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RESPONSES OF A SPINO-OLIVO-CEREBELLAR PATHWAY IN THE CAT

BY D. M. ARMSTRONG* AND R. J. HARVEY*
From the Department of Physiology, Australian National University, Canberra, Australia

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SUMMARY

1. Surface potentials, similar to those found by earlier workers, have been recorded from the vermis of the anterior lobe of the cerebellum following stimulation of muscular, cutaneous and articular nerves of the ipsilateral hind limb. The most conspicuous component of the response consisted of a positive potential succeeded by a smaller negative potential.

2. Micro-electrode recordings showed that this component coincided both with climbing fibre responses in individual Purkinje cells, and with extracellular field potentials within the cerebellar cortex which closely resembled those found by Eccles, Llinás & Sasaki (1966) following electrical stimulation of the inferior olive.

3. Stimulation of the cerebellar surface, in the region where the responses to limb nerve stimulation were largest, led to antidromic invasion of neurones of the contralateral inferior olive. The antidromic action potentials were sometimes followed by up to three orthodromic spikes. Histological techniques were used to show that these neurones were located in the caudal parts of the dorsal and medial accessory olives.

4. Stimulation of nerves of the hind limb evoked discharges of the same neurones of the dorsal accessory olive which were antidromically invaded from the vermis of the anterior lobe. The nerves used (quadriceps, gastrocnemius-soleus, sural and the posterior nerve to the knee joint) were shown to excite heavily overlapping populations of neurones.

5. Those neurones of the medial accessory olive, which were identified antidromically from the anterior lobe vermis, were not discharged by stimulation of hind limb nerves.

6. Simultaneous recording from the surface of the anterior lobe and from the dorsal accessory olive showed that the onset of olive cell discharges occurred about 5 msec before the onset of the positive potential at the cerebellar surface.

* Present address: Department of Physiology, University of Bristol.

INTRODUCTION

There are many descriptions of surface responses recorded from the ipsilateral side of the vermis of the anterior lobe of the cat's cerebellum following electrical stimulation of hind limb afferents (Dow, 1939; Grundfest & Campbell, 1942; McIntyre, 1951; Mountcastle, Covian & Harrison, 1952; Haddad, 1953; Morin & Haddad, 1953; Carrea & Grundfest, 1954; Combs, 1954; Laporte, Lundberg & Oscarsson, 1956; Morin, Catalano & Lamarche, 1957). The most striking and constant feature of the published records has been a large, positive-going potential with a latency of between 15 and 25 msec, though small responses with shorter latencies have been observed and attributed to activity in the spino-cerebellar tracts. The large potential has been termed 'Potential III' by Carrea & Grundfest (1954) while Morin (1956) and Morin, Catalano & Lamarche (1957) have distinguished a negative deflexion which succeeds the positive component, and have applied the term 'potential y' to the whole diphasic response.

This response has been shown to survive section of Flechsig's tract (Carrea & Grundfest, 1954) and indeed its latency argues strongly against generation through either the dorsal or the ventral spino-cerebellar tracts. McIntyre (1951) and Morin & Haddad (1953) showed that the response depends on impulses which ascend in the ventral quadrant contralateral to the sites of stimulation and recording. The 'spinal' part of the olive (the caudal parts of the dorsal and medial accessory olives) receives afferents which ascend from lumbar levels in the ipsilateral ventral quadrant (Brodal, Walberg & Blackstad, 1950), and this part of the olive projects to the vermis of the anterior lobe (Brodal, 1940). Since the olivo-cerebellar projection is almost entirely crossed, McIntyre (1951) and Morin & Haddad (1953) were led to suggest that the cerebellar response might depend on activity crossing in the lumbar region of the cord and ascending to relay in the inferior olive.

This suggestion was supported by the finding that appropriately timed activity could be recorded from the spinal olive with steel needles when nerves of the contralateral hind limb were stimulated (Morin, Lamarche & Ostrowski, 1957). These results have recently been confirmed and extended by a micro-electrode study of the caudal part of the dorsal accessory olive (Armstrong, Eccles, Harvey & Matthews, 1968). Evidence has also been provided by Grant & Oscarsson (1966), who have recorded mass discharges of the olivo-cerebellar fibres in the dissected inferior cerebellar peduncle.

In contrast to the other cerebellar afferent systems (e.g. spino-cerebellar and reticulo-cerebellar pathways) which terminate in the cortex of the anterior lobe of the cerebellum as mossy fibres, the olivo-cerebellar fibres end as climbing fibres (Szentágothai & Rajkovits, 1959), each of which

makes direct and extensive synaptic contact with the apical dendrites of a single Purkinje cell (Ramón y Cajal, 1911). If the large potentials recorded from the surface of the anterior lobe are indeed a result of activity in the spino-olivo-cerebellar pathway, they presumably reflect climbing fibre activation of the Purkinje cells. Eccles *et al.* (1966) have shown that such activation is revealed in extracellular recordings from individual Purkinje cells by the presence of a highly characteristic burst of action potentials (identified with the inactivation response of Granit & Phillips, 1956) which is generated by a very large unitary depolarization (the climbing fibre excitatory post-synaptic potential). They have also shown that simultaneous excitation of large numbers of Purkinje cells by their climbing fibres, following an electrical stimulus to the inferior olive, gives rise to characteristic extracellular field potentials within the cerebellar cortex. We have therefore attempted to correlate cerebellar surface potentials generated by stimulation of cutaneous, muscular and articular nerves of the hind limb, with responses recorded by micro-electrodes from within the cortex of the anterior lobe.

In addition we have made micro-electrode recordings from the inferior olive, and neurones which project to the vermis of the anterior lobe have been identified by antidromic invasion down the olivo-cerebellar fibres (cf. Armstrong & Harvey, 1966). These neurones have also been activated orthodromically by volleys in nerves of the hind limb. We have recorded simultaneously from the surface of the anterior lobe and from the spinal olive, and have used the technique of Thomas & Wilson (1965) to identify the olivary subdivisions from which we recorded.

METHODS

The experiments were performed on cats weighing between 2·0 and 3·3 kg. They were anaesthetized by intraperitoneal injection of 30–40 mg/kg pentobarbitone (Nembutal, Abbott Laboratories); supplementary doses of anaesthetic were administered intravenously.

In six experiments the vermis of the anterior lobe of the cerebellum was exposed by removing the occipital poles of the cerebral hemispheres and the bony tentorium. The craniotomy was extended caudally to the fissura prima and laterally to the junction of the anterior lobe with Crus I of the cerebellar hemisphere. The left quadriceps, gastrocnemius-soleus and sural nerves, and the posterior nerve to the knee joint were stimulated as described previously (Armstrong *et al.* 1968), and recordings were made between a lightly sprung silver ball electrode on the anterior lobe vermis and an indifferent electrode sown beneath the scalp. The ball electrode was moved over the cerebellar surface while the nerves were stimulated, until a region was located which yielded large responses to stimulation of the nerves. The electrode was then removed, the exposed area of the cerebellum was covered by a thin layer of agar gel, and micro-electrode probing was begun in the most responsive region of the cortex. The micro-electrodes were filled with 4 M-NaCl solution and those with d.c. resistances between 2 and 10 MΩ were chosen for use.

