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EFFECTS ON PURKINJE CELLS OF SURFACE STIMULATION OF THE CEREBELLUM

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From the pioneer Ferrier (1886) onwards surface stimulation of the cerebellum has been practised by many experimenters, and it is clear that the effect of excitation of the cortex must ultimately be due to events in its outgoing pathway originating in the Purkinje cells (P-cells). Some of the problems of cerebellar action and after-action have, indeed, been posed in terms of the probable mode of response of the P-cells to the different kinds of electrical stimulation employed (Moruzzi, 1950). The absence of data on the behaviour of P-cells under such circumstances is almost certainly explained by difficulties of electrical resolution coupled with formidable distortion by shock artifacts. The most accessible P-cells will actually be found to lie about 0.2 mm below the stimulating cortical electrode. Phillips (1956), with Betz cells, circumvented these difficulties by intracellular recording. The spikes are then large enough to allow of low gain amplification and thus of deriving the full benefit of balancing devices for artifact compensation.

Granit & Phillips (1956) have recently found that large amplitude spike potentials can be obtained from cerebellar cells which have been identified as P-cells by antidromic or monosynaptic stimulation. Intracellular recording is vitiated by injury discharges, but the outside approach with capillary micro-electrodes gave large enough spikes to suggest that it would be feasible to investigate P-cells below the cortical stimulating electrode and thus to supply the missing data.

Accordingly, the problems raised, and also solved, in this investigation concern direct and indirect activation of P-cells by surface stimulation. By direct activation is meant depolarization of P-cells by a surface-anodal stimulus; by indirect activation, the fact that other structures, in particular

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the stellate and basket cells in the molecular layer above the P-cells and the granular cells just below them, can hardly remain uninfluenced by the stimulating current and so must set up trans-synaptic effects on the P-cells, excitatory or inhibitory as the case may be.

Furthermore, by restricting our observations to the anterior lobe, which is probably the part most commonly studied by surface stimulation, we have been able to make a rough estimate of the number of P-cells that have to be inactivated in order to elicit a visible response in the animal. By varying distance and depth from the stimulus site in measurements of threshold effects we have endeavoured to duplicate the similar experiments by Phillips (1956) on the Betz cells of the motor cortex, due regard being paid to differences of structural organization.

METHODS

Cats were decerebrated at precollicular level by suction under Thiogental (Merck, Darmstadt; sodium salt of 5-(2-methylthioethyl)-5-(L-methylbutyl)-2-thiobarbituric acid) anaesthesia. For the purposes of mapping, the anterior lobe of the cerebellum was flooded with warm mineral oil and explored with a focal electrode (a spring-mounted silver ball 0.5 mm in diameter). The stimulating rectangular pulses were positive- or negative-going with respect to the remote earth electrode. To increase the background rigidity of the forelimbs, the spinal cord was cut in the mid-thoracic region under trichlorethylene anaesthesia after the preliminary map had been drawn. The relaxation of rigidity in the forelimb and its post-inhibitory recrudescence were observed by hand and eye. In some experiments the limb was attached to a strain-gauge myograph by a thin rubber strip. The tension in the rubber was sufficient to flex the limb passively when the extensor rigidity was relaxed. The timing of relaxation and rebound could then be read off from the myographic record.

The methods of fastigial stimulation and micro-electrode recording have already been described in full detail (Granit & Phillips, 1956), together with the criteria of P-cell identity.

Cells lying in the superficial parts of the cerebellar folia (depth up to 0.5 mm) were probed with microcapillaries whose terminal 0.5 mm of shaft was very fine (diam. about 10–20 μ): d.c. resistance was 5–10 M Ω . Such fine capillaries can be pushed through the intact pia (the blood-vessels being avoided by microscopical observation) without indenting it or breaking themselves, and with less risk of superficial cortical damage than if the pia has to be reflected. Cells lying in the deeper folds were approached through fine pial incisions, which are necessary to permit the entry of the wider (about 50–70 μ diam.) part of the micropipette shaft.

The focal electrode used for stimulating the overlying cortex was the 0.5 mm pore in the watch-glass used to control cardiovascular and respiratory pulsation (Phillips, 1956). A chlorided silver wire dipped into the pool of Ringer's solution filling the watch-glass, making the pore electrode positive ('surface positive') or negative ('surface negative') with respect to the subcutaneous reference (earth) electrode, which was also of chlorided silver. For stimulation at a short distance (about 2 mm) horizontally along the same folium, or (to control the factor of current spread) on an adjacent folium, watch-glasses with two or three pores were used. The corresponding Ringer pores were well insulated from one another with paraffined partitions. The stimulator could be connected to any pool. Designed by Dr B. Frankenhaeuser, it delivered rectangular pulses of either polarity, whose voltage, duration, repetition frequency, and train duration could be independently varied. Shock artifacts were balanced by a bridge arrangement in which the second cathode follower of the high impedance amplifier input was connected to the slider of a 100 k Ω potential divider connected across the stimulator output (Phillips, 1956).

RESULTS

A. *Motor effects of anterior lobe stimulation*

An array of papers beginning with Sherrington (1898) and Löwenthal & Horsley (1897) up to the present era (see summary by Moruzzi, 1950) has demonstrated that ipsilateral inhibition on the extensors, often followed by rebound, is a characteristic response to stimulation of the anterior lobe in the stiff decerebrate preparation. In attempting to verify this observation and to measure stimulus strength in our own preparations, which were decerebrated by suction, we found the precollicular cat, which (in our hands) has a rigidity of the soft plastic type, mostly to respond in terms of ipsilateral forelimb flexion alone. The limb tended to remain in the flexed state for a long time and had to be passively re-extended for the next observation. Maps on the anterior lobe were plotted for threshold strength and the most sensitive region was generally found to be just medial to the ipsilateral paravermian vein. A more lateral stimulus tended to give ipsilateral extension (see, for example, Moruzzi, 1950, pp. 25-26). Stronger stimuli also gave contralateral reciprocal effects. Variations of stimulus frequency and duration did not fundamentally alter the responses obtained but the threshold reached a minimum at rates somewhat above 100/sec. Effects were hardly ever obtained below 2.0 V.

In agreement with other workers (e.g. Bremer, 1922; Miller & Banting, 1922; Denny-Brown, Eccles & Liddell, 1929; Hare, Magoun & Ranson, 1936), we found ipsilateral extensor inhibition and flexor excitation to be the most regular and easily elicitable effect of threshold stimulation of the anterior lobe. We therefore tried to create optimal conditions for inhibition of extensor tonus by spinal cord section (under trichlorethylene) which produces stiff forelimbs (the well-known Schiff-Sherrington phenomenon). After some time for recovery the cerebellar cortex was re-mapped against this background. After spinal transection the characteristic extensor inhibition was regularly followed by rebound. The cortical area from which this effect was obtained at threshold underwent a large increase and at the same time the threshold also fell, suggesting that we had obtained the sensitization desired. It is impossible to know whether this change in cortical reactivity was due to a change of input pattern into the cerebellum or to a sensitization at some lower level in the neuraxis or to both. The important point in the present work was to obtain an approximate figure for threshold stimulus strength with a reproducible indicator in terms of muscular movement. We never succeeded in reaching below 0.8-1.0 V. at frequency 140/sec and stimulus duration 50 μ sec. Faster rates did not improve this threshold. The rebound had a long latency, never below a second and mostly a great deal longer. The measurements of threshold and timing of response were made under myographic control (see Methods).

