Exp. Brain Res. 15, 261—277 (1972)

Cutaneous Mechanoreceptors Influencing Impulse Discharges in Cerebellar Cortex. II. In Purkyně Cells by Mossy Fiber Input

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Received December 7, 1971

Summary. This paper gives an account of single Purkyně cell responses when three types of mechanical stimulation, as in the previous paper, are applied to the forefoot and hindfoot of the decerebrate unanesthetized cat. Attention was concentrated on the effects of brief mechanical pulses to the footpad. Recording was extracellular by glass microelectrodes and special precautions were taken in identifying the spike responses as being due to a single Purkyně cell and in securing its effective isolation for our computer averaging techniques, as described in the previous papers. All Purkyně cells were in the ipsilateral anterior lobe in the lateral vermis or pars intermedia of lobules III, IV, V, except for a few recordings in the extreme rostral zone of lobule VI.

Mechanical pulses or taps evoked responses from many Purkyně cells which were pure excitatory, pure inhibitory or admixtures thereof. The latencies of onset were usually in the range of 12—20 msec from the onset of the tap, which tends to be a little longer than the observed latencies for mossy fiber responses described in the preceding paper. There was often a considerable difference in the sizes of the responses evoked from different pads of the same foot, and the usual threshold for response was below 0.2 mm amplitude. Durations of responses were usually 10—20 msec for excitation and 50—100 msec for inhibition.

Pressure pulses to the central foot pads of 2 sec duration evoked a wide variety of responses: brief phasic at "on" and "off" that could be admixtures of excitation and inhibition; almost pure tonic excitations or inhibitions that were well maintained during the 2 sec; phasic-tonic responses in various relative degrees. Usually 500 g was maximally effective and the threshold was below 100 g.

Hair receptors were stimulated preferentially by brief air jets, there being brief excitatory or inhibitory responses much as with taps, but with rather longer latency. The effective area was usually fairly extensive over the hairy skin of the foot.

In general the effects on Purkyně cells by cutaneous mechanoreceptors acting via mossy fibers were in accord with the mossy fiber responses reported in the preceding paper and with the well-known excitatory and inhibitory effects that are exerted by mossy fiber inputs on Purkyně cells.

Key words: Cerebellum — Cutaneous mechanoreceptors — Mossy fibers — Purkyně cells — Cat

Introduction

It was first shown by Adrian (1943) and by Snider and Stowell (1944) that various kinds of cutaneous stimulation evoked responses in the cerebellum. These initial investigators were more concerned with utilizing these responses for somatotopic mapping than in attempting to make an analytical study of the manner of their production. Indeed, although there had been much subsequent investigation, the genesis of potentials by the cerebellar cortex could not be critically studied until the neuronal electrophysiology had been satisfactorily correlated with the neuronal structure (cf. Eccles, Ito and Szentágothai, 1967). It has been shown that stimulation of cutaneous nerves exercises powerful influences on the Purkyně cells via mossy and climbing fibers (Oscarsson, 1967, 1969; Larson, Miller and Oscarsson, 1969a, 1969b; Eccles, Provini, Strata and Táboříková, 1968a, 1968b; Kitai, Táboříková, Tsukahara and Eccles, 1969; Latham and Paul, 1968a, 1968b; Armstrong and Harvey, 1968; Freeman, 1970; Rubia and Phelps, 1970; Körlin and Larson, 1970; Eccles, Faber, Murphy, Sabah and Táboříková, 1971a, 1971b, 1971c). Special mention should be made to recent studies on the responses of individual units, some of them being Purkyně cells, that are evoked by stimulation of cutaneous receptors (Thach, 1967; Tarnecki and Konorski, 1970).

The principal aim of this study has been to investigate the transmission of information from cutaneous receptors to the cerebellum. It is hoped in this way to derive a clearer understanding of the manner in which the neuronal machinery of the cerebellum utilizes this information in carrying out its function in controlling posture and movement. It has been postulated that this is effected by a continuous dynamic loop control (Eccles, 1967, 1969), the pathways being from cerebellum to the on-going movement or posture, which in turn is signalled back to the cerebellum via peripheral receptors and the afferent pathways.

This present report is concerned only with exemplifying the various types of responses of Purkyně cells that are evoked by the mossy fiber input from the cutaneous mechanoreceptors. A preliminary account has been published (Eccles, Sabah, Schmidt and Táboříková, 1971). In a subsequent paper (Eccles, Sabah, Schmidt and Táboříková, 1972c) the findings of this paper will be utilized in analyzing more complex responses of Purkyně cells to cutaneous mechanoreceptor input and in considering their integration and their topography.

Methods

The technical procedures employed in stimulating cutaneous mechanoreceptors have been fully described in the preceding paper (Eccles, Sabah, Schmidt and Táboříková, 1972a), the same experimental animals being utilized in the experiments described in this paper. In addition the general experimental procedures have been described in two earlier papers (Eccles *et al.*, 1971a, 1971b).

Purkyně cells may now be identified with assurance by utilizing the criteria already described (Eccles *et al.*, 1971b). The responses evoked by cutaneous mechanoreceptors have been studied in over 400 Purkyně cells. By judicious adjustment of the recording microelectrode there has been effective isolation of the extracellular spike potentials of individual units, which have then been subjected to various averaging procedures by an on-line computer, and are displayed as poststimulus time histograms (PSTH) and cumulative frequency distributions (CFD) (cf. Eccles *et al.*, 1972a). The cutaneous mechanoreceptors were excited in a precisely determined manner at a repetition rate under control by a master timer, and the PSTHs and CFDs of the Purkyně cell responses have a high reliability because of the averaging procedures.

