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Mossy and Climbing Fibre Organization on the Anterior Lobe of the Cerebellum Activated by Forelimb and Hindlimb Areas of the Sensorimotor Cortex

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Summary. Stimulation of forelimb and hindlimb areas of the sensorimotor cortex in the cat evokes in the lobus anterior of the cerebellum an early response at latency of 3—3.5 msec due to the mossy fibre input (MF) and a later response at latency of 13—16 msec due to the climbing fibre (CF) input.

In the pars intermedia these two types of responses are organized in a somatotopic manner: the hindlimb area projects in lobuli HIV and HIII whereas the forelimb area projects to lobulus HV. In the vermis a somatotopic arrangement is less clear. Both forelimb and hindlimb areas of the sensorimotor cortex project to lobuli III, IV and V: on a maintained somatotopy in a caudo-rostral direction there is a tendency for the hindlimb area of the sensorimotor cortex to be well represented in a longitudinal strip close to the paravermal sulcus. This arrangement in the vermis is evident for the CF pathways, but more difficult to demonstrate for the MF pathways.

The forelimb area of the sensorimotor cortex projects to those areas of the lobus anterior impinged upon by the forelimb nerves through both the MF and CF systems and the same holds true for the hindlimb area and the hindlimb nerves.

Key Words: Cerebellum — Cerebrum — Somatotopy

Introduction

Information on the anatomy of the connections from the cerebrum to the cerebellum has been well summarized by JANSEN and BRODAL (1954, 1958). There are two main pathways by which the cerebral cortex may send information to the cerebellum: one is via the pontine nuclei and the other is via the inferior olive. In addition, there is a pathway relayed by the three reticular nuclei which are known to project to the cerebellum, i.e., the lateral reticular nucleus (see also BRODAL et al. 1967), the paramedian nucleus and the nucleus reticularis tegmenti pontis. There is evidence that the cortico-olivo-cerebellar pathway terminates as climbing fibres (CF) (SZENTÁGOTHAI and RAJKOVITS 1959; JANSEN and FANGEL 1961; ARM-

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STRONG and HARVEY 1966; ECCLES, LLINÁS and SASAKI 1966). The cortico-ponto-cerebellar and the cortico-reticulo-cerebellar pathways, in contrast, seem to end as mossy fibres (MF) (SNIDER 1936; SZENTÁGOTHAI and RAJKOVITS 1959; JANSEN and FANGEL 1961; SASAKI and STRATA 1967). The pathway through the pontine nuclei is faster than that through the inferior olive (DOW 1939; JANSEN 1957; JANSEN and FANGEL 1961; ARMSTRONG and HARVEY 1966).

Many papers have been devoted to the study of cerebro-cerebellar connections by applying an electrical stimulus to the cerebral hemispheres and recording the activity evoked in the cerebral cortex (DOW 1939, 1942; CURTIS 1940; ADRIAN 1943; SNIDER and ELDRED 1948, 1951; HAMPSON 1949; ALBE-FESSARD and SZABO 1954, 1955; JANSEN 1957; JANSEN and FANGEL 1961; DEURA 1961; KENNEDY et al. 1966). Only a few of these papers were devoted to studying the somatotopic organization of these connections. The first attempt to investigate this problem was made by ADRIAN (1943). He concluded that in the monkey, under barbiturate anaesthesia, the projections from the sensorimotor area of the cerebral cortex to the anterior lobe of the cerebellum showed a clear somatotopic arrangement; the hindlimb area projected to the contralateral lobulus centralis, the forelimb area to the culmen and the face area to the lobulus simplex. This type of somatotopic projection in a rostro-caudal direction has been confirmed (SNIDER and ELDRED 1948, 1951; HAMPSON 1949). In addition a somatotopic projection has also been found to the lobulus paramedianus (SNIDER and STOWELL 1944). However, no attempt has ever been made to find out whether this somatotopic organization is due to the pathway terminating in the cerebellum as CF or to those pathways terminating as MF, or to the effects of both systems. This problem would seem to be very important in understanding cerebellar function. Recent papers have pointed out that the CF responses evoked in the lobus anterior by peripheral nerve stimulation follow a somatotopic pattern organized in sagittal strips (OSCARSSON and UDDENBERG 1966; OSCARSSON 1967a, 1968). On the other hand it is known that some of the pathways terminating as MF are organized with a somatotopic pattern in a rostro-caudal direction (GRANT 1962; see OSCARSSON 1967b). As a result, a somatotopic system has been proposed, in which the somatotopy of the MF and CF inputs is orthogonal (OSCARSSON 1967b).

One of the aims of this work has been to investigate the projections from the sensorimotor area of the cerebral cortex to the anterior lobe of the cerebellum, and in particular, to study the relative organization of the two pathways ending as MF and CF. We were assisted in this aim by the known pattern of field potentials generated by a MF and CF input in the depth of the cerebellar cortex (ECCLES, LLINÁS and SASAKI 1966; ECCLES, SASAKI and STRATA 1967; SASAKI and STRATA 1967).

ADRIAN (1943) pointed out that the somatotopic organization of the cerebro-cerebellar connections is the same as that evoked by stimulation of peripheral nerves, i.e., the same area of the cerebellum receives information from both hindlimb nerves and from the hindlimb area of the motor cortex; this organization also applied to both forelimb and face nerves. Therefore, in the present investigation an attempt has also been made to correlate the results obtained in the anterior lobe by cortical stimulation with those obtained by stimulation of peripheral nerves (OSCARSSON and UDDENBERG 1966; OSCARSSON 1967a, 1968; ECCLES et al.

1968b). On anatomical grounds, an overlap between cortical and peripheral projections can be explained only for the MF system, where it is known that cortico-ponto-cerebellar pathways and the spino-cerebellar and the spino-reticulo-cerebellar pathways all project to most of the lobus anterior. In contrast the cortico-olivo-cerebellar and the spino-olivo-cerebellar pathways are held to terminate separately in the lobus anterior: the former in the pars intermedia, the latter in the vermis (JANSEN and BRODAL 1958). Our results do not support this conclusion. Instead, they show that the overlap between cortical and peripheral input is present at the level of both MF and CF systems.

A preliminary note has been published (PROVINI et al. 1967).

Methods

The experiments have been performed on 10 adult cats. They were anaesthetized with pentobarbital sodium (35 mg/kg) administered intraperitoneally; small additional doses were given intravenously as required. No paralyzing agents have been used and the animals were not artificially ventilated. The right femoral artery was cannulated in order to monitor the blood pressure, which was maintained at mean values of 100—110 mm Hg by the use of metaraminol bitartrate administered intravenously and intramuscularly. When it was not possible to maintain the blood pressure above 80 mm Hg the experiment was discontinued.

The left superficial radial and median nerves of the forelimb, as well as the tibialis nerve of the hindlimb were dissected and stimulated in a pool of warm paraffin oil with a strength 6 times the threshold, in keeping with the technique used in other investigations (ECCLES, PROVINI, STRATA and TÁBOŘÍKOVÁ 1967, 1968a). The right sigmoid gyrus was exposed by removing the overlying bone and dura. Bipolar silver ball electrodes with a 2 mm polar separation were used to stimulate the forelimb and hindlimb areas of the sensorimotor cortex. These areas were located by recording monopolarly the primary potentials evoked by stimulation of the dissected peripheral nerves. The pole used for recording was kept as a cathode, while the other pole was used as an anode and placed 2 mm above. One to three stimuli, usually two, were delivered from a condenser discharge stimulator with a time constant of 0.1 msec. A stimulus strength which was sufficiently high to give a well defined potential on the cerebellar cortex was selected at the beginning of the experiment and maintained constant throughout it. In some experiments several strengths were used in order to compare the effect of different intensities of stimulation. The stimulation of the sensorimotor cortex has always been below the threshold for evoking any detectable movement to the limb in that particular experimental condition.

