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Olivary Projections From the Mesodiencephalic Structures in the Cat Studied by Means of Axonal Transport of Horseradish Peroxidase and Tritiated Amino Acids

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ABSTRACT
By means of horseradish peroxidase (HRP) and autoradiographic methods, olivary projections from mesodiencephalic structures were studied in the cat.

Following HRP injections in various parts of the inferior olive, many cells were labeled ipsilaterally in the nucleus of Darkschewitsch, the nucleus accessorius medialis of Bechterew, the nucleus of the fields of Forel, and the subnucleus dorsomedialis and ventrolateralis of the parvocellular red nucleus. Some labeled cells also occurred ipsilaterally in the suprarubral reticular formation and a few labeled cells in the interstitial nucleus of Cajal.

After injection of tritiated amino acids in different parts of the mesodiencephalic region mentioned above, labeled fibers were found in different parts of the inferior olive, presenting a high degree of the topographic correlation within the mesodiencephalo-olivary projection, which was exclusively ipsilateral. That is, the nucleus of Darkschewitsch was found to project to the rostral half of the medial accessory olive and the dorsomedial cell column. There was mediolateral topographic relation in this projection. The nucleus accessorius medialis of Bechterew was found to project to the ventral lamella and the lateral part of the dorsal lamella as well as to a small rostromedial part of the caudal half of the medial accessory olive. The subnucleus dorsomedialis and ventrolateralis of the parvocellular red nucleus projected to the rostral and caudal halves, respectively, of the medial part of the dorsal lamella. The subnucleus ventrolateralis of the parvocellular red nucleus also sent fibers to the lateral part of the ventrolateral outgrowth. The nucleus of the fields of Forel, suprarubral reticular formation, and interstitial nucleus of Cajal appeared to project to the caudal half of the medial accessory olive, the medial part of the ventrolateral outgrowth, the rostral part of the dorsal cap, and the caudal part of the dorsal accessory olive.

Key words: **inferior olive, mesodiencephalic structures, horseradish peroxidase, autoradiography, cat**

In an early study with the Marchi method, Ogawa ('39) demonstrated ipsilateral projections in the cat from the nucleus of the fields of Forel, nucleus of Darkschewitsch, and interstitial nucleus of Cajal to the rostral parts of medial accessory olive and principal olive. Olivary projections from these areas as well as from the parvocellular red nucleus have later been described in cats by many authors using degeneration (Walberg, '56, '60, '74; Hinman and Carpenter, '59; Mabuchi and Kusama, '70), horseradish peroxidase (HRP) (Abols and Basbaum, '79; Saint-Cyr and Courville '80, '81; Walberg and Nordby, '81; Walberg, '81, '82; Condé and Condé, '82; Zuk et al., '82), and autoradi-

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ographic (Edwards, '72; Saint-Cyr and Courville, '80, '82) methods. However, using a degeneration method in the cat Walberg ('56, '74) has shown that the parvocellular red nucleus projects only to the dorsal lamella of the principal olive and that the nucleus of Darkschewitsch and the ventral central gray project to the rostral half of the medial accessory olive, the dorsomedial cell column, and the principal olive (both the dorsal and ventral lamellae). After destruction of the entire part of the interstitial nucleus of Cajal and the nucleus of Darkschewitsch as well as their neighboring structures in the cat, Mabuchi and Kusama ('70) observed degenerating nerve terminals in the entire region of the principal olive, the rostral two-thirds of the medial accessory olive, the ventrolateral outgrowth, and the dorsal cap, while no degenerations were found in the dorsomedial cell column and nucleus β.

Cells of origin of these olivary projections have recently been identified with the HRP method by several authors (Walberg, '81; Walberg and Nordby, '81; Saint-Cyr and Courville, '81; Condé and Condé, '82; Zuk et al., '82). These include the parvocellular red nucleus, the parafascicular nucleus, the subparafascicular nucleus, the nucleus of Darkschewitsch, the interstitial nucleus of Cajal, the nucleus of the fields of Forel, and parts of the mesencephalic reticular formation.

Quite recently, Saint-Cyr and Courville ('82) have studied the projection autoradiographically and found that there are olivary projections from these structures to almost the entire part of the medial accessory olive and the principal olive (except for the nucleus β and the dorsal cap) and the caudal part of the dorsal accessory olive. However, they were not able to define a detailed topographic organization within the projections.

The aim of the present study has been to identify the more precise localizations of the cells of origin and their terminations in the mesodiencephalo-olivary projections of the cat by using the HRP and autoradiographic methods.

MATERIALS AND METHODS

A total of 13 cats were used in this study. Under Nembutal (sodium pentobarbital) anesthesia, 0.1–0.6 μl of a 50% (w/v) HRP (Toyobo, Grade I-C) solution was injected in various parts of the inferior olive in eight cats. Injections were made stereotaxically (a dorsal approach) or under direct vision (a ventral approach through the pyramis), by means of a glass micropipette or a 25-gauge needle connected with a 1.0-μl Hamilton syringe. After 71–73 hours, animals were again deeply anesthetized and perfused through the heart with 0.9% saline followed by a mixture of 0.4% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer. The brains were dissected free from the skull and stored overnight in the same fixative at 4°C, and then immersed in 0.1 M phosphate buffer containing 30% sucrose for about 24 hours. The frozen sections about 50 μm thick were cut transversely from the brain stem on a freezing microtome. Every fifth section was treated with acetate-buffered p-cresol-3-3'-diaminobenzidine tetrahydrochloride (DAB) according to the method of Streit and Reubi ('77).

In the other five cats, 0.45–0.5 μl of 20–40 μCi/μl of equal amounts of L-[4, 5-^{3}H(N)] leucine (New England Nuclear, specific activity 58.5 Ci/mMol) and L-[2,3-^{3}H(N)] proline (New England Nuclear, specific activity 23.7 Ci/mMol) was stereotaxically injected under anesthesia in the parvocellular red nucleus and its adjacent structures, and the animals were perfused with 10% formalin after 4 days and then processed for the autoradiographic study. Sections were cut transversely at the thickness of 25 μm on a freezing microtome and every tenth section was mounted on chrome-alum coated slides, defatted in xylene, and dipped in Kodak NTB-2 emulsion. The sections of both injection sites and terminals were exposed in light-tight boxes for the same time (3–8 weeks) and then developed in Kodak D-19 (cf. Cowan et al., '72).

All sections of both HRP and autoradiography were counterstained with cresyl violet. Labeled cells and fibers as well as injection sites were examined under the microscope with brightfield and darkfield illuminations (Fig. 1) and the drawings of sections were made with the aid of a projection apparatus. The domain of the amino acid injection sites was characterized by darkfield illumination (Figs. 4, 5).

RESULTS

Mesodiencephalic structures in the cat (Figs. 1, 2)

In the present study, the nomenclature of the cat mesodiencephalic structures was mostly adopted from cytoarchitectural studies of Mitomo ('42) and Zuk et al. ('82). Some comments will be given below concerning the territories of the nuclei. The subparafascicular and parafascicular nuclei described by some previous authors (Saint-Cyr and Courville, '80, '81, '82; Walberg, '81, '82; Condé and Condé, '82; Zuk et al., '82) are included here in the rostral part of the nucleus of Darkschewitsch (cf. 43–46 in Fig. 2), whose lateral part is penetrated by the fasciculus retroflexus. Caudally, it tapers off and disappears before the oculomotor nucleus appears (Mitomo, '42; Zuk et al., '82).

