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ON THE CIRCUITRY OF THE INFERIOR OLIVARY NUCLEUS IN THE OPOSSUM, DIDELPHIS VIRGINIANA: I. AN AUTORADIOGRAPHIC STUDY OF MIDBRAIN-DIENCEPHALIC PROJECTIONS TO THE INFERIOR OLIVARY NUCLEUS. II. THE ORGANIZATION OF THE OLIVO-CEREBELLAR PROJECTION AS REVEALED BY RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE.

The Ohio State University, Ph.D., 1977
Anatomy

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MARTINS LINAUTS

ALL RIGHTS RESERVED
ON THE CIRCUITRY OF THE INFERIOR OLIVARY NUCLEUS
IN THE OPOSSUM, Didelphis virginiana.

I. AN AUTORADIOGRAPHIC STUDY OF MIDBRAIN-DIENCEPHALIC
PROJECTIONS TO THE INFERIOR OLIVARY NUCLEUS.

II. THE ORGANIZATION OF THE OLIVO-CEREBELLAR PROJECTION
AS REVEALED BY RETROGRADE TRANSPORT
OF HORSERADISH PEROXIDASE.

DISSERTATION
Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Martins Linauts, B.S.

* * * * *

The Ohio State University
1977

Reading Committee:              Approved By
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Professor George F. Martin, Adviser

George F. Martin
Adviser
Department of Anatomy
Dedicated to my parents,

LEONIDS and DAINUVITA LINAUTS

Kas zin kur nākošais celš mani novedīs.
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And to my family, and Sandy, without whose constant and unwavering support this educational experience would not have been the same, I am deeply grateful.
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LIST OF ABBREVIATIONS

Inferior Olivary Nucleus (OI)*

a - subnucleus "a" of medial accessory nucleus (MAO)
b - subnucleus "b" of medial accessory nucleus (MAO)
c - subnucleus "c" of medial accessory nucleus (MAO)
d - dorsal accessory nucleus (DAO)
pr - principal nucleus (PO)
pr.d - dorsal lamella of principal nucleus (DL-PO)
pr.v - ventral lamella of principal nucleus (VL-PO)

*The capital letter abbreviations (in parenthesis) are those used in the text; the lower case letter abbreviations are used in the illustrations.
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<tr>
<td>Nd</td>
<td>nodulus</td>
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<tr>
<td>ped</td>
<td>pedunculus cerebri</td>
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<tr>
<td>PFl</td>
<td>paraflocculus</td>
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<tr>
<td>PMi</td>
<td>paramedian lobule inferior</td>
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<tr>
<td>PMs</td>
<td>paramedian lobule superior</td>
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<tr>
<td>PrT</td>
<td>nucleus pretectalis</td>
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<td>PyC</td>
<td>pyramis</td>
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<td>Fyr</td>
<td>tractus pyramidalis</td>
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<td>r III</td>
<td>radix n. oculomotori</td>
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<td>fasciculus retroflexus</td>
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<td>RGc</td>
<td>nucleus reticularis gigantocellularis</td>
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<td>RN</td>
<td>nucleus ruber</td>
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<td>area interstitialis tegmenti</td>
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<td>area profunda tegmenti</td>
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<td>TgV</td>
<td>area ventralis tegmenti</td>
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<td>TrMo</td>
<td>nucleus motorius n. trigemini</td>
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<td>TrSV</td>
<td>nucleus sensorius n. trigemini ventralis</td>
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<tr>
<td>TrSo</td>
<td>nucleus tractus spinalis n. trigemini: pars oralis</td>
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<td>nucleus corporis trapezoidei</td>
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<td>Uv</td>
<td>uvula</td>
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<tr>
<td>VstI</td>
<td>nucleus vestibularis inferior</td>
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<td>nucleus vestibularis medialis</td>
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<td>xVB</td>
<td>nuclei ventralis thalami basalis</td>
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<tr>
<td>ZI</td>
<td>zona incerta</td>
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PART I: AN AUTORADIOGRAPHIC STUDY OF MIDBRAIN-DIENCEPHALIC PROJECTIONS TO THE INFERIOR OLIVARY NUCLEUS.
INTRODUCTION

The inferior olivary nucleus is considered the major, if not sole, source of climbing fibers to the cerebellar cortex (e.g. Grant, '70, Desclin, '74, Eccles et al., '66), as well as a source of collaterals to the cerebellar nuclei (Brodal, '40, Matsushita and Ikeda, '70, Beitz, '76). Anatomical (Brodal, '40, '76, Brodal et al., '75, Brodal and Walberg, '76, Hoddevik et al., '76) and physiological (Armstrong, '74) methods have shown that the olivo-cerebellar projection is highly organized and it might be assumed that the various inputs to the olive are comparably complex. The midbrain is known to be a major source of such connections, but with only a few exceptions (Edwards, '72, Frankfurter et al., '77) our knowledge of midbrain-olivary projections is limited to data obtained by degeneration methods (Ogawa, '39, Mettler, '44, Walberg, '56, '60, '74, Martin et al., '75). The limits of those methods are well known.

In an earlier communication (Martin et al., '75), organization of midbrain-olivary pathways was described in the North American opossum as they were revealed by degeneration methods. Because of the problems inherent in those methods, however, we subsequently initiated studies utilizing axonal transport techniques (LaVail and LaVail, '72). In the
first study (Henkel et al., '75) horseradish peroxidase (HRP) was injected into the olive in order to retrogradely label and identify the midbrain neurons which relay to it. We have since taken advantage of that data and made placements of tritiated leucine so as to label, in separate experiments, the midbrain neurons identified in the HRP studies, and to follow their axons by the autoradiographic method (Cowan et al., '72). The present communication reports the results of those experiments. The terminology for the opossum midbrain is taken from Oswaldo-Cruz and Rocha-Miranda, '68, while that for the olive is from Martin et al., '75.
MATERIALS AND METHODS

Tritiated leucine (New England Nuclear) was introduced into the brains of 30 adult opossums (2.5 kg average body weight). The stock solution was dehydrated in a vacuum centrifuge, reconstituted with physiologic saline to a concentration of 10-20μCi of activity per .1μl of solution, and delivered in volumes of .1 to .4μl.

Anesthesia of the animal was achieved by intravenous injection of sodium pentobarbital (65 mg/kg body weight). The head was stabilized in a stereotaxic apparatus and a craniotomy was performed to expose the midbrain tectum. Subsequently, a 5μl syringe with a 31 gauge needle was lowered to the desired position using a microdrive system, guided by coordinates derived from the atlas of Oswaldo-Cruz and Rocha-Miranda, '68. The needle was left in place for ten minutes to allow for stabilization and the injection was carried out over a period of 30-60 minutes. After completion of the injection the needle was left in place for an additional ten minutes before removal in order to minimize spread of the isotope along the needle track.

The animals were allowed to survive for 9-11 days, at which time they were sacrificed by intra-cardial perfusion. After administration of an overdose of sodium pentobarbital, a thorocotomy was performed and the pericardial sac removed. A volume of .2cc heparin was injected

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into the left ventricle, the pulmonary vessels were clamped off, and a
13 gauge needle was inserted into the left ventricle. As a 0.9% saline-
procaine flush was initiated, the right atrium was incised to allow for
drainage of the cardiovascular system. Following administration of ap-
proximately 700 ml of the saline-procaine solution, the fixative was
introduced. The fixative employed was either buffered paraformaldehyde
(4%) - gluteraldehyde (1%) or buffered 10% formalin at a volume of
1500 ml. The brain was removed immediately and immersed in the fixative
until processing of the tissue commenced.

Transverse sections of the brainstem were cut at 35μ on a
freezing microtome and mounted out of distilled water on chrom-alum
subbed slides. The slides were then coated with NTB-2 nuclear track
emulsion diluted 1:1 with distilled water, and exposed for 4 weeks at
4°C. Development of the slides was carried out with Kodak D-19 deve-
loper, and they were subsequently counterstained with cresyl violet.
Analysis of the material was carried out in both light and dark field
illumination.
RESULTS

Projection from the V ventromedial Tegmentum

In this account, the ventromedial tegmentum refers to the area roughly bounded by the midline and by a line extending from the ventro-lateral aspect of the cerebral aqueduct to the lateral portion of the basis pedunculi. Included within this region are the nucleus linearis, the ventral periaqueductal gray, part of the nucleus of Darkschewitsch, the interstitial nucleus of Cajal, the red nucleus, and the reticular formation surrounding the red nucleus.

Neurons in the ventral periaqueductal gray and the interstitial nucleus of Cajal contain reaction product subsequent to injections of HRP into the inferior olive (see figure 1-C, arrow 1 and figure 1D). Figure one provides a schematic summary of results reported previously as a short note (Henkel et al., '75). The injection of tritiated leucine in case P-417 (figure 2 inset) labelled neurons within both areas, as well as within the tegmentum dorsomedial to the red nucleus. The isotope is most heavily concentrated over the interstitial nucleus of Cajal, but there is evidence for some spread to the dorsomedial border of the red nucleus. Heavily labelled axons can be traced from the injection site into the medial longitudinal fasciculus (MLF), ipsi-laterally, and into the rubrobulbospinal tract (Martin and Dom, '70a,b),
contralaterally. However, labelled axons cannot be traced into the olive from either pathway. Rather, it appears that the terminal label in the olive, to be described below, was a result of transport through axons which are scattered diffusely within the reticular formation. At medullary levels, such axons assume a position just dorsal to the olive. In serial sections through the olive, terminal labelling is present throughout the rostral to caudal extent of the ipsilateral principal nucleus (figure 2, B-G). The density of silver grains is higher over the dorsal lamella of the principal nucleus than over its ventral counterpart, although the difference is subtle, especially at rostral levels. There is no evidence for terminal label in the accessory nuclei at any level.

In case P-418 (figure 3), the isotope injection was aimed at neurons dorsomedial to the red nucleus which are comparable to those backfilled following olivary placement of HRP (figure 1-C, arrow 2). Both the desired area and the medial red nucleus are heavily labelled in the autoradiograms (figure 3, inset), but there is no evidence that the marker spread to either the interstitial nucleus of Cajal or the ventral periaqueductal gray. The pontine and medullary location of labelled axons is essentially the same as in P-417, and labelled terminals are still restricted to the principal nucleus (figure 3, B-G). As in P-417, the concentration of silver grains is greatest over the dorsal lamella.