Finally, part of the occipital bone was removed to expose a few cerebellar folia in front of the fissura prima, which were then covered with agar 3 % in saline. Recordings were taken from the cerebellum with a glass micropipette filled with 4M NaCl and with resistance of 2—3 MΩ. The micropipette was inserted at different angles in order to explore conveniently most of the lobus anterior. One or more micropipettes were cut and left *in situ*. At the end of the experiment, the cerebellum was fixed with formalin 10%, then dehydrated and included in celloidin. Before the inclusion, the micropipettes were removed. In this way, reference tracks were left in the cerebellum, which was sectioned in slices of 100 μ thickness, cut in a sagittal plane and stained with the Nissl method. The reference tracks were easily identified under the microscope. The other tracks were usually identifiable from small lesions left by the micropipette during each exploration and from this data, in conjunction with the depth of the microelectrode at each recording, the actual points at which records were taken could be identified with considerable accuracy. The identification of the lobuli follows the criteria of LARSELL (1953). In some cases the results were mapped on an unfolded cerebellum. To do this, photographs of histological sections were taken. The surface of the cerebellum was measured with a string on some enlarged photographs from sections and the position of each sulcus dividing each lobulus was marked on the string. By plotting these positions on a plane with the string outstretched it was easy to build a map of the unfolded cerebellum and to plot the position from which the various potentials were recorded (see Fig. 5).

Results

I. Description of the Potentials Evoked in the Cerebellum by Stimulation of the Sensorimotor Cortex. The events produced in the depth of the cerebellar cortex by pure MF or CF inputs have been analyzed in detail (Eccles, Llinás and Sasaki 1966; Eccles, Llinás, Sasaki and Voorhoeve 1966; Eccles, Sasaki and Strata 1967; Sasaki and Strata 1967). A stimulus applied to a peripheral nerve produces a more complex pattern of waves which however can be recognized and identified as MF or CF-evoked field potentials. Details of this identification and of the relationship to the histological structure and to the pattern of the foliation of the cerebellum are being published (Eccles et al. 1968a).

When the sensorimotor cortex is stimulated, the pattern of the positive and negative waves is similar to that obtained by peripheral stimulation. There is an early response at a latency of 3—3.5 msec, which can be recognized as the field evoked by the MF input and a later response at a latency of 13—16 msec having the characteristics of the fields evoked by the CF input. Fig. 1 shows the field potential evoked in the left lobus anterior following stimulation of the right sensorimotor area of the cerebral cortex.

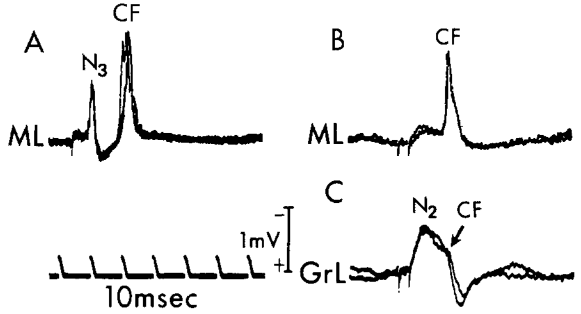


Fig. 1. *Field potentials recorded in the cerebellar cortex following stimulation of the contralateral sensorimotor cortex.* A shows a record from a molecular layer (ML); the first negative wave (N_3) has a latency of 5 msec and represents the response evoked through the MF pathways. The second negative wave (CF) has a latency of 15 msec and is mediated by the CF pathways. B and C have been recorded, in a different experiment, from a molecular (ML) and a granular layer (GrL) respectively. The MF input evokes only a small negative deflection in the ML which has the same latency of the N_2 wave of the underlying GrL. The typical field potential recorded in the granular layer is shown in C, where the MF input gives a large N_2 wave at a latency of 3.3 msec and the CF field appears as the main positive wave. Note in C the absence of a P_2 wave, which should appear following immediately the N_2 wave

In Fig. 1A the microelectrode was recording from a molecular layer (ML) and the MF input generated an unusually large N_3 wave with a latency of 4.5 msec, while the CF input generated a negative field (CF) with a latency of 15 msec. Usually, however, the MF input did not evoke any potential in the molecular layer or it gave rise to a very small negative wave with a latency of 3—3.5 msec as represented in Fig. 1B. It will be recognized that this field potential has the same latency as the N_2 wave of the corresponding granular layer (GrL) as illustrated in Fig. 1C. It will also be noted that the peaks of these negative waves have the same latency so that the small negative wave recorded in the molecular layer of Fig. 1B seems to have more of the characteristics of an N_2 wave than of an N_3. For these reasons, the negative wave recorded in the molecular layer could not be used as a measure of the MF input. Similarly, the P_2 wave generated in the granular layer by the MF input was usually small and not clearly defined. A P_2 wave, which is due to the activity of the axons of the granule cells in the molecular layer, should appear as a positivity following immediately the N_2 wave in the granular layer. In contrast, the N_2 wave of the same layer was well defined, and this field potential was taken as a measure of the MF input.

The CF input generates a negative wave in the lower part of the molecular layer (Fig. 1A and B) which reverses into a large positivity in the superficial part of the molecular layer and into a mainly positive wave in the granular layer, where it is superimposed on the MF evoked field (Fig. 1C). The negative wave in the molecular layer provides the best measure of the CF input (cf. Fig. 1A and B).

The N_2 wave in the granular layer is evoked directly by the synaptic action of the MF input and similarly the late negative wave in the molecular layer is directly

