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THE OLIVOCEREBELLAR PROJECTION TO LOBULES I AND II

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INTRODUCTION

The olivary projection to the cerebellar anterior lobe has been extensively investigated (for review, see ref. 3). However, in these studies, it has been difficult with certainty to establish the organization of the projection to lobules I and II. The main reason for this is the inaccessibility and small size of these lobules. Injections of tracer into this cerebellar region almost inadvertently leads to a spread to the neighbouring cerebellar folia, and only in one previous study (12) has it been possible to restrict the injections to these lobules.

Recently, Mori *et al.* (14) have described a technique for implantation of crystalline horseradish peroxidase (HRP). We have modified this technique and previously used it for implantation of wheat germ agglutinin-horseradish peroxidase complex (WGA-HRP) in crystalline form in the cerebellar nuclei and nodulus (see e.g., refs. 7 and 17). We describe here 15 cases with implants restricted to the anteriormost parts of the cerebellar cortex with no contamination along the track of the glass cannula.

MATERIAL AND METHODS

From a larger material 15 adult cats (weight 2.0-3.4 kg) were selected for the present study. They were operated on under deep pentobarbital anaesthesia. WGA-HRP implants were made stereotactically from a dorsal approach. (The readers are referred to Dietrichs *et al.* (7) for further methodological details). After one day, again under deep anaesthesia, the cats were perfused intracardially with physiological saline followed by a mixture of 1% paraformaldehyde and 1.25% glutaraldehyde in phosphate buffer at pH 7.4. The perfusion was terminated with a cold solution of 10% sucrose in phosphate buffer. The cerebellum was isolated and cut in parasagittal sections at 50 μm on a freezing microtome. Each section was stored separately, and they were all processed with tetramethylbenzidine as described by Mesulam (13). The two first of every five sections were mounted, the first unstained, the other stained with Neutral red. Through the implantation site also the remaining three sections were usually mounted and stained. By this procedure it was possible with accuracy to measure the mediolateral extent of the cerebellar implants.

After isolation, the brain stem was cut in transverse sections. These sections were collected in groups of five, and two sections from each group were processed as mentioned above. One series was mounted unstained, the other stained with Neutral red.

All sections were examined microscopically with bright field illumination and in polarized light. The implantation site was carefully checked to decide if staining had occurred along the needle track. Only cases which were negative in this respect were used. The location

and extent of the staining at the implantation site was indicated in drawings of the sections. Photomicrographs of two of the implants are shown in Fig. 1. With two exceptions (cats B.St.L. 1214 and 1233) the stained area at the implantation site was in all cases confined to the cerebellar cortex and folial white matter, with no spread of staining to the central cerebellar white matter. The distribution of the retrogradely labelled cells in the inferior olive was mapped and the findings were subsequently transferred to a standard diagram.

RESULTS

1. *Morphology of lobules I and II.* — Sublobule Ia, which is connected with the anterior velum, is very narrow and is separated from sublobule Ib with a rather shallow fissure. This sublobule is again delimited from sublobule IIa by the distinct precentral fissure. Figure 1 shows, however, that the configuration of sublobule Ia and b can differ, as can also the attachement of the anterior velum. The size of sublobule Ia varied considerably among our 15 cases, and this sublobule was absent in one cat. In most of his figures Larsell (10) indicated that sublobule Ib consists of two folia. However, in our material this was the case in only one animal. In the remaining 14 cases the cortical surface of sublobule b was unindented.

2. *Implants in sublobule Ia.* — The implant in cat *B.St.L. 1215* (Fig. 2) was confined to sublobule Ia. The central, intensely stained region was found on the left side, in a mediolateral direction comprising an area between the parasagittal planes 750 and 1500 μm from the midline. On both sides of this there was a weakly stained area, which on the left side could be followed 250 μm further laterally, almost to the lateral border of the folium. On the right side of the intensively stained region, the weakly stained cortex extended laterally beyond the midsagittal line including 1250 μm of the cortex on the right side. Retrogradely labelled cells were present only in the right medial and dorsal accessory olives (Fig. 2). In the former, the labelled cells appeared from level I-V. Caudally they were restricted to the medial region, but in the rostral direction gradually filled also more lateral parts, leaving only the lateral border free. In the dorsal accessory olive, the positive cells occurred only caudally at levels IV to VI, filling mainly the ventral part, but rostrally occupying also its dorsal region.

