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EXCITATORY AND INHIBITORY PROCESSES ACTING UPON INDIVIDUAL PURKINJE CELLS OF THE CEREBELLUM IN CATS

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Efferent cortical activity from the cerebellum is carried by the Purkinje cells (P-cells, see Fig. 1) which are the final common path (*P.e.*) of two major synaptic organizations. One is a complex polysynaptic network of dendrites and stellate cells in the superficial molecular or plexiform layer to which apparently both cerebellar afferent systems contribute though its main supply is from the mossy fibres (*Mo.a.*) over the granular cells (*Gr.c.*). The other afferent system is the monosynaptic one over the climbing fibres (*Cl.a.*) to P-cell dendrites along which they climb. These are held to contribute also to the polysynaptic network (Estable, 1923; Scheibel & Scheibel, 1954) and many other complexities are encountered, but to a first approximation the physiologist has to be content with a general subdivision of cerebellar afferents into mono- and polysynaptic ones along the lines suggested.

His next concern will be with the two additional systems centered on the P-cells one of which consists of two types of recurrent collaterals (*P.r.*): Cajal's (1911, fig. 12) 'plexus sous-cellulaire' spreads among the cells below the P-cells and his 'plexus sus-cellulaire' traverses the molecular layer giving off branches on its way. Both types of recurrent collaterals do, however, end up on P-cells.

Finally, it would seem highly desirable to be able to elucidate the role of the unique system of basket cells (B-cells) with dendritic receptors in the molecular layer (and possibly elsewhere) and efferent axons (*B.e.*) surrounding the P-cell itself as well as the non-medullated and part of the medullated portion of its efferent axon (for good illustrations, see Estable, 1923; also Retzlaff, 1954). This basket, the 'nid' or 'nest of Cajal' (1911, p. 23), is the other major

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complicating system to be considered in evaluating records from individual P-cells (for anatomical reviews, see Cajal, 1911; Jansen & Brodal, 1954).

The way in which we have stated the primary problems of cerebellar efferent action is a simplification, based on Cajal's work. We have, for example, left out of consideration the large stellate cells whose dendrites ramify in the molecular layer and whose short axons branch richly in the granular layer. Clearly, however, it is necessary to begin with a simplified layout of the circuits converging upon the P-cells in order not to miss statistically dominating features of cerebellar organization.

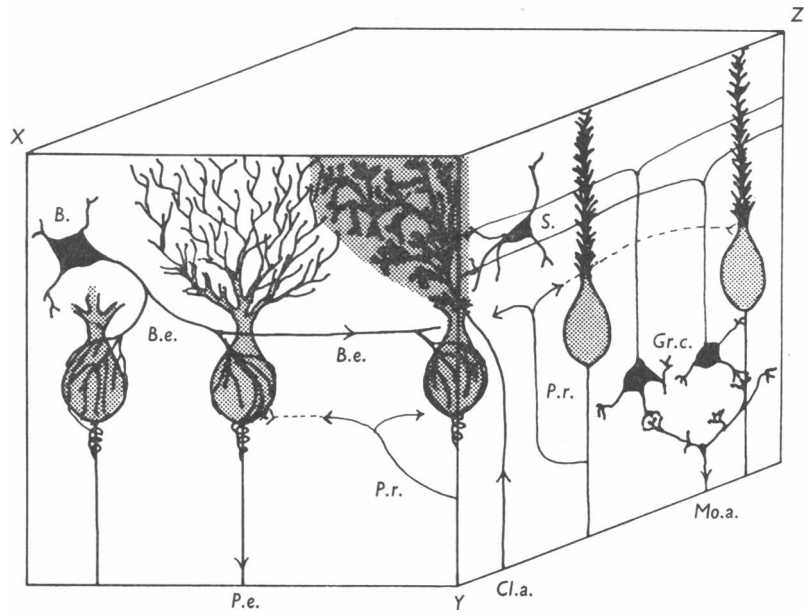

Fig. 1. Diagram of part of a cerebellar folium sliced at right angles to its long axis (XY) and parallel to its long axis (*YZ*). *P.e.*, Purkinje cell axons with *P.r.*, recurrent collaterals; *Cl.a.*, climbing fibre; *Mo.a.*, mossy fibre; *Gr.c.*, granule cells; *S.*, small star cell; *B.*, basket cell (large star cell), with *B.e.*, its efferent fibre.

Our work required strict identification of and direct recording from P-cells, for which fine capillary electrodes were used. In order to stimulate these cells antidromically or monosynaptically, tips of insulated needles were placed in the cerebellar nuclei or in the arbor vitae above them (Fig. 2). This paper deals with the identification of P-cells by such means, their general properties and what could be seen of their activity and of events in their immediate neighbourhood during excitation and inhibition. These results are of interest for the general problem of excitation and inhibition as it presents itself in a highly active unanaesthetized preparation and in an organ that has a singularly extensive dendritic network organized in a homogeneous way.

METHODS

Preparation. Thirty-five decerebrate cats were used in these experiments. They were prepared by suction, a method in regular use in this laboratory. As to its advantages, see Sprague & Chambers (1954). Under ether, the carotid arteries were tied and the trachea and one saphenous vein cannulated. The short-acting barbiturate Thiogenal (Merck) was slowly injected intravenously in doses of 5–29 mg/kg, as needed, as the ether wore off. After bilateral craniectomy reaching backwards to the edge of bony tentorium, the dura was opened on both sides and the falx cerebri divided between ligatures. The occipital lobes were removed by suction. Under deep anaesthesia the brain stem was sucked across at precollicular level, and the rest of the hemispheres removed. At the conclusion of the operation the field was usually fairly bloodless, but careful sealing of vessels was necessary to prevent later bleeding, after the anaesthetic had passed off. Stumps of basal arteries were closed with silver clips, and the cut face of the brain stem was dressed with gelatin sponge ('Spongostan', A/B Ferrosan, Malmoe, Sweden).

The results to be described below are obtainable only if the cerebellum is in perfect condition (cf. Bremer & Bonnet, 1951).

The anterior folia of the culmen were exposed on the left side, for exploration by microelectrodes, by nibbling away the bony tentorium covering them. A transverse slot, about 3 mm in length, was cut with a dental burr just behind the occipital protuberance, to admit the fastigial electrodes.

Stimulation. Paired steel needles, 2 mm apart and enamelled except at the tips, were directed through the posterior lobe and into the neighbourhood of the left fastigial nucleus or more laterally (Fig. 2) with the Horsley-Clarke apparatus. Final adjustment of their position depended on the recording of an optimum evoked potential at the cortical surface. Their tips generally lay 1 and 3 mm from the median plane. Brief rectangular shocks (duration 0·3 msec) were given, singly or as tetani, over an isolating transformer. Reversal of polarity of the stimulating electrodes allowed us to alter the locus of stimulation without moving the electrodes, and also to differentiate the response from the shock artifact (Porter, 1955).

Recording. The microelectrode technique has already been reported in detail (Phillips, 1956*a*). The micropipettes were formed in the Schuster microforge. When filled with 3 M-KCl, their d.c. resistance was 5–20 MΩ. For recording the 'giant spikes' to be described below, the optimum resistance was 5–10 MΩ.

The dendrites of P-cells being orientated in planes at right angles to the long axis of the folia (Fig. 1), it should be possible to enter obliquely in order to approach the soma without fatally wounding the dendrites. The movement of the micromanipulator was always vertical, and the angle of puncture was determined by the tilt of the head. The Horsley-Clarke apparatus was mounted on a massive stand, in which it could be bodily rotated about an antero-posterior and a transverse axis. By means of adjustable slides, the point of intersection of these axes could be placed at the point of penetration of the pia-arachnoid. Through a single small pial opening, many punctures could be made entering at different angles to reach fresh cortex lying buried in folds at greater depths than the 0·4 mm of the superficial layer of P-cell bodies. In the rat there are about 1000 P-cells per mm^2 (Inukai, 1928).

Cardiovascular and respiratory movements were reduced by light surface pressure applied through a celluloid 'watch-glass' about 8 mm in diameter. A pore in the watch-glass, 0·5 mm diam., was applied to the pial opening which was of about the same size as the pore (Phillips, 1956*a*, *b*). The microelectrode was guided through the pore under stereomicroscopic vision ($\times 16$ magnification). The watch-glass was filled with Ringer's solution and connected by a silver electrode to one input grid of an amplifier, the other grid, and the preparation, being earthed. Thus spontaneous or evoked potentials could be recorded at the area of contact of the pore.

The microelectrode was mounted in a probe containing one of the input valves of a differential cathode follower input. The other side was earthed. The input capacity was less than 3 pF. No special circuit was employed to prevent attenuation of spike potentials of brief duration. The output of this stage was led both to low-gain d.c. and variable gain condenser-coupled amplifiers. In

order to be always in readiness for intracellular recording, the cathode rays were deflected *upwards* when the microelectrode tip became positive: in the extracellular records, therefore, *negativity* is signalled by unconventional *downward* deflexions. Continuous moving-paper records of natural discharges and responses to stimulation were taken in order to monitor the background activity and delayed effects of stimulation: precise timing of latency, etc., was carried out on sweeps to which the shocks were locked, and which were photographed on the same moving paper.

