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Origin in the medial accessory olive of climbing fibres to the x and lateral c1 zones of the cat cerebellum: a combined electrophysiological/WGA-HRP investigation

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Summary. The climbing fibres to the x and lateral c1 zones of the anterior lobe of the cerebellum arise as branches of common stem olivary axons. Anatomical studies have shown that the c1 zone receives its climbing fibres from the dorsal accessory olive (DAO). It has, therefore, been assumed that the x-zone also receives its climbing fibres from this olivary subnucleus. The present study demonstrates that both the x-zone and the lateral part of the c1 zone in fact receive their climbing fibre input from the middle portion of the medial accessory olive (MAO) (approximate antero-postero-levels P10–13). Electrophysiological techniques were used to define the extent of these cerebellar zones and small volumes (15–50 nl) of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) were pressure injected into the defined zone. These small pressure injections resulted in injection sites with minimal spread to adjacent zones. The sensitive tetramethylbenzidine (TMB) reaction was used to visualize both the injection site and retrogradely labelled cells in the inferior olive. This combination of electrophysiological and neuroanatomical techniques gave extremely reproducible results. The results suggest that the zone previously named lateral c1 would be better designated cx.

Key words: Cerebellum – Inferior olive – Climbing fibres – WGA-HRP – Sagittal zones

Introduction

The organization of the climbing fibre input into longitudinal zones in the cortex of the anterior lobe of the cerebellum is now well established as a result of both physiological and anatomical studies of the olivocerebellar projection and the spinoolivocerebellar pathways (Armstrong et al. 1974; Groenewegen and Voogd 1977; Groenewegen et al. 1979; Ekerot et al. 1979). In each half of the anterior lobe 8 cortical zones can be electrophysiologically identified by the latency and other functional characteristics of their climbing fibre responses on limb nerve stimulation. From medial to lateral they are called, a, x, b, c1, c2, c3, d1 and d2 (Oscarsson 1980). Indeed, similarly named zones have been identified anatomically by Voogd and coworkers (Groenewegen and Voogd 1977; Groenewegen et al. 1979), although it is, as yet, uncertain if the boundaries of the anatomical and physiological zones are precisely identical.

More recently, Ekerot and Larson (1982) suggested that the 8 zones can be divided into two groups: 4 of the zones (a, b, c2 and d1) receive their climbing fibre input from separate and private subnuclei of the inferior olive. Thus, the a-zone receives its climbing fibres from the caudal medial accessory olive (MAO), the b-zone from the caudal dorsal accessory olive (DAO), the c2-zone from the rostral MAO and the d1-zone from the principal olive (PO) (Groenewegen and Voogd 1977; Groenewegen et al. 1979). The remaining 4 zones (x, c1, c3 and d2) are thought to be closely related and, in fact, pairs of these zones are linked because they receive climbing fibres which arise as branches of a common stem axon (Ekerot and Larson 1982). Thus, Purkinje cells in the x-zone receive climbing fibres from olivocerebellar axons which also supply climbing fibres to Purkinje cells in the lateral part of the c1-zone. The medial parts of the c1 and c3-zones are similarly linked to form a second pair, while the lateral part of the c3 and the d2-zone form a third pair.

Since it is known from anatomical studies (Groenewegen et al. 1979) that the c1 and c3 zones

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Table 1

Case	Concentration of WGA-HRP (w/v)	Zone	Number of injections	Volume (nl)	Survival time (h)
HX1LS	4%	x	2	50/25	60
HX1RS	4%	x	1	25	60
HX2LC	2%	x	1	25	70
HX2RC	2%	x	1	50	70
HX3LC	2%	c1	1	35	65
HX3RC	saline	x	1	50	65
HX5LC	2%	x	1	30	44
HX5RC	2%	x	1	25	44
HX6LC	2%	c1	1	30	70
HX6RC	2%	x	1	25	70
HX7LS	2%	c1	1	35	54
HX7RS	2%	x	1	15	54

receive fibres from the rostral DAO, it has been suggested (Brodal and Kawamura 1980 see pp 38, 106; Ekerot and Larson 1979a, 1982) that the x and d2 zones also receive their climbing fibres from rostral DAO, although it has been noted that the x-c1 pair of zones differs from the c1-c3 and c3-d2 pairs in lacking a spinoolivocerebellar input with distinct somatotopical organization (Ekerot and Larson 1982). The present investigation was carried out to determine the olivary origin of the climbing fibres supplying the x and lateral c1-zones. The results reveal that Purkinje cells in these zones receive climbing fibres from the middle portion of the MAO. Thus, it appears that with regard to their olivary input, the x and lateral c1 zones are distinct from the c1-c3 and c3-d2 pairs. A preliminary report has been presented (Campbell and Armstrong 1984).

Material and methods

The experiments were performed on 6 cats in which 8 injections of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) were made in the x-zone and 3 in the lateral c1 zone of the cerebellar anterior lobe. One injection of saline only was made into the x zone as a control: this injection produced no HRP labelling in either the cerebellum or the inferior olive. All injections were made in lobule Vb or Vc (nomenclature of Larsell 1953).

Surgical procedures

All surgical procedures were carried out using sodium pentobarbitone anaesthesia (Sagatal; initial dose 40 mg/kg ip., supplementary doses as required). A craniotomy was performed, exposing the cerebellar vermis and pars intermedia bilaterally. The exposure extended rostrocaudally from lobule Va to lobulus simplex.

Electrophysiology

Glass micropipettes (tip diameter 25–30 μm) were filled with Woods metal (fusible metal (m.p. 71° C) containing bismuth, lead, tin and cadmium) containing 13% indium and were used for surface and/or molecular layer field potential recordings. The electrode was connected to a unity gain preamplifier, the output of which was fed to a main amplifier and was band pass filtered (30 Hz to 7.5 kHz, unless otherwise stated). Signals were displayed on and photographed from an oscilloscope.

The forepaws were stimulated using needle electrodes inserted subcutaneously. The stimulus intensity was adjusted to give a reflex twitch of the forepaw and stimuli were repeated once every 2 s. The surface and/or molecular layer field potentials evoked by forelimb stimulation were mapped along a chosen folium at intervals of 100–200 μm. The zones were defined by the latency of the responses and the boundaries were defined as sites at which there were clear latency shifts.

Injections

Small volumes (15–50 nl) of WGA-HRP (Sigma; normally 2% w/v made up in 0.9% saline, but see Table 1) were injected into the electrophysiologically defined x-zone (8 cases) or the lateral c1-zone (3 cases). In one case, a 0.9% saline injection was made in the x-zone as a control. Pressure injections were made via a micropipette (tip diameter 25–30 μm) attached to a 1 μl Hamilton syringe, the plunger of which was driven via a micrometer device. The exact placement of all injections was guided electrophysiologically: for x-zone cases, the injection was placed near the middle of the identified zone; for lateral c1-zone cases, the injections were placed 500 μm medial to the boundary between the c1 and c2 zones.

