J. Physiol. (1966), **187**, *pp.* 553–574
With 2 *plates and* 11 *text-figures*
Printed in Great Britain

RESPONSES IN THE INFERIOR OLIVE TO STIMULATION OF THE CEREBELLAR AND CEREBRAL CORTICES IN THE CAT

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(*Received* 2 *May* 1966)

SUMMARY

1. Extracellular field potentials and single unit responses have been recorded from the inferior olive of the cat following stimulation of the surface of the contralateral paramedian lobule of the cerebellum, and of the ipsilateral cerebral cortex.

2. Cerebellar stimulation results in antidromic invasion of inferior olivary neurones via the climbing fibres. These responses are followed by synaptic discharges which may be generated through climbing fibre recurrent collaterals.

3. Precise histological controls have shown that these responses to stimulation of the paramedian lobule are located in the ventral lamella of the principal olive.

4. Unifocal stimulation of the sensori-motor cortex with surface-anodal pulses evokes synaptically generated discharges of neurones in the ventral lamella, with a latency of 8–9 msec. The area of cortex yielding responses has been mapped at chosen stimulus intensities and the limitations of the maps have been discussed.

5. It has been shown that the initial excitatory responses obtained from either cortex are followed by an inhibition which lasts about 100 msec, and gives way to a period of recovery or facilitation. This, in turn, is succeeded by a further period of inhibition. Possible neural substrates for these changes have been discussed.

INTRODUCTION

Anatomical studies have disclosed two routes by which the cerebellum might be activated from the cerebral cortex; that via the pontine nuclei and that via the inferior olive. The most detailed descriptions of these pathways have been provided by papers from the Oslo school (Brodal, 1940; Brodal & Jansen, 1946; Nyby & Jansen, 1951; Walberg, 1956). The

ponto-cerebellar fibres are known to end in the cerebellar cortex as mossy fibres (e.g. Snider, 1936) while the axons of the olivary neurones have more recently been shown to terminate as climbing fibres (Szentágothai & Rajkovits, 1959). Physiological investigations of cerebro-cerebellar connexions have shown that evoked potentials may be recorded from the surface of the cerebellum after single electrical stimuli to certain areas of cerebral cortex (Dow, 1939, 1942; Curtis, 1940; Adrian, 1943; Hampson, 1949; Snider & Eldred, 1951; Albe-Fessard & Szabo, 1954, 1955; Szabo & Albe-Fessard, 1954; Jansen, 1957). Jansen (1957) was able to distinguish two components of the response; an early component (latency 2–6 msec) which he attributed to activation via mossy fibres, and a later component (latency 12–25 msec) which, in a later investigation (Jansen & Fangel, 1961), was shown to be associated with 'inactivation responses' (Granit & Phillips, 1956) in the Purkinje cells. Jansen & Fangel suggested that inactivation responses were due to excitation via the climbing fibres and more recent evidence has given very strong support to this interpretation (Eccles, Llinás & Sasaki, 1966). It would have been tempting to conclude that the late component of the cerebellar response was relayed through the inferior olive, but Jansen & Fangel thought that this was unlikely as the late component survived procedures designed to interrupt the olivo-cerebellar fibres (Jansen, 1957).

The present study is limited to the cortico-olivo-cerebellar pathway and we have recorded from the inferior olive with micro-electrodes. The observation of Granit & Phillips (1957) that stimulation of the cerebellar surface gave rise in Purkinje cells to 'D potentials', since shown to be climbing fibre responses (Eccles *et al.* 1966), suggested that climbing fibres could be excited by stimulating the pia-covered cerebellar surface; hence inferior olive cells could be positively identified by the criterion of antidromic invasion: this proved to be the case. In addition this technique allowed us to make some observations on the problem of the climbing fibre recurrent collaterals which have been postulated on physiological grounds (Eccles *et al.* 1966; Ochi, 1965) though there is no anatomical evidence for their existence.

We have attempted to record activity in the ventral lamella of the principal olive because this is a main site of termination of the direct projection from the ipsilateral cerebral cortex. The cortico-olivary fibres arise mainly in the 'motor' area and are believed to descend in the pyramidal tract (Walberg, 1956). The neurones of the ventral lamella in turn send their axons to the contralateral paramedian lobule (Brodal, 1940) and we have therefore stimulated this cerebellar subdivision in order to activate the cells of the ventral lamella antidromically. Descending volleys have been produced in the cortico-olivary fibres by unifocal

stimulation of the 'motor' cortex with surface-anodal current pulses, which is a method of stimulation whose effects on cortical efferents have been very fully investigated (Hern, Phillips & Porter, 1962; Hern, Landgren, Phillips & Porter, 1962; Landau, Bishop & Clare, 1965). In some experiments we have used the method of Thomas & Wilson (1965) to establish the position of the micro-electrode tip.

METHODS

The experiments were performed on twenty-one cats weighing between 2·3 and 4·3 kg. Initial anaesthesia was secured by intraperitoneal injection of 30–40 mg/kg pentobarbitone (Nembutal, Abbott Laboratories) and supplementary doses of anaesthetic were given intravenously as required.

In order to allow both micro-electrode exploration of the medulla from the ventral surface and stimulation of the cerebral and cerebellar cortices, the animals were fixed in a head-holder (David Kopf Instruments) which was rotated so that the ear bars were approximately vertical, with the left side of the head uppermost. The animal was also supported by clamps on vertebral spine T1 and the iliac crests. Craniotomies were made and the underlying dura was reflected to expose the right paramedian lobule of the cerebellum and the pericruciate area of the left cerebral hemisphere. A bifocal stimulating electrode consisting of a pair of chlorided silver balls 2 mm apart and mounted on fine silver wires, was applied to the paramedian lobule. A Perspex 'top hat' (Hern, Landgren, Phillips & Porter, 1962) was tied into the scalp opening over the cerebral cortex and filled with warm mineral oil. The cerebral cortex was stimulated unifocally through a chlorided silver ball electrode, the location of which was plotted on an enlarged photograph of the exposed area. The electrode was made positive with respect to a diffuse electrode which was sewn beneath the scalp or inserted into the mouth of the animal.

The base of the skull was exposed by reflecting the pharynx forward and was opened to expose the ventral surface of the medulla. The dura was reflected and a small area of pia dissected off over the left olive to facilitate entry of the micro-electrode. The brain was stabilized by a Perspex pressor plate with a central pore through which the micro-electrode passed. The pressure exerted on the surface of the brain was never sufficient to halt the circulation in the pial vessels which could be seen through the transparent plate with the help of a binocular microscope.

Stimuli were either rectangular pulses of 0·2 msec duration from a Grass stimulator, or condenser discharges of total duration about 0·2 msec and were delivered through isolation transformers. Stimulus current was monitored by observing the voltage drop across a 100 Ω resistor in series with the anode of the stimulating circuit.