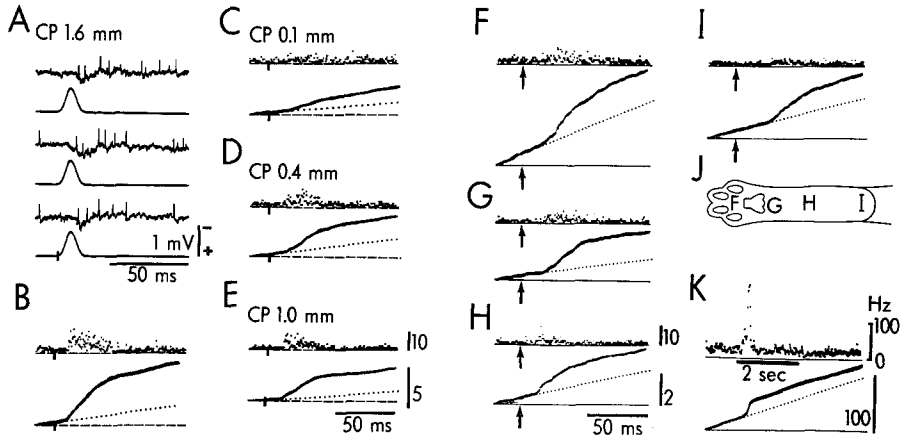

Fig. 1. *Purkyně cell excitation by cutaneous mechanoreceptors.* A gives specimen records of taps of 1.6 mm to the forelimb central pad and the evoked discharges of a Purkyně cell in the lateral vermis of lobule V, the poststimulus time histogram (PSTH) and the cumulative frequency distribution (CFD) for 64 traces being given in B. Responses of C, D and E are for smaller taps as indicated. F, G, H, I are PSTHs and CFDs for responses evoked by a brief air jet to the hairy skin at the sites marked in J. All PSTHs of B to I have same time and count scales. In B—I the count scale for the PSTH gives a measure of the counts summed in 256 bins of 0.5 msec during 64 responses. The count scale for the CFD gives a measure of the average counts for a single response, there being one scale for B—E, and another for F—I. The dotted lines are extrapolations of the initial background frequency and are useful in assessing the time course of the change produced by the stimulus. Short vertical bars in A—E indicate onset of stimulation. Arrows in F—I indicate approximate onset of air jet. In K are the PSTH and CFD for response evoked by pressure of 500 g for 2 sec as shown by the bar. 16 responses are summed in 256 bins of 20 msec duration. There is a frequency scale (Hz) for the PSTH and a count scale for a single trace of the CFD. A baseline is drawn for the PSTHs to indicate level for zero count. Decerebrate unanesthetized preparation

Results

Excitatory Actions on Purkyně Cells

It is proposed in this section to give accounts of the various types of Purkyně cell responses evoked by input from cutaneous mechanoreceptors. In the Discussion there will be a survey of all of our investigations on mossy fiber input to Purkyně cells.

In Fig. 1A are three records of the brief mechanical pulse (tap) that was applied to the central pad of the cat forefoot and of the spike responses that it induced in a Purkyně cell in lobule V. The identification as a Purkyně cell was assured by its occasional climbing fiber response (cf. Eccles *et al.*, 1971 b). In B the summed responses (PSTH and CFD) reveal that the latency from the onset of the tap (vertical bar in A) was about 12 msec, and that there was a period of increased discharge for about 35 msec, the initial low background frequency of about 15/sec being then resumed. The CFD shows that on the average there were 4 to 5 discharges for each tap, which is in agreement with the records in Fig. 1A. The series of C, D, E shows that decreased amplitude of the tap resulted in a smaller response with a more gradual onset, but there was still on the average 1.5 responses to a 0.1 mm tap.

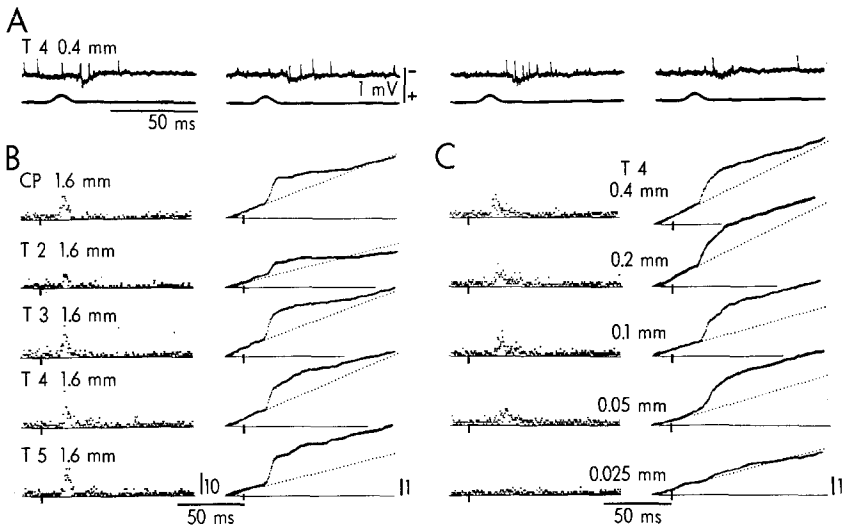

Fig. 2. *Phasic responses of Purkyně cell to cutaneous mechanoreceptors.* A gives four specimen records of responses of a Purkyně cell in pars intermedia of lobule V that are evoked by a tap of 0.4 mm to toe 4 of the forelimb. In B are the PSTHs and CFDs for summation in 256 bins of 0.5 msec of 64 responses evoked by 1.6 mm taps to the five foot pads as indicated. In C are the PSTHs and CFDs for graded amplitudes of the taps to toe 4 as indicated. All PSTHs and CFDs have the same count and time scales. Short vertical bars indicate onset of stimulation. Decerebrate unanesthetized preparation

The PSTHs and CFDs of Fig. 1F—I show that this Purkyně cell also responded to hair receptors preferentially stimulated by an air jet at the sites marked in J. The responses had longer latencies, 15 msec or more, than those evoked by tapping of the central pad. Finally in K there are the PSTH and CFD of the responses evoked by application of a weight of 500 g to the central pad for the 2 sec indicated by the bar. There was an initial large increase in discharge rate which rapidly declined to a plateau little if at all above the initial frequency for the terminal 1.5 sec of the pressure. There was a brief inhibition at "off". This large phasic and negligible tonic response was frequently observed for Purkyně cells, particularly with those that responded well to preferential stimulation of hair receptors.

