Thus it appears that, while at most one of the secondary spikes of a somatic c.f.r. is normally of sufficient amplitude to trigger the axon hillock of the Purkinje cell, the increased amplitude resulting from a preceding c.f.r. conditioning is sufficient to raise up to four to five spikes above the threshold.

For the technical reasons discussed above the material on the effects of parallel fibre conditioning on axonal c.f.r.s is somewhat meagre. Only seven axons were found where surface stimulation resulted in a spike that could confidently be ascribed to parallel fibre activation. In five of these units, a preceding surface stimulation reliably

increased the number of spikes in the test c.f.r., but the magnitude of this effect was very variable. Since it was impossible to determine the relation of the activated parallel fibre beam to the Purkinje cell under study, it is likely that the ratio of excitation to inhibition of these Purkinje cells varied. This could explain the varying effects. However, in view of the correspondence between somatic and axonal effects of inferior olive conditioning and the fact that on-beam parallel fibre conditioning increases the number of secondary spikes in the somatic climbing fibre response (Campbell & Hesslow, 1986), the data still support the contention that preceding parallel fibre input to the Purkinje cell does influence the number of secondary spikes propagated down the axon.

Functional considerations

In considering the possible effects of the Purkinje cell input to the nuclear cells, three features of the olivo-cerebellar system are particularly important. One is the electrotonic coupling demonstrated between cells in the inferior olive (Llinás, Baker & Sotelo, 1974). A second is the divergence of the climbing fibres, one olivary cell innervating several Purkinje cells in the same sagittal zone in the cerebellar cortex. These facts would be expected to synchronize the c.f.r.s in a group of Purkinje cells. Indeed, it has been observed that continuous noxious stimulation of anaesthetized cats produces highly synchronized climbing fibre discharges (Ekerot, Gustafsson, Oscarsson & Schouenburg, 1985). A third important feature of the olivo-cerebellar system is the very high degree of convergence in the Purkinje cell innervation of the interpositus nucleus. It has been estimated that more than 800 Purkinje cells may terminate on a single nuclear cell (Palkovits, Mezey & Szentágothai, 1977). This would strengthen the effect of synchronization in the climbing fibre responses which reach the nuclear cells via the Purkinje cell axons. Because of this, and the effect of climbing fibre conditioning, one would expect the second of two climbing fibre responses to result in a very powerful inhibition of the nuclear cell. This effect would be strengthened by characteristics of the excitatory input to the nuclear cells of climbing fibre collaterals. Because of the recurrent inhibition in the olivary cells, which has a duration similar to the effect of conditioning in Purkinje cells, the second of two olivary discharges will generally contain fewer spikes (Armstrong & Harvey, 1966, Crill, 1970; N. C. Campbell & G. Hesslow, unpublished observations). Thus, in the nuclear cell the second climbing fibre discharge will result in both weaker excitation and stronger inhibition.

For the anatomical reasons discussed above, the effect of parallel fibre conditioning is difficult to assess but is probably small. Because of the convergence on the nuclear cells, any effect on the c.f.r. of parallel fibre stimulation in a single Purkinje cell would have only a marginal influence on the total amount of inhibition of the nuclear cell. It is not known if there is normally sufficient synchrony in the parallel fibre excitation of a sagittal strip of Purkinje cells to have a significant effect on nuclear cell inhibition. However, it may be recalled that the duration of the conditioning effects in somatic recordings was usually twice as long (200 ms) for parallel fibre conditioning as for climbing fibre conditioning (100 ms), and that, consequently, the synchrony required is smaller than for climbing fibre conditioning (Campbell & Hesslow, 1986).

A second important question is whether climbing fibre discharges ever normally

occur at intervals sufficiently short for the conditioning effect to occur. The discharge frequency of the inferior olive is generally agreed to be 1–2 Hz (Armstrong, 1974), and it is well known that there is a strong recurrent inhibition in the olivary cells with a duration of about 100 ms. This would appear to be sufficient for preventing the occurrence of any conditioning effects. However, olivary discharges have been observed at intervals down to about 40 ms in awake walking cats (Armstrong & Rawson, 1979; Armstrong, Campbell, Edgley, Schild & Trott, 1982), and Ekerot *et al.* (1985) observed that continuous nociceptive stimulation in anaesthetized cats was accompanied by olivary discharge at frequencies exceeding 10 Hz.

Eccles *et al.* (1967) discussed the interaction between parallel fibre and climbing fibre input in terms of a ‘read-out’ hypothesis, where the number of spikes in the c.f.r. which are propagated down the Purkinje cell axon would signal the excitability level of the Purkinje cell to the cerebellar nuclei. This hypothesis was rejected by Mano (1970), who found no correlation between the number of secondary spikes and the preceding simple spike activity. This objection assumed that the spontaneous simple spike discharge rate depends only on parallel fibre input. Some evidence suggests, however, that Purkinje cells may have an intrinsic pace-maker (for references see Gruol, 1983) and thus simple spike discharge may not solely reflect parallel fibre input. It has also been shown in somatic recordings that spontaneous simple spikes did not influence the secondary spike amplitude (Campbell & Hesslow, 1986). The present results show that prior parallel fibre and climbing fibre input does influence the number of propagated secondary spikes, although the functional relevance of this interaction is unclear.

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