Moruzzi in his summary (1950) has minimal values around 0.5 V for the same indicator response. The stimulating electrodes used by most earlier workers were probably a great deal larger than the silver ball 0.5 mm in diameter that we had. As will be shown below, superficial P-cells may respond to 0.015 V. It will depend also upon the size of the contact area how many such cells are directly involved in cortical stimulation. Our figures refer to the particular conditions of our experiments and the motor threshold of the animal merely serves as a reference point to be kept in mind when precise measurements are given below for excitation of P-cells as a function of distance, depth and number of cells excited.

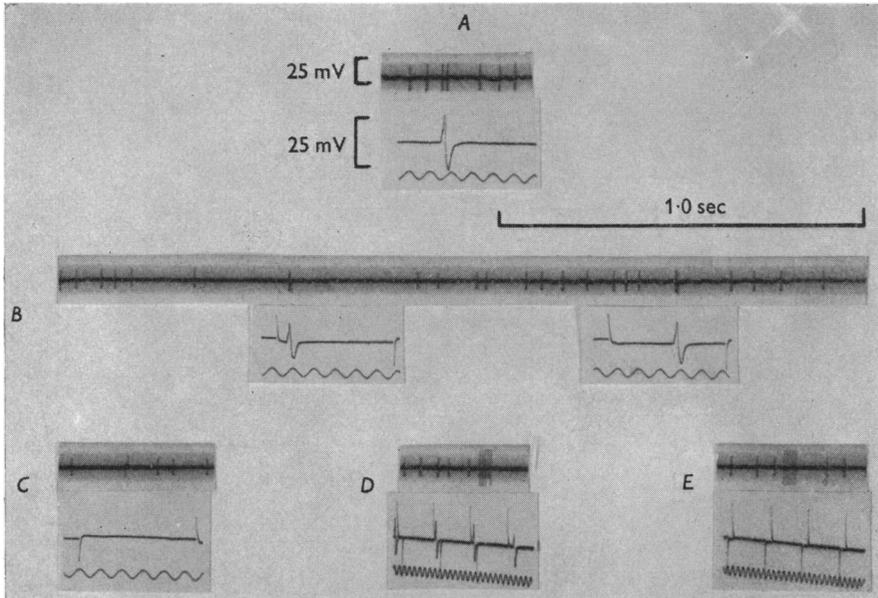
B. *Effects of single shocks*

(1) *Superficial P-cells.* An indirect index of P-cell activity has been used by Moruzzi and his collaborators (Mollica, Moruzzi & Naquet, 1953) who started or stopped the activity of individual reticular cells in the medulla by anodal polarization of the cerebellar surface. In agreement with their work we too find minimum thresholds to surface-positive pulses, provided that the P-cells are superficial and lying just below the pore, 0.5 mm in diameter, by which the Ringer's fluid makes contact with the cortex. Our values for direct excitation generally fall below 100 mV (Text-figs. 1, 2) and are lower when natural firing is in progress than when the cell is silent. Thus in Text-fig. 1 record *A* (lower) identifies the cell (depth 0.2 mm) by its response to a fastigial shock: a positive-going prepotential leading into a diphasic positive-negative spike (amplitude about 25 mV peak-to-peak), with a latent period of 0.7 msec. The records *B* are two surface-positive pulses of 100 mV strength and 5.5 msec duration. The second fired the cell with a longer utilization time owing to the delivery of this pulse very soon after a natural impulse, as shown in the continuous record above the sweep. When the pulse was made surface negative (*C*) it failed to fire the cell. Strength was then increased to 140 mV and brief tetani given. The surface-positive shocks (*D*) again fired the cell whilst the negative ones (*E*) were ineffective at the same strength.

Text-fig. 2 is a summary of our results with single pulses, a plot of depth in millimeters against threshold stimulus strength in mV. Of the cells at less than 0.5 mm depth thirty-three out of thirty-four had thresholds of the same order as that of the one illustrated in Text-fig. 1. All those were surface positive for minimum strength.

It is hardly necessary to dwell in detail upon the relation of threshold strength to firing because our results, as far as they go, are merely a replica of those recorded by Phillips (1956) with Betz cells in which satisfactory intracellular potentials were obtained. By such means it could be demonstrated directly that the thresholds became lower when the potential of the cell membrane swung towards depolarization. This would here be the case when some firing is

in progress, unless the shock happens to fall into the trough of repolarization succeeding a natural impulse (Text-fig. 1*B*, on the right). Cerebellar P-cells vary in a highly random fashion from silence to firing at modest rates of 30–60 per sec during which the threshold easily might vary from 100 to 200 mV, the high values coinciding with periods of silence. This range is illustrated by horizontal lines in Text-fig. 2. Or, again, if the positive pulse was kept at

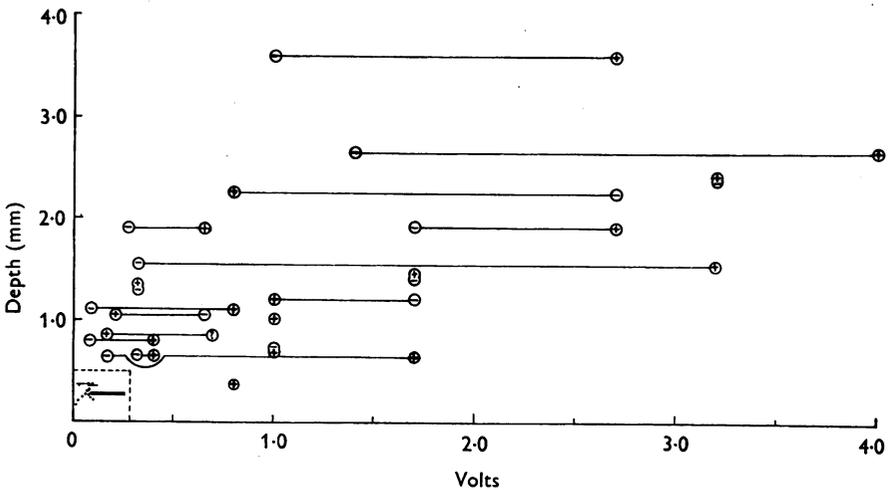


Text-fig. 1. Purkinje cell record (micro-electrode depth 0.2 mm). In this and later figures, upper records (time, 1.0 sec) show relation of stimuli and responses to background rhythm of cell. Sweeps show same shocks and responses on an expanded time scale (1000 c/s). Positivity of microelectrode signalled by *upward* deflexion (except in parts of Text-figs. 5 and 6). *A*, fastigial shock, latency 0.7 msec; *B*, single cortical shocks, strength 100 mV, surface positive, pulse duration 5.5 msec—utilization time is longer when shock is closely preceded by a natural impulse; *C*, surface negative pulses (same strength and duration) always fail to excite; *D*, shorter pulses at 140 c/s, strength 140 mV, surface positive. First shock finds cell refractory, shocks 2 to 4 fire it, with lengthening latencies, and shock 5 (not shown on sweep) fails to fire; *E*, same tetanus with reversed polarity—no effect.