A striking finding in the HRP studies is the surprisingly small number of reactive neurons within the normally defined boundaries of the
red nucleus (figure 1, C-D). The well known "rubro-olivary" projection, however, is demonstrated in case P-469, in which the isotope was injected directly into the red nucleus (figure 4, inset, and figure 11, arrow). Although the marker spread somewhat into the tegmentum immediately dorsal to the red nucleus, it should be emphasized that the tegmental areas labelled in P-417 and P-418 (see above) are spared. Rostrally, the isotope spread minimally beyond the red nucleus and into the fields of Forel. As in the previous cases, silver grains are clustered over axons in both the ipsilateral MLF and the contralateral rubrobulbospinal tract, and are present over fibers which are scattered within the reticular formation adjacent to the raphe. At rostral to mid-olivary levels, labelled terminals are essentially confined to the dorsal lamella of the principal nucleus on the side of the placement (figure 4, C-F and figure 12), with only sparse labelling of the ventral lamella. Unlike the previous cases, evidence is also present for a projection to the caudal part of the medial accessory nucleus, specifically to the ventromedial part of subnucleus "c" (figure 4-A). It appears likely that the terminal label in the latter region is due to transport from cells in the tegmentum dorsal to the red nucleus. This area will be dealt with later.

In P-467 (figure 5), a relatively large placement of the isotope was made in the midline in order to label the few cells of the nucleus linearis which are retrogradely labelled in the HRP study (figure 1-C, arrow 3). The injection site covers a roughly triangular area ventral to the central gray and encroaches upon the medial red nucleus to a greater extent on one side than the other (figure 5, inset and figure 13).
The latter observation is substantiated by the laterality of the most extensive labelling of crossed rubral fibers at pontine and medullary levels. Silver grains are clustered over the MLF and scattered over the reticular formation ventral to it bilaterally, although the density of paramedian and olivary labelling is highest on the side which shows the greatest involvement of the red nucleus at the injection site. The principal nucleus is covered with silver grains, bilaterally (figure 5, C-E), but the dorsal lamella is more heavily labelled on the side of the greatest rubral involvement (the reader's left in figure 5 and figure 14), while on the opposite side, silver grains are more evenly distributed over both lamellae (figure 5, C-D right side). Furthermore, there is evidence for labelled terminals in the caudal tip of the dorsal accessory nucleus as well as within the medial accessory nucleus (figure 5-D). In contrast to the previously described case, however, it is subnuclei "a" and "b" of the medial accessory complex which are labelled (figure 5, A-B).

**Projection from the Mesodiencephalic Junction**

Since reactive neurons were present in the subparafascicular nucleus, the nucleus of Darkschewitsch, and the fields of Forel subsequent to olivary injections of HRP (figure 1, A-B, open arrows), several cases were prepared with $^3$H-leucine deposits into each of those regions. Case P-437 (figure 6) contains a well confined injection of the subparafascicular nucleus at the level of the rostral-most tip of the interstitial nucleus of Cajal and the nucleus of Darkschewitsch (figure 6,
inset, figure 15). Spread of the marker to the aforementioned nuclei is minimal. Labelled axons descend dorsomedial to the red nucleus and assume a position adjacent to the dorsal-ventral extent of the raphe in the pons. At caudal pontine and rostral medullary levels, such fibers become confined to the gigantocellular reticular formation, pars ventralis. Analysis of grain distribution over the olive suggests that all three of its major divisions receive a projection from neurons at the injection site. Silver grains are distributed sparsely over the rostral to caudal extent of the dorsal accessory nucleus (figure 6, A-G), but are somewhat more numerous over both lamellae of the principal nucleus (figure 6, E-G). It is the medial accessory nucleus, however, that is labelled most heavily. Subnucleus "a" appears to receive a massive projection at the level illustrated in figure 6, C-D (see also figure 16), while the projection to its more rostral and caudal regions is significantly less. Labelled axons distribute to subnucleus "b", but subnucleus "c" is apparently free from label (figure 6, A-B). Additionally, some evidence is present for a light projection to the cap of Kooy (figure 6-B).

In case P-419, the placement heavily labelled cells in the fields of Forel (figure 7, inset). The injection spread dorsally and may have minimally involved a portion of the subparafascicular nucleus which does not contain reactive neurons subsequent to HRP injections in the olive. Labelled axons pass over the dorsal border of the red nucleus and occupy a position in the central portions of the reticular formation throughout the pons. Such fibers are located lateral to those labelled in the
previous case. At levels approaching the motor facial nucleus and extending into the rostral medulla, the labelled axons shift ventromedially into pars ventralis of the gigantocellular reticular formation. The distribution of terminal label in the inferior olive is comparable to that described for the subparafascicular case, but the grain density is less. The dorsal accessory nucleus is lightly labelled throughout its extent (figure 7, A-G) and the grains over the principal nucleus are most numerous over the dorsal lamella (figure 7, C-E). Subnuclei "a" and "b" of the medial accessory nucleus are both targets of labelled axons, with subnucleus "a" receiving a somewhat heavier projection at its intermediate level (figure 7-A). As in the previous case, there is little evidence for a projection to subnucleus "c".

In case P-523, the isotope was deposited in the rostral portion of the nucleus of Darkschewitsch, with spread to the adjacent interstitial nucleus of Cajal, as well as the ventral periaqueductal gray (figure 8, inset). Clusters of silver grains are present over the ipsilateral MLF throughout its course. At the level of the inferior olive, small bundles of labelled axons are seen coursing ventrally from the MLF along the raphe. There is evidence for a bilateral projection to the olive, although it is heaviest on the side ipsilateral to the injection site. Silver grains are present over the caudal 2/3 of the principal nucleus and their density is greatest over its ventral lamella (figure 8, B-C, left side). Additionally, terminal label is seen over subnucleus "a" of the rostral half of the medial accessory nucleus (figure 8, B-D).
**Projections from Dorsolateral Tegmental and Tectal Areas**

In this description, the dorsolateral tegmentum is defined as the area dorsal to a line extending from the ventral periaqueductal gray to the lateral portion of the cerebral peduncle. Injections of $^3$H-leucine into certain portions of that region result in labelling over the olive which is distinctly different from that present after either ventromedial injections or deposit of the marker into the mesodiencephalic region.

Case P-439 (figure 9, upper half) was prepared with the intent of labelling cells of the caudal pretectal complex which are comparable in position to those backfilled after injections of HRP into the olive (figure 1-B, solid arrow). In addition to labelling pretectal neurons, however, the marker spread into lateral portions of the tegmentum medial to the medial geniculate nucleus. Since there are no retrogradely labelled neurons in the latter area following HRP injections of the olive, it is tentatively concluded that the olivary labelling seen in this case reflects the transport of marked protein from pretectal neurons. In the pons, labelled axons are scattered in the ventromedial reticular formation, but the distribution of silver grains over the ipsilateral olive is quite restricted. Only a small ventromedial region of the rostral dorsal accessory nucleus (upper half figure 9-B) and the ventromedial part of subnucleus "c" of the medial accessory complex are labelled ipsilaterally (upper half, figure 9-A).

The injection in case P-480 (insert in lower half, figure 9; and figure 17) labels neurons in that part of the tegmentum adjacent to the lateral border of the central gray which contain reaction product in the
HRP experiments referred to previously (figure 1, B-C, arrow 4).

Labelled axons descend on both sides of the pons. Ipsilateral to the placement they are scattered in the gigantocellular reticular formation, pars ventralis, of the caudal pons, while contralaterally they are more compactly arranged in bundles abutting on the raphe. The autoradiograms through the olive reveal terminal label over the ipsilateral dorsal accessory nucleus (lower half, figure 9-B), as described for P-439, as well as over caudal parts of the medial accessory nuclei, bilaterally (lower half, figure 9-B). Ipsilateral to the injection the label is present over the ventromedial portion of subnucleus "c" (figure 9-A, left side; and figure 18, between arrows), as in P-439, whereas contralaterally it is concentrated over the dorsolateral portion of the comparable subnucleus.

Case P-502 illustrates the results of an injection dorsolateral to the red nucleus (figure 10, inset), with minimal spread of the marker into the red nucleus itself. Through the pons, labelled axons are identified in central portions of the reticular formation and the MLF, ipsilaterally, as well as in the crossed rubrobulbospinal tract. Through the ipsilateral medulla, labelled axons are clustered adjacent to the raphe and scattered in the ventromedial reticular formation. At olivary levels, labelled axons are seen to almost completely surround the olive. Evidence for terminal labelling is present over the ventromedial portion of the dorsal accessory nucleus, rostrally (figure 10-D) and over subnucleus "c" of the caudal medial accessory complex (figure 10, A-B) of the same side. In addition, however, there is labelling over a very
restricted portion of the rostral tip of the medial accessory nucleus where it is positioned ventromedial to the principal nucleus (figure 10, D-E).

Injections which include both the deep superior colliculus and the underlying tegmentum (not illustrated) result in label which is restricted to the dorsolateral part of subnucleus "c" on the contralateral side. Neurons are reactive in both areas after HRP is deposited within the olive. In no case of isotope injection into either the dorsolateral midbrain tegmentum or the superior colliculus is there evidence for a projection to the principal nucleus.

Midbrain Injections which did not Result in Olivary Labelling

A number of midbrain placements failed to produce olivary labelling, although the presence of silver grains over axons in the brainstem attests to the effectiveness of the injection. In case P-466 (figure 21), the marker labels neurons in the ventral tegmental area of Tsai throughout its extent, with some spread into the ventral border of the red nucleus. Serial sections through the pons and the medulla show modest silver grain accumulation over fibers of the crossed rubrobulbospinal tract, but no label is present that would indicate transport to the olive. The validity of the negative results in the olive is supported by the lack of HRP labelled cells in either the ventral tegmental area or the ventral red nucleus subsequent to injection on the enzyme into the olive.
In the cases pictured in figures 19 and 20, the isotope labels cells in the tegmentum dorsal and lateral to the red nucleus at its caudal pole. Consistent with the more rostral tegmental placements, labelled axons are present ipsilaterally in the pons and medulla, and are scattered in the ventral portion of the gigantocellular reticular formation. In P-479 (figure 19) and P-477 (figure 20) there is additional label over crossed rubral fascicles which can be accounted for by spread of the marker to the caudal red nucleus at the injection site. Labelled axons are also present, contralaterally, in the tegmentum adjacent to the raphe, corresponding to the position of labelled axons in case P-480. However, there is no evidence for marker transport to the olive in any of these cases, prompting us to suggest that tegmental areas surrounding the caudal red nucleus do not project to the olive. The negative anterograde results are substantiated by the lack of retrogradely labelled cells in caudal tegmental areas of the midbrain subsequent to multiple HRP deposits within the olive.
DISCUSSION

Axonal transport techniques were utilized in this study to reveal the organization of projections from the thalamus, subthalamus, and midbrain to the inferior olivary complex. Recent reports of degeneration studies (the cat, Walberg, '74; the opossum, Martin et al., '75) have emphasized the complexity of such connections and the authors in both instances realized the need for studies that would circumvent the fiber of passage problem. In this discussion, we will compare and contrast the current autoradiographic data with that previously obtained by degeneration techniques, as well as correlate the organization of midbrain-olivary connections with that of the projection from the olive to the cerebellar cortex.