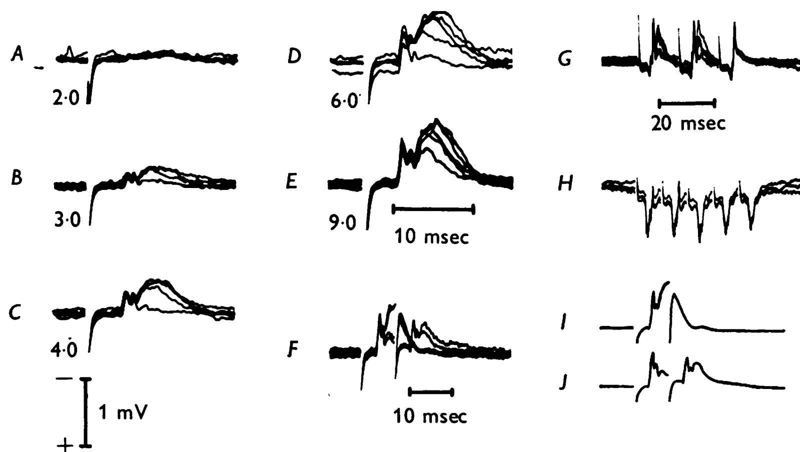
Glass micro-electrodes filled with 4 M-NaCl (DC resistance 1–10 MΩ) were used in most of the experiments. They were mounted on a three-dimensional micromanipulator angled so that the micro-electrode entered the brain stem at right angles to its surface. In four experiments the electrodes were filled with a saturated solution of the dye Fast Green FCF (National Aniline Division, Allied Chemical & Dye Corp., N.Y.) in 2 M-NaCl (DC resistance 1–4 MΩ). At a number of points in the brain stem the position of the micro-electrode tip was marked after recording the responses: dye was ejected electrophoretically into the brain by making the electrode negative with respect to earth until a charge of $5–8 \times 10^{-3}$ C had been passed (usually 10–12 μA for 10 min) as described by Thomas & Wilson (1965).

The brain stems from these experiments were fixed in 10% formol-saline, embedded in paraffin, and 15 or 25 μ serial sections were cut. Those containing dye spots were counterstained with thionin so that the location of the spots could be determined.

RESULTS

Form of the olivary mass response to cerebellar stimulation

When the pia-covered surface of the right paramedian lobule of the cerebellum was stimulated with single brief current pulses delivered through a bifocal electrode, field potentials were found in the olivary region of the left side of the brain stem during exploration with NaCl-filled micro-electrodes of about 2 MΩ resistance.



Text-fig. 1. Mass responses in the inferior olive to cerebellar stimulation. *A–E*, To a single stimulus of duration 0·2 msec; stimulus intensities in mA below each record. *F*, To two stimuli 8·0 msec apart. *I* and *J* are line drawings prepared from *F*; see text for further explanation. *G*, To three stimuli at 66/sec. *H*, To five stimuli at 100/sec (positive-going responses from a lateral penetration in a preparation with a very small second component of the response). Repetition rate for all records: one every 2 sec. Voltage calibration applies to all records. Time calibration below *E* applies to *A–E*. Time calibration below *G* applies to *G* and *H*. Time calibration for *F*, *I*, *J* is below *F*.

Whether the field potentials were positive- or negative-going, two components of the response were recognizable in all but two animals. These are clearly visible for a negative-going response in Text-fig. 1*A–E*, a sequence which demonstrates the increase in response which accompanied increase in stimulus intensity. These records are atypical in that the early sharp deflexion has two peaks; more usually there was but a single peak. The later, more prolonged wave, as the records show, was more variable in amplitude than the first. In ten experiments the latency of the first wave ranged between 3·0 and 4·0 msec (mean 3·4 msec), while the delay between the onsets of the first and second waves varied in the same experiments between 1·4 and 2·4 msec (mean 1·9 msec).

As Text-fig. 1*G* shows, the two components of the response were readily dissociated by a short train of cerebellar stimuli, when with successive stimuli the second wave rapidly declined in amplitude though the first was was little affected. In two animals the field potentials showed little sign of a second component and in these preparations there was little diminution in the responses evoked by successive stimuli in the train (Text-fig. 1*H*) which suggests their identity with the first component of the more usual response. This identification was supported by the finding that under certain stimulus conditions a typical two component response could be elicited in these animals, as explained below in the final section of Results.

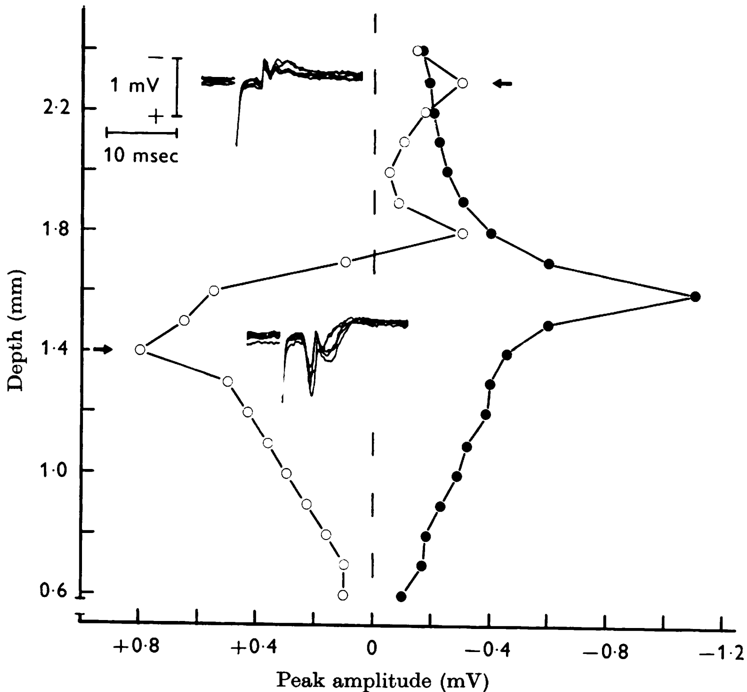
Though the two components of the field potential differed in their response to repetitive stimulation it was repeatedly found that they had the same threshold to a single stimulus which could be as small as 0·43 mA. Thus in Text-fig. 1*A* a stimulus of 2·0 mA to the paramedian lobule evoked just detectable olivary responses which nevertheless displayed both first and second components as may be seen by comparison with Text-fig. 1 *B*.

Location in medulla of olivary field potentials evoked by stimulation of contralateral paramedian lobule

The interrupted line in Pl. 1 encloses the area on the ventral surface of the medulla beneath which responses were encountered during penetrations approximately normal to the pial surface. The direction of micro-electrode advance is shown on Pl. 2, which is a photomicrograph of a thionin-stained transverse section through the inferior olive at the level indicated by the continuous line in Pl. 1.

For each of a number of micro-electrode tracks in the responsive area, a curve was constructed showing the amplitude of the first of the two components of the evoked potential plotted against depth (depth was always recorded during withdrawal of the electrode in an attempt to minimize errors due to deformation of the brain). In the medial part of the area the potentials were negative-going at all depths and reached their greatest size at a depth of about 1·5 mm as shown in Text-fig. 2, where the filled circles represent the depth profile of a typical response. Further from the mid line, under the olivary eminence and the lateral part of the pyramid, the depth profiles were of a different form. The potentials encountered were usually smaller than more medially and changed sign during the course of a penetration, being positive-going superficially, and negative-going at depths greater than 1·3–1·7 mm. Text-figure 2 (open circles) shows the usual form of the depth profile of the first component of the response, although the potentials encountered in this penetration were somewhat larger than usual.