In a further twelve experiments, micro-electrode recordings were made from the right inferior olive whilst stimulating the nerves. A general description of the techniques used has been given previously (Armstrong & Harvey, 1966). The technique of Thomas & Wilson

(1965) was used in five of the experiments to determine the position of the micro-electrode tip. The olivary neurones projecting to the vermis of the anterior lobe were routinely identified by antidromic invasion down the olivo-cerebellar fibres (cf. Armstrong & Harvey, 1966). The left side of the vermis was exposed by removing the occipital pole of the left cerebral hemisphere and the left half of the bony tentorium. The cerebellar surface was stimulated under warm mineral oil using a pair of bipolar silver ball electrodes separated by about 2 mm. The stimulus current was monitored by recording the voltage drop across a 100 Ω resistor in series with the anode of the pair. In some of these experiments cerebellar surface potentials were recorded from the anterior lobe vermis by means of a roving silver ball electrode.

RESULTS

Responses in the anterior lobe of the cerebellum to stimulation of hind limb nerves. Monopolar recording from the surface of the anterior lobe has revealed responses to stimulation of hind limb nerves which closely resemble those found by previous workers (e.g. Morin & Haddad, 1953). Figure 1*A* shows responses evoked from muscular, cutaneous and articular nerves; they are very similar in form and consist of a prominent positive deflexion which is followed by a smaller negative deflexion. Earlier responses, similar to those which Laporte *et al.* (1956) ascribed to activation via direct spino-cerebellar fibres, were sometimes present, but they were always of relatively small amplitude. We have studied only the later and much larger responses described above.

The amplitude of the responses often varied considerably from one trial to another (cf. Morin & Haddad, 1953), and this is illustrated in Fig. 1*C* which is a series of consecutive single sweeps recurring once every 2 sec. The distribution of the responses on the cerebellar surface was not studied systematically but the largest positive deflexions were recorded from the lateral part of the vermis near the junction of Larsell's Lobuli IV and V (Larsell, 1953). In four experiments the electrode position for recording the largest responses to stimulation of each nerve was determined. These 'best points' were so closely grouped that they may have been identical. The position of the 'best point' for stimulation of quadriceps nerve in a typical experiment is shown by the filled circle in Fig. 1*B*. Under the present conditions of anaesthesia the responses were quite sharply localized and electrode movements of 1 or 2 mm caused large changes in the amplitude of the positive deflexion. When the electrode was moved towards the mid line, the positive-negative responses were replaced by much smaller negative-positive responses similar to those recorded by Eccles *et al.* (1966) on stimulation of the inferior olive (see Discussion), and to those recorded by Oscarsson & Uddenberg (1966).

Micro-electrode probing was usually confined to the area over which large surface responses could be detected since activity was most readily recorded in this region. In Fig. 2*A* are shown typical extracellular field

potentials which were recorded at different depths below the surface of the cerebellar cortex and which were evoked by a strong single stimulus to the ipsilateral quadriceps nerve. The depth of the micro-electrode tip below the cerebellar surface is shown alongside each response. The values

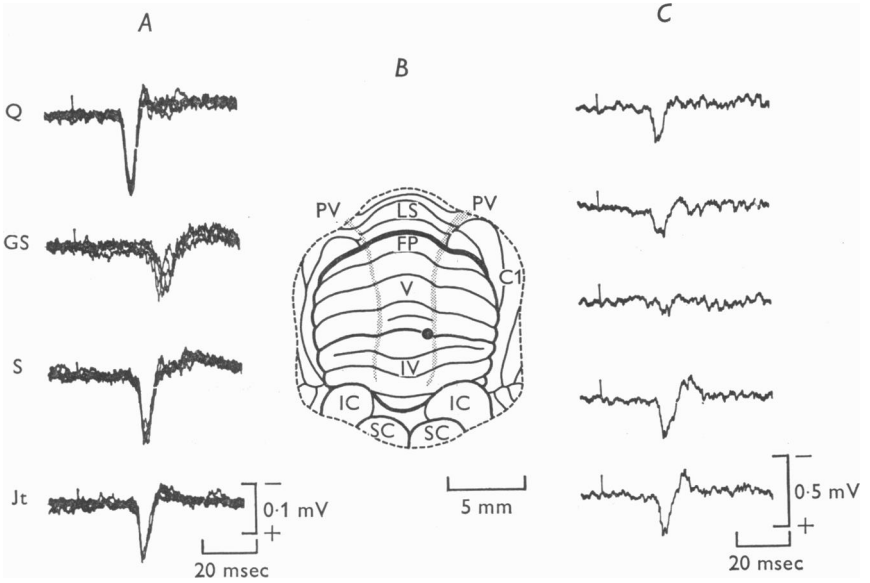

Fig. 1. Surface responses recorded from the vermis of the anterior lobe after single stimuli to nerves of the hind limb. *A*. Responses recorded from a single electrode position on stimulation of: GS, gastrocnemius-soleus nerve; Jt, posterior nerve to knee joint; Q, quadriceps nerve; S, sural nerve. Each nerve stimulated at approximately 10 × threshold intensity for the most excitable fibres (10*T*). Each record made up of five superimposed traces. Stimulus repetition rate: one every 2 sec. *B*. Frontal view of anterior lobe as exposed in a typical experiment: C1, Crus 1; FP, fissura prima; IC, inferior colliculus; LS, lobus simplex; PV, paravermal vein; SC, superior collicus. Roman numerals indicate lobuli numbered according to Larsell (1953). Filled circle indicates recording position at which the largest responses were recorded to stimulation of ipsilateral quadriceps nerve. *C*. Five successive responses to stimulation of quadriceps nerve at approximately 10*T*. Stimulus repetition rate: one every 2 sec.

given were read from the micrometer driving the electrode and may be subject to some error due to deformation of the brain, but this was minimized by making all observations during withdrawal of the micro-electrode. At 350 μ single unit discharges are visible superimposed on the field potential. This depth corresponds to the layer of Purkinje cell bodies and the single units could be identified as Purkinje cells by the presence of climbing fibre responses (Eccles *et al.* 1966). The positive deflexion of the surface response was mirrored within the cerebellar cortex by a simultaneous negative field potential which was largest about 200 μ superficial

to the level of the Purkinje cell bodies. At depths below 300 μ the negative wave diminished and was replaced by a positive wave. The profile suggests that the positive potentials on the cerebellar surface arise because the superficial dendrites of the Purkinje cells act as passive sources of current to active sinks on the deeper dendrites. These sinks presumably also draw current from still deeper passive sources on the somata and axons of the Purkinje cells. This depth profile is strikingly similar to those encountered by Eccles *et al.* (1966) following a stimulus to the inferior olive, though the potentials are more dispersed in time.

