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Descending Projections to the Inferior Olive from the Mesencephalon and Superior Colliculus in the Cat*

An Autoradiographic Study

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Summary. Descending projections from the mesencephalon and superior colliculus to the inferior olive were analyzed by an autoradiographic tracing method. Injections of tritium-labelled leucine were placed in regions which had previously been identified as sources of afferents to the olive. These were located adjacent to the central gray and extended from the rostral red nucleus to the posterior thalamus. Additional injections were made in the superior colliculus. Other injections were placed in the basal ganglia and thalamus. Injections restricted to one side of the central mesencephalon resulted in predominantly ipsilateral labelling of the olive. After injections in the caudo-medial parafascicular and subparafascicular nuclei and rostral nucleus of Darkschewitsch, deposits of grains were observed in the rostral pole of the medial accessory olive and adjacent ventral lamella of the principal olive. The medial accessory olive contained grains into its middle third. More caudal injections which involved the interstitial nucleus of Cajal as well as the nucleus of Darkschewitsch and rostral red nucleus resulted in the dense labelling of the entire principal olive (except the dorsal cap), the entire medial acessory olive (except subnucleus β and the caudo-medial pole) and the caudo-dorsal accessory olive. Injections centered in the caudal magnocellular red nucleus and extending into the rostral parvocellular division labelled the dorsal lamella of the principal olive almost exclusively. When only the caudal part of the red nucleus was involved in the injection, the olive was entirely clear of grains. Minor contralateral distributions were observed in the dorsomedial cell column, the medial tip of the dorsal lamella and in the caudal medial accessory olive. The deep layers of the superior colliculus were found to project strongly to the contralateral medial accessory olive immediately beside subnucleus β and weakly to the same area ipsilaterally.

Four pathways were identified as contributing fibers to the olivary projections. These were the medial longitudinal fasciculus, the medial tegmental tract, the central tegmental tract and tectospinal or tectobulbar fibers. The rubrospinal tract did not contribute projections to the olive. Injections in the caudate nucleus, entopeduncular nucleus and ventral anterior and ventral lateral thalamic nuclei, did not result in any labeling in the olive.

Key words: Cat – Inferior olive afferents – Mesencephalon – Superior colliculus – Autoradiography

Introduction

Cells groups in the mesencephalon and superior colliculus have recently been identified as important sources of afferents to the inferior olive in the cat (Saint-Cyr and Courville 1981). The cells projecting to the olive from the mesencephalon form a column which is adjacent to the central gray and fasciculus retroflexus (FR) and are found within the parvocellular red nucleus (RNp), the interstitial nucleus of Cajal (INC), the nucleus of Darkschewitsch (ND) and the subparafascicular nucleus (sPf). There are also a few scattered cells within the mesencephalic reticular formation (MRF) and also within the fields

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of Forel (NFF). Dorsally and laterally, other cell groups lie in the deep layers of the superior colliculus (SC) and in certain of the pretectal nuclei.

There have been numerous studies utilizing the method of anterograde degeneration to analyze the distribution of terminals from some of these regions to the inferior olive of the cat (Altman and Carpenter 1961; Bürgi 1957; Hamilton and Skultety 1969; Mabuchi and Kusama 1970; Mizuno et al. 1973; Ogawa 1939; Walberg 1956, 1960, 1974). Since the cells of origin are contained in a long column of small nuclei with ill-defined borders, concomitant destruction of adjacent structures and of fibers of passage have hampered the conclusions of these studies.

Recently in the cat, autoradiographic techniques have been applied to the study of the projections of the SC (Graham 1977; Henkel and Edwards 1978; Weber et al. 1978), the pretectal nuclei (Berman 1977), the RN (Edwards 1972), the mesencephalic reticular formation (Edwards 1975), the ventral central gray and nucleus of Edinger-Westphal (Loewy and Saper 1978). A comprehensive analysis of projections from mesencephalic and diencephalic structures has also been done in the opossum (Martin et al. 1980) and in the rat (Cintas et al. 1980).

In the cat, however, there has been no systematic study of the contributions from all mesencephalic sites of origin nor has there been a thorough analysis of distributions in the olive. The present communication reports the results of an autoradiographic study of the efferent projections from all upper brainstem regions identified with retrograde axonal transport of horseradish peroxidase (HRP) as sources of afferents to the olive (Saint-Cyr and Courville 1981) except for pretectal nuclei which were recently analyzed by Walberg et al. (1981). The differential distributions of these projections in the olive and the various pathways linking the mesencephalon and superior colliculus to the medulla are described. These findings are compared to those obtained previously in the cat and in other species.

Methods

Altogether 17 adults cats of both sexes were used in the present experimental series. Under barbiturate anesthesia (Nembutal, 35 mg/kg i.p.), ^{3}H-L-leucine (New England Nuclear, specific activity 40–60 Ci/mmoles), in saline and at concentrations of 150 to 165 μCi/μl was hydraulically injected under stereotaxic guidance with glass pipettes (tip diameter 10–25 μm) fixed to a 1 μl Hamilton syringe. The total amount of radioactivity varied from 30 to 60 μCi/injection (volumes from 0.2 to 0.4 μl). After a survival period of 5–6 days, the animals were deeply anesthetized and sacrificed by transcardial perfusion with Dextran followed by 4% buffered formalin. After 1–3 weeks post-fixation in the same fixative and in Bouin's solution, the tissue was dehydrated, embedded in paraffin and cut at 5 μm. Every 40th section was mounted on glass coated with Kodak NTB-2 emulsion, exposed for 8–12 weeks and then developed according to the method of Cowan et al. (1972). Following a light counterstaining with cresyl violet, the sections were examined under dark field illumination in order to map the injection sites, labelled fibers and deposits of silver grains over nuclear groups. The different subdivisions of the inferior olive will be referred to according to the terminolgy of Brodal (1940). For the superior colliculus and pretectal nuclei, the nomenclature of Kanaseki and Sprague (1974) was used.

Results

In the 17 cases reported here, 7 had injections placed in the mesencephalon and posterior diencephalon between the RN and parafascicular nucleus (Pf). There were 5 injections in the SC and another 5 in the basal ganglia and thalamus. These cases are presented in 3 separate groups. This communication describes only the pathways and projections to the inferior olive and will not include other sites of termination observed throughout the brainstem.

Mesencephalic Injections

In this group of 7 injections, the aim was to deposit leucine along the entire length of the cell column situated between the RN and thalamus, previously shown to project to the olive (Saint-Cyr and Courville 1981). Two of the injections were rostral and dorsal (cases DO-11 and 12), 2 were centered in the INC and RNp (cases DO-22 and 23), while the remaining 3 cases (DO-24, 25 and 26) were progressively more caudal in the RN (Figs. 1 and 6B). Cases will be presented according to the caudo-rostral position of injections.

Cat DO-26

This injection was centered immediately caudal to the RN and involved the caudal pole of that nucleus (Figs 1 and 6B). Labelled fibers travelled in the rubrospinal tract (described under cat DO-22). There was no evidence of any termination in the inferior olive.

Cats DO-25 and 24

Both of these injections were centered in the caudal RN and encroached upon the adjacent MRF. Along the pipette trajectory, a small amount of amino acid was deposited in the SC and central gray in both