The usual choice of stimulating site on the paramedian lobule was folium 4 or 5, but we sometimes stimulated 6 or 3 (numbered according to Szabo & Albe-Fessard, 1954). The responses recorded from the same site following stimulation of different folia sometimes differed in amplitude but were always similar in form. That the potentials were indeed evoked from the paramedian lobule and not through escape of the stimulus to other subdivisions of the cerebellar cortex was established in a number of experiments by shifting the stimulating electrode 2 or 3 mm to an adjacent



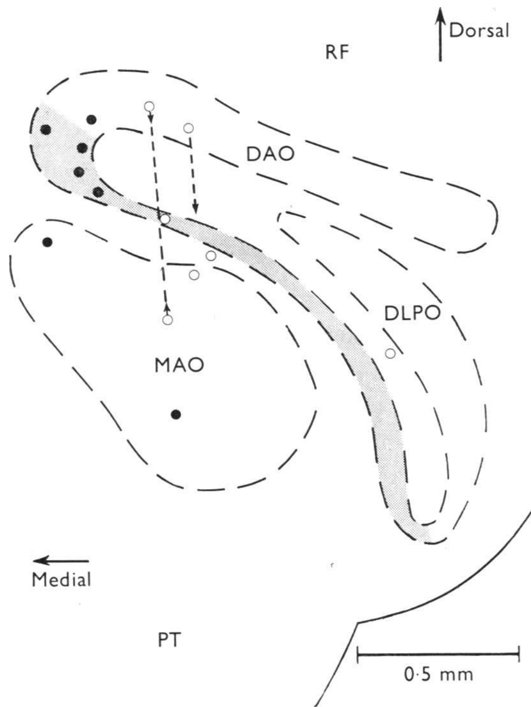
Text-fig. 2. Peak amplitude of first component of response to a single cerebellar stimulus plotted against depth below brain surface. Filled circles: typical profile for a medial penetration. Open circles: profile obtained in a more lateral penetration (see text). The inset sample responses were photographed during the lateral penetration at the depths indicated by the arrows. At these depths dye spots were also placed (see Text-fig. 3 and text). Time and voltage calibrations apply to both sample responses.

area of posterior lobe vermis while leaving the micro-electrode in the same position. This raised the threshold for evoking field potentials by a factor of three or more. The vermis of the posterior lobe receives its olivary projection from the caudal part of the medial accessory olive (Brodal, 1940) so that responses obtained while stimulating this region presumably arose through stimulus escape to the paramedian lobule. The results of this

control suggested that when the paramedian lobule was stimulated with rectangular pulses of 0·2 msec duration significant spread of current to other areas occurred only for intensities in excess of 4–5 mA.

Localization of the responses within the inferior olive

In four experiments the technique of Thomas & Wilson (1965) was used to mark positions of the micro-electrode tip at which mass responses were recorded. In our hands the method produced bright green spots only 15–30 μ in diameter, which were fairly easily found on examining serial sections



Text-fig. 3. Drawing of cross-section through olivary region of brain stem (from Pl. 2). Stippled area is ventral lamella of principal olive. DAO, Dorsal accessory olive; DLPO, dorsal lamella of principal olive; MAO, medial accessory olive; PT, pyramidal tract; RF, reticular formation. ●, Dye spots made at maximum of first component of cerebellar response when this was always negative. ○, Dye spots made in penetrations during which first component of cerebellar response changed sign (see text for further details).

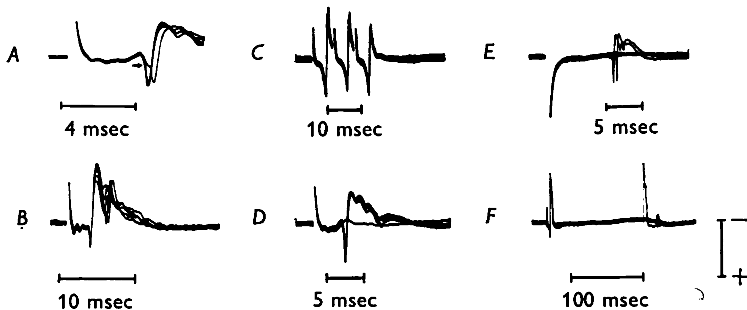
through the brain stem. Text-fig. 3 collects fourteen such spots from the four brains used. Spots represented by filled circles were made by ejecting at the depth of greatest response in penetrations during which the field potentials were at all times negative-going. In these positions unitary spikes were often evident, superimposed on the mass response. The spots

lie in or near the ventral lamella of the principal olive near its junction with the dorsal accessory olive, with the exception of one spot in the medial accessory olive for which we have no explanation.

Spots marked by open circles were made in tracks in which the field potentials underwent reversal of sign, and the four without arrows were placed at the depth where the first component of the response reversed. Of the three spots with arrows, the two joined by an interrupted line were made at different depths in the same electrode track, as shown in Text-fig. 2, and interpolation using the depth profile indicates that reversal took place in or near the ventral lamella of the principal olive. The remaining spot was placed approximately 300 μ deep to the reversal in another track, and so again the reversal occurred near the ventral lamella at the tip of the arrow. In summary, the positions of the open circles suggest that, in tracks in which the field potentials reversed sign, this change took place at the ventral lamella of the principal olive. In such penetrations also, unitary spikes were often visible in the reversal region.

Unit responses to cerebellar stimulation

Following a single stimulus to the paramedian lobule it was possible to record unitary spike responses superimposed on the olivary field potentials. With the micro-electrode close to an active unit, the extracellular spike was of the usual diphasic form with an initial positive phase. Quite commonly, the initial downstroke would show a marked inflexion on a few trials, as shown by the arrow in Text-fig. 4*A*. This inflexion probably



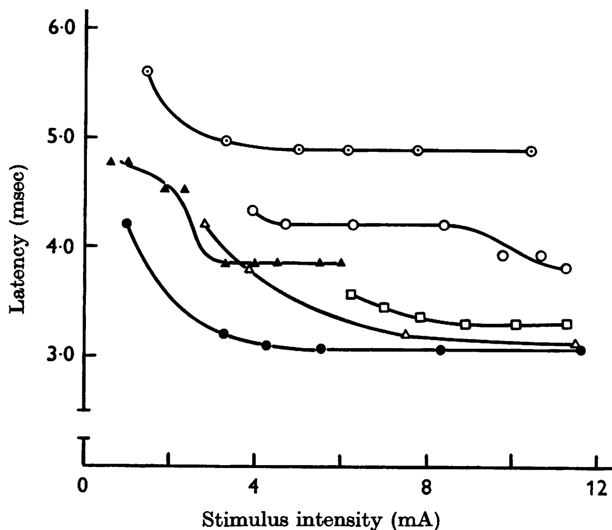
Text-fig. 4. Extracellular records from single units. Each record made up of five superimposed sweeps occurring one every 2 sec. *A*, Antidromic response to single cerebellar stimulus. Arrow indicates *A–B* transmission which is delayed on one sweep. *B*, Unit showing synaptically generated spike after antidromic spike. *C*, Antidromic response to three cerebellar stimuli at 150/sec. *D*, Unit showing ripples on falling phase of antidromic spike. Note complete failure of response on one sweep. *E*, Unit responding, in two out of five trials, to a cerebral stimulus of 2·5 mA. Note that two spikes (the second much smaller than the first) were fired in each successful trial. *F*, Unit responding to a single cerebellar stimulus with an antidromic spike and on one sweep with a late discharge 130 msec after the stimulus. Voltage calibration 2 mV for *A*, *B*, *D*, *E*, *F*; 1 mV for *C*.

signals a delay in transmission of the invading impulse as it passes from one area of cell membrane to another ('A–B transmission', see for example Phillips, 1959); in support of this interpretation, spikes were occasionally seen to truncate at the level of the inflexion. These observations suggest that we were recording from cell bodies rather than from fibres.