Two other cats had implants in the same folium, but in both the adjacent deeper part of lobulus Ib was involved. In the former (cat *B.St.L. 1214*), the implant was restricted to the left side, where it took its beginning 1 mm from the midline and extended 1/2 mm further laterally. It was restricted to the deepest parts of lobules Ia and b, and comprised the adjacent central white matter and the rostroventral tip of the fastigial nucleus. The implant was very sharply demarcated from the surroundings and consisted only of an intensively stained region. Retrogradely labelled cells were present only in the right medial and dorsal accessory olives with a distribution almost similar to that described above. The only difference was that the medial accessory olive, at caudal level III, was filled with

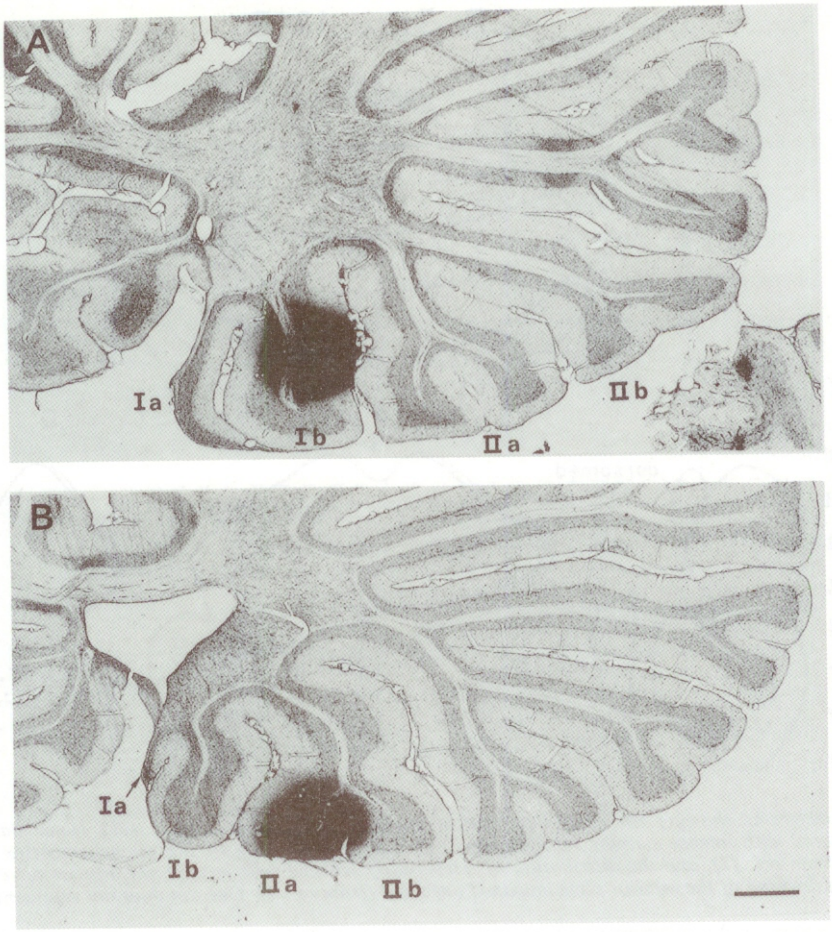

Fig. 1. – *Photomicrographs showing the implants in cats B.St.L. 1160 (A) and 1219 (B).* Note the differences in the configuration of the different lobules. Scale line 0.6 mm.

labelled cells not only in its ventrolateral part, but also dorsomedially (these dorsomedially located cells probably project to the fastigial nucleus).

The third case in this group, cat *B.St.L. 1216* (Fig. 3), had an implant with its center in the fissure between sublobules Ia and Ib, and comprised the adjacent parts of both. Along the folia, the intensely stained region measured 1050 μm, with 150 μm to the left of the midline and 900 μm to the right. On this side, the weakly stained halo continued 500 μm further laterally, on the left side the halo could be followed 700 μm in the lateral direction. Retrogradely labelled cells were present bilaterally in the medial accessory olives, slightly rostrally to those in the previous cases (see Fig. 3). The majority was located on the left side. No labelled neurons were found in the dorsal accessory olives.