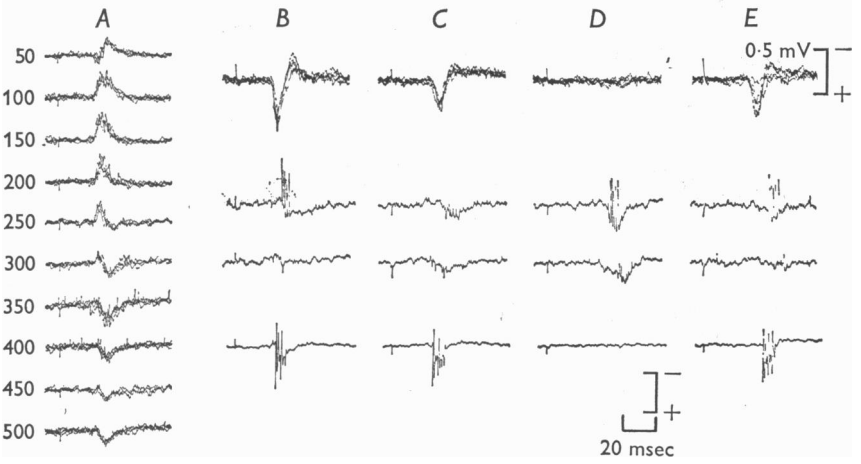

Fig. 2. Responses recorded from vermis of anterior lobe. *A*. Field potentials recorded by a micro-electrode from within the cortex of the anterior lobe, following a strong single stimulus (approx. 12 *T*) to quadriceps nerve. Records obtained from the region where the largest surface responses were recorded. Depth below brain surface shown in μ alongside each record. Each record made up of five superimposed traces. Stimulus repetition rate: one every 2 sec. *B, C, D, E*. Responses to stimulation of Q, S, GS and Jt nerves respectively. Stimulus intensity approx. 12 *T* for each nerve. Top row: surface responses recorded at a single electrode position. Each record made up of five superimposed traces. Stimulus repetition rate: one every 2 sec. Second row: responses of a single Purkinje cell discharged from Q, GS and Jt nerves. Sural nerve failed to discharge this unit, but note that a burst of spikes was fired by a neighbouring cell. Third row: residual field potentials recorded when the unit in the second row failed to discharge. Bottom row: responses of another Purkinje cell encountered in a nearby penetration. Voltage calibration at top right applies to all surface responses. Voltage calibration at bottom of figure is 0·5 mV for *A* and for second and third rows of *B, C, D, E*: 1 mV for bottom row of *B, C, D, E*.

The top row of records in Fig. 2 *B, C, D* and *E* shows responses (recorded from the surface of the cerebellum with a ball electrode) to stimulation of quadriceps, sural, gastrocnemius-soleus and the posterior nerve to the

knee joint respectively. Below each surface response are micro-electrode recordings which were obtained from within the cerebellar cortex beneath the position of the surface electrode, and which show unit responses to stimulation of the corresponding nerve. In the second row are extracellular recordings from a single Purkinje cell showing typical climbing fibre responses, while in the third row are recordings from the same locus showing trials in which the cell failed to respond. These demonstrate that the response was all-or-nothing in character. The bottom row of records shows climbing fibre responses recorded from another Purkinje cell in a nearby penetration. These and many similar records showed that climbing fibre responses are generated in large numbers of Purkinje cells at the time when the positive-going wave appears on the cerebellar surface. There is thus good evidence that the surface potentials are due to activation of many underlying Purkinje cells via their climbing fibres, and thus, presumably, to preceding activity in the inferior olive.

Responses in the inferior olive to stimulation of the surface of the anterior lobe vermis. In the region where large responses to stimulation of ipsilateral hind limb nerves were found, the surface of the vermis was stimulated, using stimulus intensities up to 5 mA, and the contralateral side of the medulla was explored with micro-electrodes. Field potentials and unit spikes were encountered in the region of the inferior olive. The mass responses were always negative-going and were similar in form to the responses found in the medial part of the ventral lamella of the principal olive when the paramedian lobule was stimulated (Armstrong & Harvey, 1966). Figure 3*A* shows that the typical response had two components, the second of which was sometimes small or absent. As with the responses in the principal olive (Armstrong & Harvey, 1966) the second component was very much more variable in amplitude than the first, and there was good agreement between the difference in latency between the first and second components which was found here (1·9–2·3 msec) and in the ventral lamella (mean 1·9 msec).

The two components of the field potential responded very differently to repetitive stimulation. The first component would follow each stimulus of a long train, although the amplitude was reduced after the first few responses (Fig. 3*D*). On the other hand, a second component of any significant size never occurred in response to more than one stimulus of a train (Fig. 3*B*, *C* and *D*). Records 3*B* and *C* also clearly show that the presence of a second component greatly diminished the response to a subsequent stimulus. The situation in Fig. 3*C*, where a second component appeared in the response to the second but not to the first stimulus, was only rarely encountered; a second component was almost always present only in the response to a first stimulus, as in Fig. 3*B* and *D*. In a few preparations,

as illustrated in Fig. 3*E*, the responses displayed no significant second component (cf. Armstrong & Harvey, 1966).

On the basis of the responses of single olivary neurones, we have been able to provide explanations for the various patterns of response described

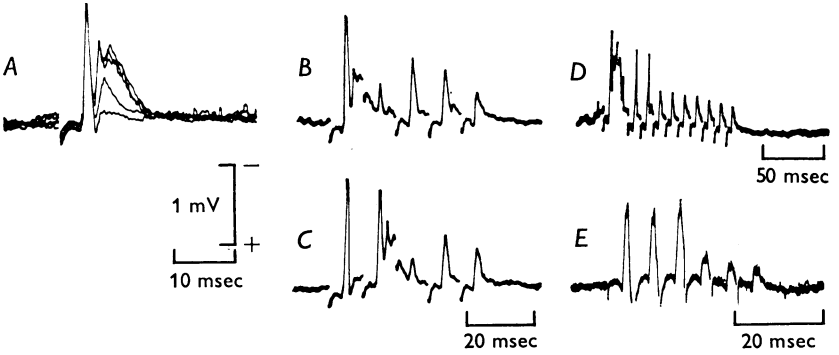

Fig. 3. Responses in inferior olive to stimulation of contralateral side of vermis of anterior lobe. *A*. Response to a single stimulus. Five superimposed traces occurring one every 2 sec. *B*, *C*. Two successive responses to five stimuli at 100/sec. *D*. Response to eleven stimuli at 100/sec. Stimulus intensity 4·0 mA. All responses recorded at same locus. *E*. Response recorded in another preparation to six stimuli at 150/sec. Stimulus intensity 3·5 mA. Five superimposed traces occurring one every 2 sec. Time calibration below *C* applies to *B* and *C*.

above. Thus, extracellular recordings from a large number of single units have shown that, due to antidromic invasion down the olivo-cerebellar fibres, impulses are discharged with a short, fixed latency following a cerebellar stimulus. This almost synchronous invasion of a large number of olivary neurones gives rise to the first component of the field potential (cf. Armstrong & Harvey, 1966). The latency of onset of the first component of the olivary mass response to a cerebellar stimulus ranged from 3·3 to 4·2 msec. This agrees well with the range encountered in the ventral lamella of the principal olive (Armstrong & Harvey, 1966) and with values found by Eccles *et al.* (1966) for the latency of climbing fibre responses appearing in Purkinje cells of the anterior lobe after a stimulus to the inferior olive.

