J. Physiol. (1972), **222**, *pp.* 357–371
With 7 text-figures
Printed in Great Britain

AN EXTREME SUPERNORMAL PERIOD IN CEREBELLAR PARALLEL FIBRES

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(*Received 14 September 1971*)

SUMMARY

1. The electrical responses produced by stimulation at the surface of the cerebellar cortex have been studied in anaesthetized cats.

2. The propagation of the underlying activity is attributed to parallel fibres, but the mode of generation of the potential changes is less certain.

3. The threshold for a second response is up to 40 % lower following a conditioning stimulus, and the responses to test stimuli less strong than the conditioning stimulus are correspondingly potentiated.

4. A second stimulus given 22 msec after the first produces a response which propagates 15–20 % faster along the folium.

5. The reduction of the threshold and the increase of the propagation velocity are as large with small (but above threshold) conditioning shocks as with large shocks.

6. Both after-effects are present from 5 to 100 msec after a conditioning stimulus, with maximal values at about 10–30 msec.

7. The fibre conduction velocity inferred from collision experiments agrees with that inferred from the propagation velocity of the responses, and shows a similar increase after conditioning.

8. A second shock does not recruit a new and faster population of parallel fibres.

9. Under the conditions of electrical stimulation the after-effects are probably restricted to those fibres which are active during conditioning.

INTRODUCTION

This paper describes new properties of the potential changes produced by electrical stimulation at the surface of the cerebellar cortex. These potential changes have been previously studied by Dow (1949) and by Eccles, Llinas & Sasaki (1966). The new properties are satisfactorily explained if the parallel fibres of the cerebellum undergo a pronounced

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supernormal period after an action potential, with a reduced threshold for excitation and an increased conduction velocity.

The apparent increase of the conduction velocity of the parallel fibres is so marked that it distinguishes them from all the other fibres in the mammalian nervous system in which the after-effects of stimulation have been studied. Experiments are described which test some of the less direct consequences which should result from such a marked supernormal period.

A preliminary account of this work has been published (Gardner-Medwin, 1971).

METHODS

Cats anaesthetized with Nembutal (*ca.* 40 mg/kg I.P.) were mounted on a heated operating table with their heads in a rigid head holder. A 10 mm length of one of the the straight transverse cerebellar folia was exposed by cutting away the bone and dura behind the cerebellar tentorium.

Platinum electrodes were used in all the illustrated experiments, for both stimulating and recording. A block containing the electrodes was made by embedding a row of fourteen 100 μ enamelled platinum wires spaced at 0·5 mm intervals in a block of Araldite (CIBA Ltd). The surface of this block in contact with the cerebellum was cut across the platinum wires so as to give a straight row of electrodes flush with the Araldite surface (Fig. 1*a*). The electrodes could be connected for stimulating and recording in any configuration, only a few of them being connected at any one time. The electrode block (2 mm wide × 8 mm long) was positioned so as to press lightly against the cerebellar surface, with its length parallel to a folium. Slight adjustments to its orientation were made until large responses could be recorded along the middle stretch of the block, capable of propagating for at least 3 mm in either direction. The exposed parts of the cerebellum around the block were then covered with an Agar gel (4% Agar in Locke solution). In this way stable recording conditions were maintained for several hours.

Stimuli were derived from 0·05 msec voltage pulses generated by Devices isolated stimulators, connected through a series 0·01 μF condenser to a pair of the electrodes. The use of a series condenser made no difference to any of the phenomena described, but helped to reduce the stimulus artifacts. The coupling time constant was approximately 20 μsec, and the electrodes were delivering two current pulses in opposite directions 50 μsec apart. The electrode which was negative first during a stimulus is referred to as the cathode. The two stimulating electrodes were always adjacent electrodes in the block, being thus 0·5 mm apart. When the polarity of the connexions to them was reversed, it could be seen from the changes in conduction delay that the cathode was the effective site of initiation of the propagated responses.

Evoked potentials were amplified with Devices amplifiers (× 1000, 0·8–30,000 Hz) and photographed on a Solartron CD 1400 oscilloscope. The potentials were recorded single-sided relative to earth, with the animal earthed through the orbit bars of the head holder. A Devices digitimer was used to trigger the oscilloscope and stimulators. All measurements of the responses were made from film.

Most of the after-effects of a conditioning stimulus were studied at a fixed interval of 20 or 22 msec. The time course of the phenomena was investigated in only three experiments. No after-effects were detected at intervals larger than about 250 msec. Sequences of stimuli repeated at rates up to one every 2 sec were regarded as independent.