If surface recordings were made, injections were made at a depth of 400–500 μm below the pial surface. For cases in which molecular layer field recordings were made, the depth at which field potentials were recorded was noted and the injections were made at that depth.

All injections were made over 10 min and the pipette was left in situ for 5 min to minimize leakage of tracer along the electrode track.

On completion of the injections, the cerebellar surface was covered with a layer of fibrin foam (Sterispon, Allen and Hanbury) and the craniotomy was sealed with acrylic cement. The wound was closed in layers and the animals were allowed to recover.

Survival times

Table 1 lists the concentrations and volumes of WGA-HRP used and the survival times for each case. Note that the case identifica-

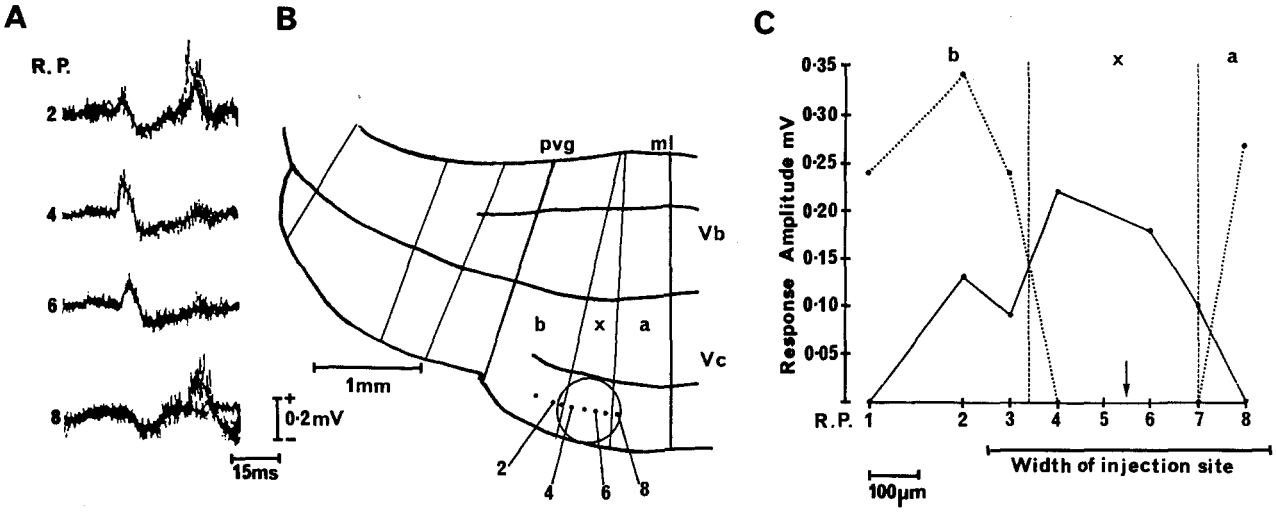

Fig. 1A–C. Electrophysiological identification of the x-zone in case HX5LC. **A** Potentials evoked at four different positions on the surface of the left hemivermis by stimulation of the ipsilateral forepaw. Stimulus coincides with beginning of sweep. Each record comprises 3 superimposed sweeps. Numbers identify the recording positions (RP). **B** Semi-diagrammatic representation of the left side of lobules Vb and c (nomenclature of Larsell 1953) in the anterior lobe of the cerebellum. P.v.g., paravermal groove; ml, midline. Thin lines indicate the boundaries of different longitudinal zones in the cortex, of which b, x and a are labelled. Dots indicate recording positions and the numbers identify the position yielding the response shown in **A.** Circle indicates the diameter of the intense WGA-HRP reaction produced as measured from processed cerebellar sections. **C** Plot showing peak amplitude of the positive-going response evoked at the eight recording positions shown in **B.** Short latency (< 20 ms) responses are connected by solid line; long latency (> 30 ms) by broken lines. Arrow indicates site of injection

tion specifies which cat is which (numeral), whether the injection was placed in the left (L) or right (R) hemicerebellum and also whether the cerebellum was sectioned sagitally (S) or coronally (C).

Perfusion

After the appropriate survival time, the animals were reanaesthetised with sodium pentobarbitone (Sagatal) and perfused intracardially with 0.9% saline followed by a phosphate buffer solution (0.1 M, pH 7.4) containing 1% paraformaldehyde and 1.25% glutaraldehyde. This was followed by a sucrose solution (10% sucrose in phosphate buffer). The brainstem and cerebellum were removed and the brainstem was placed directly in a buffer solution containing 10% sucrose, while the cerebellum was embedded in 10% gelatin before being transferred to the sucrose solution.

The brainstem was sectioned at 30 μm in the transverse plane and groups of 3 sections were collected separately. The cerebellum was sectioned at 50 μm in the transverse (8 cases) or sagittal (4 cases) plane (see Table 1) and sections were collected in groups of 2. All sections were left in phosphate buffer overnight before processing.

Processing

The processing procedure used was a modified version of the tetramethylbenzidine (TMB) method described by Mesulam (1978). The modification of this procedure comprised mounting 2 series of the brainstem sections and one series of the cerebellar sections prior to processing. These pre-mounted sections were then processed as per Mesulam (1978) except that the time for each of the stages was doubled. The remaining series of brainstem and cerebellar sections were processed 'free-floated', exactly as per Mesulam (1978). One premounted brainstem series was lightly stained, after processing, with 0.1% neutral red. All sections were then dehydrated, cleared in xylene and coverslipped with permount.

It was found necessary to mount at least one series of brainstem and cerebellar sections prior to processing because of the large shrinkage which was found to occur during 'free-floated' processing with the TMB method. This shrinkage was calculated to be 30%. In this study, it was important to have accurate measurement of the cerebellum and the premounted series provided sections which showed minimal shrinkage, this being determined by measuring the distance between the paravermal veins at the time of the surgery and then again after perfusion (maximum shrinkage due to perfusion was 10%).

Analysis

Brainstem. The stained series of brainstem sections was used to identify the olivary subnuclei. Unstained sections were examined under both bright- and dark-field illumination. Labelled cells seen under dark-field illumination on free-floated sections were plotted onto a standard series of olivary sections in the transverse plane. It should be noted that free-floated sections were used for plotting since the reaction product is more dense in these sections. However, the distribution of retrogradely labelled inferior olivary neurones was identical in free-floated and pre-mounted series.

The extent of retrograde olivary labelling was transferred to a diagrammatic representation of the 'unfolded' olivary nucleus taken from Groenewegen et al. (1979).