Figure 4*A* depicts antidromic responses of a typical unit following two cerebellar stimuli delivered at various brief intervals. The action potentials display the initial positive phase which is characteristic of spikes recorded extracellularly from single neurones when the micro-electrode tip is close to the cell membrane (e.g. Phillips, 1959). With a stimulus interval of 3·8 msec (*f*) the second stimulus produced no response, presumably because of refractoriness of the nerve fibre, but at an interval of 4·7 msec three alternative responses to the second stimulus were observed. In some

trials, there was a marked inflexion on the downstroke of the positive phase of the action potential (*e*). Such inflexions are believed to indicate the passage of the invading impulse from one area of the cell membrane to another. The electrical coupling between the two areas is such that

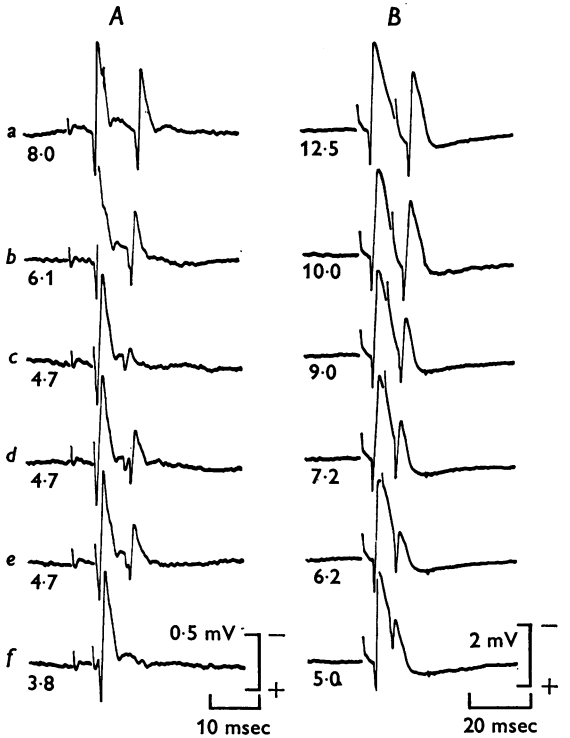

Fig. 4. Extracellular records from two single olivary neurones, showing responses to two cerebellar stimuli at various brief intervals. Stimulus intensity 1·0 mA for unit *A*, 2·5 mA for unit *B*. Stimulus interval in msec indicated alongside each record.

transmission may be delayed or blocked under certain circumstances. This delay has been termed '*A–B* delay' (Fuortes, Frank & Becker, 1957) or 'IS–SD delay' (Coombs, Curtis & Eccles, 1957). In other trials the hesitation was so marked that the *A* and *B* spikes appeared completely separated (*d*), while in yet other trials the *A* spike appeared in isolation (*c*) indicating that complete block had occurred in the transmission of the impulse from the *A* to the *B* membrane. At longer intervals (*a* and *b*), *A–B* delay was still evident, but was less marked. In Fig. 4*A* the second of each pair of antidromic spikes was reduced in amplitude even when *A–B* block did not occur. Figure 4*B* shows that this diminution in amplitude was very marked in some units and that the second spike progressively

decreased in size as the stimulus interval was reduced, though even at a stimulus interval of 5·0 msec there was an inflexion in the initial downstroke which suggests that both *A* and *B* membranes were invaded.

Single units sometimes responded to a cerebellar stimulus with one, two, or occasionally three, additional spikes following the antidromic spike. The extra spikes were not present in all trial responses and when present showed variations in latency and number. These later spikes probably arise as a result of excitatory synaptic action due to activity in the climbing fibre recurrent collaterals (Ochi, 1965; Eccles *et al.* 1966), and in the present experiments they were never seen except following an antidromic spike. Figure 5*A* shows a unit giving one extra spike while Fig. 5*B* shows five superimposed records of another unit responding in three trials with one, and in two trials with two, extra spikes of low amplitude. It was invariably found that when these spikes were present, the least interval at which a second antidromic spike could be evoked was greatly prolonged. Thus Fig. 5*C* shows that the unit of Fig. 5*A* produced no visible response to a second cerebellar stimulus 11·4 msec after the first. This failure of antidromic invasion cannot be attributed to refractoriness of either the nerve fibre or the cell membrane, since in the absence of the extra spike the unit was capable of producing a second antidromic spike at stimulus intervals much shorter (< 5 msec) than the interval between the extra spike and the expected time of arrival of the test antidromic spike. The most likely explanation is that the synaptically evoked spike gives rise to an orthodromic impulse which collides in the climbing fibre with the antidromic impulse evoked by the second stimulus. In fact, a second antidromic spike was always blocked when the interval between the second stimulus and the last spike of the first response was less than the conduction time in the axon (i.e. less than 4·4 msec for the unit of Fig. 5*A* and *C*). The synaptically generated spikes were coincident with, and presumably contributed to, the second component of field potentials evoked by cerebellar stimulation. The observation that these spikes were capable of blocking antidromic invasion in single units provides an explanation for the suppression of antidromic field potentials by a preceding second component.

In some units there was a small ripple or series of ripples on the falling phase of the extracellularly recorded antidromic action potential. Such ripples are indicated by the arrows in Fig. 5*D* and *F*. Comparison of these records with Fig. 5*E* shows that the presence of such ripples led to blockage of antidromic invasion, suggesting that even these low amplitude responses signalled orthodromic discharge. These 'spikes' were very small, but the series of Fig. 4*B* has shown that antidromic action potentials may be progressively reduced in size as the interval after a previous spike decreases. Spikes after the first were also greatly reduced in size during the

high frequency burst of action potentials which occurred spontaneously and in response to nerve stimulation (see, for example, Fig. 9*C* and Armstrong *et al.* 1968). The neurones of the ventral lamella of the principal olive showed similar behaviour (Armstrong & Harvey, 1966).