100 mV, it might fire from one to three spikes, depending upon the amount of background activity. Exactly as in the work of Phillips, higher excitability was characterized by shorter utilization periods. We conclude that the correlations between discharge and membrane potential of the Betz cells, established by Phillips, are likely to be valid for cerebellar cells too.

(2) *P-cells in deeper folds of cerebellar folia*. A striking alteration of the picture takes place when deeper-lying P-cells are picked up. Text-fig. 2 shows

that the lower thresholds now tend to accumulate around the surface-negative orientation of the stimuli whilst surface-positive shocks require very much stronger pulses. The two thresholds (+ and -) are joined by lines in the figure. The range of strength is very wide. Variation in polarity for greatest sensitivity to direct stimulation may be due to differences in orientation of the deeper-lying P-cells, whose long axes are disposed at many different angles with respect to the cerebellar surface. Evidently, too, the further away from the stimulating pore electrode the cell lies, the more numerous are the other excitable elements interposed between it and the electrode, so that the possibilities of indirect excitatory and inhibitory effects are correspondingly richer. These may mask the direct effects of the pulses on the P-cell; or, indeed



Text-fig. 2. Diagram showing surface-positive and surface-negative threshold for discharge of a single impulse by cells at different depths, i.e. in superficial and deeper folds of folia; linked symbols denote surface-positive and surface-negative threshold for each cell. Inset (bottom left-hand corner of figure) surface-positive stimulation of seventeen most superficial cells. For four of these, the range of threshold, which depends on the background activity, is shown by horizontal lines. A further sixteen superficial cells (not plotted here) had similar thresholds.

the only effects upon it may be indirect ones. The records reflect these variations by being mixtures of short utilization times indicating direct excitation with various inhibitory phenomena, among them responses of the 'inactivation type' described by Granit & Phillips (1956).

(3) *Stimulus spread sideways.* A somewhat similar situation arises when a superficial P-cell is picked up and the stimulating pore is shifted sideways. The major interest of this arrangement will be that it is possible to achieve some measure of differentiation between physiological and physical stimulus spread. A cortical electrode 2 mm distant along the long axis of the folium will excite,

for example, the granular cells (just below the P-cells) which are so numerous and possess highly developed horizontal conduction pathways towards the cell under the micro-electrode. If the stimulating pore is at the same distance but on an adjacent folium the factor of physical spread will be relatively more important, if in both cases the same amount of excitation be achieved. Actually, with five experiments in which excitation along the axis of the folium proved easy enough to elicit, shocks of similar strength would not start the P-cell from the adjacent folium. Table 1 summarizes the observations on thresholds at distances of 2-2.25 mm along the folium.

TABLE 1. Threshold voltages for superficial Purkinje cells (depth less than 0.5 mm.)

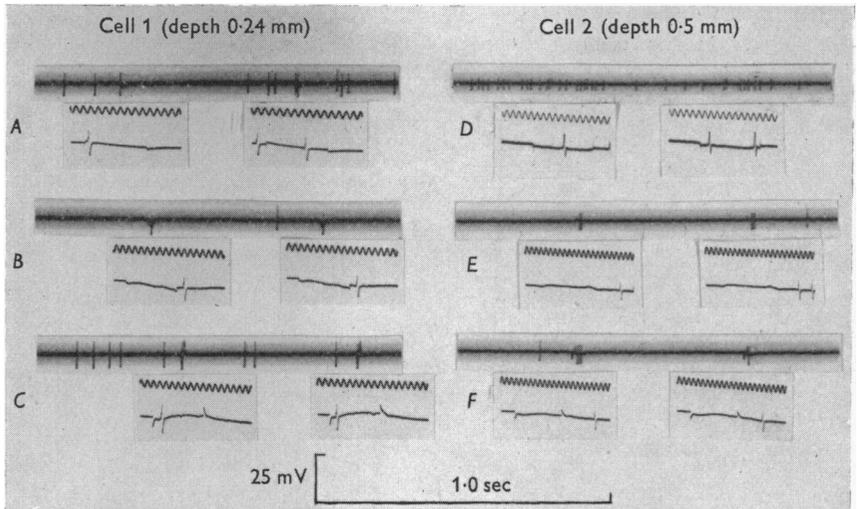
Unit no.	A. Home threshold, surface-positive (mV)	B. Distant thresholds (2.0-2.5 mm from A)	
		Surface-negative (V)	Surface-positive (V)
1	80	0.65	1.0
2	32	0.65	1.0
3	80	0.5	>1.0
4	80	0.8	*
5	14	*	1.7
6	16	0.5	>0.5
7	100	0.5	>1.0
8	100	0.5	1.0
9	21	0.4	1.0
10	17	0.5	1.0
11	80	0.8	1.4
12	17	0.5	1.7
13	32	0.8	1.7
14	40	1.0	1.0
15	65	0.5	*
16	32	0.65	0.8

* Not measured.

The table shows that the lowest threshold pulses were surface negative, but analysis of the records proved this response to be a discharge at 'break' (Text-fig. 3) and not of the highly variable type seen when distance was increased vertically as 'depth'. It should be recalled that histologically 'depth' introduces more complications than 'distance' longitudinally on the surface. The P-cell-granular layer is folded in a complex, and, from place to place, unpredictable fashion. As the microelectrode strikes layer after layer, the folds bring in a factor of 'distance' and at the same time several layers with a variety of cells (basket, stellate, granular, P-cells with recurrent collaterals). 'Distance' longitudinally on the surface should always be dominated by the efficient conduction system provided by the horizontally-running axons of the granule cells.