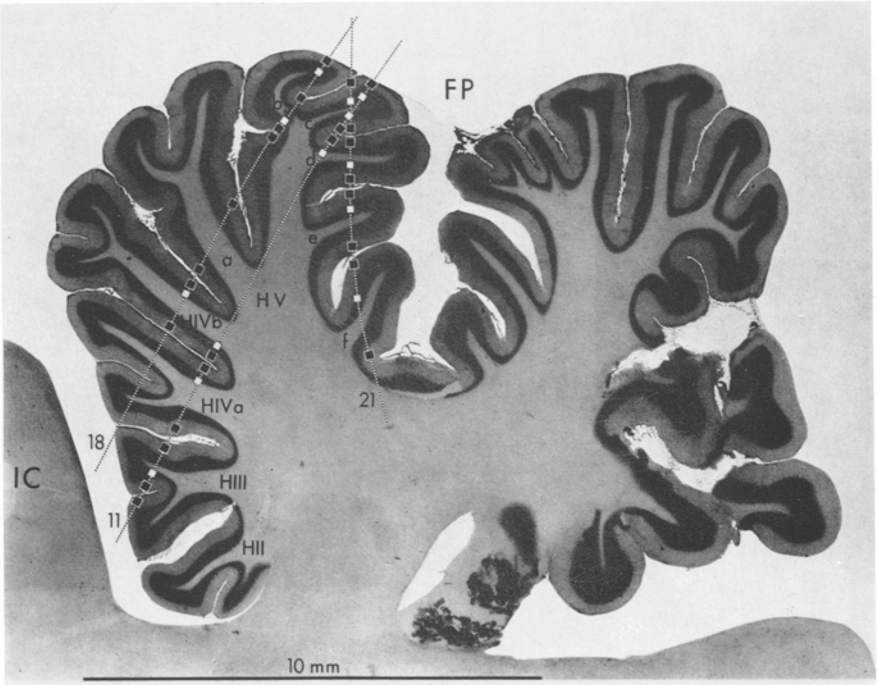


Fig. 2. *Sagittal section of a cerebellum with the position of three tracks as reconstructed from the small lesions left by the microelectrode during the penetration.* The section was taken in a sagittal plane passing 0.8 mm from the lateral edge of the left paravermal vein in correspondence of lobulus HVc. It shows the position of three tracks performed during the experiment which provided the results of Fig. 4. All tracks of Fig. 4A—B, as well as those of Fig. 4C—D, were made in the same transverse planes as tracks 11 and 18 respectively. The vertical track 21 was made, together with four others, in the same transversal plane, in order to explore lobuli HVc, HVd, HVe and HVf. The tracks have been slightly deformed because of the change in shape of the cerebellum post mortem before fixation or during the histological preparation. On each track the positions are indicated where the negative field produced by the CF (filled squares) and the N_2 wave evoked by the MF (empty squares) were recorded and measured. The cerebellar folia are labeled following Larsell's nomenclature. IC: inferior colliculus; FP: fissura prima

produced by the CF input. Since these potentials thus measure the first post-synaptic event generated by the two input systems they provide an exact location of their projection. The latency for the N_2 wave is rather consistently around 3—3.5 msec, while that for the CF negative wave is around 13—16 msec. Occasionally small potentials with the feature of a CF response were found at a much longer

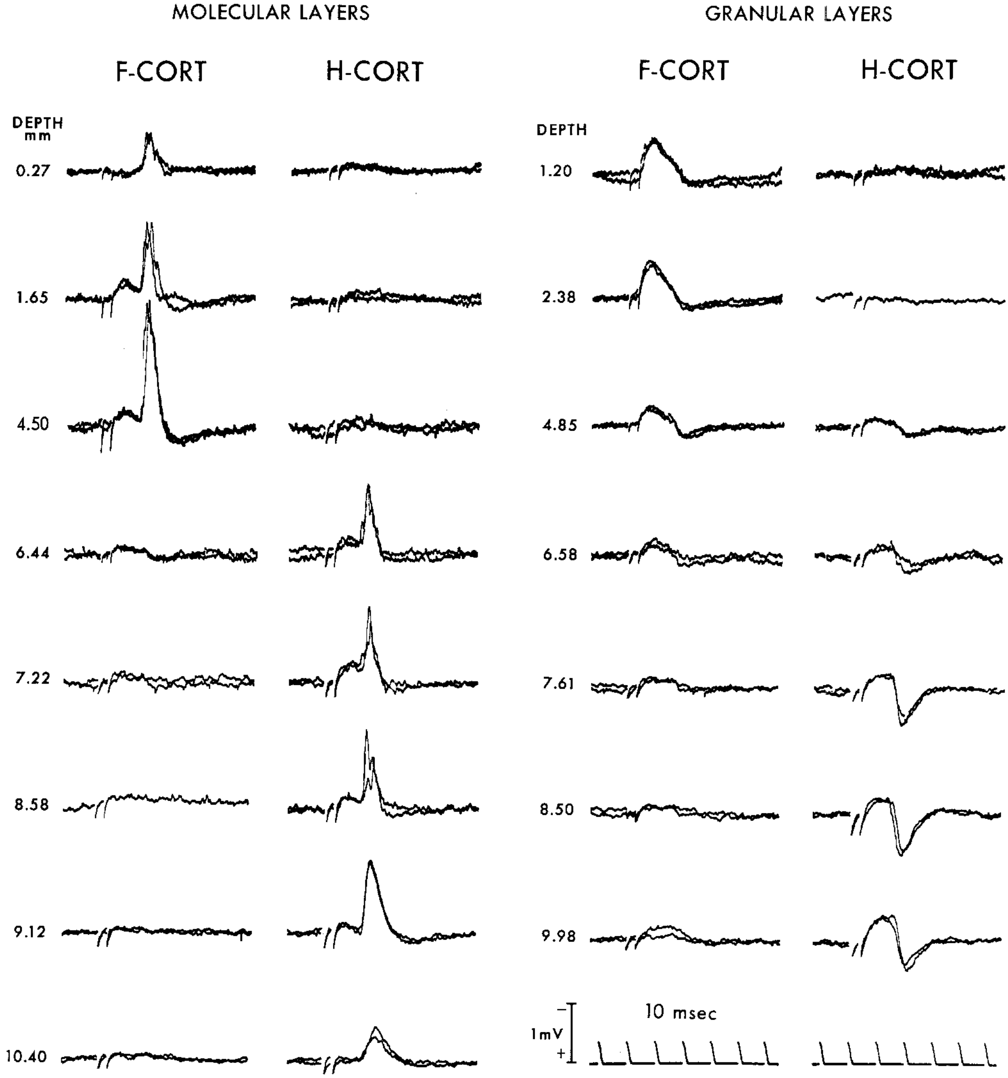
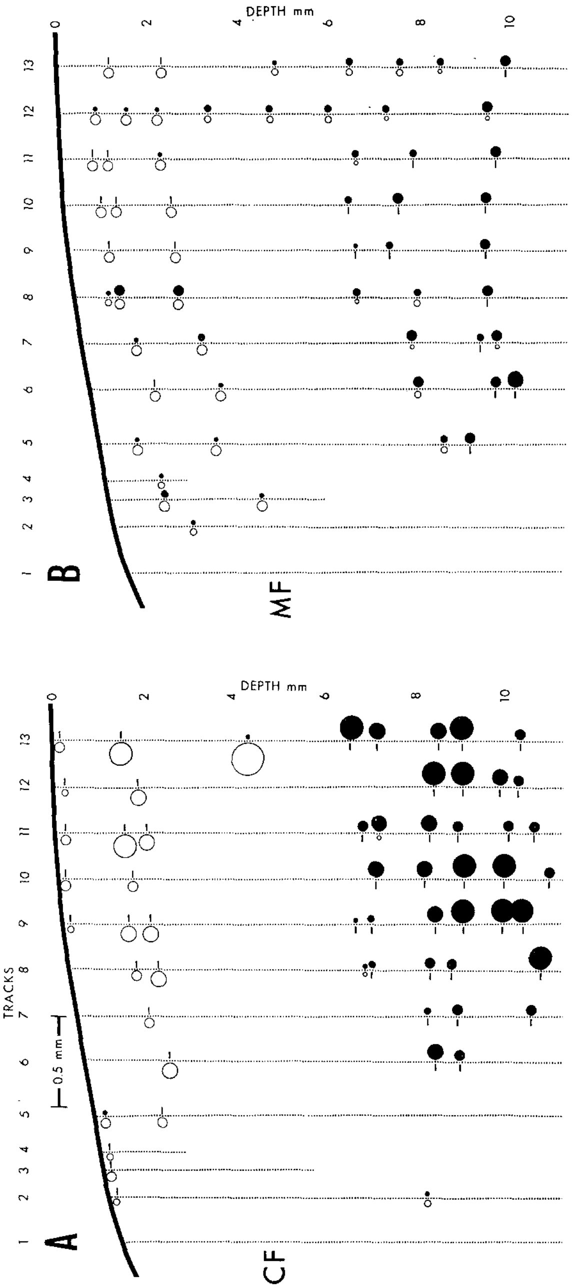


Fig. 3. *Field potentials recorded during the penetration of a microelectrode track.* The field potentials recorded in track 13 of Fig. 4, in the pars intermedia of the anterior lobe, are displayed with the actual depths in mm from the cerebellar surface at the point of entry. The field potentials recorded in molecular and granular layers have been separated into two different sequences, as indicated. At each depth the forelimb area (F-CORT) and hindlimb area (H-CORT) of the sensorimotor cortex were stimulated. Note that the forelimb sensorimotor area evoked field potentials through both climbing and mossy fiber inputs in the superficial layers of the track, whereas the hindlimb area projected only deeper

latency of 25—30 msec; they have not been taken into consideration because of the possibility that they represent transmission through less direct pathways.



