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THE ACTION OF ANTIDROMIC IMPULSES ON THE CEREBELLAR PURKINJE CELLS

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SUMMARY

1. Antidromic impulses have been set up in the axons of Purkinje cells of the cerebellar vermis by stimulation in the juxta-fastigial (J.F.) region. Most experiments were performed on the normal cat cerebellum, but in nine the cerebellum was chronically deafferented by bilateral pedunculotomy 9–23 days previously.

2. Intra- and extracellular recording from Purkinje cells both showed a characteristic inflexion on the rising phase of the spike potential (the characteristic IS–SD inflexion) that presumably signals a delay in invasion between the axon and the large soma-dendritic expansion.

3. Laminar field analysis of the antidromic spike potentials showed that the antidromic impulses invaded at least 200 μ of the main dendrites as well as the soma, there being then a steep decrement to the surface. At superficial levels there was even an inverse antidromic spike potential. There appeared to be a synchronous invasion of the soma-dendritic complex, perhaps due to trigger zones of low threshold on the dendrites.

4. Antidromic soma-dendritic invasion was modified in the expected manner by a volley in the parallel fibres; there was inhibition of transmission into the soma and up the main dendrites (maximum effect at 200–300 μ depth) due to the inhibitory action of the basket and superficial stellate cells that are excited by the parallel fibres; there was facilitation of transmission in the dendrites at levels superficial to 200 μ due to the direct excitatory action of parallel fibres. Both the inhibitory and excitatory actions had a duration in excess of 100 msec.

5. In the chronically deafferented cerebellum a second J.F. stimulation evoked a full size antidromic spike potential at an interval of 3 msec. There was a gradual decline in size down to intervals of about 2 msec, and at briefer intervals, to 1 msec, there was a small residual spike potential

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that possibly is due to transmission into the Purkinje cell axon collaterals at intervals too brief for soma-dendritic invasion. With repetitive stimulation there was a well maintained soma-dendritic invasion at a frequency as high as 300/sec.

6. In the chronically deafferented cerebellum an antidromic volley in the Purkinje cell axons caused a brief inhibitory silence of rhythmically discharging Purkinje cells. It is suggested that this is a direct inhibitory action of the Purkinje axon collaterals, that parallels their direct inhibitory action that has been demonstrated by Ito and collaborators (1964) on the intracerebellar nuclei and Deiters nucleus.

7. In the chronically deafferented cerebellum an antidromic volley in the Purkinje axons produced not only the large negative spike potential indicative of antidromic soma-dendritic invasion, but also a later small and slow positive wave that appeared to be closely linked with the negative spike. It is shown how this would arise by current flow into the dendrites that had been depolarized but not excited by the initial antidromic invasion.

INTRODUCTION

The dendritic tree of a Purkinje cell branches widely in the transverse plane of a folium (Ramón y Cajal, 1911; Fox & Barnard, 1957); but, when the whole population in a considerable area of a folium is simultaneously activated, the transverse components of the individual cells would cancel each other, and the net result would be equivalent to the arrangement in which each cell was merely a core conductor element perpendicular to the folial surface. This great anatomical simplification of the Purkinje cell population gives hope that the extent and time course of antidromic propagation of impulses in the soma and dendrites of Purkinje cells can be revealed by a systematic study of the laminated field potentials produced by synchronous antidromic activation. Granit & Phillips (1956, 1957) studied the antidromic activation of Purkinje cells, but they focused their attention on the individual cell potentials, particularly on the giant spikes with their positive–negative configuration, and on the firing of impulses by Purkinje cells under diverse experimental conditions, and not on the details of the propagation of antidromic impulses and the field potentials produced thereby.

In the present paper the antidromic propagation of impulses in Purkinje cells has been investigated by intracellular and extracellular recording and in particular by a systematic study of the field potential profiles and of their modification by repetitive stimulation and by inhibitory action on the Purkinje cells. This investigation has been performed not only on the normal cerebellum, but also on the chronically deafferented cerebellum in

order to eliminate complications produced by stimulation of climbing and mossy fibres at the same time as the Purkinje cell axons. The chronic deafferented preparation also provides ideal conditions for discovering any synaptic actions exerted by impulses in the numerous axon collaterals of Purkinje cells (Ramón y Cajal, 1911; Jakob, 1928).

It will be shown that antidromic impulses normally propagate over the soma and larger dendrites, but the safety factor is not high because this propagation is readily blocked by post-synaptic inhibition; the smaller dendrites are not invaded, but depolarization by synaptic action can enhance dendritic propagation. There is evidence also for the antidromic propagation of impulses in the axon collaterals and for a slight and variable inhibitory action of these impulses on the Purkinje cells.

METHOD

A complete description of the experimental methods has been given in a preceding paper (Eccles, Llinás & Sasaki, 1966). The only new experimental procedure is that employed in chronically deafferenting the cerebellum.

For this purpose a bilateral pedunculotomy was performed in nine cats, the post-operative survival time ranging from 8 to 23 days. The surgery was done under heavy Nembutal (sodium pentabarbitone) anaesthesia (45 mg/kg) and the cerebellum was approached from its caudal end by removal of the squamous plate of the occipital bone; the craniotomy was extended to the lambdoidal ridge. In order to obtain an ample exposure of the floor of the fourth ventricle, the caudal region of the vermis of the posterior lobe, from the fissura praepyramidalis to the velum medullare posterior, was removed by suction. The ventrolateral part of both cerebellar hemispheres was then removed until a clear view of the inferior colliculi was obtained. Care was taken not to injure the medulla, the pons or the quadrigeminal plate. At this stage it was possible, by means of a blunt edged spatula, to cut through all three peduncles bilaterally under direct vision, and thus to spare as much as possible the circulation to the cerebellum. Following the pedunculotomy the neck muscles, clavotrapesius and occipito-scapularis of both sides were approximated in the mid line and joined with the caudal edge of the temporal muscles, which had been previously severed at their origins, so that a protecting muscle sheath was created over the craniotomy. The skin was closed using metal clips.

All the cats operated upon underwent the typical decerebellate syndrome (Dow & Moruzzi 1958) with a 'dynamic stage' lasting from 2 to 3 days during which extensor rigidity and opisthotonos was observed. Following this stage the characteristic atonic period appeared. The animals showed intentional tremor with dysmetria and, in two cases, nystagmus. Nystagmus was present especially during psychical excitation, e.g. when presented with food. All through the post-operative period, lasting up to 23 days, the cats were completely ataxic and unable to stand. A week after the operation, however, they were all able to support their weight, and even to climb the wire screen on the sides of their cages, thus showing that their flexor responses were not as impaired as their antigravity mechanisms. The cats were fed through an oesophageal cannula during the first week, after which they began to eat unaided.

Following the electrophysiological studies the cerebellum and medulla were fixed in formalin. Both Marchi and Nauta stain techniques were used to study the degeneration pattern produced by the pedunculotomy. These techniques demonstrated that for the most part the degenerating fibres were restricted to the white matter of the cerebellum, leaving intact the actual cerebellar cortex. Also small portions of cerebellar cortex were removed

at the end of the experiment, before the death of the animals, fixed in 4% osmic acid and imbedded in 'Vestopal' for electron microscope (E.M.) observations which were kindly performed for us by Professor C. A. Fox and Dr D. E. Hillman of the Department of Anatomy, Marquette University. The E.M. studies demonstrated degeneration of mossy fibres leaving the granule cells intact. A striking observation previously described by Hámori (1964), by Fox (personal communication) and by Szentágothai (personal communication) was the survival of the cerebellar glomeruli of the granule layer which then consisted only of the dendrites of the granule cells and the axonal terminals of Golgi cells. There appears to be an increase in the synapses made by these axons on the dendrites of granule cells.

RESULTS

Antidromic responses of single Purkinje cells

With intracellular recording the antidromic spike potential of Purkinje cells resembles that of motoneurones and of most other neurones in displaying an inflexion on the rising phase (arrows in Fig. 1*A*, *B*) that is now generally recognized (Coombs, Curtis & Eccles, 1957; Terzuolo & Araki, 1961; Eccles, 1964) as being due to a delay in the antidromic invasion between the initial axonal segment (IS) and the soma-dendrite membrane (SD). However, Fig. 1*C*, *D* shows that Purkinje cells differ from motoneurones in that, even at the shortest response interval, there was no blockage of the antidromic IS–SD propagation, but merely an increased IS–SD delay and a slightly reduced size, which recovered with a small further increase in the stimulus interval (Fig. 1*E*, *F*). Actually, IS–SD blockage was observed in only a few Purkinje cells. Since the antidromic responses of Fig. 1*A*–F were evoked by a stimulus in the juxta-fastigial region (J.F.), impulses in mossy and climbing fibres are likely to produce post-synaptic potentials and superimposed spike potentials after the initial antidromic spike; hence these records cannot be used for the study of any after-potentials that may follow the initial spikes.

With the chronically deafferented cerebellum the potentials recorded extracellularly from single Purkinje cells are not complicated by potential fields produced by climbing fibres and mossy fibres, which in the normal cerebellum would be excited by the J.F. stimulus along with the Purkinje cell axons. In Fig. 1*G*–*K* the initial antidromic spike potential shows a slight inflexion on the initial positive wave that indicates a delay in invasion, which presumably corresponds to that recorded intracellularly in Fig. 1*A*, *B*. The extracellular responses to the second J.F. stimulation (Fig. 1*H*–*K*) also correspond to the intracellular (Fig. 1*D*–*F*) in showing an increased delay at the presumed IS–SD inflexion (arrows), but at the shortest response interval there was no IS–SD blockage. Later it will be reported that the field potential evoked by an antidromic volley at very brief stimulus intervals gives evidence of impulse propagation in some

component of Purkinje cells that has a specially rapid recovery (cf. Figs. 8, 9).