Projections from the Ventromedial Midbrain

In a previous study (Henkel et al., '75) we showed that the interstitial nucleus of Cajal, the ventral periaqueductal gray, the nucleus linearis, and the tegmentum dorsomedial to the red nucleus are retrogradely labelled following HRP injections of the inferior olive (see figure 1). Since that earlier report, generally comparable results have been obtained in the cat (Bishop et al., '76) as well as in the rat and monkey (Brown et al., '76). The autoradiographic results described
herein demonstrate that the principal nucleus of the olive is the predominant target of axons from such areas. Comparison of the different cases (figures 2-5) suggests that the projection to the principal nucleus is topographically organized so that cells within the interstitial nucleus and the ventral periaqueductal gray relay rather evenly to both lamellae, while neurons within the tegmentum dorsal and medial to the red nucleus project mainly to the dorsal lamella. It is interesting to note that it is the tegmentum dorsal to the red nucleus in the opossum that receives cerebellar input from the nucleus lateralis (Martín et al., '74). Our results generally support those of Walberg ('74) who reported that certain lesions in the ventral periaqueductal gray of the cat result in degeneration in both lamellae of the principal nucleus and that the degeneration was heaviest in the ventral lamella. The red nucleus itself projects to the dorsal lamella in agreement with Edwards' ('72) results in the cat.

The accessory nuclei of the olive also receive a small input from some of the midbrain areas referred to above. It appears that neurons in or near the nucleus linearis relay to the caudal tip of the dorsal accessory nucleus and to subnuclei "a" and "b" of the caudal medial accessory nucleus, while neurons dorsal to the red nucleus (actually considered as part of the dorsolateral tegmentum described later) project to subnucleus "c" of the caudal medial accessory nucleus.

Lesions of ventromedial midbrain areas in the opossum, excluding the ventral periaqueductal gray (see figure 26, Martín et al., '75), yield extensive degeneration throughout the principal olive, as well as
within the rostral medial accessory and caudal dorsal accessory nuclei. It now appears clear, however, that the degeneration in the rostral medial accessory nucleus resulted from interruption of fibers passing dorsal and medial to the red nucleus from more rostral origins (see below). The lesion also encroached upon the nucleus linearis and closely adjacent cells, apparently accounting for the degeneration within the caudal dorsal accessory nucleus. The lack of degeneration in the caudal medial accessory nucleus after ventromedial midbrain lesions (figure 26, Martin et al., '75) is in contrast to the light labelling seen in subnuclei "a" and "b" in the present study (figure P-467). The negative results in the degeneration material may have been a result of inappropriate survival time, but in any case, the results reported herein serve as a testimonial to the sensitivity of the autoradiographic method.

It is apparent that the ventral periaqueductal gray, the interstitial nucleus of Cajal, the area dorsomedial to the red nucleus and the red nucleus itself project strongly to that portion of the olive, namely the principal nucleus, which relays to the cerebellar hemispheres (crus I, II and the paraflocculus), as well as to the paramedian lobule (Brodal, '40, Brodal et al., '75, Armstrong, '74, as well as Linauts and Martin, '77). Neurons within the nucleus linearis and/or adjacent areas exert an additional influence over olivary cells projecting to certain direct spinal zones of the cerebellum (the anterior lobe and the paramedian lobule, Brodal, '40, Armstrong, '74, Brodal and Walberg, '76, Brodal et al., '75, as well as Linauts and Martin, '77).
Projections from the Mesodiencephalic Junction

Our data indicate that cells of the nucleus of Darkšchewitsch, the subparafascicular nucleus, and the fields of Forel provide terminals within the principal and medial accessory nuclei of the olive. The nucleus of Darkschewitsch has often been mentioned as a possible source of olivary input and our case P-523 (figure 8) provides evidence that it projects to the principal nucleus, mainly its ventral lamella, as well as to the rostral half of the medial accessory nucleus, particularly subnuclei "a" and "b". Our findings are in agreement with the degeneration results of Walberg, '74, although he could not state with certainty whether it was the nucleus of Darkschewitsch or the adjacent central gray that sent fibers to the olive. Correlation of our earlier HRP data (Henkel et al., '75) with the present autoradiographic results indicates that both regions contribute to the projection.

Prior to our earlier report (Henkel et al., '75) the subparafascicular nucleus had not been implicated as a source of fibers to the olive, whereas the fields of Forel had been reported to provide terminals within the ventral lamella of the principal nucleus as well as the rostral medial accessory nucleus (Ogawa, '39; Mabuchi and Kusama, '70). The present autoradiographic results (figures 7 and 8) reveal that both nuclear areas project moderately to the principal nucleus and provide substantial input to intermediate parts of the medial accessory nucleus. Since axons from both nuclei pass dorsal and medial to the red nucleus, their interruption accounts for the heavy degeneration seen in the medial accessory nucleus in figure 26 of Martin et al., '75. The
lesion in figure 3 of Walberg's '74 paper appears to destroy cells in both regions and probably accounts for the degeneration seen in both the principal and medial accessory nuclei.

The regions of the olive which receive fibers from the nucleus of Darkschewitsch, the fields of Forel, and the subparafascicular nucleus project to Crus I and II, the paraflocculus, as well as to parts of the anterior lobe and paramedian lobule (Brodal, '40, Armstrong, '74, Linauts and Martín, '77). Since the ventral periaqueductal gray, the interstitial nucleus of Cajal, the red nucleus and the areas dorsomedial to it also project to some of the same olivary regions, considerable convergence must exist. This, together with the fact that axons from most of those regions end within olivary synaptic clusters (King et al., in preparation), suggests that complex informational processing takes place.

**Projections from the Dorsolateral Tegmentum and Deep Superior Colliculus**

Olivary connections from the midbrain tegmentum have been difficult to specify by degeneration techniques (Martin et al., '75; Walberg, '74; Mizuno et al., '73). It is obvious from the autoradiographic experiments, however, that cells of the midbrain tegmentum and deep tectum project to restricted portions of the dorsal and medial accessory nuclei, with no input to the principal nucleus (figures 9-10).

Neurons within the deep superior colliculus and/or closely adjacent tegmentum project to a part of the contralateral medial accessory nucleus referred to as the dorsolateral part of subnucleus "c". Other authors have referred to the apparently comparable region in other
species as subnucleus "b" (Frankfurter et al., '77). The dorsolateral part of subnucleus "c" also receives a projection from certain areas of the pontine reticular formation and relays specifically to visual-auditory areas of the cerebellar vermis (Martin et al., '77).

In contrast, tegmental neurons located deeper and/or more laterally project ipsilaterally to the ventromedial bend of the dorsal accessory nucleus, rostrally, and to the ventromedial part of subnucleus "c", caudally. Although degeneration was obvious in the comparable areas of the opossum medial accessory nucleus after tegmental lesions (Martin et al., '75), that within the dorsal accessory nucleus was not emphasized (see, however, figure 24, Martin et al., '75). The ventral extreme of the dorsal accessory nucleus (DAO) also receives input from the interpositus nucleus (the opossum, Martin et al., '75; the cat, Tolbert et al., '76), the cuneate nucleus (the opossum, Martin et al., '75; the cat, Boesten and Voogd, '75, Berkley and Hand, '76), and the spinal trigeminal complex (Berkley and Hand, '76: unpublished results in opossum). The spinal trigeminal and cuneate projections suggest that this region of the DAO is a point of convergence of somatosensory information, modulated by midbrain tegmental and interpositus inputs. The results of such processing are relayed specifically to Crus I and II of the cerebellar hemisphere (Brodal, '40, Armstrong, '74, as well as Linauts and Martin, '77). In addition to receiving input from the deep tegmentum of the midbrain, the ventromedial part of subnucleus "c" (the marsupial counterpart of the B-nucleus) also receives projections from the pretectal complex (Mizuno et al., '73, Graybiel et al., '73, Martin et al.,
'75 and present data), and relays to the nodule, pyramid, and uvula of the cerebellum (Alley et al., '75, Brodal, '76, as well as Linauts and Martín, '77).

In the cat, Walberg ('56, '60, '74) has provided degeneration results which may be explainable by our autoradiographic data. Although no degeneration was reported in the rostral part of the dorsal accessory nucleus after tegmental lesion, the distribution of injured fibers to the caudal medial accessory nucleus is in agreement with our findings. The degeneration in the B-nucleus of the caudal medial accessory complex (figure 3, Walberg, '74) is apparently a result of destroying tegmental cells adjacent to the central gray at caudal levels of the lesion (area of isotope injection in case P-480, figure 9 this report). Assuming a general inter-species comparability, the degeneration seen in the rostral medial accessory and principal nuclei of the cat (figures 3 and 4, Walberg, '74) can now be attributed to interruption of fibers originating in the mesodiencephalic junction.