The most simply observed qualitative result in the paper, the earlier propagated response after a second shock, has been seen in every experiment (eleven cats). It has

in fact never been absent, either at the beginning or at the end of an experiment. Each of the more complicated and quantitative studies has been performed on at least two of the animals.

RESULTS

Responses to single stimuli

The main characteristics of the responses produced by local surface stimulation, described by Dow (1949) and by Eccles *et al.* (1966), were verified and are summarized here.

A surface potential change can be recorded up to about 4 mm from a site of stimulation, but only within a beam of points about 0·5 mm wide in line along the folium with the stimulus site. The potential change recorded at the surface is a mainly negative wave, preceded at short recording

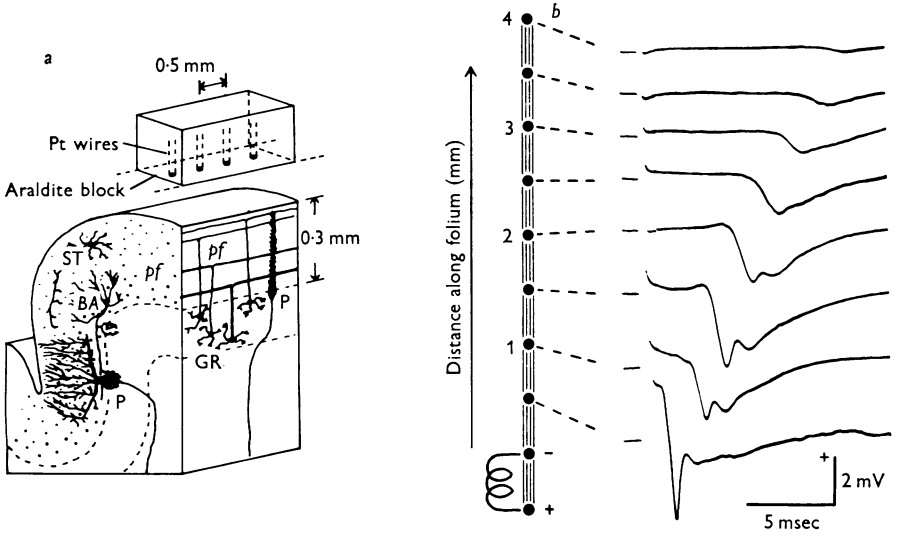

Fig. 1. *a*. Diagrammatic drawing of some structures of the cerebellar cortex, with part of the block of electrodes shown raised off the cerebellar surface. P, Purkinje cells; GR, granule cells; ST, stellate cell; BA, basket cell; pf, parallel fibres. *b*. Responses to stimuli of 20 V, recorded with electrodes at each of the indicated distances along the folium from the site of stimulation. The oscilloscope time base was started in each case 1 msec before the stimulus. Retouched records from the oscilloscope photographs in Fig. 4*a*.

distances by a small brief positive component (Fig. 1*b*). The negative wave recorded up to about 2·5 mm from the stimulus site contains two peaks, an early sharp peak with a duration of about 2 msec, and a later slow peak lasting about 10 msec. At distances larger than 2·5–3 mm the wave form does not generally show two separate peaks.

The times to both the onset and the peak of the first negative wave increase linearly with the distance from the stimulus site, corresponding to a propagation velocity of 0·3–0·4 m/sec. Response amplitudes up to about 10 mV can be recorded at short distances, falling steadily to zero at about 4·5 mm.

The threshold and amplitude of a second response

The response to the second of two equal stimuli shows potentiation of the second component, with little change in the size of the first component (Eccles *et al.* 1966). In the experiments described here, it was found that when the first shock was larger than the second, both components of the second (or 'test') response were potentiated. The threshold for a second response was reduced (Fig. 2*a*), by up to 40% at 22 msec after the conditioning stimulus.