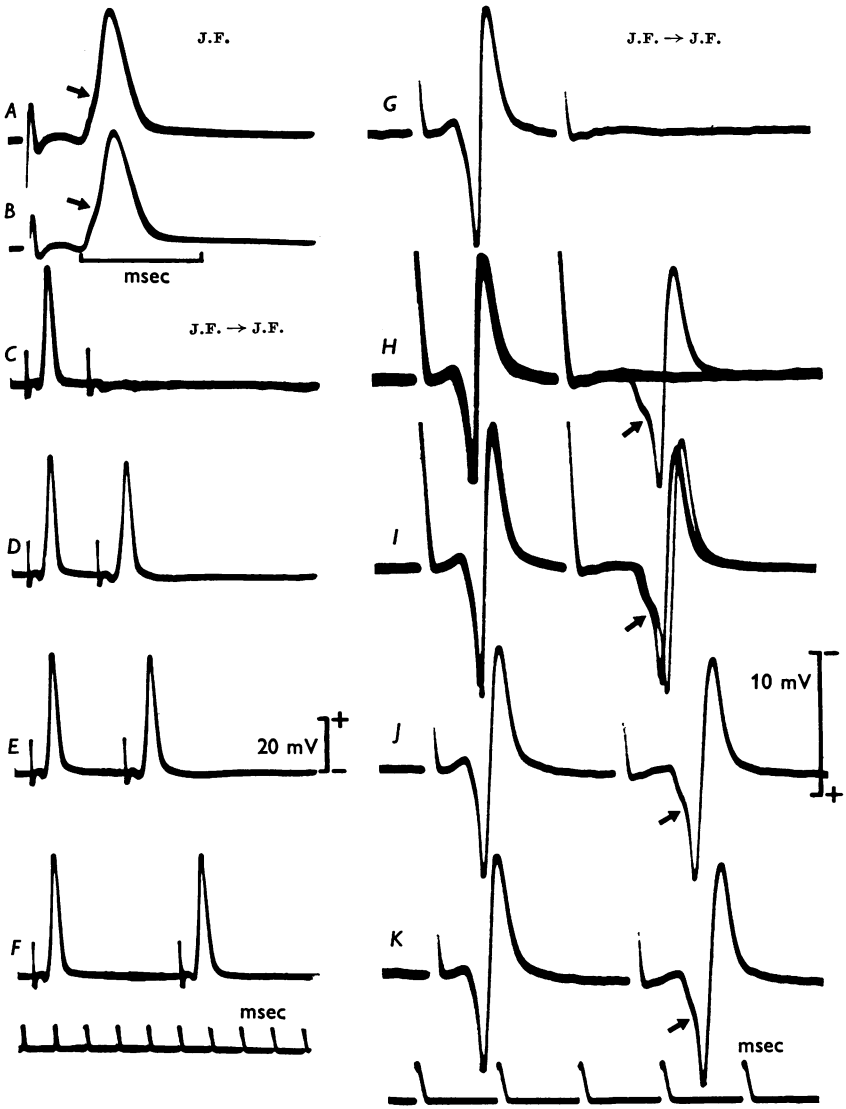

Fig. 1. Antidromic spike potentials evoked in Purkinje cells by juxta-fastigial (J.F.) stimulation. *A–F*: intracellular recording at fast (*A*, *B*) and at slower sweep speeds (*C–F*), where there was double J.F. stimulation. The spike potential in *B* is smaller and slower than in *A* because of progressive deterioration of the Purkinje cell. *G–K*: extracellular recording of giant spike potentials evoked by double stimulation in a single Purkinje cell of a chronically deafferented cerebellum. Note superimposed traces of *H* and *I*. Arrows indicate the inflexion due to delay in IS–SD transmission.

Field potentials produced in the cerebellar cortex by juxta-fastigial stimulation

A J.F. stimulus produces a complex potential field in the cerebellar cortex. For example, in Fig. 2*A*, *C* at a depth of 330 μ from the surface there is an initial diphasic spike (positive–negative) followed by a complex sequence of negative waves that begin at about 1·2 msec after the stimulus. The initial diphasic wave is so early that it must be produced by the propagation of impulses that are directly excited by the J.F. stimulus

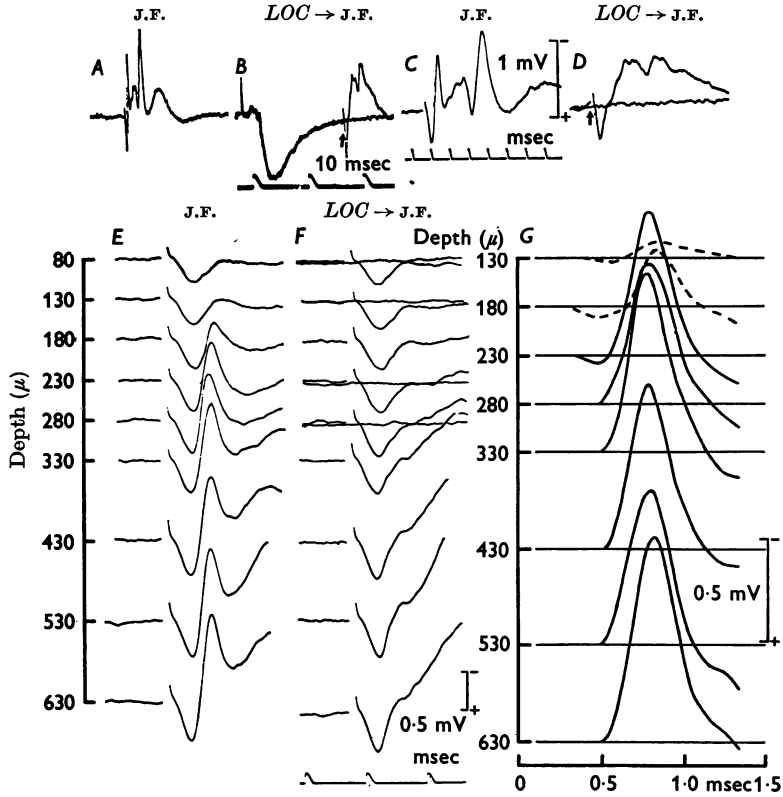

Fig. 2. Field potentials produced in the normal cerebellar cortex by an antidromic volley in the Purkinje cell axons. *A* and *C* show potential evoked at a depth of 330μ by a J.F. stimulus and recorded at slow and fast speeds, the initial diphasic (positive-negative) spike being due both to impulses in directly stimulated nerve fibres and to the antidromic spike of Purkinje cells. In *B* the antidromic response (at arrow) was inhibited by a parallel fibre (*LOC*) volley 18·3 msec earlier, and in *D* this inhibited response is shown as the fast sweep superimposed on the base line to the conditioning stimulus alone. In *E* the antidromic spike potential is shown at a still faster speed and at the indicated depths below the cortical surface, while in *F* it was inhibited by the preceding parallel fibre volley as in *B* and *D*. In *G* there are plotted at each depth the time courses of the spike potential removed by inhibition i.e. the difference between the records of *E* and *F*. At depths of 130 and 180μ the subtracted curves are shown as interrupted lines.

some 10 mm distant. These impulses could be in climbing fibres and mossy fibres as well as the antidromic impulses in the axons, the axon collaterals, the somata and the dendrites of Purkinje cells. On histological considerations it can be suspected that the antidromic invasion of the enormously extensive Purkinje cells would be dominant in generating the negative phase of the initial diphasic potential. The later potentials presumably are produced by transynaptic action and will be the subject of a later paper.

Figure 2*B* and *D* shows that a preceding parallel fibre stimulation caused almost complete suppression of the negative component of the initial diphasic potential, while the positive component was unchanged. The large prolonged positive wave produced by the conditioning stimulation in Fig. 2*B* has been attributed to the hyperpolarization arising from the post-synaptic inhibitory action of basket cells on Purkinje cell bodies (Andersen, Eccles & Voorhoeve, 1964); hence the virtual suppression of the negative component can be attributed to the action of the inhibitory hyperpolarization in blocking the antidromic propagation into the Purkinje cell somata. There is no known way in which the conditioning stimulation of Fig. 2*B* and *D* could depress propagation in mossy or climbing fibres. Evidently, therefore, the antidromic propagation of impulses in the Purkinje somata and dendrites generates almost all the negative component of the initial diphasic potential in Fig. 2*A* and *C*.

The details of the antidromic spike potentials and of the inhibitory actions thereon are shown in the very fast records of Fig. 2*E* for all depths from 80 to 630 μ. The diphasic potential continued to be large as superficially as 230 μ, but at still more superficial levels, 180, 130 and 80 μ, there was a rapid and progressive decline in size, particularly of the negative component. It was surprising to find that from 630 to 230 μ the peak of the negative wave showed no detectable (less than 0·01 msec) trace of a progressive delay such as would be expected for a propagating impulse. This fixed latency for the negative peak of the antidromic potential has been observed in all the experiments in which there has been a sufficiently fast recording for significant measurements, as was the case in Figs. 2 and 3*B*. However, at more superficial levels the rapidly diminishing negative wave always exhibited a progressively delayed summit which in Fig. 2*E* was 0·06 and 0·13 msec later at 180 and 130 μ, respectively.

By superimposed plotting of the inhibited and uninhibited responses, the time courses of the inhibited components can be calculated by subtraction at each depth and are plotted in Fig. 2*G*. For levels from 630 to 230 μ the summit of the inhibited component occurred with the same latency, within 0·02 msec, and there was only a small decline in size. However, there was a significant change in the onset at 230 μ and super-

ficially thereto, there being an initial positive phase, i.e. the inhibition caused some diminution of the initial positive wave. Also at 180 and 130 μ the subtracted negative wave had a later summit, which corresponds to the later summit of the uninhibited response (Fig. 2*E*). Evidently, the potential wave removed by inhibition has the same general character as the uninhibited negative wave. The potential waves of Fig. 2*G* may be recognized as being produced by antidromic invasion of the Purkinje somata and dendrites, for only this component of the initial spike response could be depressed by the inhibitory action of a parallel fibre volley.

Similar investigations to those illustrated in Fig. 2 have been performed in twenty-one experiments and uniformly all have given similar results, though usually the degree of inhibition was less (cf. Figs. 5*A*, *B*; 6*A–F*). At a depth of about 200 μ the summit of the negative component of the diphasic wave has had the same latency as at 400–600 μ, and its size has been reduced to 40–80 % of that occurring at the soma level. There has been a rapid decline more superficially, and also there was always the progressive increase in latency.