Midbrain and thalamic projections to the olive are quite complex, and where comparisons can be made they appear to be comparable in the opossum and cat. Certainly data derived from both species speak to the various components of the so-called central tegmental tract as that term is used in primates and man. The complexity of the connections described herein is underscored by the interactions suggested from ultrastructural analysis (King et al., in preparation) as well as the highly ordered precision characteristic of olivocerebellar projections (Armstrong, '74, Brodal, '40, Brodal et al., '75a,b, Brodal, '76, Brodal and Walberg, '76,
Hoddevik et al., '76, as well as Linauts and Martin, '77). The significance of the connectional patterns described in this study to cerebellar function remains obscure and offers a challenge to the neurophysiologist.
Neuronal labelling subsequent to multiple injections of HRP into the inferior olive is illustrated in this figure (summarized from Henkel, Linauts and Martin, '75). In selected sections through the midbrain from rostral (A) to caudal (D), reactive neurons are identified ipsilaterally at the midbrain-diencephalic junction (A and B) in the subparafascicular nucleus (A, open block arrow), the adjacent nucleus Darkschewitsch (level A, corresponding area is indicated as Dk on opposite side), the interstitial nucleus of Cajal (level B, IFLM), the fields of Forel (level B, open block arrow), the caudal pretectal complex (level B, small solid arrow), as well as the tegmentum adjacent to the central grey (level B, arrow 4). At more caudal levels (C), reactive neurons are present in the deep tectum (CS), ventral periaqueductal grey (arrow 1), the tegmentum dorso-medial to the red nucleus (arrow 2), the nucleus linearis (arrow 3), and the dorsal tegmentum (arrow 4). At the level of the interpeduncular nucleus (level D, IP), only a few scattered neurons are reactive in the ventro-medial tegmentum.
Figure 1
Figure 2

The results of an injection of tritiated leucine into the region dorsomedial to the red nucleus are illustrated. The injection site is drawn in the upper right inset, where the stippling indicates the highest concentration of the marker and the crosses represent lighter labelling at the periphery of the injection site. In selected sections through the ipsilateral inferior olive (rostral-G to caudal-A), the dots represent silver grains in the emulsion overlying the section, indicating presence of transported protein in the axonal terminals. For the sake of clarity, the silver grains outside borders of the olivary nucleus are not indicated. The outlines of the inferior olivary nucleus, as well as the midbrain sections (injection site), are traced from a tri-simplex projection image. This same format of representation is used in all subsequent schematic illustrations unless otherwise specified.
Figure 2
Figure 3  An injection of $^3$H-leucine into the medial red nucleus and the tegmentum dorso-medial to it is drawn in the upper right inset. Terminal labelling is present throughout the rostral (G) to caudal (A) extent of the principal nucleus.
Figure 3
Figure 4: Drawing of transverse sections through the inferior olive ipsilateral to the injection of $^3$H-leucine into the red nucleus (inset), showing terminal label from rostral (G) to caudal (A).
Figure 4
Figure 5  Bilateral terminal labelling is indicated in the inferior olive (rostral E to caudal A) following injection of tritiated leucine into the midline region of the midbrain (inset).
Figure 5
Figure 6  Illustrated are the findings in case P-437, a tritiated leucine injection of the subparafascicular nucleus (inset). The resulting terminal labelling in the ipsilateral inferior olive (rostral pole at level G) is indicated as described for figure 2.
Figure 7  The ensuing terminal labelling subsequent to injection of $^3$H-leucine into the fields of Forel (inset) is illustrated in transverse sections through the ipsilateral inferior olive (rostral G - caudal A).
Figure 8  Illustrated is the bilateral terminal labelling in the inferior olive (from rostral E to caudal A) following isotope injection into the nucleus of Darkschewitsch and adjacent central gray (inset).
Figure 9

Drawing of transverse sections through the rostral (B) and caudal (A) olivary nucleus showing distribution of silver grains following tritiated leucine injections into the lateral (UPPER HALF, inset) and medial midbrain reticular formation (LOWER HALF, inset).
Figure 9
Figure 10  Illustrated are the results of a tritiated leucine injection into the tegmentum dorsal to the red nucleus (inset). Terminal labelling in the ipsilateral inferior olive from its rostral pole (E) to its caudal pole (A) is indicated as described for figure 2.
Figure 10
Figure 11  A low power photomicrograph of a Nissl stained section through the injection site in case P-469 (see figure 4) (4 week exposure).

Figure 12  Dark-field photomicrograph through the inferior olive showing terminal labelling subsequent to a tritiated leucine injection into the red nucleus (case P-469, figures 4 and 11). For orientation to the arrangement of the olivary nuclei, the inset shows a Nissl stained section at a closely comparable level.

Figure 13  Photographed at low power is a transverse section through the injection site in case P-467 (see figure 5).

Figure 14  Terminal labelling in the principal nucleus of the inferior olive following a midline injection in the midbrain (case P-467) as seen in dark-field illumination. The photomicrograph corresponds to the section illustrated in figure 5, level D on the reader's left (scale is same as in figure 12).
Figure 15  A low power photomicrograph of a Nissl stained section from case P-437 showing the injection site (open arrow) of tritiated leucine in the subparafascicular nucleus (SPF).

Figure 16  A dark-field photomicrograph of terminal labelling in the medial accessory nucleus ("a" and "b") subsequent to injection of tritiated leucine into the subparafascicular nucleus (figure 15). The micrograph corresponds to level C in figure 5 (scale is same as indicated in figure 18). For orientation, a Nissl stained section through the corresponding level of the olive is shown in the inset.

Figure 17  The injection site (arrow) from case P-480 (see also lower half, figure 8, inset) is shown in a low power micrograph. The marker labels cells of the midbrain tegmentum (Tgl) which correspond in position to the reactive neurons shown at arrow 4, figure 1-B and C.

Figure 18  Terminal labelling following injection of tritiated leucine in the midbrain tegmentum (case P-480, figure 17) is seen under dark-field illumination in the ventromedial part of subnucleus "c" (between arrows) of the caudal medial accessory olive (MAO). The micrograph corresponds to level A (left side) of figure 8, lower half. The inset is a Nissl stained section showing the caudal inferior olive for orientation.
Figure 19  Photomicrograph of Nissl stained section through the injection site (open arrow) in case P-479 (negative findings in inferior olive).

Figure 20  The injection site in case P-477 dorso-lateral to the red nucleus at its caudal pole is shown at low power (negative results in inferior olive).

Figure 21  Deposit of the isotope into the ventral tegmental area of Tsai (open arrow) is seen in this low power photomicrograph (negative results in inferior olive).
Figure 22  Schematic summarization of findings. COLUMN 1 shows the organization of the projection from the ventromedial tegmentum. The solid circles represent the injection site and terminal labelling in case P-467 (figure 5); the squares represent the area covered by the injection in cases P-417 (figure 2) and P-418 (figure 3); triangles indicate case P-469 (figure 4). COLUMN 2 illustrates the results of caudal diencephalic injections and the ensuing olivary terminal labelling. Solid circles represent case P-437 (figure 6); squares indicate case P-419 (figure 7); and triangles represent case P-523 (figure 8). COLUMN 3 shows the projection from the dorsolateral tegmentum to the inferior olive; case P-439 (upper half, figure 9) is represented by the circles, while the squares represent case P-480 (lower half, figure 9). The solid arrow in section A indicates the dorsolateral part of subnucleus "c" which is labelled on the contralateral side following the medial (triangles) injection.
Figure 22
PART II: THE ORGANIZATION OF THE OLIVO-CEREBELLAR
PROJECTION AS REVEALED BY RETROGRADE TRANSPORT
OF HORSERADISH PEROXIDASE.
INTRODUCTION

Cerebellar regulation of movement is dependent upon information obtained through both mossy and climbing fiber channels. Climbing fibers are particularly significant and have been shown to produce a strong depolarizing effect on the Purkinje cell, as well as an excitatory effect on Golgi cells, basket cells, and neurons within the deep cerebellar nuclei (see Armstrong, '74 for review). It has been suggested (Eccles, '77) that climbing fibers exert both trophic and plastic influences on the cerebellum which are important in determining overall function. Indeed, recent physiologic data provides evidence that during learning of motor tasks the CF activity can alter the firing pattern of the Purkinje cell in response to concurrent parallel fiber input (for review see Gilbert and Thach, '77).

Considering the multiple and diverse inputs to the inferior olive, the major source of climbing fibers (Grant, '70, Desclin, '74, Eccles et al., '66), the possibilities for informational integration before relay to the cerebellum are overwhelming. To establish a secure foundation for functional speculation, details of the olivo-cerebellar projection are essential.

Anatomical and physiologic data (Brodal, '40; Armstrong, '74) indicate that olivocerebellar connections are highly organized in the
cat. Since the map derived by electrophysiological methods was considerably more complex than that obtained by the retrograde degeneration technique, the Norwegian workers reinvestigated olivocerebellar circuits with the more sensitive HRP method. The results of their studies to date are generally in accord with those obtained by physiological means.

In our laboratory we have undertaken an HRP study of olivocerebellar projections in the North American opossum. Such connections have not been examined in any marsupial and the results should provide data of comparative interest, particularly since the opossum is often considered to be a generalized mammalian prototype. Of additional interest is the fact that the opossum is an excellent model for developmental studies (see Martin et al., '75 for review) and we intend to use it for future investigations of olivary development. Obviously, meaningful interpretation of developmental material necessitates some knowledge of the end point, i.e. the adult organization.
MATERIALS AND METHODS

Injections of 0.1–0.6 μl of a 50% suspension (wt/vol. saline) of horseradish peroxidase (Sigma type VI) were made in the cerebellar cortex of 29 adult opossums (2.5 kg average body weight). Following anesthesia by sodium pentobarbital (65 mg/kg body weight) the head of the animal was stabilized in a stereotaxic apparatus and a craniotomy was performed to expose the desired region of the cerebellum. Using a Hamilton syringe attached to a microdrive system, a 31 gauge needle was placed in the cortex by visual guidance, or in the case of ventral folia, by coordinates derived from our material and the atlas of Oswaldo-Cruz and Rocha-Miranda, '68. Injection of the HRP was carried out over a period of 20–40 minutes, and the needle was left in place for 10 minutes before and after the injection.

After a survival time of 24 hours, the animals were sacrificed under deep anesthesia by intracardiac perfusion. Their cardiovascular systems were flushed with a saline-procaine solution followed by a mixture of 1% paraformaldehyde - 1.25% gluteraldehyde in 0.1M phosphate buffer - 1% sucrose solution. The brains were removed immediately and post-fixed for 6 hours in the fixative followed by a 48 hour rinse in a 0.1M phosphate buffer - 30% sucrose solution (pH 7.4).
Serial sections were cut in the transverse plane (sagittal plane for the cerebellum in some cases) on a freezing microtome at 40μ and treated according to the method of Graham and Karnovsky (‘66). The sections were incubated in a solution of 3,3-diaminobenzidine tetrahydrochloride in tris buffer for 30 minutes, after which dilute hydrogen peroxide was added. The reacted sections were mounted and counterstained with cresyl violet. Examination of the sections for both spread of the marker in the cortex and the presence of reaction granules in olivary neurons was carried out in both light and dark field illumination.
RESULTS

In the following account the cases are grouped according to known functional and/or anatomical areas of the cerebellar cortex. The spinal areas of the opossum cerebellum are defined as the regions receiving axons from the spinal cord (Hazlett et al., '72), but our designation of the intermediate vermis as a visual-auditory area represents extrapolation from the feline literature. The terminology employed for the different areas of the cerebellum (see figure 1) was taken from the atlas of Oswaldo-Cruz and Rocha-Miranda, '68 (with some modification), whereas that used for the inferior olive is from Martin et al ('75).

THE SPINAL CEREBELLUM

(ANTERIOR LOBE, PARAMEDIAN LOBULE, PYRAMIS)

Anterior Lobe

Several cases were prepared with injections of the anterior lobe. Case P-485 (figure 2) was subjected to an injection of .3µl of HRP into the medial vermis. The darkest staining of all cortical layers is virtually restricted to the vermis on the left side and includes several adjacent folia of the culmen. Significantly lighter staining spreads within the intermediate zone of the anterior lobe on the side of the
injection as well as across the midline (figure 2, inset). Light stain is present also in the white matter over a considerable area of the anterior lobe and it can be followed to the deep nuclei.