Cerebellum. Cerebellar sections were examined under both dark- and bright-field illumination. Corresponding sections in free-

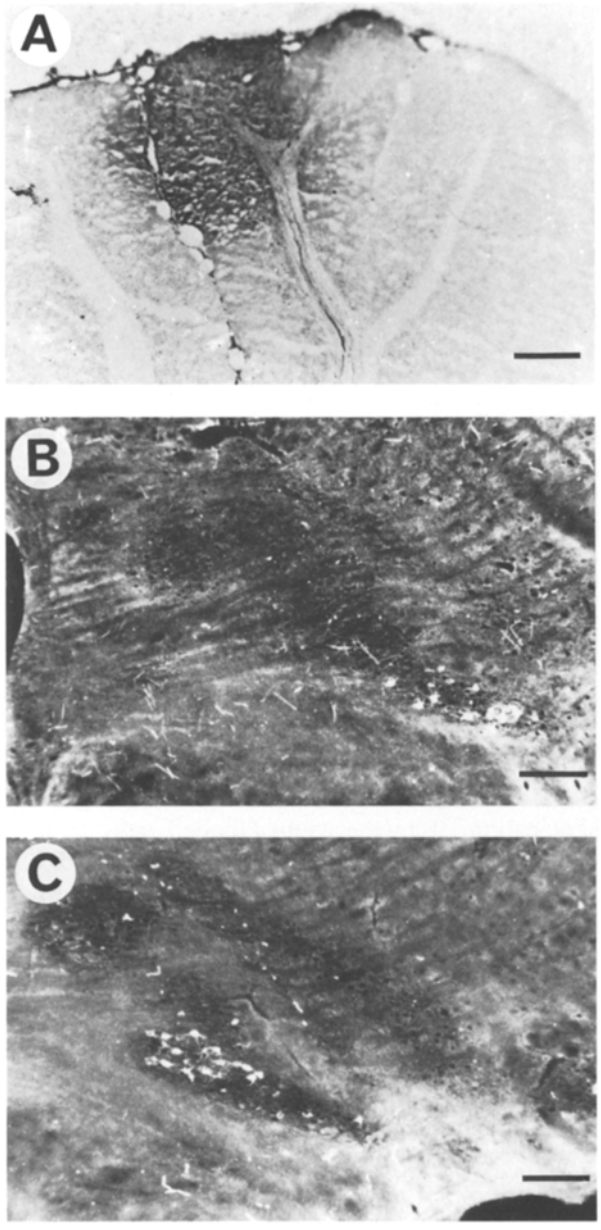

Fig. 2A–C. Photomicrographs showing typical histological findings. **A** x-zone injection site from case HX7RS. Unstained sagittal section; bright-field. Note approximately circular area of reaction product and labelled axons in the subcortical white matter. Calibration bar 500 μm. **B** Retrograde labelling in right inferior olivary nucleus in case HX5LC. Unstained transverse section at AP level 12.25; dark-field. Brainstem midline is near left-hand edge of photograph. Note labelled olivary neurones confined to lateral part of medial accessory olive. Calibration bar 250 μm. **C** As **B** but at AP level 11.75. Note labelled olivary neurones confined to medial accessory olive and mainly in its medial half. Calibration 250 μm

floated and pre-mounted series were compared and the mediolateral extent of intense HRP reaction product was estimated by counting sections in sagittally sectioned cases or by direct measurement from the premounted sections in the coronal cases. These values were corrected for shrinkage and the result was then taken as the width of the 'effective' injection site. In two of the cases, HX1RS and HX5RC, the injection sites could not be measured. However, the injections were placed in the centre of the x-zone and the position of the retrogradely labelled olivary cells was fully consistent with the other x-zone cases.

Results

Injections in the x-zone

In 8 cases, WGA-HRP injections were made near the centre of the electrophysiologically defined x-zone. A typical case (HX5LC) will be described in detail.

Figure 1A illustrates surface climbing fibre responses (3 superimposed sweeps) from 4 of the 8 recording positions in the anterior lobe vermis (lobule Vc) on stimulation of the ipsilateral forepaw. The recording positions are indicated in the diagram in Fig. 1B. At recording position 2, two positive-going responses are visible, a small response at latency 17 ms and a larger response at latency 35 ms. The early response is due to climbing fibre activity in the x-zone while the late response is due to climbing fibre activity in the b-zone (cf. Andersson and Eriksson 1981). 200 μm more medially (recording position 4) only the short latency response is present. This is also the case for recording position 6. At recording position 8, the positive short latency response has been replaced by a negativity and a more variable and longer latency response is also present. This late response is presumably due to activity in the a-zone.

Figure 1C shows the amplitude of positive short latency responses (< 20 ms, solid line) and long latency responses (> 30 ms, dotted line) at the 8 recording positions in the vermis. The vertical lines indicate the estimated borders of the x-zone. The arrow indicates the position at which the injection was made. The circle in Fig. 1B and the horizontal bar in Fig. 1C indicate the extent of the intense HRP reaction product measured from the cerebellar sections after coronal sectioning and processing. In this case, the mediolateral extent of this staining was 600 μm. Comparing this value with the electrophysiologically defined extent of the x-zone, it can be seen that the injection site encompassed the whole width of the x-zone at this cerebellar level, and encroached, albeit minimally, on the a and b zones.

Figure 2A is a bright-field photomicrograph of a sagittal cerebellar section showing an x-zone injection site (case HX7RS). It can be seen that although this site is one of the largest made it nevertheless has a diameter of only 1200 μm. The smaller sites in the series had a markedly lower density of reaction