The parvocellular red nucleus used by some authors, e.g., Walberg and Nordby ('81) and Condé and Condé ('82), largely corresponds to the domain of the subnucleus ventrolateralis of the parvocellular red nucleus as described in the present paper. A group of cells extending dorsomedially

Abbreviations

CTT	Central tegmental tract
d.cap.	Dorsal cap
d.l.	Dorsal lamella of principal olive
dm.c.col.	Dorsomedial cell column
dm pNr	Subnucleus dorsomedialis of parvocellular red nucleus
Dtn	Dorsal terminal nucleus of accessory optic tract
ED	Edinger-Westphal nucleus
FR	Fasciculus retroflexus
l	Lateral
L	Left
m	Medial
MLF	Medial longitudinal fasciculus
mNr	Magnocellular red nucleus
MTT	Medial tegmental tract
NB	Nucleus accessorius medialis of Bechterew
ND	Nucleus of Darkschewitsch
NF	Nucleus of the fields of Forel
Nint	Interstitial nucleus of Cajal
Npa	Nucleus pretectalis anterior
Pf	Parafascicular nucleus
Ph	Perihypoglossal nuclei
R	Right
sPf	subparafascicular nucleus
suprarub RF	Suprarubral reticular formation
v.l.	Ventral lamella of principal olive
v.l.o.	Ventrolateral outgrowth
vl pNr	Subnucleus ventrolateralis of parvocellular red nucleus

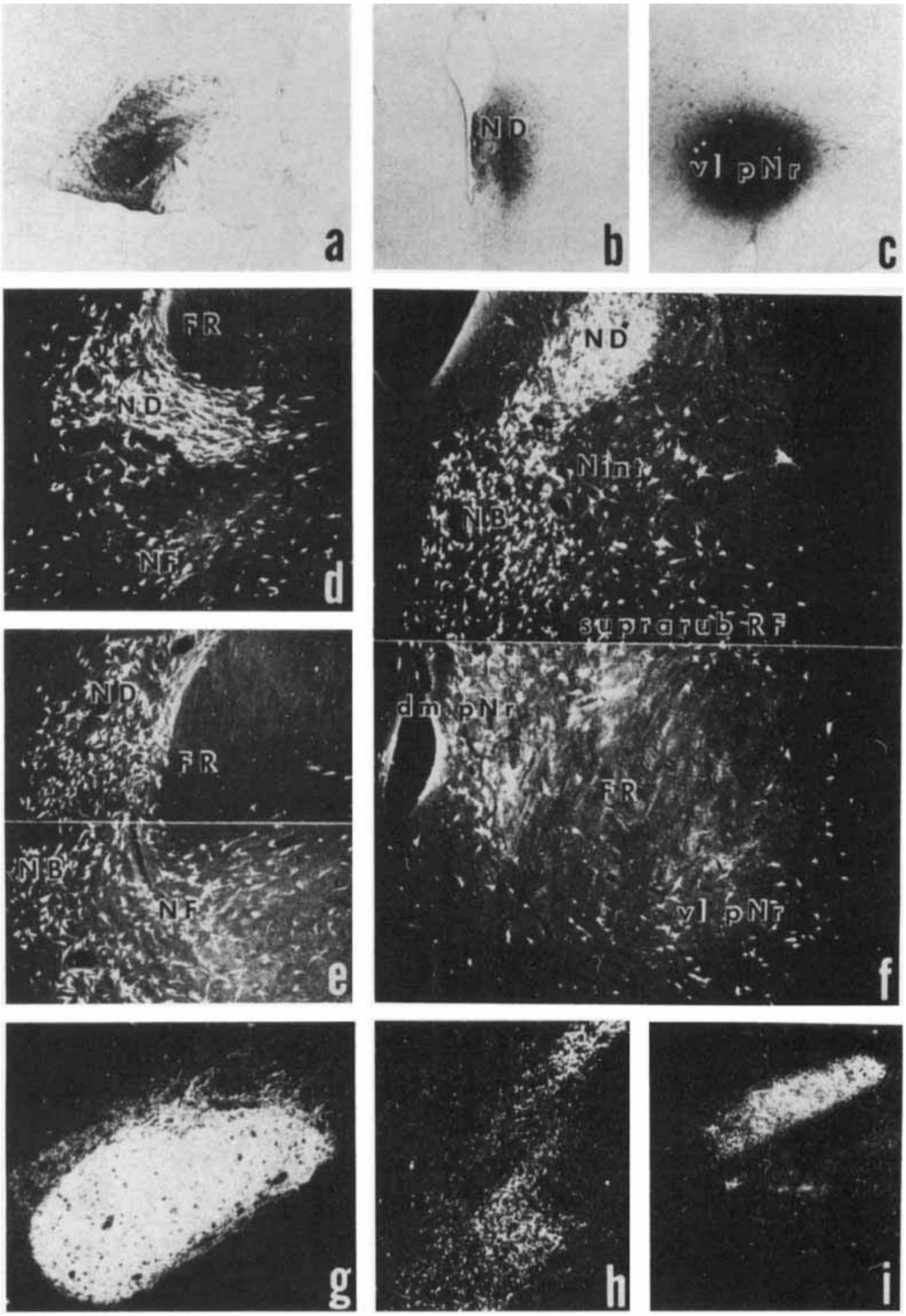
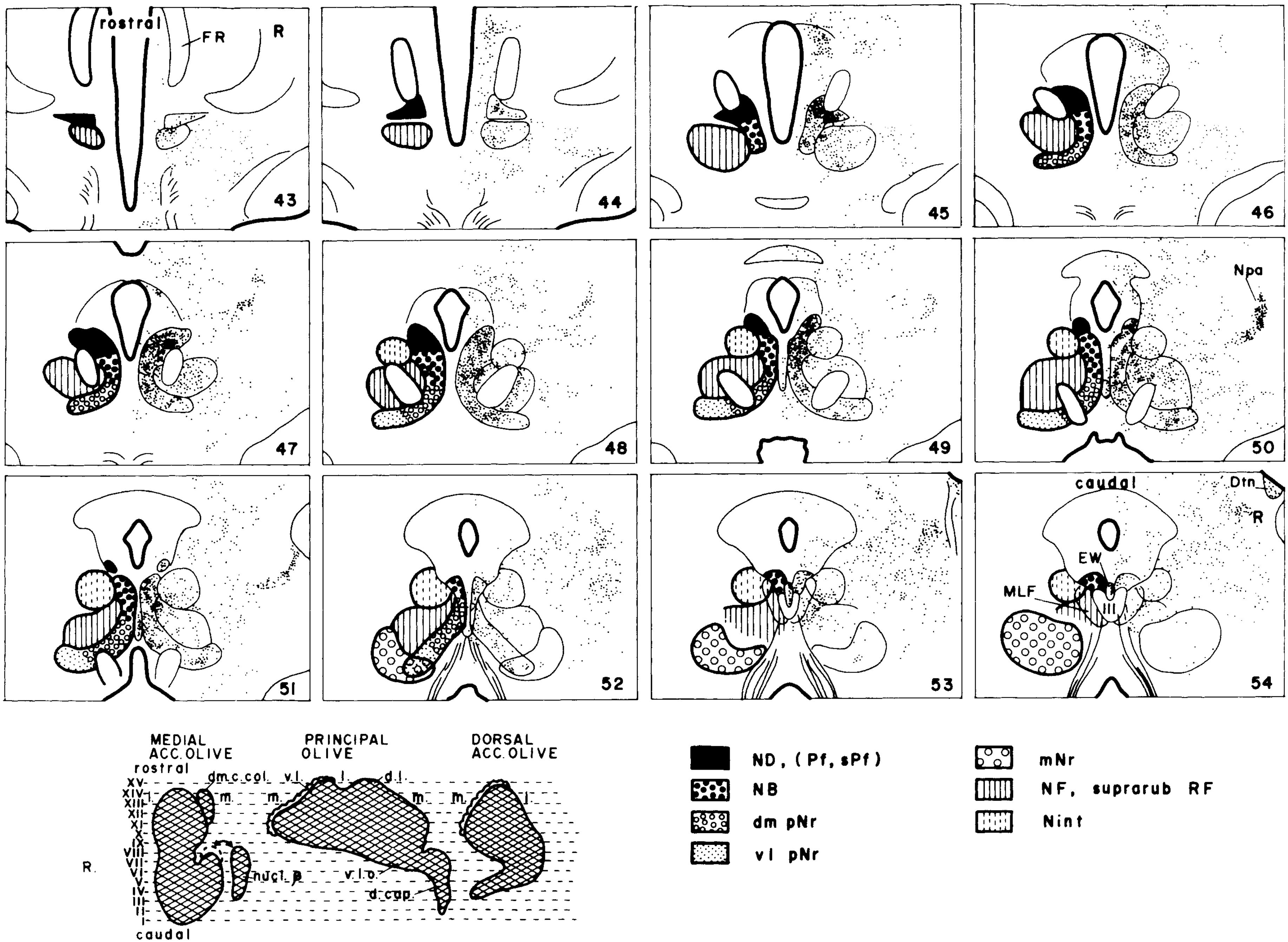