When the mossy and climbing fibres of the cerebellum were eliminated by the degeneration resulting from bilateral pedunculotomy, J.F. stimulation always produced the greatly simplified potentials illustrated in Fig. 3*A*. The triphasic spike potential at 800 μ is attributable to the recording in volume of the propagating impulses in the Purkinje cell axons. At depths from 400 to 150 μ there was an initial diphasic wave and it was followed by a slow positive wave having a total duration of about 15 msec. The initial diphasic (positive–negative) potential resembles that observed in the intact cerebellum (Fig. 2) except that the positive phase was relatively smaller (Fig. 4*A*, *C*; 8). Experimental evidence has been adduced to support the postulate that this diphasic wave is due to the antidromic propagation of impulses up the axons and into the somata and dendrites of Purkinje cells, and this identification has been corroborated by the demonstration of a similar diphasic potential in each of our nine successful experiments on the chronically deafferented cerebellum. In such a cerebellum no structures other than the axons of Purkinje cells would be available for conducting impulses from the J.F. region up to the cerebellar cortex. However, the small size of the initial positive component of the diphasic spike indicates that in the intact cerebellum much of this component is generated by impulses in mossy and climbing fibres. The manner of production of the slow positive wave of Fig. 3*A* will be considered in the Discussion.

The very fast records from another deafferented cerebellum (Fig. 3*B*) give opportunity for studying in detail the temporal relations of the extracellular spike potentials throughout the whole thickness of the cerebellar

cortex. As shown in Fig. 3*C* (filled circles) the plotted measurements of the spike summits in Fig. 3*B* were synchronous within the limits of measurement at all depths from 700 μ up to 250 μ, and synchronism was also observed for the antidromic spike potentials (crosses in *C*) set up by another J.F. stimulating electrode in this same experiment. However, this synchronism did not obtain for the rising phases of the spike potentials, which were progressively delayed along the antidromic pathway from 600 to 250 μ depth, as may be seen by the vertical line in Fig. 3*B* at 0·35 msec latency and by the plotted points (open circles) in Fig. 3*C*. Thus the early rising phase of the spike potential exhibits a conduction velocity indicative of the progressive invasion of propagating impulses.

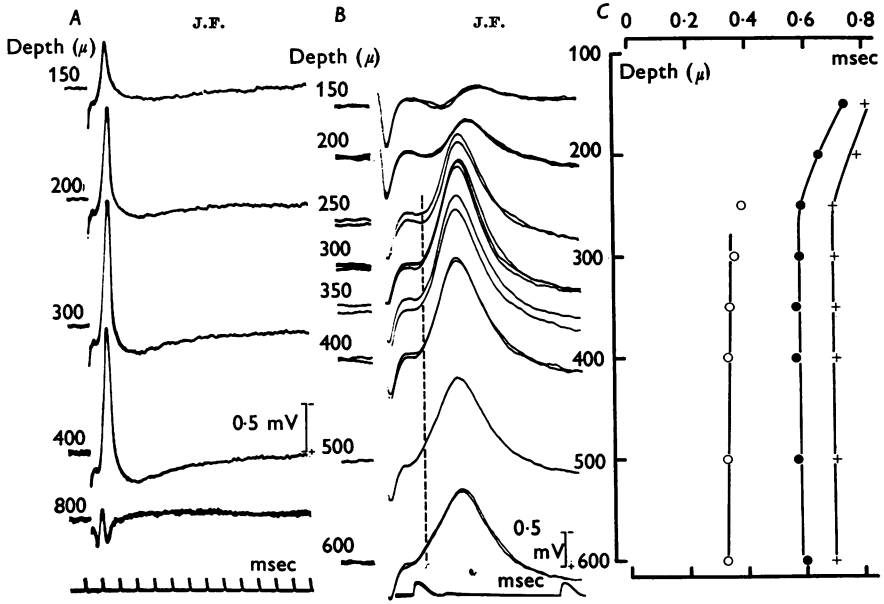

Fig. 3. Field potentials produced in the chronically deafferented cerebellar cortex by an antidromic volley in Purkinje cell axons. *A*. Potentials at the indicated depths below the cortical surface showing the typical positive-negative spike and the slow positive wave at depths of 150–400μ. *B*. Spike potentials in another experiment at very fast sweep speed in order to allow accurate comparison of the rising phases and summits at the different depths. The vertical line at a latency of 0·35 msec shows the progressively delayed onset of the negative spike at more superficial levels. *C*. Measurements from *B* are plotted to show no significant difference in time to summit (filled circles) from depths of 600 to 250μ, and the progressive increase more superficially. On the other hand (open circles) there was a progressively longer latency from 600μ to the surface when it was measured to a fixed voltage (a negative deflexion of 0·15 mV) on the rising phase. The crosses also show the constant latency of the antidromic spike summit when set up by another J.F. stimulus in this same experiment.

An approximate conduction velocity of 5–10 m/sec is indicated. These findings of a progressively delayed rising phase and of a synchronized summit in the deafferented cerebellum exactly match those on the normal cerebellum (cf. Fig. 2*E*, *G*).

Figure 3*B*, *C* shows that, just as in Fig. 2*E*, *G*, more superficially than 250 μ the antidromic spike potential declined rapidly in size and was progressively more delayed both in its rising phase and summit. In some experiments there was actually a complete reversal of potential at the most superficial levels. For example, in Fig. 4*A* at 250–50 μ depth the antidromic spike appeared as a small positive deflexion. There was also a gradual transformation of the slow positive wave so that the wave form at 150–50 μ was virtually a mirror image of that below 250 μ. In Fig. 4*A* the antidromic potential profile indicated that antidromic propagation ceased at an unusually deep level of the cortex—about 300 μ; hence presumably the prominence of the mirror reversal of the antidromic potential complex. Nevertheless, this reversal was also observed to occur to a smaller extent in several experiments where the antidromic propagation continued to the usual level.

Conditioning of the antidromic potential complex by a parallel fibre volley

In Fig. 4*B* parallel fibre stimulation produced in the chronically deafferented cerebellum a P-wave having a potential profile in depth resembling that of a normal cerebellum (Andersen *et al.* 1964). For depths below 250 μ the antidromic spike potential in Fig. 4*A* had the same configuration as in Fig. 3*A*, a negative spike followed by a slow positive wave. In Fig. 4*B* the negative spike was depressed by the preceding parallel fibre volley as in Fig. 2*B*, *D* and the later slow positive wave suffered a larger depression.

The sequence of potentials from 250 up to 50 μ in Fig. 4*A* displays the reversal of the antidromic potential complex (spike plus slow positive wave) that was referred to above. Figure 4*B* shows that the parallel fibre volley effected a re-reversal of this antidromic potential. A similar re-reversal was also observed in experiments on the normal cerebellum. An explanation of this effect will be given in the Discussion.

Figure 4*C* is a series resembling that of Fig. 4*A*, but in another experiment on a chronically deafferented cerebellum in which the antidromic complex became progressively smaller towards the surface, but did not reverse. In Fig. 4*D* a parallel fibre volley had the usual inhibitory effect at depths from 600 to 200 μ. By contrast, the much smaller antidromic spike complex from 100 μ to the surface actually was facilitated by the parallel fibre volley. This facilitation at superficial levels was also observed

in several experiments on the normal cerebellum. In both the normal and chronically deafferented cerebellum the facilitation was observed only when the recording was in proximity to the beam of excited parallel fibres.

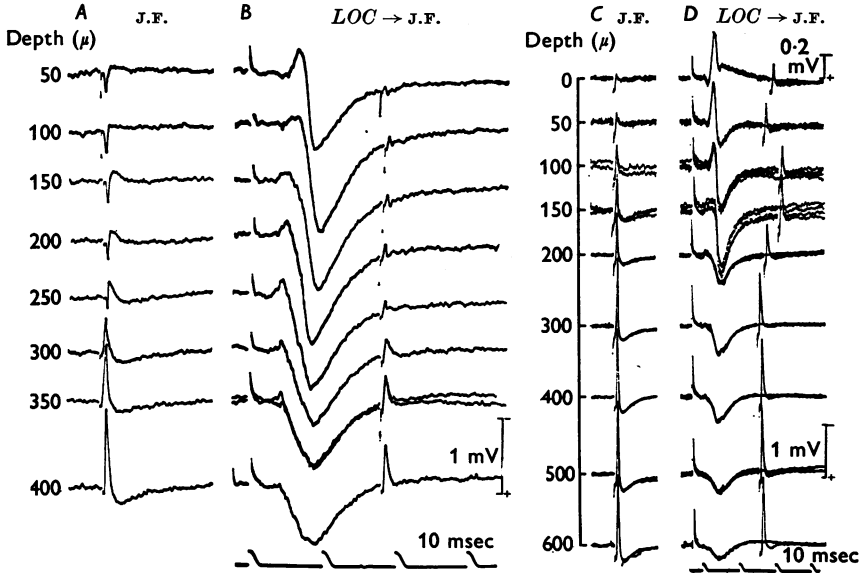

Fig. 4. Inhibitory and facilitatory influences of a parallel fibre (*LOC*) volley on the antidromic spike potential in the cortex of a chronically deafferented cerebellum. *A* shows potential fields set up by a J.F. stimulus at the indicated depths below the cortical surface. Note inversion at superficial levels as described in text. In *B* this potential was conditioned by a parallel fibre volley 18 msec earlier, facilitation was signalled by a re-reversal (see text). *C* and *D* were obtained in another deafferented cerebellum just as *A* and *B* except that the stimulus interval varied from 18 to 24 msec.