II. Somatotopic Projections from the Cerebral Cortex to the Anterior Lobe of the Cerebellum. The organization of the projections to the pars intermedia of the lobus anterior has been found to be rather different to that of the vermis. For this reason the results will be presented separately.

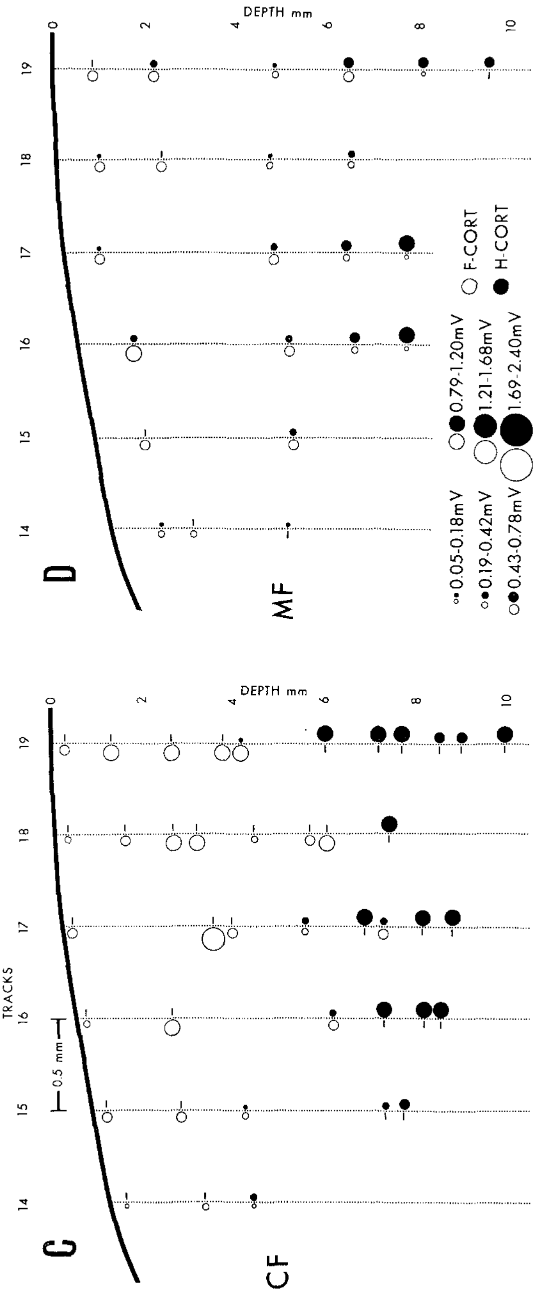


Fig. 4. *Organization of climbing fibre (CF) and mossy fibre (MF) inputs to the left pars intermedia of the anterior lobe of the cerebellum from the right sensorimotor cortex.* The two plotted series of tracks (1—13 and 14—19) were made in the same experiment in the two transverse planes of tracks 11 and 18 of Fig. 2. CF evoked responses are displayed in A und C, while MF responses are displayed in B and D. The circles mark the size and the depth, in mm from the cerebellar surface, of the recorded field potentials evoked from the forelimb (F-CORT, empty circles) or the hindlimb (H-CORT, filled circles) area of sensorimotor cortex

a) Pars Intermedia: it has been a consistent finding in all five experiments in which the pars intermedia has been investigated that forelimb and hindlimb areas of the cerebral cortex project to well defined areas of the contralateral pars intermedia. This holds true for the projections of both the MF and CF systems. In particular the forelimb area projects to the more caudal part corresponding to lobulus HV, while the hindlimb area projects to a more rostral part, corresponding to lobuli HIII and HIV. Lobuli I and II have a very small representation, particularly in the pars intermedia and very few records have been obtained from them in this investigation.

The results of a typical experiment are documented in Figs. 2—5. Fig. 2 shows a sagittal section of a cerebellum passing in the lobulus HVc 800 μ lateral to the edge of the vein which lies on the left paravermal sulcus. The dotted lines indicate the position and the direction of 3 tracks (Tracks 18, 11, 21), which were all made in this plane. Other tracks were made in directions parallel to each of the 3 tracks shown and along the same folia with separations of usually 250 μ or 500 μ. By this tracking method most of the pars intermedia of the lobus anterior could be conveniently explored. When the recording electrode was penetrating the cerebellum, a series of molecular, Purkinje and granular layers were encountered and recognized on the basis of their typical evoked activity. The filled squares of Fig. 2 indicate the places where the microelectrode recorded fields from molecular layers and the empty squares indicate where the fields of granular layers were recorded.

The fields recorded in a track which was made in the same transverse plane as track 11 of Fig. 2, but 500 μ medially are reported in Fig. 3. This shows that microelectrode recordings were first obtained from 3 molecular layers at depths of 0.27, 1.65 and 4.50 mm, and from 3 granular layers, at depths of 1.20, 2.38 and 4.85 mm, which were receiving respectively CF and MF projections from the forelimb area of the sensorimotor cortex. At all the other depths CF and MF responses were evoked almost exclusively by the hindlimb area except for the granular layer at 6.58 mm depth, where the projections from both cortical areas were overlapping.

This type of distribution with the forelimb cortex projecting more superficially and the hindlimb cortex more deeply was typical of all tracks made in the transverse planes of tracks 18 and 11 shown in Fig. 2. The results are summarized in Fig. 4. Fig. 4A and B display the responses recorded along a series of 13 tracks in the transverse plane of track 11 of Fig. 2. Another series of 6 tracks was made in the transverse plane of track 18 and the results are shown in Fig. 4C and 4D. Five tracks were also made in the transverse plane of track 21 and in all of them the MF and CF responses were almost exclusively evoked from forelimb areas.

Figures 4A and 4C show the depths at which CF negative waves in molecular layers were encountered following stimulation of the contralateral sensorimotor cortex. The empty circles indicate the sizes and the positions of the CF responses evoked by the forelimb area of the sensorimotor cortex, while the filled circles indicate the size and the position of the CF responses evoked by the hindlimb area. Similarly Fig. 4B and D show the depths and the sizes of the N_2 waves evoked by the forelimb and hindlimb areas of the cerebral cortex, and recorded in the same tracks as those of Fig. 4A and 4C. It is evident that responses from the forelimb area of the cerebral cortex are distributed in the more superficial portion of each track, while those from the hindlimb area are distributed more deeply. This is evident for both CF and MF evoked potentials. It should be noted however, that the separation of the two regions is sharper for the CF responses than for the MF responses. In this particular experiment a total of 24 tracks was made and a total of 118 CF responses was recorded in different positions of the pars intermedia of the lobus anterior. The position of all these CF responses has been mapped in the unfolded cerebellum of Fig. 5. This map gives a clear demonstration of the somatotopic arrangement in a rostro-caudal direction and shows that forelimb cortex projections mainly to lobuli HV, while hindlimb cortex projects to lobulus HIV and HIII.