Fig. 1. a. Photomicrograph to show the maximal extent of injected horseradish peroxidase (HRP) at olivary level II in cat KEN 110. ×12. b, c. Photomicrographs of the injection sites of tritiated amino acids in cats KH 95 (at the section level 49 in Fig. 4) and KH 110 (at the section level 51 in Fig. 5), respectively. ×12. d–f. HRP-labeled cells in some mesodiencephalic structures at section levels 45, 46, and 49 in Figure 2, respectively, after injection of HRP in the inferior olivary complex of cat KEN 73. ×35. g. Darkfield photomicrograph from cat KH 101 to show terminations in the rostral part of the medial accessory olive at level X in Figure 4. ×35. h. Darkfield photomicrograph from cat KH 102 to show terminations in the caudal part of the medial accessory olive and the ventrolateral outgrowth at level VII in Figure 5. ×35. i. Darkfield photomicrograph from cat KH 110 to show terminations in the caudal part of the dorsal lamella of the principal olive at level X in Figure 5. ×35.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ND, (Pf, sPf)
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dm pNr
vl pNr
mNr
NF, suprarub RF
Nint

Figure 2

from the rostral red nucleus to the ventral part of the mesencephalic central gray are regarded as the cells of the reticular formation by Saint-Cyr and Courville ('80, '81, '82), Walberg and Nordby ('81), Walberg ('82), and Zuk et al. ('82), and as the cells of the subparafascicular nucleus by Condé and Condé ('82). These can be considered here as being composed of two parts: 1) the subnucleus dorsomedialis of the parvocellular red nucleus located in the reticular region and 2) the nucleus accessorius medialis of Bechterew in the ventral central gray. The nucleus accessorius medialis of Bechterew is located immediately ventral to the nucleus of Darkschewitsch (Mitomo, '42), and extends caudally to the region dorsal to the rostral level of the oculomotor nucleus. The nucleus accessorius medialis of Bechterew in this paper covers part of the interstitial nucleus of Cajal described in Walberg's ('82) paper.

The nucleus of the fields of Forel is assigned to the area situated ventrally to the nucleus of Darkschewitsch and laterally to the nucleus accessorius medialis of Bechterew and subnucleus dorsomedialis of the parvocellular red nucleus (Mitomo, '42). It appears to correspond to the nucleus prestitialis (Hassler, '72), or the nucleus of prerubral field (Graybiel, '77). In the present study, caudal continuation of the nucleus of the fields of Forel is called the "suprarubral reticular formation" (cf. Hartman-von Monakow et al., '79, in the monkey) situated ventrally to the interstitial nucleus of Cajal and laterally to the nucleus accessorius medialis of Bechterew (Fig. 1f). The suprarubral reticular formation and subnucleus dorsomedialis of the parvocellular red nucleus are called the "interstitial nucleus medialis" by Mitomo ('42) who also gave the term "interstitial nucleus lateralis" to the interstitial nucleus of Cajal. The interstitial nucleus of Cajal, located ventrally to the nucleus of Darkschewitsch (Fig. 1f), is seen in transverse sections between the levels of the fasciculus retroflexus and the rostral part of the oculomotor nucleus (Mitomo, '42; Pompeiano and Walberg, '57; Walberg, '82; Zuk et al., '82). The domain of the interstitial nucleus of Cajal corresponds well to that described by Zuk et al. ('82). All of these nuclei mentioned above fuse with each other, and their borders do not always remain apparent (cf., e.g., Mitomo, '42).

HRP studies

Cat KEN 73R (0.6-μl injection, killed after 71 hours, Fig. 2). After injection of HRP, the entire part of the right inferior olive was heavily stained and extended as well to the medullary reticular formation. In addition, there was diffusion of the enzyme to the facial nucleus, small ventral parts of the vestibular complex, and the contralateral inferior olive.

Almost all of the cells were labeled in both the nucleus of Darkschewitsch and nucleus accessorius medialis of Bechterew (Fig. 1d–f). These appeared to form a cell column in the ventral central gray, directly rostrocaudally. A number of labeled cells were also found in the parvocellular red nucleus (Fig. 1f). The main body of the parvocellular red nucleus is the subnucleus ventrolateralis of the parvocellular red nucleus, and the number of labeled cells occurring therein was 274 (counted in every 5th section). Many labeled cells also occurred in the nucleus of the fields of Forel, and some cells in the suprarubral reticular formation. In the interstitial nucleus of Cajal, more labeled cells were found in the ventral part.

In addition, some labeled cells were observed in the mesencephalic reticular formation and the magnocellular red nucleus.

Cat KEN 74R and L (0.42-μl injection, killed after 73 hours, not illustrated). After injection of HRP in the right inferior olive, almost the entire olive was heavily stained with the partial involvement of the surrounding reticular formation. The dorsomedial cell column and a small rostromedial part of the left medial accessory olive were also stained. The distribution pattern of labeled cells in the right mesencephalon was almost identical to that found in cat KEN 73R (Fig. 2). On the left side, many labeled cells were found only in the nucleus of Darkschewitsch except the rostromedial part, and a few cells were found in the nucleus accessorius medialis of Bechterew. As for the number of labeled cells in the subnucleus ventrolateralis of the parvocellular red nucleus, 308 were found on the right side and none on the left.

Cat KEN 107R and L (0.2-μl injection on either side, killed after 72 hours, Fig. 3). The same amount of HRP was injected in either side of the inferior olive; the rostral and central parts on the right side, and the caudal part on the left. There was some spread of the enzyme to the reticular formation on both sides. On the right side, a number of labeled cells were found in the nucleus of Darkschewitsch and nucleus accessorius medialis of Bechterew, as well as in the subnucleus dorsomedialis and ventrolateralis (237 labeled cells) of the parvocellular red nucleus, the suprarubral reticular formation, and the nucleus of the fields of Forel. Some cells were also labeled in the rostral part of the interstitial nucleus of Cajal. On the left side, labeled cells were found in the nucleus of the fields of Forel, the suprarubral reticular formation, the caudal part of the nucleus accessorius medialis of Bechterew, and the interstitial nucleus of Cajal. A few labeled cells occurred as well in the rostral part of the nucleus accessorius of Bechterew, the nucleus of Darkschewitsch, and the parvocellular red nucleus.