Figure 5*C* gives a typical depth profile of the action of a parallel fibre volley on the antidromic spike potential, specimen records being shown in *A* and *B* for the normal series and the series with the stronger inhibition (filled triangles in *C*). The antidromic spike potential for this normal cerebellum resembles in general that for the deafferented cerebellum in Fig. 4, but of course the specimen records show the complex potentials that immediately follow the antidromic spike (cf. Fig. 2*A–D*). The degree of inhibition was much less than that of Fig. 2, which gives favourable conditions for comparing the inhibitory depressions of the antidromic spike potential at different levels of the cerebellar cortex. The depth profile (open circles) in Fig. 5*C* shows typically that there was not a large decrement of the uninhibited antidromic spike until it was more superficial than 200 μ. On the other hand the inhibited spikes (filled circles and triangles) were quite small at 200 μ, there being a continuous severe

decrement in spike size from the deepest level. The depth profiles of the inhibitory diminution of the antidromic spikes (Fig. 5*D*) show typically that, from a relatively small amount of inhibition at the deepest level, there was a progressive increase to a maximum at 250–200 μ depth.

For the most part the rapid decrement in the amount of inhibition more superficially than 200 μ is attributable to the rapid decrease in size of the uninhibited response (Fig. 5*C*), but a contributory factor would also be the replacement of inhibition by facilitation at the most superficial levels, as illustrated in Fig. 4. The depth profiles of the uninhibited and inhibited spikes for the experiment illustrated in Fig. 4*C*, *D* are plotted in Fig. 5*E* for a testing interval of 25 msec, and in Fig. 5*F* is the depth profile for the

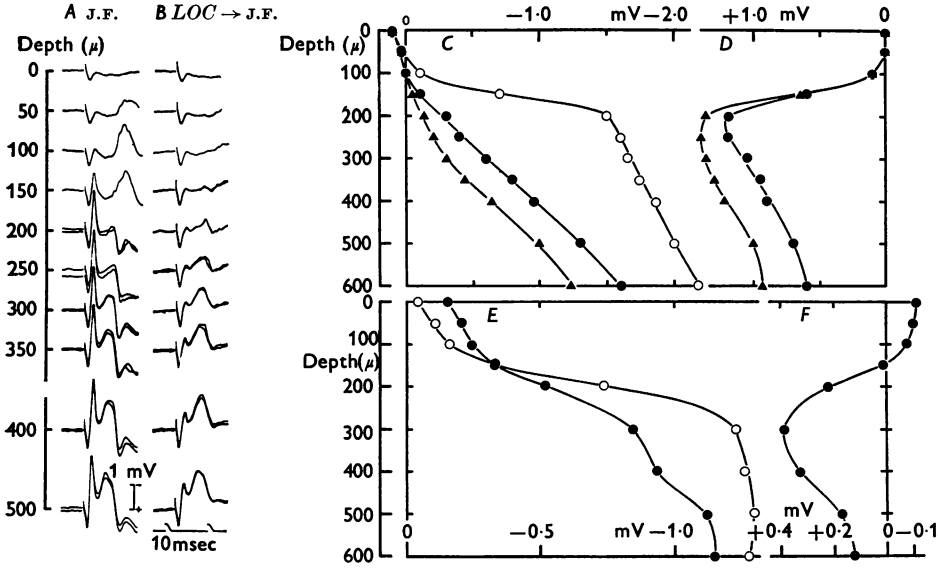

Fig. 5. Depth profile of the field potential generated by an antidromic volley in Purkinje axons. In *A* a J.F. stimulus evoked a complex potential wave at the indicated depths below the cortical surface. The initial diphasic (positive–negative) component is the antidromic spike potential of Purkinje cells (cf. Fig. 2) plus the spike potentials in the directly stimulated mossy and climbing fibres. The observations of *B* were produced concurrently with those of *A* by the same J.F. stimulus, but were conditioned by a parallel fibre volley 18 msec earlier, just as in Fig. 2*B* and *D*. In *C* the heights of the negative components of the antidromic spike potentials are plotted against the depths for the series of *A* (open circles) and *B* (filled trangles) and also (filled circles) for a series similar to *B*, but with conditioning by a parallel fibre volley evoked by a weaker stimulus (half strength). In *D* there are similar depth profile plots for the amount of inhibition by these two sizes of parallel fibre volley, which is expressed as the difference between the control and the inhibited responses. In *E* there is a similar plot for the depth profile of the control and the inhibited antidromic spike potentials as in *C* and in *F* of the amount of inhibition as in *D* for a similar investigation on a chronically deafferented cerebellum. Specimen records are illustrated in Fig. 4*C* and *D*.

actual changes in the spike heights. In general this latter curve resembles those of Fig. 5*D*, but differs in that there is the clear facilitatory phase superficial to 150 μ.

The actions of a parallel fibre volley were tested in Figs. 4 and 5 at intervals of about 20 msec, which is approximately the time of maximum inhibition and facilitation. In Fig. 6 are a series of specimen records of an antidromic spike potential at a depth of 400 μ in a normal cerebellar cortex (*A*) and at various intervals after a conditioning parallel fibre volley (*B–F*). At the two briefest testing intervals (*B*, *C*) there was no detectable inhibition, and in *D–F* the inhibition increased progressively with increasing test interval. The full time course of the conditioning action of a parallel fibre volley is plotted in Fig. 6*G* from a comparable series of

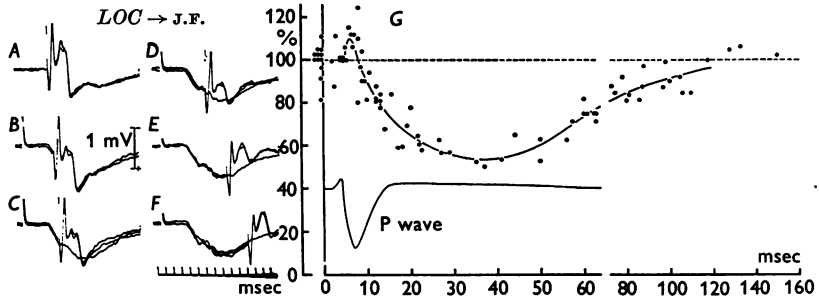

Fig. 6. Time course of the action exerted by a parallel fibre volley on the antidromic spike potential in the cerebellar cortex. *A* shows the typical complex potential produced by a J.F. stimulus in a normal cerebellum and recorded at a depth of 500μ. In *B–F* this response was conditioned by a parallel fibre volley at various stimulus intervals, there being superimposed in *C–F* a trace of the response to the conditioning volley alone in order to aid measurements of the size of the negative component of the antidromic spike. The sizes of similar potentials in another experiment are expressed as percentages of the mean control responses and are plotted against the stimulus intervals in *G*. Note the change in abscissal scaling at the interruption of the base line at 70 msec. In *G* there is also a tracing showing on the same time scale the *P* wave that was produced by the conditioning parallel fibre volley as in *C–F*.

observations in another experiment. The inhibition was maximum at test intervals of 20–50 msec and full recovery did not occur until about 120 msec. Furthermore, Fig. 6*G* gives an indication of a slight and transient facilitation at about 7 msec test interval.

A similar brief and slight facilitation has been observed in several other experiments, and may be correlated with the intracellularly recorded potentials from Purkinje cells, which sometimes showed an EPSP and even a spike potential just preceding the onset of the IPSP generated by a parallel fibre volley (Andersen *et al.* 1964; Eccles, Llinás & Sasaki, 1964*a*, Fig. 2*D*). The prolonged time course of these IPSPs matches the time

course of the inhibition of the antidromic spike potentials in Fig. 6 *G*. On the other hand the positive waves (P-waves) recorded extracellularly at the same time as the inhibitory curve (cf. Figs. 6*C–F*, and 6*G*, traced record) always have a very different time course. The P-wave onset was several milliseconds earlier, the maximum occurred early on the incrementing phase of the inhibition and the P-wave actually terminated while the inhibition was still increasing. The complex problems involved in the attempt to relate the extracellularly recorded P-wave to inhibitory action on Purkinje cells will be treated in a later paper.

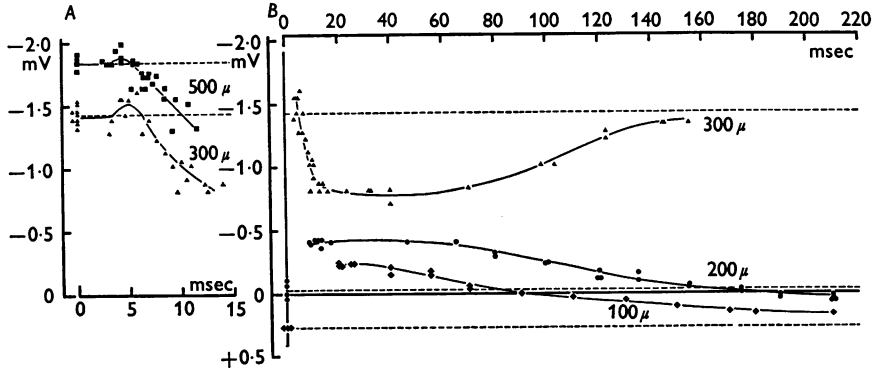

Fig. 7. Time courses of the facilitatory and inhibitory actions exerted by a parallel fibre (*LOC*) volley on the antidromic spike potentials at various levels of a chronically deafferented cerebellar cortex. The antidromic spike potentials were similar to those of Fig. 4*A*, *B* in that the dominant negative phase of the antidromic spike potential reversed to a positive spike potential at superficial levels such as 100μ, the 200 μ depth being transitional, but the experiment was on another chronically deafferented cerebellum. In *A* the sizes of the conditioned antidromic spike are plotted as in Fig. 6*G* against stimulus intervals of up to 14 msec for depths of 500 μ (filled squares) and 300 μ (filled triangles). In *B* there is a similar plotting of the conditioned antidromic spike potential for intervals up to 210 msec and for depths of 300μ (filled triangles), 200μ (filled circles) and 100μ (filled squares). Note that the mean control potentials in *B* were about zero at 200μ and positive at 100μ. Note also that at 100μ the facilitatory influence converts the positive spike to a negative spike, from below to above the zero line, as is illustrated at the superficial levels of Fig. 4*B*.