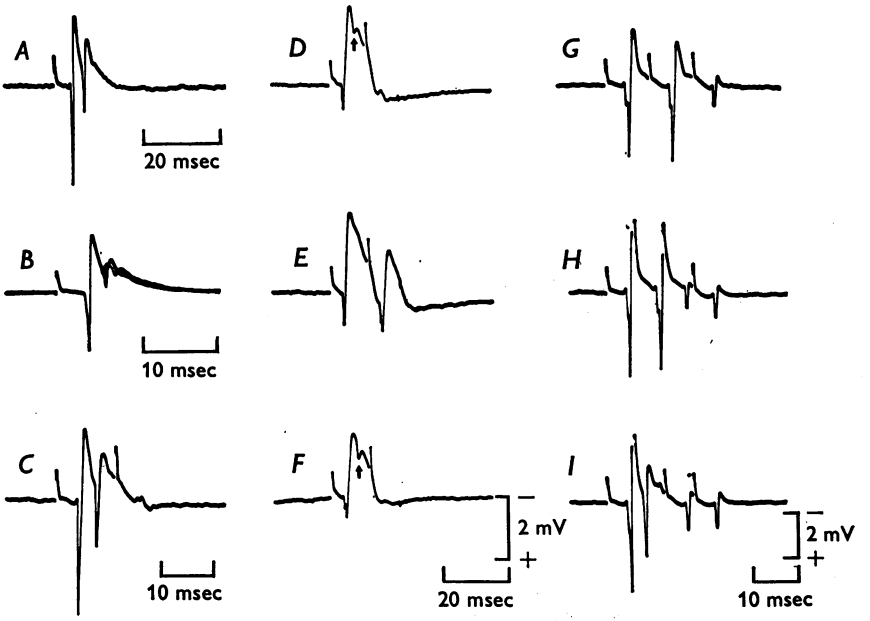

Fig. 5. Extracellularly recorded responses of single olivary neurones to stimulation of vermis of anterior lobe. *A*. Response to a single stimulus. *B*. Five superimposed responses of another unit. *C*. Response of unit in *A* to two stimuli 11·4 msec apart. *D*, *E*, *F*. Responses of another unit to 2 stimuli 11·0, 11·0 and 12·0 msec apart respectively. *G*. Response of unit in *A* to three stimuli at 100/sec. *H*, *I*. Two successive responses of unit in *A* to four stimuli at 150/sec. Voltage calibration beside F applies to *A*, *C*, *D*, *E* and *F*; beside *I* applies to *B*, *G*, *H* and *I*. Time calibration below *F* applies to *D*, *E* and *F*; below *I* applies to *G*, *H* and *I*.

On repetitive stimulation of the cerebellum all units studied showed failure of the *B* component of the antidromic spike after the first two or three responses. This is illustrated in Fig. 5*G* and *H* which show responses of the unit of Fig. 5*A* to trains of stimuli at 100 and 150/sec respectively. Figure 5*I* shows a response to four stimuli at 150/sec in which the second stimulus failed to evoke an antidromic spike because a synaptically generated spike followed the antidromic spike evoked by the first stimulus. The failure of the *B* component of the antidromic spikes of single units provides an explanation for the reduced amplitude of all but the first two or three antidromic field potentials evoked by repetitive stimulation (see Fig. 3*D*).

Location within the inferior olive of antidromic responses to stimulation of the vermis of the anterior lobe. In each of five experiments, a series of micro-electrode tracks was made on a grid to cover the area on the ventral surface of the medulla bounded medially by the basilar artery, caudally by the vertebral artery, laterally by the rootlets of the hypoglossal nerve and rostrally by the level of the most rostral rootlet of the hypoglossal nerve (see Fig. 7*E*). Low resistance (1–2 MΩ) micro-electrodes filled with a saturated solution of Fast Green in 2 M-NaCl were used

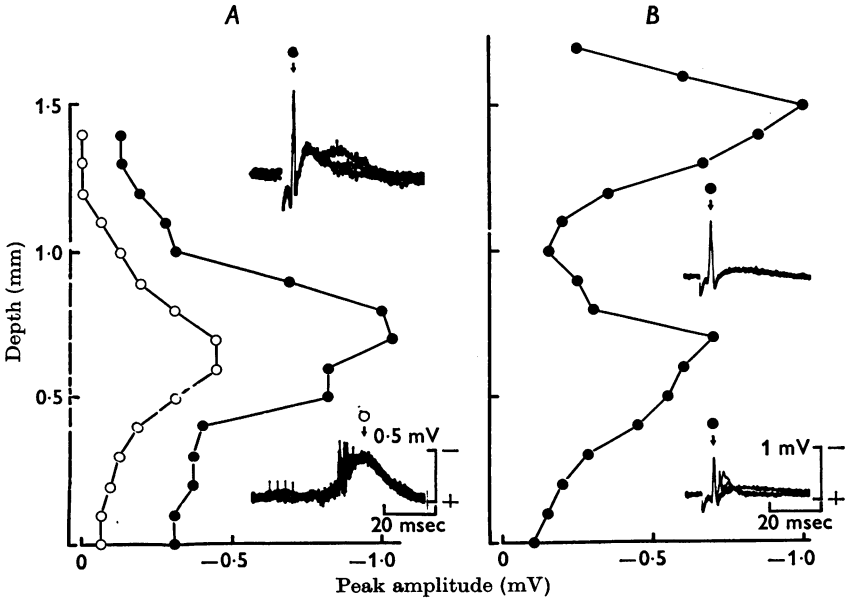

Fig. 6. Peak amplitude of olivary mass responses plotted against depth below brain surface. *A*. Filled circles: first component of a response to a single stimulus to anterior lobe vermis. Open circles: response in same penetration to stimulation of contralateral quadriceps nerve with four stimuli at 300/sec, intensity approx. 12*T*. Inset sample records are responses recorded at depth 0·7 mm. Arrows indicate point at which response amplitude was measured. *B*. First component of response to stimulation of anterior lobe vermis in another preparation. Inset sample records show responses recorded at 0·7 and 1·5 mm depth.

(Thomas & Wilson, 1965). During each penetration the extracellular field potentials evoked by a cerebellar stimulus were recorded at points 100 μ apart in depth, and, for those penetrations where any response was found, the amplitude of the first component of the response was plotted against depth below the surface of the brain. At depths where the response was largest, dye was ejected electrophoretically from the micro-electrode. The locations of the dye spots were later determined histologically, as described in a previous paper (Armstrong & Harvey, 1966).

In most penetrations, the depth profile of the antidromic response showed a single peak (filled circles in Fig. 6*A*) at a depth which varied, in different parts of the grid, between 0·5 and 1·2 mm. In a more restricted area of the grid, the profile showed two peaks at different depths (Fig. 6*B*). In four penetrations yielding this type of profile, a dye spot was made both at the deep and at the superficial maximum. Figure 7*A*, *B*, *C* and *D* are a series of diagrammatic drawings from transverse sections through the caudal part of the olive. The sections were not at equal distances from one another, but they illustrate the main changes which occur in the configuration of the olive between approximate Horsley–Clarke levels P 13·5 and P 15·5 mm (D. M. Armstrong & R. J. Harvey, unpublished). The position of each dye spot produced in the present experiments has been indicated by a circle on the diagram corresponding most closely to the level at which the spot was located. The four pairs of spots joined by lines were made in penetrations which showed peaks of response at two separate depths as described above.