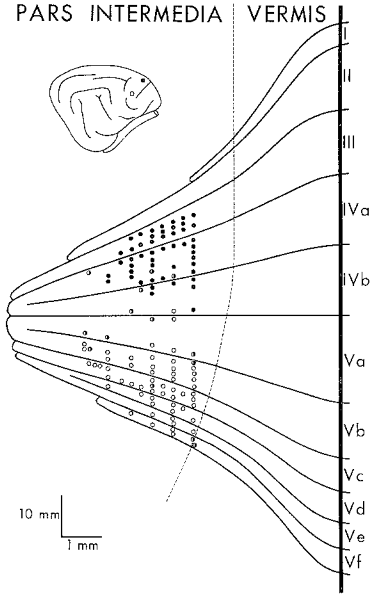
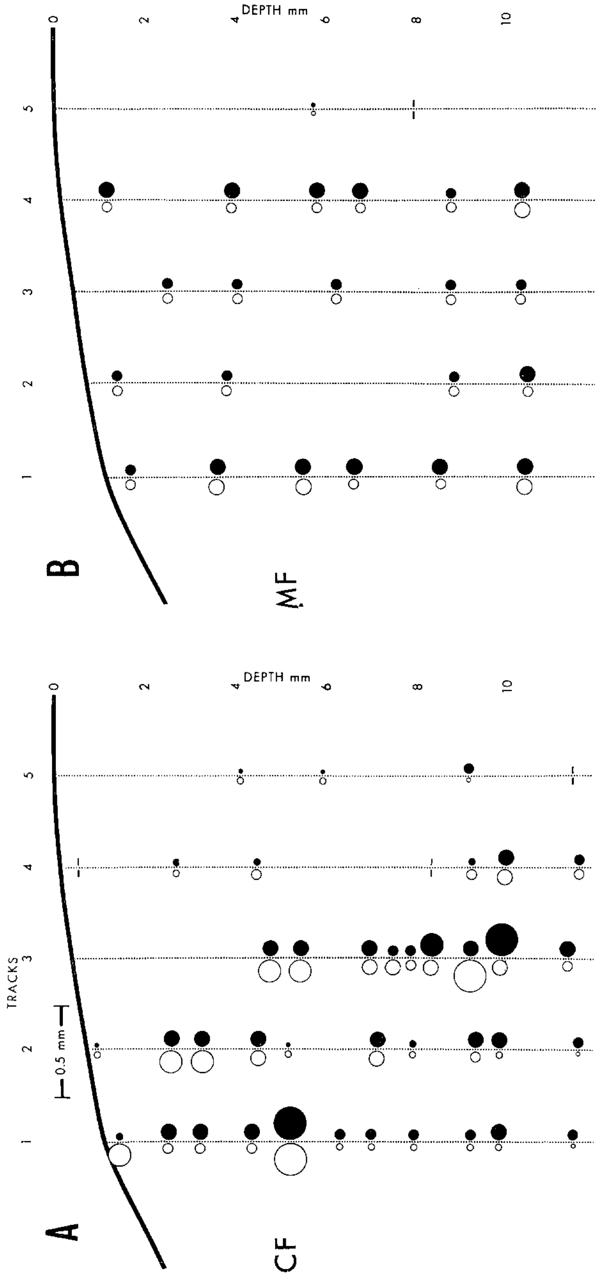


Fig. 5. *Unfolded cerebellar cortex of the left anterior lobe showing the climbing fibre projections from the right sensorimotor cortex to the pars intermedia.* The division between the five Larsell's lobuli is indicated as well as the position of the left paravermal vein (dotted line) which divides the vermis from the pars intermedia. On the latter, the position of each recorded field potential is marked by an empty circle or by a filled circle when the climbing fibre input was from the forelimb or the hindlimb sensorimotor area respectively. Overlapping projections are indicated by a half-filled circle

b) Vermis: with methods similar to those used in the pars intermedia, a series of microelectrode explorations have been performed in the vermis in eight experiments. Here the projections from forelimb and hindlimb areas of the cerebral cortex are not sharply separated and both project to lobuli III, IV and V. The projections from the hindlimb area tend to predominate in a longitudinal strip close to the paravermal sulcus, while those from the forelimb area are relatively larger in a longitudinal strip located 0.5—1 mm more medially. Within this strip, however, the forelimb area is better represented in lobulus V than in lobuli IV and III. In the region of the vermis close to the midline the evoked potentials are usually small and consequently, it is difficult to draw any conclusions on the relative distribution of the cortical afferents.

Figure 6 shows the results obtained in the left vermis following stimulation of the contralateral sensorimotor cortex. A total of 16 tracks was made along two transverse planes with an orientation similar to that of the two series of tracks documented in Fig. 4 for the pars intermedia. Fig. 6C shows that the CF projections evoked from the hindlimb area of the sensorimotor cortex predominate at almost all depths in tracks 6—8 (see also Fig. 7) which are the closest to the paravermal sulcus; on the contrary the forelimb area projections predominate at the majority of depths in tracks 9—12, but particularly in the more superficial section of each track. Fig. 6A shows similar results obtained in a series of tracks placed in a transverse plane 1 mm caudal to the tracks of Fig. 6C.

Figures 6B and 6D show the size and the distribution of MF fields as recorded in the same explorations. The MF somatotopy in the vermis is less sharp than the CF somatotopy, which also occurred for the pars intermedia. However, a tendency to repeat the type of arrangement seen for the CF distribution was usually noted. Another representation of the somatotopic arrangement of the sensorimotor cortex input via the CF pathway is given in Fig. 7 where for each depth of Fig. 6C only the predominant projection — either from the hindlimb area (filled diamonds) or from the forelimb area (empty diamonds) — is indicated. This emphasizes the areas where the hindlimb area or forelimb area of the sensorimotor cortex predominate. It should be noted, however, that while the predominance shown in



tracks 6—8 and 9—12 has been consistent in all the experiments, that of more medial tracks was variable.