The curve in Fig. 6 for the time course of inhibition was obtained at the depth of 500 μ. In Fig. 7*A* there are plotted the early parts of the inhibitory curves at depths of 500 and 300 μ in a chronically deafferented cerebellum. Just as in Fig. 6*G* there was probably at 300 μ a small initial facilitation at 4–6 msec intervals, and there may even have been a trace in the 500 μ depth. However at depths of 200 and 100 μ there was facilitation (Fig. 7*B*) resembling that illustrated in Figs. 4*B*, *D* and 5*E* for testing intervals of about 20 msec. In Fig. 7*B* this facilitation had a time course as prolonged as the inhibitory time courses at 300 μ depth in

Fig. 7*B* and at 500 μ depth in Fig. 6*G*. In Fig. 7*B* it can be seen that the inhibitory and facilitatory curves have an approximate mirror-image relation. In the Discussion explanations will be developed for these mixed inhibitory and facilitatory actions of parallel fibre volleys.

Conditioning of the antidromic potential complex by a preceding antidromic volley

In the normal cerebellum it is impossible to set up an antidromic volley in the Purkinje cell axons without at the same time exciting mossy fibres and climbing fibres, which themselves have profound influences on the excitability of Purkinje cells. On the other hand the chronically deafferented cerebellum is ideal for this investigation because the conditioning J.F. stimulus influences the Purkinje cells under examination solely by the antidromic impulses in their axons and axon collaterals.

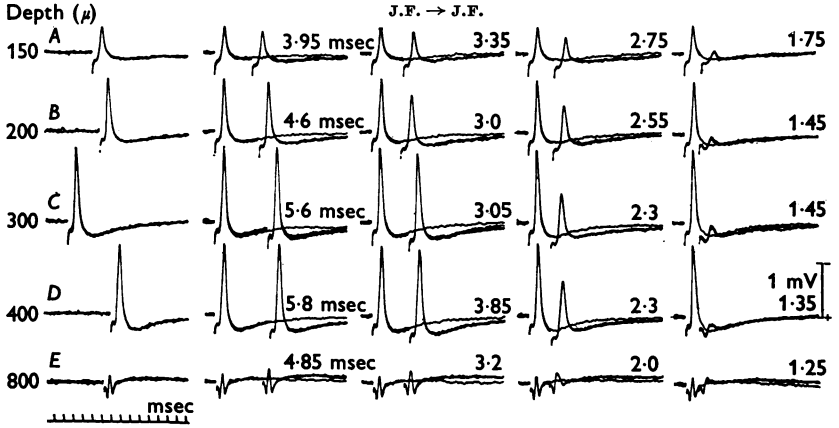

Fig. 8. Field potentials evoked by double J.F. stimulation in the chronically deafferented cerebellum and recorded at the indicated depths below the cortical surface. The initial traces at each depth were evoked by the second stimulus alone. The stimulus intervals in milliseconds are indicated on the double stimulus records, and superimposed on each of these records is a trace of the response evoked by the first stimulus alone.

In Fig. 8 the testing J.F. stimulus evoked the typical antidromic potential complex at levels of 400, 300, 200 and 150 μ, while much deeper, at 800 μ, the triphasic spike potential is attributable to the recording in volume of the antidromic volley as it passed the electrode en route to the cerebellar cortex. At the two longest testing intervals the testing J.F. stimulus added on to the conditioning response a potential complex closely resembling the control. With testing intervals from 2·3 to 2·75 msec there was at depths of 400, 300, 200 and 150 μ a diminution of the negative spike and the later slow positive wave. Finally, at the briefest intervals (1·35–1·45 msec) there was only the same very small negative spike at all

depths from 400 to 200 μ. Correspondingly, at 800 μ depth the triphasic response at the longer test intervals (2·0–4·85 msec) changed to a diphasic (positive–negative) at the briefest interval (1·25 msec). This change signifies that at that brief interval the antidromic impulses were very ineffectively transmitted into the cerebellar cortex. Presumably there was blockage of many impulses that normally invade the Purkinje cell somata and dendrites. It is essential to recognize that the field potentials evoked by the antidromic impulses are produced by the summed responses of many Purkinje cells.

Since the two stimuli were of similar strength and were applied through the same electrode, relative refractoriness of the Purkinje axons would be expected to diminish the response to two stimuli at the intervals of less than 3 msec. There was a considerable diminution of the triphasic response at a 2·0 msec test interval at the 800μ depth. However, the diphasic response at 1·25 msec shows that at brief intervals there was failure of propagation along the normal antidromic path for impulses set up early in the relatively refractory period. And this failure is also indicated at 2·0 msec by the relatively small size of the terminal positive component of the triphasic spike. The failure of antidromic invasion at a test interval of 2·1 and 1·7 msec was illustrated for single Purkinje cells in Fig. 1 for the intracellularly and extracellularly recorded antidromic spike potentials, respectively. These test intervals were critical for invasion, which occurred at the same intervals in Fig. 1*D*, *H* and *I*. The antidromic spike potentials were then a little reduced in size and had a more prominent IS–SD inflexion.

It would be expected that there would be a relatively low safety factor for propagation of an antidromic impulse into the enormously expanded surface membrane of the soma and dendrites of a Purkinje cell; hence an explanation is provided for the failure of antidromic propagation during the relatively refractory period, and for the greater prominence of the inflexion in Fig. 1*D* and *H–K*, which resembles the IS–SD spike potential complex of a motoneurone (Brock, Coombs & Eccles, 1953; Coombs *et al.* 1957; Terzuolo & Araki, 1961). However, account has also to be taken of an additional pathway for the antidromic impulses, namely, along the collaterals of the Purkinje axons, that have been described by Ramón y Cajal (1911) and by Jakob (1928) as forming a fairly dense meshwork both deep and superficial to the layer of the Purkinje cell somata. Possibly propagation along these collaterals is solely responsible for the small spike potentials in Fig. 8 at such test intervals as 1·35 and 1·45 msec in the records at 400, 300 and 200 μ depth.

This suggestion has been tested by a detailed examination of the responses evoked by the second antidromic volley when the stimulus interval was very finely adjusted over the range between failure of response

and the least interval for a full-sized response. In another experiment on a chronically deafferented cerebellum (Fig. 9*A*) the least stimulus interval for a small spike as in Fig. 8 was 1·1 msec, and increasing the interval to 1·7 msec was associated with only a relatively small increase in size, but, with a further increase to 2·0 msec, the antidromic spike potential had almost doubled, and by 3 msec it was full-sized. This phase of a small spike response over a considerable range of brief stimulus intervals is exactly according to expectation if the small spike were generated by impulse conduction in some components of the Purkinje cells that had a higher safety factor for transmission, as has been postulated above for the

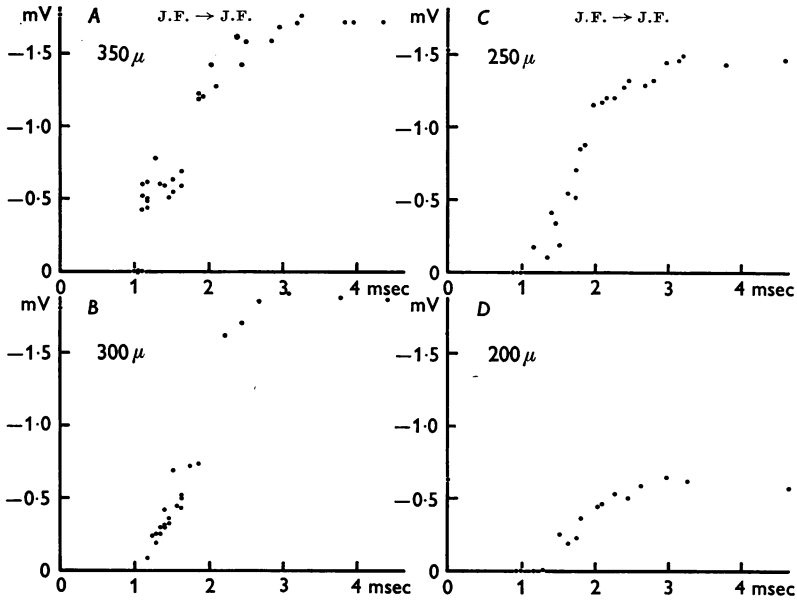

Fig. 9. Time course of recovery of the antidromic spike potential produced by a second J.F. stimulation in a chronically deafferented cerebellum. In a series similar to that of Fig. 8 there were large numbers of observations at intervals giving all stages of recovery and at depths of 350μ (*A*), 300μ (*B*), 250μ (*C*) and 200μ (*D*) below the cortical surface. The sizes of the negative spike potentials in millivolts were plotted against the stimulus intervals.

axon collaterals. The identification of this small spike as due to impulses in the supraganglionic plexus of Purkinje axon collaterals is supported by the observation that the small spike decreased in size very rapidly at levels more superficial than that of Fig. 9*A*, as may be seen at 300, 250 and 200 μ in *B*, *C* and *D*, respectively. This rapid decrement towards the surface would be expected for potentials generated in the supraganglionic plexus that is illustrated by Ramón y Cajal (1911, Fig. 12).