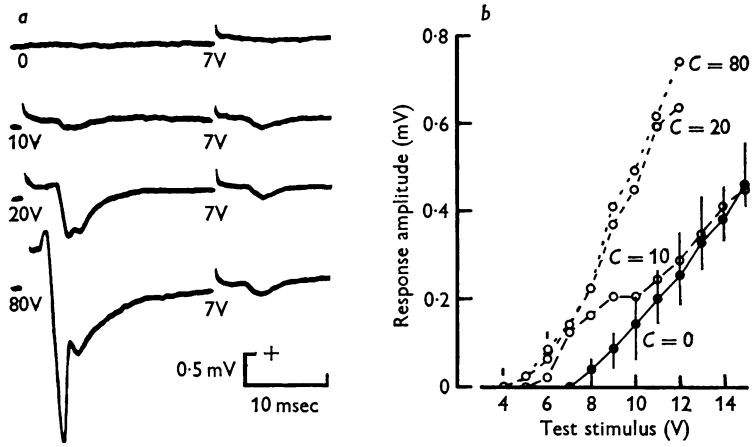

Fig. 2. *a*. The effect of conditioning stimulation on the responses to a subthreshold stimulus. A constant (7 V) stimulus was presented with no prior stimulation, and with stimuli of 10, 20 and 80 V, 22 msec before. The recording electrode was 1·5 mm from the stimulus cathode. *b*. The total amplitude of the negative wave plotted against the stimulus voltage, for test stimuli presented alone (continuous line) and 22 msec after conditioning stimuli having the various indicated strengths (dashed lines; stimulus strengths in volts). Values for $C = 0$ are the averages of four measurements repeated during the experiment, with the total range indicated by vertical lines. Same experiment as *a*.

The threshold was measured by plotting the response amplitude as a function of stimulus strength. The effects of various strengths of conditioning stimulation on such plots are shown in Fig. 2*b*. The conditioning stimuli were given 22 msec before the test stimuli. For each conditioning strength a single set of responses was recorded as the test strength was

gradually increased. Alternate sequences were taken with the conditioning strength zero ($C = 0$). The plot for $C = 0$ shows the average of the four values obtained, with the total range of values indicated by the vertical bars. The responses to stimuli near threshold cannot generally be separated into a first and second component (Fig. 2*a*), so the amplitude from the base line to the peak of the total response was measured. A conditioning stimulus only just above threshold always produced almost as big a reduction of threshold as larger stimuli, though the corresponding potentiation was seen only for the small range of stimuli less than the conditioning strength. This can be seen for the 10 V conditioning stimulus in Fig. 2*b*, for which the stimulus–response curve has a double knee shape.

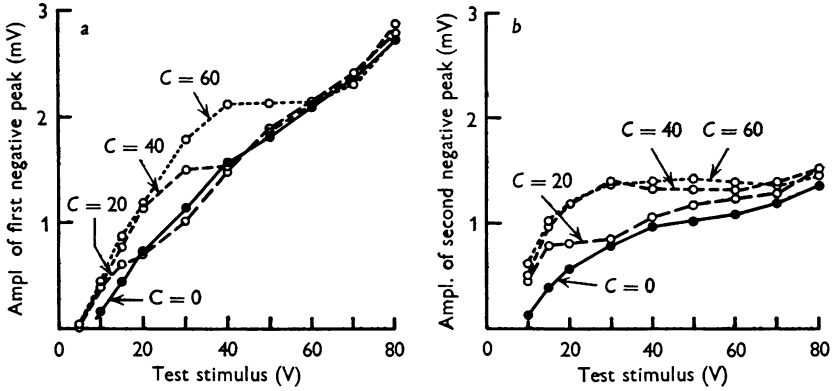

Fig. 3. *a*. The amplitudes of the first negative peak of the responses to test stimuli given 22 msec after conditioning stimuli of various strengths. The amplitudes are plotted as functions of the test stimulus strength for various fixed conditioning strengths (labelled in volts). The recording electrode was 1 mm from the stimulus cathode. *b*. The amplitudes of the second negative peak of the responses plotted as in *a* from the same set of responses.

When the stimulus–response plots are continued to strengths well above threshold, it can be seen that for all amplitudes of conditioning stimulation the curve shows the same characteristic double knee shape (Fig. 3*a*). The height of the first negative peak from the base line is plotted in Fig. 3*a*, with responses recorded 1 mm from the stimulus site. With each conditioning strength there was potentiation only when the test shock was weaker than the conditioning shock. The curves are of the form which would be expected if only active fibres have their threshold reduced after conditioning (see Discussion).

Stimulus–response curves as in Fig. 3*a* were found in each of two experiments in which recording was 1 mm from the site of stimulation. In two further experiments in which recording was at distances of 2 and 3 mm the

same characteristic shape of the curves was seen, but the conditioned curves were displaced upwards. Thus some potentiation was present even with equal shocks. In these experiments the measurements of the first peak could not have been independent of the process underlying the late component of the responses, since at the larger distances the second peak was not distinct from the first peak (Fig. 1*b*).

The potentiation of the second component of the responses has quite different input–output characteristics from that of the first peak. Fig. 3*b* shows measurements of the heights of the second peak plotted in the same way as Fig. 3*a* from the same set of responses. A double knee is still present in those curves for which there were low conditioning strengths. But there was potentiation with equal shocks, and at large stimulus strengths the second component of the responses reached a saturation amplitude.

