Afferents to the Cerebellar Lateral Nucleus. Evidence from Retrograde Transport of Horseradish Peroxidase after Pressure Injections through Micropipettes [1]

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ABSTRACT HRP was injected by pressure from glass capillary micropipettes unilaterally into the lateral nucleus of rat so as to encompass the entire nucleus, but without spread into the interpositus nuclei. The cells of origin of the afferents to the lateral nucleus were studied after retrograde transport of the HRP. The reticulotegmental nucleus of the pons was labelled bilaterally and is the major source of crossed and uncrossed reticular inputs. The pontine nuclei also provide extensive crossed and uncrossed afferents. The inferior olive gives a large crossed olivo-lateral nucleus projection and a minor uncrossed input. The trigeminal nuclear complex — the nucleus of the spinal tract and the mesencephalic, principal sensory, and motor nuclei — all provide uncrossed afferents. The rostral portion of the lateral reticular nucleus gives a small crossed and uncrossed projection while the perihypoglossal nuclei and the dorsal parabrachial body give crossed afferents to the lateral nucleus. The norepinephrine afferent system from the locus coeruleus is represented by one or two heavily labelled cells and the serotonin raphe systems come from at least five raphe subgroups, the dorsal, superior centralis, pontis, obscurus and magnus nuclei. No evidence was found for commissural fibers between ipsilateral or contralateral cerebellar nuclei, or afferent axons from the spinocerebellar nuclei and the paramedian reticular nucleus. The significance of these sources of afferent inputs to the lateral cerebellar nucleus is discussed. The question is raised of the direct relationship between size of terminal axonal arborization and the quantity of HRP granules present in a cell after retrograde transport. The limitations of the HRP method for detecting subtle local differences in the distribution of afferents within the heterogeneous groups of neurons in the lateral nucleus are discussed.

The cerebellar cortex has been a favorite location in the central nervous system for the study of nerve cell organization, synaptology and connections. Its various cellular components have been minutely investigated with regard to its structure, physiological function, and circuitry (Eccles et al., '67; Mugnaini, '72; Palay and Chan-Palay, '74). The deep cerebellar nuclei, however, have suffered in comparison; until recently, they received only sporadic attention. This neglect is exceeded only by their importance, in that these cerebellar nuclear neurons are the ultimate recipients of the entire cortical outflow, except for a small contingent of Purkinje cell axons that proceed to the vestibular nuclei. In an attempt to fill in this void of information, an extensive series of investigations have been made into the morphology, organization and connections of the lateral cerebellar nucleus in the rat (Chan-Palay, '73a–i) and the dentate nucleus in the rhesus monkey (Chan-Palay, '75b). Concurrently, there has been a resurgence of interest in the cerebellar nuclei by physiologists, attracted by the important discovery by Ito et al. ('64, '69, '70) that nu-

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clear neurons can maintain a high frequency discharge despite inhibition from cortical Purkinje cells. These studies imply that nuclear cells, unless they are spontaneously active pacemaker cells, must have excitatory inputs from extracerebellar sources in order to overcome the tonic inhibitory input from Purkinje cells. From these initial observations have arisen a number of studies, mainly by physiologists, in the quest for the sources of the mossy fibers and climbing fibers which send collaterals to the cerebellar nuclei on their way to the cortex.

The afferent pathways to the cerebellar cortex have been extensively studied by anatomists and physiologists (reviewed in Evarts and Thach, '69; Bloedel, '73; Allen and Tsukahara, '74; Armstrong, '74; Lafleur et al. ('74). There are far fewer studies of afferent fiber systems to the deep cerebellar nuclei. Two reasons may account for this: (1) the nuclear groups are far less accessible than cortex, and (2) for the morphologists, studies of nuclear connections using degeneration techniques invariably incur difficulties with disruption of the overlying cortex and the fibers of passage surrounding the nuclei. Furthermore, comparisons between investigations are difficult because, at least in some studies, the nuclear groups involved are not precisely identified.

A review of the anatomical and physiological literature on afferent sources to the three deep cerebellar nuclei, the dentate (lateral), interpositus, and fastigial (medial) groups in a variety of species is summarized in table 1. Only those systems identified with certainty have been included. Briefly, Szentágothai suggested, in support of physiological studies, that the pontine nuclei probably project to the cerebellar nuclei (Eccles et al., '67). The inferior olive is implicated as a source of afferents as well (Brodal, '40; Matsushita and Ikeda, '70a; Szentágothai, in Eccles et al., '67). An early study by Pearson ('49) showed axons to the dentate nucleus from the mesencephalic nucleus of the fifth cranial nerve. Rubro-cerebellar fibers to the contralateral interpositus nucleus have been shown by Courville and Brodal ('66). Collier and Buzzard ('03) postulated fibers to the dentate nucleus from the spinal cord in Marchi degeneration studies. The interpositus and fastigial nuclei have been shown to receive axons from the spinal cord (Matsushita and Ikeda, '70b; Szentágothai, in Eccles et al., '67; Ebbesson, '67; Jacobs, '68), although some authors (Grant, '62; Voogd et al., '69) were not able to demonstrate them. In a degeneration study, Cohen et al. ('58) suggested that commissural fibers between the nuclei of two sides in the cerebellum existed, and Deura ('66) provided physiological data in support of them. Jansen and Jansen ('55) earlier, however, found no evidence for them in a study with hemisection of the cerebellum. Recent interest in monoaminergic pathways to the cerebellum has resulted in the discovery of a sparse norepinephrine and serotonin input to the nuclei (fluorescence, Hökfelt and Fuxe, '69; electron microscopic studies, Chan-Palay, '73h). The norepinephrine input is from the locus coeruleus (Olsen and Fuxe, '71) and the serotonin from certain raphe cell groups (Chan-Palay, '75a,b). In earlier studies of retrograde cell change in raphe neurons of cat, Brodal et al. ('60) suggested that the raphe pontis gives afferents to the dentate nucleus and that the raphe pontis, obscurus, and pallidus supply the fastigial and interpositus nuclei.