Repetitive stimulation of Purkinje cell axons

As explained in the preceding section this investigation can be performed only in the chronically deafferented cerebellum. In this preparation antidromic propagation of impulses continues to produce during a tetanus the large negative spike potential characteristic of soma-dendritic invasion. For example, in Fig. 10 at a frequency of 310/sec there was a small reduction in the height of the first few responses, but thereafter a fairly steady level was maintained for the terminal 25 spikes of the longest

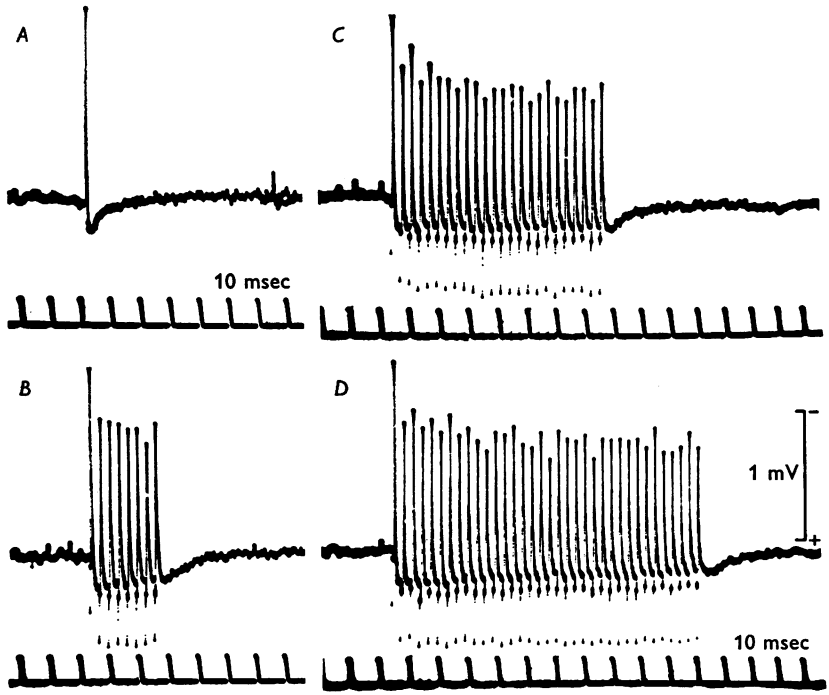

Fig. 10. Field potentials evoked in the chronically deafferented cerebellar cortex by repetitive J.F. stimulation. *A* shows control response recorded at a depth of 500μ and in *B–D* there was progressively longer tetanic stimulation at a frequency of 310/sec.

tetanus (35 impulses). In all tetani (*B–D*) each negative spike declined to a positive potential comparable with that following a single response (*A*). There was little or no increase in this background positive potential during the whole tetanus, and on cessation of the tetanus in *B–D* the slow positive wave was not noticeably larger or longer than after a single stimulus. Certainly there is very little cumulative effect during an antidromic tetanus. The significance of this observation will become apparent in the Discussion

Inhibitory action of antidromic impulses on the rhythmic responses of Purkinje cells

Since Purkinje cells have been shown to have a direct inhibitory action on the cells of Deiters nucleus (Ito & Yoshida, 1964) and of the intracerebellar nuclei (Ito, Yoshida & Obata, 1964), a similar synaptic inhibitory action would be expected for the axon collaterals of Purkinje cells. The chronically deafferented cerebellum gives opportunity for testing this inference. There is histological evidence that these collaterals contribute synapses to the large dendrites of Purkinje cells (Szentágothai, 1964, 1965 and personal communication; C. A. Fox, personal communication). However, testing by a second antidromic volley revealed no depression of Purkinje cells beyond the very brief depression (Figs. 8, 9) that is satisfactorily explicable by refractoriness; nor is there any accumulation of depression during repetitive stimulation (Fig. 10), as would be expected for inhibitory synaptic action. It could, however, be objected that the safety factor for antidromic invasion of Purkinje cells is such that weak synaptic inhibitions are ineffective as depressants. For this reason it is important to investigate the more sensitive index of inhibitory action that is provided by the spontaneous rhythmic discharge of a Purkinje cell in a chronically deafferented cerebellum.

Figure 11*A* gives a consecutive series of records in which single J.F. stimuli were applied during the rhythmic response of a Purkinje cell in a chronically deafferented cerebellum. In each of these five records the stimulus evoked an antidromic response marked by the arrows, and this was followed by a silence of 52–146 msec, which is much longer than the longest cycle (35 msec) of the rhythm before the stimulation. However, this silence was much briefer than the silence produced by the inhibitory action of a single parallel fibre volley, the range being 325–380 msec for this rhythmic response, one example (325 msec silence) being illustrated in Fig. 11*A* (cf. Anderson *et al.* 1964, Fig. 6). In the deafferented cerebellum the mode of generation of the spontaneous rhythm is unknown; nevertheless, the typical inhibitory action of the parallel fibre volley establishes that it is a sensitive indicator of post-synaptic inhibitory action on Purkinje cells. Hence, it can be concluded that the antidromic volley in the Purkinje axons exerts a considerable inhibitory action on this Purkinje cell. The variability in the duration of the silence may be attributed in part to variations in the rhythmic mechanism itself, as disclosed by the irregularities in the rhythm before the silent periods, and in part to variations in a weak inhibitory mechanism.

The most regular rhythmic discharge that we have observed is illustrated in Fig. 11*B*, *C* from another chronically deafferented cerebellum. In series

B the J.F. stimulation was just at threshold for the axon of the Purkinje cell under observation, setting up a discharge in three examples and failing to do so in four others. In the former the interpolated antidromic impulse was followed by a pause in the rhythm which in each case was a little longer than compensatory, i.e. the sum of the intervals before and after

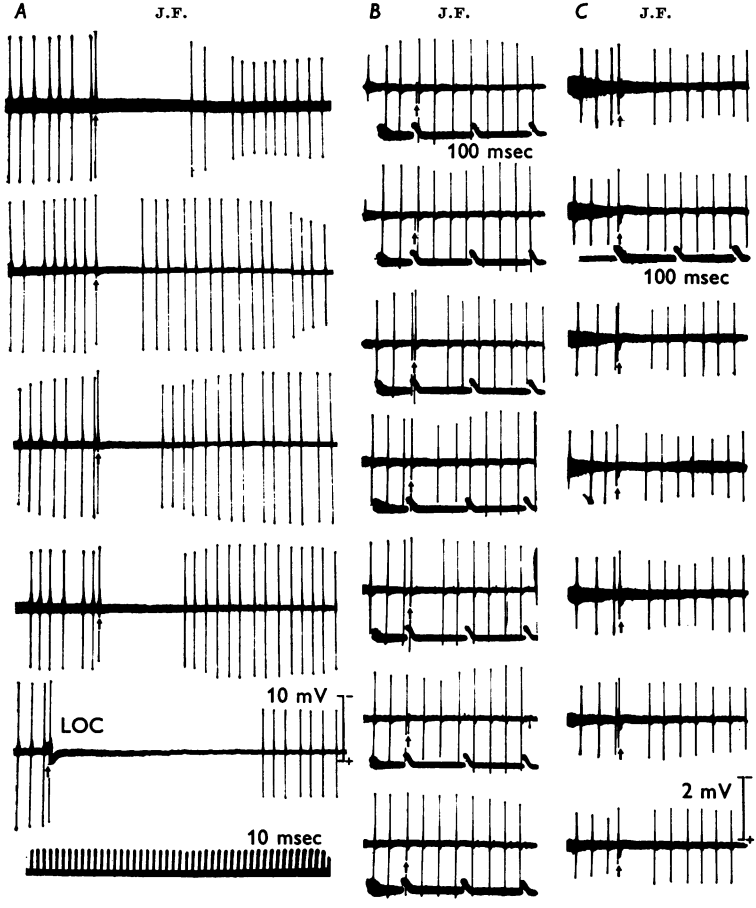

Fig. 11. Inhibitory action on the spontaneous rhythmic discharges of Purkinje cells in the chronically deafferented cerebellum. In the four upper records of *A* a single J.F. stimulus was applied at the arrows and evoked an antidromic response of the Purkinje cell followed by a silence of variable duration. In the lowest record a much longer silence was produced by a single parallel fibre volley. The variations in the spike heights are due to slight movements caused by respiration. *B* and *C* illustrate the weaker inhibition that a J.F. stimulus exerts on a rhythmic Purkinje cell in another chronically deafferented cerebellum. In *B* the stimulus was just at threshold and in this consecutive series of traces it excited the Purkinje cell antidromically only in the middle three. In *C* the J.F. stimulus was 2·5 times the threshold strength and on every occasion an antidromic spike was set up followed by a fairly long silence.

the interpolated response was longer than two normal cycles. On the other hand, in the latter group of four, the rhythmic discharge appeared to be unaffected; yet as shown by a small potential wave (marked by arrows) this stimulus had excited other Purkinje axons. The plotted points of Fig. 12*D* for the whole series including the examples of Fig. 11*B*, shows that stimuli failing to excite the axon of the observed Purkinje cell usually caused a slight delay of the rhythmic response that was just statistically significant (mean value 2·09, $P = 0{\cdot}05$), whereas there was a longer delay (mean value 2·19, $P < 0{\cdot}01$) if the stimulus were effective (Fig. 12*A*). When the J.F. stimulus strength was increased so that antidromic impulses were set up in a larger number of Purkinje axons, it is seen in the plotted points of Fig. 12*B* and *C* for two increases in stimulus strength that the silences in the rhythm were longer (Fig. 11*C*), the pauses being always more than compensatory. In general the stronger the stimulus, the longer the silence, the mean values being 2·49 and 2·31 for *B* and *C* which were highly significant ($P < 0{\cdot}01$). It can be concluded that in this experiment also antidromic impulses in the Purkinje axons exerted a weak inhibitory action on Purkinje cells.

Altogether, thirteen rhythmically discharging Purkinje cells of the deafferented cerebellum have been tested for the inhibitory action of impulses in Purkinje cell axons, and inhibition has been demonstrated in nine, including the two illustrated in Figs. 11 and 12, and it probably occurred in the four other Purkinje cells, but their rhythmic discharges were too irregular to allow a convincing demonstration. It is important to recognize that this inhibitory action is very weak relative to the inhibition generated by parallel fibre volleys; hence the double antidromic volley technique failed to demonstrate any inhibitory depression beyond that attributable to refractoriness (Figs. 8, 9), though the antidromic spike potential wave was very effective in demonstrating the inhibition produced by a parallel fibre volley (Figs. 2, 4, 5, 6, 7).