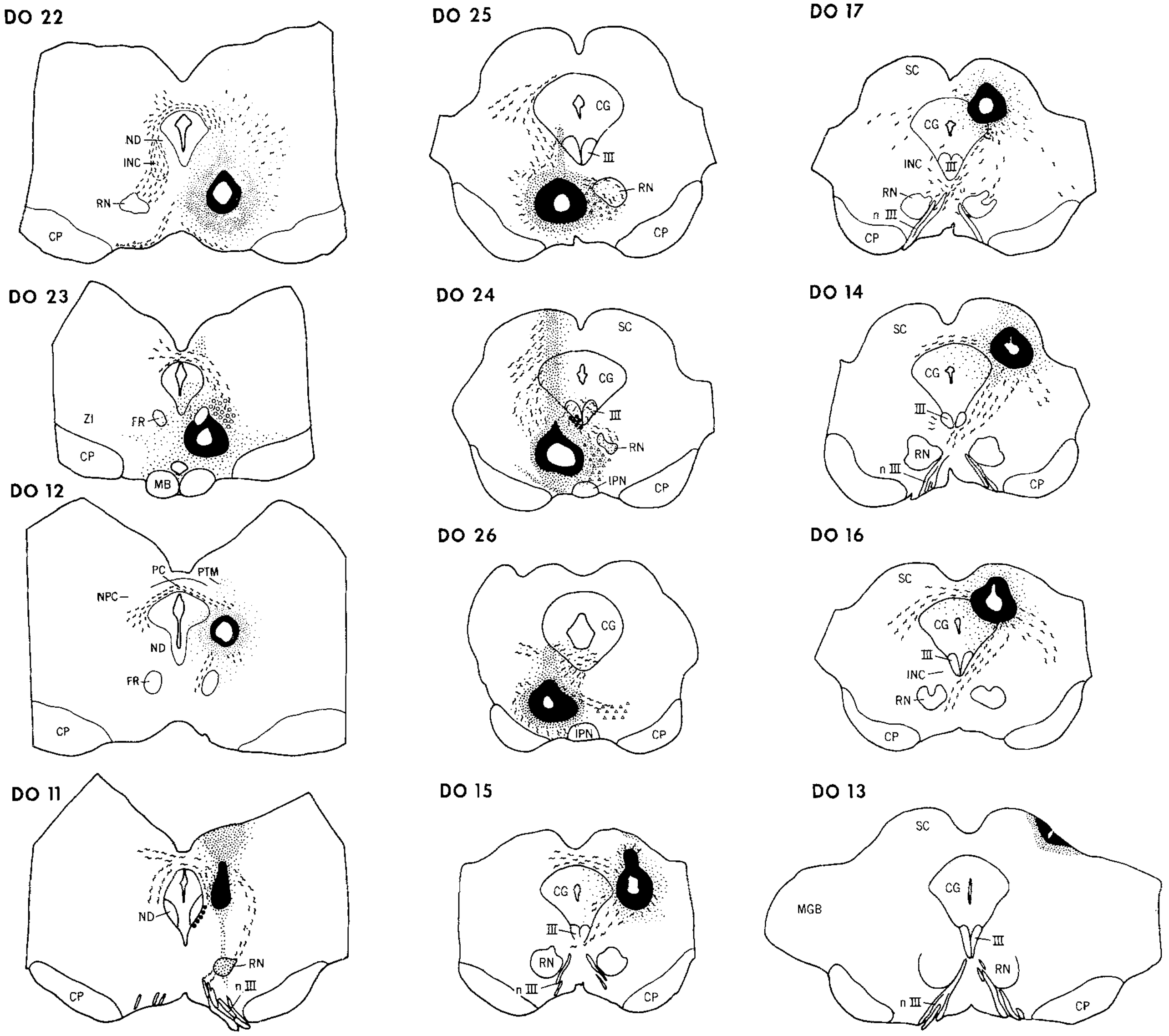

Fig. 1. Sections showing the centers of all injections placed in the mesencephalon and superior colliculus. White areas indicate the extent of damaged tissue, black areas delimit regions containing heavily labelled somata and stipplings outline zones of light deposit of label. Labelled fiber tracts issuing from the injections are shown as wavy lines. Black dots mark the position of labelled fibers in the medial longitudinal fasciculus, triangles those within the rubrospinal tract and circles the labelled fibers descending in the central tegmental tract. Abbreviations listed separately on page 346

cases. This marking was greater in DO-24 than in DO-25. The injection in DO-25 was slightly more rostral than that made in DO-24 (Figs. 1 and 6B). In both cases, the RST and central tegmental tract (described under cat DO-22) were the only labelled pathways. In DO-25, a weak deposit of grains was present in the dorsal lamella of the principal olive (dlPO) and in the rostral portion of the medial accessory olive (MAO), ipsilaterally (Fig. 6A). In DO-24, there were very small amounts of label in the ipsilateral ventro-lateral outgrowth (vlo) and caudal MAO (Fig. 6A). In addition there was a small amount of label in the contralateral caudal MAO adjacent to β. This was attributed to the involvement of the SC (see section on superior colliculus injections).

Cat DO-22

This injection was centered in the rostral pole of the RN and included part of the adjacent INC (Figs. 1 and 2). The lateral part of the ND, the nucleus of the posterior commissure and the caudal portions of the sPf and NFF and NFF and parafascicular (Pf) nuclei were also heavily labelled. The caudal RN, the

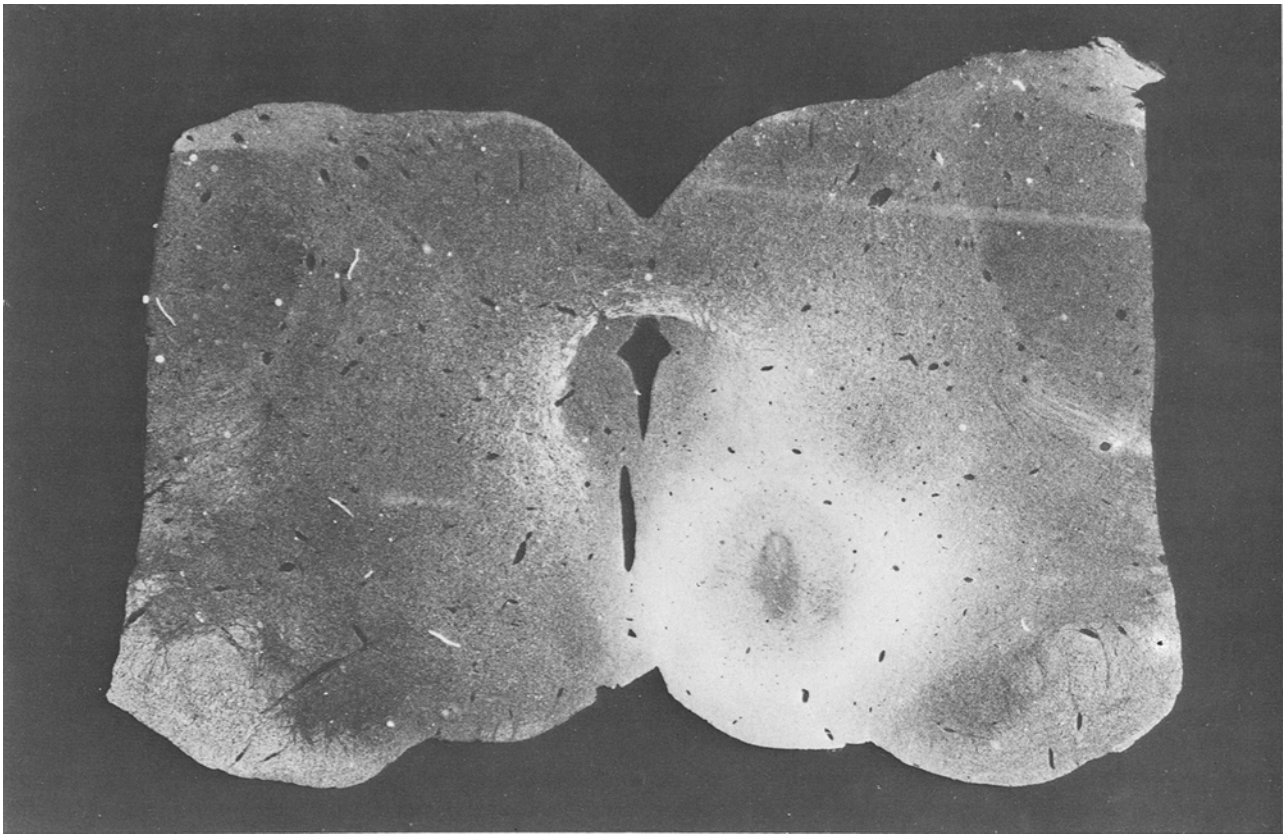

Fig. 2. Photograph of a section through the center of injection in cat DO-22. Compare with Figs. 1 and 6B

surrounding MRF and the ventral tegmental area of Tsai also contained a fairly dense accumulation of grains. Along the pipette trajectory, a small amount of leucine was also deposited in the SC.