The present investigation was aimed at clarification of the sources of input to a single cerebellar nucleus. A unilateral injection of horseradish peroxidase (HRP) was precisely placed within the lateral nucleus. The amount injected was large enough to encompass most of the lateral nucleus but without noticeable diffusion into the neighboring interpositus or surrounding white matter (fig. 1). HRP is carried by retrograde axonal transport to the cell somata of origin of these fibers (Kristensson and Olsen, '71; LaVail and LaVail, '72). These afferent sources were sudied in the brainstem and compared to other results from a control injection in the interpositus nucleus. The great advantage of this technique is its precise localization of HRP to the terminal axonal fields within the one nucleus.

MATERIALS AND METHODS

A total of 24 male Sprague-Dawley rats (150–200 g) were injected unilaterally

TABLE 1

Review of afferents to cerebellar nuclei. A summary of the literature dealing with afferents to the cerebellar nuclei in several experimental species on the basis of anatomical and physiological methods

Source/Tract	Author	Methods	Species	Dentate (lateral)	Interpositus	Fastigius (medial)
1. Pontine nuclei	Szentágothai (in Eccles et al., '67)	Degeneration, Nauta	cat	(+)	(+)	(+)
	Tsukahara et al., '68	Physiology	cat		+ contra	
	Ito et al., '70	Physiology	cat	+ ipsi		
	Hoddevik, '75	HRP	cat		+	
2. Inferior olive	Szentágothai (in Eccles et al., '67)	Degeneration, Nauta	cat	(+)	(+)	(+)
	Ito et al., '70	Physiology	cat	+ contra		
	Matsushita and Ikeda, '70a	Degeneration, Nauta	cat	+ contra	+	+
3. Mesencephalic V	Pearson, '49	Degeneration, pyridine silver	human, cat, opossum	+ ipsi	−	−
4. Lateral reticular nucleus	Ito et al., '70	Physiology	cat	+ ipsi		
	Eccles et al., '72	Physiology	cat		+ ipsi	
	Eccles et al., '74	Physiology	cat			+ ipsi
5. Locus coeruleus	Hökfelt and Fuxe, '69	Fluorescence	rat	(+)	(+)	(+)
	Chan-Palay, '73	EM, fluorescence	rat	+		
	Mugnaini and Dahl, '75	Fluorescence	chicken	−	−	−
6. Raphe nuclei	Hökfelt and Fuxe, '69	Fluorescence	rat	(+)	(+)	(+)
pontis	Brodal et al., '60	Degeneration, retrograde	cat	+ contra	+	+
obscurus, pallidus dorsalis, sup.				−	+	+
centralis, pontis, obscurus, magnus	Chan-Palay, '75c	EM, autoradiography, HRP	monkey	+	+	
7. Spinocerebellar tracts	Collier and Buzzard, '03	Degeneration, Marchi	human	+		
	Ebbeson, '67	Degeneration, Nauta	lizard	(+)	(+)	(+)
	Szentágothai (in Eccles et al., '67)	Degeneration, Nauta and Golgi	cat	+	+	+
	Jacob, '68	Degeneration, Nauta	lizard	−	−	+
	Matsushita and Ikeda, '70b	Degeneration, Nauta	rabbit, cat	−	+ bilat	+
	Eccles et al., '72, '74	Physiology	cat		+ ipsi	+ ipsi
8. Red nucleus	Courville and Brodal, '66	Degeneration, Nauta	cat	−	+ contra	−
9. Commissural nucleus	Cohen et al., '58	Degeneration, Nauta	cat	+	+	+
	Deura, '66	Physiology	cat	+	+	+

Key: + reported present; − reported absent; (+) cerebellar nucleus not specified.