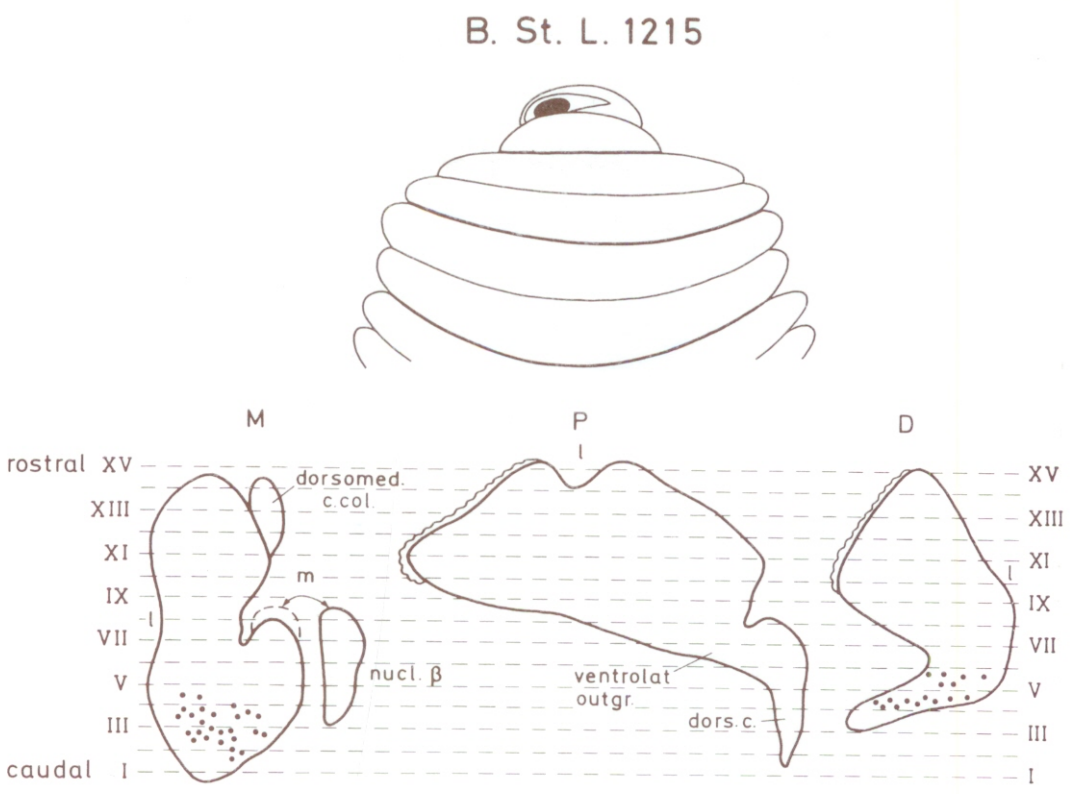

Fig. 2. – *Diagrams showing the cerebellar cortical implant (top) in cat B.St.L. 1215 (total stained area indicated, area with intense staining labelled black) as seen in a drawing of the cerebellar cortex imagined unfolded (from ref. 11), and the distribution of retrogradely labelled cells in the right inferior olive (bottom) as seen in a drawing of the inferior olive imagined unfolded (from ref. 2). One dot does not represent one cell.*

Abbreviations: D, dorsal accessory olive; dors. c., dorsal cap; dorsomed. c. col., dorsomedial cell column; l, lateral; M, medial accessory olive; m, medial; nucl. β, nucleus β; I-XII, caudo-rostral levels of the inferior olivary complex.

3. *Implants in sublobule Ib.* — In cat *B.St.L. 1160* (Figs. 1A and 4) the intensely stained region was rather circumscribed and reached the precentral fissure. Its folial extension was from 250 μm to the right of the midline to 1 mm to the left. The weakly stained area continued 250 μm on both sides. Retrogradely labelled cells were present in the right medial and dorsal accessory olives, with a distribution and caudorostral extension as shown in Fig. 4. On the left side, however, only three positive cells were found in the medial accessory olive, all located ventromedially in its caudal region.