Many painstaking attempts were needed to convince us that there was little prospect of regular and durable impalement of active cerebellar neurones with the technique we have described. Stable resting potentials, rising abruptly to -60 to -80 mV as the microelectrode penetrated the elements responsible for them, were, however, very frequently encountered. These showed neither oscillations nor spikes (Coombs, Eccles & Fatt, 1955*a*; Frank & Fuortes, 1955; Phillips, 1956*a*). Our experiments have thrown no light on the nature of such elements.

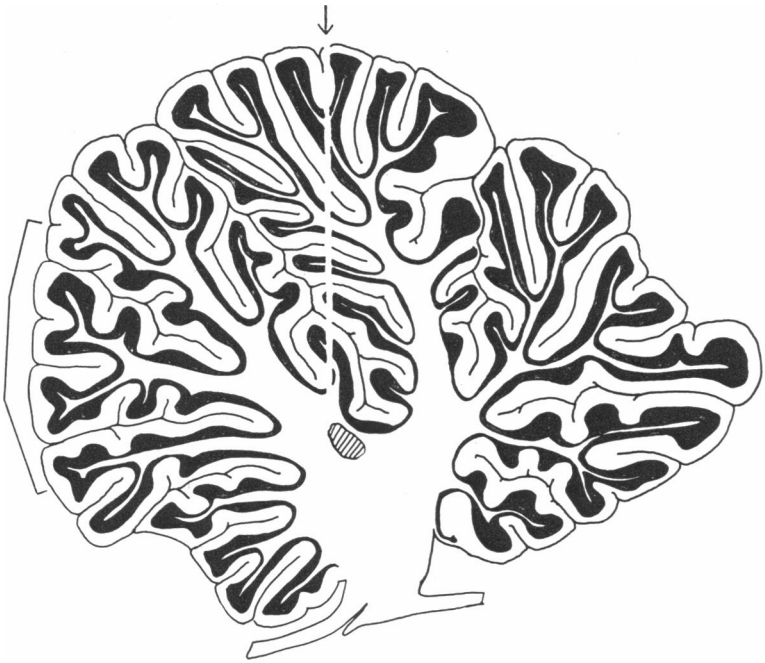

Fig. 2. Tracing of parasagittal section of cerebellum stained with thionin. The Purkinje-granular layer is shown in black. The position of the stimulating electrode tips in the arbor vitae is marked by Hess's Prussian blue method. In all these experiments microelectrode exploration was confined to certain folia of the anterior lobe, indicated by a bracket. Scale of figure is given by the length of the needle tract (whose direction is here reconstructed from the evidence of several sections): the tips were 10 mm from the needle entry in the fresh specimen.

We were readily able to record resting and action potentials from single fibres of the lumbar dorsal roots in the manner described by Frank & Fuortes (1955), so that the adequacy of our manipulating arrangements and microelectrodes is not in question, and the difficulties may, perhaps, be ascribed to the expansile pulsation of the cerebellum with the heart beat. Although its surface is immobilized at the area of contact of the watch-glass, the amplitude of its movement must increase as a function of depth. Alternatively, the double membranes of the Purkinje cells (Cajal, 1911, p. 4) may set special difficulties in the way of penetration. We had no more success

with the rat's cerebellum in which pulsations are easily controllable. Buser & Rougeul (1954) recording from pigeon P-cells, report similar experiences.

Anatomical control. The position of the stimulating cathode, or, in some experiments, of both stimulating electrodes, was marked by Hess's (1949) method (Fig. 2). Alternate 30 μ parasagittal paraffin sections were stained with thionin.

RESULTS

Identification by antidromic or monosynaptic activation

It has to be admitted at the outset that the antidromic identification of P-cells by fastigial stimulation is inevitably less certain than the antidromic identification of spinal motoneurones by stimulation of ventral roots. For in their course through the cerebellar substance, the Purkinje axons are inextricably mixed with afferent fibres, and notably with the climbing fibres which enter into monosynaptic relationship with the P-cell dendrites. Rivalry between antidromic and monosynaptic activation is therefore to be expected.

Activation at the shortest latencies (0·35–0·6 msec, Fig. 4) can hardly be other than antidromic; the longer ones (0·6–0·8 msec), on the other hand, may represent either antidromic or monosynaptic firing. Monosynaptic activation in itself, however, provides a strong presumption of P-cell identity, in that the climbing fibre–Purkinje dendrite nexus is such a conspicuous feature of cerebellar cortical architecture. The alternative possibility, that the impulses with monosynaptic latencies are due to firing of granule cells by mossy fibres, is hardly likely. The granule cell population is so densely packed that, if these large spikes originated in granule cells, one would expect to encounter several such spikes in the course of every puncture. The relative rarity of our large early spikes is in favour of their origin from the P-cells, which form only a single layer in each fold of cortex and are less closely spaced.

When fastigial stimulating and cortical recording electrodes have been correctly placed relative to one another, each shock sets up an early positive surface wave (Fig. 3 (1) and (2)), preserving its sign down to the white matter. This is used for general orientation of the electrodes. In Fig. 3 it is recorded at a depth of 0·48 mm among the P-cells. Its latency is from 0·17 to 0·30 msec. As the microelectrode approaches the layer of P-cells the later portion of this wave turns negative with a latent period around 0·5 msec and then, when the tip comes near enough to a cell, an all-or-none spike arises from some part of the negative wave. Fig. 3 (1), demonstrates this spike at the threshold (0·4 V). Use of a 10 V stimulus in (2) emphasizes the early wave, but has no influence on spike size. Three more negative-going spikes (3)–(5) from different experiments serve to illustrate similar responses, all of which have a latent period which but rarely is below 0·5 msec and mostly somewhat longer (Fig. 4). With threshold stimuli and a well-placed fastigial electrode the negative wave is very small but can, nevertheless, be seen in the superimposed pictures of the

figure whenever the shock just has failed to excite a spike. The purely negative spikes hardly ever exceed 5 mV and are mostly between 2 and 3 mV.

Fig. 3 (6)–(8) illustrates what happens when, a unit spike having been picked up, the microelectrode is cautiously moved onwards. The early spike then

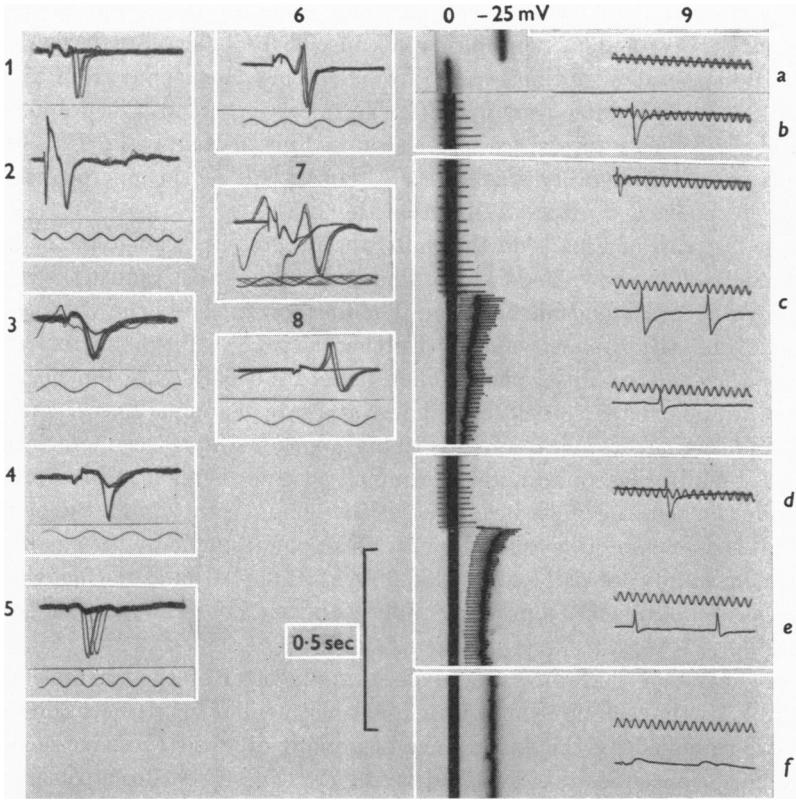

Fig. 3. In sweeps in this and all other figures (unless otherwise stated), negativity of the microelectrode tip is signalled by *downward* deflexions, positivity by *upward* deflexions. Time 1000 c/s. Vertical continuous records to be read from above downwards: negativity towards sweeps, positivity away from sweeps. Time 0·5 sec. (1) to (8), superimposed sweeps showing unit responses to fastigial shocks. (1), threshold shocks (0·4 V). Shock artifact followed by small positive-negative wave. Unit spike (amplitude 5·0 mV) fired in some sweeps. (2), same unit is always fired by stronger shocks (10·0 V); spike size unchanged. (3) to (5), three further negative-going spikes responding to near-threshold fastigial stimulation in a proportion of trials. (6), diphasic spike (similar amplification), showing positive-going prepotential. (7) and (8), giant spikes (less amplification, d.c. records), 33·0 and 23·0 mV peak-to-peak. (9), closer approach to cell membrane (d.c. records). Calibration (top left) −25 mV. (*a*), voltage zero; (*b*), extracellular giant spike (fluctuating amplitude in continuous record is due to faulty immobilization); (*c*) first attempt to penetrate membrane (spikes still diphasic); (*d*), withdrawn; (*e*), second attempt (low membrane potential, small positive-going spikes); (*f*), deteriorated spikes, persisting low membrane potential.

develops a positive initial part consisting of prepotential and spike (see below, Fig. 6). At this moment one does well not to push the microelectrode deeper because left to itself, by some process not understood in detail, the spike after a while very often begins to increase in size, as if the brain tissue, retreating in front of the pressure wave, now again had had time to advance and readjust itself for close contact. When this occurs it is generally possible to record the spike on the low-gain d.c. amplifier because it becomes very large, rarely below 10 mV, and in optimal cases around 60 mV, measured from positive to negative peak. Values around 20–40 mV are very common. The prepotential definitely becomes positive, as better seen in Figs. 6–8 and Fig. 11. Similar findings on a considerably smaller scale (larger tips of the microelectrodes) have been reported by Rose & Mountcastle (1954).