Fiber Pathways

In this cat, four bundles of fibers were observed to leave the injection site and descend to the medulla. The largest of these issued from the medial aspect of the RN, crossed the midline among the fibers of the third cranial nerve (Fig. 1, triangles see also cats DO-24, 25 and 26) and gradually took up a lateral position within the pons. In the medulla, these fibers were located ventral to the spinal trigeminal tract and dorsolateral to the lateral reticular nucleus (Fig. 3, triangles). These fibers which did not contribute olivary projections were identified as the rubrospinal tract (Fig. 4, RST). Fascicles of labelled fibers oriented rostrocaudally were seen to emerge from the injection site and course in a position dorsal to the RN (Fig. 1, DO-23, circles). These descended to the olive ipsilaterally as a rather loose bundle occupying the center of the reticular formation. These fibers progressively moved ventrally to pass above the trapezoid body. In the medulla, they were located dorsal and lateral to the olive and entered the nucleus from that approach (Fig. 3, circles). They correspond to the classical central tegmental tract (Fig. 4, CTT). Numerous labelled fibers also travelled within the ipsilateral medial longitudinal fasciculus (Fig. 4, MLF) down to the level of the medulla where some descended ventrolaterally towards the olive (Fig. 3, black dots in MLF and wavy lines in levels 16–24). Labelled fibers within the MLF continued further caudally. These correspond to the interstitio-spinal tract. Due to their proximity to injection sites, the fibers of the MLF could not always be identified at their origin (see Fig. 1, cats DO-11 and 24, black dots). A separate fascicle of labelled fibers became gradually detached from the MLF in the pons. These fibers were located near the midline and were distributed throughout the vertical extent of the pontine tegmentum. In the medulla, they occupied the same position and entered the olive through its dorsomedial regions (Fig. 3, black dots near the midline and wavy lines through dorsomedial olive). These fibers correspond to the medial tegmental tract (Fig. 4, MTT).

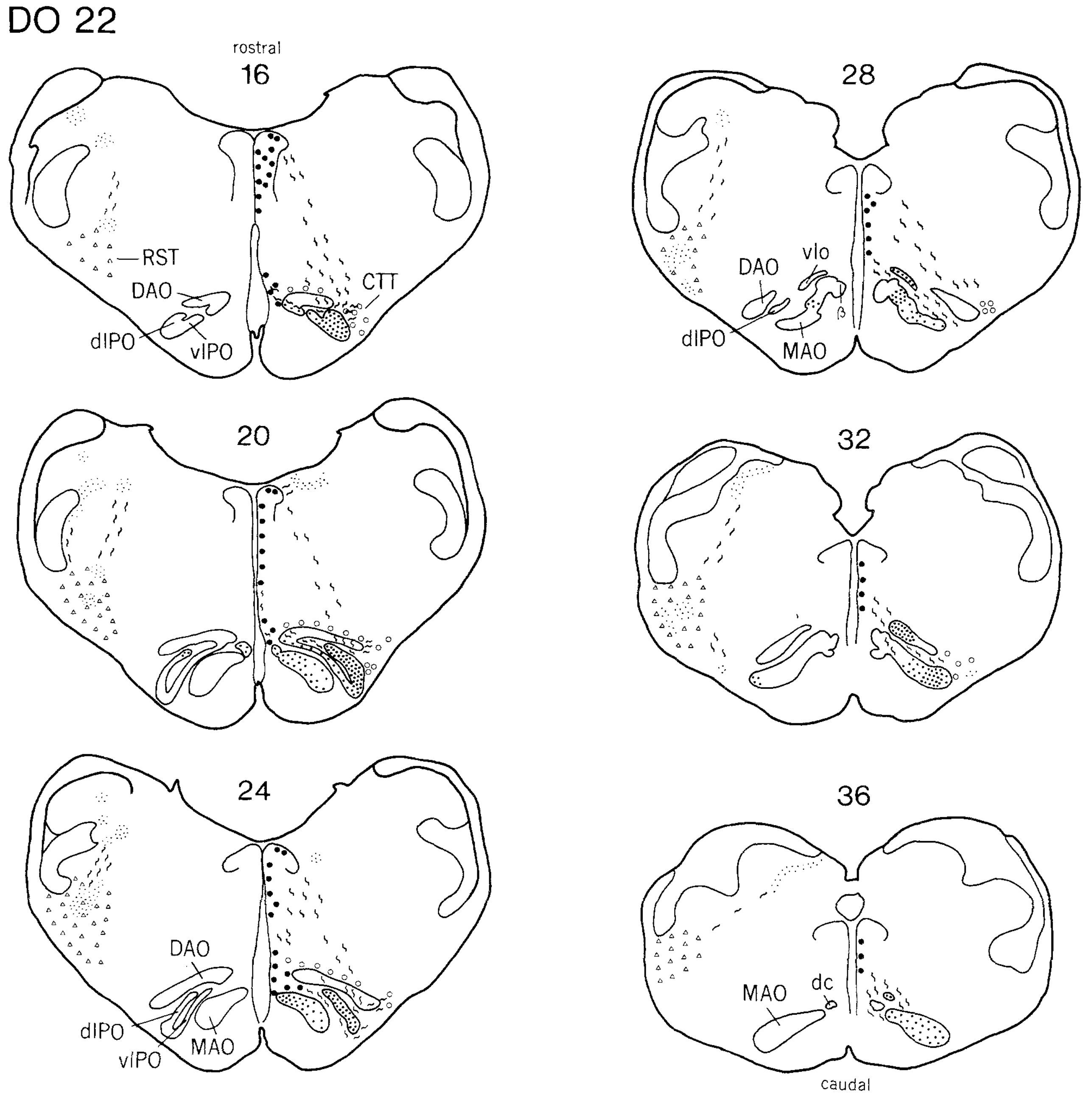

Fig. 3. Series of equally spaced sections through the inferior olivary nucleus in cat DO-22. Small black dots show the distribution and different densities of silver grain accumulations in the olive. Labelled fibers in the central tegmental tract are marked by circles and in the rubrospinal tract by triangles. Large black dots indicate labelled fibers either in the medial longitudinal fasciculus or in the medial tegmental tract. Fibers given off from these two tracts and running obliquely towards the olive are shown as wavy lines. Other wavy lines and fine dots indicate labelled fibers and terminals in other regions of the medulla. Abbreviations listed separately on page 346

Extensive projections were found ipsilaterally in the principal olive (PO) and MAO (Figs. 3, 5, and 6A). In the PO, dense marking was seen in the dlPO and adjacent vlo. The vlPO was clearly but less heavily labelled. Rostrally, the dorsomedial cell column (dmcc) and adjacent medial part of the MAO were densely labelled with fewer grains being present laterally. In the caudal MAO, deposits were located in central and lateral portions of this subgroup. There were no deposits in subnuclei β, dorsal cap (dc) nor in the parts of MAO adjacent to β. With the exception of its caudal pole, the dorsal accessory olive (DAO). was free of silver grains. Contralaterally there were light distributions in the dmcc. in the medial tip of the dlPO, and in the central and lateral portions of the caudal MAO. These contralateral projection sites