In another cat, *B.St.L. 1204*, the implant in a ventrodorsal direction comprised a greater part of sublobule Ib than in the preceding case. The folial extension of the heavily stained region was 750 μm and symmetrical on the two sides, on the right side the weakly stained area continued 1 mm further laterally, on the

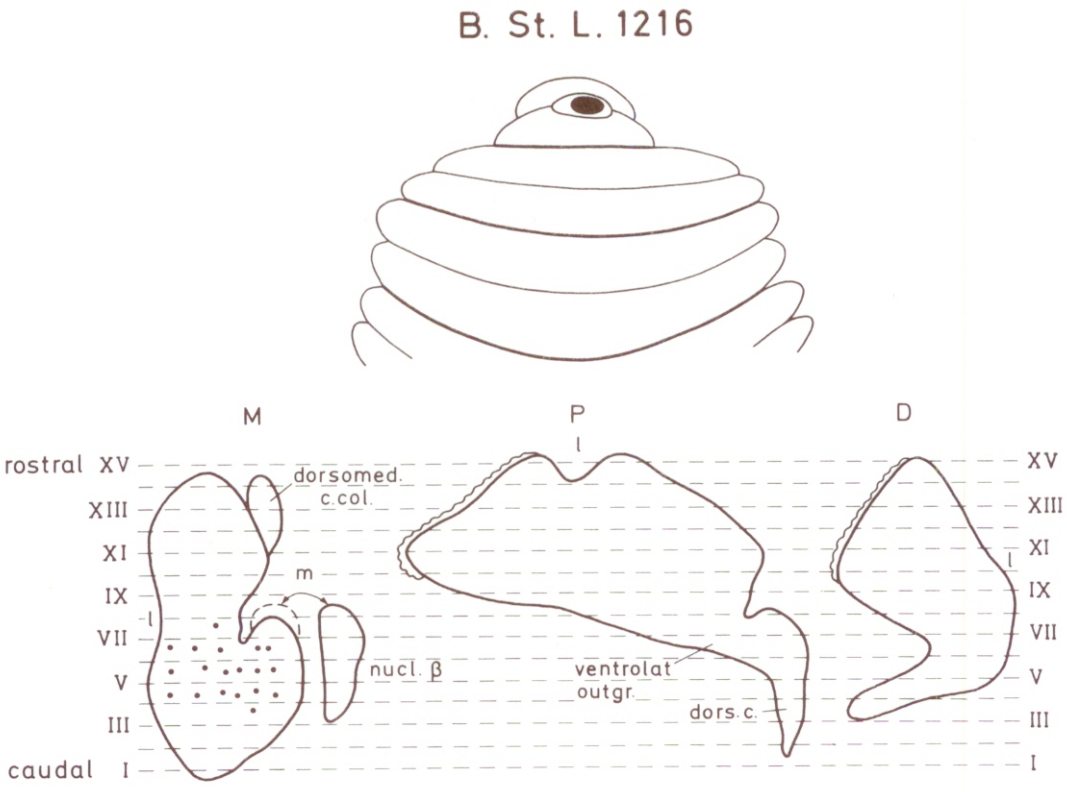

Fig. 3. – *Diagrams showing the cerebellar cortical implant (top) in cat B.St.L. 1216 (total stained area indicated, area with intense staining labelled black) and the distribution of retrogradely labelled neurons in the left inferior olive (bottom). One dot does not represent one cell.*

For abbreviations, see Fig. 2.

left side 1250 μm. This cat had retrogradely labelled cells bilaterally restricted to the medial accessory olive (levels I-VII) with the majority on the right side. Except for at the caudalmost levels, the cells were found medially in this subnucleus.

In a third case, cat *B.St.L. 1217*, the implant was located deeper in sublobule Ib and almost restricted to the midline. On the right side the heavily stained area could be followed 200 μm laterally, on the left side 150 μm. The lightly stained region continued 150 μm further on the right, 100 μm on the left. Again, the retrogradely labelled cells were restricted to the medial accessory olives, with a majority on the left side. Their distribution was as in the previous cases.