In the last row of Fig. 3 (on the right) the progressive change of the spike, described above, is shown in d.c.-recording, both in the sweeps and in the vertical continuous record alongside the film. The first sweep shows the voltage zero, the next one the diphasic spike. Further advance of the microelectrode enlarges the positive initial phase. Then puncture occurs with damage to the membrane and excites the cell to discharge at a rate of about 120/sec. The spike is still diphasic. At its maximum it is 19 mV. Withdrawal of the microelectrode follows. Finally, a second, more determined advance is made. Now, with the tip inside the cell, the spike becomes positive-going even though the penetration in this case cannot have been a very clean one to judge by the small value of the membrane potential (calibration above, on the left) and the fact that the spike was of modest size from the beginning and soon deteriorated (last sweep, bottom).

It is concluded that precise and close application of the microelectrode, preferably by the technique of waiting for adjustment of the tissue, as described above, is signalled by the appearance of a giant diphasic (positive-negative) spike. This, sometimes with small adjustments, can be kept for a considerable length of time under the microelectrode and behaves just as the negative spikes which are recorded a little farther away, yet near enough to appear uncontaminated by other spikes.

The microelectrode may often penetrate the cell membrane spontaneously, owing to some movement within the cerebellum (vascular, occasionally respiratory). We have never seen this happen in these relatively small cells encased in double membranes without signs of an injury discharge (for illustration see Fig. 7; cf. also Buser & Rougeul, 1954). Yet microcapillaries of similar dimensions could be kept within Betz cells (Phillips, 1956*a*, *b*) or dorsal root axons for a considerable time without signs of obvious injury. Despite this, inside records may be revealing, especially with respect to precise and fast events, though so far hardly useful in the analysis of general P-cell behaviour. In the circumstances we have used the negative and the

giant diphasic spikes (Fig. 3 (1)–(8)) in the attempt to identify P-cells. In the cerebellar preparation small spikes which from the beginning have been positive have not been found worth studying because of their comparatively fast deterioration.

A curious histological feature of the Purkinje cells, according to Cajal (1911, p. 4), is the double membrane ('ciment') or outer 'cement'. It may well be that the tip of the electrode comes into close contact with this structure, creating, in some manner not fully understood, optimum recording conditions in which large voltage changes appear at the microelectrode tip. That the tip remains on the outside of the cell membrane is proved by the absence of 'resting' membrane potential (in the d.c. records).

Dr B. Frankenhaeuser has pointed out to us that the giant spike is a registration of the flow of membrane current at the site of recording. The first flow is outwards (tip positive), signalling distant activity (the prepotential). The second, inward flow (tip negative) occurs as the membrane becomes active at the recording site. The recording resistance, whatever its nature and location, must be large in order to account for the large recorded voltage changes.

There can be no question of serious injury being a prerequisite for diphasic responses, because such spikes may occasionally be lost on by-passing the cell on the inward track without exploding its membrane and then fully retrieved on withdrawal of the electrode. It therefore suffices for the approach to be close, without actually being penetrating. When this is the case the events around the Purkinje cell and its membrane, in optimal cases, assume an order of magnitude—the giant spikes—that has proved to be of the utmost value in our work (see below). No doubt the same events, perhaps on a smaller scale, occur with all kinds of cells (cf. Rose & Mountcastle, 1954), but the P-cells may well—for the reasons discussed—be particularly favourable for their demonstration.

The statistical distribution of the latent periods of early spikes is shown in Fig. 4. This includes giant spikes with prepotentials as well as negative spikes without them. In the former case the starting-point of the prepotential has been taken to indicate the moment of invasion. It will be shown below that the spike may arise earlier or later on the prepotential (e.g. in Fig. 6, latency of prepotential is 0·47 msec, and the take-off of the spike varies between 0·75 and 1·1 msec). Thus the distribution curve of Fig. 4 may include a number of late spikes whose prepotentials, if available, might have pushed the maximum to the left. It is now seen to be in the 0·6–0·7 msec range. For the purpose of antidromic or monosynaptic identification of P-cells, how far to the right of this figure is one entitled to go? Reference to the histological structure (Fig. 1) is necessary for discussion of this question.

There cannot be much difference between the times of arrival of an antidromic impulse, having to traverse the non-medullated part of the P-axon, and an impulse in the monosynaptic climbing afferents contacting the large dendrites of the same P-cell. The total conduction distance is of the order of 10 mm. This leaves little room for differentiation on the basis of fibre size, both fibres being of much the same order of thickness (Cajal, 1911, p. 64). Thus, if for some reason excitability is low at the moment of arrival of the antidromic impulse to the first of the two regions of low safety factor (medullated to non-medullated, and non-medullated to cell body), the ensuing low degree of depolarization may nevertheless suffice to facilitate the entrance of the impulse from the

nearby thick dendrites activated almost simultaneously by climbing afferents. At least with some electrode placements, these are likely to be co-activated by the fastigial shock. It may, therefore, be impossible to ascertain when one process begins and the other ends. Stimulation of the brachium pontis, held to be purely afferent (cf. Jansen & Brodal, 1954), has actually given latencies of the same order. Since the maximum in Fig. 4 is between 0·6 and 0·7 msec, we have taken the upper limit of antidromic activation to be 0·8 msec. According to Brock, Coombs & Eccles (1953) the antidromic invasion of the ventral horn cells may vary between time limits as wide as 0·35 msec, their index having been the discharge of a full-sized impulse.

Fig. 5 serves to emphasize the physiological aspects of the histological questions raised. Spike (1) had a shortest latency of 0·5 msec and is thus, on our evidence, a P-cell, but there is a latency play of about 0·3 msec which need not, but may well, signify alternative activation over climbing afferents. Spike (2) varied in latency from 1·2 to 2·0 msec, and cannot therefore on our criterion be identified as a P-cell even if it were one. The latency play is still greater for spike (3), and its minimum is 1·0 msec. It cannot either be included among identified P-cells, yet its voltage, 14 mV on d.c.-recording, and its depth, 0·42 mm, strongly suggest that it belongs in this group but that the stimulating shock either did not excite its efferent fibre or that the cell itself failed to be invaded by antidromic impulses in the way ventral horn cells (e.g. Lloyd (1943) by extracellular and Brock *et al.* (1953) by intracellular recording) and the retinal ganglion cells (Granit, 1955) often fail to respond unless properly facilitated orthodromically. Our preparation, however, does not permit unequivocal differentiation of antidromic from monosynaptic activation.

The two remaining strips of film in Fig. 5 show spontaneous activity. Brookhart, Moruzzi & Snider (1950, 1951), in unidentified cells within the Purkinje-granular layer, observed very high rates of spontaneous firing. In our experience P-cells often are silent, then suddenly start firing and again lapse into silence for no obvious reason and also in a highly random fashion. Caught fresh or in the unstimulated state by a microelectrode they have modest firing frequencies, from next to nothing up to 30 impulses per sec, more rarely 50–60. But in the course of our experiments the P-cells were stimulated, repetitively and by single shocks, in order to obtain the information needed, and then, after a while, the cerebellar 'drive' often rose to a new level distinguished by a higher rate of discharge, which again but rarely was above 60/sec. Such effects are long-lasting and probably correspond to the long after-effects of stimulation described by Clark (1939) with implanted cerebellar cortical electrodes and by Chambers (1947) with nuclear electrodes in intact animals.

Individual variations were considerable, no doubt largely owing to variations in the number and nature of afferents co-excited by the fastigial electrodes. Considering the wealth of dendritic

skeins around the P-cells and their own widespread trellis-work (see Fig. 1) it seems very likely that microelectrodes of the coarser varieties cause destruction within the plexiform layer. Rose & Mountcastle (1954) have come to the same conclusion in their study of thalamic cells. Ionic leakage may therefore set up multiple trigger zones (artificial synapses) which could account for the high frequencies seen with such electrodes (as it seems likely that P-cells must have occurred among the ones recorded from by Brookhart *et al.* 1950, 1951).