Figure 2 illustrates the responses of a Purkyně cell that was highly sensitive to taps. The specimen records in A show that a 0.4 mm tap to toe 4 evoked an impulse discharge, the PSTHs and CFDs for these responses being in the upper row of C. The shortest latency was 16 msec and the average duration of the discharge was about 10 msec. In B are the PSTHs and CFDs for responses evoked by taps of 1.6 mm amplitude to the central pad and the 4 toes. The small response evoked by the taps to toe 2 was duplicated in a second recording. In C is a remarkable series of PSTHs and CFDs showing that a tap of only 0.05 mm to toe 4 evoked a response as large (about 2 discharges on the average) as that to the 1.6 mm tap in B, though the latency was longer. Even the 25 μm tap in C evoked a just detectable response. This high sensitivity of the pathway from pad cutaneous mechanoreceptors to Purkyně cell was observed in several experiments. Such responses must be due almost entirely to preferential stimulation of Pacinian corpuscles (Jänig, Schmidt

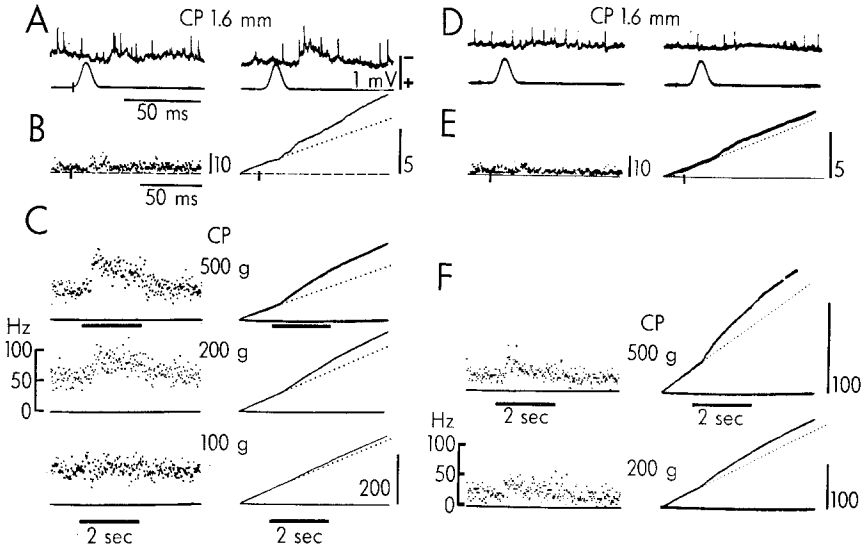

Fig. 3. *Responses of two Purkyně cells to taps and pressure.* In A are specimen records of responses of a Purkyně cell in the lateral vermis of lobule III evoked by taps of 1.6 mm to the central pad of the hindfoot, the PSTH and CFD for summation of 64 responses in 256 bins of 0.5 msec being in B. C gives the PSTHs and CFDs evoked by application of the pressures given in grams and at the times indicated by the 2 sec bars. Summation of 16 responses is in 256 bins of 20 msec. Same scales for all three responses, that for the PSTHs being given in Hz, and for the CFDs by the counts for a single response. D, E, F similar to A, B, C, but are from another experiment on the central pad of the hindfoot, the Purkyně cell being in the pars intermedia of lobule III. Same Hz scale for both PSTHs of F, but each CFD has its own scale. Both preparations were decerebrate and unanesthetized

and Zimmermann, 1968; Eccles, Sabah, Schmidt and Táboříková, 1972d). The cell of Fig. 2 was excited by air jet stimulation. Pressure was not tried.

In Fig. 3 there are examples of the tonic excitatory effect of pressure on two Purkyně cells. The specimen records of A and the PSTH and CFD of B show that a tap of 1.6 mm to the central pad evoked an initial response of almost 2 impulses on the average, and a long continued later response of about the same size. Application of a weight of 500 g in C approximately doubled the frequency from 50/sec to 100/sec (cf. the PSTH), there being a slow decline of frequency during the terminal 1.5 sec of the pressure. A return to the initial frequency occurred on cessation of the pressure. A weight of 200 g evoked a smaller response of the same general character, but 100 g was only slightly effective. Figure 3C gives a good example of a tonic excitatory action with but little phasic admixture, thus contrasting with the dominant phasic response of Fig. 1K.

Figure 3D—F illustrates the responses of a Purkyně cell that gave an intermediate response to pressure (F) with a phasic excitation falling to a moderate tonic plateau, particularly with the 500 g response. A small excitatory response was evoked by taps of 1.6 mm to the central pad, both in the specimen records of D and in the PSTH and CFD of E.

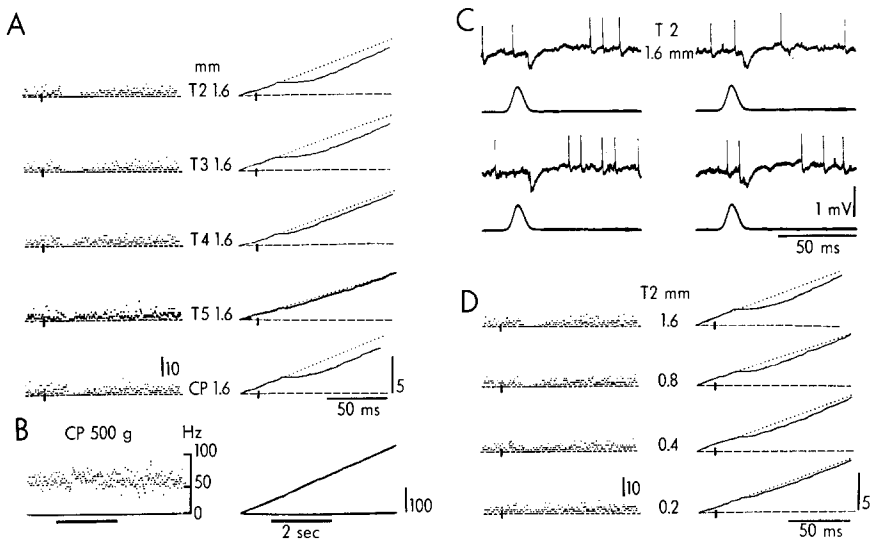

Fig. 4. *Phasic inhibition of a Purkyně cell.* Specimen records of responses of this Purkyně cell in the pars intermedia of lobule V are shown in C for taps of 1.6 mm to toe 2 of the forefoot. In A are PSTHs and CFDs for taps of 1.6 mm to the five pads of the forefoot as indicated, each being the sum of 64 traces in 256 bins of 0.5 msec. In B pressure of 500 g was applied for the 2 sec of the bars, the scales for the PSTH and CFD being in Hz and counts for a single trace respectively. There was summation of 16 responses in 256 bins of 20 msec. The PSTHs and CFDs of responses evoked by taps to toe 2 of the indicated amplitudes are shown in D. Same scales of counts and time for A and D. Decerebrate unanesthetized preparation

Inhibitory Actions on Purkyně Cells

In the specimen records of Fig. 4C taps of 1.6 mm amplitude to toe 2 are seen to effect a brief silence of the spontaneous discharge of the Purkyně cell, the time course being illustrated in the PSTH and CFD of the top row of A. The latency of onset was 17 msec and the duration was in excess of 40 msec, including about 12 msec of total silence. Figure 4A gives a good illustration of differential effects from the different pads: toes 2 and 3 were most effective, then in diminishing series central pad, toe 4, toe 5. Despite the considerable size reduction, the time courses of these inhibitory effects were comparable. This was also the case in D with the reduction of inhibition effected by reducing the sizes of the taps to toe 2 from 1.6 to 0.2 mm. The 0.2 mm tap gave a small inhibition comparable with the 1.6 mm tap to toe 5, i.e. toe 2 was approximately 8 times as sensitive as toe 5 in converting a mechanical stimulus to inhibitory action on this Purkyně cell. In B it is seen that pressure of 500 g to the central pad had no tonic action, there being in the PSTH merely a small phasic inhibitory-excitatory effect at onset.