Text-fig. 3, with two P-cells, demonstrates the threshold break response to cathodal pulses for a longitudinal distance of 2 mm (*B*, *E*) compared with stimulation through the pore containing the micro-electrode (*A*, *D*). The two lowermost records (*C*, *F*) demonstrate the effects of the stronger positive pulses

needed to excite the cell from the distant pore. Only 20–30 mV were required to excite these cells at their site by surface positive pulses, while between 1000 and 2000 mV were needed to excite them from a distance of 2 mm. The mechanism of distant excitation was clearly different in the two cells. In the first (Text-fig. 3, *B, C*) excitation occurred shortly after the make or break of the stimulating pulse, depending on the polarity. In the second (Text-fig. 3, *E, F*) it occurred after the break of both positive and negative pulses, and took the form of delayed repetitive firing.

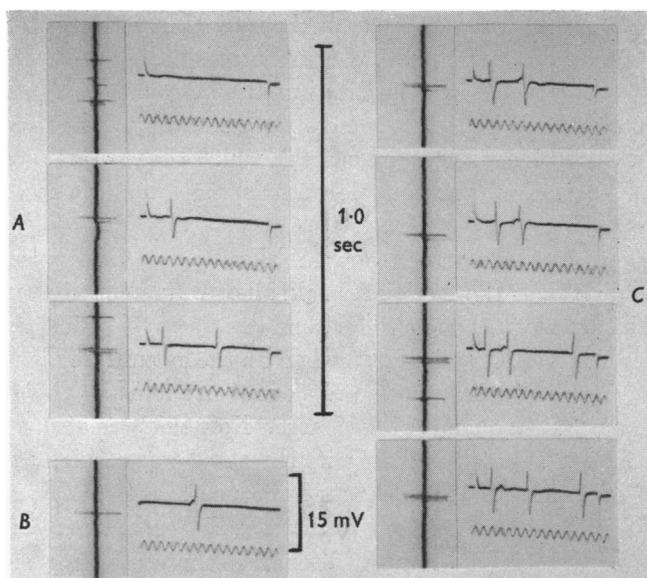


Text-fig. 3. Responses of two superficial Purkinje cells (micro-electrode depths 0.24 and 0.5 mm) to stimulation at the overlying pore electrode (*A, D*) and at the distant pore electrode, 2 mm along the same folium (*B, C; E, F*). Sweep scale 1000 c/s. *Cell 1. A*, surface-positive pulses at overlying ('home') electrode, strength 32 mV, fires cell early, unless closely preceded by a natural impulse. *B*, surface-negative pulse at distant electrode, strength 0.8 V, excites after break of current; latency about 1.2 msec. *C*, surface-positive pulses, strength 1.7 V (distant), excite after make of current, latency about 1.2 msec. *Cell 2. D*, 'home' electrode, surface-positive, threshold 21 mV. *E*, distant electrode, surface-negative, threshold 0.4 V—brief burst of impulses follows break of current; latency about 7.0 msec. *F*, distant electrode, surface-positive, threshold 1.0 V—brief burst after break, latency 5.0–7.0 msec.

Since, as a rule, the threshold current at 2 mm distance only excites at break and does it longitudinally but not transversely across a folium, it is probably mainly an indirect excitation prevented from appearing at 'make' because of the anodal polarization surrounding the region of a cathodal pulse.

In terms of our threshold for movement which was of the order of 1.0 V under optimal conditions (sensitive cortex, repetitive pulses), a highly restricted positive pulse of that order is likely to excite from one to three layers of P-cells within an area with a radius of the order used (2.0 mm).

(4) *Inhibition in response to single pulses.* Over and above the excitation of P-cells to the discharge of one or more impulses, single square-wave pulses sometimes have an inhibitory effect, manifested in slowing or arrest of the natural impulse trains, or detected by experimental interference between single impulses, as evoked from the fastigial electrode, and cortical shocks. Such inhibition is sometimes seen as the sole response to the cortical pulses.



Text-fig. 4. Purkinje cell, micro-electrode depth 0.35 mm. Interaction between cortical and fastigial shocks. In this figure the strips of continuous record are arranged vertically, to the left of the corresponding sweep (time, 1000 c/s). *A*, surface-positive stimulus, strength in top fires one or two impulses, unless sandwiched between inactivation responses (as 125 mV; record). *B*, fastigial stimulus, latency 0.8 msec; the unit spike is preceded by the massed P-cell potential, which is to be distinguished from the unit prepotential (see also Text-fig. 5). *C* (above downwards), fastigial stimulus made progressively earlier in relation to cortical stimulus; fastigially evoked impulse fails when shock falls at end of first cortically evoked impulse (bottom record); the massed positive-going P-cell potential evoked by the fastigial stimulus alone survives. In middle two sweeps note increasing latency of fastigially evoked spike.

The evidence for the statements which follow is based on examination of records of more numerous trials than can be illustrated here. Full information is difficult to obtain on account of the great variation in natural activity from one cell to another, and in one and the same cell from time to time. Natural firing may be absent altogether, or too slow and irregular to reveal inhibitory effects.

Mere blocking by refractoriness is easily demonstrated. Text-fig. 4*A* shows three responses of a superficial P-cell to near-threshold (125 mV) surface

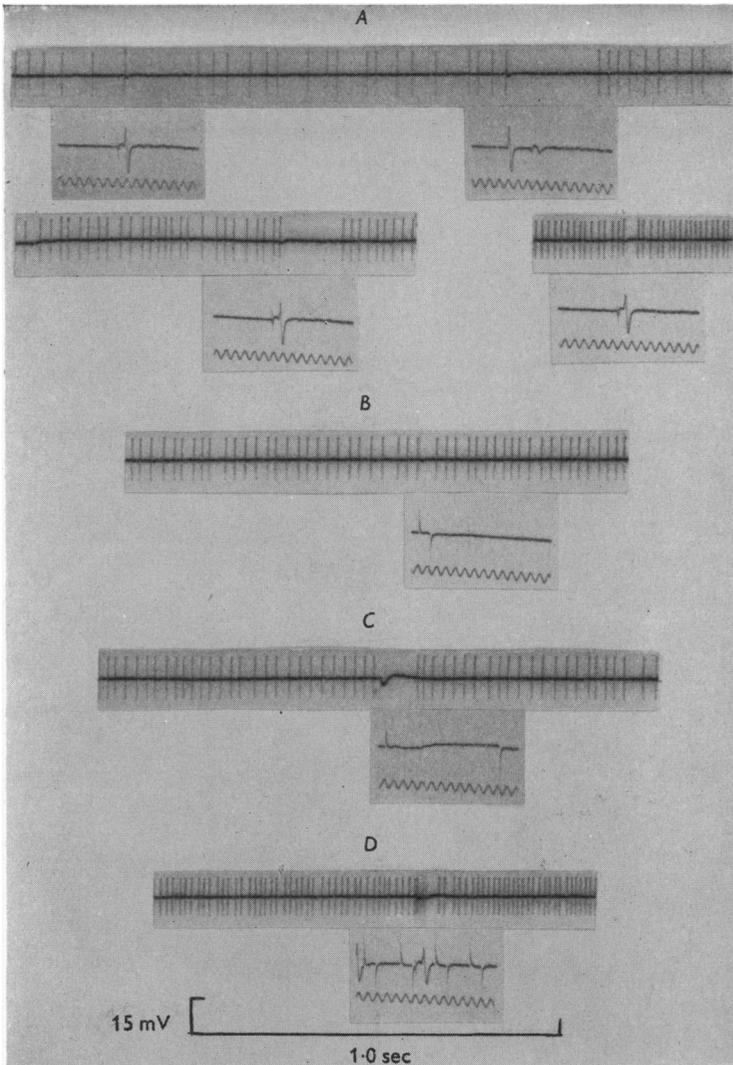
positive shocks. The first is sandwiched between two inactivation responses (Granit & Phillips, 1956) and has no effect, but the others fire one or two impulses. Text-fig. 4*B* is the response to the fastigial shock alone. It begins with a small positive hump which is the massed response from several P-cells, and should be distinguished from the subsequent specific prepotential proper to the discharging cell lying under the micro-electrode, which (on the compressed time scale used) appears as a thickening of the foot of the spike (cf. Granit & Phillips, 1956). In the records *C* the fastigial shock is shifted up towards the spike elicited by the cortical pulse. In the bottom record it is near enough to fall in the refractory phase of the first impulse and accordingly the fastigial spike fails to appear. What is left is merely the massed positive response. By contrast true inhibition was seen in cases in which the cortical pulse was sub-threshold for excitation, yet capable of stopping a spike elicited from the fastigial electrode.