Within the contralateral inferior olive, retrogradely labelled neurons are restricted to the accessory nuclei. Lightly labelled cells, discernable only under dark field illumination, are found in central and lateral regions of the dorsal accessory olive (DAO). Such cells are most numerous in the middle 1/3 of the rostral-caudal extent of the complex (figure 2, D), although some extend more caudally (figure 2, B-C). The heaviest labelling, however, is present in caudal parts of the medial accessory olive (MAO) where reactive neurons mainly occupy sub-nucleus "a" (figure 2, B-C). It can be noted from figure 2A that the caudal ends of both accessory nuclei are unlabelled. Although the marker spread across the midline, its concentration apparently is not heavy enough to result in labelling of the olive on the side of the needle track.

In case P-487 (figure 3), the injection (.3μl) labels all layers of several folia within the lateral culmen. There is some spread of the marker medially, but not to the vermal zone (figure 3, inset). The distribution of labelled neurons in the olive is similar to that seen after vermal injections, but obvious differences are present. Heavy labelling is found in the DAO, beginning at its rostral pole and continuing caudally for approximately 2/3 of its rostral-caudal extent (figure 3, C-F and figure 15). The reactive neurons are somewhat more medially placed than in the vermal case, but there is overlap in the central part of the
nucleus (compare section D in figures 2 and 3). Additionally, backfilled neurons are present in the MAO. They appear more rostrally than in the vermal case (figure 3, D), although they still occupy its lateral extreme (subnucleus "a") at intermediate levels (figure 3, C). Such cells are both more lightly labelled and less numerous than after injections of the anterior lobe vermis. A third area of labelling, not seen in the vermal case, is located within the dorsal lamella of the principal nucleus (DL-PO) (figure 3, D-E). The reactive neurons contained therein are few in number and so lightly labelled that they can be seen only under dark field illumination.

The injection of HRP (0.3μl) in P-489 (figure 4, inset) labels the cortex of the anterior lobe at a position intermediate to that in the cases just described. The injection is limited to the culmen and the spread of the marker encroaches only slightly upon the vermis and the lateral zone of the anterior lobe. The ensuing labelling of olivary neurons is distributed within the same general regions described for the previous cases. Heavily labelled cells are present, as they were in the lateral placement, in the rostral pole of the DAO (figure 4, F), and as that nucleus enlarges more caudally, they extend across much of the nucleus (figure 4, C-E). Only a small ventromedial part of the rostral DAO is free of labelling. Further caudally, reactive cells are still present in the lateral DAO (figure 4, B), although they do not extend to its caudal tip (figure 4, A).

Unlike the other anterior lobe cases, a few lightly labelled cells are seen in the medial tip of the rostral MAO (figure 4-D).
Slightly more caudally, in the intermediate 1/3 of the olivary complex, heavily labelled cells appear abruptly in the lateral part of the MAO, occupying mainly subnucleus "a" (figure 4-C). The number of cells and the intensity of their labelling decreases rather rapidly as the caudal 1/3 of the nucleus is approached (figure 4-B). There is no evidence for labelling in the principal nucleus at any level.

In one case (not shown) the preculmen was heavily labelled with no spread to the culmen and relatively little to the lingula. Reactive neurons are restricted to the accessory nuclei where they are most heavily labelled in the lateral extreme of the dorsal nucleus and in subnuclei "a" and "b" of the medial accessory complex. Lightly labelled neurons are present, however, within the central part of the dorsal accessory nucleus at rostral levels where they overlap with those which react after the injections described above.

Paramedian Lobule

The paramedian lobule of the opossum cerebellum consists of a large superior folium and a smaller inferior one. On the left side of case P-490 (figure 5, inset) an injection of .4μl of the enzyme resulted in labelling of all cortical layers in the medial 1/3 of the inferior folium, while a .3μl injection into the right side folium (figure 5, inset) labelled the cortex most heavily in the central part of the superior folium, with spread both medially and laterally. At rostral levels through the superior injection site (right side) there is spread of the enzyme to the inferior folium. As will be described below, each of the injections resulted in a different pattern of olivary labelling.
On the side opposite the injection of the superior folium heavily labelled neurons are abundant in the rostral half of the DAO and are confined to progressively more ventromedial parts of the nucleus as one moves from rostral to caudal (left side, figure 5, B-D). The medial accessory nucleus is also labelled, and the reactive neurons are located in the medial 2/3 (subnucleus "b" and part of the adjacent subnucleus "a") of the nucleus at intermediate levels of the complex (left side, figure 5, B-C). Restricted regions of the principal nucleus also contain back-filled neurons. A tightly clustered group of moderately reactive cells is seen in the rostral 2/3 of the dorsal lamella (left side, figure 5, C-E), while a relatively small number of faintly positive neurons is located in the medial portion of the ventral lamella (VL-PO) at approximately its middle third (left side, figure 5, C-D).

In the olive opposite the small injection of the inferior folium evidence for transported protein can be seen in lateral (right side, figure 5, D) and central (right side, figure 5, B-C) regions of the DAO, beginning at a level considerably caudal to that labelled on the opposite side. The relative position of HRP positive cells in the DAO can be appreciated best by comparing the two sides at level D of figure 5. Additionally, a few positive cells are found in the medial part (subnucleus "b") of the MAO at an intermediate rostral-caudal level (right side, figure 5, B-C). No positively labelled neurons could be discerned, even under dark field illumination, in either lamella of the principal nucleus.
In case P-460 (not illustrated) the injection is also within the inferior lobule, with only minimal light spread into the adjacent nodulus. The olivary labelling is similar to that just described, although some also is present in the dorsal lamella of the principal nucleus and the reactive cells within the MAO are located more laterally, within sub-nucleus "a".

Case P-459 (figure 6) contains a large placement (.6μl) which heavily labels both paramedian folia as well as parts of Crus II and the intermediate vermis. The distribution of reactive neurons in the opposite DAO covers the same rostral to caudal extent seen in P-490 (figure 6, B-E) and as would be expected, the labelled neurons are present over nearly the entire medial to lateral extent of the nucleus. Moderately reactive cells also are found in the rostral 2/3 of the dorsal lamella of the principal nucleus (figure 6, C-E) as well as in a restricted area of its ventral lamella (figure 6, D). The medial accessory nucleus, however, contains labelled neurons over a more extensive area than is the case after injections confined to the paramedian lobule alone (figure 6, A-E). The most heavily labelled cells are found at intermediate levels in the lateral 2/3 of the complex (figure 6, B-C), while the medial region at this level (subnucleus "b") is almost free of label. This is in contrast to case P-490 (figure 5, B-C), where it is the medial half of the MAO which contains positive cells, but it does correspond to the results obtained in case P-460 (described above). Labelled neurons are additionally seen in the rostral tip of the MAO (figure 6, E) as well as subnucleus "c" of the caudal MAO (figure 6-A).
As will be seen later such labelling can be explained by spread of the marker to Crus II (rostral MA0) and the paravermal zone of the auditory-visual vermis (subnucleus "c").

Pyramis

Case P-493 (figure 7) contains an injection which heavily stains all cortical layers of the pyramis and encroaches slightly upon the adjacent uvula. The latter labelling is most likely due to pulsations and respiratory movements during the injection (see Walberg, '76 for discussion of these problems). In the rostral olive of the opposite side, lightly labelled cells are seen in the medial portion of the VL-PO (figure 7, E-F). In addition, heavily labelled cells appear in central regions of the DA0 at the level shown in figure 7-E. Reactive DAO cells are located more medially at slightly more caudal levels (figure 7-D). Lightly reactive neurons are also present in two areas of the caudal MA0. One cluster bridges subnuclei "a" and "b" (figure 7, B-C) and a second group is located in the ventromedial part of subnucleus "c" (figure 7, B).

THE VERMAL VISUAL-AUDITORY AREA

Injections into visual-auditory areas of the vermis result in backfilling of neurons only in the caudal MA0. Case P-497 (top, figure 8) was subjected to a .3μl injection aimed at the folium and tuber (lobule VII). The reacted sections reveal spread of the marker to both
sides of the midline and for some distance in the rostral to caudal direction. The exact extent of the spread is difficult to determine, however, since the sections were cut in the transverse plane. In the inferior olive, there is nearly symmetrical, bilateral labelling of neurons in the caudal half of the MAO. Reactive cells are apparent most rostrally in the medial part of the nucleus, specifically subnucleus "b" and the dorsolateral part of "c" (top, figure 8-B and figure 17). Progressing caudally, however, another group of positive cells appears within subnucleus "a" (top, figure 8-A). At the latter level the most heavily labelled neurons are located in the dorsolateral part of "c" and within the lateral part of "a", although faintly labelled cells are scattered in subnucleus "b". Reactive neurons are present in the above regions to the caudal tip of the MAO. Additionally, on the side opposite the needle tract, several reactive neurons are present in the ventromedial part of subnucleus "c" (B-nucleus of the cat).

Case P-498 (lower half, figure 8) contains a smaller injection which is limited to the central parts of the vermal visual-auditory area. In the contralateral olive, heavily labelled neurons are present only in the dorsolateral part of subnucleus "c" (lower half, figure 8-B).

In another case (P-475, not illustrated) the enzyme was injected into the declive (lobule VI) and staining of the cortex is restricted to a very narrow zone slightly to the left of the mid-line. However, in transverse sections, it is difficult to ascertain the precise rostral to caudal spread of the marker. In any case, olivary labelling (contralateral only) is located mainly within the dorsolateral part of
subnucleus "c" as described above with some spread into the adjacent
subnucleus "b" (corresponding to lower figure 8-B). As in P-498 there
are no reactive cells visible in the lateral part of the MAO. Labelling
in the latter region evidently is dependent on cortical staining of more
posterior folia.

THE UVULA

The results of a small injection into the uvula (lobule IX) are
illustrated for P-419 in figure 9. The diffusion of the marker is
limited to cortical layers of the uvula (figure 9, inset). Within the
rostral half of the olive, only a few lightly labelled cells are present
in the ventral lamella of the principal nucleus (figure 9-C). Within
its caudal half, however, reactive neurons are localized in two areas of
the MAO. One cluster of moderately labelled neurons occupies a position
in subnucleus "a" (figure 9-B), while slightly more caudally, reactive
cells are also located in the ventromedial part of subnucleus "c"
(figure 9-A).