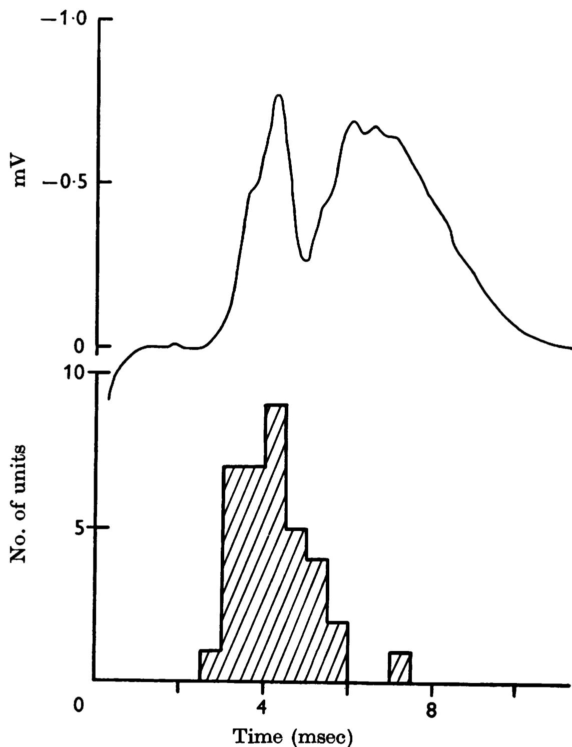
Extracellular records were obtained from a total of thirty-six units, each responding to a cerebellar stimulus with a spike which had a short invariant latency. This spike would fail to appear when a spontaneous discharge of the unit happened to occur shortly before the evoked spike was expected. When brief tetani were applied to the cerebellum some units responded to each stimulus in the train until the frequency exceeded 250/sec, though others would not follow above about 100/sec. Text-fig. 4*C* shows a unit responding to each of three stimuli at 150/sec. The maximum following-frequencies were not very high, but in view of the small diameter of the climbing fibres (1–5 μ, Szentágothai-Schimert, 1941) we have felt justified in regarding the spikes as being due to antidromic invasion of olivary neurones projecting to the paramedian lobule.

Though the latency of each spike response was constant at any one stimulus strength, there was some change when the strength was varied. This is shown for six representative units in Text-fig. 5. When the stimulus was increased from the just threshold value there was in all cases a graded decrease in spike latency and, as the figure shows, a plateau value was reached with strong stimuli. Some of the latency change can be explained as being due to shortening of utilization time as the stimulus was increased from the threshold value, but increases in intensity could also lead to excitation of the climbing fibres further from the terminals. In view of the slow conduction velocity of these fibres near their termination (Eccles *et al.* 1966), spread of the stimulus only 1 or 2 mm below the cerebellar surface could account for the observed decrease in latency of antidromic responses recorded in the olive. The existence of a latency plateau for each unit suggests that stimuli of a certain intensity spread to initiate impulses at a region of the fibre (perhaps the first node) which is especially excitable, so that impulses continue to be generated there even when the stimulus is further increased. A few units, one of which is illustrated in Text-fig. 5, showed a decrease in latency from the plateau value, but this only occurred with stimuli so strong that impulses may have been initiated deep in the cerebellar white matter.

The results of single-unit recording suggest that the first component of the olivary field potential signals the antidromic invasion of numbers of cells. Thus in Text-fig. 6 the latency and time course of this wave are seen to correspond well with the histogram of the antidromic spike latencies for the thirty-six single units from which we recorded. In construction of



Text-fig. 5. Effect of stimulus intensity on latency of antidromic response of six single olivary units to stimulation of contralateral paramedian lobule.



Text-fig. 6. Top: tracing of a typical mass response in olive to single cerebellar stimulus of intensity 2·8 mA. Bottom: histogram of plateau values (see text) of latency of antidromic response for the thirty-six units studied (bin width, ½ msec).

the histogram the latency chosen for each unit was the plateau value reached with strong stimuli. With the single exception of a unit with latency 7·2 msec, which nevertheless obeyed the criteria used to identify spikes as antidromic, the antidromic latencies lay between 2·8 and 5·5 msec.

Text-figure 6 gives no evidence as to the origin of the second component of the field potential except to show that antidromic spikes in the olivary cells could play only a small role in its production. There are, however, several indications that potential changes in the antidromically invaded units give rise to the wave. Thus the open circles in Text-fig. 7*A* show that the second component has the same distribution in depth as the first (filled circles). More direct evidence is offered by the finding that in single units the antidromic spike was often followed by one or more spikes which were slightly variable in latency and presumably arose as a result of synaptic excitation. In some units these spikes were quite well developed (Text-fig. 4*B*) but in others they were severely stunted. In thirteen cells showing a clear second spike it occurred 1·6–2·0 msec after the antidromic spike. In some cells the only indication of activity following the antidromic spike was the occurrence of rhythmic ripples with a period of 1·4–2·0 msec (Text-fig. 4*A* and *D*). Movements of the micro-electrode tip sometimes led to changes in recording conditions which showed that these ripples in fact represented a series of partially developed spikes. Text-figure 4*D* is interesting in that on one trace the stimulus failed to produce an antidromic spike and on this occasion the ripples were also absent. We were never able to evoke a synaptically generated spike in the absence of antidromic invasion, even when using units with a high antidromic threshold and stimuli which were just subthreshold for these cells but which fired many other units as judged from the field potential.

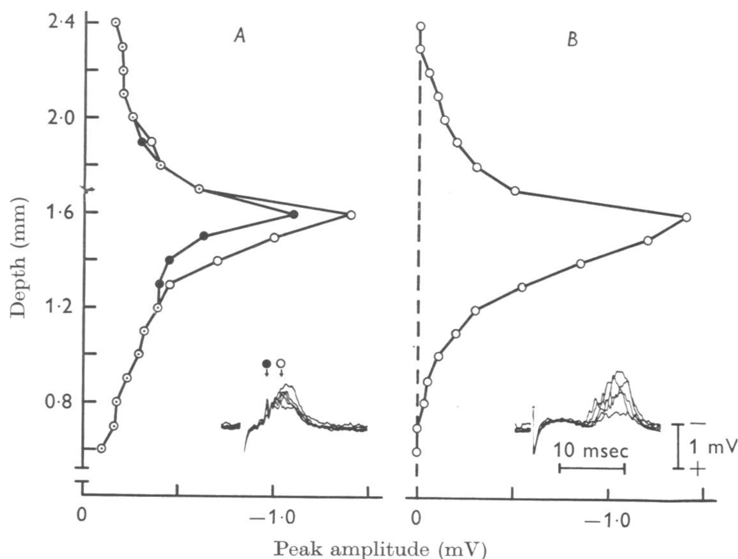
Though there was usually a large difference in amplitude between the first spike and subsequent spikes or ripples, we are confident that all were due to activity in the same unit: repetitive responses occurring spontaneously or in response to stimulation of the cerebral cortex were similar in form; the different components all increased in size together as the tip of the micro-electrode approached the unit, and the whole response would disappear if the electrode impaled the cell and destroyed it. The later spikes are reduced in size, presumably because of partial inactivation of the spike mechanism. The data presented here do not exclude the possibility that the repetitive discharge of the cells might be due to a depolarizing after-potential following the first spike (cf. Kandel & Spencer, 1961), but some intracellular records obtained from olivary neurones of the ventral lamella which were too depolarized to support spike activity showed undoubted excitatory post-synaptic potentials evoked by cerebellar stimulation.