Cat KEN 81R (0.3-μl injection, killed after 56 hours, Fig. 3). In the right inferior olive, the injected HRP was restricted to the rostrolateral part of the medial accessory olive and the ventral lamella of the principal olive, without involvement of the surrounding reticular formation. Many labeled cells were found in the rostromedial part of the nucleus of Darkschewitsch and some in the nucleus accessorius medialis of Bechterew. No cells were labeled in the parvocellular red nucleus, the nucleus of the fields of Forel, the interstitial nucleus of Cajal, or the suprarubral reticular formation.

Cat KEN 112L (0.1-μl injection, killed after 72 hours, not illustrated). The medullary reticular formation as well as small rostral parts of the principal olive and dorsal accessory olive were stained on the left side. Labeled cells were found ipsilaterally in the nucleus accessorius medialis

Fig. 2. Diagrammatic representation to show the distribution of HRP-labeled cells in the mesodiencephalic structures in cat KEN 73. The labeled cells were found in the right side of the mesodiencephalic structures as indicated by dots in the drawings of 12 successive serial sections (43–54). These drawings are rostrocaudally arranged, i.e., the section 43 is the most rostral one. One dot represents one labeled cell. In this experiment (KEN 73), it has been verified that the injected HRP has been spread into all the area from caudal (I) to rostral (XV) of the right inferior olive, as shown in the lower-left corner of the figure. The Roman numerals (I–XV) represent the levels of sections from caudal to rostral. The crossed stripes in the unfolded olivary complex indicate the heavy HRP-stained area. Divisions and names of the mesodiencephalic structures as used in the present study are illustrated on the left side of the drawings.

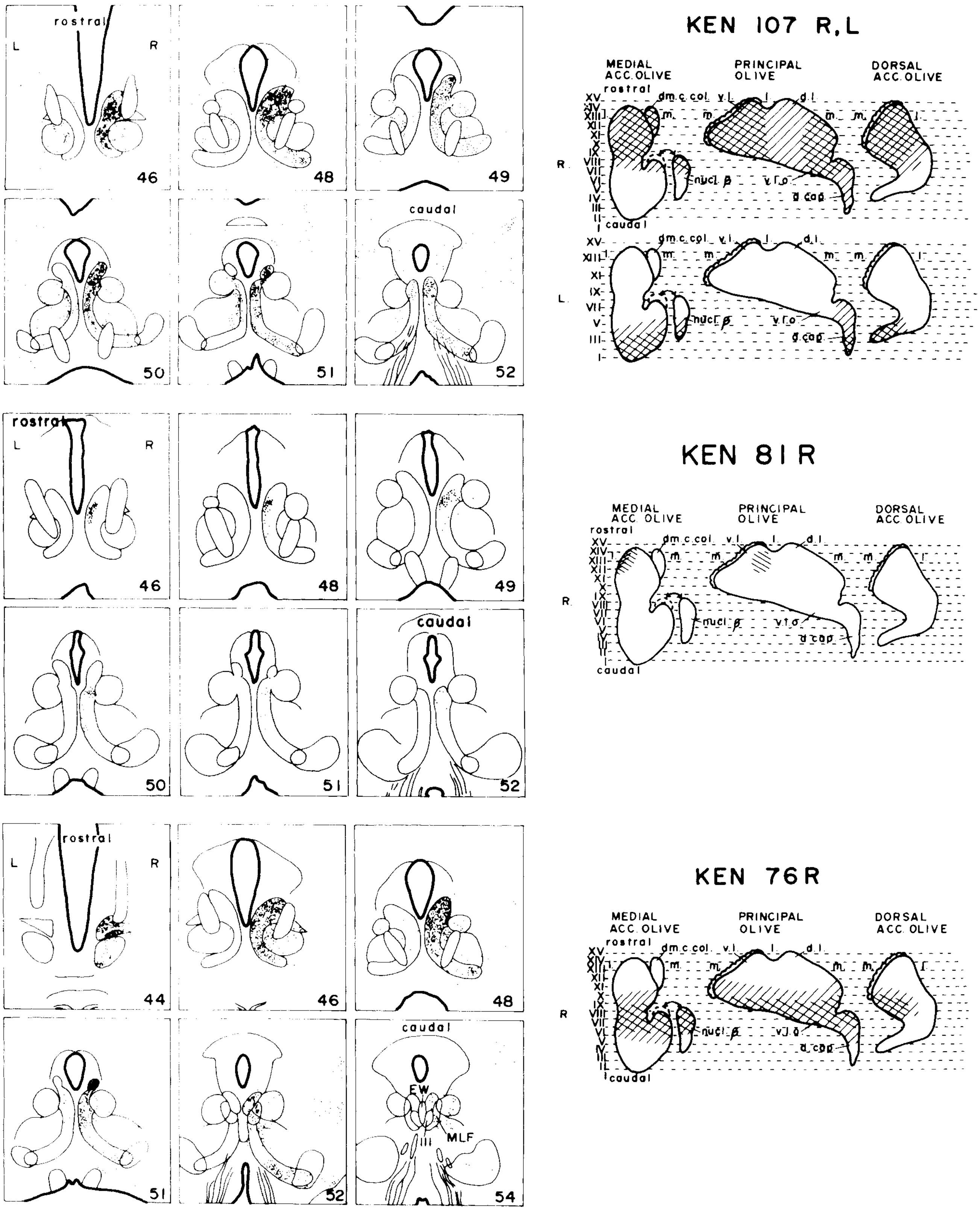

Fig. 3. The left three columns are the diagrammatic representation of the distribution of labeled cells (dots) in the mesodiencephalic nuclei after olivary injections of HRP in cats KEN 107 (group above), KEN 81 (middle group), and KEN 76 (group below). Areas stained with HRP are shown in the standard unfolded olivary map in the right side of the figure. Cross stripes denote heavy staining of HRP, and hatches indicate varying degrees of lighter staining. The number indicated in each drawing of mesodiencephalic sections corresponds to that in Figure 2.

of Bechterew, the subnucleus dorsomedialis of the parvocellular red nucleus, the nucleus of the fields of Forel, and the interstitial nucleus of Cajal.

Cat KEN 76R (0.2-μl injection, killed after 73 hours, Fig. 3). The middle parts of the right inferior olive were stained with HRP. Many labeled cells were found in the nucleus of Darkschewitsch, nucleus accessorius medialis of Bechterew, and subnucleus ventrolateralis of the parvocellular red nucleus. Some cells were also labeled in the nucleus of the fields of Forel, suprarubral reticular formation, interstitial nucleus of Cajal, and mesencephalic reticular formation. No labeled cells were observed in the central gray except the nucleus of Darkschewitsch and nucleus accessorius medialis of Bechterew. The area between the red nucleus and substantia nigra also contained some labeled cells.

Cat KEN 110L (0.2-μl injection, killed after 71 hours, not illustrated). Only the caudal parts of the inferior olive were stained with HRP (Fig. 1a). There was minimal diffusion of the enzyme into the medullary reticular formation. Many labeled cells were found in the nucleus of the fields of Forel, the suprarubral reticular formation, the interstitial nucleus of Cajal, and the caudal one-fourth of the nucleus accessorius medialis of Bechterew. A few labeled cells were also found in the nucleus of Darkschewitsch, the parvocellular red nucleus, the mesencephalic reticular formation, and the periaqueductal gray.

Cat KEN 118L (0.1-μl injection, killed after 71 hours, not illustrated). The dorsal part of the left medullary reticular formation, perihypoglossal and solitary tract nuclei, and the dorsal nucleus of the vagus were stained with injected HRP. There was no involvement of the inferior olive. A small number of labeled cells were found ipsilaterally in the nucleus of the fields of Forel and interstitial nucleus of Cajal. No cells were labeled, however, in the red nucleus, nucleus accessorius medialis of Bechterew, and nucleus of Darkschewitsch.