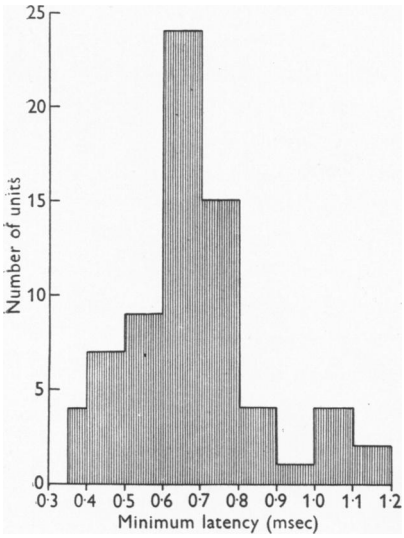

Fig. 4

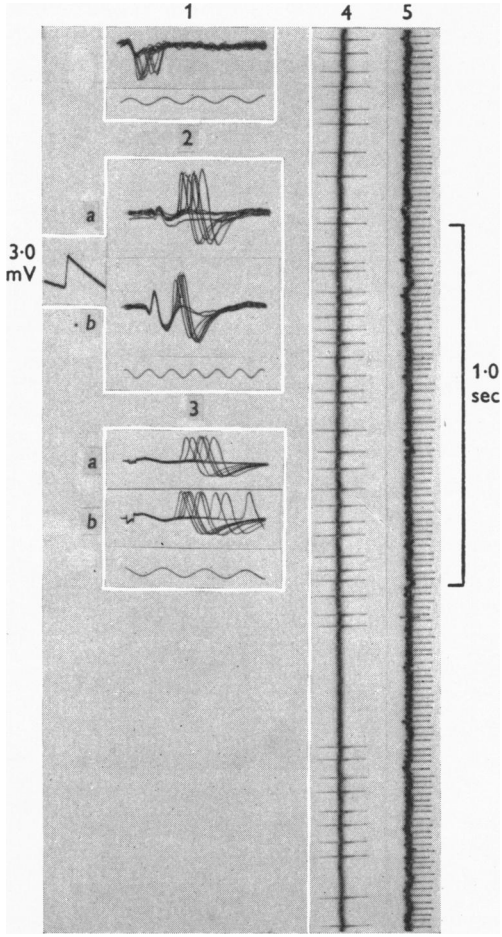

Fig. 5

Fig. 4. Minimum latencies of 70 units of cerebellar cortex in response to single shocks to fastigial nucleus or overlying white matter. The latencies are measured to the beginning of the prepotential in cases in which this was clearly marked.

Fig. 5. (1) and (2), units responding to fastigial shocks with longer and variable latency. Superimposed sweeps. (*a*), at threshold strength; (*b*), at twice threshold strength. Time 1000 c/s. Calibration 3·0 mV. (3), giant spike (amplitude 14·0 mV). (*a*), near threshold; (*b*), ×8 threshold. (4) and (5), natural firing of two cells whose latencies to fastigial stimuli were 0·62 and 0·54 msec respectively. Time 1·0 sec.

Inhibition by inactivation

At this stage a new type of inhibitory process will be described, lest it otherwise should prove confusing to the reader to find it unrecognized in the P-cell records. It will be called the 'inactivation response' and is very common in our records. It is also to be seen in fig. 2 (*b*, *g*), of Buser & Rougeul (1954), which they assume to be a P-cell record. The long vertical film strip of Fig. 6 presents a practically silent P-cell with an initial shock on the two first sweeps.

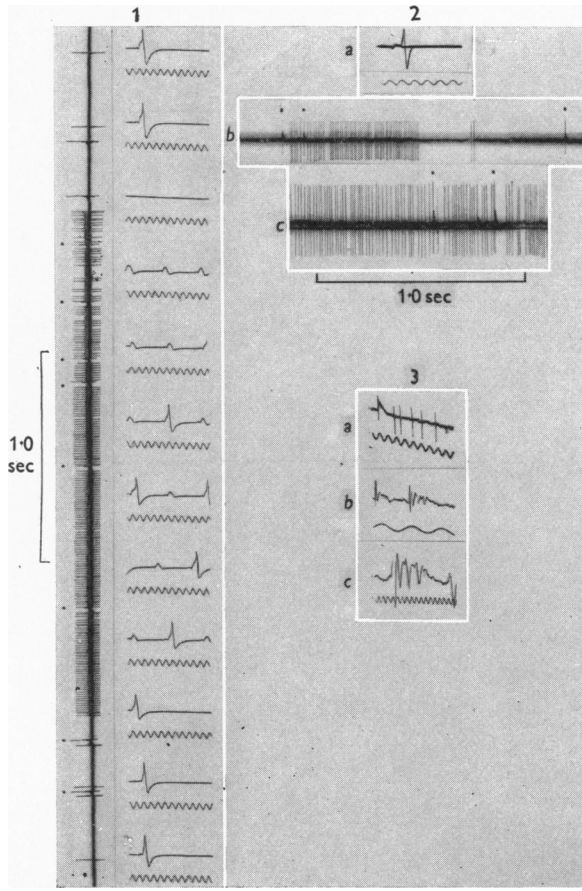

Fig. 6. (1), giant spike (44 mV peak-to-peak). Single near-threshold fastigial shocks in first and last two sweeps, separated by a burst of the same shocks at 188 c/s. Inactivation responses marked by dots. Latency of prepotentials 0·47 msec; of spikes, 1·1 msec before tetanus, 0·75 msec after tetanus. (2), (*a*), diphasic spike, latency 0·7 msec to prepotential; (*b*), inactivation responses during a tetanus; (*c*) higher gain; inactivation responses during a period o natural firing. (3), naturally occurring inactivation responses from another experiment recorded at different sweep speeds. In (*a*) and (*b*), time 100 c/s. In (*c*), higher gain, and time 1000 c/s.

Then follows one sweep without shock as the stimulator is switched over to repetitive stimulation at a rate of 185/sec. The cell is stirred to activity, as best seen on the continuous (vertical) record, but most of the shocks only elicit the positive prepotential generally preceding the large diphasic spikes. (For similar experiences with ventral horn cells, see Brock *et al.* 1953.) The samples seen on the sweeps further demonstrate that the spike occupies slightly varying positions on this positive wavelet (cf. Brock *et al.* 1953; Eyzaguirre & Kuffler, 1955*b*). The earliest positions, giving a spike latency of 0·75 msec, occur *after* stimulation. The latency of the prepotential was constantly 0·47 msec.

The curious event called inactivation is seen as a thick brief positive-going wave interrupting for a while the discharge of the P-cell. Each inactivation is marked by a dot. The records (2) refer to another P-cell which in the middle record (standing spot on running film) responds to a brief tetanus. Three records of inactivation responses occur on this strip. The P-cell then was stirred to fast activity followed by a long-lasting after-discharge; amplification was increased (2*c*) and two clear-cut positive-going inactivation responses were recorded.

In order to record the inactivation response spread out in time, a very large number of sweeps were taken in an experiment where it occurred spontaneously. Successful exposures are shown in (3), the two uppermost records at time 100 c/s, the lowermost one at higher gain and time 1000 c/s. The uppermost record happened to catch the inactivation response in the midst of an intense spontaneous discharge of the P-cell and thus provided a good background for the inhibitory suppression. The length of the pause varies with the amount of original drive but is generally of the order of 15–30 msec. Occasionally we have seen longer pauses. The lowermost record illustrates a high-frequency spike discharge on top of the slow positive wave of the inactivation response. This discharge cannot always be seen and apparently requires favourable location of the electrode tip. As has been pointed out above, the positive direction relative to the negative-going spikes does not as such permit far-reaching conclusions. Sometimes the inactivation response has actually been negative in extracellular records (e.g. Fig. 12 (1)). Only in intracellular records (see below) can the direction of the change of potential be used for inferences about the change in the membrane potential of the P-cell during inactivation.

For further analysis it was necessary, in the first instance, to be able to drive the inactivation response from the fastigial electrode. This was the case in the experiment of Fig. 7. On the vertical strip the spontaneous firing rate of a P-cell was interrupted by a tetanic series giving a large number of inactivation responses, thus suggesting that they might also be driven by the shocks. This proved to be the case at slower rates of repetition, as shown in (2) in d.c. records, which illustrate that the inactivation response reached 6·5 mV

positive and returned to the base line within about 14 msec. The small spikes on it were now positive. Records (3) repeat this on a faster sweep with d.c. amplifier alone. The d.c. amplifier beam was then shifted to the zero position indicated in the calibration scale in (4) and penetration attempted. This led to the usual high-frequency discharge and a relatively stable level of −46 mV membrane potential. On both sweeps and continuous records it can now be seen that the characteristic positive-going spikes are stopped and interrupted in their falling phase by the inactivation response (samples on sweep) which therefore must represent a cathodal depression or inactivation (Frankenhaeuser, 1952; Hodgkin & Huxley, 1952), i.e. an inhibition by a depolarization going beyond the firing capacity of the membrane. As repolarization takes place, one can (vertical records in (4), cf. also Figs 9 (2) and 12 (1)) see the spike gradually increase in size, provided that the rate of discharge has been sufficiently high.