Further support for the view that synaptically evoked spikes contribute to the second component of the field potential comes from interactions between responses to two cerebellar stimuli given at short intervals. Text-figure 1*F* shows the field potentials evoked by two cerebellar stimuli

8 msec apart. It can be seen that there are two distinct patterns of response which have been separately drawn in Text-fig. 1 *I* and *J*; when the synaptically evoked component of the response to the first stimulus is large, the response to the second stimulus is very small (*I*), but when the former is small the latter is well developed (*J*). More commonly, cases were seen which were intermediate between the two extremes shown. This effect was present for stimulus intervals up to about 15 msec, which suggests that it is largely due to collision of the second antidromic volley with orthodromic impulses in the climbing fibres. This in turn implies that the second component of the cerebellar field potential is made up, at least partially, of spikes generated synaptically in those cells which undergo antidromic invasion.

Olivary responses to stimulation of the ipsilateral cerebral cortex

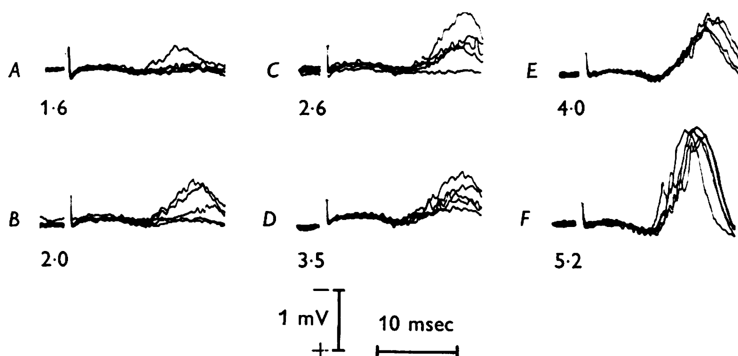
When responses to cerebellar stimulation were found, field potentials and unit spikes were also seen when the ipsilateral pericruciate cortex was stimulated by a focal anode delivering brief (0·2 msec) rectangular current pulses. Comparison of Text-figs. 7 *A* and *B* shows that, in medial tracks the distributions in depth of the fields evoked from the cerebral and cerebellar cortices were strikingly similar, which suggests that the fields were



Text-fig. 7. *A*, Peak amplitude of first (●) and second (○) components of response to a single cerebellar stimulus plotted against depth below brain surface. *B*, Peak amplitude of response to a single cerebral stimulus (4·0 mA, 0·2 msec) in the same penetration. Inset, sample responses photographed at depth 1·4 mm. Time and voltage calibrations apply to both records.

generated by activity in the same region. In lateral tracks the cerebral and cerebellar responses were of the same sign at all points, both potentials undergoing reversal at the same depth.

As illustrated in Text-fig. 8, the amplitude of the field potential evoked from the cerebral cortex increased with stimulus intensity, though at any one strength there were often considerable fluctuations in the size of the response. Text-figure 8 also shows that the latency of the response was not detectably affected by stimulus strength. In the records presented the latency was 8·3 msec and in our experiments the range of latencies encountered was from 8·3 to 9·4 msec.

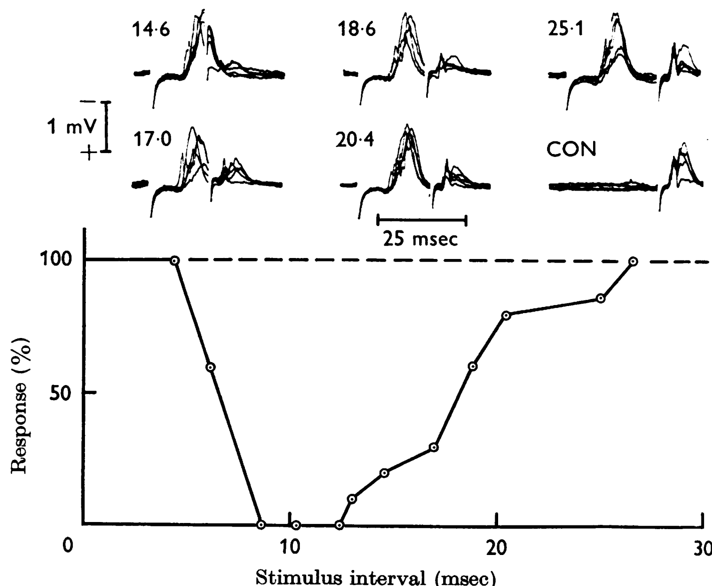


Text-fig. 8. Responses to single cerebral stimuli, duration 0·2 msec. Stimulus intensity in mA shown below each record. Each record comprises four or five superimposed sweeps. Stimulus repetition rate: one every 2 sec.

When a cerebral response preceded a cerebellar stimulus by a short interval, there was a depression of the antidromic component of the cerebellar response. Typical records showing this interaction, together with the complete time course of the effect as plotted from similar records, are shown in Text-fig. 9. The duration of this depression suggests strongly that it arose through the collision of impulses in the climbing fibres, and therefore that a single stimulus to the cerebral cortex was capable of discharging olivary neurones. Complete suppression of the antidromic field potential was sometimes observed and may be taken to indicate that all the units contributing to the antidromic wave were discharged by the cortical stimulus. Single unit studies confirmed that the field potential evoked from the cortex was made up, in part at least, of cell discharges. We recorded from many antidromically identified units which were fired by a single cortical shock. As with the unitary responses to cerebellar stimulation, those evoked from the cerebral cortex were usually multiple discharges (Text-fig. 4*E*); when two or more spikes were evoked the inter-spike intervals were between 1·3 and 2·0 msec. It is interesting that spon-

taneous discharges of the identified cells were also commonly multiple with similar interspike intervals.

The cortico-olivary projection arises mainly from the sensori-motor cortex (Walberg, 1956) and our cerebral exposures were therefore limited to the area shown in the line drawings of Text-fig. 10. These drawings were prepared by tracing from the photographs on which the position of the stimulating electrode was charted during the experiment. For several preparations, the position of the electrode was determined at which the threshold for evoking an olivary field potential was lowest. In each brain this point lay at the junction of the coronal and anterior sigmoid gyri; a

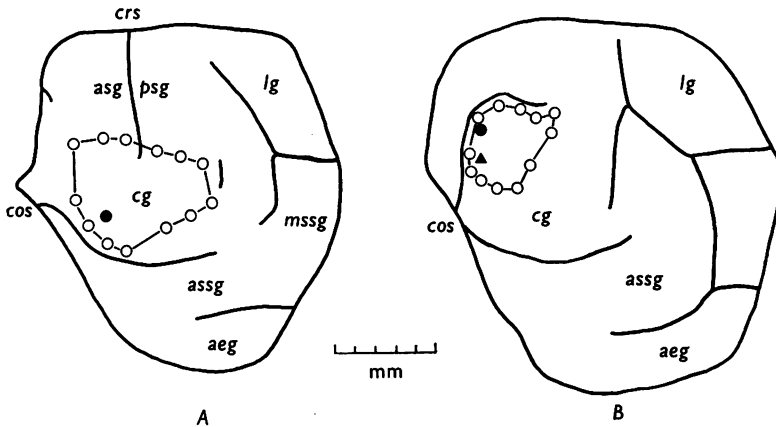


Text-fig. 9. Top: responses to a single cerebellar stimulus (3·9 mA) conditioned by a preceding response to a single cerebral stimulus (4·0 mA). Stimulus interval (msec) is indicated beside each record. CON, control response to the cerebellar stimulus. Bottom: size of antidromic component of cerebellar response plotted against stimulus interval.

typical result is shown by the filled circle in Text-fig. 10*A*. In four experiments we accurately measured the current required at the lowest threshold position to evoke the smallest olivary response which was detectable, using a technique of inspecting many successive traces displayed on a long-persistence oscilloscope screen at high amplification. The values obtained were 0·6, 0·5, 0·8 and 0·7 mA.