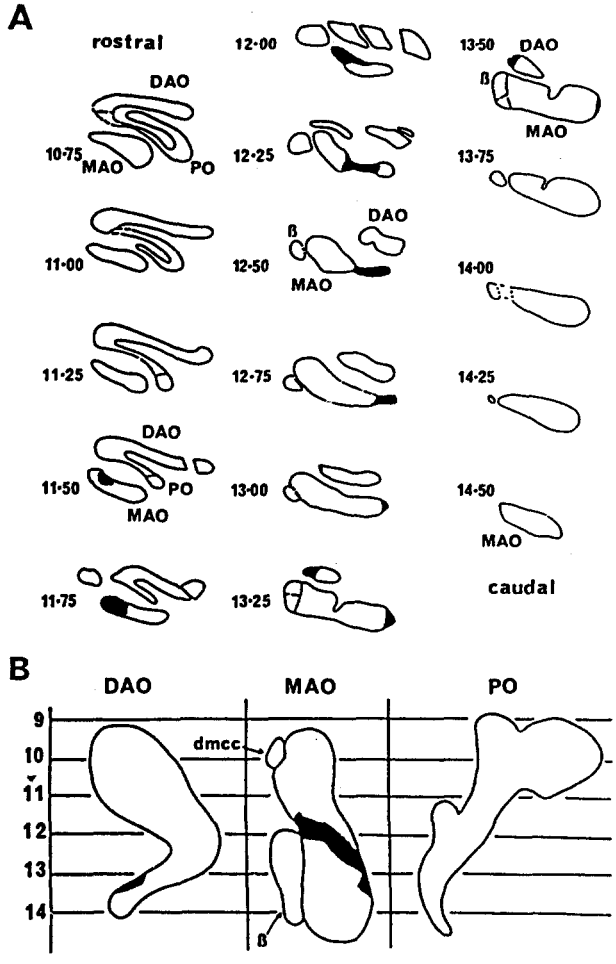

Fig. 3A and B. Summary diagram showing location of retrogradely labelled inferior olivary neurones following x-zone injection of WGA-HRP in case HX5LC. **A** Transverse sections of right inferior olivary nucleus. Numerals indicate AP level and intersection interval is 250 μm. Note that no sections are shown from the caudal- and rostral-most part of the nucleus. Filled regions contained retrogradely labelled neurones. **B** Results in **A** transferred to an 'unfolded' representation of the inferior olive, adapted from Groenewegen et al. (1979). MAO, medial accessory olive; DAO, dorsal accessory olive; PO, principal olive; d.m.c.c., dorsomedial cell column; β, nucleus β

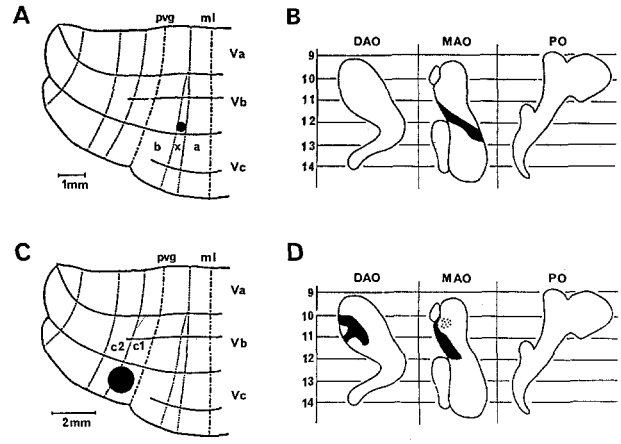

Fig. 4A–D. Summarized results from two cases. **A** Location of an x-zone injection of WGA-HRP in case HX2LC. Note size of filled circle indicates extent of intense reaction product which was entirely confined to the x-zone. **B** Location of olive cells retrogradely labelled after the injection shown in **A. C** Location of an injection centred in the lateral c_1 zone in case HX6LC. **D** Location of olive cells retrogradely labelled after the injection shown in **C.** Conventions and abbreviations as in Fig. 1B and Fig. 3B

product and consequently photographed badly although their dimensions could usually be determined with confidence.

Figure 2B is a dark-field photomicrograph of the inferior olivary nucleus at approximately A–P level 12.25 (case HX5LC). Retrogradely labelled cells are clearly seen and are confined to a small cluster in the ventro-lateral portion of MAO. Figure 2C shows the inferior olivary nucleus from the same case (HX5LC) at a more rostral level (approx. AP 11.75), again in dark-field. Here, the retrogradely labelled olive cells are still confined to MAO, but are located mainly in the medial half of the nucleus.

Figure 3A is a standard series of transverse sections through the inferior olivary nucleus showing the location of retrogradely labelled cells in case HX5LC. It can be seen that most of the labelled cells are found in a narrow strip which starts laterally in the caudal half of MAO and moves medially and rostrally. There are additional areas of labelled cells more caudally in MAO and DAO in which the cells were more scattered, more weakly labelled and were considerably fewer in number. The weak labelling in caudal MAO is consistent with spread of tracer from the x-zone to the lateral part of the a-zone while the labelling in caudal DAO is consistent with spread of injectate to the medial part of the b-zone (Groenewegen and Voogd 1977). Figure 3B shows the location of the retrogradely labelled cells when transferred to a summary diagram of the 'unfolded' olivary nucleus (from Groenewegen et al. 1979): such summary diagrams facilitate comparison between cases.

Results from another x-zone injection (case HX2LC) are summarized in Fig. 4A and B. In this case, the electrophysiology revealed that the x-zone was less than 500 μm wide. The injection, centred in the x-zone in lobule Vb (Fig. 4A), resulted in the smallest 'effective' injection site in the series (400 μm as measured from coronal sections) and the retrogradely labelled olivary cells were entirely confined to MAO (Fig. 4B). The pattern of labelled cells is again a narrow strip in the middle of the MAO, starting caudally and laterally and moving rostrally and medially (cf. Fig. 4B and Fig. 3B).

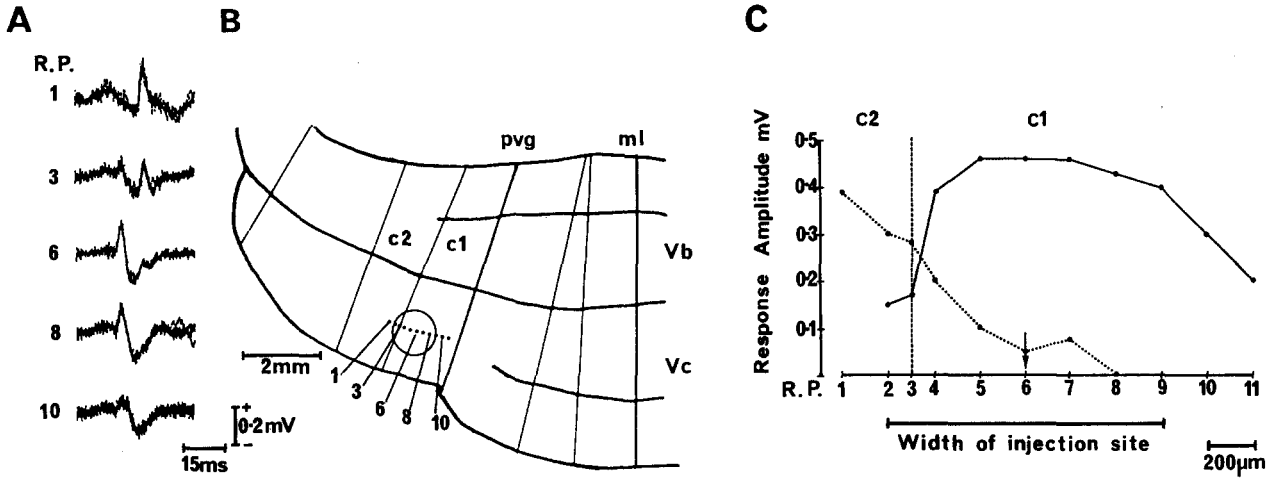

Fig. 5A–C. Electrophysiological identification of the lateral c_1 zone in case HX3LC. **A** Potentials evoked at five different positions on the surface of the pars intermedia after stimulation of the ipsilateral forepaw. **B** Semidiagrammatic representation of lobules Vb and c. Dots indicate 11 recordings points including the five shown in **A**. Circle indicates diameter of intense reaction product. **C** Plot showing peak amplitude of the positive-going responses evoked at the recording positions shown in **B**. Short latency (< 20 ms) responses connected by solid line; long latency (> 20 ms) responses by broken line. Arrow indicates site of injection. Abbreviations as in Fig. 1