Occasionally there is a 'rebound' increase in the frequency of discharge following the pause of an inactivation response (Fig. 9 (4)).

This clearly is an inhibition different from the one described by Brock *et al.* (1952) and Coombs, Eccles & Fatt (1955*b*) in cat spinal motoneurones, which is an inhibition by hyperpolarization. Their variety of inhibition is also seen in Fig. 7 (4), in which two transitory spontaneous hyperpolarizations lead to a period of complete silence of the discharge. Such inhibitory hyperpolarizations are also seen in the Betz cells (Phillips, 1956*a*, *b*) in which inactivation responses are notably absent (these records have since been reviewed by C. G. P. from this point of view).

Fig. 7 (1) also emphasizes that inhibition by inactivation does not necessarily require a high rate of discharge of the cell itself (cf. also Fig. 6 (1) and (2)), and all our records demonstrate its general property of being a discrete and specific transient event. This suggests a specific systemic governor capable of causing an abrupt inhibitory strangulation of the P-cell. The briefest latent period of the inactivation response at its minimum (Fig. 7 (4)) is the latent period of the spike *plus* the time it takes for it to reach its top and somewhat less than half-value, in this case only 1·5 msec, and varies with the latency of the spike, partly, perhaps, only because masked by the latter. Its latent period will, of course, like other cellular latencies, depend on the level of the membrane potential. It is short enough to indicate a fairly simple circuit.

Since inactivation is common in itself and very rarely obtainable in response to the fastigial shock, the recurrent P-collaterals would seem to be excluded. On this system inactivation responses would have to be practically inevitable both by backfiring as well as by orthodromic excitation. More probable seems a depolarization beginning from the bottle-neck of the P-cell where it, together with part of its axon, is surrounded by the synapses of its own specific basket

axons, the 'nid' or nest of Cajal (1911, p. 23). This is a regular and yet singular enough feature of P-cells to lead one to expect a correspondingly unique, but for these cells common, type of reaction. A synaptic loop through the basket cells which are large cells, in size second only to the P-cells, should be given first rank in a list of possible working hypotheses.

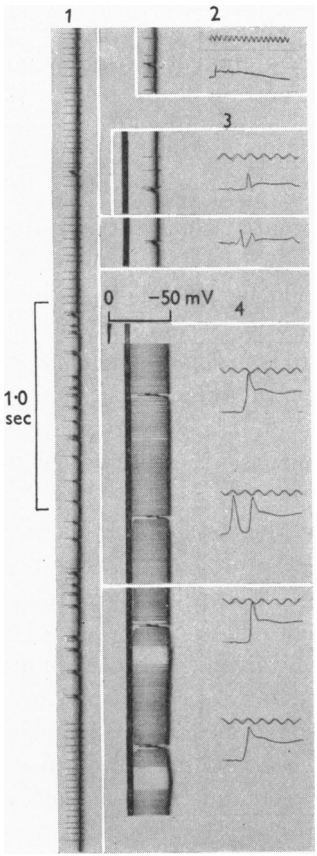

Fig. 7

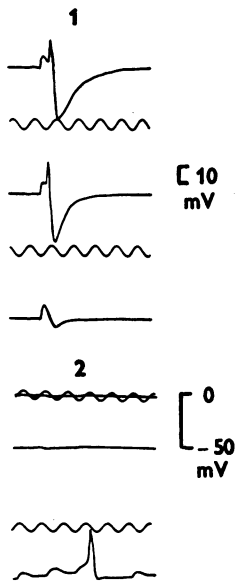

Fig. 8

Fig. 7. (1), part of a continuous extracellular record showing inactivation responses during a fastigial tetanus, interrupting the natural cellular rhythm. d.c. amplification, calibration as in (4). (2) and (3), inactivation responses to single fastigial shocks, at different sweep speeds (time 1000 c/s). (4), intracellular recording, showing contrast between inhibition by inactivation and inhibition by hyperpolarization, as explained in text.

Fig. 8. (1), giant spike with prepotential, and (lowest sweep) prepotential alone, initially positive-going. a.c. amplification. (2), penetration of the same cell with brief survival. d.c. record. Spontaneous positive-going prepotentials, one of which generates an impulse.

Investigation of the process of impulse generation by fastigial stimulation at critical frequencies

In records of giant spikes (first section), in which the excitatory prepotentials are clearly visible, it was possible to observe the failure of the prepotentials to generate impulses when the intervals between excitatory volleys were reduced below critical values. And when in the absence of electrical stimulation a series of naturally occurring prepotentials follow one another too closely, a similar failure of impulse generation is also seen.

Thus, it appeared in Fig. 6 (1) that the giant spike of 44 mV arose from a positive wavelet which itself had a constant latency. Sometimes the positive wavelet occurred by itself. Another similar giant spike (threshold stimulus) is shown in Fig. 8 (1) in which the prepotential alone turns up in the third sweep. Fig. 8 (2) begins with a calibration of the d.c. amplifier followed by a penetration of the cell membrane. The spike now is seen to be positive-going and starts from a similarly positive-going wavelet, the largest of a series of spontaneous ones. The purpose of this record is to demonstrate that sufficiently close to the membrane the prepotential has the same sign (positive-going) as within the cell. These initial positive prepotentials are regularly seen with sufficiently large spikes, so that for many purposes there is no need for penetration of the cell in order to study them.

In sending in two successive shocks from the fastigial electrodes we have in a considerable number of cases measured the probability of response to the second shock at different shock intervals. Exceptionally this value at an interval of 3 msec has been 1·0, the average would be of the order of 0·4. Below 2·2 msec interval the second shock has merely given the wavelet of prepotential by itself. High probabilities for an impulse to the second shock have begun to occur between 3 and 4 msec intervals but sometimes considerably longer times have been required. There is no further need to discuss these facts because, inasmuch as they can be followed by our technique, they confirm the results of Brock *et al.* (1953) in every detail (cf. also Eyzaguirre & Kuffler, 1955*a*, *b*; Frank & Fuortes, 1955). Thus, for instance, at brief intervals both prepotentials and spikes decrease in size, as found by these authors.

The long interval means that very few cells can follow faster rates than 200 c/s for any length of time, as indeed was confirmed by repetitive stimulation. Most cells were barely able to follow 200 shocks/sec. It is unlikely that the stimulating electrodes excite efferent fibres alone, so that there is always in our preparation (cf. Fig. 1) a chance for polysynaptic inhibition to arise when, in a double-shock test, the interval exceeds, say, 2 msec. Many cells have also failed to respond twice to stimuli farther apart than 5–10 msec. This is also seen with ventral horn cells (Brock *et al.* 1953). Only the shortest

intervals can in our preparation be significant for antidromic or monosynaptic excitation and these, for a probability of 1·0, fall between 3·2 and 3·5 msec. In unidentified cells of the kind likely to have been antidromically inaccessible P-cells we have seen intervals as brief as 1·8 msec. By definition the excitation then must have been afferent, over synapses, possibly climbing fibres. It is well known that orthodromic activation of ventral horn cells may have briefer latent periods than antidromic excitation (Coombs *et al.* 1955*a*; Frank & Fuortes, 1955).

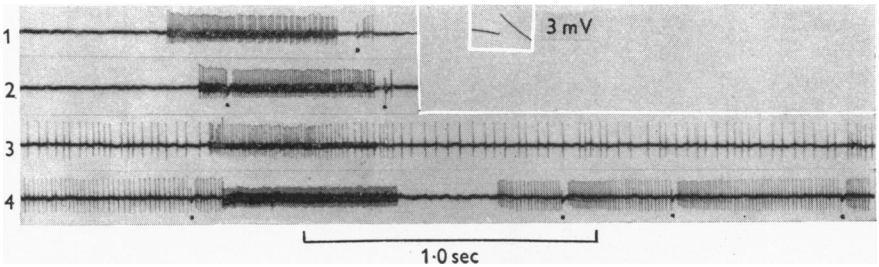

Fig. 9. Responses of unit (latency 0·6–0·7 msec) to fastigial stimulation at different strengths and frequencies. Negativity in records (1) to (4) is signalled by *upward* deflexion. (1), stimulation at 180 c/s, at twice threshold strength. (2), 280 c/s, at twice threshold strength. (3), 500 c/s, at threshold strength. (4), 500 c/s, at four times threshold strength. Inactivation responses marked by dots.

The cell of Fig. 9, when stimulated with shocks somewhat above threshold strength at a rate of 180/sec, followed this rate well for some time (1), but responded with its own rhythm (2) to 280 shocks/sec. At 0·5 V, near threshold (3), it fired at a rhythm of its own to 500 shocks/sec. At this rate of stimulation using 1·6 V, the shocks set up inhibition during stimulation followed by rebound afterwards (4). Black dots mark the characteristic inactivation responses interrupting the discharge and followed by rebound increase in firing frequency. While these observations were made, the firing rate of the cell was stirred up. The cell had been silent from the beginning except in response to the shocks.