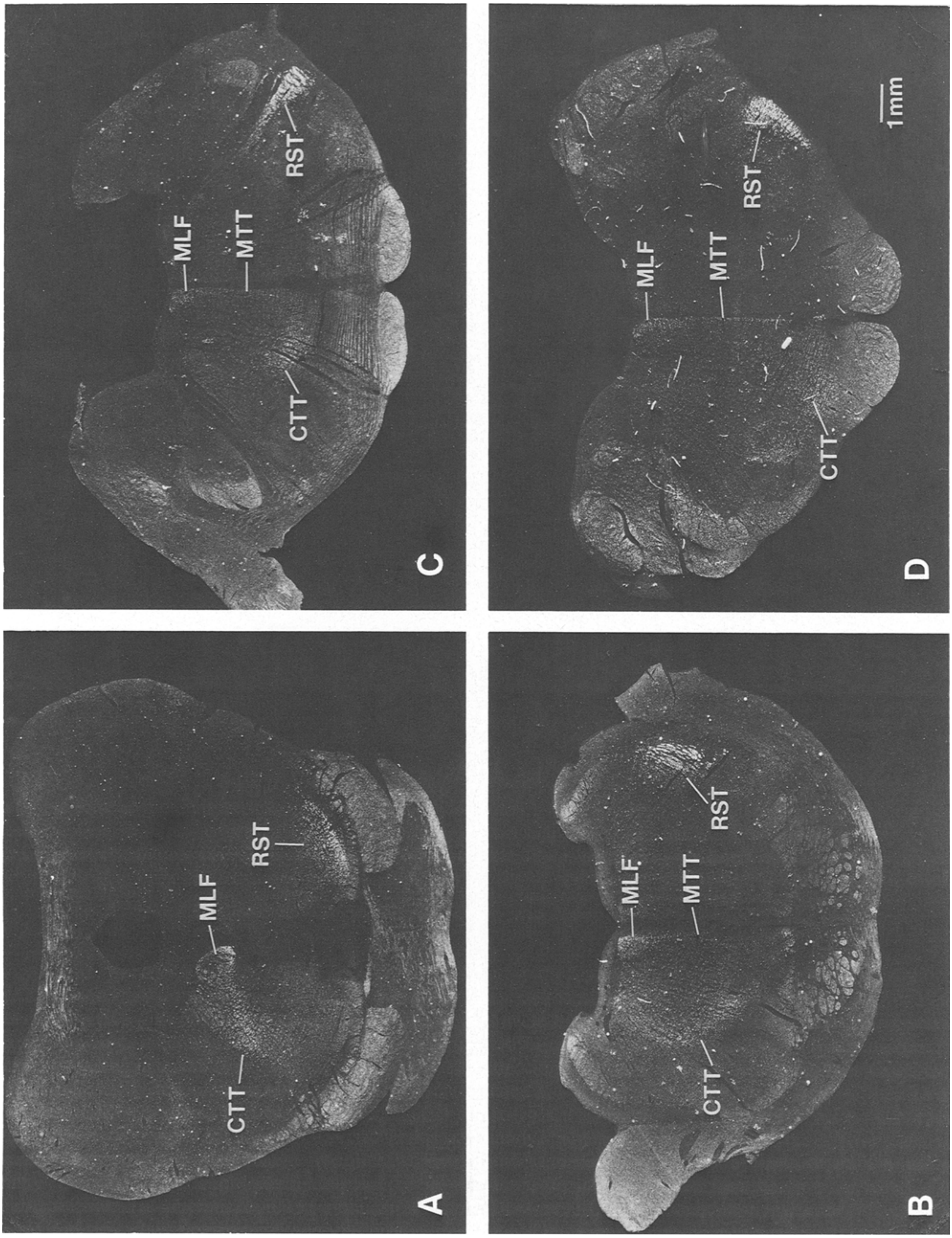

Fig. 4A–D. Microphotographs of 4 sections from cat DO-22 from **(A)** upper pons, **(B)** lower pons, **(C)** level of trapezoid body and **(D)** level of rostral pole of the olive illustrating the position of labelled fibers running within the medial longitudinal fasciculus (MLF) the medial tegmental tract (MTT), the central tegmental tract (CCT) and the rubrospinal tract (RST)

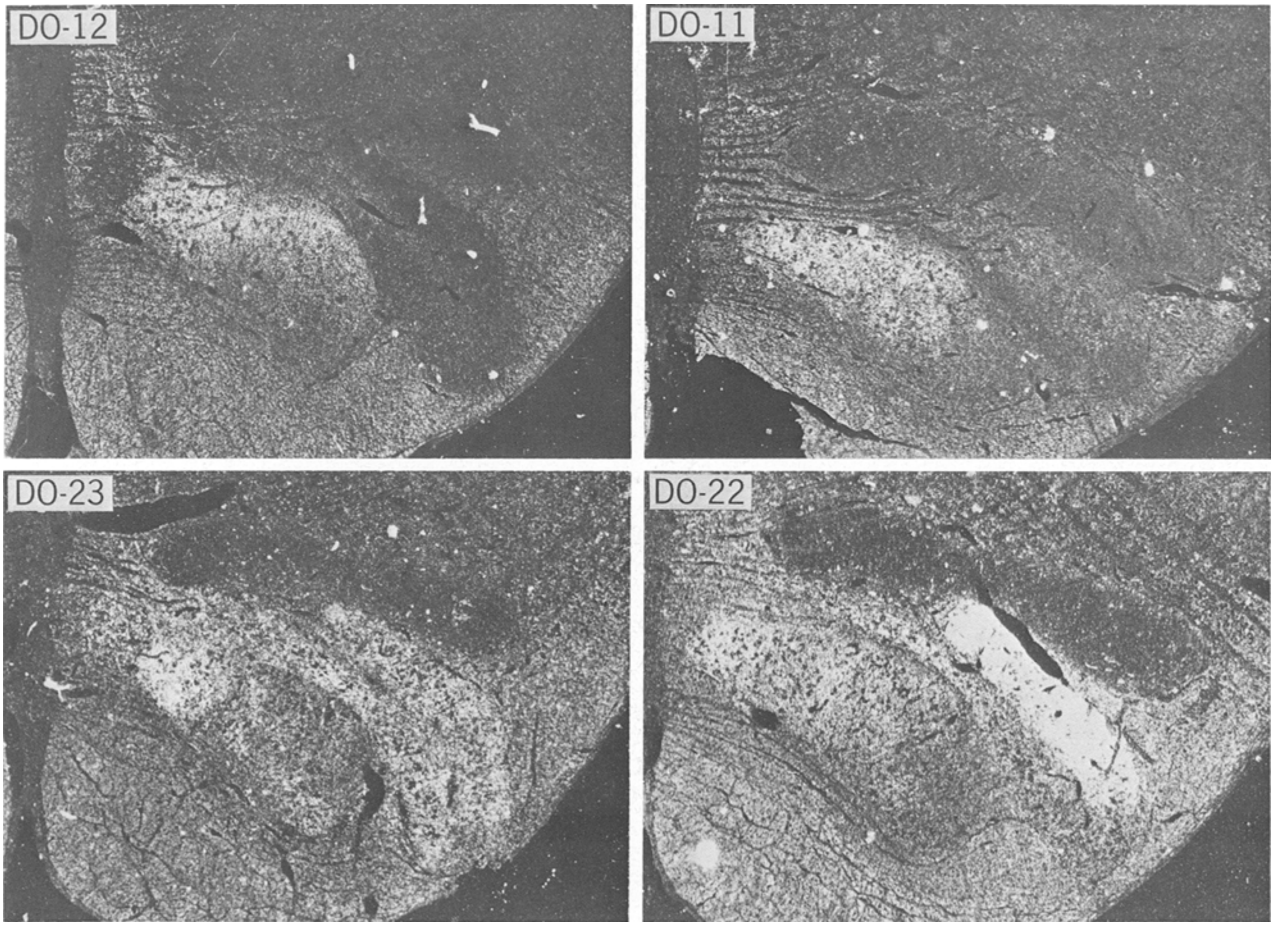

Fig. 5. Microphotographs illustrating the distributions of silver grains in the inferior olivary nucleus in 4 cases. For cat DO-22, the photograph corresponds to a level a little rostral to level 24 of Fig. 3

were reached by fibers which crossed the midline at the level of the olive and emanated apparently from the MTT.

Cat DO-23

The injection in this case was centered in the sPf and nucleus of the fields of Forel (NFF, Figs. 1 and 6B). Rostrally, it extended into the dorsocaudal hypothalamus. Caudally, the injection extended to the rostral pole of the RN as well as to the reticular cells situated dorsally and rostrally to that portion of the RN. The ventral portion of the INC was included along with the ventral tegmental area of Tsai. Along the trajectory of the pipette, some silver grains were found in the nucleus of the posterior commissure but the ND was not touched by the injection. The labelled fiber tracts coursing to the olive were the same as those reported for cat DO-22 and except for the RST descended only ipsilaterally. In the olive, the sites of distribution were closely comparable to those of cat DO-22 with minor differences in the density of grains in several regions (Figs. 5 and 6A). Very few silver grain deposits were observed in the dmcc contralaterally.