The reduction of threshold cannot be attributed simply to a property of the stimulators used, or to a local physical or chemical change at the site of the electrodes used for conditioning. First, there was no detectable influence of a prior conditioning pulse on the stimulating current, when this was measured. Secondly, a threshold reduction was still found when different pairs of electrodes were used for conditioning and testing. In two experiments the stimulating sites were 3 mm apart, and the recording electrode was half way in between. Stimulus–response plots (as in Fig. 2*b*) showed a reduction of threshold of more than 15%. Thus possible local changes cannot wholly account for the threshold reduction.

The time course of the threshold reduction after a single shock was studied in two animals, by measuring the response to a test shock presented at the same electrodes at a strength only just above the unconditioned threshold. After a conditioning shock, such a response may be several times larger than normal as a result of the threshold reduction. This provides a simple way of measuring the time course of the threshold reduction. Potentiation of the response was maximal between 10 and 30 msec, and was still present up to about 100 msec. These results are illustrated (Fig. 5*c*) together with the time course of the latency reduction to be described in the next section.

The propagation velocity of a second response

Fig. 1*b* shows how the latency of a single response increased with the recording distance. In Fig. 4*a* the responses to two equal shocks given 22 msec apart are superimposed for each recording distance. The response to the second shock is in each case marked with an arrow. Responses at different distances were recorded by connecting the recording amplifier to each of the platinum wires in turn. In this way no electrodes had to be moved, and during the 10 min or so needed for a set of recordings there was

always complete reproducibility of the responses when recording from any one electrode was repeated.

It can be seen from Fig. 4*a* that at each recording distance the latency of the response to a second shock was reduced, and that the latency reduction was larger at large distances. In Fig. 4*b* the times to the onset of the negative wave in the first and second responses, and also the difference between the two, are plotted as functions of the recording distance for the data of Fig. 4*a*. For each set of points a linear regression line is drawn. The propagation velocities of the first and second responses were calculated as the

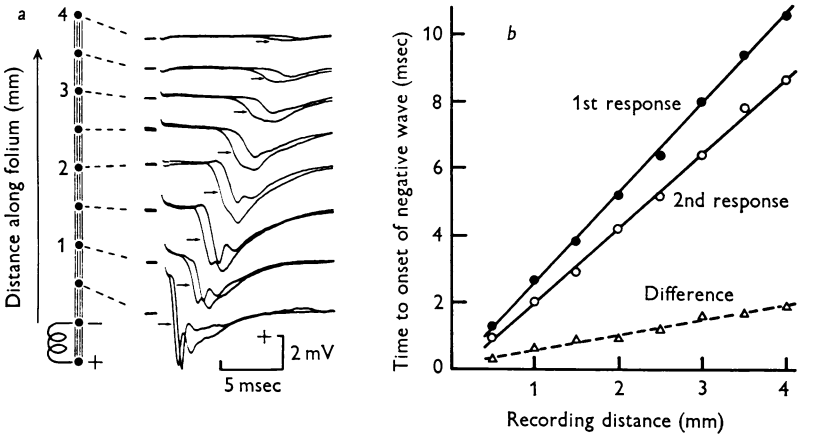

Fig. 4. *a*. The responses to two equal stimuli of 20 V (22 msec apart) superimposed on the oscilloscope, with the recording electrode at the various indicated distances along the folium from the stimulus site. The time base started 1 msec before the stimulus each time. Arrows mark the response to the second of the two stimuli in each superimposed pair. *b*. The times from the stimulus to the onset of the large negative wave in the first and second responses of *a*, together with the difference between the two times, plotted as functions of the recording distance.

reciprocals of the gradients of the first two plots. The two velocities were 0·374 and 0·450 m/sec and the increase from the first to the second was thus 20·3 %. The plot of the difference between the two times shows that the latency reduction increased linearly with distance, up to about 2 msec. Measurements of the propagation velocities were made in six animals, always using at least three recording distances spaced over a range of at least 2 mm. The mean velocity for the first response was $0{\cdot}356 \pm 0{\cdot}021$ m/sec, for the second response was $0{\cdot}422 \pm 0{\cdot}027$ m/sec, and the mean percentage increase in velocity from the first to the second response was $18{\cdot}5 \pm 1{\cdot}4$ %. The standard error of the mean is given along with each mean value.