Figure 5 illustrates an investigation into the inhibitory action of a second tap at various intervals after the first, there being two specimen records, together with the PSTH and the CFD for each test interval. The PSTHs and CFDs show that the second stimulus was almost fully effective in producing inhibition at the longest test interval (144 msec). As well shown by the PSTHs its inhibitory action decreased progressively with the test intervals of 131 and 108 msec, and was greatly diminished at the two briefest intervals of 67 and 40 msec.

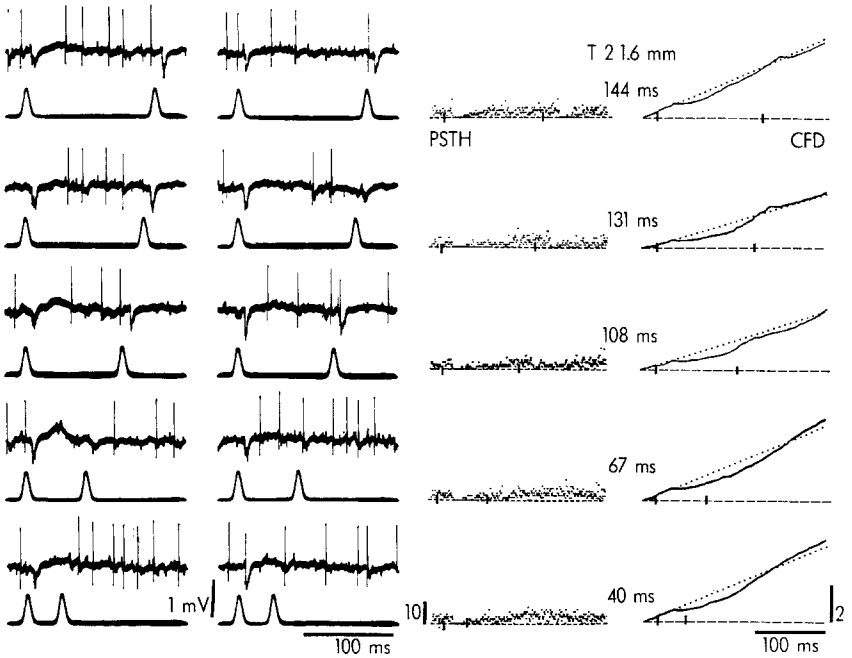

Fig. 5. *Phasic inhibition of Purkyně cell by double stimulation.* Same Purkyně cell as in Fig. 4, but with double stimulation of toe 2 by 1.6 mm taps at the indicated intervals. For each test interval, there are two specimen records, the PSTH and the CFD. Same scales throughout for times and counts. There was summation of 64 responses in 256 bins each of 1 msec duration

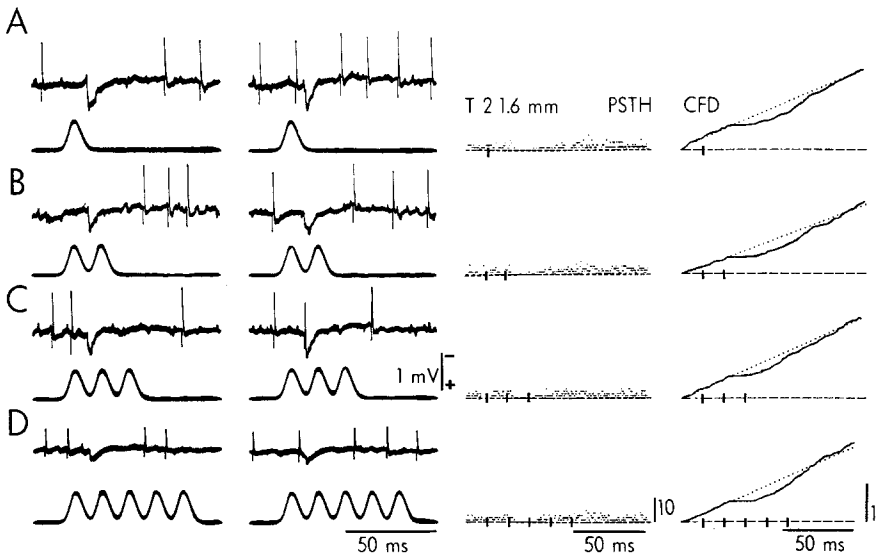

Fig. 6. *Phasic inhibition of Purkyně cell by repetitive stimulation.* In A are specimen records and the PSTH and CFD evoked in the same Purkyně cell as in Figs. 4 and 5 by a single tap of 1.6 mm to toe 2. In B, C and D there are 2, 3 and 5 taps at a frequency of 67 Hz as indicated in the specimen records. All PSTHs and CFDs have the same time and count scales, and represent the summation of 64 responses in 256 bins of 0.5 msec. The short vertical bars show onsets of mechanical stimulations