Text-fig. 5 serves to illustrate true inhibition by a cortical pulse. It begins with four records *A* of the fastigial shock, all of which caused a pause in the natural discharge, even though the second one failed to elicit a spike. The length of the silent period varied inversely with the natural frequency. Record *B* shows that a brief surface positive pulse of 400 mV had a doubtful inhibitory effect on the natural frequency of discharge. Now this was a superficial spike which behaved exceptionally because its threshold was as high as 800 mV, a fact in itself suggesting that the shocks happened also to activate inhibitory elements. When the 400 mV pulse was increased in duration (record *C*), there was a definite pause in the natural discharge, confirming the surmise that some inhibitory structure was responsible for its unusually high threshold. When at this strength the pulse was made repetitive at a duration of 1 msec (record *D*) it failed to block the spike (in the middle), set up by a fastigial shock. The fastigial latency also remained unchanged, as measured to the foot of the prepotential, but the take-off from the prepotential was definitely delayed (compare with the records *A*). The brief tetanus caused a short pause in the natural discharge. Similar inhibitory effects were also seen with surface negative pulses as well as with 'distant' stimulation of P-cells.

C. Repetitive stimulation

(1) *P-cells below stimulating pore electrode.* In most papers on stimulation of the cerebellar cortex repetitive shocks have been used. These are likely to bring in greater complexity of indirect activation than single pulses can ever do. Our results with repetitive stimulation bear out this surmise.

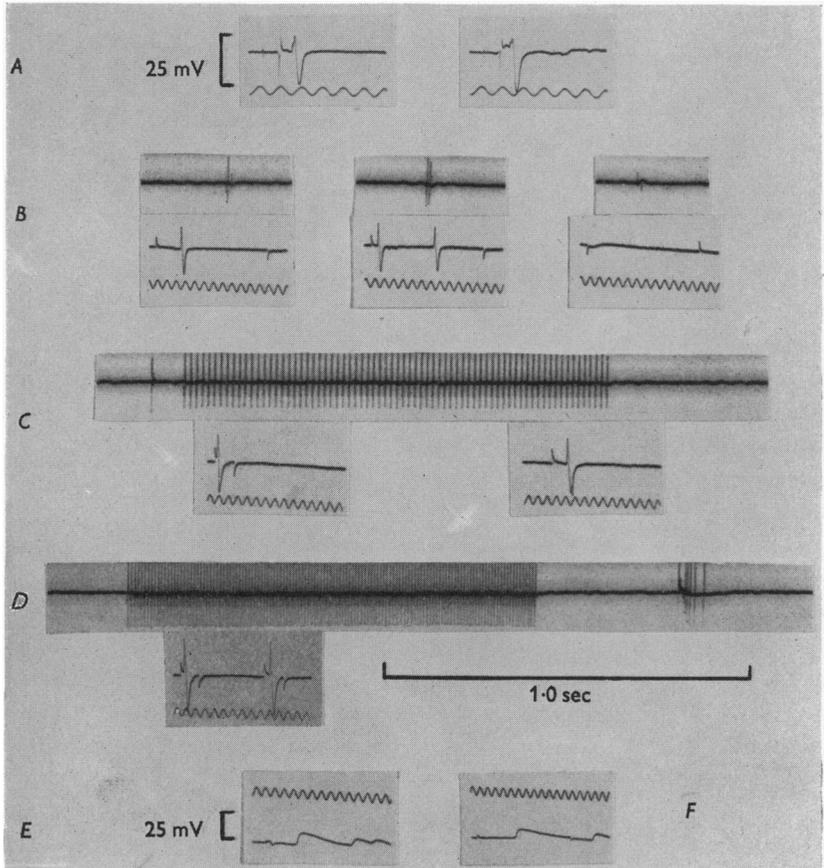
Text-fig. 6 shows the simplest experimental situation, in which an idle P-cell is stimulated to follow the frequency of the incident shocks. This is defined as 'driving' for the purposes of this paper. The only natural responses seen in the figure (and throughout the records from which it is taken) are



Text-fig. 5. Purkinje cell, micro-electrode depth 0.36 mm; sweep scale 1000 c/s. *A*, responses to four fastigial shocks, with different excitatory backgrounds: natural rhythm interrupted, even when unit firing does not occur (second sweep and continuous record). Note, in second sweep, the massed positive-negative P-cell potential due to fastigial stimulation, and in other sweeps, that it is clearly distinguished from the positive-going unit prepotential. *B*, surface-positive cortical pulse, strength 400 mV, duration 1.0 msec; no excitation, doubtful inhibitory effect. *C*, ditto, but duration 12.0 msec; no excitation, but pause in natural firing. *D*, short tetanus of surface-positive pulses, strength 400 mV, does not inhibit fastigially evoked impulses, but causes pause in natural rhythm. Threshold for excitation by single pulses was unusually high, 800 mV surface positive (cf. Text-fig. 2).