Cat DO-11

The injection in cat DO-11 was small (Figs. 1 and 6B). The pipette track passed through the lateral habenular nucleus and Pf leaving a small deposit of leucine along the course of its trajectory. The major portion of the injection was centered in the caudal pole of the nucleus of the posterior commissure with marginal involvement of the rostral ND, the adjacent sPf and the rostrolateral border of the INC. The injection extended caudally to the rostral RN and into the root fibers of the third nerve but fewer silver grains were found in that part. The pipette had been lowered to that level and then pulled back. A few of

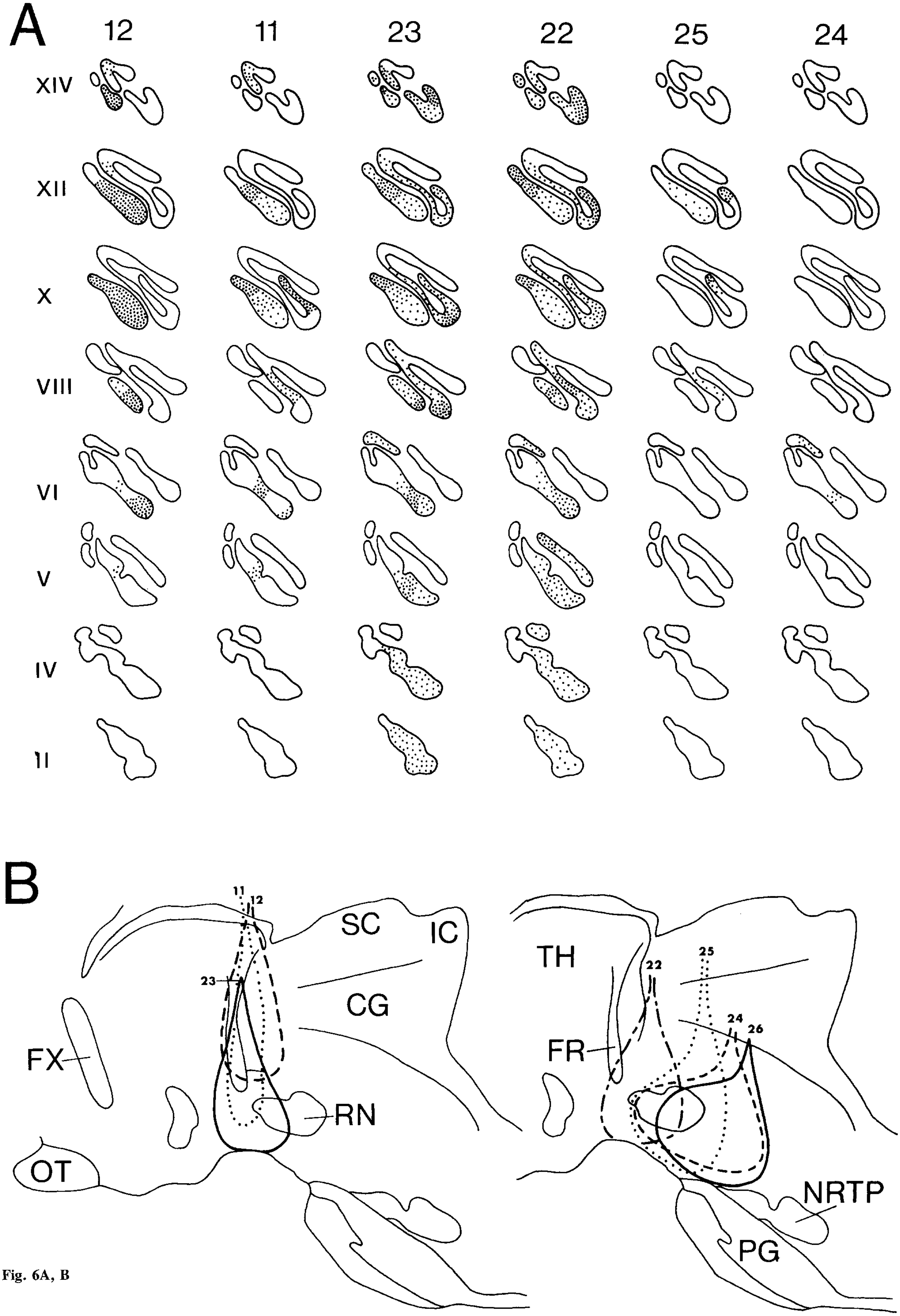
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Fig. 6A, B

the cells of the rostral RN were heavily labelled. Two ipsilateral caudally directed contingents of labelled fibers could be followed to the ipsilateral inferior olive. One of these travelled in the MLF which remained well labelled to the medulla. The other fibers detached from the MLF and descended in the MTT.

Silver grain deposits were limited to the ipsilateral olive. In the PO, there was an accumulation of grains in the rostromedial junction of the vlPO and the DAO. Part of the dlPO was labelled and this deposit of grains continued to the caudal limit of the dlPO. The MAO showed a pattern of distribution similar to that observed in cats DO-22 and 23 (Fig. 5) although less extensive caudally (Fig. 6A). Caudal to the olive, a few fascicles of labelled fibers continued to be present in the MLF as reported under cat DO-22.

Cat DO-12

In this cat the pipette track passed in the vicinity of the medial pretectal nucleus rostral to the SC and the deposit of leucine was centered around and immediately caudal to the vertical limb of the FR (Figs. 1 and 6B). The injection involved the following structures: sPf, a portion of the PF, rostral central gray, ND, nucleus of the posterior commissure and the reticular formation dorsal to the rostral RN. Of the various labelled fiber bundles emanating from the injection site, only one contingent descended caudally. It joined the ipsilateral MLF ventromedially to the injection. While many of the labelled fibers could be followed within that tract to the rostral pole of the olive, a separate fascicle of fibers detached from the MLF and descended in the MTT. In the olive, labelling was only located on the side ipsilateral to the injection. The greatest accumulation of grains was found in the rostral part of the MAO (Fig. 6A). In the dmcc, there were few if any silver grains (Fig. 5). Weak accumulations of grains were also present in the medial portion of the vlPO rostrally. In the middle third of the MAO, there was a modest and uniform distribution of grains which became separated into a central band and a small lateral deposit. No other portions of the olive appeared to contain silver grains above background level. Furthermore, there were no labelled fibers caudal to the olive.

Superior Colliculus Injections

Cat DO-13. The injection in this case was very superficial and restricted to the upper two laminae of the SC (Fig 1). No labelled descending fiber pathway was observed in this case and there was no evidence of label in the inferior olive.

Cat DO-15. The injection in this case was fairly deep in the lateral part of the SC (Fig. 1). It involved the deep laminae of the SC (IV to VII) and bordered on the central gray and dorsal MRF. The majority of fibers leaving the injection site arched ventrally in a fan-like radiation (fountain radiation of Meynert) through the MRF adjacent to the central gray (Fig. 1, wavy lines). These fibers formed into a compact bundle (predorsal bundle), crossed the midline immediately beneath the oculo-motor complex and descended to the medulla as scattered fascicles located beneath the MLF and running immediately adjacent to the midline. They were identified as the tectospinal tract and, according to the description above, ran within the MTT. Within the olive, there were two sites of termination (Fig. 7). The densest of these was contralateral to the injection. It was located in the caudal MAO adjacent to but not including subnucleus β and silver grain deposits extended to the central portion of the MAO (Fig. 7 level 17). Ipsilaterally, a weaker and less extensive deposit was present in the MAO adjacent to β. Descending fibers to these sites recrossed the midline at the level of the olive. Rostrally a weak deposit was present in the medial vlPO at the border of the DAO (Fig. 7 level 31) and more caudally silver grains were also found near the center of the DAO (level 21).

Cat DO-14. This injection was slightly more caudal and lateral than in the previous cat (Fig. 1). It involved the deeper laminae (IV to VIII) of SC and the dorsal MRF. It also included the dorsal border of the posterior commissure, the central gray and barely touched the posterior pretectal nucleus.

Labelled fibers descended only in the crossed tectospinal tract as in cat DO-15. Olivary terminations on the contralateral side were identical to those described for the previous case, that is, in the caudal MAO next to β (not illustrated) while the ipsilateral projections were very scarce.