Figure 7*E* is a diagram of the ventral surface of the brain stem. The arrows indicate the approximate levels of the transverse sections in Fig. 7*A–D*. The two areas outlined by interrupted lines in Fig. 7*E* and stippled in *A–D* indicate those portions of the dorsal and medial accessory olives which Brodal (1940) reported as giving rise to fibres projecting to Lobuli 4 and 5 of the anterior lobe vermis. These areas also receive the great majority of the afferents to the olive from the lumbar spinal cord (Brodal *et al.* 1950; Mizuno, 1966). In Fig. 7*E* the circles show the approximate positions of those penetrations in which the dye spots shown in Fig. 7*A–D* were made. The positions are necessarily somewhat approximate because the spots were collected from five experiments and the precise position of surface landmarks varied a little from cat to cat, as also did the location and form of the olive. It can be seen that the spots all lie in the caudal parts of the dorsal and medial accessory olives (i.e. in the spinal olive) and the present results, using antidromic responses, agree very well with the anatomical observations of Brodal (1940). The explanation for those depth profiles with two maxima (Fig. 6*B*) is also apparent from the diagrams in Fig. 7: there is a region where the medial accessory olive lies directly superficial to the dorsal accessory olive so that the micro-electrode passes through both subdivisions as it is advanced into the brain stem.

Antidromic responses in the spinal olive could also be evoked by stimulating the surface of the hemispheral part of the anterior lobe, though stimulus intensities in excess of 4 mA were required. This region of the anterior lobe derives its olivo-cerebellar fibres from the rostro-medial part of the dorsal accessory olive (Brodal, 1940), so that the responses observed presumably arose as a result of stimulus spread to the vermis.

Responses in the inferior olive to stimulation of nerves of the contralateral hind limb. In penetrations where antidromic mass responses to stimulation of the surface of the anterior lobe were found, an attempt was made to

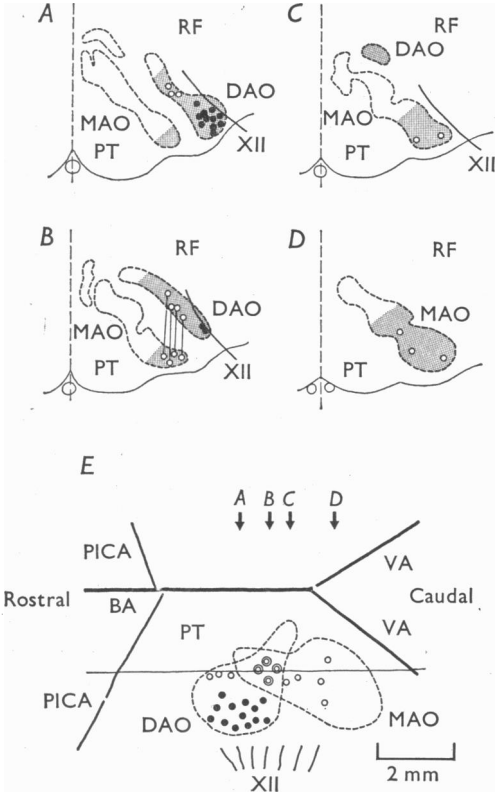

Fig. 7. Location of dye spots placed within the inferior olive. *A*, *B*, *C*, *D*. Drawings of transverse sections through the caudal part of the olivary region of the brain stem. Sections are at the successively more caudal levels indicated by the arrows in *E*. Dashed line indicates mid line. BA, Basilar artery; DAO, dorsal accessory olive; MAO, medial accessory olive; PT, pyramidal tract; RF, reticular formation of brain stem. Stippled areas of dorsal and medial accessory olives project to vermis of anterior lobe and receive afferents from lumbar spinal cord. See text for further explanation. *E*. Drawing from a photograph of ventral surface of brain stem. PICA, posterior inferior cerebellar artery; VA, vertebral artery; XII, rootlets of hypoglossal nerve. Continuous line indicates lateral border of pyramid. The projections on to the brain surface of those areas of the dorsal and medial accessory olives which project to the vermis of the anterior lobe and which receive spinal afferents are enclosed by interrupted lines.

activate the olivary neurones orthodromically by stimulating nerves of the contralateral hind limb. The open circles in Fig. 6*A* illustrate the finding that, in each track in which mass responses to nerve stimulation were

found, these responses had a similar distribution in depth to the antidromic response. The filled circles in the diagrams of Fig. 7 represent dye spots placed at points where, in addition to antidromic responses, field potentials and unitary responses were evoked by stimulation of one or more hind limb nerves. It can be seen that such responses were confined to the dorsal accessory olive, and, in addition, it appeared that not all of that part of the dorsal accessory olive which could be activated antidromically by stimulation of the anterior lobe vermis could be activated from the hind limb nerves. The largest orthodromic responses were recorded from the lateral part of the dorsal accessory olive, and the contralateral quadriceps nerve provided the most effective input (Armstrong *et al.* 1968).

Significant field potentials in response to nerve stimulation were never seen in the medial accessory olive, even when the quadriceps nerve or the whole sciatic nerve were stimulated with trains of strong stimuli at 300/sec. This finding was surprising, as Brodal *et al.* (1950) have stated that fibres ascending from the lumbar spinal cord terminate in the caudal parts of both the medial and the dorsal accessory olives. However, although stimulation of the hind limb nerves used did not give rise to responses in the medial accessory olive, stimulation of the spinal cord in the upper lumbar region, or of dorsal roots from L 3 to S 1 inclusive, did produce responses here; hence presumably the medial accessory olive is activated from regions other than the hind limb.

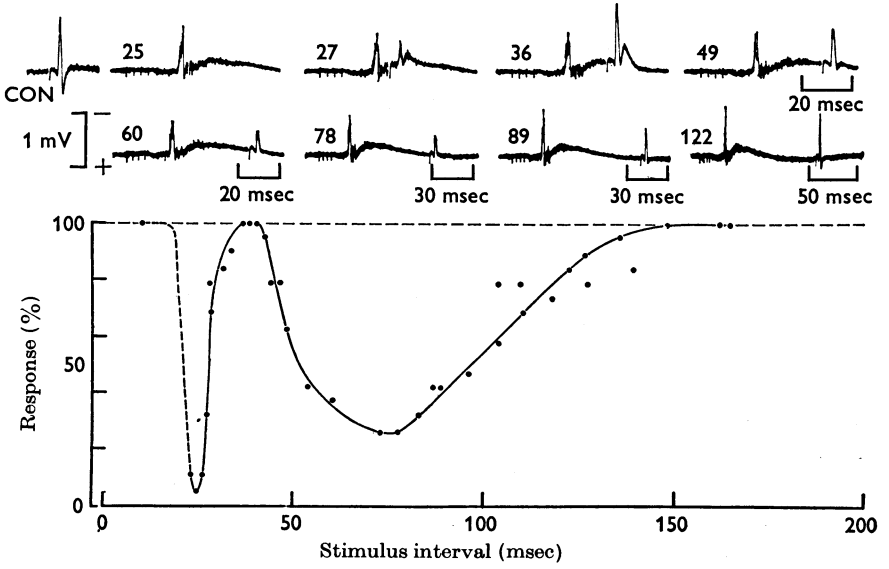

Fig. 8. Effect of a response to stimulation of quadriceps nerve (four stimuli at 300/sec; approx. 12*T*) on a subsequent antidromic response evoked by a single cerebellar stimulus (intensity 4·2 mA). Graph shows relation between stimulus interval (measured from first stimulus to nerve) and amplitude of first component of response to cerebellar stimulation. The records were selected from those on which the graph is based. CON, control response to cerebellar stimulus. Stimulus interval (msec) is indicated alongside other records. Time calibration at top right applies to all records in top row.