The remaining 6 x-zone cases (see Table 1) also showed retrogradely labelled cells in this same narrow band in the middle portion of MAO (see Fig. 8). In addition, scattered and more weakly labelled cells were also found in caudal MAO and caudal DAO. The extent to which labelling occurred in these subnuclei corresponded well with the estimated spread of injectate beyond the x-zone. Thus, in the two starred cases in Fig. 8, the largest volumes of most concentrated WGA-HRP (50 and 25 nl of 4% solution; see Table 1) resulted in extensive labelling in caudal MAO, presumably due to spread to the a-zone, and also to labelling in caudal DAO (not illustrated) due to spread of injectate into the b-zone. In fact, in all except case HX2LC (Fig. 4A and B), injections in the x-zone resulted in some weakly labelled cells in caudal DAO, although the variability and intensity of labelling suggested that these cells were labelled from the extremity of the injection site.

Injections in the lateral c1 zone

In 3 cases, injections were made 500 µm medial to the boundary between the c2 and c1 zones in order to label cells in the lateral c1-zone. The boundary was determined by using ipsilateral forepaw stimulation and noting where the climbing fibre response latency shifted from long latency responses (> 20 ms) characteristic of the c2 zone to short latency c1 responses (< 20 ms) (cf. Ekerot and Larson 1979a). Similar results were obtained in all 3 cases (see Fig. 8) and case HX3LC will be described in detail.

Figure 5A shows typical surface climbing fibre responses (3 superimposed sweeps) recorded at 5 of the 11 recording positions in the pars intermedia of the anterior lobe (lobule Vc) on stimulation of the ipsilateral forepaw. The recording positions are indicated in Fig. 5B. At recording position 1, there is a large positive long latency response (27 ms) due to climbing fibre activity in the c2 zone. 300 µm medially (recording position 3), the long latency response has diminished and a positive short-latency response (18 ms), due to climbing fibre activity in the c1-zone, has appeared. 500 µm more medially (recording position 6), the short-latency response has grown in amplitude and the long latency response has virtually disappeared. Thereafter (recording position 8 and 10), only the short latency response is present. Figure 5C shows the amplitude of positive short latency responses (< 20 ms, solid line) and long latency responses (> 20 ms, dotted line) at all 11 recording positions in the pars intermedia. The vertical line indicates the estimated border between the c1 and c2 zones. The arrow indicates the position at which the injection was made. The circle in Fig. 5B and the horizontal bar in Fig. 5C indicate the extent of the intense HRP reaction product, in this case 1.2 mm. Deducing, from the electrophysiology, that the c1/c2 boundary occurs at, or near, recording position 3, this injection site must have extended to the lateral edge of the c1-zone and just into the c2-zone.

Figure 6A is brightfield photomicrograph of a transverse cerebellar section showing a typical lateral c1-zone injection (case HX3LC, as in Fig. 5). The

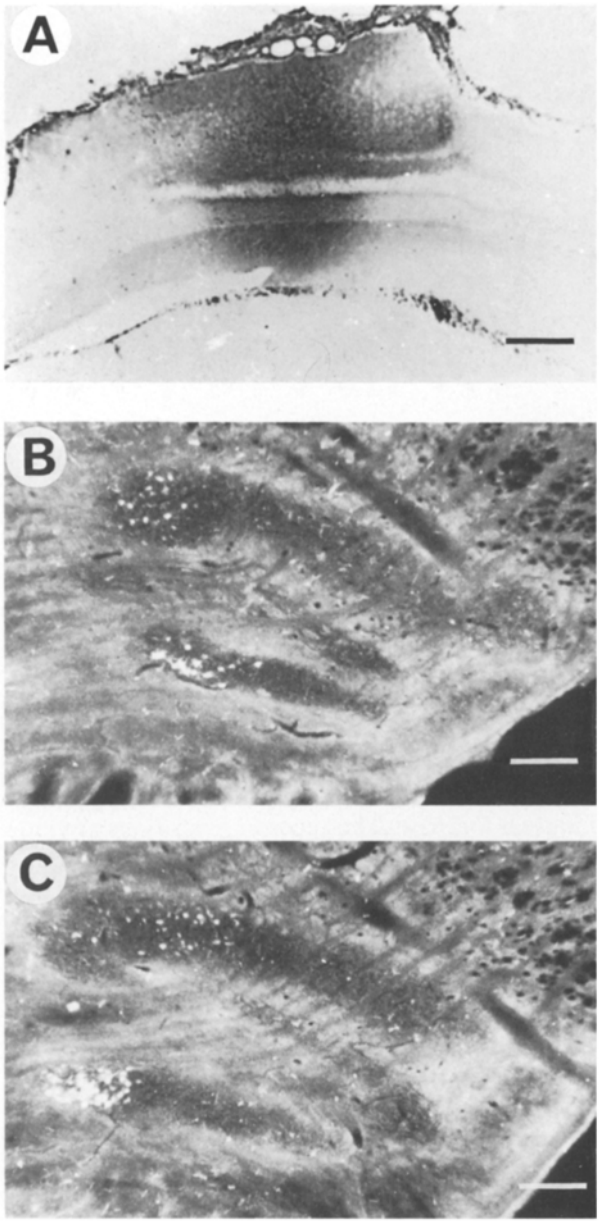

Fig. 6A–C. Histological findings from the experiment illustrated in Fig. 5 (case HX3LC). **A** Injection site in the lateral c_1 zone. Unstained coronal section; bright-field. Calibration bar 500 μm. **B** Retrograde labelling in right inferior olivary nucleus. Unstained transverse section at AP level 11.5; dark-field. Note heavily labelled olivary neurones in medial half of MAO and also less densely labelled cells in medialmost part of DAO. Calibration bar 250 μm. **C** As **B** but at AP level 11.0. Calibration 250 μm