Cat DO-16. This injection was situated in the deep laminae (IV to VII) of the central SC and encroached upon the dorsolateral part of the central gray

Fig. 6. A Distributions and relative densities of silver grain deposits in 6 cases with injections in the medial part of the mesencephalon. Numbers at the top are case numbers and roman figures refer to the standard levels of the olive by Brodal (1940). **B** Projections on a parasagittal section of the brainstem of the position and extent of 6 injections in the medial mesencephalon

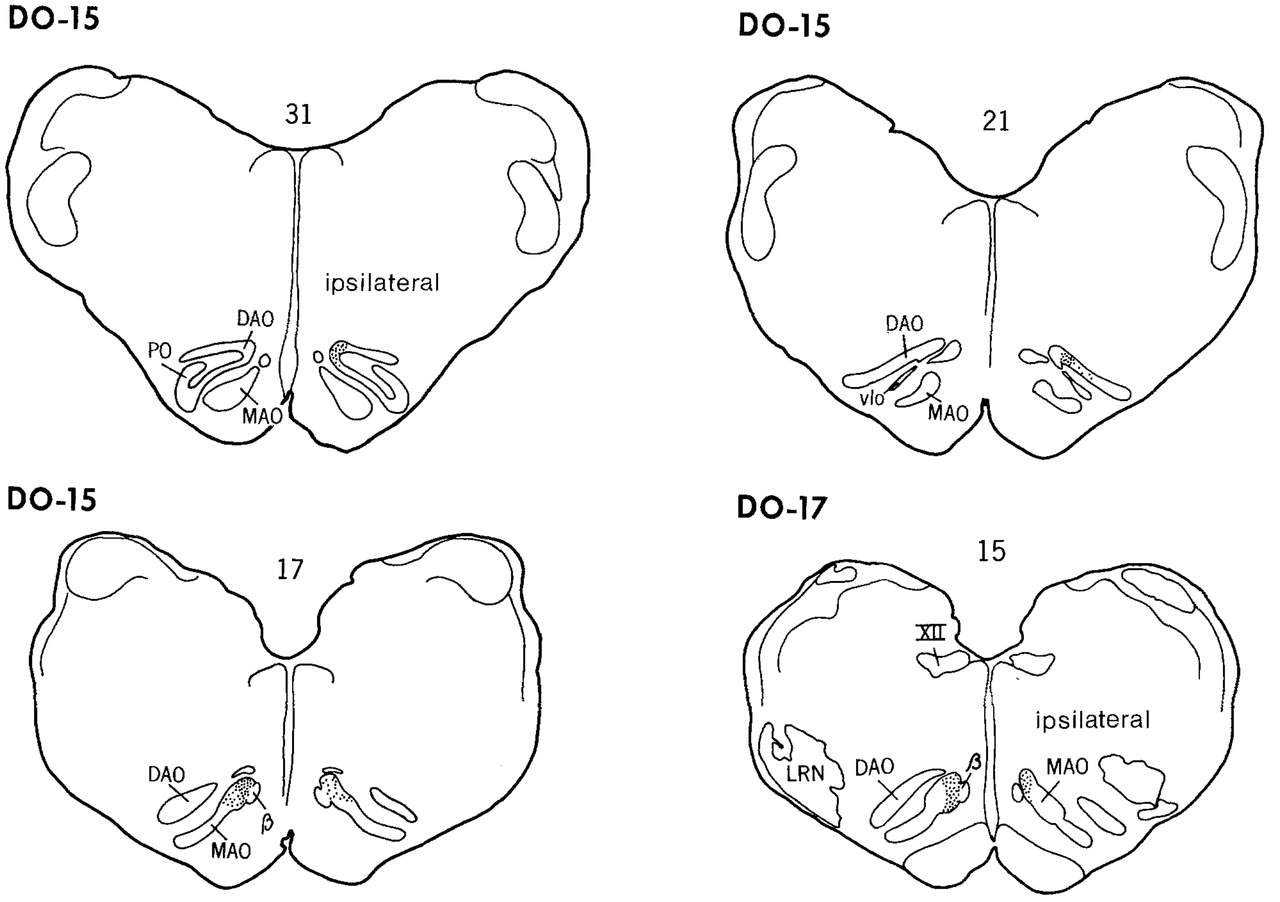

Fig. 7. Drawings of histological sections showing the distributions of silver grains in the inferior olivary nucleus in cats DO-15 and 17. Compare the position of deposits in the medial part of caudal MAO (two lower drawings) with clearly different pattern of termination present at similar levels in cat DO-22 (Fig. 3, levels 28 and 32). Ipsilateral indicates injection side. Abbreviations are all listed on page 346

(Fig. 1). The tectospinal fibers were also labelled. Silver grain deposits in the olive were restricted to the contralateral MAO adjacent to β and were far more restricted in extent than in previous cases.

Cat DO-17. In this case, an injection in the center of SC (laminae II to VII) also encroached upon the central gray. Again the tectospinal fibers were labelled. Within the olive, there was a moderate but restricted deposit of grains in the contralateral caudomedial MAO and a weaker but symmetrical ipsilateral deposit (Fig. 7).

Control Injections

This group of 5 injections comprised 2 in the head of the caudate nucleus (cases DO-29 and DO-30), 1 in the entopeduncular nucleus (case DO-32) and 2 in the ventral lateral (VL) and ventral anterior (VA) nuclei of the thalamus (DO-33 and DO-34). Two of these injections (DO-30 and DO-32) are illustrated in Fig. 8. Although these injections were successful in that appropriate fiber pathways and terminal fields were revealed, none resulted in labelling of the inferior olive.

Discussion

Injections Sites

The first group of injections presented here covered the mesencephalic region adjacent to the central gray and involved the nuclei Pf, sPf, INC, ND, RN and the neighboring reticular formation. Although these injections extended beyond the nuclear groups identified after HRP injections in the olive (Saint-Cyr and Courville 1981), the assumption is made that the terminal distributions in the olive result from the uptake of leucine by neurons in these nuclear groups. Other midbrain nuclei (ventral central gray, linearis raphe and EW) which have been suggested as sources

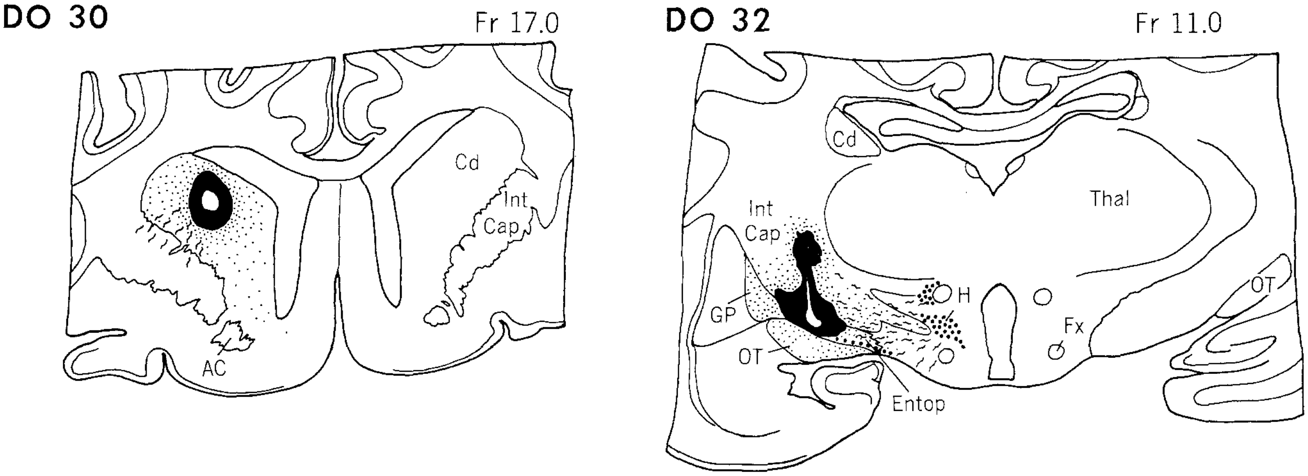

Fig. 8. Two sections through the caudate and entopeduncular nuclei illustrating the centers of injections within those structures. Wavy lines represent labelled fibers issued from the injections and black dots indicate the position of labelled fiber tracts which could be followed for some distance from the injection sites. Abbreviations are all listed on page 346

of afferents to the olive in HRP studies (Henkel et al. 1975; Brown et al. 1977; Loewy and Saper 1978) were included in some injections. Injections in cats DO-22-24-25 labelled the linearis raphe marginally. In numerous cases the dorsolateral or ventral portions of the central gray (CG) were included while the EW was not labelled (Fig. 1). The contributions of the CG and linearis raphe therefore cannot be evaluated on the basis of the present material. As discussed more fully elsewhere (Saint-Cyr and Courville 1981), it appears unlikely that either the linearis raphe or EW project to the olive. A contribution from the central gray, if at all present, can only be minor.