THE FLOCCULONODULAR LOBE

Injections of HRP which involve the flocculonodular lobe result
in backfilling of cells in several circumscribed regions of the olive.
Case P-543 (top, figure 10) contains an injection which is centered in
the white matter between the hemisphere and vermis. However, the marker
spread to cortical layers of the flocculus, as well as to Crus I, making it useful for our purposes. Moderately reactive neurons are found throughout the contralateral cap of Kooy (top, figure 10, A-B) as well as within the dorsal lamella of the principal nucleus (top, figure 10-C). Comparison with case P-508 and P-511 (figures 11 and 13, a Crus I injection with no involvement of the flocculus) leads us to believe that the backfilled cells in the PO most likely incorporated the marker from the cortex of Crus I.

Following deposit within the nodulus (bottom, figure 10, right), with some spread to the adjacent uvula, heavily labelled neurons are present both in the rostral tip of the MAO (bottom, figure 10-C) and the ventromedial part of subnucleus "c" caudally (bottom, figure 10-A and figure 18). Additionally, lightly reactive cells are evident in central portions (subnucleus "b") of the caudal MAO (bottom, figure 10-A).

THE HEMISPHERES
(CRUS I, CRUS II, LOBUS SIMPLEX, PARAFLOCCULUS)

Crus I and II, Lobus Simplex

Several cases were prepared with injections into Crus I and II of the hemispheres. In case P-508 (figure 11, inset) .3μl of HRP labels the lateral half of Crus I, with very little spread caudally into Crus II. There is no evidence for diffusion of the marker to the paravermal zone or to the paramedian lobule. In the contralateral olive, reactive cells are identified in the rostral half of all major divisions, with
the heaviest labelling located rostrally and diminishing in intensity as
the midpoint of the complex is approached. Reactive cells are found
throughout the rostral tip of the DAO (figure 11-F), but in progressively
more caudal sections they occupy a rapidly diminishing area which becomes
limited to its ventromedial extreme (figure 11, D-E and figure 16). Cells
with abundant reaction product are also present throughout the rostral
half of the principal nucleus (figure 11, C-F and figure 16), with the
greatest intensity at the level shown in figure 11-E. Additionally,
neurons are lightly to moderately labelled in subnucleus "a" of the
rostral MAO (figure 11, D-E).

In another case (P-520, not illustrated) the injection covers
essentially the same area as in P-508. Olivary neurons are backfilled
in all of the regions identified above, but in addition, some are
labelled in subnucleus "c" of the caudal MAO. The additional labelling
may be accounted for by light spread of the marker to the nucleus
fastigius.

Case P-504 (figure 12) contains an injection which stains the
cortex of lateral Crus II (inset, figure 12). The distribution of back-
filled neurons within the contralateral olive is similar to that de-
dscribed for Crus I (figure 11, P-508), although it is not as widespread
and individual cells are not as heavily labelled. A few HRP positive
neurons are present in the ventromedial extreme of the rostral DAO
(figure 12, D-F) and within subnucleus "a" of the rostral MAO (figure 12,
D-E). Within the principal nucleus labelled neurons are located in two
restricted regions. The first region is located in the rostral half of
the dorsal lamella (figure 12, C-E), while the second is found in a more restricted zone of the rostral ventral lamella (figure 12, D-E).

Results similar to those described above are seen in several cases where the injection was made into the paravermal zone and case P-511 (figure 13) serves as an example. As in the previous cases, heavily labelled cells are present in the ventromedial region of the rostral DAO (figure 13, D-F), and lightly labelled cells are located in the rostral portion of the VL-PO (figure 13, F). In another similar case (P-541, not illustrated), positive cells are also present in the dorsal lamella at the level where the ventral lamella is labelled in figure 13-F. In contrast to lateral injections of the hemisphere, however, paravermal deposits label additional cells in intermediate and caudal portions of the MAO. Such cells are located in central portions (subnuclei "a" and "b") of the MAO at levels shown in figure 13, B-D, although more caudally (figure 13, A) they occupy subnucleus "c" and the caudal tip of the DAO.

The rostral most folium of the hemisphere, the lobus simplex, was injected in one case (P-476, not illustrated) and the reacted sections reveal that only the ventral lamella of the principal nucleus is labelled.

The Paraflcoccus

Injections of HRP into lateral and medial paraflcoccus of separate animals produced quite different results from those obtained after deposits within Crus I and II. In the case with the lateral placement (top, figure 14) retrogradely filled neurons are located only in the
rostral portion of the MAO (top, figure 14, B–C). In contrast, the medial placement (bottom, figure 14) resulted in neuronal labelling which was most heavy in the PO, specifically the lateral half of its ventral lamella (bottom, figure 14, A–C). Only a few lightly labelled cells are found in the rostral MAO (bottom, figure 14, C). Labelling is not present in the caudal half of the olive in either case.
DISCUSSION

The horseradish peroxidase method (Kristensson and Olson, '71, LaVail and LaVail, '72) was utilized to study olivo-cerebellar projections in the North American opossum (Didelphis virginiana). In the following discussion we will analyze our data as they relate to those obtained from other species (as well as other techniques) and summarize briefly the various afferent connections of the olive in terms of olivo-cerebellar connections.

THE SPINAL CEREBELLUM

Anterior Lobe

The anterior lobe of the cat cerebellum has been divided into vermal and intermediate zones, each with its own characteristic projection from the olive (Brodal, '40). Within the vermis, a medial and a lateral zone are further distinguished. Anatomical data obtained by the HRP method in the cat indicate that the medial-vermal zone receives input from the caudal MAO, while the lateral one obtains projections from the lateral DAO (Brodal and Walberg, '76). The present results suggest that olivary projections to the anterior lobe of the opossum are organized in a similar fashion. In the most medial vermal injection
(figure 2), the heaviest labelling is found in the caudolateral MAO, although neurons in the dorsal accessory nucleus are lightly reactive. It is assumed that cells which are labelled most heavily are those which have terminals at the center of the injection site (medial vermal zone), while the labelling of neurons in the DAO may be a result of enzyme incorporation at the lateral edge of the stained area (lateral vermal zone). In such a way, our data indirectly supports the conclusion of Brodal and Walberg ('76) that spatially separate populations of olivary neurons project exclusively to a single zone of the vermis. At the same time, however, our results corroborate those obtained by electrophysiological methods in the cat which indicate that at least a proportion of the neurons projecting to the two zones of the vermis are co-extensive within the accessory nuclei (Armstrong, '74). The small size of the opossum cerebellum, coupled with the limits of the HRP technique (see Walberg et al., '76), precludes a definitive statement concerning this question.

Following injections into the intermediate anterior lobe, labelled neurons are located more medially in the DAO as well as more rostrally in the MAO (figure 4, D). Such results are in excellent accord with those reported by Brodal and Walberg, '76. Furthermore, the most lateral zone of the anterior lobe is indicated by both Armstrong, '74, and Brodal and Walberg ('76) as receiving terminals from the dorsal lamella of the PO, and concordantly, it is only after lateral placements that reactive cells are found in the comparable region in the opossum (see figure 3, D-E). Contrary to the physiologic data,
however, the HRP method does not reveal a projection in either species from the ventral lamella of the principal nucleus to the anterior lobe-intermediate zone.

There appear to be differences in the opossum and cat as regards the anterior lobe projections of the caudal MAO. All of our cases (figures 2-4) show some degree of neuronal labelling in lateral portions of the MAO at intermediate and caudal levels. In the cat only the medial vermis receives input from the apparently comparable region (Brodal and Walberg, '76). This may reflect a species difference, but it must be borne in mind that the arrangement of olivary nuclei is somewhat different in the opossum and cat (Martin et al., '75).

It is of interest to consider the afferent connections of those parts of the olive which relay to the anterior lobe. The spinal cord, especially lumbar levels, projects strongly to the lateral DAO (cat, Boesten and Voogd, '75, Worden and Berkley, '75; the opossum, Martin et al., '75), as does the gracile nucleus (the cat, Boesten and Voogd, '72, Berkley and Hand, '76; the opossum, Martin et al., '75). In contrast, more medial parts of the DAO receive input from the cuneate nucleus (the cat, Boesten and Voogd, '75, Berkley and Hand, '76; the opossum, Martin et al., '75). In simplistic terms, this arrangement suggests a "hindlimb dominated" channel via the lateral DAO to the vermis and a "forelimb pathway" to the intermediate anterior lobe by way of the medial DAO. It should be remembered, however, that the anterior lobe is also organized so that hindlimb stimulation activates the lingula and preculmen, while forelimb stimulation results in
evoked potentials from the culmen (e.g. Adrian, '43, Snider and Stowell, '44, Combs, '54). In that regard, it is interesting that the part of the dorsal accessory nucleus (lateral) which receives input from the caudal cord and nucleus gracilis relays heavily to the preculmen, and the region which receives cuneate fibers (medial DAO) projects to the culmen.

Axons from the caudal division of the spinal trigeminal complex project to the ventromedial extreme of the dorsal accessory nucleus (unpublished data in the opossum and Worden and Berkley, '75 in the cat) where they appear to overlap somewhat with input from the nucleus cuneatus (Martin et al., '75). Neurons in the same area backfill after certain injections of the anterior lobe and the superior folium of the paramedian lobule as well as after placements into Crus I and II. One is tempted to speculate that the latter regions contain zones which are responsive to face input via climbing fiber channels.

Caudal areas of the MAO which project to the anterior lobe receive their inputs from a number of sources. Not surprisingly, there are spinal projections from lumbar and cervical levels (e.g. the cat, Boesten and Voogd, '75, Worden and Berkley, '75; the opossum, Hazlett et al., '72, Martin et al., '75) and from the lateral cervical nucleus (the cat, Worden and Berkley, '75, Mizuno, '66) as well as input from the dorsal column nuclei (the opossum, Martin et al., '75 and the cat, see summary by Boesten and Voogd, '75). In addition, parts of the same nucleus receive afferent connections from the caudal spinal trigeminal complex (opossum, unpublished observations; the cat, Worden and
Berkley, '75), the nucleus of the tractus solitarius (the opossum, unpublished results; the cat, personal communication from Burton and Loewy of Washington University, Saint Louis) as well as projections from the reticular formation of the caudal brainstem (Martin et al., '77) and midbrain (Linauts and Martin, '77). Although there also is such extensive overlap of inputs to parts of the caudal MAO (subnuclei "a" and "b" of Martin et al., '75), the afferent connections of the DAO are more segregated and limited (Martin et al., '75, Linauts and Martin, '77).

It is possible that the type of information relayed to the spinal cerebellum from the two nuclei is quite different.

Intermediate rostral-caudal levels of the MAO also project to the anterior lobe, but they receive little spinal input (Martin et al., '75). Rather, they receive a strong projection from the subparafascicular nucleus of the thalamus and from the fields of Forel (Linauts and Martin, '77).