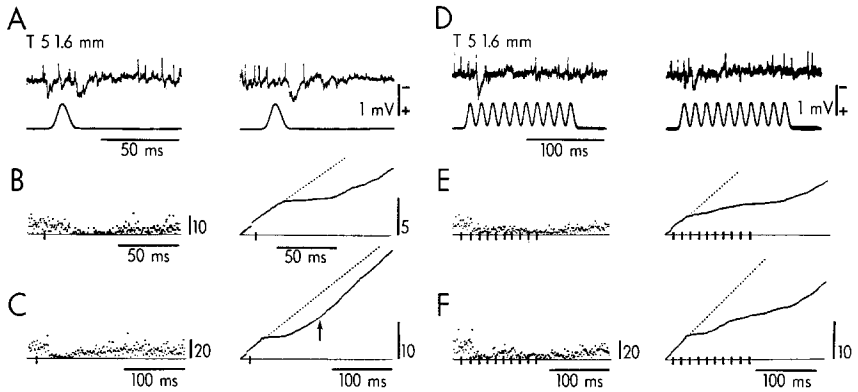

Fig. 7. *Inhibition of Purkyně cell by single and repetitive stimuli.* In A are specimen records of Purkyně cell inhibition by single 1.6 mm taps to toe 5 of the hindfoot. The PSTH and CFD for summation of 64 responses in 256 bins of 0.5 msec are shown in B, and in another test series at a slower sweep in C, there being summation of 64 responses in 256 bins of 1 msec. In D are specimen records of effect of a rhythmic series of 10 taps at 65 Hz. E and F give PSTHs and CFDs for summation of 64 responses in 256 bins of 1 msec in two separate tests, E being before C and F afterwards. Times of onset of mechanical stimuli are shown by short vertical bars. C, E and F have same scales for both PSTHs and CFDs, and B has separate scales as shown. The Purkyně cell was in lobule IV of the lateral vermis and the preparation was decerebrate and unanesthetized

A related investigation is illustrated in Fig. 6 on the same Purkyně cell, where, with repetitive stimulation at 67 Hz, two, three and five taps were not appreciably more effective than one. Investigations on mossy fiber discharges (Eccles *et al.*, 1972a, Figs. 1B, 3E, F, 5) showed that there was a reduction of the number of impulses in the successive responses, but not as a rule failure to respond to the successive taps. Presumably there is a further depression in the inhibitory pathway from mossy fiber to granule cell to basket cell to Purkyně cell, a probable site of depression being at the synapses from mossy fibers to granule cells with their powerful inhibition by Golgi cells (Eccles, Ito and Szentágothai, 1967).

In contrast to Fig. 6, repetitive mechanical stimulation produced in Fig. 7 a continuation of the inhibitory action of the first stimulus, though at a reduced level. The inhibitory action of a single tap to toe 5 is seen to be at least 50 msec in duration in the specimen records of A and the PSTH and CFD of B. The PSTH and CFD of C show at a slower sweep speed the full duration of the inhibition. The initial background frequency was resumed at the arrow 95 msec after the onset of the inhibition. D shows specimen records of the effect of 10 taps at 65 Hz, as indicated in the lower traces. There appears to be a relatively effective depression of discharge throughout the whole stimulation period. Sure confirmation is given by the PSTHs and CFDs of E and F, which are the averages of two separate tests of 64 sweeps each. Comparison with the CFD of C at the same sweep speed shows that the inhibition produced by the first stimulus was continued by subsequent stimuli, though at a diminished level. It was maintained throughout the whole duration of the stimulation and full recovery did not occur until about 100 msec after the last tap of the repetitive series. This limited ability of repetitive stimulation to produce a maintained depression of a Purkyně cell has been observed also

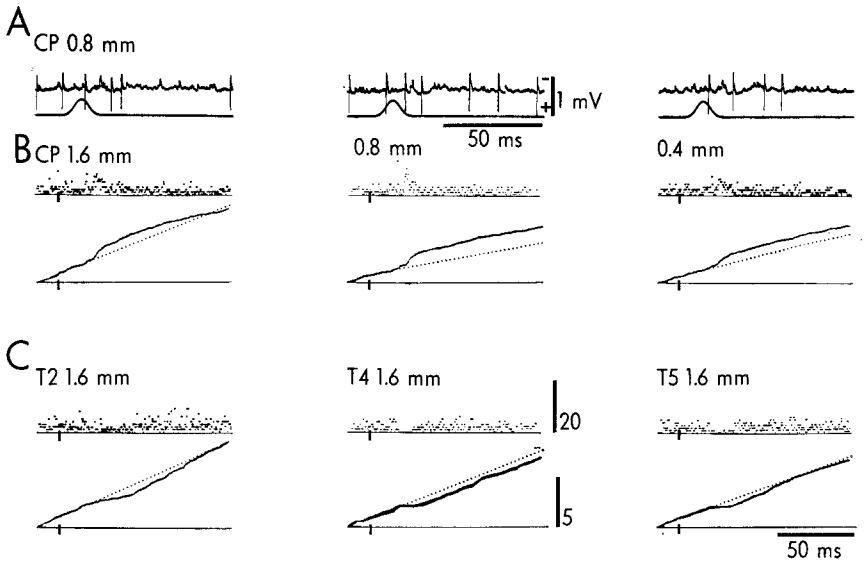

Fig. 8. *Excitatory and inhibitory responses of Purkyně cell.* A gives specimen records of responses of Purkyně cell to 0.8 mm taps to central pad of the hindfoot, the PSTHs and CFDs being given in the central frame of B. In B are also the PSTHs and CFDs for 1.6 and 0.4 mm taps. In C are responses to taps of 1.6 mm to toes 2, 4 and 5 as illustrated. Toe 3 responses were similar but are not illustrated. Purkyně cell was in lobule IV of lateral vermis. All averages are for 64 traces in bins of 0.5 msec. The preparation was decerebrate and unanesthetized

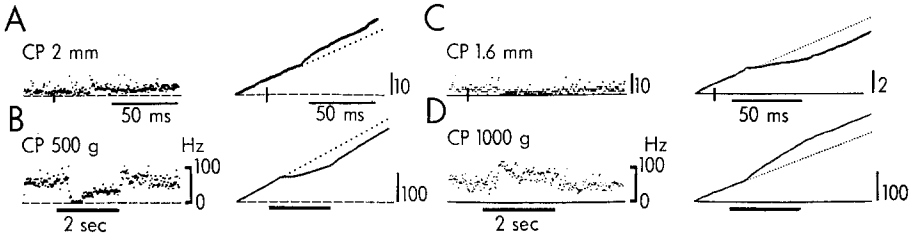

Fig. 9. *Responses of two Purkyně cells to tap and to pressure.* In A are the PSTH and the CFD for the summation of 64 responses to a tap of 2.0 mm to the central pad of the hindfoot and in B are the PSTH and the CFD for the summation in 256 bins of 20 msec of the responses to a pressure of 500 g applied for 2 sec, as shown by the bar. C and D are similar to A and B but from another experiment. The amplitude of the taps to central pad was 1.6 mm and the pressure was 1000 g. Both Purkyně cells were in lobule III of the lateral vermis, and both preparations were decerebrate unanesthetized

with repetitive nerve stimulation (Eccles *et al.*, 1971b, Figs. 9A, B, E—H, 10). The failure of a maintained inhibition in Fig. 6 and also in one illustration of nerve stimulation (Eccles *et al.*, 1971b, Fig. 9C, D) may in part at least be due to an admixture of an excitatory action submerged beneath the dominant inhibition.

