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The Trigemino-olivary Projection in the Cat as Studied with Retrograde Transport of Horseradish Peroxidase

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Summary. The trigemino-olivary projection was studied in cats which from a ventral approach were injected with horseradish peroxidase into various parts of the inferior olive. Retrogradely labelled cells of all sizes were present in all three divisions of the spinal sensory nucleus: nucleus caudalis, nucleus interpolaris and nucleus oralis. The projection is bilateral with the highest number of labelled cells on the contralateral side. No retrogradely labelled cells are found in the principal nucleus, but some cats showed a few retrogradely labelled cells within the ipsilateral mesencephalic nucleus.

The findings are discussed and related to recent observations concerning the distribution of the direct trigemino-cerebellar fibres.

Key words: Trigemino-olivary projection – Mammals – Retrograde transport of HRP

Previous experimental anatomical studies have shown that in the cat there is a trigeminal input to the inferior olive (Stewart and King 1963; Kawamura 1971; Boesten and Voogd 1975; Berkley and Hand 1978). More specifically, these observations which have been performed with autoradiographic and anterograde degeneration techniques have given evidence that the fibres are given off from the pars caudalis of the spinal trigeminal nucleus, and that they reach very restricted parts of the olivary complex.

Recent investigations in mammals where horseradish peroxidase has been used as a retrograde tracer, have provided new details in the projection from the trigeminal nuclei to the superior colliculus, the thalamus, the cerebellum, various brain stem nuclei and the spinal cord (Baleydier and Mauguiere 1978; Hockfield and Gobel 1978; Karamanlidis et al. 1978; Burton and Craig 1979; Burton et al. 1979; Fukushima and Kerr 1979; Kruger et al. 1977; Shigenaga et al. 1979; Somana et al. 1980; Matsushita et al. 1980, 1981). A similar detailed tracer study has, however, until now not been performed for the trigemino-olivary connection.[1] The present report which is based on horseradish peroxidase used as a retrograde cellular marker, shows that the trigeminal nuclear efferent fibres take their origin from more widespread areas of the complex than has hitherto been known.

Material and Methods

Altogether 20 cats have been used.[2] The animals were operated upon under Mebumal anaesthesia, and free[3] horseradish peroxidase (HRP) (4% Serva, with poly-L-ornithine (50 µg/ml) added, Itaya et al. 1978) was injected iontophoretically in the left inferior olive from a ventral approach.[4] One cat (B.St.L. 916) was injected with Sigma peroxidase type VI labelled lectin (wheat germ agglutinin). A current of 5 µA was used for 20 s – 10 min. Two days later the animals were deeply anaesthetized with Mebumal and perfused with 0.1 M phosphate buffer at pH 7.4, 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), and finally with 10% sucrose. Blocks from the brains were then immediately cut in serial transverse sections at 50 µm on the freezing microtome. The sections were reacted according to the technique of Mesulam (1978). One series was left unstained, another weakly stained with neutral red. In some cases all sections through the injection sites were mounted to allow mapping of the localization and extent of the HRP. The mapping showed that the HRP staining was restricted to parts of the olivary complex in 13 of the cats. Circumscribed areas of the reticular formation dorsal to the olive were included in the injections in the seven other cats (see below). The sections from the trigeminal

1 Brown et al. (1977) in their HRP study in the rat mention fibres from nucleus interpolaris to the contralateral inferior olive

2 The majority of the cats presented here were used for a study of the pretectal afferents to the inferior olive (Walberg et al. 1981)

3 The term free horseradish peroxidase when used in this communication denotes the Serva type of HRP

4 Cat B.St.L. 833 was injected on the right side

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sensory nuclei were studied with bright field and with interference contrast microscopy.