With regard to the series of injections in the SC, only in cases that resulted in deposits of leucine in the deeper layers (VI–VII) of the SC was there evidence of olivary terminations. This result is in keeping with the position of labelled cells in the SC after HRP injections in the olive (Saint-Cyr and Courville 1981; see also Weber et al. 1978). The pretectal and accessory optic tract nuclei were not included in the present analyses. Finally, a third group of injections involved the caudate and entopeduncular nuclei which had previously been anatomically (Walberg 1956) and physiologically (Sedgwick and Williams 1967; Fox and Williams 1968) identified as sources of projections to the olive. Since there is no evidence of an olivary termination in these cases, in confirmation of HRP studies (Saint-Cyr and Courville 1981; Brown et al. 1977), an explanation for the positive results must be sought. The physiological observations can be explained by relays between the caudate, entopeduncular and nigral nuclei (Nauta 1979) and projections from the reticular portion of the substantia nigra to the deep layers of the superior colliculus (Graybiel 1978). As observed in the present material and reported elsewhere (Altman and Carpenter 1961; Graham 1977; Saint-Cyr and Courville 1980), the SC projects not only to the olive directly but also to the INC, thereby activating large areas of the olive. Two injections in the VA and VL thalamic nuclei served as additional controls and both of these proved negative with regard to olivary terminations.

Pathways. Four pathways were traced to the olivary complex from injection sites in the midbrain. The most rostrodorsal injections (Cats DO-11 and DO-12, Figs. 1 and 6B) resulted in the labeling of fibers which were strictly ipsilateral and initially all travelled within the MLF. At the level of the pons, part of the fibers gradually detached from the MLF and entered the olive through its dorsomedial aspects. This pathway corresponds to the medial tegmental tract (MTT) first described by von Economo and Karplus (1909, mesencephalo-olivary tract) and later described in detail by Ogawa (1939). The latter authors attributed its origin to the NFF, ND and INC. That these nuclei contribute fibers to the MTT is confirmed in the present study by the fact that injections involving these structures (cats DO-11, 12, 22 and 23; Figs. 1 and 6B) all labelled this pathway. It appears likely that the caudomedial sPf and Pf nuclei also project to the olive via the MTT, Bürgi and Bucher (1956) identified this pathway in Marchi material and cited Hassler in attributing its origin to the pre-interstitial nucleus. Recent evaluation of the nomenclature applied to these regions has resulted in the suggestion that this nucleus in the cat corresponds to the rostral portion of the INC and may also include the caudal sPf. In the cat, this area has been named the nucleus of the pre-rubral field (Graybiel 1977) while in the monkey, the term rostral interstitial nucleus of the MLF has been used (Büttner-Ennever and Büttner 1978).

Injections centered in and around the INC and rostral RN (Cats DO-22 and DO-23) labelled not only the MTT but also a conspicuous aggregate of fibers which corresponds to the central tegmental tract (CTT). This has also been called the anulo-olivary pathway (Mettler 1944). In view of the paucity of contributions from the central gray itself, it is suggested that the term anulo-olivary should be abandoned. Since the CTT fibers were not labelled after injections sparing the rostral pole of the RN (Cat DO-12) but were well demonstrated in cats DO-22 and 23 and partially labelled in cats DO-24 and 25 which barely touched the rostral part of the RN (Figs. 1 and 6B), it is concluded that the CTT originates in the INC, RNp and adjacent cells. This is in agreement with the general location of the cells of origin reported by Mettler (1944) and Walberg (1956, 1974). This tract does not appear to receive contributions from the sPf-Pf and NFF regions which are related to the MTT. Previous HRP studies and the present observations (Cat DO-26; Figs. 1 and 6B) permit the conclusion that the major portion of the RN (magnocellular elements roughly the caudal two-thirds) does not contribute to the CTT while the parvocellular elements (RNp) do so. The conspicuous RST labelled after RN injections contributes no fibers to the olive. The conclusions regarding the CTT and RST are in agreement with previous observations in various species (Brown et al. 1977; Edwards 1972; Henkel et al. 1975; Linauts and Martin 1978; Mabuchi and Kusama 1970; Mettler 1944; Ogawa 1939; Strominger et al. 1979; Verhaart 1949). As suggested by Bebin (1956) and recently confirmed in autoradiographic studies (Graybiel et al. 1973; Tolbert et al. 1976), other fibers originating in the cerebellar nuclei and joining the crossed descending limb of the brachium conjunctivum blend into portions of the CTT along its course. The CTT is therefore an heterogenous bundle of fibers with diverse origins and a common termination in the inferior olive. This tract also gives off collaterals to adjacent regions of the medulla.

Bürgi (1957) in summarizing the work done in collaboration with Bucher mentioned a thalamo-pretecto-tegmental fasciculus as being afferent to the olive. From the description given, it woud appear that he was referring to tectospinal or tectobulbar fibers, many of which are intermingled with those of the MTT. The cases of SC injections reported here clearly show these efferents, most of which cross the midline to form the predorsal bundle which continues caudally as the tectobulbar and tectospinal tracts. This pathway has often been described in the past (Altman and Carpenter 1961; Graham 1977; Henkel and Edwards 1978; Kuru et al. 1961; Weber et al. 1978). It would appear that these fibers were interrupted in some of Walberg's (1956, 1960) lesion experiments. (See further).

In summary, the four pathways which have been identified as afferent to the inferior olivary complex are the MTT, the CTT, the MLF (Fig. 4) and the tectospinal or tectobulbar pathways.

Distributions Within the Major Subdivisions of the Olivary Complex

A. Principal Olive. The dorsal lamella of the principal olive (dlPO) and its caudal continuation the vlo were only labelled when the RNp and dorsomedially adjacent cells were included in the injections (Cases DO-11, 22, 23, 24, 25; Figs. 1 and 6B). Cats DO-22 and DO-23 showed a dense ipsilateral projection to these regions and light and less extensive distributions, contralaterally. Cat DO-11 presented a dense ipsilateral projection only while in cats DO-24 and DO-25, there was a light ipsilateral distribution. It is therefore concluded that this projection is largely ipsilateral. The results obtained with HRP injections (Saint-Cyr and Courville 1981) did not lead to firm conclusions on this point since it was suspected that many of the injections involved the olives on both sides. As discussed in Saint-Cyr and Courville (1981) the division between the parvo and magnocellular portions of the RN is not clear. Nevertheless, the densest labeling in the dlPO was observed in cases in which injections involved the rostral pole of the RN (Fig. 6). These findings are in fairly close agreement with the observations of Walberg (1956), Edwards (1972) and Loewy and Saper (1978) in the cat. The delineation between the magno- and parvocellular portions of the RN is especially clear in primates where a relation between the RNp and dlPO has been repeatedly demonstrated (Courville and Otabe 1974; Hinman and Carpenter 1959; Miller and Strominger 1973; Strominger et al. 1979).

The dorsal cap which is immediately caudal to and continuous with the vlo in the principal olivary nucleus remained free of silver grains in all of the present cases. This is consistent with the fact that the nuclear group known to project to this region (nucleus of the optic tract, Mizuno et al. 1973) was not involved in the cases presented.

The ventral lamella (vlPO) was overlaid with silver grains in numerous cases. An analysis of the injections suggests that the projections to the vlPO originate in nuclear groups extending from the level of the sPf to the rostral RNp. In the case of an injection which barely touched the RNp such as in cat DO-24, there was no evidence of a distribution in the vlPO and this region therefore probably does not participate in the projection. These results are compatible with those reported by Walberg (1974) and Loewy and Saper (1978), although the nuclei of origin specified in recent HRP studies are different (Saint-Cyr and Courville 1981) from those identified by those authors.

B. Medial Accessory Olive. Various parts of the MAO were labelled in the present study. Injections in cats DO-12, 11, 23 and 22 all resulted in deposits of grains in the MAO. Cases with rostrodorsal injections such as in cat DO-12 and 11 (Fig. 6B) labelled the rostral MAO preferentially while caudoventral injections (cats DO-23 and DO-22, Fig. 6B) labelled nearly the entire length of the MAO. All projections to the MAO were ipsilateral except for light distributions in the contralateral caudal MAO in cats DO-22 and 24. In cat DO-22 it was concluded that the distributions observed in the central and lateral portions of the caudal MAO likely correspond to a contralateral projection from medial mesencephalic cell groups. However, in cat DO-24, the deposit of grains was adjacent to subnucleus β and therefore comparable to the contralateral distribution seen after SC injections. Thus, the small deposit of leucine in the SC along the pipette trajectory in cat DO-24 was likely responsible for this labelling.