This inhibitory action that may be postulated for impulses in the axon collaterals of Purkinje cells is so weak that it may have but little functional importance as a negative feed-back control of Purkinje cell activity; nevertheless it is highly significant because it corroborates the general postulate that all the synapses made by the axonal branches of a neurone have the same function: in this case there is inhibitory action both by the synaptic terminals of the Purkinje cells in the intracerebellar nuclei (Ito *et al.* 1964) and in Deiters nucleus (Ito & Yoshida, 1964) and at the synapses of the Purkinje axon collaterals on to Purkinje cells. There is the further finding (Fox, 1962, personal communication; Szentagothai, personal communication) that Purkinje axon collaterals make many synapses on the somata and dendrites of basket and Golgi cells. Inhibitory action on these

cells by such axon collaterals has also been demonstrated (Eccles, Llinás & Sasaki, 1965*a*). It is an example of a positive feed-back pathway having a disinhibitory function (cf. Wilson & Burgess, 1962), the discharge of impulses by Purkinje cells inhibiting both the basket cells that inhibit

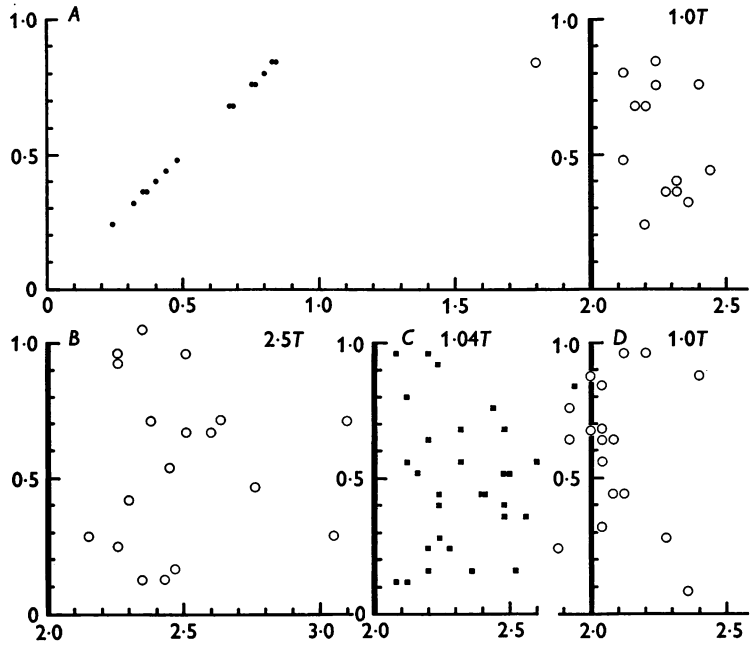

Fig. 12. Plotting of the effect of a J.F. stimulus on the rhythmic responses of a Purkinje cell. In Fig. 11*B* are the specimen records for some of the points plotted in *A*. All measurements are reduced to fractions of the mean cycle time for the spontaneous rhythm. Since the interpolated antidromic response would be expected to be followed by a partial or complete compensatory pause, the effect due to this can be separated from any silent period due to superimposed inhibition by the method of plotting adopted in *A*. The lengths of the cycles interrupted by the antidromic response expressed as a fraction of the mean cycle time are plotted (filled circles) on both the horizontal and vertical co-ordinates so that the antidromic responses lie strictly on a 45° line as shown. The subsequent cycle for each of these antidromic responses is plotted (open circles) on the same abscissal scale. The thick vertical line at 2·0 gives the time at which this cycle would terminate if the subsequent cycle were fully compensatory, i.e. if the original phase of the rhythm was resumed. It is seen that the silence is longer than compensatory in all but one case, the mean increase being to 2·19. This J.F. stimulus was just at threshold as shown in Fig. 11*B*, and the cases where it failed to set up an antidromic spike are plotted in *D* using the same convention as in *A* and measuring to the next spike but one. The J.F. stimulus produced little or no delay in this spike, the points falling as a rule very close to the 2·0 line, the mean value being 2·09. *B* shows a similar plotting for the series of Fig. 11*C* for a J.F. stimulus of 2·5 times (*T*) the threshold strength (mean increase was to 2·49), and in *C* with a J.F. stimulus only 1·04 times threshold the mean increase was to 2·31.

Purkinje cells (Andersen *et al.* 1964), and the Golgi cells that inhibit the granule cells that excite Purkinje cells (Eccles, Llinás & Sasaki, 1964*b*.)

DISCUSSION

Interpretation of laminated potential fields produced by antidromic invasion of Purkinje cells

Figure 13*A* shows diagrammatically the flow of current at about 0·3 msec after the J.F. stimulation, when the somata and adjacent dendrites of the Purkinje cells would be acting as sources in the external circuits for the sinks at the regions of the impulses. Since the circuit loops are so extensive vertically relative to the transverse dimensions of the surface folium under investigation, the circuits correspond to the well-known features for parallel core conductors immersed in a conducting medium. At depths well below the superficial cortical layer (Figs. 3*A* and 8*E*), the typical triphasic spike potential is observed as the volley of antidromic impulses passes by the tip of the recording electrode. This triphasic spike was rarely observed with a deeply placed micro-electrode, presumably because the electrode was close to the cortical layers of deeper folia. At the more superficial levels represented in Fig. 13*A* there is similarly an initial positive potential (Fig. 2), and the time courses of these potentials are almost identical at depths of 500–200 μ, which would correspond to the somata and major dendrites. However, careful measurement of fast records discloses that the terminal phase of the positive wave becomes progressively later at more superficial levels, so that in Fig. 2, for example, the crossing of the zero potential line occurs at 0·61 msec at 600 μ, and at 0·62, 0·635, 0·64, 0·665 msec at each successive 100 μ towards the surface. Hence the virtual absence of current flow over this range of levels during the first 0·4 msec is changed at 0·1–0·25 msec later to a considerable flow from sources in the more superficial dendrites to the axons and somata of the Purkinje cells. Similarly, there is evidence for this current flow in Fig. 3*B*, *C*. This is of course the current that flows as the Purkinje dendrites are being depolarized by electrotonic spread from the regions already invaded by the antidromic impulses.

It has been a remarkable finding that, despite its later onset, the summit of the extracellular antidromic spike potential is not appreciably ($< 0{\cdot}02$ msec) later at the level of the Purkinje dendrites at 200 μ than at the deeper levels of the somata and axons at 400–600 μ (Figs. 2*E*, 3*B*, *C*). Furthermore, the negative antidromic spike has been well maintained in size up to 200 μ depth (Figs. 2*E*, 3*A*, 5*A*, *C*). More superficially the size rapidly declined and the summit was progressively more delayed, and close to the surface there was often a reversal of its polarity (Fig. 4*A*). The laminated

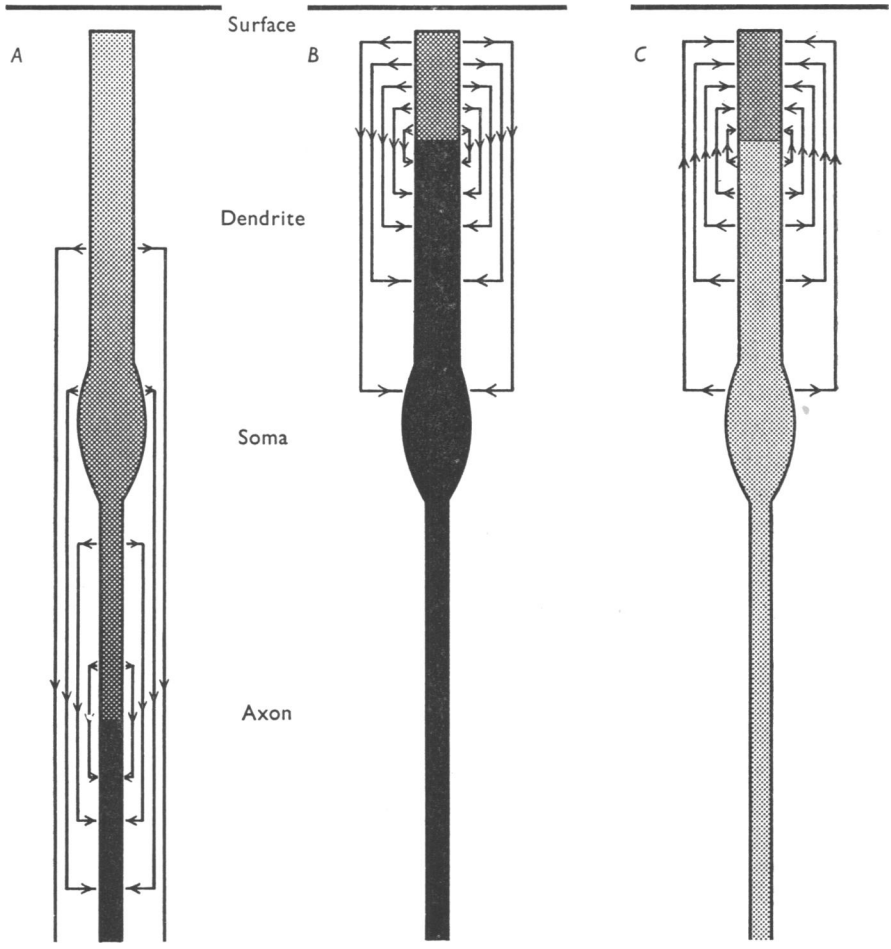

Fig. 13. Diagrams to show field potentials generated by the antidromic propagation of an impulse into a Purkinje cell. The grey shading indicates depolarization, the darker the grey the more intense the depolarization. The zones occupied by the impulse are shown black. A single Purkinje cell only is shown in *A*, *B* and *C*, but the lines of extracellular current flow are drawn confined to the immediate surround, as they would be in the situation where all Purkinje cells in an area are being simultaneously invaded. In *A* the antidromic impulse is propagating up the axon and there is a graded electrotonic depolarization of the soma and dendrites. In *B* the impulse has invaded the soma and dendrites to the maximum extent, there being a terminal dendritic zone not invaded, but merely deeply depolarized by the electrotonic currents as shown. In *C* the axon, soma and the invaded part of the dendrites have almost completely recovered from the impulse, with the consequence that those regions are less depolarized than the uninvaded dendritic zone; hence the reversed current flow. Further description in text.

potential field at the time of the antidromic spike potential (Figs. 2*E*, 3*B*) suggests that the flow of current in the cerebellar cortex is as shown in Fig. 13*B*. The slight potential gradient at depths from 600 to 200 μ indicates that there is little vertical current flow, but on the other hand from 200 μ to the surface there is a large current flowing from sources near the surface to sinks as deep as 200 μ, but not so much to deeper sinks, as is roughly indicated by the density of vertical current lines in Fig. 13*B*. The sources at the superficial levels may actually be positively directed spikes as in Fig. 4*A*, but more frequently are only relatively less negative than at deeper levels as in Fig. 4*C*.