Note: micro-electrode positivity is signalled by *upward* deflexions in the sweeps, but by *downward* deflexions in the continuous records.

inactivation responses (e.g. at the end of record *D*). The three records *B* are: (i) the surface positive threshold response to 80 mV, followed by (ii) a double response to 100 mV, and (iii) failure of surface negative shock of same strength and duration (12 msec). In *C* strength was raised to 140 mV surface positive and duration reduced to 2 msec. These pulses drove the cell at 62 c/s through-



Text-fig. 6. Purkinje cell, micro-electrode depth 0.25 mm; no natural activity, but occasional inactivation response (cf. beginning of continuous record *C* and end of continuous record *D*). *A*, responses to fastigial shocks at two strengths, latency 0.75 and 0.6 msec, sweep scale 1000 c/s. *B*, responses to single pulse stimulation, surface-positive, strength 80 and 100 mV; surface-negative pulse, strength 100 mV, without effect. *C*, driving by surface-positive pulses, strength 140 mV, duration 2.0 msec, at 62 c/s; sweeps (below continuous record), taken at beginning and end of tetanus, show lengthening utilization time as tetanus proceeds. *D*, driving by same pulses at 110 c/s. *E*, D-wave elicited by fastigial stimulus (latency 3.1 msec) after puncture of cell. *F*, D-wave elicited by surface positive cortical pulse, threshold strength 270 mV, latency 4.8 msec. *Note*: micro-electrode positivity is signalled by *upward* deflexions in the sweeps, but by *downward* deflexions in the continuous records.

out a train lasting over a second, with progressive increase of a utilization time as shown by the early and late samples on the sweeps below the train of spikes. In *D* driving occurred at 110 c/s and was still obtained at 130 c/s when cell penetration occurred before the experiment was completed. This led to loss of the spike and appearance, in *E*, of the slow D-wave of Granit & Phillips (1956).

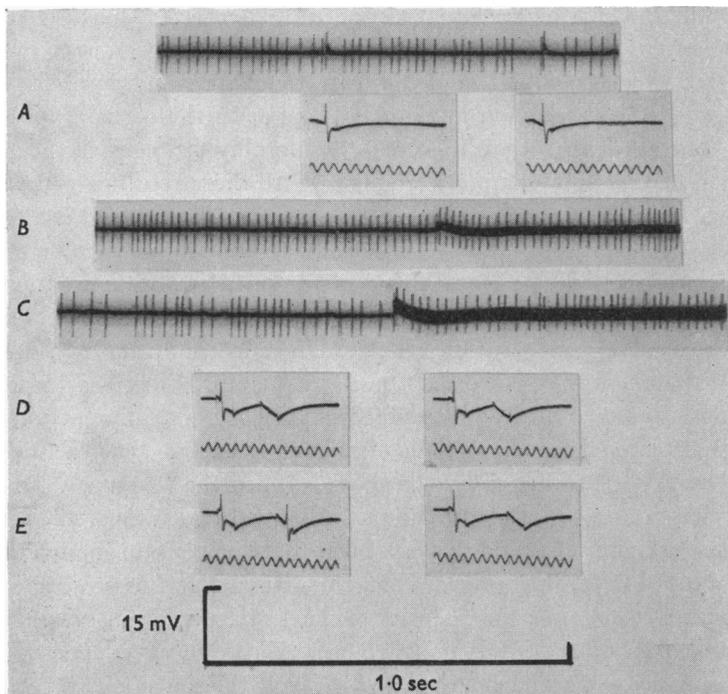
It is characteristic of the D-wave, and illustrated in record *E*, that it has a long latency, 3.1 msec, to a fastigial shock, showing that it could not be the decaying spike which had a latent period of 0.75 msec (cf. record *A*). Also, surface-positive stimulation of as much as 270 mV was necessary to evoke the D-potential, which then had the long utilization time of 4.8 msec (record *F*). D-waves were fairly common in these, as well as in the earlier experiments, and, without exception, they required considerably stronger surface stimuli than spikes at the same micro-electrode site. All these results strengthen our earlier conclusion (Granit & Phillips, 1956) that the D-potential is a response type in its own right and not a deteriorating spike potential.

At times during which natural firing is present, the effects are more complicated, as illustrated by Pl. 1. The threshold for short single pulses (surface positive) was only 17 mV, and Pl. 1 *A* shows the effects of trains of these pulses at 23, 50, 133 and 167 c/s. Their action is most simply described by the term 'triggering'. The over-all frequency of firing is not very different during the tetanization periods and the periods of natural discharge, but the individual impulses tend to be triggered by the shocks. When the frequency the experimenter seeks to impose is below the prevailing natural rate (*A*, 23/sec) each shock triggers an impulse, and the gaps between them contain enough impulses to maintain the natural average. When the artificial and natural frequencies are more nearly the same, the majority of shocks trigger impulses, and there is less natural firing in the intervals (*A*, 50/sec). At higher frequencies (*A*, 133 and 167/sec) it can be seen from the sweeps (below the continuous records) that every impulse is triggered by a shock, although of course not every shock triggers an impulse. The average frequency is here a little higher during the tetani than before or after.

Column *B*, in which 50 mV shocks were used, illustrates the transition between triggering and driving of the cell. At 23/sec every shock is effective, except during refractoriness due to a previous natural impulse. At 50/sec the appearance of driving is obvious. Only a few extra impulses are interpolated during this tetanus, and some of these so closely preceded a shock, that the cell was made refractory to it. At 133/sec the first five shocks drove, the rest triggered. Initial driving was also evident at 167/sec. Measurements of the records showed that the 50 mV shocks were unable to drive the cell to follow frequencies greater than the prevailing natural rhythm, which varied from 48 to 60/sec, but by triggering these shocks succeeded in stirring up the firing rate to a

faster rhythm than the natural one, tending in fact to fire it at about half the stimulating frequency.

Finally column *C* shows that shocks of twice the previous strength (100 mV) drive the cell to follow stimulating frequencies up to 133/sec, with but a rare failure. It also followed 266/sec with rare failures. The fluctuating spike amplitude is a manifestation of underlying waves of polarization and depolarization of the cell membrane (Granit & Phillips, 1956). Inhibitory pauses followed the



Text-fig. 7. Purkinje cell, micro-electrode depth 0.24 mm. *A*, excitation by single surface positive pulses, strength 50 mV. *B*, beginning of tetanus of same pulses, at 180 c/s; no driving. *C*, beginning of tetanus of 100 mV pulses, at 180 c/s; no driving. *D*, paired 100 mV shocks, surface-positive, interval 4.8 msec; second shock ineffective. *E*, same shocks, interval 6.2 msec; second shock sometimes fires. Sweep scale 1000 c/s.

tetani at rates above 100/sec. It is rare to find a cell following as high frequencies as 266/sec. To judge by the results of Granit & Phillips (1956) an absolute limit would be around 300/sec.