The time to onset of a single response can be reduced by increasing the

stimulus strength. Fig. 5*a* shows the times to onset for the responses to the first and second of two equal shocks, plotted as a function of the stimulus strength. The results are plotted for three recording distances. At the lowest shock strength used (10 V) the responses were large enough to allow a latency measurement only at the shortest recording distance. It can be seen from Fig. 5*a* that the amount by which the latency fell as the stimulus strength was increased was the same for each recording distance, and the same for the first and second responses.

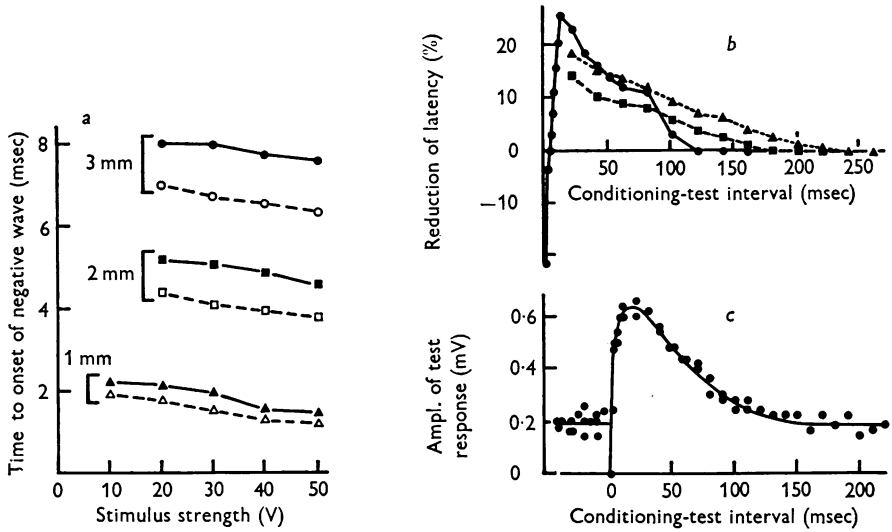

Fig. 5. *a*. The times to the onset of the large negative wave in the responses to two equal stimuli 22 msec apart, plotted as functions of the stimulus strength for the first responses (filled symbols) and second responses (open symbols) at the various indicated recording distances. *b*. The percentage reduction of the time to the onset of the second response when two equal stimuli were given at various intervals apart, in three different animals. *c*. The amplitude of the responses produced by a near threshold test stimulus (13 V) when presented at various intervals before and after a larger conditioning stimulus (40 V).

Fig. 5*a* shows that not only did large shocks fail to recruit faster fibres to contribute to the first response, but that the size of the increase of propagation velocity from the first to the second response was just as large with pairs of weak shocks as with pairs of strong shocks. The latency of the response to a weak stimulus, only just above threshold, was in fact found to be as much reduced by an equal conditioning shock as by a much larger conditioning shock.

The reduction in latency of a test response was also seen when conditioning and test shocks were given at different sites 3 mm apart, with the recording electrode half way in between.

The time course of the reduction of latency of a second response was studied in three animals, by recording responses to two equal shocks presented at the same site at various intervals. The reduction of the time to onset of the second response was expressed as a percentage of the time to onset of the first response, and is plotted for each animal in Fig. 5*b*. The after-effect can be seen to last for at least 100 msec.

The recovery of excitability at a distance

In the experiments described in this section the conduction velocity was measured by a collision technique, which does not require an assumption about the time relation between the propagating activity and the evoked potential.

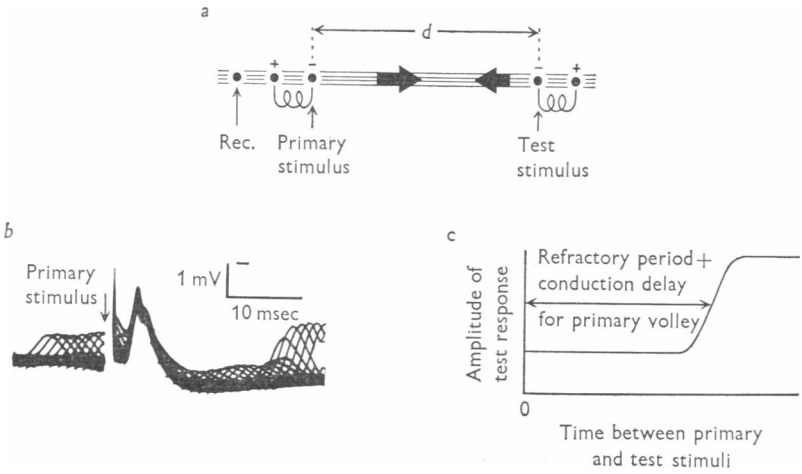

Fig. 6. *a*. Diagram showing the arrangement of electrodes for studying the recovery of excitability of the fibres at a distance from the site of the primary stimulus. *b*. A set of superimposed responses, with the test stimulus given 3 mm from the primary stimulus (d = 3 mm, in Fig. 6*a*) at various intervals (in 1 msec steps) before and after the primary stimulus. The recording site was 4 mm from the test stimulus site. *c*. A diagrammatic plot showing how a value for the sum of the conduction delay and refractory period of the primary volley is derived from results such as those of *b*.