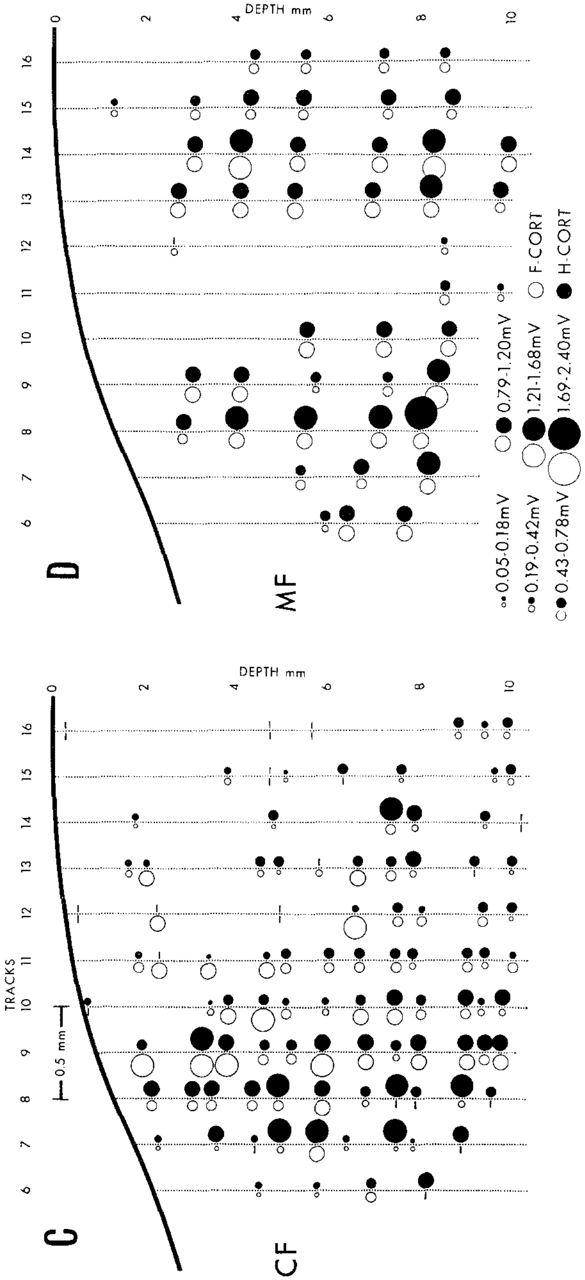


Fig. 6. *Distribution of climbing fibre (CF) and mossy fibre (MF) input to the left vermis of the anterior lobe of the cerebellum evoked from the right sensorimotor cortex.* Two series of tracks (1—5 and 6—16) have been placed with an orientation similar to the two series of tracks shown in Fig. 4, but in another experiment and in the vermis of the lobus anterior. The CF responses are shown in A und C, and the MF responses are shown in B and D. Symbols as in Fig. 4

III. Correlation Between Cortical and Peripheral Input to the Anterior Lobe. The results so far reported indicate that the topographical distribution of forelimb and hindlimb areas of the sensorimotor cortex on the cerebellar anterior lobe have some common features to those reported following stimulation of ipsilateral forelimb and hindlimb nerves (Oscarsson and Uddenberg 1966; Oscarsson 1967a, 1968; Eccles et al. 1968b). In order to obtain more information on the correlation between cortical and peripheral projections to the anterior lobe, we have compared their relative distributions by recording the evoked field potentials for both cortex and periphery. We chose the tibial nerve in the hindlimb because this nerve has the most powerful action on the cerebellar anterior lobe (Eccles, Provini, Strata and Táboříková 1967, 1968a, b). For the same reason we chose the ipsilateral median and superficial radial nerves in the forelimb.

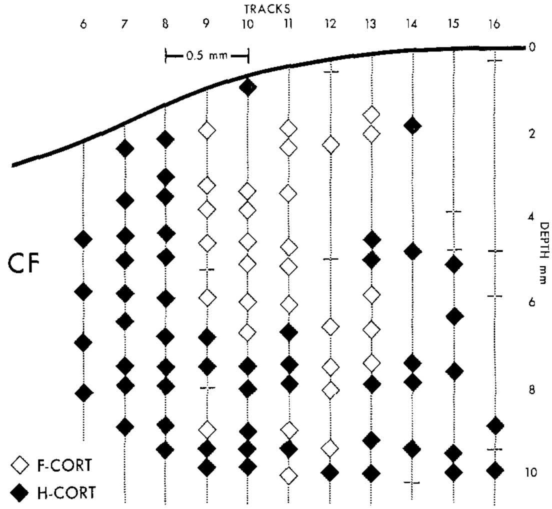


Fig. 7. *Simplified map of climbing fibre (CF) responses to indicate dominant response evoked in the vermis of the lobus anterior following stimulation of the forelimb and hindlimb areas of the sensorimotor cortex.* The tracks of Fig. 6C have been redrawn and at each depth a filled or an empty diamond indicates respectively whether the CF response evoked by the hindlimb area of the sensorimotor cortex was larger or smaller than that evoked by the forelimb area. The symbol — — indicates the depths at which no predominance was found

It has been a consistent finding that the forelimb area of the sensorimotor cortex projects to the same regions of the contralateral anterior lobe which are impinged upon by the ipsilateral forelimb nerves. This holds true for either the pars intermedia or the vermis.

Figure 8 gives a documentation for the pars intermedia. The series of tracks are the same as those shown in Fig. 4A. In Fig. 8A the empty circles indicate the sizes and the positions where CF responses were evoked by the forelimb area of the contralateral cerebral cortex, while the empty squares indicate the CF responses evoked by the ipsilateral median nerve. Fig. 8B shows the correlation between the contralateral hindlimb area of the sensorimotor cortex and ipsilateral tibial nerve represented respectively by filled circles and filled squares. At almost all depths where a CF response was recorded from the forelimb area of the cerebral cortex a CF response from the forelimb nerves could also be recorded. The same

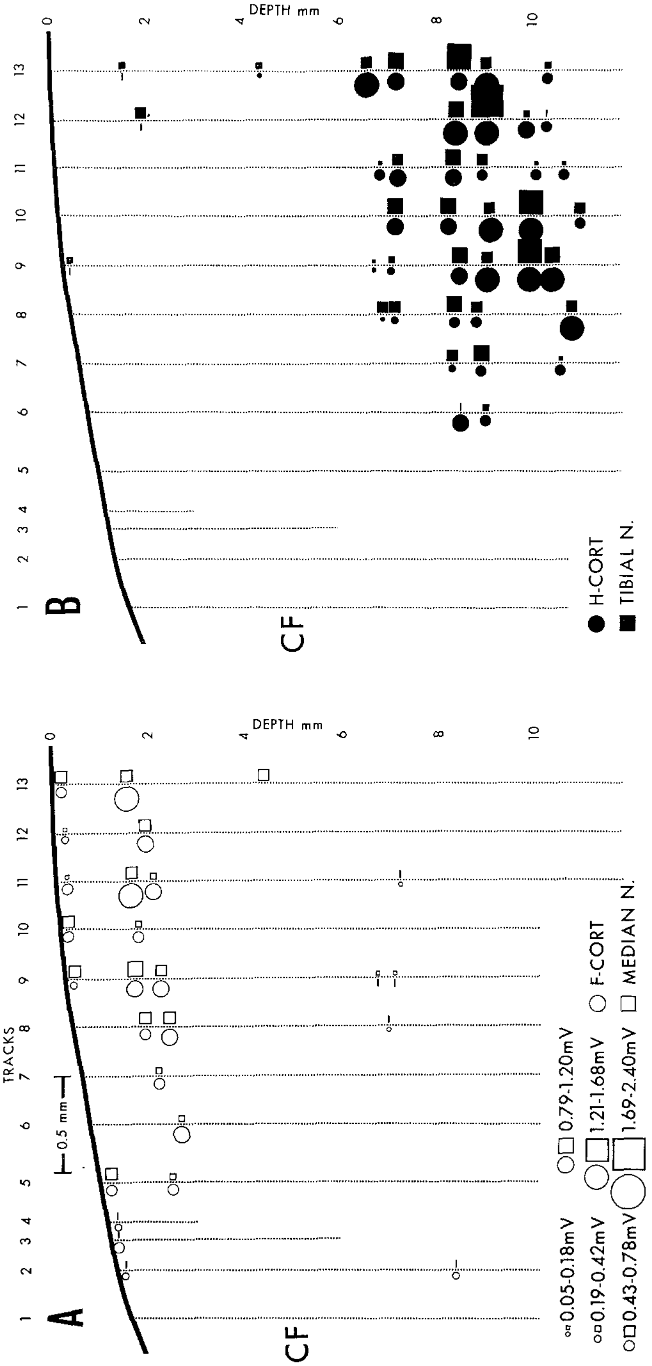


Fig. 8. *Convergence on the anterior lobe of cerebellum of climbing fibre (CF) projections from sensorimotor cortex and peripheral nerve stimulation.* A shows tracks 1—13 of Fig. 4A with the size of the field potentials evoked from forelimb area (F-CORT, empty circles) of the cortex compared with the responses to stimulation of the median nerve (MEDIAN N., empty squares) as recorded at the same microelectrode position. The potentials evoked from hindlimb area (H-CORT, filled circles) and from tibial nerve (TIBIAL N., filled squares) recorded during the same tracks, are displayed in B

was found for cerebral hindlimb area and hindlimb nerves. A similarly good correlation has been found for the MF responses evoked by cortical and peripheral nerve stimulation and recorded both in the pars intermedia and the vermis.