Excitation is a great deal more common than inhibition, at least in the anterior lobe region, but occasionally powerful concurrent inhibition is stirred up by stimulus repetition, preventing driving of a cell by shocks which never fail to excite it when delivered singly. In the cell of Text-fig. 7 *A*, single 50 mV

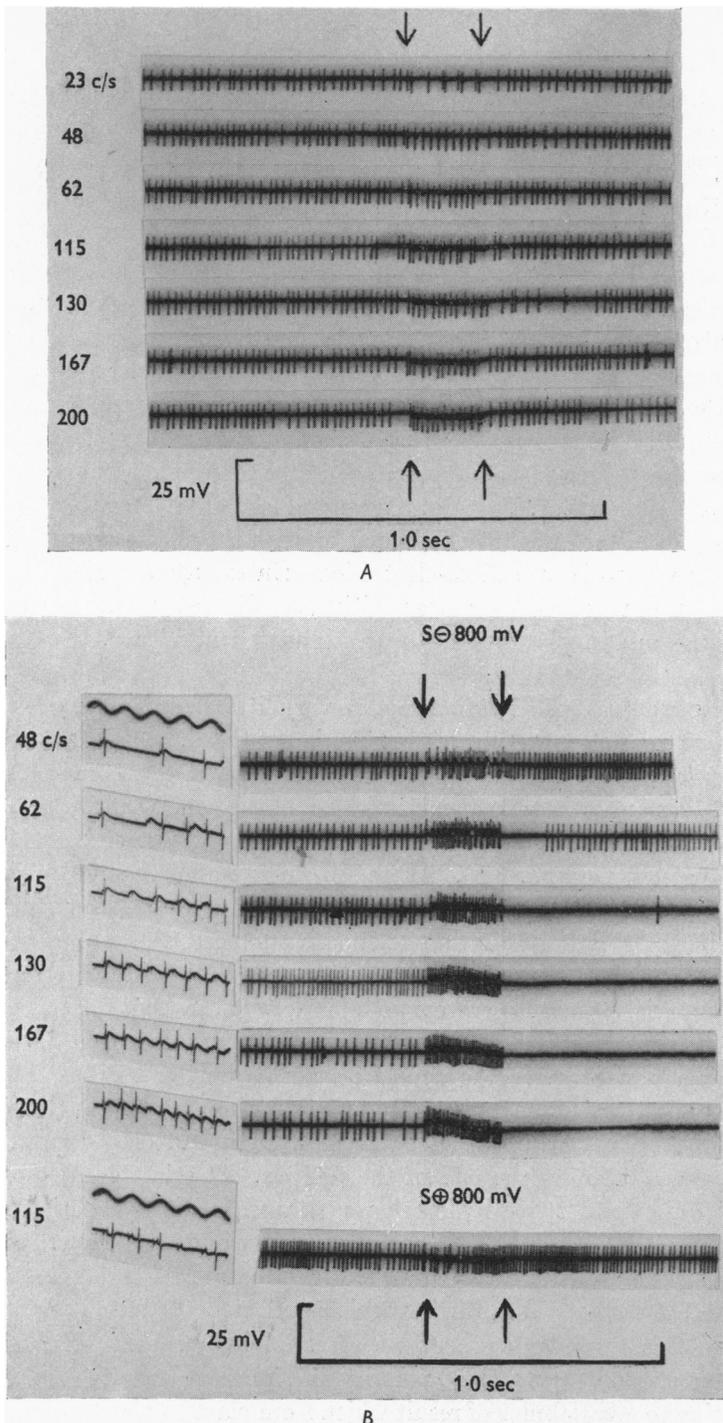
shocks fired impulses, followed by short inhibitory pauses in the natural rhythm. Tetani of the same pulses at 180 c/s, whether of strength 50 mV (Text-fig. 7B) or 100 mV (C) did not drive this cell. In Text-fig. 7D and E the inhibitory effect of a single 100 mV shock was tested by a second 100 mV shock at different intervals (see legend).

(2) *P-cells at some distance from stimulus.* It is not to be expected that repetitive electrical stimulation at some distance from the cell under the microelectrode would do more than emphasize indirect effects at the expense of direct ones and thus contribute to a variety of behaviour that already goes beyond the range of prediction. Stronger stimuli will, as we have seen, be needed and so, if driving is the effect, it should be improved; if, on the other hand, inhibitory phenomena happen to be in the foreground, they will tend to become more prominent. Text-fig. 8 is reproduced chiefly for the sake of the comparison of distant repetitive stimulation with the effects of stimulation at the pore through which the micro-electrode was introduced. The local threshold for single pulses was 32 mV surface positive, the distant 650 mV surface negative. In the tetani, the local site was stimulated at 50 mV, the distant at 800 mV.

The effects of local tetanization require very little description, as they were very similar to those described in connexion with Pl. 1. The natural firing frequency lay between 45 and 55/sec. Stimulation at 48/sec caused obvious driving, lower and higher frequencies triggered the cell (Text-fig. 8A).

Very different results were obtained by distant stimulation with surface negative pulses (Text-fig. 8B). At 48/sec single impulses were fired at the breaks of the first two shocks, but the intervals between the later shocks were filled by bursts of impulses so that 10 shocks produced 26 impulses at a mean frequency of 130/sec. The mean natural frequency was 56/sec before the tetanus, and after it, 80/sec. There were no inhibitory pauses, but the spikes at and after the end of the tetanus were small in size and grew again as if the cell were repolarizing after an excessive depolarization (Granit & Phillips, 1956). At 62/sec the effect was similar: mean frequency during stimulation 150/sec, but a pause now followed it. At 115/sec the cell was stirred to 155–190 spikes/sec. Abrupt silence followed the end of the tetanus. At stimulus frequencies of 130/sec, 167/sec and 200/sec the cell was stirred to discharge rates of 170–180/sec. The ensuing pauses were sudden in onset and lasted over 3 sec. By contrast, surface positive stimulation (which fired during the shock to single pulses) at 115/sec caused a stirring which outlasted the stimulation period.

Thus in this single experiment we see stimulus strength, distance from stimulating electrode, frequency, polarity and inherent activity of the cell, all contributing to a variability of result which from place to place and cell to cell may follow very different rules depending upon how these factors are combined.



Text-fig. 8. Purkinje cell, micro-electrode depth 0.1 mm. *A*, tetanization (between arrows) at overlying pore electrode, surface-positive pulses, strength 50 mV; frequencies shown at left of figure. *B*, tetanization at pore electrode 2.0 mm distant along the folium; sweeps (left of figure, time 100 c/s) show responses to first few shocks of each tetanus; surface polarity, strength, and frequency as marked on figure. Full explanation in text.

DISCUSSION

We are not aware of any previous study of the response of identified Purkinje cells to cortical stimulation and so it is of interest to distinguish the general from the particular before comparisons are made with observed motor responses in animals.