The responses to repetitive stimulation varied too much to make it possible to review all the details. Only one generalization seems safe: if the cell had not been stirred up by repeated tests—sometimes even if it had—the characteristic response to a period of repetitive stimulation of modest strength and frequencies up to some 200/sec was of the type shown in Fig. 9 (1) and (2), i.e. a discharge of but slightly diminishing frequency abruptly ending with a pause after which the cell, discharging an occasional after-burst, settled down to its spontaneous rate whatever that happened to be. The bursts, when they occurred, often showed a start with initially smaller spikes indicating remaining or otherwise maintained depolarization (see below). In a number of animals

with good rigidity this was accompanied by fore-limb relaxation during the stimulation period and rebound when the discharge returned to its previous level.

As stated, stirred-up cells often became hyperactive and then there were also ample opportunities for observing the effects of intense spontaneous dendritic

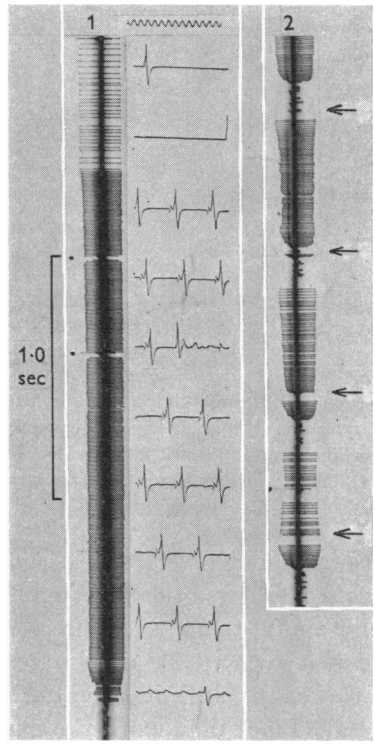

Fig. 10. (1), giant spike (28 mV peak-to-peak) stimulated at 180 c/s. Inactivation responses marked by dots. Note diminished spike height, and high-frequency membrane oscillation, following end of tetanus. (2), another giant spike (26 mV peak-to-peak). Bursts of grouped natural discharges following end of fastigial tetanization. Arrows indicate interpolated shocks.

bombardment. Fig. 10 (1) is a P-cell firing to a stimulation rate of 180/sec, except when interrupted by the characteristic positive wave of the inactivation response (marked by dots). The cell did not return to its original firing rate. Instead there occurred very small positive wavelets, best visible on the sweeps and looking like diminished prepotentials. These came at a rate far beyond the firing frequency of the cell. Before this happened, the spikes, first gradually, then suddenly, grew smaller. Record (2) is from a stirred-up cell firing spontaneously at a very high rate. Each time paired shocks were put in, there was a pause (arrows on record), unless the cell had already silenced itself by the

spontaneously and irregularly occurring process of gradual spike diminution, so different from the sudden onset of the inactivation response stopping the discharge. The membrane change, however, appears to be the same. Spikes on the falling wave of the inactivation response of Fig. 10 (1) were also smaller, as they should be in a highly depolarized cell (see above, section 2 and Fig. 7). Similarly, retinal ganglion cells, depolarized by long-lasting repetitive stimulation at high frequencies, exhibit a diminution of spike size (Granit, 1955), though recovery is less rapid than in the cerebellum. The diminution of spike height in highly depolarized cells has since been demonstrated with inside records from ventral horn cells (Coombs *et al.* 1955*a*) and crayfish stretch receptors (Eyzaguirre & Kuffler, 1955*a*, *b*).

In Fig. 11 (1) is shown the end of a long-lasting stimulation of a P-cell (a.c.- and d.c.-recording) discharging the characteristic giant spike which in this case follows a rate of about 200 shocks/sec. During stimulation it stopped firing and, soon afterwards, stimulation was switched off. The reason for cessation of spike activity seems clear: the generating positive wavelet of prepotential had grown small and frequent. After some time of silence (2) these wavelets spontaneously began to slow down in frequency and, at the same time, to increase in size. First small, and then somewhat larger spikes are now seen to arise on top of these prepotentials, and the cell soon started firing spontaneously at a rate of about 110 spikes/sec. At this lower rate of discharge spike size also settled down to a larger value than before. However, the cerebellar cortex was by and by stirred to high activity by several long-lasting fastigial stimulation periods. A persisting excitatory state outlasted these stimulation periods for several seconds with the cell firing at a higher rate than in the long run it could carry. The spikes again disappeared. Records (3) and (4) show the cell discharging spontaneous positive prepotentials or wavelets at varying rates. Whenever the rate grew sufficiently low, the wavelet increased in size and a spike arose on top of it. Similar records have been obtained by Eyzaguirre & Kuffler (1955*b*) from crayfish stretch receptors whose dendrites had been depolarized by stretch.

This process of varying excitability is studied in eleven sweeps (Fig. 11, records (3) and (4)), which well illustrate the behaviour of the membrane in a rhythmically discharging cell, activated by a live (unanaesthetized) dendritic (plexiform) organization. Even without intracellular records, it seems safe to conclude, in view of the earlier results presented and the experiences of many other authors, that the diminution of the spike is a sign of cell depolarization. The principle of this mechanism of inhibition is the same as that of the inactivation response, with the difference only, that the latter is a sudden, transitory choking of the P-cell by a specific system, while in this case the incessant dendritic activation has led to a long-lasting state of hyper-depolarization. In this case dendritic activity is stirred up by repeated periods of stimulation,

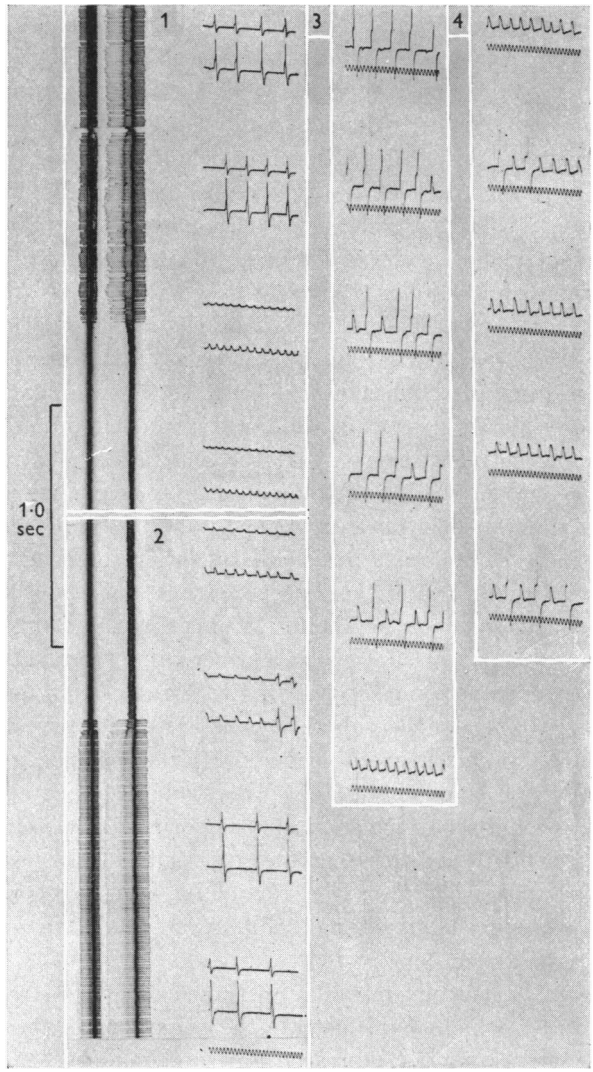

Fig. 11. Giant spike (13 mV peak-to-peak). (1), records at two different amplifications showing the ending of a fastigial tetanus. Stimulation ended after spike stopped firing. Note subsequent increased frequency and subliminal amplitude of prepotential. (2), continuation of (1), showing spontaneous resumption of impulse discharge as frequency of prepotentials declines and their amplitude is gradually restored. (3) and (4), increased amplification. Sweeps showing prepotentials generating impulses whenever their rate of discharge declines so as to allow them to grow to sufficient size.

whilst Eyzaguirre & Kuffler (1955*a*) produced the same state of permanent dendritic hyper-depolarization by stretch. Their records, however, do not show the rhythmically recurring prepotentials so characteristic for the P-cells.

It is further very interesting to note that in a live dendritic network the natural mechanism of activation of its effector cells consists of a translation of the dendritic input into a rhythmic process of recurring prepotentials, as if the P-cells were sense organs run by dendritic terminals. With a larger cell than the P-cell it should be possible to obtain intracellular records relating the frequency and size of the rhythmic wavelets to the level of membrane potential. Yet these measurements would not be absolute unless the microelectrode could be inserted into the actual dendritic site of excitation (Eyzaguirre & Kuffler, 1955*a*).