Three cats served as controls (cats B.St.L. 866, 880, 881). The first of these was injected from a ventral approach and had an HRP deposit restricted to the reticular formation just dorsal to the olive. The two other cats were from a dorsal approach injected into the left reticular formation somewhat more dorsally. The injections in these three cats covered the areas of the reticular formation included in the injections in cats B.St.L. 836, 841, 867, 868, 872, 900 and 902. Of the control cases only cat B.St.L. 880 was positive and showed four labelled cells within the contralateral nucleus interpolaris of the spinal trigeminal nucleus. Several unoperated cats served as normal controls; none of these animals had endogenous peroxidatic activity in the trigeminal nuclear complex.

The mapping of the olivary injections was made in drawings of the olive made by means of a projection apparatus. Every fifth section of the olive was drawn. In order to facilitate the comparison of the level of the injections in the various cases, the map obtained from an individual case was transferred to a standard diagram of fifteen equally spaced transverse sections through the olive. After this, the stained olivary areas were entered in a diagram of the olive unfolded into one plane (both diagrams from Brodal 1940). This made possible a comparison of the accurate level and site of injection in the individual cases. Figure 1 shows the standard diagram of the unfolded olive. Table 1 gives the number of labelled cells in the trigeminal nuclei in each injected case. The labelled cells were counted from the stained series in every fifth consecutive serial section.

Results

The subdivision of the trigeminal sensory nuclei (TSN) used in this study is based on the description given by Taber (1961). The reader is referred to this publication for details, also concerning cell types.

The TSN in the cat constitutes a cell column taking its beginning at the rostral end of the dorsal horn of the first cervical segment and terminating at the level of the posterior commissure. The spinal sensory nucleus (Vsp) can be subdivided in a nucleus caudalis (Vc), nucleus interpolaris (Vi), and nucleus oralis (Vo). The Vc, which can be divided into three subnuclei, can rostrally be followed to the level of the obex, proceeds as Vi to the level of the rostral pole of the hypoglossal nucleus and then continues as the Vo. At the upper pole of the facial nucleus Vo fuses with the principal nucleus (Vp) which stretches to where the caudal pole of the medial parabrachial nucleus takes its beginning. The mesencephalic nucleus (Vme), situated somewhat more medially, begins at the rostral pole of the trigeminal motor nucleus and can be followed as a strand of cells to the rostral end of the complex.

Experimental Findings

The hatched areas within the olivary complex in Fig. 1 show the regions which Berkley and Hand (1978) in

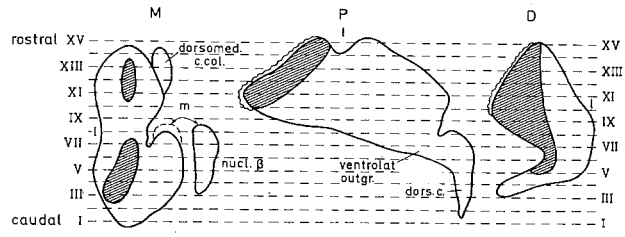

Fig. 1. Digram modified from Berkley and Hand (1978) showing the four areas within the inferior olive (hatchings) by these authors found to be the terminal regions for the trigemino-olivary fibres. The diagram of the unfolded inferior olive in this figure and in Fig. 2 is taken from Brodal (1940). Abbreviations: D, dorsal accessory olive; dors.c., dorsal cap; dorsomed.c.col., dorsomedial cell column; l, lateral; m, medial; M, medial accessory olive; nucl. β, nucleus β; P, principal olive; ventrolat outgr., ventrolateral outgrowth; I–XV, transverse sections through the inferior olive from caudal (I) to rostral (XV)

their autoradiographic and fibre degeneration study indicate as the terminal regions for the trigemino-olivary fibres. These regions comprise two areas within the medial accessory olive (M), one caudally, the second rostrally, a third in the ventral lamella (vl), and a fourth in the medial part of the dorsal accessory olive (D). Eleven of the 20 cats presented here have olivary injections including one or more of these areas (Fig. 2A–E, I, K, M, N, P, S). The nine other cats (Fig. 2F–H, J, L, O[5], Q, R, T) have HRP injections in areas of the olivary complex outside the hatched regions shown in Fig. 1.