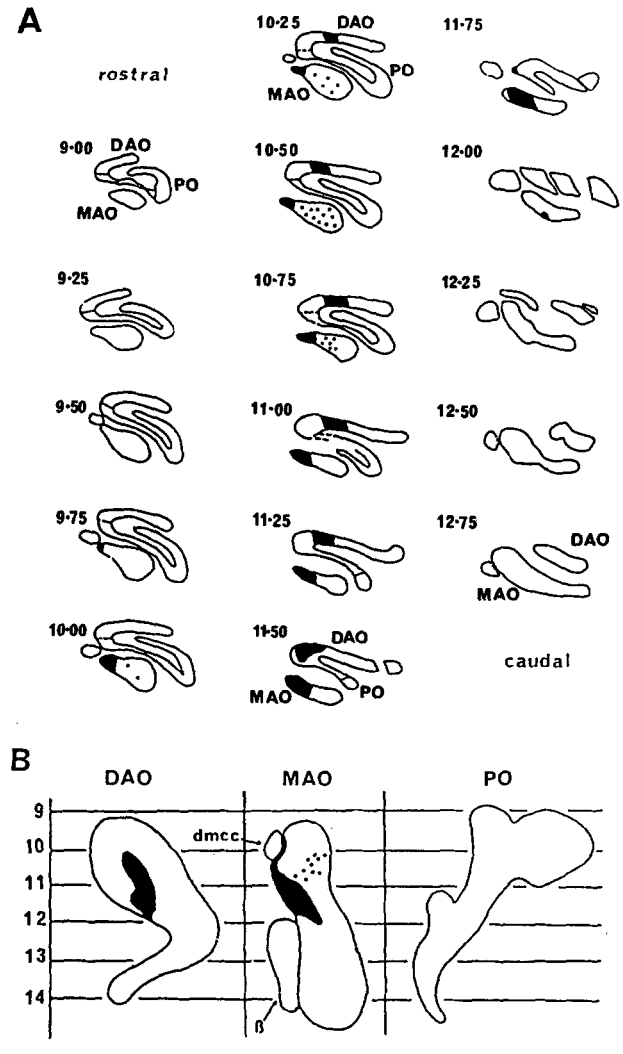

Fig. 7A and B. Summary diagram showing distribution of retrogradely labelled olivary neurones from same case as in Figs. 5 and 6 (case HX3LC; injection into lateral c_1 zone). **A** Transverse sections of inferior olive between AP levels 9.0 and 12.75. Note no sections are shown from the caudalmost part of the nucleus. Filled regions contained retrogradely labelled cells. **B** Results in **A** transferred to unfolded olivary nucleus (cf. Fig. 3)

'effective' injection site is seen as an intensely labelled circle in the centre of the field. Figure 6B is a darkfield photomicrograph of the inferior olivary nucleus of case HX3LC at approximately AP level 11.5 (cf. Fig. 2C, case HX5LC x-zone injection). Retrogradely labelled cells are clearly seen at the medial edge of the MAO. There are also weakly labelled cells at the very medial tip of DAO. Figure 6 shows a darkfield photomicrograph of the inferior olivary nucleus from the same case (HX3LC) at a more rostral level (approx. AP 11.0). The retrogradely labelled cells in MAO still occupy a medial position in the nucleus and a second, more scattered population of cells is seen in the medial half of DAO.

Figure 7A is, again, a standard series of transverse sections through the inferior olive (cf. Fig. 3A) showing the distribution of retrogradely labelled cells in case HX3LC. Three populations of cells can be seen. In rostral DAO, a column of labelled cells is

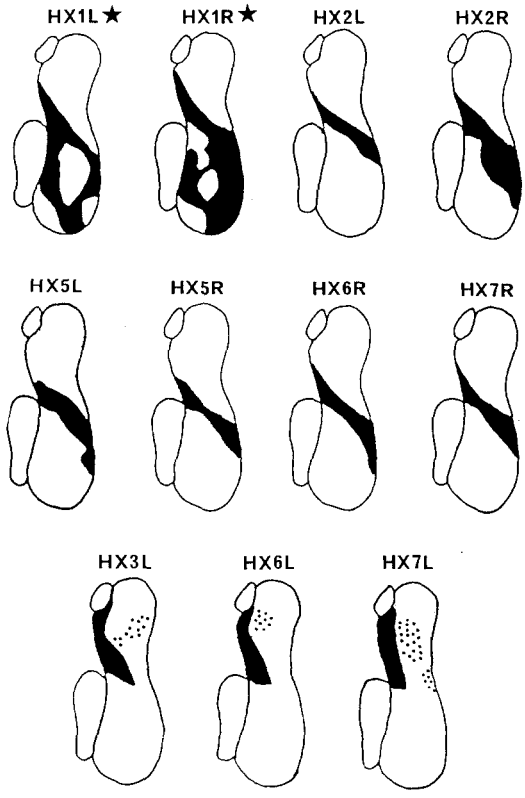

Fig. 8. Distribution in medial accessory olive of neurones retrogradely labelled in 11 different cases. Cases in upper two rows involved injections into the x zone. In the two starred cases large injection sites resulted from the use of 4% WGA-HRP (see Table 1). Patchy labelling in caudal MAO presumably arose because the injection site involved the a as well the x zone. Bottom row, three cases involving injections in the lateral c_1 zone

found near the medial edge of the subnucleus and extends in a rostro-caudal direction for 1.75 mm. In rostral MAO, two populations of cells are observed. Starting at the caudal edge of the rostral MAO, a very localized group of retrogradely labelled cells extends from the middle of the MAO to the medial edge of the subnucleus. More rostrally, this population of cells moves medially and at its most rostral point occupies only the very medial tip of the MAO. The second MAO population is much more dispersed, comprises fewer cells and occupies the lateral half to two-thirds of the nucleus between AP levels 10.75 and 10.00. Figure 7B is a summary diagram of the distribution of retrogradely labelled cells in the inferior olive of case HX3LC.

A second lateral c1 case (HX6LC) is presented in Fig. 4 (C and D). Figure 4C shows the position and extent (1.6 mm) of the injection site in the pars intermedia (lobule Vc), while Fig. 4D shows the location of retrogradely labelled olivary cells. Again, three populations of labelled cells can be distinguished: one in rostral DAO and two in rostral MAO. A comparison of Figs. 4D and 7B shows that the medial MAO population occupies an almost identical position in both cases (see also Fig. 8 case HX7LS), while the other two populations (DAO and more rostral/lateral MAO) vary slightly between the two cases. The occurrence of a separate population of retrogradely labelled cells in rostrolateral MAO is consistent with the spread of tracer from the lateral part of the c1 zone into the c2 zone while the column of labelled cells in rostral DAO is consistent with spread of injectate to the medial part of the c1 zone (Groenewegen et al. 1979).