Despite inhibitory complications, the over-all effect of cortical stimulation is P-cell excitation by direct depolarization. Stimuli of the kind used in physiological experiments will be much suprathreshold for the cells nearest the stimulating electrode (cf. the similar conclusion for Betz cells, Phillips, 1956) so that within a considerable area the P-cells will be 'driven' at the imposed frequency, while outside this area or a couple of layers below it, they will be 'triggered'. After cessation of stimulation some cells will have been stirred to long-lasting discharges, gradually petering out into the original random behaviour of silence and firing described by Granit & Phillips (1956). Other cells will pause for a long while, and in our material such pauses were more common with stimulation at high rates and great strength. It is not possible to point to any single characteristic property by which cortical stimulation would definitely differ from white matter stimulation by fastigial electrodes, as studied in our previous paper. However, it is our impression that the P-cells were more effectively stirred to long-lasting states of activity by the fastigial stimulus which, of course, involved not only antidromic stimulation of P-cell axons but also trans-synaptic excitation through adjacent afferents (mossy and climbing fibres).

Every P-cell, from the point of view of electrical stimulation, must be regarded as an individual proposition, particularly with the repetitive mode of stimulation used in physiological experimentation. The variability is beyond description, but it is clear that what we have to consider is the most probable average response of an assembly. How many cells are concerned in the characteristic extensor inhibition followed by rebound of the stiff decerebrate animal? With our electrode diameter of 0.5 mm the optimum threshold was of the order of 1 V, more commonly around 2 V. Considering Text-fig. 2 and Table 1, it is clear that the surface effect then would spread beyond the 2.25 mm which was our longest distance, and also that at 2 mm depth most cells still respond to surface positive shocks below 1 V. Downwards this effect may, however, shrink laterally. The width of the folia and the pattern of cortical folding vary a great deal from place to place, but taking Inukai's (1928) figures for the rat cerebellum (1000 cells/mm²), a rough calculation shows that such shocks can hardly excite fewer than 20,000 P-cells, and may very well excite more. This is for a just suprathreshold effect observed in an animal sensitized by spinal section, as described above. Indirect triggering may well bring in a very much larger number of cells. All these cells may not

necessarily be directed towards the same output channel and some may be inhibited. On the other hand, the organization of the cerebellar cortex seems to be designed for the very purpose of spread and mass action.

The cerebellar cortex is a very homogeneous structure and so it is likely that all cerebellar areas behave in the same way, because cortical stimulation always involves a strong component of direct depolarization. This means that differentiation is achieved on the basis of connexions made below the level of the P-cells. Having used the anterior lobe, however, we can fall back upon a body of physiological knowledge about stimulation of this portion which it seems desirable to bring into line with our evidence. Thus the ipsilateral extensor inhibition means that, on an average, P-cell excitation has this effect. When, as often is the case, there is simultaneous flexor excitation, it is not necessary to place this reciprocal organization into the cerebellum. We found a certain amount of stiffness of the limb necessary for good rebound. A return to this state of rigidity is the obvious consequence of the gradual return of the excited P-cells to their previous rate of firing. In agreement with this is the long latent period of the rebound. Sometimes the limb rebounds over and above its previous state of tonic contraction. It is wholly unnecessary to invoke any different explanation of this case, because so many of the focal and thus highly excited P-cells will be silent for a while after stimulation. This means temporary removal of the tonic restraint that they had exercised before stimulation upon tonic extensor excitation, in other words, a release of the system whose activity the P-cells had been damping while in activity. In line with this interpretation is the well known fact that removal or cooling of the cortex of the anterior lobe leads to very great rigidity (Bremer, 1922; Miller & Banting, 1922; Pollock & Davis, 1927; Stella, 1944*a, b*; Moruzzi, 1950; Granit, Holmgren & Merton, 1955).

The other type of reaction reported from stimulation of the anterior lobe consists in persistence of the motor response for a long while. This has been seen in intact animals, with implanted electrodes, by Clark (1939) and by Chambers (1947), in decerebrate animals by Hare *et al.* (1936) and now in this paper in several animals with precollicular severance of the brain stem by aspiration (before spinal section). We suggest that the basis of this response is the long-lasting stirred-up activity of P-cells, noted also with the stimulating needles in the fastigial nuclei or overlying arbor vitae.

While the results of electrical stimulation of the cortex have been dominated by excitation of the P-cells, this is not to be taken to mean that inhibition is insignificant for cerebellar action under physiological circumstances. Rather does it imply criticism of the electrical method as a wholly inadequate instrument for the analysis of performance in a central structure, even when it is as homogeneous as the cerebellum. Its role is to supplement anatomy by showing in a rough general way what distant structures can be activated from any

given place (when positive results are obtained) and to aid physiological analysis by setting up certain states or acts of excitation or inhibition which the physiologist may want to analyse in some other manner. Thus in a central structure, function cannot be sensibly understood from the organizational point of view by electrical stimulation, particularly not when gross movement is taken as indicator. In refined myographic and electromyographic experiments, Denny-Brown *et al.* (1929) found that stimulation of the cerebellar cortex invariably produced mixed excitatory and inhibitory effects on the spinal motoneurons. Both in the cerebrum (Phillips, 1956) and in the cerebellum a considerable number of cortical cells are stimulated with pathologically strong stimuli (about $10 \times$ threshold) when a visible effect is beheld. These strong shocks are necessary in order to excite the large number of cells which in both these structures are necessary to bring on movement. With the cerebellum it is perhaps better to use relatively large surface electrodes and abandon the notion that very small electrodes could improve differentiation.

SUMMARY

1. Single units of the cat's cerebellar cortex (anterior lobe) were closely approached with fine KCl-filled microcapillaries, and identified as Purkinje cells by their giant-spike responses to fastigial (antidromic and monosynaptic) stimulation.

2. The cerebellar cortex was then stimulated by rectangular currents flowing between a focal electrode (0.5 mm in diameter) and a remote electrode of large area. Anodal and cathodal shocks were used, singly and in repetitive trains.

3. Cells of the layer of cortex lying nearest to the surface (depth up to 0.5 mm) were selectively stimulated by surface-positive shocks, at thresholds of 20–100 mV.

4. Cells in the deeper folds needed stronger shocks (threshold up to 4.0 V). Here, surface-negative stimulation was usually, but not always, effective at weaker strengths than surface-positive. Inhibitory as well as excitatory effects were produced.

5. Stimulation at a focal electrode 2.0–2.5 mm distant along the same folium was effective at strengths of 0.4–1.0 V.

6. It is estimated that at least 20,000 Purkinje cells are likely to be involved in the inhibition of decerebrate rigidity by electrical stimulation of the anterior lobe of the cerebellum at the current strengths generally used.

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EXPLANATION OF PLATE

Purkinje cell, microelectrode depth 0.16 mm. Stimulation by surface-positive pulses at three different strengths (vertical columns, *A*, 17 mV; *B*, 50 mV; *C*, 100 mV) at frequencies indicated in margins of figure; stimulation periods marked by arrows; sweeps below continuous records (time 100 c/s) show responses to first few shocks of some of these tetani. Full explanation in text.