Autoradiographic studies

Cat KH 90L (20 μCi/0.5 μl injection, killed after 4 days, Fig. 4). Tritiated leucine and proline were injected in the left nucleus of Darkschewitsch. The entire part of the nucleus of Darkschewitsch and the considerable part of the central gray were heavily labeled with partial involvement of the interstitial nucleus of Cajal. The nucleus accessorius medialis of Bechterew was scarcely labeled. A group of labeled fibers descended ipsilaterally through the medial tegmental tract and reached the left inferior olive. Some labeled axons could be traced from the injection site to the ventral tegmental area. Labeled fibers were seen in the medial longitudinal fasciculus. Within the inferior olive, dense terminal labeling was seen ipsilaterally in the rostral half of the medial accessory olive.

Cat KH 95R and L (10 μCi/0.5 μl injection, killed after 4 days, Fig. 4). Labeled amino acids were injected in the medial parts of the right nucleus of Darkschewitsch and nucleus accessorius medialis of Bechterew with involvement of small medial parts of the left nucleus of Darkschewitsch and nucleus accessorius medialis of Bechterew (Fig. 1b). There was no diffusion of the isotope to the surrounding reticular formation and interstitial nucleus of Cajal. On the right side, many labeled fibers descended through the medial tegmental tract and reached the rostral half of the medial accessory olive, the dorsomedial cell column, the ventral lamella of the principal olive, the lateral part of the dorsal lamella of the principal olive, and a small rostromedial part of the caudal half of the medial accessory olive. It may be noted that a small medial side of the rostral part of the medial accessory olive showed less dense labeling. On the left side, there was some labeling in the ventral lamella of the principal olive and the lateral part of the dorsal lamella of the principal olive, as well as in the lateral edge of the rostral part of the medial accessory olive.

Cat KH 101L and R (20 μCi/0.5 μl injection, killed after 4 days, Fig. 4). After injection of labeled amino acids in the left nucleus of Darkschewitsch, the entire part of the nucleus of Darkschewitsch and nucleus accessorius medialis of Bechterew as well as parts of the interstitial nucleus of Cajal, central gray, and reticular formation were heavily labeled. The medial part of the right nucleus accessorius medialis of Bechterew was also slightly involved. On the left side, labeled fibers were seen in the medial tegmental tract and medial longitudinal fasciculus. Many labeled terminals were found in the rostral half of the medial accessory olive, the ventral lamella of the principal olive, the lateral part of the dorsal lamella of the principal olive, and the rostromedial part of the caudal half of the medial accessory olive (Fig. 1g). Labeled terminals of lesser density were also found in other parts of the caudal half of the medial accessory olive as well as in the rostral part of the dorsal cap and the caudal part of the dorsal accessory olive. On the right side, light labeling of fibers was seen in the ventral lamella and bend region of the principal olive. In the mesencephalic region, many labeled fibers were seen to pass through the posterior commissure and terminated in the contralateral nucleus of the fields of Forel, interstitial nucleus of Cajal, and suprarubral reticular formation.

Cat KH 102L (20 μCi/0.5 μl injection, killed after 4 days, Fig. 5). Tritiated amino acids heavily labeled the entire part of the nucleus of the fields of Forel, the most parts of the interstitial nucleus of Cajal and suprarubral reticular formation, and parts of the subnucleus dorsomedialis of the parvocellular red nucleus with slight involvement of small lateral parts of the nucleus of Darkschewitsch and nucleus accessorius medialis of Bechterew. Many labeled fibers ran through the medial longitudinal fasciculus and terminated in the caudal half of the medial accessory olive (Fig. 1h), the rostral part of the dorsal cap, the medial part of the ventrolateral outgrowth, and the caudal part of the dorsal accessory olive. Fibers also traveled through the central tegmental tract, ending in the rostral part of the dorsal lamella of the principal olive. Weakly labeled terminals were observed in the rostral part of the medial accessory olive, particulary in the medial part, as well as in the ventral lamella and bend region of the principal olive.

Cat KH 110L (17 μCi/0.45 μl injection, killed after 4 days, Fig. 5). On the left side, the almost entire part of the subnucleus ventrolateralis of the parvocellular red nucleus and the rostral part of the magnocellular red nucleus were heavily labeled after injection of the amino acids (Fig. 1c). The subnucleus dorsomedialis of the parvocellular red nucleus, nucleus accessorius medialis of Bechterew, and interstitial nucleus of Cajal were not involved. Labeled fibers were seen to run in the left-central tegmental tract and the right Monakow's bundle, which projected to the inferior olive and the spinal cord, respectively. The fibers in the central tegmental tract entered the inferior olive from the lateral side and terminated in the caudal half of the dorsal lamella and the lateral part of the ventrolateral outgrowth (Fig. 1i).

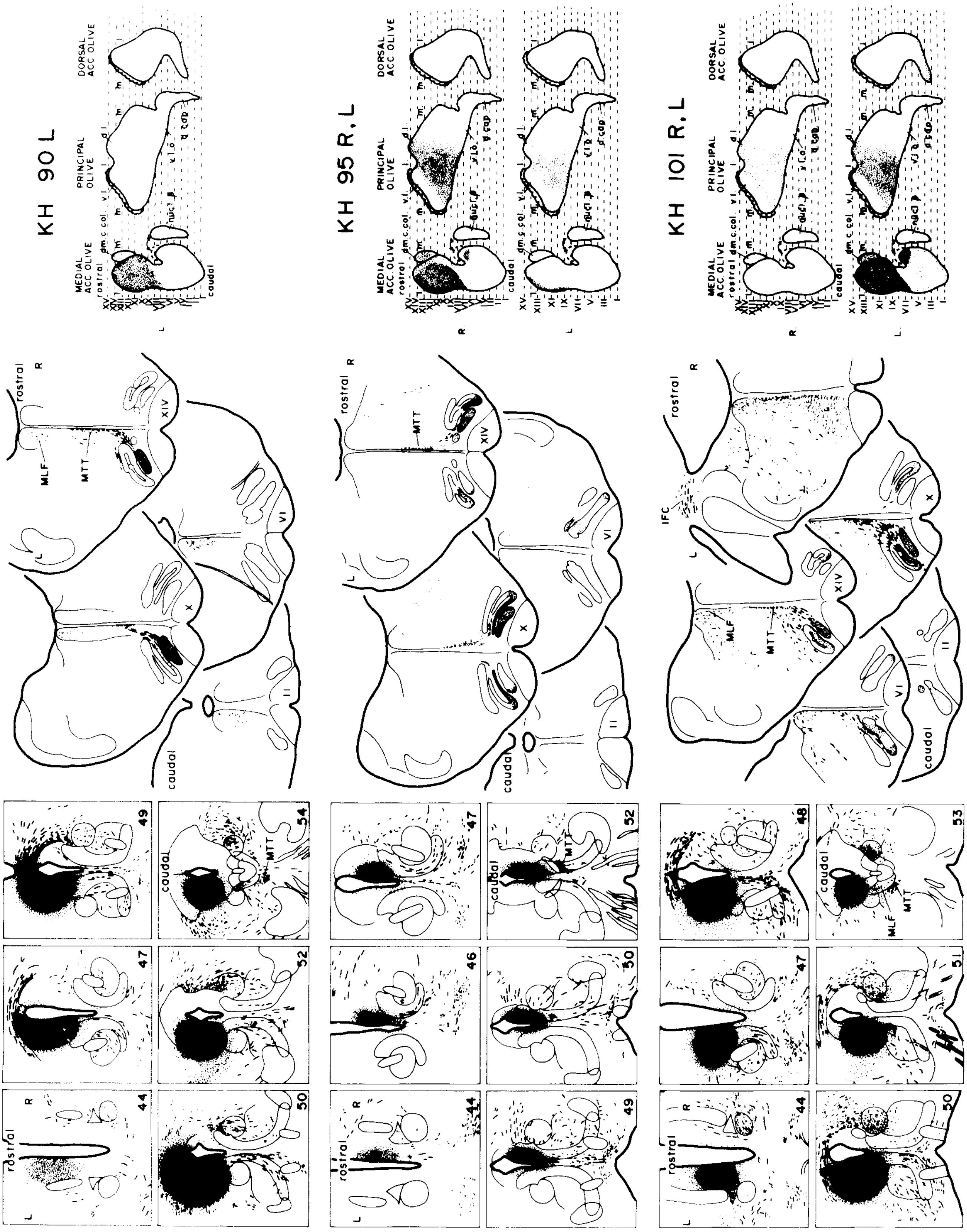
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Figure 4