Table 1 gives the number of retrogradely labelled TNS cells in the 20 cases presented here. Cells of all sizes are labelled (Fig. 3), and there is no topical difference in their nuclear distribution in the various animals. The trigemino-olivary projection is bilateral, with the contralateral connection being the most important, and the retrogradely labelled trigeminal cells present within Vc (its magnocellular part), Vi, Vo, and Vme. The contralateral Vi has the heaviest projection to the inferior olive, there is a weaker projection from the contralateral Vc, and a more inconspicuous projection from the Vo. Noteworthy is furthermore that four of the cats have a projection from the Vme to the ipsilateral olive (Fig. 2A, B, D, F, and Table 1).

Cat B.St.L. 863 (Fig. 2K) is the animal with the highest number of retrogradely labelled TSN cells (82). The injection in this animal covers parts of three of the four areas which Berkley and Hand (1978) concluded were the olivary regions for the termination of the efferent TSN fibres (see Fig. 1). A high number of retrogradely labelled TSN cells are

5 The injection in the ventral lamella encroaches slightly upon the hatched region shown in Fig. 1

Table 1. Table showing the number and distribution of retrogradely labelled cells in the trigeminal sensory nuclei in 20 cats with HRP injections into various parts of the left inferior olive. See Fig. 2 for injection sites

	Ipsilateral (left) side					Contralateral (right) side					
Cat B.St.L.	Vc	Vi	Vo	Vme	Total	Vc	Vi	Vo	Vme	Total	Total
833				5	5	4				4	9
834				2	2		3			3	5
836					0		3	1		4	4
837				3	3		5			5	8
841		1	5		6	14	8			22	28
847[a]				1	1		1			1	2
849[a]					0					0	0
857[a]					0	3				3	3
859	3	14			17	8	18	1		27	44
862[a]					0	2	13			15	15
863		15			15	3	55	9		67	82
866[a]					0					0	0
867					0	3				3	3
868		4			4	1	10			11	15
872[a]	1	16	4		21	15	14	1		30	51
873		5			5	7	12			19	24
877[a]					0		1			1	1
900[a]	4		1		5	3				3	8
902	2	4			6						6
916[a]					0		11			11	11

[a] Indicate cats with HRP injections in olivary regions outside those shown in Fig. 1

likewise present in cats B.St.L. 841, 859, and 873 (Fig. 2E, I, P), also with HRP injections covering parts of the hatched areas shown in Fig. 1.

Seven of the nine cats with olivary injections outside the hatched areas shown in Fig. 1 have retrogradely labelled TSN cells (Fig. 2F, H, J, O, Q, R, T, and Table 1). One of these, cat B.St.L. 872 (Fig. 2O) had a very high number of positive TSN cells (51 cells).

Two cats (Fig. 2G, L, and Table 1) were negative with respect to retrogradely labelling of TSN cells. Both these cats had olivary HRP injections outside the hatched regions shown in Fig. 1.

Discussion

Origin of the TSN Efferent Fibres

The present observations show that it is the Vc, Vi, Vo, and Vme, but not the Vp which in the cat give origin to the fibres reaching the olivary complex. Boesten and Voogd (1975, fibre degeneration) described a contralateral projection from Vi only, and Kawamura (1971, fibre degeneration) and Berkley and Hand (1978, fibre degeneration, autoradiography) found fibres from the Vc to the olive on both sides. Stewart and King (1963, fibre degeneration) traced fibres from the Vc to the ipsilateral olive. These partly conflicting observations are probably due to the techniques used; the method of studying the retrograde transport of HRP is superior for the demonstration of the origin of a relatively scanty projection. This is especially demonstrated by the observation that in the cat there is a small but clearcut projection also from the Vme to the inferior olive. This projection is ipsilateral only and appears to reach the M and P (see Fig. 2A, B, D, F, and Table 1). In this context it should be noted that recent HRP studies have given evidence for a rather widespread origin also of the trigeminocerebellar fibres (Ikeda 1979; Somana et al. 1980). The latter authors found that in the cat the majority of the cerebellar TSN efferents originated in the Vi, with Vo as the second most important region, and with a clearcut projection also from the Vp, Vc and Vme, but that contrary to what is observed here, the trigeminocerebellar projection was almost exclusively ipsilateral.