Discussion

Methodological considerations

In the present series of experiments, electrophysiological and neuroanatomical techniques have been combined to solve an anatomical problem. This combination of techniques is particularly suitable for cerebellar studies, where the organization of the system is so well defined in terms of physiological inputs. The organization of the climbing fibre input, for example, in sagittal zones in the cerebellar cortex is well established (see Oscarsson 1980, for references). However, the exact position of the zones relative to landmarks on the cerebellar surface (e.g. the midline, the paravermal groove and especially the paravermal veins) varies significantly between experimental animals. Thus, in studying the afferent and efferent pathways to any one zone using purely anatomical techniques, variable results are liable to be obtained. In the present study, however, the mediolateral extent of the x-zone and the position of the lateral c1-zone could be determined using precise electrophysiological techniques. Small injections of the retrograde tracer WGA-HRP could then be made within a defined zone (with minimal spread to adjacent zones) and the tissues processed with the sensitive TMB method (Mesulam 1978). The results obtained using this combination of techniques are very reproducible. The top two rows of Fig. 8 show the location of retrogradely labelled cells in the MAO of the 8 cases in which injections were centred on the electrophysiologically defined x-zone. The 2 starred cases were injections of 4% WGA-HRP (see Table 1). In all 8 cases, the retrograde labelling included the same narrow band of cells in the middle portion of MAO. By contrast, the extent of labelling further caudally in MAO was very variable. The different injection sites varied in size, which is not surprising because the volume of injectate varied

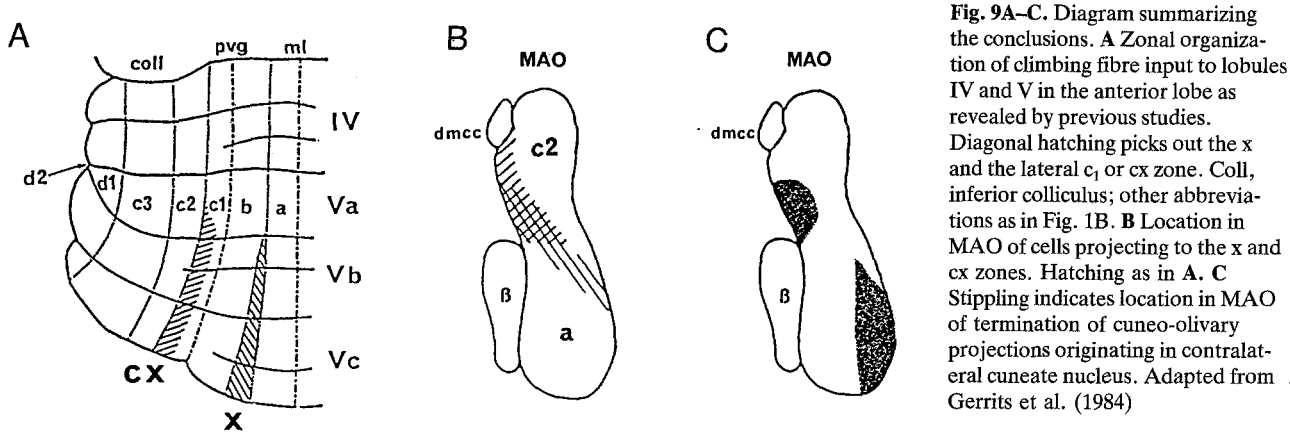

Fig. 9A–C. Diagram summarizing the conclusions. **A** Zonal organization of climbing fibre input to lobules IV and V in the anterior lobe as revealed by previous studies. Diagonal hatching picks out the x and the lateral c_1 or cx zone. Coll, inferior colliculus; other abbreviations as in Fig. 1B. **B** Location in MAO of cells projecting to the x and cx zones. Hatching as in **A. C** Stippling indicates location in MAO of termination of cuneo-olivary projections originating in contralateral cuneate nucleus. Adapted from Gerrits et al. (1984)

between cases (see Table 1) and presumably also because pressure injections of HRP are inherently variable. The degree to which there was labelling in caudal MAO in fact correlated well with the extent to which the corresponding injection site encroached on the neighbouring a-zone which is known to receive its climbing fibres from caudal MAO (Groenewegen and Voogd 1977). The bottom row of diagrams shows the similarly high degree of reproducibility obtained with injections in the lateral part of the c1-zone.

Locations in the inferior olivary nucleus of cells supplying climbing fibres to the x and lateral c1 zones

Anatomical evidence has shown that the c1 and c3 zones of the anterior lobe pars intermedia receive climbing fibres from the rostral DAO (Groenewegen et al. 1979). Because the x-zone climbing fibre responses have similar functional characteristics (latency, receptive fields, etc.) to those of the c1 and c3 zones (Ekerot and Larson 1979a, b), and, because the x-zone shares climbing fibre collaterals with the lateral c1 zone (Andersson and Eriksson 1981; Ekerot and Larson 1982), it has been assumed that its climbing fibres also arise from DAO (Brodal and Kawamura 1980; Ekerot and Larson 1979a, 1982). However, the results of the present study reveal that the x-zone and the lateral c1 zone receive climbing fibres from MAO (see Figs. 2–4, 6–8). A similar although less precise conclusion regarding the source of x-zone climbing fibres has been reached by Voogd (1983 and personal communication) through reinterpretation of previously published autoradiographic/degeneration experiments (Groenewegen and Voogd 1977).

It could be argued that the lateral c1-zone also receives climbing fibres from rostral DAO since in all cases in which injections were made in the lateral part of the c1 zone, some retrogradely labelled cells were found in rostral DAO. However, the DAO labelling was not exactly consistent between cases (cf. Fig. 4C and D, and Fig. 7B). Thus, although the present results do not exclude the possibility that the lateral c1 zone receives some climbing fibres from rostral DAO they are nevertheless consistent with the hypothesis that rostromedial MAO sends climbing fibres to the lateral c1 zone, while rostral DAO sends climbing fibres to the medial c1 zone, which was always encroached upon by injections intended to cover the lateral c1-zone.

Since the lateral and medial parts of the c1 zone receive climbing fibres from different olivary subnuclei, and have somewhat different somatotopical organizations (Ekerot and Larson 1979b, 1982), these two cerebellar subdivisions must be considered to be separate zones. It is, perhaps, reasonable to rename the lateral c1-zone as the cx-zone. Retention of the designation c serves to emphasize the location of the zone among the other c zones in the pars intermedia while the x emphasises its linkage with the vermal x-zone.

Figure 9A and B is a summary diagram showing the findings from the present study. Figure 9A shows a hemicerebellum with the 9 identified zones (i.e. a, x, b, c1, cx, c2, c3, d1, d2). The diagonal-hatching in the cx and x-zones matches the hatching in the diagram of the unfolded MAO (Fig. 9B), showing the location of the cells of origin of the climbing fibres to these zones.

Comparison of the olivary sources of the x and cx zones

As discussed above, the x-zone and the cx zone (previously called lateral c1 zone) are supplied by olivary axons which divide to give climbing fibres to both zones (Andersson and Eriksson 1981; Ekerot

and Larson 1982). Indeed, Ekerot and Larson (1982) have suggested that all of the Purkinje cells in the x-zone share climbing fibre collaterals with Purkinje cells in the cx zone (although the reverse is not true). Thus, one would expect a considerable overlap in the olivocerebellar projection to these zones. Indeed, if the distribution of retrogradely labelled cells in MAO following WGA-HRP injections in the x-zone (Figs. 3B and 4B) is compared with the distribution of labelled cells following injections in cx (Fig. 4D and 7B; see also Fig. 9B), it is clear that an olivary region lying medially and caudally in rostral MAO is retrogradely labelled from both zones. However, if it is true that all the x-zone climbing fibres are collaterals of cx-zone climbing fibres, one might expect a larger area of overlap.