DISCUSSION

By using the methods of axonal transport of HRP or tritiated amino acids, Saint-Cyr and Courville ('80, '81, '82) have recently found in cats that the mesencephalic nuclei (red nuclus and its neighboring structures) project to the almost entire parts of the medial accessory olive and principal olive as well as to the caudal part of the dorsal accessory olive and stated "it appears impossible to define a strict topographical organization of the projection from the mesencephalon to the olive" (see p. 346 of their paper, '82). Difficulties in obtaining a clear-cut topographic pattern of organization of the mesencephalo-olivary projection might be due to the paucity of detailed reexamination of the mesencephalic structures on the basis of terminal sites in the olivary complex.

The present study with the HRP and autoradiographic methods demonstrates ipsilateral projections from the mesodiencephalic nuclei to the inferior olive. Only in some HRP experiments, labeled cells appeared on the contralateral side, which can be attributable to the diffusion of the injected enzyme to the other side (e.g., cat KEN 76R, Fig. 3). Although Saint-Cyr and Courville ('82) found some labeled terminals in the contralateral inferior olive (see cat DO 22 in their Figs. 1 and 3) after injection of tritiated leucine in the mesencephalon, such fibers were not observed in the present materials.

Olivary projections from the nucleus of Darkschewitsch and nucleus accessorius medialis of Bechterew via the medial tegmental tract

The present HRP study has shown that the nucleus of Darkschewitsch and nucleus accessorius medialis of Bechterew have the strongest olivary projections among mesodiencephalic nuclei (cats KEN 73R, KEN 74R, KEN 107R, KEN 76R, Figs. 2, 3). It is further suggested that there are projections from the nucleus of Darkschewitsch to the rostral part of the medial accessory olive and the dorsomedial cell column (cats KEN 74L and KEN 81R, Fig. 3), and that projections from the nucleus accessorius medialis of Bechterew to the rostral parts of the principal olive and dorsal accessory olive (cat KEN 112L, not illustrated) as well as to the part of the caudal part of the medial accessory olive (cat KEN 110L, not illustrated). On the other hand, when both the nucleus of Darkschewitsch and nucleus accessorius medialis of Bechterew are labeled with tritiated amino acids, labeled fibers are found in the medial tegmental tract which enter the rostral half of the inferior olive: Dense labeling is found in the rostral half of the medial accessory olive, and the moderate labeling is in the dorsomedial cell column, ventral lamella, lateral part of dorsal lamella, and the rostromedial part of the caudal half of the medial accessory olive (cats KH 95R and KH 101L, Figs. 1b,g, 4). In cat KH 90L (Fig. 4), where the nucleus of Darkschewitsch and its adjoining areas are labeled without involvement of the nucleus accessorius medialis of Bechterew, grains representing terminals are found in the rostral half of the medial accessory olive as well as in the dorsomedial cell column on the ipsilateral side. In addition, when parts of the nucleus accessorius medialis of Bechterew are labeled with the isotope, there are always labeled terminals in the ventral lamella and the lateral part of the dorsal lamella (Figs. 4, 5), whether the labeled area in the nucleus accessorius medialis of Bechterew is large or small. Therefore, it is concluded that the nucleus of Darkschewitsch projects mainly to the rostral half of the medial accessory olive and moderately to the dorsomedial cell column via the medial tegmental tract, and that the nucleus accessorius medialis of Bechterew projects to the ventral lamella and the lateral part of the dorsal lamella via the same tract. The present findings are essentially in agreement with, and at the same time reveal more detailed topographical projections than, previous studies (Ogawa, '39; Mabuchi and Kusama, '70; Saint-Cyr and Courville, '80, '82). Walberg ('74) and Cintas et al. ('80) have used cats and rats, respectively, and reported that the nucleus of Darkschewitsch and the ventral central gray project to the rostral half of the medial accessory olive, dorsomedial cell column, and principal olive. On the other hand, Saint-Cyr and Courville ('80, '82) have concluded that the rostral part of the medial accessory olive and a small part of the ventral lamella receive fibers from the rostral part of the nucleus of Darkschewitsch, parafascicular, and subparafascicular nuclei, whereas the main part of ventral lamella receives fibers from the nuclear groups that extend from the levels of subparafascicular nucleus to the rostral part of the parvocellular red nucleus. Within the nuclear groups, the nucleus accessorius medialis of Bechterew as defined in the present study appeared to be included. It may be mentioned here that, although Saint-Cyr and Courville ('82) have concluded that the medial part of the caudal half of the medial accessory olive is labeled only after involvement of the superior colliculus, silver grains are always found there in the present experiments (cats KH 95R, KH 101L, and KH 102L in Figs. 4, 5) in which the labeled amino acids are injected into the caudal part of the nucleus accessorius medialis of Bechterew without involvement of the colliculus. It can therefore be concluded from the present findings that the rostromedial part of the caudal half of the medial accessory olive receives fibers from the caudal part of the nucleus accessorius medialis of Bechterew.

With respect to the olivary projection from the nucleus of Darkschewitsch, it is indicated from the present data that the medial part of the nucleus of Darkschewitsch projects to the lateral part of the medial accessory olive (cf. cats KEN 81R and KH 95R, Figs. 3, 4), while the lateral part of the nucleus of Darkschewitsch projects to its medial part (cf. cats KEN 74L and KH 102L, Fig. 5). Thus, there is a certain degree of mediolateral topographic correlation within the projection, whose tendency is apparently shown in Saint-Cyr and Courville's ('82) findings (cf. their Figs. 1, 6).

Olivary projections from the subnucleus dorsomedialis and ventrolateralis of the parvocellular red nucleus via the central tegmental tract

The present study clearly demonstrates that there is a topographic correlation within the projection. Thus, the

Fig. 4. These diagrams indicate the mesodiencephalic sites of injection of tritiated amino acids (left three columns), and the silver grains in the inferior olivary complex (middle drawings, representative four sections). The right drawings show the silver grains on the unfolded olivary complex. The upper row is KH 90, the middle row is KH 95, and the lower row is KH 101. Black areas in the drawings of the left column show heavy labeling of the amino acids in the mesodiencephalic regions, and the number in each drawing corresponds to that in Figure 2.