A stimulus was given to initiate the propagating activity for which the conduction velocity was to be measured. This is called the 'primary' stimulus (Fig. 6*a*). A second ('test') stimulus was then delivered at a distance along the folium, and the time interval which had to elapse before this second stimulus could produce a full sized response propagating in the reverse direction was measured. This interval was taken to be equal to the time required for the primary volley to propagate to the test site, plus the refractory period of the fibres (Fig. 6*c*).

Fig. 6*b* shows a set of superimposed responses, in which the test stimulus was given at various intervals before and after the primary stimulus. The test responses recorded before the primary stimulus were, of course, unaffected by it and provide a 'control' amplitude. Responses to stimuli shortly before and shortly after the primary stimulus were reduced, and then rose sharply to a level above the control level. The depression was attributed to collision of impulses, and the increase above control level was

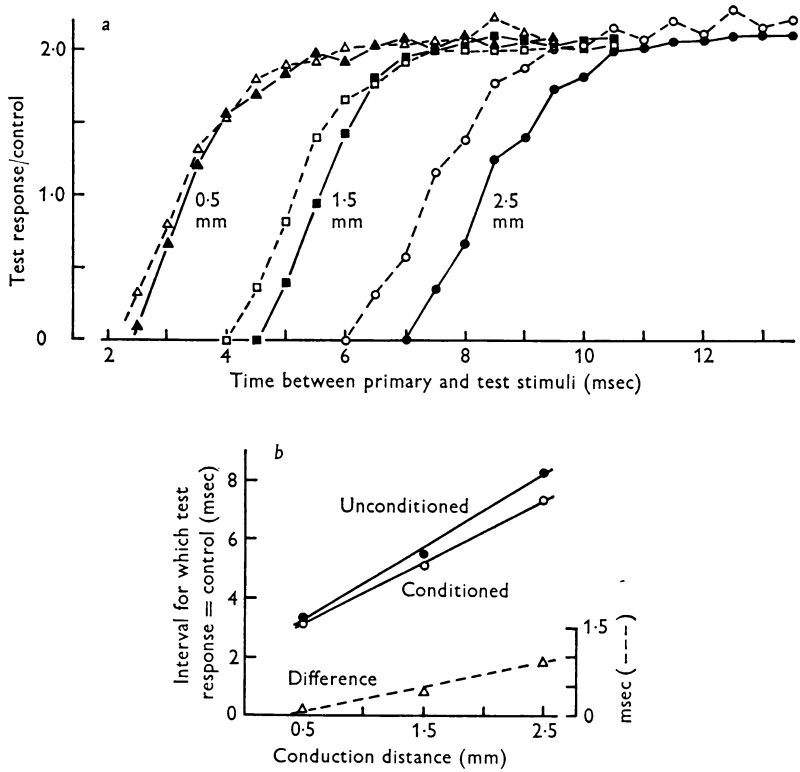

Fig. 7. *a*. The amplitude of the responses to 30 V test stimuli, expressed as a fraction of the control amplitude (obtained with no primary stimuli), and plotted against the interval between the primary and test stimuli. The primary stimulus (40 V) either was single (continuous lines and filled symbols) or was the second of two equal shocks 20 msec apart (dashed lines and open symbols). The recording point was 3·5 mm from the test stimulus cathode. The pair of electrodes used for the primary stimulus was varied to give conduction distances (*d* in Fig. 6*a*) of 0·5 mm (triangles), 1·5 mm (squares) and 2·5 mm (circles). *b*. The intervals in *a* which gave responses equal to the control amplitude are plotted as functions of the conduction distance. The values are plotted for the single primary stimulus (filled circles) and for the conditioned primary stimulus (open circles). The differences between the times under the two conditions are plotted (dashed line) at twice the vertical scale.

attributed partly to the lowering of threshold after propagation of the primary volley, and partly to the potentiation of the second component of the evoked potential (which at this recording distance (4 mm) was not distinct from the first component). This interpretation is assumed in the analysis of the more complex results in Fig. 7.