The open circles in Text-fig. 10*A* demarcate, for a typical experiment, the area outside which olivary responses could not be evoked with a stimulus intensity of 1·0 mA. Along the coronal sulcus, the responses at the

points marked by the circles were quite large, but no responses could be obtained from the other lip of the sulcus, while the circles along the other borders of the area mark points from which responses were obtained that were just detectable by the above technique. It is notable that the point from which the largest response to a 1·0 mA stimulus was obtained (which was also the lowest threshold point) is eccentrically placed within the effective area.



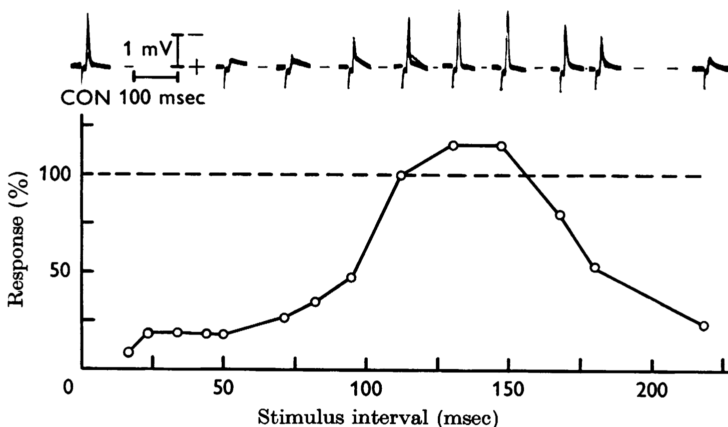
Text-fig. 10. Line drawings from photographs of the pericruciate area of the left cerebral cortex as exposed in two experiments. The mid line lies along the upper border of the exposed area in each case. *aeg*, Anterior ectosylvian gyrus; *asg*, anterior sigmoid gyrus; *assg*, anterior suprasylvian gyrus; *cg*, coronal gyrus; *cos*, coronal sulcus; *crs*, cruciate sulcus; *lg*, lateral gyrus; *mssg*, middle suprasylvian gyrus; *psg*, posterior sigmoid gyrus. For further explanation see text.

Text-figure 10*B* is a drawing prepared from the photograph of a brain which showed an anomalous pattern of sulci. The map was plotted with a surface-anodal stimulus of 1·5 mA. In this brain also, the best point (filled circle) and lowest threshold point (filled triangle) were eccentrically placed within the effective area.

Inhibition and facilitation of responses in the inferior olive

A single stimulus to either the cerebral or the cerebellar cortex produces in the olive a state of inhibition which lasts for about 90 msec. Following a cerebral or cerebellar stimulus there was a reduction in the amplitude of synaptically evoked mass responses, i.e. responses to cerebral stimuli or the second component of responses to cerebellar stimuli. The duration of this reduction (Text-fig. 11) was much longer than that of the short-lived depression of the antidromic wave which has been discussed above, and which lasted not longer than 25 msec (but see below).

It was not possible to determine the time of onset of inhibition when a cerebellar response was used as test because the olivary discharges evoked by the conditioning stimulus reduced the incoming volley through impulse collision in the climbing fibres. When the response to cerebral stimulation was used as test, the onset of inhibition appeared to be rapid and inhibition was fully developed by the end of the olivary response produced by the conditioning stimulus. Following the period of inhibition, which was often complete, the test response returned to the control value at an interval of about 100 msec, but a second period of inhibition began at about 150 msec. At intervals between 100 and 150 msec some preparations showed



Text-fig. 11. Effect of a preceding cerebral stimulus of 4·0 mA on the olivary mass response to a second cerebral stimulus of the same intensity. Graph shows relation between stimulus interval and amplitude of second response expressed as a percentage of the control value. Records of second response selected from those on which the graph is based. The stimulus artifacts are vertically above the points on the graph to which the specimen records belong. Note that the time scale of the records is much slower than that of the graph.

facilitation of the test response (see for example records and graph in Text-fig. 11). This facilitation of the olivary neurones was sufficient to cause late firing of some single units between 100 and 150 msec after a single stimulus to either cortex (Text-fig. 4*F*). These spikes would presumably collide with antidromic impulses and this would account for the small reduction seen at this time in the amplitude of the antidromic component of a test cerebellar field potential. In the two preparations which showed very little synaptic response to a single cerebellar stimulus, responses to a second cerebellar stimulus during this period showed a well-developed second component.

DISCUSSION

Our experiments have shown that stimulation of the paramedian lobule of the cerebellum produces field potentials in the region of the contralateral olivary complex, and that the first component of these potentials is produced by antidromic invasion of the olive cells. The method of Thomas & Wilson (1965) enabled us to locate the micro-electrode tip with great accuracy by producing dye spots little larger than the soma of an olivary neurone (see Pl. 2). It was found, using this technique, that the negative-going field potentials encountered in our medial penetrations were maximal near the compact group of cells at the junction of the ventral lamella of the principal olive with the dorsal accessory olive. The responses declined rapidly in amplitude both deep and superficial to this region. Only near the maximum were unitary responses seen superimposed on the mass response. In a study on another compact group of brain stem neurones, the hypoglossal nucleus, Porter (1965) has also found that antidromic invasion produced negative-going field potentials in the region of the cell bodies.

The depth profiles encountered more laterally (e.g. Text-fig. 2, open circles) are not easily explained, though here again unitary responses were seen near the depth at which the ventral lamella is situated. It is interesting that profiles showing reversal were only encountered where the ventral lamella forms a thin lamina containing many cells which have their dendritic fields extending towards the cavity of the principal olive while bundles of their axons are directed medially between the ventral lamella and the medial accessory olive (Scheibel & Scheibel, 1955). It is possible that the positive-going responses, maximal about 300 μ superficial to the ventral lamella, arise because the axons act as current sources to the sinks produced by active depolarization of the cell bodies and dendrites. It is at any rate unlikely that the positivities were a consequence of any injury to the olivary neurones because the responses remained constant over many hours. Dissecting off the overlying pial vessels should not significantly interfere with the blood supply to the olive as this is derived mainly from deep vessels fanning out from the ventral part of the mid line raphe (Scheibel & Scheibel, 1955).

In numerous penetrations when dye-filled electrodes were not used, similar depth profiles were found and unitary responses were seen at depths below the surface which correspond with those found in experiments in which dye spots were made. It seems certain that the potentials observed have been at least very largely due to activity in cells of the ventral lamella of the principal olive, a finding which confirms Brodal's (1940) histological evidence that the ventral lamella projects to the contralateral

paramedian lobule of the cerebellum. We have routinely stimulated only folium 4 or 5 of the paramedian lobule so that our experiments can provide no evidence for a detailed localization within the ventral lamella such as Brodal (1940) has described for the rabbit but not the cat.