The fourth cat in this group, *B.St.L. 1213*, had a very superficial implant in sublobule Ib (mainly in the molecular layer). The heavily stained region reached 950 μm from the midline on the left side, 50 μm on the right. On the left side the weakly stained area continued 500 μm further laterally, on the right side 250 μm. Retrogradely labelled cells were also in this animal restricted to the medial

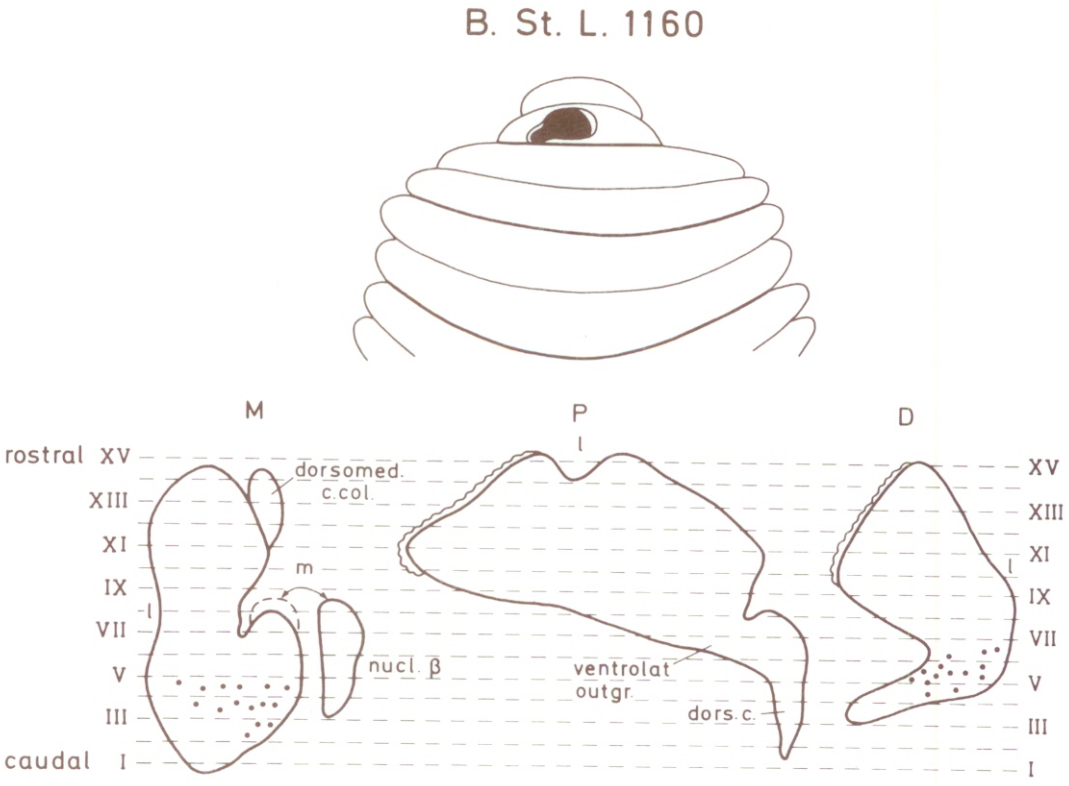

Fig. 4. – *Diagrams showing the cerebellar cortical implant (top) in cat B.St.L. 1160 (total stained area indicated, area with intense staining labelled black) and the distribution of retrogradely labelled neurons in the right inferior olive (bottom). One dot does not represent one cell.*

For abbreviations, see Fig. 2.

accessory olives, with all but one cell found on the right side, and with a distribution as previously described.

The two last cases with implants in lobule Ib (cats *B.St.L. 1221* and *1233*) had staining of the lateralmost part of this lobule. In cat B.St.L. 1221 the implant was located deep in the precentral fissure, with staining of the posterior part of lobule Ib as well as the anteriormost part of lobule IIa. The heavily stained region occupied an area between the parasagittal planes 1400 and 2000 μm to the left of the midline. The lateral edge of lobule Ib was also found in the latter plane. An area with weak staining extended 250 μm further laterally in lobule IIb. On the medial side, the weak staining could be traced for another 150 μm. Retrogradely labelled neurons were only found in the right dorsal accessory olive, at levels IV to VI. Cat B.St.L. 1233 had an implant just under the cortex of lobule Ib. The heavily stained area included the adjacent part of the central white matter and also extended slightly into sublobules Ia and IIa. The lateral margin

was 1900 μm to the left of the midline, the medial margin 1000 μm to the left. A lightly stained ara continued 500 μm further medially. Retrogradely labelled cells were found in both accessory olives on the right side. In the dorsal accessory olive neurons were found at levels III-VII, in the medial accessory olive at levels II-VII (only medially at rostral levels) and throughout nucleus β.