Discussion

MF and CF Inputs. It is known that stimulation of the cerebral cortex evokes in the cerebellum an early and a late response (Dow 1942; Jansen 1957; Jansen and Fangel 1961; Deura 1961). Jansen (1957) suggested that the early response at 2—6 msec latency could be due mainly to activity in the granule cell layer and consequently mediated by the MF system, while the long latency response at 12—15 msec latency could be due to the direct excitation of Purkinje cells and therefore mediated by the CF system. Jansen and Fangel (1961) gave support to this suggestion by showing that local stimulation of the cerebellum was able to block almost completely the early response, while the later response was largely unaffected.

The present study confirms that stimulation of the sensorimotor area of the cerebral cortex evokes in the contralateral lobus anterior of the cerebellum an early response, at a latency of 3—3.5 msec in our experiments, and a late response at 13—16 msec. In addition it shows that the early response has the depth profile in the cerebellar cortex typical of that evoked by a MF input (Eccles et al. 1967; Sasaki and Strata 1967) whereas the late response has a depth profile typical of that evoked by a CF input (Eccles, Llinás and Sasaki 1966; Eccles, Llinás, Sasaki and Voorhoeve 1966). These results give direct evidence that the early response is due to the MF input and the late one to the CF input.

While it is likely that the CF responses are mediated by the cortico-olivo-cerebellar pathway (Szentágothai and Rajkovits 1959; Eccles, Llinás and Sasaki 1966; Armstrong and Harvey 1966), the question whether one or both of the cortico-ponto-cerebellar and the cortico-reticulo-cerebellar pathways are responsible for the MF responses has not been considered in this investigation.

It was remarkable to find that in our experimental conditions the P_2 and the N_3 waves were usually very small or absent, despite the presence of a good N_2 wave. Since P_2 and N_3 waves are due to activity in parallel fibres and Purkinje cells while N_2 wave is due to activity in the granule and Golgi cells, the conclusion should be drawn that a marked excitatory action on the granule cells is not followed by a comparable initiation of impulse discharges. This conclusion is supported by our observations that in response to stimulation of the contralateral sensorimotor cortex Purkinje units were fired only occasionally by the MF input, whereas the CF input was very effective in evoking discharges. A possible explanation of this finding is given in another paper where similar results have been obtained by stimulation of peripheral nerves (Eccles et al. 1968a).

Somatotopy. A somatotopic arrangement with a caudo-rostral orientation has been reported as characteristic of the lobus anterior of the cerebellum following stimulation of different areas of the sensorimotor cortex (Adrian 1943; Hampson 1949; Snider and Eldred 1951). Our results show that this type of organization is clearly present only in the pars intermedia where on the other hand the lobus HV is almost entirely dominated by cortical projections from the forelimb area and on the other the lobuli HIV and HIII from the hindlimb area (Fig. 5). In addition the results demonstrate that this type of somatotopy obtains for both the MF and CF inputs.

In the vermis, the projections from forelimb and hindlimb areas are not sharply separated and both occupy lobuli III, IV and V. There is a tendency for the forelimb area of the sensorimotor cortex to give larger responses in lobulus V than in lobuli IV and III, but an organization may also be recognized in a latero-lateral orientation. Projections from the hindlimb area dominate in a longitudinal strip of 0.5—1 mm in width, extending in lobuli III, IV and V, whereas the forelimb area dominates in a more medial longitudinal strip, but wider in lobulus V and narrower in III. Occasional observations with unitary recording from Purkinje cells activated by CF were in good agreement with this type of topographical distribution (Provini, Redman and Strata, unpublished observations). As already pointed out in the Results, there is a wide strip close to the midline where the projections from the cerebral cortex, particularly through the CF system, are poor, and the results inconsistent. It seems likely that the main input to this area comes from sources other than those used in the present study.

In the vermis, a somatotopic organization for the CF, more distinctly organized in longitudinal strips than we have described, has been first reported by Oscarsson and Uddenberg (1966) and Oscarsson (1967a, b), following stimulation of peripheral nerves. In the pars intermedia Oscarsson (1967a, b), has also described an organization in longitudinal strips, superimposed on a caudo-rostral somatotopy for a new spino-olivo-cerebellar pathway. In our experiments however, the longitudinal strips have not been found in this region of the anterior lobe.

Some overlap exists in the pars intermedia and in the vermis between the regions receiving projections from the forelimb and hindlimb areas of the cerebral cortex. In the vermis, where these regions are in narrow strips, the overlap seems to be prominent and diffuse. That most of this overlap may be due to spreading of stimulation in the cortex seems unlikely for several reasons: I) the strength of the stimulation of the sensorimotor cortex was always well below the threshold for evoking detectable movements; II) in the same experiment, the same cortical stimulation always gave in the pars intermedia, regions impinged upon by only the forelimb or hindlimb area of the cortex; III) decreasing the strength of cortical stimulation greatly reduced the size of the CF responses, but still left a considerable amount of overlap; IV) an overlap is present also from peripheral nerve stimulation. Some of this overlap is certainly due to the fact that we were recording fields generated by many cells. A more precise localization of the projection areas should be made with unitary recording. In fact, quite often we observed Purkinje cell spikes responding to only one cerebral area, though the fields were generated by both areas (Provini, Redman and Strata, unpublished observations).

Although electrophysiological investigations of the lobus anterior have generally revealed a somatotopic organization in the cerebro-cerebellar projections, no evidence was available to decide whether it was due either to the MF or to CF system, or to both. In addition Deura (1961) did not find any somatotopy for his long latency cerebellar responses, which may be considered the equivalent of our CF response. Indeed anatomical investigations failed to reveal any somatotopic organization in the cortical projections to the inferior olive (see Jansen and Brodal 1958). Our findings demonstrate that the somatotopy is present in both the MF and CF pathways, and it is very well defined in the pars intermedia. It was unexpected to find CF responses in the vermis evoked by the sensorimotor cortex as well as the strict correlation between cortical and peripheral projection mediated by the CF, since the part of the olive that is impinged upon by cortical fibres is known to project to the pars intermedia, while that part receiving from the spinal cord is known to project to the vermis (see Jansen and Brodal 1958).

Convergence of cortical and peripheral input was not only detected with field recording but also was seen on occasional recording from Purkinje units (Provini, Redman and Strata, unpublished observations). Since only one climbing fiber synapses with a Purkinje cell, the observed convergence must be present on the single cells of the inferior olive. Evidence for this has been recently provided (Sedgwick and Williams 1967).

Correlation Between MF and CF Somatotopy. The correlation in the distribution between MF and CF systems has been documented in Figs. 3, 4 and 6. A remarkable degree of convergence appears in the pars intermedia, though the somatotopy of the MF pathways is less sharp than that of the CF. This finding could be expected because it is known that MF input terminates in a less localized manner than the CF input: one mossy fiber terminates on a large number of granule cells, even on different folia (Ramón y Cajal 1911; Eccles, Ito and Szentágothai 1967). In the vermis, where the forelimb and hindlimb areas of the sensorimotor cortex project to overlapping regions, it is not surprising that the MF somatotopy appears to have very little distinguishable pattern.