The dmcc was only well labelled in cases in which the injections were centered in the INC and rostral RN (Cats DO-11, 22, 23). Since the dmcc was not labelled on cats DO-24 and 25 wherein the injections involved the RNp, it is concluded that the INC projects to the dmcc. Projections to the dmcc were distributed bilaterally in cats DO-22 and DO-23 and ipsilaterally in cat DO-11. Subgroup β and the adjacent medial portion of MAO were typically free of grains in the cases of injections limited to medial mesencephalic structures (Figs. 1 and 6A). The medial MAO region was only labelled after SC injections (Cats DO-14, 15, 16, 17). In those cases, grain deposits were dense contralaterally and weak ipsilaterally. These distributions reconcile the present data with those of Walberg (1956, 1960) who described bilateral projections to β and adjacent MAO from the central gray and red nucleus. Lesions in that study interrupted the fibers from the SC as noted in the section on pathways in the Discussion. Similar observations to those obtained in the present material have been reported in the cat (Altman and Carpenter 1961; Graham 1977; Henkel and Edwards 1978; Kuru et al. 1961; Weber et al. 1978) and the monkey (Frankfurter et al. 1976). In the opossum, Linauts and Martin (1978) also described bilateral projections to subnucleus c of the caudal MAO which they considered homologous to nucleus β of the cat. Unless this is due to an involvement of the SC and if the homology is correct, this would indicate differences in the projections between the opossum and cat. In a study of descending reticular projections, Edwards (1975) illustrated a projection to the contralateral caudal MAO and β. However, in the illustration of these cases, it appears that the injection may have included the deep laminae of the SC. With regard to subnucleus β it has been reported to receive fibers from the caudolateral SC (Graham 1977) and the adjacent dorsolateral MRF (Linauts and Martin 1978).

C. Dorsal Accessory Olive. This portion of the olivary complex received the least input from mesencephalic centers. In 2 cases only (cats DO-22 and 15) terminations were found ipsilaterally within the DAO. These 2 injections were located differently (Fig. 1) and the distribution were found in distinct regions of the DAO (Figs. 3, 6A and 7). Silver grain deposits in the DAO have been seen in the opossum and the cat after injections in the vicinity of the posterior pretectal nuclei and adjacent MRF (Linauts and Martin 1978; Loewy and Saper 1978). It can be recalled that in HRP studies on the cat (Saint-Cyr and Courville 1981), the posterior pretectal nucleus was the only labelled cell group in that region. In the opossum (Linauts and Martin 1978), the caudomedial DAO was also weakly labelled after injections which included either the rostral RN and adjacent cell groups or the sPf and NFF. These results suggest that the MRF and adjacent regions may be at the origin of small projections to the DAO. If the deposits located in the rostromedial junction of the vlPO and DAO are interpreted, as in the present text, to belong to vlPO, it can be stated that there is no evidence for projections from the mesencephalon to the rostromedial DAO.

In their autoradiographic study in the cat, Loewy and Saper (1978) reached the conclusion that it was the EW nucleus and the adjacent MRF which project to the caudal DAO. Their identification of the EW nucleus as an origin was based on a single case of retrograde transport of HRP from the olive. As discussed in a previous HRP study (Saint-Cyr and Courville 1981), this marking of EW cells could have been due to uptake and transport by fibers near the olive en route to the spinal cord since no labelled cells were ever located in the EW nucleus. In addition, in the present cat DO-22 in which the injection did not encroach upon the EW, labeling of the caudal DAO identical to that reported by Loewy

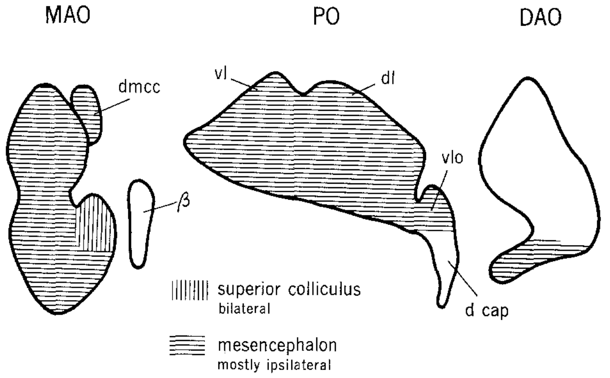

Fig. 9. Diagram of the unfolded olive after Brodal (1940), illustrating the extent of the projection from the medial mesencephalon and superior colliculus

and Saper (1978) was observed. From this finding it is suggested that the projection to the caudal DAO originates from a portion of the INC. It has also been suggested that the nucleus linearis raphe projects to the caudal DAO. This nucleus was shown to harbor a few HRP positive cells in previous studies (Henkel et al. 1975; Saint-Cyr and Courville 1981). As this nucleus was also involved in some of our injections, the possibility of a projection from linearis raphe to the caudal DAO cannot be excluded.

In summary, it appears impossible to define a strict topographical organization of the projection from the mesencephalon to the olive. Differences in the distributions obviously occur in relation to the positions of the injections but they cannot always be related to individual nuclei. The overall distributions to the inferior olive from the mesencephalon and SC are illustrated in Fig. 9.

Functional Implications. The most striking feature of the mesencephalo-olivary projection demonstrated in this study is that it covers nearly all regions of the MAO and PO and involves some parts of the DAO. This means that widespread areas of the cerebellar cortex are influenced by these midbrain centers via the climbing fiber system. As discussed in previous papers (Saint-Cyr and Courville 1980, 1981), many of the mesencephalic nuclei are intimately related to the control of eye and head movements. Studies with electrical stimulation (Faulkner and Hyde 1958; Hassler 1972; Hyde and Eason 1959), lesions (Carpenter et al. 1970; Mabuchi 1970; Sanó et al. 1972) and unit recording (Büttner et al. 1977; King and Fuchs 1979; King et al. 1980) have related the sPf and INC to certain torsional, rotational and vertical movements of the eyes and head. In contrast, the action of the ND may be largely inhibitory for all types of eye movement (Scheibel et al. 1961; Szentágothai and Scháb 1956). With regard to the SC, its critical role in the control of coordinated eye and head movements is well established (Roucoux et al. 1980). These results suggest that large portions of the inferior olive are in receipt of information concerning eye and head movements which may play a rather global role in the organization of motor control via the cerebellum.

List of Abbreviations

AC	anterior commissure
Cd	caudate nucleus
CG	central gray
CP	cerebral peduncle
CTT	central tegmental tract
DAO	dorsal accessory olive
dc	dorsal cap of Kooy
dmcc	dorsomedial cell column of the inferior olive
dlPO	dorsal lamella of the principal olive
Entop	entopeduncular nucleus
EW	nucleus of Edinger-Westphal
FR	fasciculus retroflexus
Fx	fornix
GP	globus pallidus
H	H field of Forel
HRP	horseradish peroxidase
IC	inferior colliculus
INC	interstitial nucleus of Cajal
Int Cap	internal capsule
IPN	interpeduncular nucleus
LRN	lateral reticular nucleus
MAO	medial accessory olive
MB	mammillary body
MGB	medial geniculate body
MLF	medial longitudinal fasciculus
MRF	mesencephalic reticular formation
MTT	medial tegmental tract
ND	nucleus of Darkschewitsch
NFF	nucleus of the fields of Forel
NPC	nucleus of posterior commissure
NPP	posterior pretectal nucleus
NRTP	nucleus reticularis tegmenti pontis
n III	third cranial nerve fibers
OT	optic tract
PC	posterior commissure
PF	parafascicular nucleus
PG	pontine gray
PO	principal olive
PTM	medial pretectal nucleus
RNp	parvocellular red nucleus
RN	red nucleus
RST	rubrospinal tract
β	subnucleus beta of the inferior olive
sPf	subparafascicular nucleus
SC	superior colliculus
TH	thalamus
vlPO	ventral lamella of the principal olive
vlo	ventral lateral outgrowth of the principal olive
VTA	ventral tegmental area of Tsai
ZI	zona incerta
III	nucleus of third cranial nerve
XII	nucleus of twelfth cranial nerve

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