Fig. 7 shows the results of an experiment in which the time of the recovery of excitability was measured at three conduction distances (*d* in Fig. 6*a*), and for both a single primary stimulus and a primary stimulus preceded by an equal conditioning stimulus 20 msec earlier at the same site. The primary stimulus was sufficiently large to depress the test responses completely.

Fig. 7*a* shows the amplitude of the test response, expressed as a fraction of the control value, plotted against the interval between the primary and test stimuli. At each conduction distance the curve was displaced to the left when the primary stimulus had been preceded by a conditioning stimulus (dashed lines), by an amount which increased with the conduction distance. The times at which a test stimulus could produce a response equal in amplitude to the control value are plotted against conduction distance in Fig. 7*b*, for the single and conditioned primary stimuli. The gradients of these plots give conduction velocities of 0·402 and 0·478 m/sec for the unconditioned and conditioned primary volleys. This represents an increase of 18·8 % from a first to a second volley. Each of these values is within the range of values inferred in the last section from measurements of the propagation velocities of the responses.

In deriving the points for Fig. 7*b* the times were used at which a test response became equal to the control amplitude. Any other fixed amplitude could equally well have been chosen. But the fact that the curves of Fig. 7*a* are nearly parallel shows that the results are hardly affected by this arbitrary choice. It indicates that there was little spread of conduction velocities within a single volley, both with and without conditioning.

DISCUSSION

The structures underlying the responses

The responses described in this paper can be recorded only along a narrow beam parallel to a cerebellar folium. They are therefore almost certainly propagated by the parallel fibres (Eccles, Ito & Szentagothai, 1967). Since there are many other elements in the cerebellar cortex, however, one cannot infer that the measured electrical threshold is the threshold of the parallel fibres, or that the recorded responses are recorded directly from the parallel fibres.

In the collision experiments (Figs. 6 and 7) the parallel fibres must have

been directly activated by the stimuli, for in these experiments impulses are propagated in opposite directions from widely separated sites in the same fibres. But even though direct activation can occur, the lowest threshold mechanism for activating the parallel fibres might involve the granule cells or the proximal part of their axons. Involvement of mossy fibres is ruled out, because activation of them produces a response which propagates fast in all directions over the folium (Eccles *et al.* 1966).

It has been argued by Eccles *et al.* (1966) that the first component of the recorded responses is the action potential of the parallel fibres, and that the second component is a post-synaptic potential. This interpretation may be correct; but since none of the arguments on which it is based is conclusive, it will not be assumed in this paper. The arguments of this discussion are intended to take account of the possibilities that each component of the responses may be either presynaptic or post-synaptic.

The change of threshold after conditioning

The threshold was reduced as much by near-threshold conditioning stimuli as by large conditioning stimuli (Fig. 2*b*). This is evidence against there being any contribution to the threshold reduction from a local physical or chemical change at the electrodes. It also shows that recruitment of extra fibres in the conditioning activity did not affect the threshold of the lowest-threshold fibres. The mechanism must be one by which the less excitable fibres do not influence the threshold of the more excitable fibres. This conclusion is supported by the characteristics of the potentiation following conditioning (discussed in the next section).

These results would be explained if the threshold of any one parallel fibre is reduced only when it has been active. This interpretation seems the most likely to be correct for these experiments, but it does not follow that the restriction of the after-effects to active fibres is necessarily true also under physiological conditions of activation. With electrical stimulation different adjacent fibres may tend to be activated together, especially perhaps those fibres which run together in a bundle (see e.g. Fox, Hillman, Siegesmund & Dutta, 1967). If, for example, the after-effects are a result of changes in the extracellular environment of the fibres, then under physiological conditions this would be a mechanism by which one active fibre could affect adjacent inactive fibres almost as much as itself. But with electrical stimulation an active fibre might be nearly always surrounded by active fibres, and an inactive fibre by inactive ones. Thus changes in the extracellular environment might locally be practically all-or-none. If this hypothesis is true, then the supernormal after-effects in a fibre which is active under physiological conditions may be less than has been found in these experiments.

The potentiation after conditioning

The potentiation of the second component of the responses occurred with two equal shocks, and may be attributable to synaptic potentiation (Eccles *et al.* 1966). Its characteristics are not directly relevant to discussion of the supernormal period.

Both the first and the second components of the responses were potentiated when the test stimulus strength was below the conditioning strength. When the test stimulus strength was varied, the stimulus–response curves for the first peak showed a characteristic double knee shape, with a plateau below the level of the conditioning strength. At a short conduction distance (1 mm) there was no potentiation with equal shocks (Fig. 3*a*). At larger conduction distances some potentiation was found with equal shocks, perhaps attributable to a contribution from the second component or to increased synchrony of the activity in individual fibres.