Admixtures of Excitatory and Inhibitory Actions on a Purkyně Cell

Several Purkyně cells exhibited excitatory and inhibitory responses when taps were applied to different pads of the same foot. For example in Fig. 8 taps to the

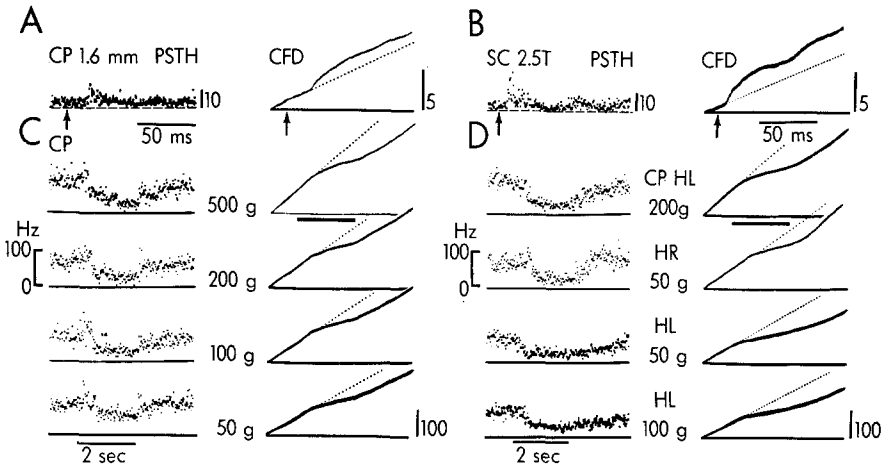

Fig. 10. *Purkyně cell responses to cutaneous mechanoreceptors.* A. Post-stimulus time histogram (PSTH) and cumulative frequency distribution (CFD) for Purkyně cell spike discharges evoked by a tap to the central pad of the hindfoot (onset at arrow) and summed for 64 traces in 256 bins of 0.5 msec. B. Similar to A, but evoked by a single stimulus (at arrow) to the sciatic nerve at 2.5T strength. C. PSTHs and CFDs for spike discharges evoked in the same cell by the indicated weights applied for 2 sec at the times shown by the thick bars below the lowest PSTH and the uppermost CFD, the summation being for 16 traces in 256 bins of 20 msec. D as in C, but for the indicated weights applied midway between central pad and heel (CP-HL), nearer still to heel (HR) and at heel (HL), where both 50 and 100 g were applied. Same count scale for bins of PSTH in A and B, and for average of single sweep of CFD in A and B. Same frequency scale in Hz for all PSTHs of C and D, and same count scale for CFDs of C and D, being the average for a single sweep. Same time scales for A, B and for C, D respectively. The Purkyně cell was in lobule III of lateral vermis, and the preparation was decerebrate and unanesthetized

central pad of the hindfoot excited the Purkyně cell, as seen in the specimen records of A and the PSTHs and CFDs of B, much as in Figs. 1—3. Yet taps to the pads of toes 2, 4 and 5 are seen to have an inhibitory effect resembling that in Figs. 4 and 7.

In Fig. 9A the PSTH and CFD show that a single tap to the central pad evoked a delayed excitatory response of the Purkyně cell, the latent period being 25 msec. In B this same cell was profoundly inhibited by application of a weight of 500 g to the central pad. There was a trace of a brief excitation at onset, as would be expected from the response in A, then a severe inhibition causing complete silence for 400 msec. During the last second of the pressure, the frequency was steady at 25/sec, which was less than half the initial background of about 60/sec. A slight rebound excitation occurred at off.

The opposite admixture is illustrated in Fig. 9C, D. In the PSTH and CFD of C a 1.6 mm tap to the central pad is seen to cause a brief excitation passing over to an inhibition that was maintained at a fairly steady level for 45 msec. In contrast a weight of 1000 g effected a tonic excitatory action (D), there being probably a trace of an inhibition at onset, corresponding to the phasic inhibition in C. The slow decline of frequency during the initial third of the pressure is the common type of tonic response of Purkyně cells, rather than the almost steady response

illustrated in Fig. 3 for 200 g pressure. It is to be noted that the discharges of the slowly adapting receptors show a similar decline in frequency during the application of a steady pressure (Jänig *et al.*, 1968).

A particularly interesting example of the admixture of excitatory and inhibitory actions of cutaneous mechanoreceptors is illustrated in Fig. 10. In A the PSTH and CFD show a typical phasic excitation (latency 18 msec) by a 1.6 mm tap to the central pad. In B stimulation of the sciatic nerve also gave a typical excitation of short latency (9 msec), but there was in addition a small later inhibition. By contrast, in C weights of 500 g to 50 g all gave a strong tonic inhibition. However closer examination of the responses of C reveals that excitation was superimposed on the dominant inhibition. Not only was a brief excitation at "on" shown in all PSTHs, but during the pressure there was a slow increase in the tonic inhibition, particularly with the 500 g pressure. Moreover there was even a brief interlude of relative excitation superimposed on the tonic inhibition about the middle of the applied 200 g pressure. Evidently the Purkyně cell was responding to a mixture of excitatory and inhibitory influences. In D there are a series of tonic inhibitions produced by application for 2 sec of the indicated weights to various regions of the hairy skin of the foot as indicated by the symbols. The admixture of excitation is also indicated, particularly in the two upper responses.

The examples of admixture illustrated in Figs. 9 and 10 can be regarded as rather extreme examples of the situation that must usually occur with a mossy fiber input to any Purkyně cell. For example, even if there is a relatively sharp delimitation of the mossy fibers projecting to a particular area of the cerebellar cortex, the parallel fibers excited thereby will activate Purkyně cells along a beam extending for 3 mm along a folium, and the basket cells also activated by that beam have an inhibitory projection up to 1 mm on either side of that beam (cf. Eccles, Ito and Szentágothai, 1967). It should be even the occasion for surprise that the cutaneous mechanoreceptors from the foot have an action on individual Purkyně cells with the apparent purity of the excitatory or inhibitory actions illustrated in Figs. 1—7.