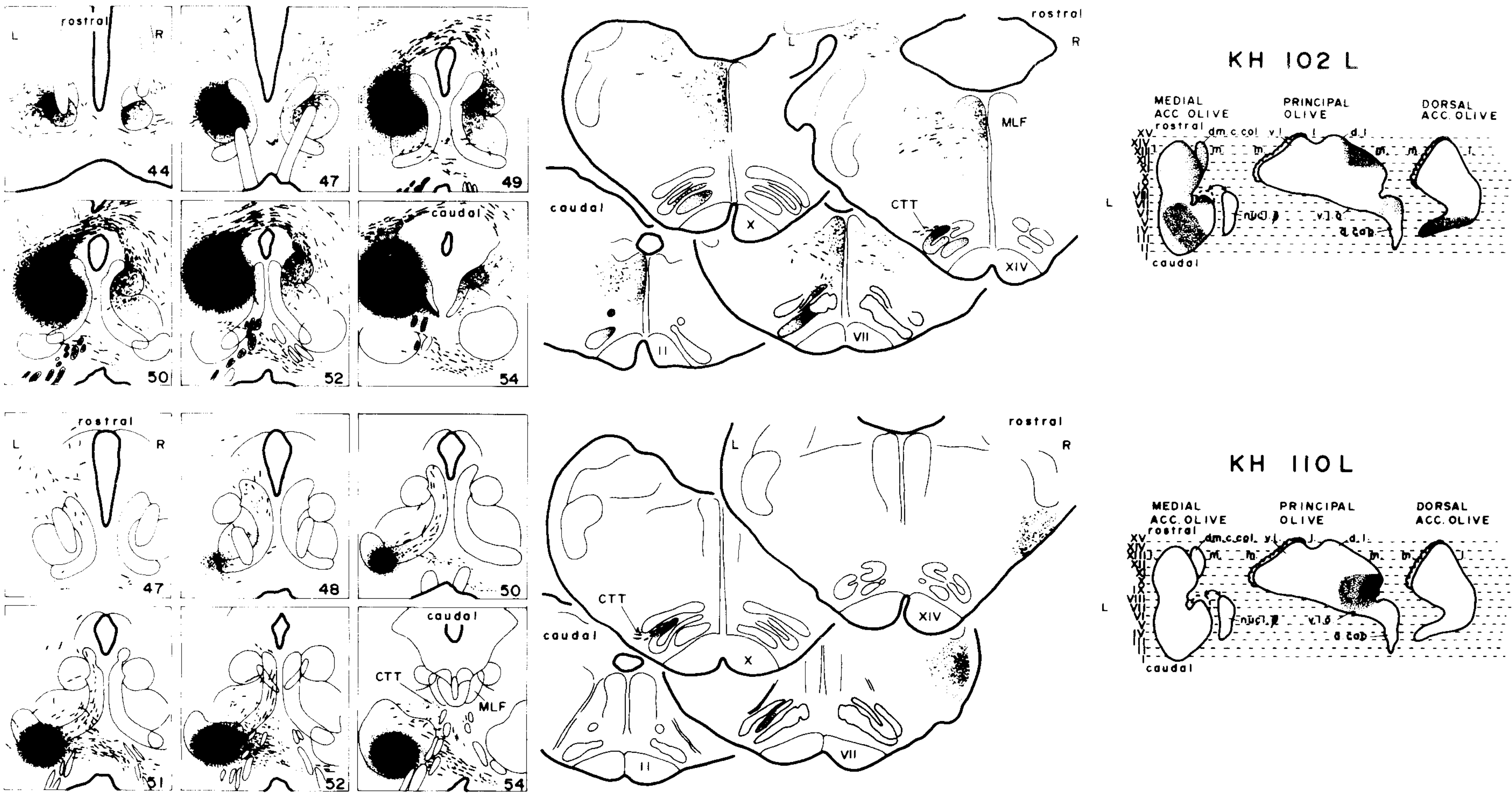

Fig. 5. Left three columns show the extent of injected tritiated amino acids in the mesodiencephalic regions, and the distribution of labeled terminals in the inferior olive is depicted in the middle drawings, and also in the unfolded inferior olive (right side of the figure). The upper row is KH 102 and the lower one is KH 110. See Figure 4 for the explanation of the presentation.

subnucleus dorsomedialis and ventrolateralis[1] of the parvocellular red nucleus project to the rostral and caudal halves, respectively, of the medial part of the dorsal lamella of the principal olive via the central tegmental tract (Figs. 1c,i, 3, 5). The subnucleus ventrolateralis of the parvocellular red nucleus also gives off some fibers to the lateral part of the ventrolateral outgrowth (cat KH 110L, Fig. 5). The projection from the subnucleus ventrolateralis of the parvocellular red nucleus to the caudal part of the dorsal lamella of the principal olive is in agreement with the data of Saint-Cyr and Courville ('82) (cf. their Figs. 1 and 6).

In the monkey, Strominger et al. ('79) have demonstrated with the autoradiographic method that the lateral and medial parts of the parvocellular red nucleus project to the dorsal and ventral lamellae of the principal olive, respectively, and that the intermediate part projects to the bend region of the principal olive. In their study, this medial part of the parvocellular red nucleus contains some part of the nucleus accessorius medialis of Bechterew. The present study has demonstrated that the nucleus accessorius medialis of Bechterew should be distinguished from the parvocellular red nucleus, judging from the difference in their part projecting to the inferior olive. Based upon a detailed examination of monkey findings with degeneration techniques, Courville and Otabe ('74) have interpreted the data as "genuine rubro-olivary fibers" that might terminate in parts of the dorsal lamella of the principal olive alone. From these considerations, it may be safe to consider that the rubro-olivary projections in cats and monkeys have essentially the same pattern, although the parvocellular red nucleus of monkeys seems to be different from that of cats.

Although a few cells in the magnocellular red nucleus are labeled in some experiments (e.g., see cat KEN 76R in Fig. 3) in which the injected HRP is spread to the surrounding medullary reticular formation, no labeled cells occurred in the magnocellular red nucleus (e.g., see cats KEN 107 and KEN 81, Fig. 3) when the reticular formation is free of HRP. This is in agreement with the recent autoradiographic findings of Saint-Cyr and Courville ('82).

Olivary projections from the nucleus of the fields of Forel, suprarubral reticular formation, and interstitial nucleus of Cajal via the medial longitudinal fasciculus

As described above, the nucleus of the fields of Forel, suprarubral reticular formation, and interstitial nucleus of Cajal are closely located and sometimes difficult to delineate border lines. None of the present autoradiographic experiments are able to involve only one structure. Therefore, precise topographic relations between the mesodiencephalic structures and the inferior olive have not emerged from the isotope study alone. However, in cats KEN 107L (Fig. 3) and KEN 110L (Fig. 1a) in which the caudal part of the inferior olive is stained with HRP, many labeled cells are found in the nucleus of the fields of Forel and suprarubral reticular formation, and some labeled cells in the interstitial nucleus of Cajal. On the other hand, when the almost entire part of the nucleus of fields of Forel, suprarubral reticular formation, and interstitial nucleus of Cajal is heavily labeled with tritiated amino acids (cat KH 102L, Fig. 5), labeled terminals are found in the caudal half of the medial accessory olive, the caudal part of the dorsal accessory olive, the medial part of the ventrolateral outgrowth, and the rostral part of the dorsal cap (Fig. 1h). From these data it can be concluded that there are common projections from the nucleus of the fields of Forel, suprarubral reticular formation, and interstitial nucleus of Cajal to the caudal parts of the inferior olive. Grains appearing in the rostral parts of the medial accessory olive and principal olive in cat KH 102L (Fig. 5) can not be taken as the terminal sites from these areas, since no labeled cells are found in these three nuclei in cat KEN 81R (Fig. 3), in which only the rostral parts of the inferior olive are stained with HRP.

The present findings are almost in agreement with those of Saint-Cyr and Courville ('81, '82). However their consideration ('82) that the interstitial nucleus of Cajal[2] is the site of the cells of origin projecting to the dorsomedial cell column is not compatible with the present study. Although Walberg ('82) identified many labeled cells as interstitial nucleus of Cajal, after HRP injections in the rostral part of the inferior olive (cat B.St.L 916, Figs. 1L, 2, 3A–D) in their study, these labeled cells of the interstitial nucleus of Cajal correspond to the cells of the nucleus accessorius medialis of Bechterew described in the present study (see cat KEN 81R, Fig. 3). With the HRP method in the cat, Zuk et al. ('82) have concluded that the interstitial nucleus of Cajal, especially its rostral part, projects to the caudal half of the inferior olive. Thus, after injections of HRP in the caudal part of the inferior olive, labeled cells are found in the interstitial nucleus of Cajal throughout its entire rostrocaudal extent, and the largest concentration of such cells is found in the ventral and rostral parts of interstitial nucleus of Cajal. Our negative data of the mesodiencephalo-olivary projections to the caudal part of dorsal cap are in accordance with those of Saint-Cyr and Courville ('82).