4. *Implants in sublobule IIa.* — Cat *B.St.L. 1209* had a superficial implant with heavy staining restricted to the left side of the folium. It started 50 μm lateral to the midline and was 900 μm wide. The weakly stained halo continued 450 μm laterally on the left side. On the right side it reached 200 μm beyond the midline. Retrogradely labelled cells were observed only on the right side, in the medial as well as the dorsal accessory olives, at levels II-V and IV-VI, respectively. The neurons were distributed as in the previous cases.

In a second cat, *B.St.L. 1208*, the implant was located deeper and confined to the part of sublobule IIa facing sublobule Ib. The heavily stained region could be followed for 750 μm on the right side, but only for 400 μm on the left side of the midline. On the right side the weakly labelled area extended 1250 μm further laterally, on the left side only 400 μm. Positive cells were found bilaterally, but restricted to the medial accessory olives at levels II-VI.

The intensely stained area at the implantation site in cat *B.St.L. 1219* (Fig. 1B), extended 700 μm to the left and 550 μm to the right of the midline, and it also involved a small part of lobule IIb. The weakly stained region stopped 1250 μm from the midline on the left side and 1000 μm from the midline on the right. Retrogradely labelled cells were found in both accessory olives on the right side and in the left medial accessory olive. In the medial accessory olive retrogradely labelled neurons were found centrally at levels I-VI, in the dorsal accessory olive at levels IV-VI.

The last animal with an implant in lobule IIa (cat *B.St.L. 1235*, Fig. 5), had an intense staining covering the lateralmost part of this sublobule. The staining (and the folium) stopped 2350 μm to the left. In the medial direction the intensely stained region reached to 1900 μm from the midline, while some light staining could be traced for another 150 μm. Retrogradely labelled neurons were found laterally in the right dorsal accessory olive at levels IX to XII.

5. *Implant in sublobule IIb.* — We have only one cat, *B.St.L. 1212*, in this group. The implant lay around the intracentral fissure, and included the adjacent parts of sublobule IIb and IIIa. The heavily stained region took its beginning 200 μm to the left of the midline and continued 650 μm on the right. The pale halo extended 250 μm further laterally to both sides. Positive cells were present centrally in the left medial accessory olive at levels I-V, and centrally and laterally in the left dorsal accessory olive at levels VI-VII.

6. *Implant in sublobule IIIa.* — Also one case with an implant in sublobule IIIa was included in this study. Cat *B.St.L. 1232* had a very minor spot-like