This strict correlation in the topography of the MF and CF seems to be a basic feature in the organization of the input to the cerebellar cortex, which cannot be without significance, and it suggests that the integration of these two types of input to the cerebellar cortex is importantly concerned in its operation.

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References

Adrian, E.D.: Afferent areas in the cerebellum connected with the limbs. Brain **66**, 289—315 (1943).

Albe-Fessard, D., et T. Szabo: Observations sur l'interaction des afférences d'origines péripherique et corticale destinées à l'écorce cérébelleuse du Chat. J. Physiol. (Paris) **46**, 225 to 229 (1954).

— — Activités enregistrées au niveau des cellules de Purkinje dans le cortex cérébelleux du Chat. C.R. Soc. Biol. (Paris) **149**, 1090—1093 (1955).

Armstrong, D.M., and R.J. Harvey: Responses in the inferior olive to stimulation of the cerebellar and cerebral cortices in the cat. J. Physiol. (Lond.) **187**, 553—574 (1966).

Brodal, P., J. Maršala and A. Brodal: The cerebral cortical projection to the lateral reticular nucleus in the cat, with special reference to the sensorimotor cortical areas. Brain Res. **6**, 252—274 (1967).

Curtis, H.J.: Cerebellar action potentials in response to stimulation of cerebral cortex. Proc. Soc. exp. Biol. (N.Y.) **44**, 664—668 (1940).

Deura, S.: Long latency cerebellar responses in cerebellar pedunculi and cortex. Neurology **11**, 940—949 (1961).

Dow, R.S.: Cerebellar action potentials in response to stimulation of various afferent connections. J. Neurophysiol. **2**, 543—555 (1939).

— Cerebellar action potentials in response to stimulation of the cerebral cortex in monkeys and cats. J. Neurophysiol. **5**, 121—136 (1942).

Eccles, J.C., M. Ito and J. Szentágothai: The cerebellum as a Neuronal Machine, p. 335. Berlin-Heidelberg-New York: Springer 1967.

—, R. Llinás and K. Sasaki: The excitatory synaptic action of climbing fibres on the Purkinje cells of the cerebellum. J. Physiol. (Lond.) **182**, 268—296 (1966).

— — — and P.E. Voorhoeve: Interaction experiments on the responses evoked in Purkinje cells by climbing fibres. J. Physiol. (Lond.) **182**, 297—315 (1966).

ECCLES, J.C., L. PROVINI, P. STRATA and H. TÁBOŘÍKOVÁ: Pattern of climbing fiber input to the cerebellar anterior lobe. Brain Res. **5**, 425—430 (1967).

— — — — Analysis of electrical potentials evoked in the cerebellar anterior lobe by stimulation of hindlimb and forelimb nerves. Exp. Brain Res. **6**, 171—194 (1968a).

— — — — Topographical investigation on the projection of forelimb and hindlimb afferents to the cerebellar anterior lobe. Exp. Brain Res. **6**, 195—215 (1968b).

—, K. SASAKI and P. STRATA: Interpretation of the potential field generated in the cerebellar cortex by a mossy fibre volley. Exp. Brain Res. **3**, 58—80 (1967).

GRANT, G.: Spinal course and somatotopically localized termination of the spinocerebellar tracts: An experimental study in the cat. Acta physiol. scand. **56**, Suppl. 193, 1—45 (1962).

HAMPSON, J.L.: Relationship between cat cerebral and cerebellar cortices. J. Neurophysiol. **12**, 37—50 (1949).

JANSEN, J.: Afferent impulses to the cerebellar hemispheres from the cerebral cortex and certain subcortical nuclei. Acta physiol. scand. **41**, Suppl. 143, 1—99 (1957).

—, and A. BRODAL: Aspects of Cerebellar Anatomy, p. 423. Oslo: John Grundt Tanum 1954.

— — Das Kleinhirn. In: Handbuch der mikroskopischen Anatomie des Menschen, Bd. 4/8 Nervensystem, p. 323. Hrsg. von W.V. MÖLLENDORFF u. W. BARGMANN. Berlin-Göttingen-Heidelberg: Springer 1958.

JANSEN, J.K.S., and C. FANGEL: Observations on cerebrocerebellar evoked potentials in the cat. Exp. Neurol. **3**, 160—173 (1961).

KENNEDY, T.T., R.J. GRIMM and A.L. TOWE: The role of cerebral cortex in evoked somatosensory activity in cat cerebellum. Exp. Neurol. **14**, 13—32 (1966).

LARSELL, O.: The cerebellum of the cat and the monkey. J. comp. Neurol. **99**, 135—199 (1953).

OSCARSSON, O.: Termination and functional organization of a dorsal spino-olivo-cerebellar path. Brain Res. **5**, 531—534 (1967a).

— Functional significance of information channels from the spinal cord to the cerebellum. In: Neurophysiological Basis of Normal and Abnormal Motor Activities. 3rd Symposium of the Parkinson's Disease Information Center. Ed. by M.D. YAHR and D.P. PURPURA. Hewlett, N.Y.: Raven Press 1967b.

— Termination and functional organization of the ventral spino-olivo-cerebellar path. J. Physiol. (Lond.) **196**, 453—478 (1968).

—, and N. UDDENBERG: Somatotopic termination of spino-olivo-cerebellar path. Brain Res. **3**, 204—207 (1966).

PROVINI, L., S. REDMAN and P. STRATA: Somatotopic organization of mossy and climbing fibres to the anterior lobe of cerebellum activated by the sensorimotor cortex. Brain Res. **6**, 378—381 (1967).

RAMÓN Y CAJAL, S.M.: Histologie du Système Nerveux de l'Homme et des Vertébrés. II. Paris: Maloine 1911.

SASAKI, K., and P. STRATA: Responses evoked in the cerebellar cortex by stimulating mossy fibre pathways to the cerebellum. Exp. Brain Res. **3**, 95—110 (1967).

SEDGWICK, E.G., and T.D. WILLIAMS: Responses of single units in the inferior olive to stimulation of the limb nerves, peripheral skin receptors, cerebellum, caudate nucleus and motor cortex. J. Physiol. (Lond.) **189**, 261—279 (1967).

SNIDER, R.S.: Alterations which occur in mossy terminals of the cerebellum following transection of a branchium pontis. J. comp. Neurol. **64**, 417—435 (1936).

—, and E. ELDRED: Cerebral projections to the tactile, auditory and visual areas of the cerebellum. Anat. Rec. **100**, 714—714 (1948).

— — Electro-anatomical studies on the cerebro-cerebellar connections in the cat. J. comp. Neurol. **95**, 1—16 (1951).

—, and A. STOWELL: Receiving areas of the tactile, auditory and visual systems in the cerebellum. J. Neurophysiol. **7**, 331—358 (1944).

SZABO, R., et D. ALBE-FESSARD: Répartition et caractères des afférences somesthésiques et d'origine corticale sur le lobe paramédian du cervelet du Chat. J. Physiol. (Paris) **46**, 528—531 (1954).

SZENTÁGOTHAI, J.V., u. K. RAJKOVITS: Über den Ursprung der Kletterfasern des Kleinhirns. Z. Anat. Entwickl.-Gesch. **121**, 130—141 (1959).

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