Termination of the TSN Efferent Fibres

Stewart and King (1963) concluded that the fibres from the TSN reached the M and the dorsal cap (dc), Kawamura (1971) claimed that the fibres reached the D, and Berkley and Hand (1978) presented evidence for a termination of the TSN efferent fibres within

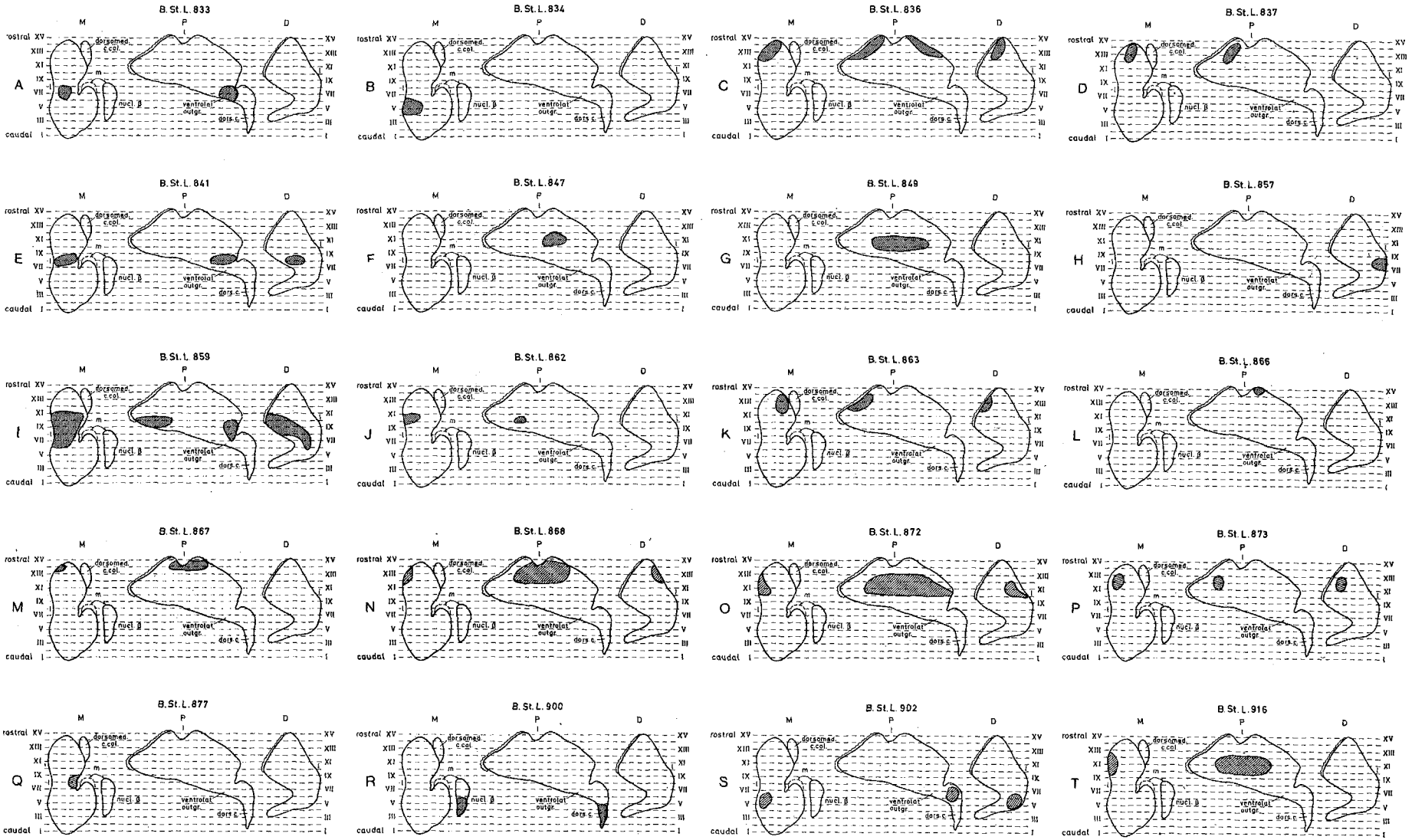

Fig. 2. Diagrams of the HRP injection sites in the inferior olive in the 20 cats presented in the text. Table 1 gives the number of retrogradely labelled trigeminal cells in the illustrated cases. Abbreviations, see legend to Fig. 1

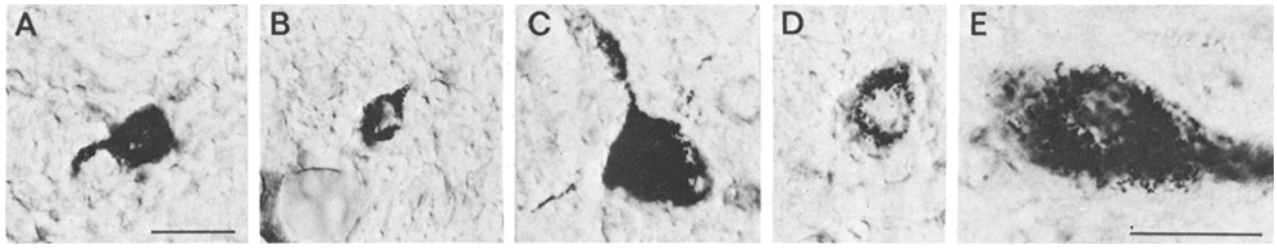

Fig. 3. A–D Retrogradely labelled cells in right trigeminal spinal sensory nucleus in cat B.St.L. 863. **A** Medium-sized cell in nucleus caudalis. **B** Small cell in nucleus interpolaris. **C** Medium-sized cell in same nucleus. **D** Medium-sized cell in nucleus oralis. **A–D** same magnification. **E** Large cell in left mesencephalic trigeminal nucleus in cat B.St.L. 833. Scale lines 20 μm

the four regions depicted in Fig. 1. The present observations confirm all these findings, especially those made by Berkley and Hand (1978). Thus, a high number of retrogradely labelled TSN cells are found in all those cases where the olivary HRP injections cover parts of the three or four olivary areas mentioned above (see especially the cats shown