The present study of the principal olive has revealed a mass response to cerebellar stimulation which is apparently similar to that found by Ochi (1965) in the spinal olive (the caudal parts of the medial and dorsal accessory olives). He recorded a response to stimulation of the cerebellar white matter which had two components, the first attributable to antidromic invasion of the olivary neurones and the second to repetitive re-excitation of the same cells at 1·2 msec intervals. He has interpreted this synaptic re-excitation as being due to activity in climbing fibre collaterals directly excitatory to the olive cells. This explanation is presumably based on the absence of histological evidence for any connexion between the cerebellum and the olive other than the climbing fibres.

Climbing fibre collaterals excitatory to the olive cells have also been postulated by Eccles *et al.* (1966) as a possible basis for the climbing fibre reflex and other features of their results. They found that stimulation using an electrode deep in the cerebellar white matter ('juxta-fastigial') would sometimes evoke synaptic discharge of an olive cell (as judged by the climbing fibre excitatory post-synaptic potentials (EPSPs) in a Purkinje cell) in the absence of direct excitation of the climbing fibre. We did not observe any olive cells which could be discharged synaptically in the absence of antidromic invasion following a stimulus to the cerebellar cortex, but this may be due to some difference between the spinal part of the olive and the ventral lamella. The delays found here between the two components of the field potential evoked from the cerebellum and between single unit antidromic and synaptic spikes are in good agreement with the delay (2 msec) observed in the cerebellum between the successive climbing fibre EPSPs evoked in Purkinje cells by a single stimulus to the inferior olive (Eccles *et al.* 1966). The possibility does exist, however, that our synaptic responses were initiated by an axon reflex involving collaterals of mossy fibres to the paramedian lobule. Certainly there are endings in the principal olive which morphologically resemble the mossy fibre endings in the cerebellar cortex (Scheibel & Scheibel, 1955). However, no collaterals to the inferior olive have been described for the mossy fibres to the paramedian lobule and our finding that the antidromic and synaptic discharges had the same threshold suggests that they were both evoked via the climbing fibres.

Field potential and single unit studies both suggest that a single stimulus to the sensori-motor cortex gives rise to discharges of the neurones of the ventral lamella. This is not inconsistent with Walberg's (1956)

demonstration that the major part of the cortico-olivary projection arises in the sensori-motor area and that the projection has its densest termination in the ventral lamella. We have no evidence that the olivary discharges were monosynaptically produced, other than our inability to shorten the latency of onset of the mass response by increasing stimulus intensity. The responses could therefore have been mediated by a polysynaptic pathway, but none has been described. The only projections to the ventral lamella, other than the direct cortical pathway, arise in the periaqueductal grey matter of the mesencephalon (Walberg, 1956), and in the mesencephalic reticular formation (Walberg, 1960). If the responses were monosynaptic, the descending fibres must be of small calibre, with a conduction velocity of about 5 m/sec. There is, however, much evidence for the presence of fine fibres in the pyramidal tract; in the medullary pyramid of the cat some 70 % of the axons are 2 μ or less in diameter and most of these have a diameter of about 1 μ (Van Crevel & Verhaart, 1963) and therefore probably conduct at about 6 m/sec.

Surface anodal stimuli of duration 0·2 msec and of similar intensities to those employed here have been shown to excite pyramidal neurones of the neocortex only by a direct electrical action and not synaptically via intracortical systems afferent to the cortico-fugal cells (Hern, Landgren, Phillips & Porter, 1962; Phillips & Porter, 1962; Landau *et al.* 1965). Our stimuli should therefore have produced only single cortico-fugal volleys. It is perhaps surprising that olive cells were so readily caused to discharge by a single volley; the motoneurones of the baboon cervical cord are never discharged by volleys in the direct projection from the motor cortex even when these are produced by cortical stimuli as strong as 10 mA (Phillips & Porter, 1964). Our cortical maps cannot be claimed to define accurately the spatial extent of the cortico-fugal cell population which excites the ventral lamella even though the parameters of stimulation were chosen to ensure that the maps were not significantly complicated by excitation of intracortical systems feeding on to the cortical efferents. Thus a stimulus of only 1·0 mA may directly excite some pyramidal cells situated up to 5 mm from the point of stimulation (Hern, Landgren, Phillips & Porter, 1962). Furthermore, we could probably only detect effects which were large enough to cause discharges of the olivary cells so that we cannot exclude the presence of cells in areas outside those shown in Text-fig. 10, which were excited by our stimuli but in numbers sufficient to cause only subthreshold depolarizations of the olivary neurones. Another limitation of the maps is indicated by the consistent location of the 'best point' near the forward edge of the effective area mapped at the same stimulus intensity. This suggests that the population of cells projecting to the olive extended into the wall of the coronal sulcus. In the anomalous brain

(Text-fig. 10*B*) the 'best' and lowest threshold points were again placed eccentrically and near a sulcus.

The present investigation shows that many olivary neurones begin to discharge impulses 8–9 msec after a single cerebral stimulus; the impulses travel up the climbing fibres to arrive at the cerebellar cortex 3–5 msec later. The consequent excitation of many Purkinje cells in the paramedian lobule will thus occur with a latency corresponding to the late response seen by Jansen (1957) and the inactivation responses seen in single Purkinje cells by Jansen & Fangel (1961). No significant number of climbing fibres arising outside the inferior olive has been found in a careful study by J. Szentágothai (personal communication to Sir John Eccles) so it is difficult to escape the conclusion that the responses seen by Jansen (1957) and by Jansen & Fangel (1961) were relayed via the inferior olive.

If the olive contains excitatory recurrent collaterals (Ochi, 1965; Eccles *et al.* 1966), which is consistent with the present results, then in order to curtail 'avalanche conduction' (as suggested by Ramón y Cajal, 1909) some inhibitory mechanism would be required. The present experiments have shown that there is indeed a powerful inhibitory mechanism. The olive is, however, said to contain no cells which do not project to the cerebellar cortex (Brodal, 1940), i.e. no purely internuncial cells; and the only direct projections into the ventral lamella which have been described are from the cerebral cortex, the periaqueductal grey matter and the mesencephalic reticular formation. The first connexion we have shown to be excitatory, and there is no known direct connexion from the olive to the periaqueductal grey matter or the mesencephalic reticular formation, or indeed to any other region of the brain stem, which could complete the necessary circuits for the inhibition to arise outside the olive. The olive shows some resemblance to the ventro-basal nucleus of the thalamus (Andersen, Brooks, Eccles & Sears, 1964; Andersen, Eccles & Sears, 1964) where a single stimulus is followed by a rhythmic alternation between periods of inhibition and facilitation. Since the above physiological studies were published, interneurones have been described in the thalamus, (Tombol, 1965); it is possible that in the olive also, physiological evidence for the existence of interneurones has been found before they have been indentified anatomically.

The authors wish to thank Professor Sir John Eccles, who suggested this study, for his constant encouragement and advice extended freely at all stages of the investigation. They are also indebted to Dr D. R. Curtis for advice on histological procedures.