Despite the slightly later onset at more superficial levels, there was a virtually synchronous attainment of the peak negatively at depths 600–200 μ (Figs. 2*E*, 3*B*, *C*); hence, it can be envisaged that the whole complex of soma plus large dendrites of a Purkinje cell tends to fire almost synchronously, presumably on account of so-called 'trigger zones' which are located at strategic sites on the dendrites—perhaps at main branching sites—and which generate impulses at a lower threshold of depolarization, as has been postulated, for example, for hippocampal pyramidal cells (Spencer & Kandel, 1961*b*). These trigger zones may arise on account of background synaptic activation on the Purkinje cell dendrites. The distribution of sources and sinks in Fig. 13*B* shows that the most superficial levels of the Purkinje cell dendrites are heavily depolarized by electrotonic currents flowing into the larger dendrites. The small and later spike potentials at levels of 150 and 100 μ (Figs. 2*A*, 3*B*, *C*) show that there was at least some antidromic invasion after the instantaneous invasion up to about 200 μ, but the rapidly diminishing spike indicates that for the most part impulse invasion would fail for dendrites at such levels, and there can be but little invasion more superficially than the 100 μ level. If the fine superficial dendrites are in this way merely passively depolarized without spike initiation, it can be presumed that the small Purkinje dendrites that are densely packed between the larger dendrites at deeper levels are also not invaded antidromically.

Over a wide range of levels (100–600 μ) in the chronically deafferented cerebellum the sharp negative potential that signals antidromic invasion of the Purkinje somata and dendrites is always followed by a slower positive wave (Figs. 3*A*, 4*A*, *C*, 8, 10) of 10 to 20 msec duration. At more superficial levels than 200 μ this positive wave declines in parallel with the initial spike potential. There may even be reversal at the surface, which again parallels the reversal of the initial spike (Fig. 4*A*). The laminar profile for this slow positive wave (Figs. 3*A*, 4*A*, *C*, 8) shows that the generating extracellular current corresponds to the diagrammatic illustration (Fig. 13*C*), which in fact is an exact reversal of that during the

negative spike. It is remarkable that most experimental procedures link these two potentials together, so that a common mechanism of generation seems likely at least for part of the positive wave. For example, in Fig. 8, with antidromic responses depressed by a preceding antidromic volley, there was in general a good correlation between the size of the negative spike and the subsequent positive wave. In some cases, however, at depths of 500–300 μ, conditioning by a parallel fibre volley depressed the positive wave more than the initial negative spike. If the positive wave were due to an active hyperpolarizing process, a differential depression of this kind would occur during the hyperpolarization of the IPSP produced by the parallel fibre volley. Thus the differential depression suggests the possibility that the positive wave might be in part due to inhibitory synaptic currents produced by the synapses that the axon collaterals of Purkinje cells give to the soma and the main dendrites of the neighbouring Purkinje cells (Figs. 11 and 12) and possibly also to Golgi and basket cells (Eccles, Llinás & Sasaki, 1965*a*).

We have already postulated that the antidromic impulse does not invade the finer dendritic branches, which merely are passively depolarized thereby. On analogy with other nerve cells and axons (Hodgkin, 1964; Ito & Oshima, 1964) it can be assumed that in membrane invaded by an impulse the initial phase of high sodium conductance leads to a phase of high potassium conductance that rapidly restores the membrane potential; and this rapid restoration of membrane potential is illustrated by the intracellular spikes in Figs. 1*A*, *B*. In those regions of the dendrites not invaded by the antidromic impulse there is no such rapid mechanism of restoration of the passively depolarized membrane. The only intrinsic mechanism of recovery depends on the resting ionic permeabilities that effect a recovery with the resting time constant of the membrane, which similarly with other nerve cells (Coombs, Eccles & Fatt, 1955; Coombs, Curtis & Eccles, 1959; Spencer & Kandel, 1961*a*; Creutzfeldt, Lux & Nacimiento, 1964) may be assumed to be several milliseconds in duration. This recovery will therefore lag far behind the recovery of the activated regions of the membrane; and consequently there is a reversal of electrotonic current flow as the active regions recover to a higher level of polarization than the passive. This reversal of current (Fig. 13*C*) would of course generate most of the slow positive wave.

There is thus a satisfactory explanation of the observed positive waves at all depths and of the correlation between the initial fast negative wave and the later slow positive wave. The latter part of this time course would give an approximate measure (actually an underestimate) of the time constant of the membrane potential of the finest dendrites. This postulated diphasic current flow between the activated and passive components of a

single core-conductor element has been demonstrated in the spinal motoneurone when the antidromic impulse in the initial segment failed to invade the soma-dendritic membrane (Coombs *et al.* 1957, Fig. 8).

It will be recognized that a very great simplification has been introduced into this theoretical discussion by assuming that the small dendrites (the spiny branchlets) are concentrated in a layer superficial to the large invaded dendrites. Actually these small dendrites are also inextricably mixed up with the larger Purkinje dendrites at all the deeper levels of the molecular layer. However, the currents flowing in this mixed zone will be completely randomized, and so will not contribute to the general field potential. On the other hand, more superficially than 200 μ, the large dendrites will be rapidly terminating by profuse branching; consequently there will be a progressive preponderance of small dendrites; hence there will be a laminated arrangement in the integral of the currents generated by the individual elements, which gives justification for the construction of the diagram in Fig. 13*A–C*.

The potentials produced by antidromic propagation into the hypoglossal and oculomotor nuclei exhibit a configuration closely resembling those for Purkinje cells, there being an initial large negative spike and a later slow positive wave (Lorente de Nó, 1947, 1953). These potentials were there interpreted on the basis of a closed field system for these nuclei with a concentration of somata in the centre and a surround preponderantly of dendrites, which is essentially the condition for the laminated arrangement of the Purkinje cells with the dendrites oriented to the free surface of the cortex. However, in part at least the interpretation of the later slow positive wave differed radically from ours, it being postulated that it was due to continued invasion of the finer dendritic terminals that were concentrated in the peripheral surround of the nuclei. We agree with Lorente de Nó that during this positive wave the current is flowing from the somata towards the dendritic terminals, but we attribute this, not to a delayed invasion of the dendrites, but to their delayed repolarization after their initial passive depolarization. It is not conceivable that the dendritic invasion by the antidromic impulses could continue for more than a fraction of a millisecond. For example, when the dendritic invasion was facilitated by excitatory synaptic action on the Purkinje dendrites (Fig. 4*B, D*), the spike so produced was not appreciably later than the soma spike.

Inhibitory and excitatory synaptic action on antidromic impulse conduction

When the inhibitory action of a parallel fibre volley on the antidromic spike potential was at a moderate level, as in Figs. 4, 5, and not almost

total as in Fig. 2, it provided information on the range of levels at which the inhibitory synapses were effectively acting on the Purkinje cells. It was an almost invariable finding of such experiments that the depth profile of the spike diminution reached a maximum at the 200–300 μ level (Figs. 4, 5*D*, *F*). It was considerably less at 400 μ and was progressively less at deeper levels. The time course of this inhibitory action (Figs. 6*G*, 7*B*) corresponds to the time course of the intracellular IPSP that is recorded in the Purkinje cell soma (Andersen *et al.* 1964), and that was attributed by them to the inhibitory action of the basket cell synapses on the Purkinje cell somata. Such an IPSP would block the propagation of antidromic impulses into all those somata where the safety factor was low, and so would cause the observed depression of the antidromic spike potential.

If the blockage were due solely to an inhibitory action concentrated on the somata, it would be expected that there would be a rapid diminution in the number of antidromically propagated impulses at and more superficially than the soma level, i.e. at about 400 μ. If the impulses were able to propagate through such an inhibitory barrage at the soma level, it would be expected that they would continue to propagate in a normal manner up the dendritic trees. However, the depth profile of the antidromic spike potential indicates that the inhibition increased considerably in effectiveness during this phase of dendritic propagation, and in some cases there was even a sign of a second zone of effective inhibition at a depth of 250–200 μ. There will be discussion elsewhere (Eccles, Llinás and Sasaki, 1965*b*) of the hypothesis that this dendritic inhibition is due to the inhibitory action of the synapses that the superficial stellate cells form on the Purkinje dendrites (Szentágothai, 1964, 1965).

In addition to this depressant action on the antidromic propagation of impulses, parallel fibre stimulation provided two demonstrations of facilitation of antidromic propagation at depths of about 300–400 μ. A small transient facilitation sometimes occurred at test intervals of about 6 msec (Figs. 6*G*, 7*A*), which was just before the onset of the inhibition; at superficial levels (200 μ to surface) there was often a facilitation or a re-reversal of the small antidromic spike (Figs. 4*B*, *D*; 7*B*). Both of these facilitation phenomena appear to be explicable by the direct excitatory action of parallel fibres on the Purkinje dendrites, and, as would be expected, they are observed only when the recording electrode is in or close to the beam of excited parallel fibres. So far as has been investigated (Fig. 7*B*), the time course of the antidromic spike facilitation at superficial levels resembles that for inhibition at deeper levels. At these levels the slightly earlier onset of the monosynaptic excitatory action gives it a brief initial advantage over the disynaptic inhibition (Figs. 6*G*; 7*A*), and presumably

it continues as a submerged background during the dominant inhibition.

Figure 7*B* shows that, despite the inhibitory depression of the antidromic spike at deeper levels and its facilitation superficially, there continues at all times to be a progressive decrement towards the surface, as is shown by the curve through the filled circles in Fig. 5*E*. Inhibition accentuates the decrement at deeper levels (Fig. 5*C*, *E*), and the superficial excitatory action of the parallel fibres merely slows down the rate of the decrement at that level (Fig. 5*E*). These observations can be interpreted as due to the opposed actions of synaptic inhibition and excitation on antidromic propagation in the Purkinje dendrites: the net inhibition at deeper levels lowers the safety factor and causes blockage; at the more superficial levels the net excitation raises the safety factor above normal and so enhances above normal the dendritic propagation of the surviving impulses as in Fig. 4*B*, *D*.

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