The characteristics of the potentiation of the first peak can be explained if the thresholds of only those fibres which were active during conditioning were reduced after conditioning. A test shock weaker than the conditioning shock would then have been able to recruit all the fibres active during conditioning, and further increases in test strength would not have recruited more fibres until the test strength had been exceeded.

The increased propagation velocity after conditioning

It is possible that the recorded responses might be post-synaptic in origin. But even if this is so, the reduction of latency of the responses to a second shock cannot be attributed to a reduction in a synaptic delay. The latency reduction increased linearly with the conduction distance (Fig. 4), and reached values of almost 2 msec. Furthermore, the results of experiments in which the recovery of excitability at a distance was studied show conclusively that there was an increase in the speed of the propagation process.

Could new parallel fibres with a higher conduction velocity have been recruited by a second shock? Three considerations make this unlikely. First, the early component of a second response would always be expected to be potentiated if its reduced latency were to be explained by recruitment of extra fibres, whereas with equal shocks there was no potentiation at short recording distances (Fig. 4*a*). Secondly, it was not possible to recruit faster conducting fibres by using larger single shocks, though even small shocks after conditioning produced faster conduction (Fig. 5*a*). Thus, any hypothetical faster fibres must somehow have been unavailable for single shock activation. Thirdly, the results of the collision experiments

show that if a faster population of fibres was recruited, then the slow fibres must have dropped out of the second volley.

It must be concluded that a second shock can activate the same fibres which were active after a first shock, and that these fibres then conduct faster.

Are the parallel fibres different from other fibres?

Supernormal periods have been described in various peripheral nerve fibres. Mammalian C-fibres are in size and conduction velocity the most similar of these to the parallel fibres. In C-fibres of the hypogastric nerve of the cat there is an increase in excitability of up to about 30 %, occurring between 14 and 60 msec after the conditioning stimulus (Grundfest & Gasser, 1938). This change in excitability has the same time course as the depolarizing after-potential, which is probably the result of changes in external potassium concentration (Greengard & Straub, 1958). Grundfest & Gasser (1938) found no depolarizing after-potential and no supernormality in C-fibres of the saphenous nerve.

There do not seem to be any descriptions of changes in conduction velocity during supernormality in mammalian C-fibres. Increases of 10–20 %, equal to those described in the present paper, have been seen after single action potentials in the lateral giant fibres of the earthworm (Bullock, 1951). Smaller increases after single conditioning shocks have been seen in frog sciatic nerve fibres (Graham, 1934; Bullock, 1951), although there may be no increase at all when sciatic nerves are freshly excised or kept in good condition (Graham & Lorente de Nó, 1938). A constancy of conduction velocity to within 1 or 2 % seems to be the rule in most fibres, apart from a brief subnormal period associated with the relative refractory period. The supernormal period of the parallel fibres therefore requires some special explanation.

The parallel fibres are unmyelinated, and have diameters ranging from 0·2 to 1·0 μ, with the larger fibres deeper in the molecular layer (Fox & Barnard, 1957). The average diameter is probably much closer to 0·2 μ than to 1·0 μ. The fibres dilate every few microns along their course to form synaptic contacts. The dilated portions are typically 0·5–1·0 μ in diameter (Fox *et al.* 1967).

The packing density of the parallel fibres is very high. Fox & Barnard (1957) arrived at figures which correspond to an average of about 3·2 fibres per square micron crossing through the dendrites of the Purkinje cells. Within the parallel fibre bundles the densities will be higher; and rough measurements from published electron micrographs of cross-sections of parallel fibre bundles (Palay *et al.* 1962; Fox *et al.* 1967; Gobel, 1968) indicate packing densities of 10–20 fibres per square micron. Similar rough

measurements made from published electron micrographs of unmyelinated fibres in the rabbit vagus nerve (Keynes & Ritchie, 1965) and in the cat sural and hypogastric nerves (Gasser, 1955) give packing densities of 1·5–2·5 fibres per square micron within bundles. Thus the parallel fibres are considerably more densely packed than mammalian peripheral C-fibre bundles.

The small size of the parallel fibres, their high packing density and their numerous synaptic enlargements make them exceptional in the nervous system. But there is not sufficient evidence to say which, if any, of these properties accounts for the extreme supernormal period. Perhaps the most likely hypothesis is that as a result of their close packing there is a large change in the extracellular environment of the fibres after an action potential.

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