The dorsal lamella of the PO projects to the lateral-most anterior lobe and is a major target of fibers from both ventromedial midbrain and diencephalic regions, particularly from the red nucleus and adjacent areas (see Linauts and Martin, '77 for details).

Recent data indicate that the olive receives a highly ordered feedback from the deep cerebellar nuclei. The fibers to the dorsal accessory nucleus arise mainly (the opossum, Martin et al., '76), if not exclusively (the cat, Tolbert et al., '76), from the anterior interpositus nucleus, whereas those to the medial accessory nucleus take origin with the interpositus posterior (to intermediate rostral-caudal levels;
Martin et al., '76, Tolbert et al., '76) and fastigial nuclei (to the caudal MAO, Martin et al., '76). The projection from the fastigial nucleus to the caudal MAO is limited in its extent and density (Martin et al., '76). In contrast, the principal nucleus receives a strong input from the lateral nucleus of the cerebellum (Martin et al., '76, Tolbert et al., '76). Electron microscopic studies (King et al., '76) reveal that the deep cerebellar axons terminate within the olivary synaptic cluster (glomerulus) where their influence is integrated with input from midbrain thalamic regions (King, '77, in preparation). Interestingly, however, direct spinal input overlaps regionally with the cerebello-olivary input, but terminates on dendritic shafts, not in glomeruli (King et al., '75).

The Paramedian Lobule

The present results reveal that the paramedian lobule receives input from essentially the same olivary regions which also project to the anterior lobe, supporting the concept of divergent olivo-cerebellar connections. It is apparent that there are distinct differences in the area of the DAO which project to superior or inferior regions of the paramedian lobule. As also shown by Brodal et al., '76, and Armstrong, '74, the lateral half of the DAO projects to the inferior (caudal) region, while its medial half projects to the superior (rostral) folium. With regard to the other regions of the olive which relay to the paramedian lobule, a topographical difference also was evident. Although both lamellae of the PO are labelled following injection of the superior
folium, the two cases with inferior folium injection reveal differing results. In one (P-490, figure 5) no labelled cells are present in the PO, while in the other (P-460, not illustrated), a few reactive neurons are present in the DL-PO. These results are supportive of those reported for the cat (Brodal et al., '76) which suggest that the caudal 1/3 of the paramedian lobule (which we believe to be equivalent to the inferior folium of the opossum) receives input from the DL-PO, but not from the VL-PO.

The medial accessory nucleus, specifically its medial 2/3 at intermediate rostral-caudal levels, projects to the superior folium while the lateral 1/3 of the MAO at the same level projects to the inferior folium. The same arrangement is seen in figure 7 of Brodal et al., '76. The electrophysiological map of Armstrong, '74, indicates four separate receiving zones in the paramedian lobule (essentially its rostral 2/3 only) and the areas of the olive projecting to these zones are closely comparable in their total extent to the areas identified by the HRP method (Brodal et al., '76, present report).

The regions of the olive which project to the paramedian lobule receive input from the spinal cord (Boesten and Voogd, '75, Worden and Berkley, '75, Hazlett et al., '72) and dorsal column nuclei (Boesten and Voogd, '75, Berkley and Hand, '76, Martin et al., '75). Since in the cat at least, the paramedian lobule is organized so that the inferior (caudal) region responds to hindlimb stimulation and the superior (rostral) area to forelimb and face manipulation (see for example Combs, '54), it is not surprising that their inputs from the accessory nuclei of the
olive are topographically organized. As mentioned before, the principal
nucleus which relays to the paramedian lobe obtains most of its projec-
tions from the midbrain (Linauts and Martin, '77). Again, the olivary
nuclei relaying to the paramedian lobule are the recipients of organized
feedback loops from the cerebellar nuclei as described for the anterior
lobe.

The Pyramis

The pyramis, the third division of the spinal cerebellum (Hazlett
et al., '72), receives input from two regions of the olive which also
supply other spinal areas. One region is present in the medial half of
the DAO and it also projects to the intermediate anterior lobe and the
superior (rostral) paramedian lobule. The second is located within the
ventral lamella of the principal nucleus and it relays also to the para-
median lobule. Although cell labelling is present in the caudal MAO
after pyramis injections, comparison with case P-519 (uvula, figure 9)
suggests that it may result from spread of the marker to the uvula.
Currently, there is no HRP data on olivary projections to the pyramis
in the cat, and it is difficult to interpret Armstrong's ('74) drawing
with respect to the pyramis.

Sources of input to the medial DAO have been discussed with the
anterior lobe. The ventral lamella of the PO specifically receives in-
put from several midbrain and caudal thalamic regions, including the
nucleus of Darkschewitsch, the interstitial nucleus of Cajal, the fields
of Forel, and the nucleus linearis (and/or closely adjacent cells)
(Linauts and Martin, '77).
VERMAL VISUAL-AUDITORY AREA

A topographically organized projection from the caudal MAO to the vermal visual-auditory area exists in the opossum and it appears similar to that reported in the cat (Hoddevik et al., '76, Armstrong, '74). Briefly, the decline (lobule VI in the cat) receives most of its input from central regions of the caudal MAO (subnucleus "b" of the opossum). The folium-tuber (lobule VII) obtains its fibers exclusively from the caudomedial MAO (dorsolateral "c" in the opossum), and the posterior-most vermis (lobule VIII) receives its major projection from the lateral extreme of the MAO (subnucleus "a") (compare our figure 8 with Hoddevik et al., '76 - figure 5).

It has been established that the above regions of the vermis respond to visual and auditory stimuli (Snider and Stowell, '44) and that their stimulation results both in eye movements (Ron and Robinson, '73) and turning of the head (Hampson, '49). Since the deeper layers of the superior colliculus respond to both visual and auditory stimuli (cat, Stein and Arigbede, '72; monkey, Gordon, '73, Updyke, '74), it is probable that the visual-auditory properties of the vermis are generated by tectal projections to those parts of the pontine grey, reticulotegmental nucleus, and the inferior olive which project to the region of the cerebellum in question (e.g. Frankfurter et al., '77, Harting, '77).

With regard to the olive specifically, the superior colliculus distributes to precisely that part of the olive (dorsolateral part of subnucleus "c" in the opossum, and subnucleus "b" in the monkey) which
relays to the center of the visual-auditory vermis (Frankfurter et al., '77, Linauts and Martin, '77). It is of interest that the nucleus gigantocellularis and/or the nucleus gigantocellularis pars ventralis of the caudal brainstem (terminology for opossum from Oswaldo-Cruz and Rocha-Miranda, '68) projects to the same olivary target (Martin et al., '77) and both regions receive input from the superior colliculus (Martin, '68) as well as the underlying tegmentum (Linauts and Martin, '77).

The significance of projections to the so-called visual-auditory vermis from subnuclei "a" and "b" is not as readily apparent. As mentioned previously, subnuclei "a" and "b" receive projections from the spinal cord and the dorsal column nuclei (Martin et al., '75, Boesten and Voogd, '75, Worden and Berkley, '75), the caudal spinal trigeminal nucleus (opossum, unpublished results; the cat, Worden and Berkley, '75), the nucleus of the tractus solitarius (unpublished results, and communication from Burton and Loewy of Washington University, St. Louis) and certain parts of the brainstem reticular formation of the opossum (Martin et al., '77). Subnuclei "a" and "b" also relay to known spinal regions of the cerebellum. However, since the parts of the vermis in question are at the anterior and posterior edges of the visual-auditory vermis and thus close to known spinal regions (anterior lobe and pyramis) it may be that they also have some "spinal" properties. It should be emphasized, however, that the heaviest labelling of cells in the olive following intermediate vermal injections of HRP is found in the dorso-lateral part of subnucleus "c", a region which does not appear to receive spinal information (Martin et al., '75).
THE UVULA

The regions of the olive which project to the uvula in the opossum (figure 9) appear to be generally comparable to those which distribute to the comparable area in the cat (Brodal, '76). In the opossum, such neurons are found in the VL-PO, the lateral MAO at intermediate levels, and the ventromedial part of subnucleus "c" of the MAO. Brodal, '76, emphasized especially the B-nucleus of the cat (the region comparable to the ventromedial part of "c" of the opossum) as well as the dorsal-median cell column, while physiologic data in the same species implicate only the caudal MAO (Van Gilder and O'Leary, '70).

Functionally, the uvula has been shown to respond to stimulation of the retina and the optic chiasm (Simpson et al., '74). It is probable that visual information reaches the uvula by way of projections from pretectal regions to the ventromedial part of subnucleus "c", the counter-part of the feline B-nucleus (Martin et al., '75, Linauts and Martin, '77). Our material also provides evidence that the ventromedial part of subnucleus "c" receives input from the midbrain tegmentum (Linauts and Martin, '77) and it is of interest that this midbrain region is a target of both tectal (Martin, '69) and pretectal fibers (unpublished results in the opossum; see also Berman, '77 in the cat).

The VL-PO receives input from midbrain and thalamic regions (Linauts and Martin, '77) and in addition to projecting to the uvula, it also sends fibers to the paramedian lobule (Brodal et al., '76; present results), the pyramids (current data) and the hemispheres (present
results; Armstrong, '74). The lateral MAO receives descending afferents, especially from the subparafascicular nucleus and the fields of Forel (Linauits and Martín, '77) as well as ascending spinal and dorsal column nuclei projections (opossum, Martin et al., '75; cat, Boesten and Voogd, '75, Worden and Berkley, '75). It in turn projects to parts of the anterior lobe, the paramedian lobule, the posterior vermis, the uvula, and the hemispheres.

The above data serve to reinforce Armstrong's ('74) conclusion that considerable divergence exists in olivocerebellar projections. It could be argued that there are specific populations of neurons within any one olivary subnucleus which project to widely separate targets, but comparison of the amount of neuronal labelling in the different experiments suggest that is not the case. Although the backfilling of cells in several different olivary subnuclei after single HRP placements suggests convergence in one sense, it may be that axons from each subnucleus occupy a separate zone at the injection site.

The Flocculonodular Lobe

Our material indicates that the cap of Kooy in the opossum distributes axons to the flocculus. Such results are in accord with those of Alley and co-workers in the rabbit (Alley et al., '75) who reported evidence for a climbing fiber pathway to the flocculus via a link involving the cap of Kooy. The labelled cells seen in the principal nucleus after flocculus injection may be due to spread of the HRP to Crus I (see, however, Brodal, '40).
Although evidence for a pretectal projection to the dorsal cap of Kooy is still controversial (compare Mizuno et al., '73, Frankfurter et al., '77, Maekawa and Simpson, '73, Berman, '77), it is generally agreed that there is a pathway which conveys visual information to the flocculus (Alley et al., '75). Our material (Linauts and Martin, '77) is inconclusive relative to the origin of midbrain afferents to the cap of Kooy although a projection appears to exist (Martin et al., '75).