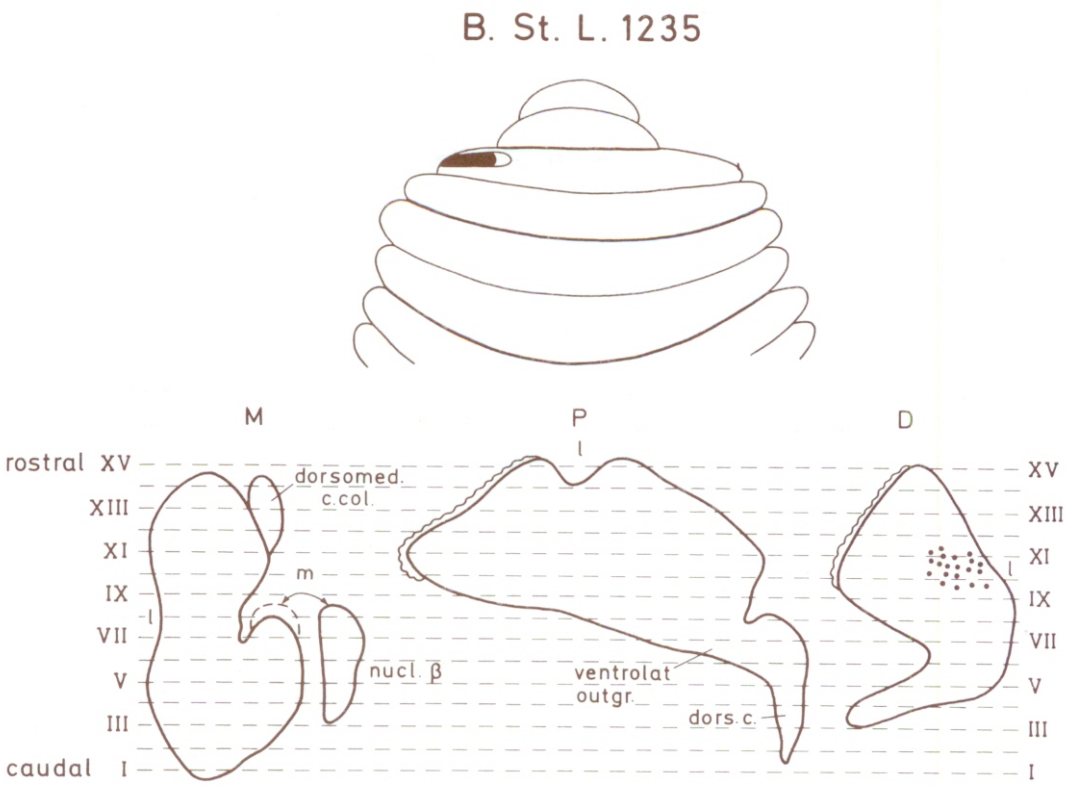

Fig. 5. – *Diagrams showing the cerebellar cortical implant (top) in cat B.St.L. 1235 (total stained area indicated, area with intense staining labelled black) and the distribution of retrogradely labelled neurons in the right inferior olive (bottom). One dot does not represent one cell.*

For abbreviations, see Fig. 2.

implant in the lateralmost part of this folium, 2750 μm from the midline. The only retrogradely labelled cell in this case was found laterally in the medial accessory olive at level VIII.

7. *Interpretation of findings.* — The implants in the cases described above comprise sublobules Ia through IIIa. The retrogradely labelled olivary cells are found either unilaterally or on both sides, and are restricted to the dorsal and/or medial accessory olives. With minor differences, the nuclear location is similar in all cases. It is well known that in the cat, the olivocerebellar projection is entirely crossed. This is confirmed by the findings made in our five cats (B.St.L. 1214, 1221, 1232, 1233 and 1235) with unilateral (left) implants, where positive cells are only found in the right inferior olive. It is not clear whether uptake and transport of the tracer in our material has occurred only from the intensely stained area at the implantation site, or also from the weakly stained surrounding area.

However, it is noteworthy that in our two cases with unilateral intense and bilateral weak staining (cats B.St.L. 1209 and 1215), retrogradely labelled olivary neurons were only found on one side (contralaterally to the intensely stained region).

Previous tracer studies have shown that the caudal part of the medial accessory olive sends its cerebellar afferent fibres to Voogd's A zone, and that the caudal part of the dorsal accessory olive projects to the B zone, the rostral part of the dorsal accessory olive to the C_1 and C_3 zones, and the rostral part of the medial accessory olive to the C_2 zone (for details, see ref. 3). Retrogradely labelled neurons were found in the olivary A and B regions after implants in lobule I, but labelling in the C_1/C_3 area was not seen even after the lateralmost implants in this lobule (cf. cats B.St.L. 1221 and 1233). It therefore appears justified to conclude that the A and B, but not the C_1/C_3 zones are present in lobule I. The exact border between the A and B zones can not be determined from the present material, but it is noteworthy that both A and B regions are extensively labelled in cats B.St.L. 1160 and 1214, the former with a medial intense staining ending 1000 μm from the midline, the latter with a lateral implant sparing the medialmost 1000 μm of the lobule. These observations indicate that the position of the border may vary, but that it is located about 1000 μm lateral to the midline. This seems plausible also from the observations in our other cases.

As concerns the medial border of the B zone in lobule II, the findings in cases B.St.L. 1209, 1212 and 1219 tend to indicate that this lies more medially than in lobule I. In cat B.St.L. 1212 the intensely stained area extended no more than 650 μm laterally (and the weakly stained area another 250 μm), still a substantial number of labelled neurons were found in the olivary B region. The lateral border of the B zone is probably located 1900-2000 μm from the midline. The olivary labelling was confined to the B region in cat B.St.L. 1221, with intense staining extending laterally to the 2000 μm parasagittal plane, whereas only C_1/C_3 labelling was found in cat B.St.L. 1235, with intense staining extending medially to the 1900 μm parasagittal plane.