In the present experiments, the retrogradely labelled cells in the MAO arising from injections in the x-zone always occurred in a strip extending across the middle of the MAO in a caudolateral to mediorostral direction. The cells lying caudally and laterally in this strip were never labelled by injections in the cx-zone. Two explanations are possible. The first is that the caudolateral group of cells are not olivary cells supplying the x-zone but cells supplying the adjacent a-zone, since this zone is known to receive climbing fibres from caudal MAO (Groenewegen and Voogd 1977). This explanation is, however, unlikely since the presence of this caudolateral group of cells was a consistent finding between cases, even when the precise position and size of the injection site varied (cf. Figs. 1B/2B, 4A and B and 8). A more plausible explanation is that the climbing fibres in the cx-zone which arise from this caudal portion of the olivary nucleus innervate Purkinje cells which lie in cortex buried in the depths of the cerebellar fissures or in cortex more rostral or caudal than the restricted part of the cerebellar surface (lobules Vb and c) accessible in the present experiments.

It should be noted that the whole rostro-caudal extent of the x-zone was not studied in the present experiments. The x-zone extends from around the middle of lobule V into lobule VI (Andersson and Eriksson 1981; Ekerot and Larson 1982) but only lobules Vb and c were used in the present study. Thus, definitive comment cannot be made regarding the total extent of MAO which projects to the x-zone. However, it is interesting to note that injections in lobule Vb and in lobule Vc both result in labelling in the same olivary region (cf. Figs. 3B and 4B). This is consistent with sagittal branching of the climbing fibres within the x-zone (Ekerot and Larson 1982) and may suggest that the area of MAO which supplies the x-zone is likely to be approximately as delimited in Fig. 9.

Correlation with other studies

In the present investigation the boundaries of the cerebellar cortical zones have been delimited using electrophysiological techniques and for this reason we have used lower case letters as identifiers for each zone (cf. for example Oscarsson 1980). This usage acknowledes the fact that it is not yet definitely established whether the 'electrophysiological' zones are precisely congruent with those which have been revealed by purely anatomical studies of localisation in the olivo-cerebellar projection. The latter are normally identified with upper case letters (see for example Groenewegen and Voogd 1977; Brodal and Walberg 1977; Groenewegen et al. 1979). Congruence is, however, likely – particularly as a retrograde HRP study (Voogd 1983) has recently demonstrated the existence of an 'anatomical' X zone in the cat which apparently coincides with the electrophysiological x zone.

In regard to this zone it is noteworthy that Voogd (1982) made the following comment: "Intermingling of A- and C_2-projecting cells at the border region of the rostral and caudal halves of the MAO seems to be slight and the existence of double projecting cells with axons to both zones has not yet been investigated. As pointed out before, the occurrence of cells with branching axons in this region would offer an alternative explanation for the origin of the branched climbing fibers terminating in the x and c_1 zones."

This comment in a real sense anticipates our findings but it also carries the implication that the x and cx zones should not be seen as forming anatomically distinct zones. It suggests that the x zone should be regarded as a lateral subdivision of the A zone and the cx zone as a medial subdivision of the C_2 zone. Such a view is partially undermined by Voogd's own subsequent demonstration of a distinct X zone (Voogd 1983) but in regard to the cx zone it would provide one possible explanation for the fact that the heaviest olivary labelling, after our injections in this zone, was in a (medial) part of the rostral MAO which was found in a previous HRP study to be included in the olivary region projecting to the C_2 zone (Brodal and Walberg 1977, see especially cases B.St.L. 625 and 629). However, the injection sites in those cases are likely to have involved the cx as well as the C_2 zones so that the currently available HRP evidence seems insufficient to establish whether the cx zone is or is not a subdivision of the C_2 zone.

Other evidence bearing on this problem is provided firstly by the observation that the Purkinje cells in the C_2 and X zones are slightly smaller than those in the nearby A, B, C_1 and C_3 zones and have axons slightly smaller in diameter. This has been taken as

indicating some linkage between the two zones (Voogd 1983). Secondly, in an orthograde tracing study (primarily using ^{3}H-leucine) it was concluded that the whole of rostral MAO projects to the C_2 zone (Groenewegen et al. 1979). However, it is not impossible that the labelling of climbing fibres in that study extended to both the cx and the C_2 cortical zones, although to be tenable this interpretation would seem to require an assumption that the olivary axons to both zones reach the cortex via the same (C_2) compartment of the subcortical white matter.

Finally, it is worthwhile returning to the electrophysiological evidence: both the x and cx zones by definition function as termination zones for forelimb inputs mediated via a dorsal funiculus spino-olivocerebellar path and in this they differ sharply from the c_2 zone which receives no direct input from this pathway (see Oscarsson 1980).

Afferent inputs to the MAO region projecting to the x and cx zones

Ekerot and Larson (1979a) demonstrated that the olivary cells projecting to the x and cx (lateral c1) zones receive inputs from the dorsal column nuclei (DCN). They assumed, on the evidence of Groenewegen et al. (1975) and Berkley and Hand (1978), that the cuneo-olivary projection giving rise to the short-latency climbing fibre responses in the x and cx zones terminated in the DAO. In light of the present results, demonstrating a MAO source of climbing fibres to these zones, a re-examination of the literature on cuneate projections to this olivary subnucleus is necessary.

Boesten and Voogd (1975), using degeneration techniques, demonstrated a crossed cuneo-olivary projection terminating at the medial edge of the rostral MAO just rostral to nucleus β (see their Figs. 4–7 and 13). This termination area was not detected by Groenewegen et al. (1975) using anterograde transport of ^{3}H-leucine, nor by Berkley and Hand (1978) using a combination of lesion and autoradiographic tracer techniques. Recently, however, a detailed autoradiographical study of the projections from the cuneate nucleus to the cerebellum and the inferior olivary nucleus (Gerrits et al. 1984) has again revealed the existence of a projection from the contralateral cuneate nucleus to the rostral MAO (see Fig. 9C). The position of this termination matches with the area of MAO found, in the present study, to project to both x and cx zones.

Thus, it appears from both electrophysiological studies (Ekerot and Larson 1979) and anatomical evidence (Boesten and Voogd 1975; Gerrits et al. 1984) that the MAO region projecting to the x and cx zones in the anterior lobe of the cerebellum receives spinal input relayed through the cuneate nucleus.

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