In the opossum, the ventromedial part of subnucleus "c" backfills after HRP placement limited mainly to the nodule, confirming the report of Alley et al., '75, indicating an apparently comparable projection in the rabbit. A word of caution is in order, however. Since the uvula also receives input from the ventromedial part of subnucleus "c" (present results, Brodal, '76), and the marker spread to the uvula in our nodule placements, it is possible that the ventromedial "c" projects to the uvula, but not the nodule (Alley also reports the same problem in the rabbit). Comparison of the extent of labelling in the olive following uvula (figure 9) and nodulus (lower half, figure 10) injections, however, suggests rather strongly that both areas receive input from subnucleus "c" (B-nucleus). Furthermore, responses to visual stimuli can be recorded in both the nodule (Maekawa and Simpson, '73) and the uvula (Simpson et al., '74). Possible sources of visual input to the ventromedial part of subnucleus "c" include the pretectum and the midbrain tegmentum (see discussion of uvula results). In the opossum and the rabbit the rostral MAO also provides input to the nodule as suggested for the cat by Brodal ('40).
Crus I and II

Horseradish peroxidase studies of olivary projections to the cerebellar hemispheres have not been reported to date. Our material indicates that the principal nucleus of the opossum olive projects heavily to both Crus I and II, and that rostral parts of the dorsal and medial accessory nuclei also contribute. Such results are more supportive of the physiologic data (Armstrong, '74) than they are of those obtained by the retrograde degeneration method (Brodal, '40). Armstrong does not show the dorsal accessory nucleus as projecting to the lateral hemisphere, but he does indicate a contribution from the medial accessory complex. Our injections of the intermediate zone produce a different pattern of olivary labelling than those which are more laterally situated, providing possible verification for the longitudinal zones illustrated by Armstrong, '74.

The regions of the olive projecting to Crus I and II receive strong projections from ventromedial midbrain areas as well as certain thalamic and subthalamic regions (Martin et al., '75, Linauts and Martin, '77). In addition, that part of the dorsal accessory nucleus which relays to both crura receives input from the nucleus cuneatus (Martin et al., '75, Boesten and Voogd, '75, Berkley and Hand, '76) and the spinal trigeminal complex (Berkley and Hand, '76; and unpublished data in the opossum). It should be remembered that the part of the dorsal accessory nucleus in question also provides fibers to the
paramedian lobule and pyramis and that the principal nucleus distributes
axons to the lateral anterior lobe, the paramedian lobule and the pyra-
mis, as well as Crus I and II. Physiologic studies are necessary to
shed light on the possible meaning of such an extremely complex organiza-
tion.

The Paraflocculus

To our knowledge, HRP studies of olivary projections to the para-
flocculus have not been reported for any species. Injections in the
opossum paraflocculus (figure 14) revealed a sharp difference in olivary
inputs to lateral and medial zones. Following a lateral injection,
labelled neurons are found only in the rostral MA0. In contrast, the
injection in the medial half of the paraflocculus resulted in neuronal
labelling in the lateral part of the ventral lamella of the principal
nucleus. The labelled cells in the principal nucleus are in approxi-
mately the same area which contains reactive cells after paraflocculus
ablation (Brodal, '40). Each of the above regions receives a strong
input from the midbrain and project also to regions of the cerebellum
distant from the paraflocculus.
CONCLUSIONS

The retrograde transport of horseradish peroxidase reveals an extremely complex organization of olivo-cerebellar projections in the opossum. Where comparisons can be made (the anterior lobe, the paramedian lobe, the pyramis, the uvula, the visual-auditory cerebellum and the flocculonodular lobe) olivo-cerebellar circuits of the marsupial opossum and those of certain placental mammals (cat, rabbit) are similar.

Collaterals of climbing fibers were reported in Golgi studies (Fox, '69), and suggested by electrophysiologic data (Armstrong, '74). The horseradish peroxidase technique further supports the presence of collaterals and reveals that specific nuclei of the olive relay to several areas of the cerebellar cortex which, in some cases, are widely separated (e.g. dorsal accessory nucleus projections to both the anterior lobe and the pyramis). These results provide anatomical collaboration for the degree of divergence suggested by Armstrong ('74).

On the other hand, our results also indicate that convergence may exist. Axons from spatially separate subnuclei of the olive project to apparently single functional or anatomical areas of the cerebellar cortex. For example the dorsal and medial accessory nuclei both project to the anterior lobe. Such anatomical data, however, do not constitute
unequivocal evidence for functional convergence, as axons from different subnuclei may distribute to selective longitudinal zones within a given region (Groenewegen and Voogd, '77, Armstrong, '74).

It is clear that the exchange of information between the inferior olive and the cerebellar cortex is of a highly integrated and specifically organized nature. The presence of synaptic clusters and gap junctions (King, '76, Llinas et al., '74) in the olive also suggests that a high degree of modulation is possible within the olive before information is even relayed to the cerebellum. As Brodal ('76) so concisely stated, "the complexity in the anatomical and functional organization of the inferior olive appears to be overwhelming". Clearly more physiological investigations are necessary to elucidate the meaning of the highly ordered climbing fiber input to the cerebellar cortex.
ILLUSTRATIONS
To the left is a photograph of the brain of an opossum, while on the right is a slightly enlarged drawing of the same, focusing on the divisions of the cerebellum. The open block arrow indicates the primary fissure, separating the anterior lobe from the posterior lobe. The solid arrow indicates the secondary fissure, separating the visual-auditory vermis from the pyramis. The flocculonodular lobe and the preculmen are not visible in this dorsal view.

Abbreviations:

- Cn  =  culmen
- Cr. I  =  crus I
- Cr. II  =  crus II
- Dcl  =  declive
- Fol  =  folium
- PF1  =  paraflocculus
- PMi  =  paramedian lobule inferior
- PMs  =  paramedian lobule superior
- PyC  =  pyramis
- Tbr  =  tuber
- Uv  =  uvula
Figure 2  

The results of an HRP injection into the medial anterior lobe vermis are illustrated. In the inset, the upper drawing is a transverse section through the injection site, where the black area indicates the heaviest concentration of HRP while the stippled gray represents spread of the marker at a lighter concentration. Very light spread of the marker in the white matter is not indicated. The lower figure in the inset shows the area of cortical labelling transposed to a drawing of the gross cerebellum. The white dot in the injection site represents the point of entry of the needle. In selected sections through the olive contralateral to the injection site (rostral-F, caudal-A), reactive neurons are indicated as dots. This same format of representation is used for all subsequent schematic illustrations unless otherwise specified.
Figure 3  Reactive neurons in the contralateral inferior olive are indicated (rostral-F to caudal-A) subsequent to an injection of HRP into the lateral anterior lobe (inset).
Figure 4

The injection site for case P-489 is drawn in the inset. In transverse sections through the contralateral olive from rostral (F) to caudal (A), the distribution of reactive neurons is indicated by the dots.
Figure 4
Figure 5

The results of a double injection of HRP are illustrated (made possible by the complete crossing of olivo-cerebellar axons). In the inset, the injection site is drawn in the inferior paramedian folium on the left and in the superior paramedian folium on the right side. The location of HRP positive cells resulting from the inferior folium injection is drawn on the right side of the midline, while the reactive neurons with terminals in the superior folium are shown on the left side.
Figure 5
Figure 6  The injection site of HRP into both folia of the paramedian lobule is drawn in the inset and the ensuing olivary labeling in the contralateral olive is shown from rostral (E) to caudal (A).
Figure 6
Figure 7  Following injection of HRP into the pyramid (inset), the resulting olivary labelling is indicated in select transverse sections (rostral F - caudal A) of the contralateral olive.
Figure 7
Figure 8 The results of HRP injections into the visual-auditory region of the posterior vermis are illustrated. In the upper half (P-497) the injection spreads over a wide area (stippled region on transverse and dorsal cerebellar drawings). Case P-498 (lower half) is a more limited injection as indicated in the sagittal and dorsal views. In both cases, the caudal half of the olive is drawn bilaterally, with the most caudal level at A.
Figure 8
Figure 9  The location of reactive neurons in the contralateral olive is drawn at rostral (C) and caudal (A) levels subsequent to injection of HRP into the uvula. The injection site is shown in sagittal and dorsal views in the right half of the drawing.
Figure 9
Figure 10  Olivary labelling following HRP injections in the vesti-bulo-cerebellum is illustrated by two cases. The upper half (P-543) shows the limited distribution of reactive neurons following staining of the flocculus. In case P-539 (lower half of figure) the injection of HRP stained the cortex of the nodulus, and the bilateral labelling is shown in the olive at rostral (C) to caudal (A) levels.
Figure 11 In transverse sections through the contralateral inferior olive (rostral F to caudal A), the location of reactive neurons is indicated subsequent to HRP injections into the lateral portions of Crus I (inset).
Figure 11
The distribution of HRP labelled neurons in the olive is illustrated following injection of the enzyme into the lateral part of Crus II (inset). Transverse sections of the olive are drawn from rostral (F) to caudal (A).
Figure 13  The results of an injection of HRP into the medial hemisphere (inset) are shown in transverse sections through the contralateral inferior olive from its rostral (F) to caudal (A) poles.
Figure 13
Figure 14  Differential labelling of olivary neurons following injections of the lateral (P-513) and medial (P-545) paraflocculus is illustrated in this drawing.
Figure 15  Dark-field photomicrograph of the inferior olive (corresponding to level D of figure 3) showing labelled neurons in the contralateral dorsal accessory nucleus (d) following HRP injection in the lateral anterior lobe (inset). For orientation, refer to inset in figure 16. Scale is same as indicated in figure 17.

Figure 16  Dark-field photomicrograph of reactive neurons following injection of HRP into Crus I (see figure 11-E). The open block arrow indicates the division between the dorsal accessory nucleus and the ventral lamella of the principal nucleus (see inset for orientation).

Figure 17  Reactive neurons are seen in the dorsolateral part of subnucleus "c" (arrow) following HRP injection of the visual-auditory vermis (see case P-497, figure 8-B).

Figure 18  Reactive neurons are seen in the ventro-medial part of subnucleus "c" (B-nucleus) subsequent to HRP injection in the nodulus (see case P-539, figure 10). Comparison of figures 17 and 18 clearly shows the sharp localization of labelled neurons. The open block arrow in the inset corresponds in position to the arrows in figures 17 and 18.
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