The form and properties of the responses in the dorsal accessory olive have been described in a previous paper (Armstrong *et al.* 1967), but it is important to note that they involved discharges in the same neurones that were antidromically invaded from the cerebellar surface. The records and the graph of Fig. 8 show that, at stimulus intervals of between 20 and 30 msec, an antidromic mass response was almost completely blocked by a preceding response to strong stimulation of quadriceps nerve. At slightly longer intervals (30–40 msec) the antidromic response recovered completely, but a second phase of depression began at a stimulus interval of about 40 msec (see below). The time course of the first depression was consistent with its being produced by the collision of the antidromic volley with orthodromic impulses set up by nerve stimulation, and single unit studies have supported this interpretation. Figure 9*A* and *B* show responses of a typical unit to a quadriceps stimulus followed by a cerebellar stimulus. In *A*, the quadriceps stimulus failed to fire the cell, and an antidromic spike followed the cerebellar stimulus. In *B*, the quadriceps stimulus evoked an orthodromic spike; the antidromic spike then failed to appear. This blockage of antidromic invasion was absolutely dependent on the presence of appropriately timed orthodromic spikes.

It was shown in several preparations that the same mass response evoked from the cerebellum could be powerfully blocked by the orthodromic response to stimulation of each limb nerve used (quadriceps, sural and gastronecmius-soleus nerves and the posterior nerve to the knee joint). This confirms the conclusion, reached by Armstrong *et al.* (1968), that the different nerves discharge heavily overlapping populations of olivary neurones.

It has been demonstrated that orthodromic responses in the dorsal accessory olive are followed by a period during which a second response is profoundly depressed (Armstrong *et al.* 1968). The second phase of depression in Fig. 8 indicates that, during this period, a test antidromic field potential was also reduced in amplitude. This decline in the amplitude of the field potential was also investigated by recording from single olivary neurones. Figure 9*C* shows a typical unit discharged by a quadriceps volley and then responding with a very small antidromic spike to a cerebellar stimulus delivered 41 msec after the quadriceps stimulus. Figure 9*D* shows this antidromic spike recorded at a much faster sweep speed, while *E* shows the antidromic spike which was recorded in the absence of the conditioning stimulus to quadriceps nerve. These records show clearly that the conditioning stimulus brought about a failure of *A*–*B* transmission of the antidromic action potential. This phenomenon was observed in all units investigated for its occurrence; the probability of block occurring in successive trials was greatest for stimulus intervals of 50–80 msec and

declined at longer intervals. It seems likely that the depression of the antidromic field potential can be attributed to the occurrence of *A–B* block in many individual olivary neurones, presumably as a consequence of hyperpolarization produced by the inhibitory synaptic actions which are exerted on the cells during this period (Armstrong *et al.* 1968).

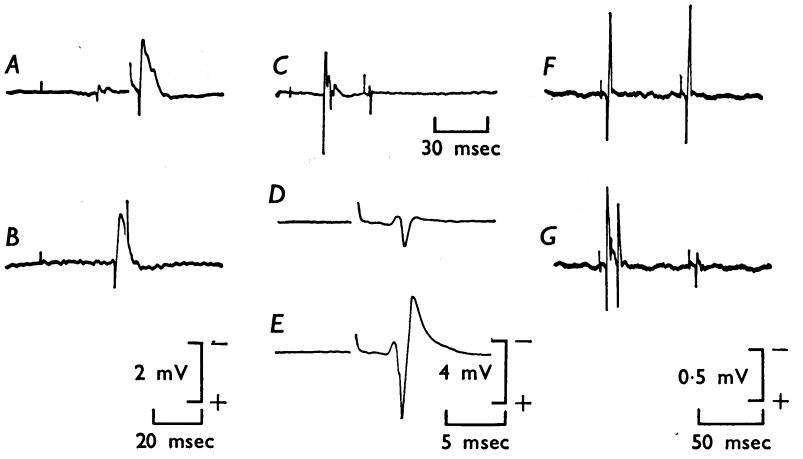

Fig. 9. *A*, *B*. Responses of a single olivary neurone to a single cerebellar stimulus preceded by a single quadriceps stimulus. In *A*, the quadriceps stimulus failed to fire the unit, but in *B* a spike evoked by the quadriceps stimulus blocks the antidromic spike. *C–E*. Responses of another single olivary neurone. *C*. Response to a quadriceps stimulus giving rise to *A–B* block in the antidromic spike produced by a cerebellar stimulus 41 msec later. *D*. The antidromic spike of *C* on a faster time scale. *E*. Control antidromic spike. *F*, *G*. *A–B* block produced in an antidromic spike by two preceding cerebellar simuli (*G*) but not by one (*F*). Time calibration below *E* applies to *D* and *E*.

Figure 9*F* and *G* illustrate the finding that *A–B* block could also be produced by conditioning stimuli delivered to the cerebellum. In the unit shown, a full sized antidromic spike was evoked when the test stimulus was preceded at an interval of 61 msec by a single conditioning stimulus. When, however, two conditioning stimuli (9 msec apart) were employed, the test stimulus evoked only an *A* spike. When mass antidromic responses were used as test, it was found that a single conditioning shock did in fact produce some depression of the antidromic field potential, indicating that *A–B* block occurred in some cells, but the depression was much larger following a pair of conditioning stimuli.

Simultaneous recording from the dorsal accessory olive and the vermis of the anterior lobe. If the cerebellar surface responses are due to activation of Purkinje cells by the climbing fibres, then the onset of olivary discharges should precede the onset of the cerebellar surface waves by an interval of

3–4 msec. A number of determinations were made by Armstrong *et al.* (1968) of the latency of the onset of olivary discharges following stimulation of the nerves used here, and the following ranges were obtained: c14–23 msec for stimulation of quadriceps nerve; 24–29 msec for gastrocnemius-soleus nerve; 21–30 msec for sural nerve, and 24–33 for the posterior nerve to the knee joint. It is obvious that the variations in