A summary diagram of the topographic correlation within the mesodiencephalo-olivary projections as revealed in the present study is shown in Figure 6.

Functional implication of mesodiencephalo-olivary projections

The accurate topographical projections from the inferior olive to the longitudinal zone of cerebellar cortex and at the same time to the cerebellar nuclei have been demonstrated in many studies (Groenewegen and Voogd, '77; Groenewegen et al., '79; Kawamura and Hashikawa, '79; Brodal and Kawamura, '80; Gerrits and Voogd, '82), indicating that the individual nuclei in the mesodiencephalic area project via inferior olive to the particular longitudinal zones of cerebellar cortex as well as to the cerebellar nuclei. On the other hand, the recent studies have suggested the existence of topographical projections from cerebellar nuclei to mesodiencephalic area, including the nucleus of Darkschewitsch, interstitial nucleus of Cajal (Sugimoto et al., '82; Hirai et al., '82), and parvocellular red nucleus (unpublished data). Therefore, the present and other recent studies suggest the existence of some topographical organization in the mesodiencephalo-olivio-cerebellar circuits.

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[1] After injections of HRP in the inferior olive of the cat, Walberg and Nordby ('81) and Condé and Condé ('82) counted a total number of ca. 100 labeled cells (examined in every fifth section) and ca. 1,500 (examined in every section), respectively, in the subnucleus ventrolateralis of the parvocellular red nucleus. In the present study, the number of labeled cells therein is ca. 300 (examined in every fifth section of cats KEN 73R, KEN 74R, KEN 76R, and KEN 107R).

[2] The interstitial nucleus of Cajal described in their paper appears to be a little more extensive in area than that considered in the present paper.

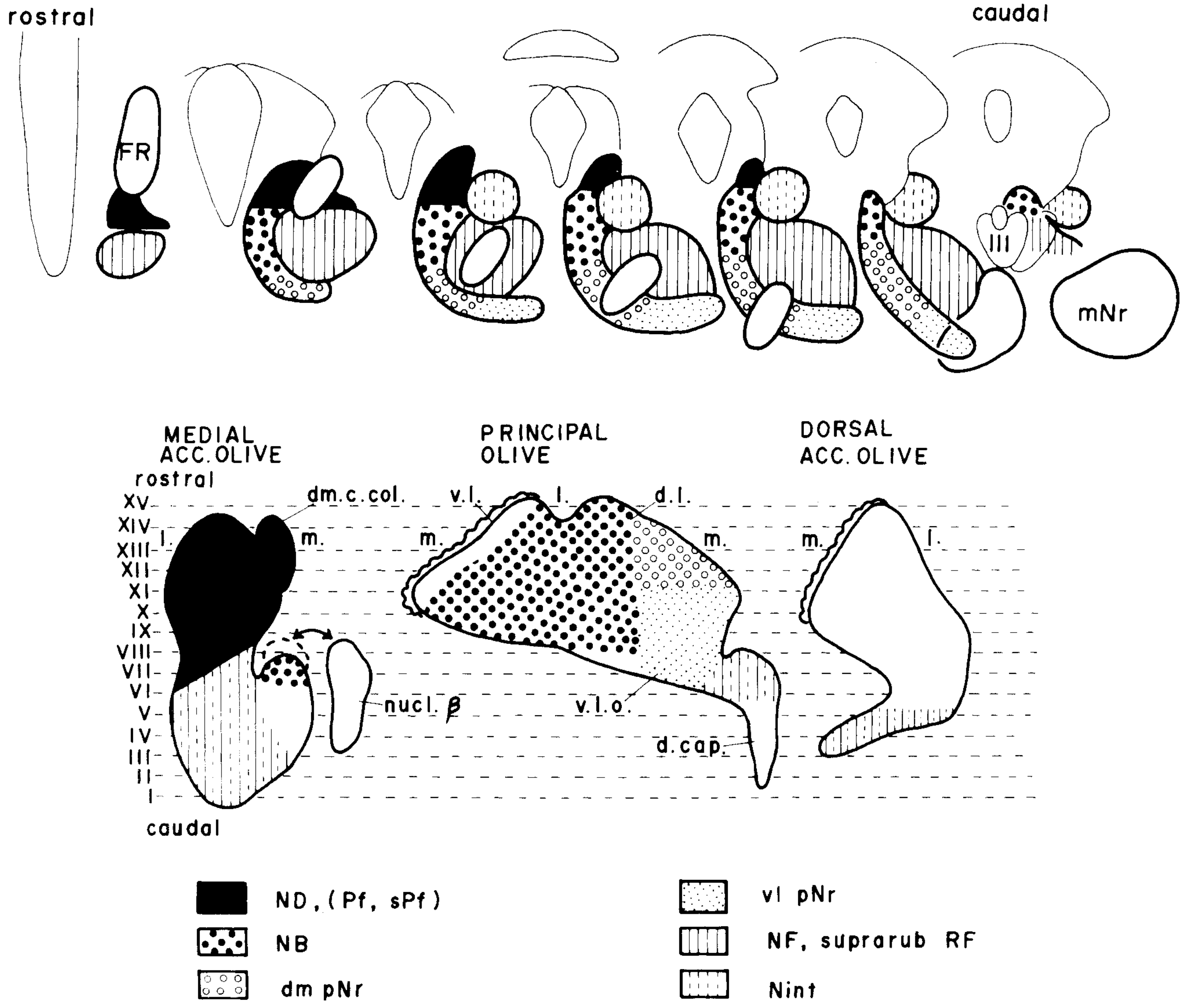

Fig. 6. Diagram summarizing the topographic relations between the mesodiencephalic nuclei (above) and the inferior olive (below) as demonstrated in the present study. Six symbol designs represent the origins and their corresponding terminations in the mesodiencephalo-olivary projection.

The thought that the nucleus of Darkschewitsch has fiber connections via olive with the flocculus (zone C_2) is compatible with the physiological fact presented by Szentágothai and Scháb ('56) and Scheibel et al. ('61) that the nucleus of Darkschewitsch is related to the inhibitory regulation of the contraction of extraocular muscle, including vestibulo-ocular reflex. The interstitial nucleus of Cajal and nucleus of the fields of Forel, which receive fibers from vestibular nuclei, project to the oculomotor nuclei (Mabuchi and Kusama, '70; Graybiel, '77; Büttner-Ennever and Büttner, '78). These nuclei are considered to be related to the coordinated reflex movements of eye and head (Hassler, '72) through the circuits of vermis (zone A and zone B) and flocculus (zone F) as described above. The nucleus accessorius medialis of Bechterew and parvocellular red nucleus have fiber connections via the principalolive with zones D_1 and D_2 of the cerebellar hemisphere in cat. The stimulation of the corresponding cerebellar area evokes eye and limb movements in monkey (Ron and Robinson, '73). Moreover, the nucleus accessorius medialis of Bechterew and the parvocellular red nucleus receives fibers from prefrontal cortex (Leichnetz, '82), and from motor cortex (Hartman-von Monakow et al., '74), respectively, in monkey. These evidences indicate that the mesodiencephalo-olivo-cerebellar circuits may be at least associated with the reflex eye movements in coordination with the body.

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