in Fig. 2E, I, K, and P). However, it should be noted that seven of the nine cats with olivary injections outside the hatched areas shown in Fig. 1 have retrogradely labelled trigeminal cells (Fig. 2F, H, J, O, Q, R, T, and Table 1), and one of these cats (B.St.L. 872, Fig. 2O) has 51 positive cells. Only cat B.St.L. 863 (Fig. 2K) shows a higher number of labelled cells. This could indicate that the trigeminofugal fibres may reach olivary regions outside those shown in Fig. 1, but the technique with retrograde transport of HRP is not well suited for a demonstration of details in the termination of a fibre tract.

The HRP injections are confined to one olivary subdivision only in cats B.St.L. 847, 857, and 877 (Fig. 2F, H, and Q). These three cases give evidence that TSN efferent fibres reach the ventral bend of P, the lateral D and the medial M, but it should be noted that the number of retrogradely labelled TSN cells is very low in all these three cases. Since cat B.St.L. 849 (Fig. 2G) has a larger injection in the ventral bend than cat B.St.L. 847 and is negative with respect to labelled TSN cells, and since cat B.St.L. 862 (Fig. 2J) has 15 labelled cells but the lectin injected cat B.St.L. 916 (Fig. 2T) only 11 labelled cells[6], it is possible that the rostrolateral M is a more important region for the TSN efferents than the ventral bend.