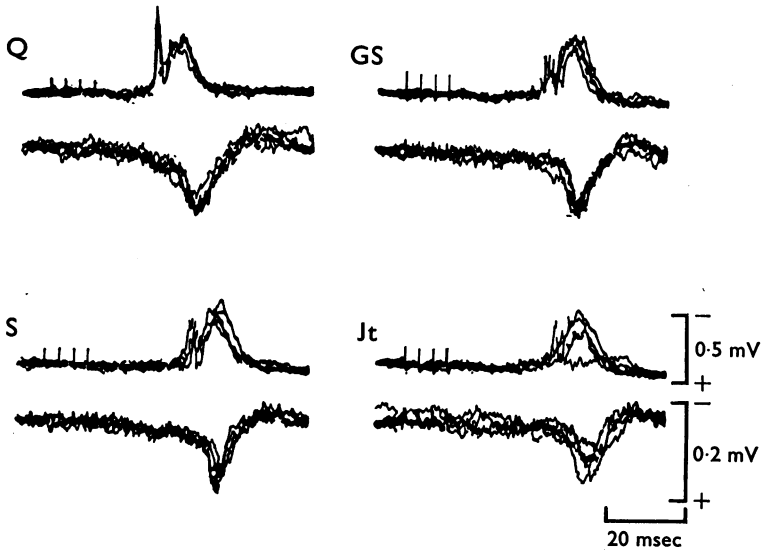

Fig. 10. Simultaneous recordings from dorsal accessory olive (upper traces) and contralateral side of vermis of anterior lobe (lower traces). Nerves stimulated with four stimuli at 300/sec. Stimulus intensity approx. 10 *T*. Upper voltage calibration applies to upper traces, lower calibration to lower traces

latency from one preparation to another were such that very many experiments would be required to establish reliably the predicted difference between the means of the above latencies and the mean latencies determined for the corresponding cerebellar responses in separate preparations. Therefore, in four experiments, we have recorded simultaneously from the olive with a micro-electrode, and from the surface of the cerebellum with a silver ball electrode. Figure 10 shows the responses obtained in a typical preparation by stimulating quadriceps, gastrocnemius-soleus and sural nerves and the posterior nerve to the knee joint. The upper traces are olivary responses and the lower traces are the simultaneously recorded cerebellar responses. The onset of the olivary activity evoked from each nerve precedes the corresponding positive potential on the cerebellar surface by an interval of about 5 msec.

DISCUSSION

The field potentials which we have recorded from the caudal parts of the dorsal and medial accessory olives, following stimulation of the vermis of the anterior lobe, were very similar in form and latency to those evoked in the ventral lamella of the principal olive by stimulation of the paramedian lobule (Armstrong & Harvey, 1966). The accessory olives form fairly compact groups of neurones, as does the medial part of the ventral lamella, and, in these three regions, the antidromic field potentials were always negative. Positive responses, such as those recorded by Armstrong & Harvey (1966) near the lateral part of the ventral lamella, where the neurones form a thin lamina, were not seen in the accessory olives.

Antidromic action potentials evoked in single units during periods when the olivary neurones were subject to inhibitory synaptic actions often consisted only of *A* spikes. In this, the dorsal accessory olive differs from the ventral lamella of the principal olive, where neither antidromic spikes of single units, nor antidromic field potentials were significantly changed by inhibitory influences (see Armstrong & Harvey, 1966). This difference in the safety factor for *A–B* transmission may be associated with morphological differences between the cell types in these two subdivisions of the olive. According to Scheibel & Scheibel (1955), cells in the accessory olives are mainly of a type with long unramified dendrites while those in the principal olive have a very compact and extensively branched dendritic arbor.

Antidromic field potentials evoked by stimulation of the anterior lobe vermis were found in the caudal parts of both the dorsal and medial accessory olives, and the responsive regions correspond well with the description by Brodal (1940) of the areas projecting to the vermis of Larsell's Lobuli IV and V (Larsell, 1953), which together make up Lobulus IV in Brodal's diagrams. Our failure to find orthodromic responses to nerve stimulation in the medial accessory olive was unexpected in view of the anatomical results of Brodal *et al.* (1950), and the responses which Morin, Lamarche & Ostrowski (1957) found here. Anderson & Berry (1959), stated that the majority of afferents from the lumbar spinal cord terminate in the dorsal accessory olive, but, more recently, Mizuno (1966) has confirmed the findings of Brodal *et al.* (1950). We have found, however, that stimulation of the spinal cord or dorsal roots in the lumbar regions evoked field potentials in both accessory olives, so that it is possible that the medial accessory olive is excited by afferents from the posterior primary rami of the spinal nerves. Further investigation of this problem is obviously required. The largest responses to nerve stimulation were found in the lateral portion of the dorsal accessory olive, and this is consistent with the

observations of Brodal *et al.* (1950) and Anderson & Berry (1959) who state that terminations of spinal afferents are more numerous in the lateral part of this subdivision.

In the present investigation, we have stimulated hind limb nerves and have recorded responses from the surface of the anterior lobe of the cerebellum. However, we have only studied that component of the responses which corresponds to 'potential *y*' of Morin (1956) and Morin, Catalano & Lamarche (1957). We consistently found that the latency of the response from quadriceps nerve was several milliseconds shorter than the latency from the other nerves tested, but we did not find any consistent latency differences between responses from the cutaneous, muscular and articular branches of the sciatic nerve. This is in contrast with the results of Morin & Haddad (1953) who found that 'the latency of the potentials elicited from nerves to muscles and joints is consistently larger than the latency of the potentials evoked by stimulation of other mixed and cutaneous nerves'. Our latencies agree well with those found by McIntyre (1951), Combs (1954) and Laporte *et al.* (1956), and with those reported by Morin & Haddad (1953) and Haddad (1953) for stimulation of muscle and joint nerves. However, the values given by the last authors for cutaneous nerves, and the values given by Dow (1939), Grundfest & Campbell (1942) and Carrea & Grundfest (1954) are less than those which we have found.

In addition to recording from the surface of the anterior lobe, we have used micro-electrodes to record extracellular field potentials at different depths within the cerebellar cortex, and also to record from individual Purkinje cells. The results show that 'potential *y*' coincides in time both with climbing fibre responses in individual Purkinje cells and with a pattern of extracellular field potentials within the cortex very similar to that set up by electrical stimulation of the inferior olive (Eccles *et al.* 1966). The potentials set up on the surface of the cerebellum by electrical stimulation of the inferior olive closely resemble 'potential *y*', the negative-positive surface waves illustrated by Eccles *et al.* (1966), being typical of responses recorded peripheral to the main focus of activity (J. C. Eccles & K. Sasaki, personal communication). Stimulation of the region of the anterior lobe vermis where 'potential *y*' is maximal leads to antidromic invasion of the same olivary neurones which are discharged orthodromically by nerve stimulation. Simultaneous recording from the dorsal accessory olive and the anterior lobe vermis shows that the onset of olivary activity precedes the onset of 'potential *y*' by about 5 msec, which is comparable with the conduction time in many olivo-cerebellar fibres, as indicated by the latency of the antidromic responses in the olive. The above evidence leads us to support the conclusion of Morin, Catalano & Lamarche (1957) that 'potential *y*' signals simultaneous excitation of

many Purkinje cells, and the suggestion by McIntyre (1951) and by Morin & Haddad (1953) that this excitation is relayed in the inferior olive.

We would like to thank Sir John Eccles for his continued advice and encouragement.

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