It appears from our material (cf. cat B.St.L. 1235, Fig. 5) that the C_1/C_3 zone reaches the lateralmost part of lobule IIa, and that there is no trace of a C_2 zone in this sublobule. The findings in cat B.St.L. 1232 show that the C_2 zone is present in lobule IIIa, but it is not possible from our material to determine whether the C_2 zone ends in lobule IIIa or IIb.

DISCUSSION

The most detailed previous retrograde tracer study of the olivo-cerebellar projections to lobules I and II is that by Matsushita and Okado (12). After pressure injections of HRP into these lobules, they found that the retrogradely labelled cells were restricted to the medial and dorsal accessory olives, an observation confirmed by us. At the injection sites, Matsushita and Okado (12) distinguish between a strong, moderate and weak HRP reaction, but they do not relate their

observations in the olive to the folial width of these stained areas. Therefore, it is impossible to compare their data as regards the exact laterality and distribution of the various olivocerebellar terminal cortical zones with our findings.

The present study has shown that only the A and B zones are present in lobule I, whereas also the fused C_1/C_3 zone is found in lobule II. This is largely in accordance with the results of previous anterograde and retrograde tracer studies (see Fig. 17 in Brodal and Kawamura's (3) review article), and also seems to fit with the observations made by Matsushita and Okado (12). In their figures Brodal and Kawamura (3) indicate that the B zone may not be present in lobule Ia, but our material has shown beyond doubt that the B zone is found even in this lobule (see especially cat B.St.L. 1215). Similar observations were made also in some of the autoradiographic cases of Kawamura and Hashikawa (9). In his illustrations Voogd (16) indicates that the x zone is interposed between the A and B zones also in the anteriormost lobules, whereas other authors indicated that the x zone disappears already in the rostral part of lobule V (1). Our material can not give a definite answer to this problem, but it is noteworthy that most of our cases with involvement of both the A and B zones (see especially cats B.St.L. 1160, 1209, 1212, 1215, 1219 and 1233; Figs. 2 and 4) had no labelling in the olivary region previously reported to project to the x zone (15). Also as concerns the rostral end of the C_2 zone, there are some uncertainties. Groenewegen *et al.* (8) illustrate three cases in which a distinct autoradiographically labelled C_2 band can be followed into lobule IIIa, but not further. Courville *et al.* (5), on the other hand, illustrate a small patch of cortex also in lobule IIb receiving fibres from the olivary C_2 region (their Fig. 14C). In the present study we have confirmed that the C_2 zone is present in lobule IIIa and absent in lobule IIa, but we can not comment on its possible presence in lobule IIb.

The present study is the first attempt to measure the widths of the olivocerebellar terminal zones in lobules I and II. Previously, the extension of the cortical zone projecting to the vestibular nuclei has been calculated (see e.g., refs. 4 and 6). Comparing these results with those of the present study, one reaches the rather unexpected conclusion that most of the Purkinje cells in lobule I projecting to the vestibular nuclei lie medially to the olivocerebellar terminal B zone. The above mentioned studies (4, 6) place the lateral border of the vestibular projecting zone about 1000 μm from the midline, and according to the present material this is where the B zone starts in lobule I. In this context one should, however, keep in mind that the Purkinje cells projecting to the vestibular nuclei show a widespread distribution and are not found as a sharply demarcated sagittal band (6). Nonetheless, if most of the corticovestibular fibres from this lobule originate outside the olivocerebellar terminal B zone, this may have major functional implications and may indicate that we should not consider cerebellar cortical processing as schematic as we do today.

SUMMARY

The olivocerebellar projection to lobules I and II was studied by means of retrograde transport from implants of the crystalline WGA-HRP complex. Retrogradely labelled neurons were found in the medial and dorsal accessory olives. Judged from the distribution of labelled cells, we conclude that parasagittally the olivocerebellar terminal zones A and B (i.e., the cerebellar cortical strips receiving axons from the olivary A and B regions) extend anteriorly into lobule Ia, whereas the fused olivocerebellar terminal C_1/C_3 zones reach lobule IIa. The olivocerebellar terminal C_2 zone extends into lobule IIIa but not into lobule IIa.

In lobule I the medial border of the B zone lies about 1 mm from the midline, in lobule II the B zone extends somewhat more medially. The lateral border of this zone is 1.9-2 mm from the midline. Compared to previous results, it appears that most of the Purkinje cells in lobule I projecting to the vestibular nuclei lie medially to the olivocerebellar terminal B zone.

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