6 Gonatas et al. (1979) claim that lectin labelled peroxidase is about forty times more sensitive than free HRP

The present observations indicate that there is an ipsilateral trigemino-olivary projection. This conclusion is based on the observation that unilateral HRP injections restricted to the olive result in labelled cells bilaterally within the trigeminal nuclei. Such a conclusion is warranted only if the crossing of the trigemino-olivary fibres occurs dorsally to the ipsilateral olive and not within the olivary complex itself. In the latter case, a unilateral olivary injection would probably result in an uptake of HRP into the crossing fibres and a retrograde transport of HRP to cells in the trigeminal nuclei on the injected side. No data appear to be available as regards the medullary location of the crossing trigemino-olivary fibres. However, the observations by Stewart and King (1963, fibre degeneration), Kawamura (1971, fibre degeneration) and Berkley and Hand (1978, fibre degeneration, autoradiography) demonstrate a unilateral trigemino-olivary connection. The present observations, like those by Berkley and Hand (1978) give evidence that this is less dense than the contralateral projection.

Another factor to consider is that seven of the cats (Fig. 2C, E, M, N, O, R, and S) had olivary injections which had spread dorsally and in most of them included a circumscribed part of the immediately adjacent reticular formation (nucleus reticularis ventralis or nucleus reticularis gigantocellularis). Several authors have shown that there is a projection from the TSN to the ipsi- and contralateral reticular formation, and that it is the nucleus gigantocellularis which is the main recipient for the fibres (for references, see Stewart and King 1963). It could therefore be argued that in the seven cases mentioned here, the retrogradely labelled TSN cells send their axons to the reticular formation and not to the ventrally lying inferior olive. However, only one of the three control cases with injections into the

reticular formation without encroachment on the olive showed retrogradely labelled TSN cells, and this cat had only four positive cells in the contralateral Vi (see Material and Methods). Furthermore, the HRP injections were restricted to the inferior olive in thirteen of the animals, and eleven of these animals had retrogradely labelled TSN cells. All these observations make it probable that the majority of the retrogradely labelled TSN cells in the seven cats are labelled as a result of the olivary injection.

Recent experimental studies give evidence that the seven cerebellar cortical zones of Voogd receive their afferents from separate parts of the olivary complex (for references to the literature, see Brodal and Kawamura 1980). The relation is as follows: the caudal part of the medial accessory olive (levels I–VIII in Fig. 1), the nucleus β and the dorsomedial cell column (dm.c.col.) send their fibres to the A zone, the caudal part of the dorsal accessory olive (levels III to approximately IX) projects to the B zone, the rostral part of the medial accessory olive (levels VI-XV) projects to the C_2 zone, the rostral part of the dorsal accessory olive (approximately levels IX–XV) projects to the C_1 and C_3 zones, and the principal olive projects to the D_1 and D_2 zones, with its caudomedial extension, the vlo and the dc projecting to the flocculonodular lobe. Figure 2 shows that all olivary areas mentioned (except for the dm.c.col.) have been included in the HRP injections, and this observation indicates that all the seven cerebellar cortical zones of Voogd may receive trigeminal impulses via the inferior olive. Since, however, as mentioned above, very few of the injections are confined to one olivary subdivision, it is difficult to draw conclusions about a possible topical pattern in this projection or concerning differences in the projection to different parts of one cerebellar cortical zone. It is evident, however, that in the cat the trigemino-olivo-cerebellar impulses, obviously mediated by climbing fibres, may be conveyed to rather large cortical fields, although probably with quantitative differences between regions. Most of the direct trigemino-cerebellar fibres, on the other hand, presumably by way of mossy fibres, reach the intermediate-lateral part of lobulus simplex with the adjacent lobule V, the rostralmost folia of the paramedian lobule with the surrounding part of crus I and II, and lobule IX (Somana et al. 1980). The two former terminal fields belong to what is considered to be the cerebellar face areas. The observations mentioned therefore indicate that the direct and indirect trigemino-cerebellar projections are arranged according to the principle of a dual input to the cerebellar cortex (see e.g., Strata 1975), and that they may subserve different functions.

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