Discussion

The great majority of the observations considered in this paper and all of the illustrations have been on decerebrate unanesthetized preparations. As already reported (Eccles *et al.*, 1971b), the climbing fiber responses are greatly depressed by the decerebration, hence conditions are specially favorable for recording the mossy fiber responses, which are very active in the absence of anesthesia. Continuous monitoring of the Purkyně cell responses has given assurance that in this paper all illustrations except the histograms of Fig. 11 are for mossy fiber responses uncontaminated by climbing fiber responses. Conjoint mossy and climbing fiber responses will be fully treated in a later paper (Eccles *et al.*, 1972c).

Excitatory Actions on Purkyně Cells

Good correlation would be expected between two types of excitatory response evoked by stimulation of cutaneous mechanoreceptors: impulses recorded in the mossy fibers to any particular area of the anterior lobe (Eccles *et al.*, 1972a) and the impulse discharges evoked from Purkyně cells of that area. However, when

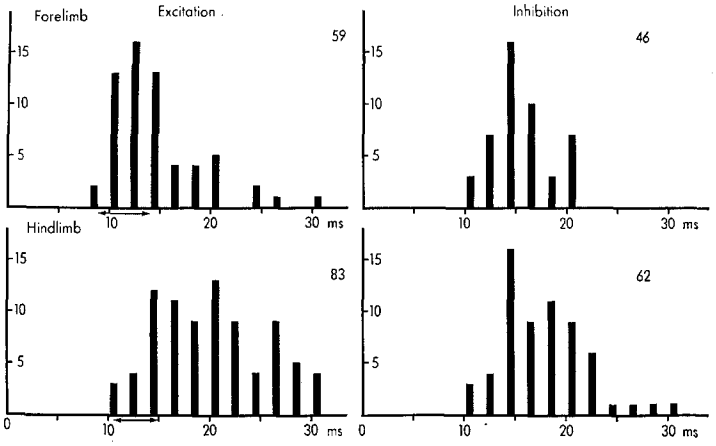

Fig. 11. *Histograms showing latencies of onsets of excitatory and inhibitory responses of Purkyně cells.* The onsets of taps with a time course of onset as illustrated are at zero and the numbers of Purkyně cells with latencies of response in 2 msec bins are shown by the column heights. The two upper frames are for the excitatory and inhibitory responses to taps of the pads of the forefoot, and the two lower frames similarly for the hindfoot. The numbers on each frame show the number of cells. However, when the responses were combinations of excitation and inhibition as in Figs. 8 and 9C, a cell was plotted in both excitatory and inhibitory frames. No cell was tested for responses with taps to forefoot and hindfoot. As would be expected weaker responses and responses to weaker taps usually had latencies longer by several milliseconds. Only the briefest latency was plotted for each cell

attempting this correlation, it must be appreciated that impulses in mossy fibers have both an excitatory and an inhibitory action on Purkyně cells. Furthermore, the important mossy fiber pathway via the lateral reticular nucleus (Grant, Oscarsson and Rosén, 1966; Oscarsson and Rosén, 1966) presumably is concerned in many of the responses with rather long latent periods.

Latent periods can best be compared for the responses evoked by single taps to foot pads. Thus in Figs. 1B and 2B the shortest latencies for the Purkyně cell discharges were 12 and 14 msec respectively after the onset of the tap. In the left upper frame of Fig. 11 is the histogram of the latencies of responses of the 59 Purkyně cells that responded by a clear excitation out of a total of 101 that were tested by taps to the pads of the forefoot. Similarly in the lower left frame is the histogram for the latencies of the 83 Purkyně cells that responded to hindfoot taps out of the total of 253 that were so tested. There is the expected tendency towards longer latencies for the hindfoot taps. Many of the responses at the latencies above 25 msec, particularly for the hindfoot, were for responses evoked by climbing fiber inputs.

There is reasonable agreement between these latencies and those determined for mossy fibers in close proximity to the Purkyně cells. These latencies were 9—14 msec and 10.5 to 15 msec respectively for the mossy fibers excited from forefoot pads (5) and hindfoot pads (16) (double-headed arrows in Fig. 11; cf. Eccles *et al.*, 1972a). A time differential of no more than 2 msec would be expected between the shortest latencies for mossy fibers and Purkyně cells. The few very short latencies

in Fig. 11 can be attributed to mossy fiber responses still earlier than the relatively few we investigated.

The shortest latencies in the histograms of Fig. 11 presumably are attributable to the fast mossy fiber pathways via the dorsal spinocerebellar tract and the cuneocerebellar tract (Oscarsson, 1965; Eccles *et al.*, 1971a), but with latencies in excess of 15 msec the slower spinoreticular cerebellar pathway (Grant *et al.*, 1966; Oscarsson and Rosén, 1966) would also be effective.

Inhibitory Actions on Purkyně Cells

In accordance with the basic physiology of the cerebellar cortex (Eccles, Ito and Szentágothai, 1967), several explanations can be offered for the inhibition of Purkyně cell discharge illustrated in Figs. 4—7: mossy fiber excitation of basket and stellate cells (via granule cells and parallel fibers) with a consequent inhibition of Purkyně cells; mossy fiber excitation of Golgi cells either directly or via granule cells and parallel fibers, the consequent inhibition of granule cells giving depression of their background excitatory action on Purkyně cells; inhibition of the background mossy fiber discharge by the cutaneous mechanoreceptors as in Fig. 7 of the preceding paper (Eccles *et al.*, 1972a), with the consequent diminution of excitatory action on Purkyně cells. It would be expected that the most effective inhibitory mechanism on a Purkyně cell would be provided by the direct inhibition of the basket and stellate cells; nevertheless Golgi cell inhibition of granule cells must also be considered because the observed inhibitions of Figs. 4, 5, 6, 7 and 9C have a shorter duration (not more than 100 msec) than the Purkyně cell inhibition produced by a parallel fiber volley in the unanesthetized preparation (Eccles, Faber and Táboříková, 1971). In Fig. 11 the histograms to the right display the shortest latencies for inhibition of Purkyně cells. Of the 101 tested by forefoot taps 46 displayed significant inhibitions at the latencies plotted in the upper frame. The inhibitory latencies tend to be a little longer than for excitation (upper left frame) which would be expected on account of the extra synapse in the cortical pathway. Similarly the lower right frame gives the histogram for inhibition of 62 Purkyně cells out of the 253 tested from hindfoot pads. Unlike the forefoot, there is no general tendency for a longer latency, but there is a considerable distortion of the lower left frame by the admixture of long latency climbing fiber responses.