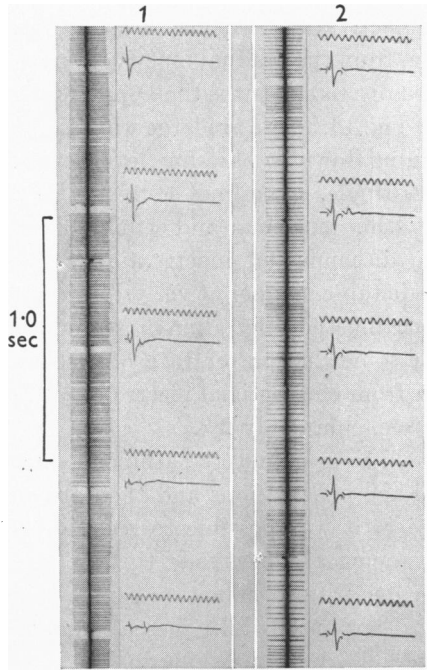

Fig. 12. (1), giant spike (28 mV peak-to-peak). Note pauses in natural rhythm following paired fastigial shocks, whether or not the first of each pair fires an impulse. Note also *negative-going* inactivation response (marked by a dot). (2), giant spike (25 mV peak-to-peak). Each stimulus interpolates an impulse into the natural train, and the single inactivation response (marked by dot) is typically positive-going.

Post-excitatory pause

Least analysed of the inhibitory phenomena noted is the one illustrated in Fig. 12. Two different giant spikes are shown, firing at different spontaneous rates. Record (1), in which the spontaneous rate is faster, presents a better

background for the pause. There this inhibition follows after each pair of test shocks to the fastigial nuclei and lasts about 30 msec. In record (2) the test consists of paired shocks of which only the first elicits a spike. It is clear that the post-excitatory pause after the shock is not identical with the inactivation response, of which a characteristic sample occurs in each record. After the inactivation response, here as elsewhere, the spikes gradually increase in size, whereas after the pause, the spikes start full-size or slightly supernormal with the fresh onset of the discharge. This suggests that the post-excitatory pause, reminiscent of the same phenomenon in motoneurones, is a brief period of hyperpolarization, but at the moment we have no intracellular records by which to support this view.

The D-potential

The spike and the inactivation response are two discrete events, as distinguished, for instance, from the gradual inactivation by a massive, incessant dendritic input. A third discrete event is the D-potential, not so far described. Examples are shown in Fig. 13. These are large waves, from 4 to 20 mV, lasting from 6 to 10 msec running down to base-line level in d.c.-recording as shown by Fig. 13. Sometimes single, sometimes notched, they occur at all depths from 0·1 mm inwards within molecular and cellular layers. To a first approximation they resemble disconnected inactivation responses, occurring like those in a highly unpredictable manner at very slow irregular rates. Occasionally one succeeds in stirring them by afferent stimulation (touch, shocks to saphenous nerve) to a somewhat faster rate of discharge, more often it is possible to drive them from the fastigial electrodes at latencies which never have been below 2·5 msec, commonly 2·8.

Initially they were held to be definitely pathological responses because they often occur on low-voltage penetrations and then are always positive-going. However, in several cases it was possible to record them as negative-going events merely by advancing the electrode tip. It was also observed that destruction of a cell or dendrite by puncture generally ended up in a slowly deteriorating rhythmical response, whilst the considerably bigger D-potential lasted very much longer, slowly diminishing in size. Its curious discreteness and the large size, maximum 20 mV with *external* approach, lend support to our final standpoint that the D-potential is a response type in its own right belonging to dendrites or Golgi II cells. The discovery of the very similar and perfectly normal inactivation response showed that slow waves of the D-type do occur as discrete events and not only as summated slow mass effects. Of the latter there was a large variety which, in this connexion, we do not intend to discuss. We have no evidence to identify the D-potential with the inactivation response.

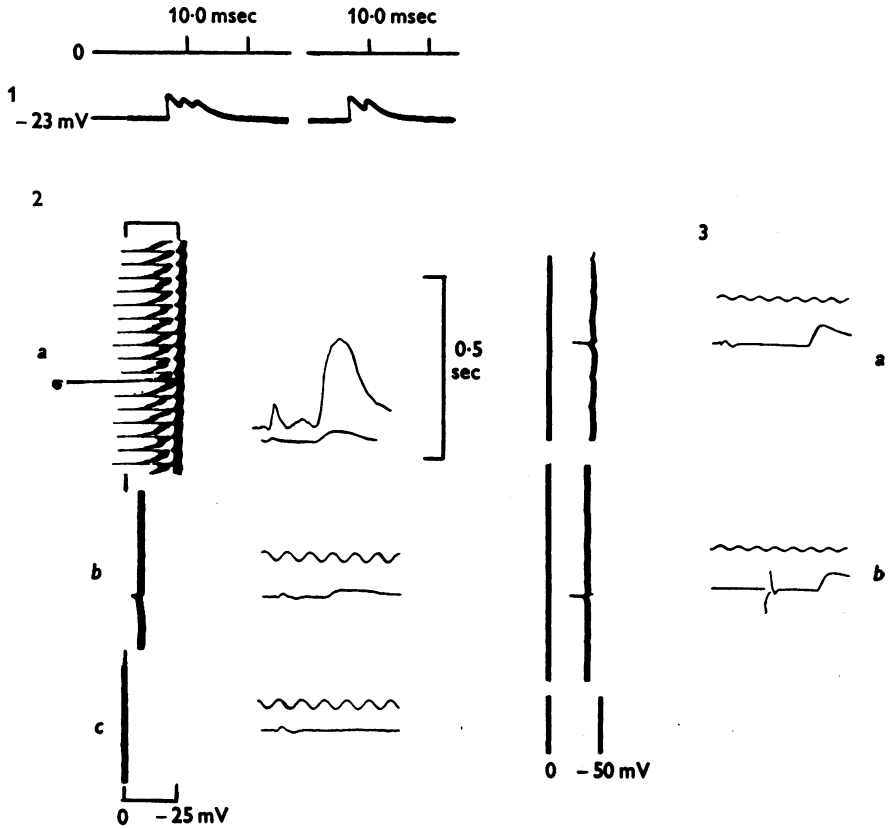

Fig. 13. (1), D-potentials (d.c. records). Two examples from a series elicited by pinches of foot. Membrane potential, initially more than −50 mV, declined rapidly before record was taken. (2), stages of deterioration of a D-response. (*a*), within a few seconds of penetration. *Vertical record* has d.c. record of membrane potential (right), and higher gain a.c. record (left) showing contrast between declining spikes and the D-potential evoked by a fastigial shock (marked by a dot). Sweeps show same response, a.c. on upper, d.c. on lower beam. (*b*) 5 min later. Spikes have ceased. Membrane potential and D-potential (d.c. record) have decayed. (*c*), microelectrode withdrawn to give zero voltage record. Time 1000 c/s. (3), d.c. record of D-potential responding (*a*) to stimulation of brachium pontis, (*b*) to stimulation of n. fastigii. Time 1000 c/s. In (*a*) note contrast between deteriorating spikes and D-potential alongside record.

DISCUSSION

The conclusion that the spikes presented are from P-cells is based on five lines of evidence: (i) no earlier spikes have ever been seen in responses to fastigial (antidromic or monosynaptic) activation; (ii) these are the largest responses obtained, and the P-cells are the largest cells available at (iii) the correct depth. We have found such responses occasionally at depths between 0·2 and 0·3 mm, though more commonly a little deeper, 0·4–0·6 mm. As fold after fold

of buried cortex is penetrated, new layers of P-cells are approached, distinguished by characteristic variations of the massed initial wave and by the occurrence of good-sized spikes. (iv) The results of Brock *et al.* (1953) on antidromic stimulation of ventral horn cells with single or double shocks are in such perfect agreement with our findings that we have been able to shorten description by referring to their paper (cf. also Eyzaguirre & Kuffler, 1955*a*, *b*; Frank & Fuortes, 1955). To these arguments might be added (v) the gradually accumulating experience of the relative uniformity of behaviour of P-cells.

From the point of view of function, the cerebellum seems to be an organ setting up states or levels of activity suitable for tonic regulations. The finding that under the conditions of our experiments its P-cells are naturally active at most times is therefore hardly surprising. For it is presumably this tonic activity that prevents the soft plastic rigidity of the precollicular decerebrate cat from turning into the extreme extensor rigidity brought about by added lesion or by cooling of the anterior lobe (Bremer, 1922; Pollock & Davis, 1930; Stella, 1944*a*, *b*; Moruzzi, 1950; Granit, Holmgren & Merton, 1955).

There is an obvious difference between this cerebellar situation and the state of the spinal motoneurones in the important experiments of Eccles and his collaborators. In those experiments the motoneurones are silent at all times, unless stimulated antidromically or trans-synaptically. Indeed, motoneurones (except those taking part in postural activity) differ from other central neurones in that they are normally silent when the body is at rest. This silence has been encouraged by deep anaesthesia and spinal transection. Reflex excitation by well-synchronized afferent impulses has been shown to be due, under these conditions, to the building-up of the excitatory post-synaptic potential (e.p.s.p.), which decays with the time-constant of the post-synaptic membrane (about 4 msec). It tends to be assumed that excitation elsewhere in the central nervous system, and under conditions of continuously graded as well as of abrupt afferent stimulation, is due to the same fundamental process as that revealed in the spinal motoneurone.