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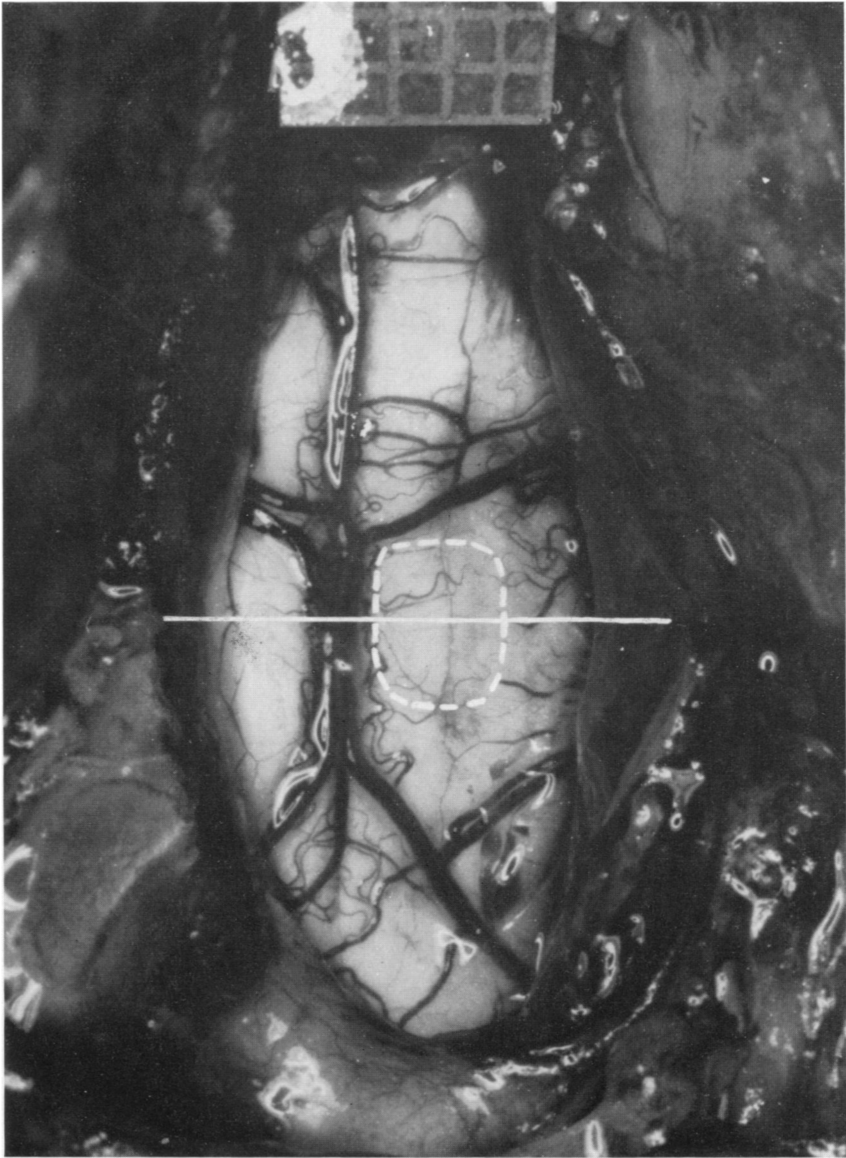
EXPLANATION OF PLATES

PLATE 1

Photograph of ventral surface of brain stem. Scale of 1 mm at rostral end of exposure. Interrupted line encloses area from which responses were obtained. Continuous line shows approximate level of section illustrated in Pl. 2.

PLATE 2

Photomicrograph of transverse section through olivary region of left brain stem at level indicated in Pl. 1. Reference to Text-fig. 3 will identify olivary subdivisions and orientation of section. Interrupted line shows direction of micro-electrode penetrations. Lines at borders of section are co-ordinates of two dye spots. Longer lines intersect at position of a spot in medial accessory olive; shorter lines intersect at a spot in ventral lamella of principal olive near junction with dorsal accessory olive. Calibration: 500 μ.



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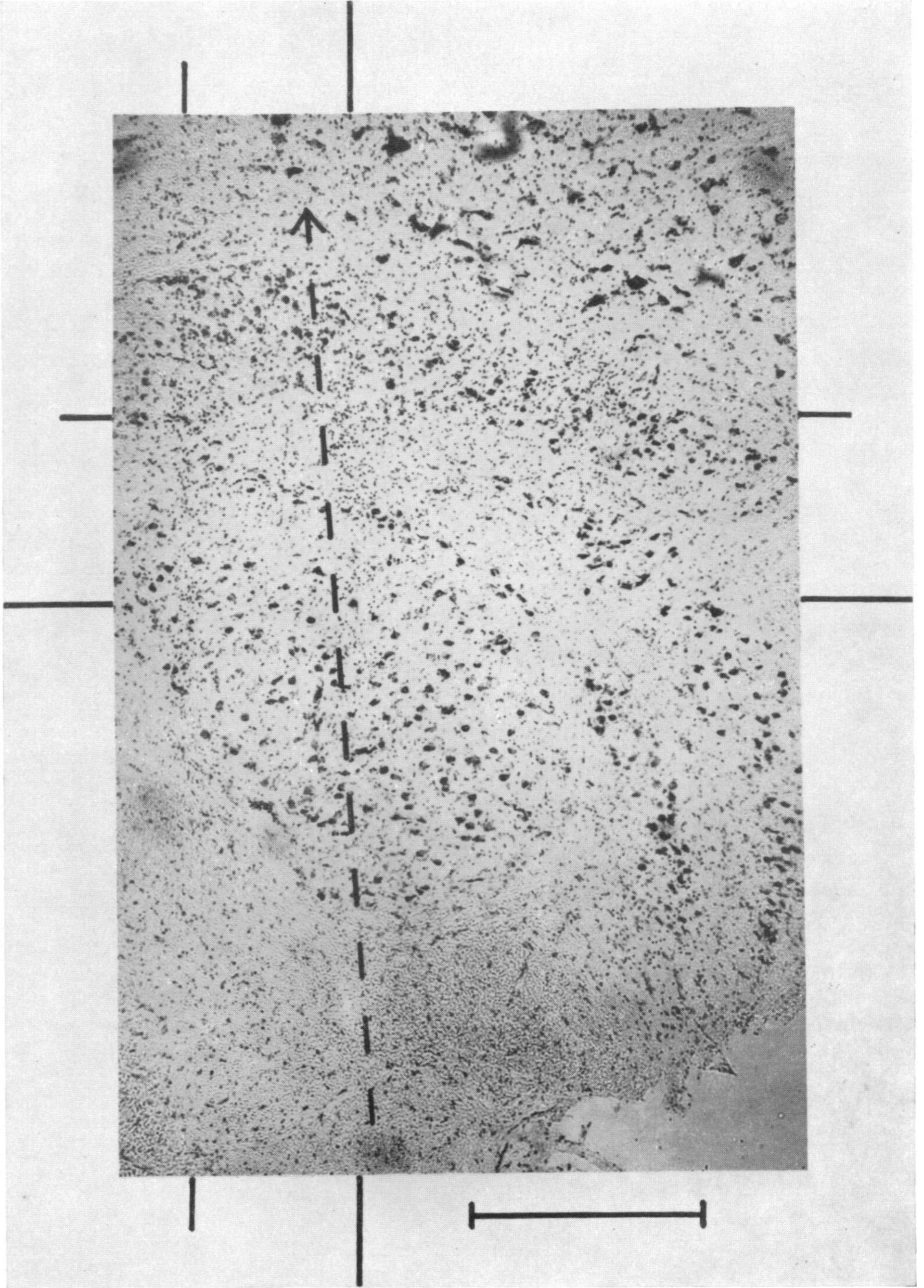

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