Phasic and Tonic Responses

Figure 1K illustrates the most common response to a steady pressure of 2 sec duration. After a transient effect at "on", the initial frequency is almost exactly resumed, only to be transiently disturbed again at „off". The "on" and "off" transients may be in any combination of excitation and inhibition. For example in Fig. 1K there is on-excitation and off-inhibition and in Fig. 4B merely at "on" an inhibition-excitation sequence. All gradations exist between such pure phasic responses and the virtually pure tonic excitation of Fig. 3C. The PSTHs of Fig. 3F, particularly that for 500 g, show an initial phasic excitation declining to a steady tonic excitation, and a similar response is seen in Fig. 9D. Corresponding phasic and tonic responses have been exhibited by mossy fibers. For example in Fig. 6C of the preceding paper (Eccles *et al.*, 1972a) pressure evoked no tonic response,

there being a brief phasic excitation at "on" and "off". In Figs. 2A and 4D of that paper the phasic excitatory responses of the mossy fiber dominated the relatively small tonic excitation, and in Fig. 3C, the tonic excitation was strong and steady during the pressure, though there was a phasic excitation at "on" and a small "off" inhibition. In general the mossy fiber responses have been characterized by stronger tonic excitation than the Purkyně cells. This difference can be attributed to the negative feedback control that Golgi cells exercise on the mossy fiber excitatory pathway to Purkyně cells.

Tonic inhibitory action on Purkyně cells has been observed as frequently as tonic excitation. In Fig. 9B the inhibition was so strong that it silenced the cell for about 400 msec, there being a later steady inhibition for the full duration of the pressure. In Fig. 10C there was a tonic inhibition that even increased during the pressure. As already suggested, this is probably the result of a superposition of an initial semitonic excitation, after which the full tonic inhibition was revealed.

Some Purkyně cells reacted to the steady pressure in what we may term a semitonic manner. The initial increase in frequency slowly declined over 500 msec or even longer. All of these variants parallel responses observed for the mechanoreceptors of the foot pads (Jänig *et al.*, 1968). Pacinian corpuscles would respond very briefly at "on" and "off", whereas the rapidly adapting receptors (RAR) discharge for even 0.5 sec in response to a steady pressure. Finally the slowly adapting receptors (SAR) respond by a very slowly decaying discharge. The terminal 1.5 sec of our 2 sec pressure stimuli would be evoking only SAR responses. The variations observed in the tonic responses to pressure in Figs. 3C, F; 9B, D; 10C, D can be attributed to the variations in the effectiveness with which the SAR activates the excitatory and inhibitory pathways to the particular Purkyně cell under observation.

In our 25 experiments on unanesthetized decerebrate cats 149 Purkyně cells were tested by steady pressure in addition to the taps. Of these almost half (72) responded by a tonic excitation or inhibition as in Figs. 3C, F, 9B, D, 10C, the remainder (77) giving only phasic on- and off- as in Figs. 1K; 4B.

Hair Stimulation

It was reported (Eccles *et al.*, 1972a) that those mossy fibers very effectively activated from hair receptors exhibited good phasic but poor or absent tonic responses from pads. Purkyně cells exhibit a comparable differentiation. For example in Fig. 1 there was a strong excitation from hair receptors, but no tonic action from the central pad. Unfortunately, in the illustrated examples of strong tonic excitation or inhibition, hair stimulation was not tried. However it should be noted that in Fig. 10 pressure on the hair skin produced a tonic inhibition, which presumably is attributable to the slowly adapting tactile corpuscles (Iggo, 1966). The Purkyně cell of Fig. 8 was very effectively inhibited by hair stimulation with a latency of about 20 msec (not illustrated). In general the latencies of responses to hair stimulation were a few milliseconds longer than to taps, the usual values being in the range 15—25 msec from the onset of the airjet. Probably this difference is merely technical — the delay involved between the onsets of the airjet and of the hair movement.

In our 25 decerebrate unanesthetized preparations 67 of the Purkyně cells responding to taps to the pads of forefoot or hindfoot were tested by both pressure and hair stimulation. Of the 36 that responded to hair stimulation via a mossy fiber input only 10 gave a detectable tonic response. Of the remaining 31, 12 gave a tonic response but no hair response, 16 failed in both and in 3 the hair response was by a climbing fiber input and there was also a tonic response via mossy fibers.

Sensitivity of Purkyně Cells to Cutaneous Mechanoreceptors

There is a good correlation between the sensitivities observed for mossy fiber responses to taps and pressure (Eccles *et al.*, 1972a) and those observed for Purkyně cells. The threshold level for taps has usually been below 0.2 mm (Figs. 1C, 4D) for either excitation or inhibition, with extreme sensitivities as low as 0.025 mm (Fig. 2C). Taps of 0.05 mm or less would preferentially excite Pacinian corpuscles (Eccles *et al.*, 1972d), so it seems likely that Purkyně cells can be excited by the Pacinian input. Some mossy fibers had still lower thresholds — 0.01 mm in Fig. 1C (Eccles *et al.*, 1972a), but again 0.2 mm was a usual level as in Figs. 3B, 4B of that paper. Likewise for tonic action on Purkyně cells the threshold was usually below 100 g (Figs. 3 and 10) and there was little or no increase with pressure above 500 g, which correlates well with the observations on mossy fibers (Figs. 2A and 3C of Eccles *et al.*, 1972a).

The sensitivity of the mechanoreceptor action by the different toe pads on Purkyně cells sometimes exhibited a striking differential, and sometimes very little. For example in Fig. 2B the weak response to toe 2 was only about one third of that to the other pads, and a tap of only 0.05 mm to toe 4 produced a larger phasic excitation than a tap of 1.6 mm to toe 2. The inhibitory effects of toe taps in Fig. 4A also showed a significant differential, inhibitory actions by taps of 1.6 mm to toes 4 and 5 being approximately the same as for a 0.2 mm tap to toe 2. Differences of this order were observed with mossy fibers. For example in Fig. 1C of the preceding paper (Eccles *et al.*, 1972a) a tap to central pad of 0.02 mm evoked much the same mossy fiber discharge as 1.0 mm taps (D) to any of the four toe pads. On the other hand in Fig. 6B of that paper there was very little differential with the four pads that were tested.

Acknowledgements: This work was supported by a grant from the National Institute of Neurological Diseases and Stroke, Grant No. ROlNB0822101,2,3 and by generous research support by Dr. Henry C. and Bertha H. Buswell Fund to J.C. Eccles, R.F. Schmidt and H. Táboříková. We wish to thank Miss Tecla Rantucci for her technical assistance and Mr. J. Waldron for the photography.

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