In our experiments, with the cerebellum in a state of incessant paradendritic activity, we have found that the steady pressure of natural excitation is translated into rhythmic wavelets, the prepotentials (Figs. 6, 8, 11), which are very brief events of the order of 1 to 2 msec duration. Their fast decay, in particular, contrasts with the slower decay (time constant 4 msec) of the e.p.s.p. Their sign is positive in extracellular records, and has remained positive in the few successful penetrations of the membrane, so that they cannot have been generated by the membrane at the recording site, but must have arisen in membrane at some distance from the electrode tip. In contrast, the main negative-going component of the spike has always reversed its direction on penetration, showing that it is an active response of the membrane at the recording site.

At present we do not know why these prepotentials emerge as rhythmic fluctuations of brief duration. We can only register their definite connexion with the process of excitation as it ultimately leads to the discharge of an impulse. Their exact nature remains a matter for speculation.

In our experiments their size has been uniformly graded, and the spikes have arisen near their crests with some latency variation, as already described. They have not been clearly 'quantal', as in the experiments of Katz (1950*a*) on frog muscle spindles. Katz interpreted these quantal prepotentials as all-or-nothing impulses in nerve branches, blocked at junctions where a low safety factor prevails. He suggested that a similar state of affairs might be found at the dendritic branchings of central neurones: the main branchings of the Purkinje dendritic tree, which springs from a single pole of the cell and branches dichotomously, are inviting from this point of view, but no evidence of quantal prepotentials has been obtained in our experiments.

An alternative interpretation would ascribe the prepotentials to excitation in the region of the axon hillock, at which region of the spinal motoneurone Eccles (1956) now gives reasons for supposing that all impulses, whether antidromic or orthodromic, actually arise (cf. Gesell, 1941). On this interpretation our giant spikes would be soma-dendrite spikes, invading the soma and dendrites in retrograde fashion from the axon hillock. The presence of prepotentials in all of our giant spikes, whether responding naturally to the steady excitatory dendritic pressure, or artificially excited antidromically or monosynaptically, is less difficult to understand on this than on any other interpretation.

It is clear, at any rate, that our records are derived from a region at some distance from the graded 'generator potential' which by analogy with sense organs (Granit, 1947; Katz, 1950*b*; Eyzaguirre & Kuffler, 1955*a*, *b*), must be presumed to intervene between the steady paradendritic activity and cellular firing. This region must lie in the profuse fine terminal extensions of the large Purkinje trellis-work. Our extracellular d.c. records show no trace of events of longer duration than the prepotentials, except the D-waves (see below), and in this connexion our inability to obtain intracellular records of good quality and stability has been especially disappointing. In lobster stretch receptors (Eyzaguirre & Kuffler, 1955*a*, *b*), although the intracellular recording site is at a distance from the fine dendritic terminals, some trace of the generator potential can be registered, attenuated by electrotonic transmission so that the impulses appear to arise at a 'firing level' spuriously low. Each spike is preceded by a slow graded depolarization, ending in a steeper upcurving at the foot of the spike. A similar picture is seen in naturally active Betz cells (Phillips, 1956*a*, *b*). At the moment it seems reasonable to ascribe the differences between Kuffler's findings in lobster stretch receptors and cerebellar P-cells to geometrical differences rather than—in the first instance—to proceed to postulate different types of membranes.

The actual locus of impulse generation—lying somewhere between dendritic terminals and axon hillock—may well be different in different types of neurones. Differences in site of impulse origin in antidromic and orthodromic excitation are evident, for example, in 'fast' and 'slow' receptor cells of Eyzaguirre & Kuffler (1955*a*, *b*).

Another discrete event, the slow D-potential, seems more akin to the postsynaptic potential of Eccles and his collaborators, though we have no evidence justifying precise identification. Its nature remains for further work to elucidate. A large number of authors (e.g. Adrian, 1937; Chang, 1951) have recorded slow potential changes as mass effects and have had good reasons for ascribing them to dendrites. Our D-waves differ from the dendritic responses of Tasaki, Polley & Orrego (1954) in being monophasic in d.c. records. The dendritic responses from Mauthner cells (Tasaki, Hagiwara & Watanabe, 1954) are in better agreement with our D-waves. If postsynaptic potentials, they would represent massive synchronous activation of dendrites of individual cells or of Golgi II cells.

Inhibition has shown particularly interesting features. There has been the inhibition by hyperpolarization described by Brock *et al.* (1952), Coombs *et al.* (1955*b*), Kuffler & Eyzaguirre (1955) and Phillips (1956*a*, *b*), but this organ seems to have made especial use of what has been known for a long time in peripheral nerve as cathodal depression (cf. Frankenhaeuser, 1952; Hodgkin & Huxley, 1952). In ventral horn cells Coombs *et al.* (1955*a*) found that artificial depolarization to about -50 mV from a resting level around -80 mV prevented antidromic spike discharge. This process has here occurred naturally in two varieties, the first of which was unexpected: this was the special brief inactivation wave or response, precise in time and space, so as to suggest a likewise special system to run it. In looking for a histological substratum our first suggestion has been to locate this system to the basket cells with efferent axons around P-cell and the bottle-neck of its exit point. This, at the moment, is an hypothesis based largely on the necessity of relating a unique, yet common, finding to a likewise unique system common in the cerebellum.

When the P-cell is firing spontaneously at a high rate under intense synaptic bombardment a similar cathodal depression or inactivation may develop, during which it merely discharges fast prepotentials, too small to activate a spike. This in particular, we believe, is a process that requires live and well-circulated cerebellar tissue so that activation from the extensive paradendritic synaptic organization in the plexiform layer can reach the necessary level of intensity. Inactivation by gradual depolarization has been seen both in the large retinal ganglion cells (Granit, 1955) and in stretch receptors of the crayfish (Eyzaguirre & Kuffler, 1955*a*, their fig. 9). Their records may be compared with our Fig. 11. Drugged or asphyxiated organs can hardly be expected to respond in this fashion. Indeed, unless the cerebellum is in excellent condition,

it is difficult to do anything at all with its P-cells. The spinal cord often gives good reflexes when the cerebellum for no obvious reason is clearly abnormal.

It is perhaps not out of place to emphasize what would be a biological advantage of inhibition by inactivation as compared with inhibition by hyperpolarization. A persistent excitatory inflow will tend to counteract hyperpolarization but would maintain inhibitions by inactivation as a kind of self-strangulation of the discharging mechanism of the cell.

The inhibitory process described above as the post-excitatory pause, so reminiscent of the similar pause in the ventral horn cells, is believed to be a hyperpolarization but the evidence is still missing. If akin to the corresponding process in ventral horn cells, it would be run by a loop over P-cell recurrent collaterals.

SUMMARY

1. Micropipettes of the kind used for internal recording from nerve cells and fibres have been applied to single Purkinje cells of the cerebellum of decerebrate cats. These cells have been identified by the short latency (0·8 msec or less) of their response to antidromic or monosynaptic activation from cerebellar nuclei or neighbouring arbor vitae.

2. At the membrane large diphasic positive-negative spikes (20–50 mV peak-to-peak) can be recorded ('giant spikes'). These, with the positive-going prepotentials which initiate them, are the most useful responses in the analysis of Purkinje cell behaviour. Puncture of the cell membrane leads to rapidly deteriorating 'resting' and action potentials, permitting only brief periods of observation. Large stable resting potentials (-60 to -80 mV), without oscillations or impulses, are very common in the cerebellar cortex.

3. In the absence of stimulation the Purkinje cells generally fire at slow rates, with spells of silence, but tetanization from the cerebellar nuclei or arbor vitae is outlasted by persisting excitatory states during which the rate of firing is greatly increased.

4. Under such persisting excitatory pressure, Purkinje cells generate rhythmic prepotentials (cf. (2) above) of graded amplitude. At high frequencies these become too small to initiate impulses. At lower frequencies they grow larger and initiate normal giant spikes.

5. The well-known inhibition by hyperpolarization has been seen in inside recording.

6. A second type of inhibition by excessive depolarization (cathodal depression, inactivation) is a regular feature of Purkinje cell behaviour. It has occurred in two varieties: (*a*) as a transient event, stopping the discharge for 15–30 msec, and suggesting a specific inhibitory apparatus; such transient inactivations occur spontaneously as well as in response to single fastigial shocks; (*b*) as a gradual process in which increasing frequency and diminishing

size of the prepotentials leads to reduced spike size and ultimately to cessation of firing.

7. Another discrete wave of depolarization (the 'D potential') is reported because of its very common occurrence in both inside and outside records. Its nature is not understood.

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Note added in proof

Our attention has since been drawn to a paper by A. Arvanitaki & N. Chalazonitis (1955, Potentiels d'activité du soma neuronique géant (*Aplysia*). *Arch. Sci. physiol.* **9**. 115–144), in which intracellular records from the excised visceral ganglion of *Aplysia*, in addition to the typical spike, occasionally gave another type of spike succeeded by a phase of maintained depolarization, reminiscent of our inactivation response. Excitation in this cell also reminds one of P-cell behaviour in that it takes the form of oscillatory prepotentials.

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