Abbreviations

DPB, Dorsal parabrachial body
I, Interpositus
IO, Inferior olive
L, Lateral nucleus of cerebellum
LC, Locus coeruleus
llv, Ventral nucleus of the lateral lemniscus
LRN, Lateral reticular nucleus
MNv, Motor nucleus of V
n IV, Trochlear nucleus
n XII, Hypoglossal nucleus
NMT, Nucleus of the mesencephalic tract of V
NST v, Nucleus of the spinal tract of V
PN, Pontine nuclei
PSN v, Principal sensory nucleus of V
RCS, Raphe centralis superior
RD, Raphe dorsalis
RP, Raphe pontis
RTP, Reticulotegmental nucleus of pons
TB, Trapezoid body

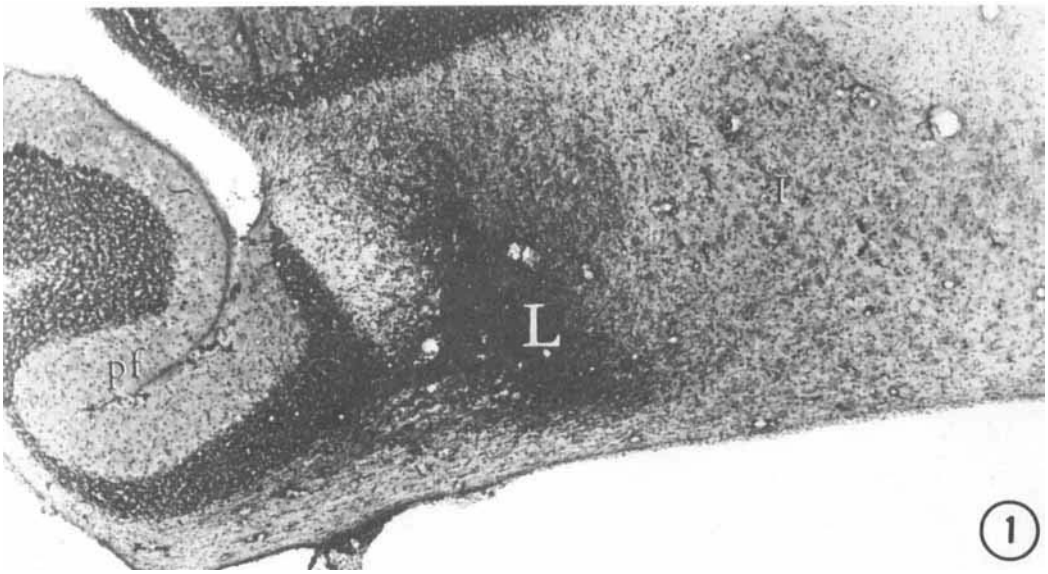


Fig. 1 Injection site, rat's cerebellum, lateral nucleus (0.2 μl HRP). The injection is well localized within the boundaries of the nucleus (L) and is distinct from the adjacent paraflocculor cortex (pf) and interpositus nucleus (I). A few groups of diffusely stained Purkinje cells and their axons can be seen extending towards the cortex (see text). × 35.

with HRP, of which 12 were suitable for study of the afferents to the dentate nucleus. One rat with an injection into the interpositus nuclei was prepared as control. The rats were anesthetized with chloral hydrate (35 mg/100 g body wt) i.p. and placed in the stereotaxic apparatus. Coordinates from Pellegrino and Cushman ('67) and the system of De Groot were used in all cases. Suitable coordinates for the dentate nucleus are A-3.80, L-3.75; V-3.25; and for the interpositus nucleus A-3.80; L-2.75; V-3.25.

An initial series of seven rats was injected with 0.05 to 0.2 μl of 33% solution of HRP (Sigma, type VI) in 0.9% saline through a 1 μl syringe (Unimetrics #1001) connected to a beveled, 60 mm, 34 gauge needle (Bradford Scientific, Inc., Marblehead, Mass.) by a short length of polyethylene tubing (Intramedic #7405). The syringe was mounted on an infusion pump (Harvard, Model 975) and the HRP was injected over a period of 10 to 30 minutes.

The remainder of the rats were injected by means of glass micropipettes (Kimax #46485, O.D. 0.7–1.0 mm) which were drawn on a Kopf vertical pipette puller (Model 700C). All pipettes used for injection had tips of 10–15 micron diameter which were broken or beveled on a rotating Arkansas stone disc. A 7% solution of HRP in normal saline was used for these injections. The solution was filtered before

use (#AAWP01300, 22 micron, Millipore Corp., Bedford, MA) and the filled pipettes were stored with their tips in saline to prevent clogging. The injections were made with the aid of a pressure injection system (Kater et al., '73). Using a pressure of 2–4 psi the HRP was smoothly injected over a period of 10–20 minutes. The volume of HRP injected was monitored by watching the descent of the meniscus in the capillary tube. After the injection, the pipette was left in situ for 15 minutes to allow time for diffusion of the HRP into the brain. With the addition of a suitable amplifier and oscilloscope, this injection system allowed simultaneous physiological recording (Kater et al., '73) through the same pipette. When used in conjunction with accurate stereotactic coordinates this was valuable in ensuring that the electrode was in fact in the cerebellar nucleus. Whereas the system was perfected in the rat, it was most effective in studies of the primate brain, results of which will be reported separately (Chan-Palay, '75b,c).

Twenty-four to 48 hours after injection, the rats were perfused through the heart with a solution of 1% formaldehyde and 1% glutaraldehyde in 0.05 M phosphate buffer at pH 7.4 (Palay and Chan-Palay, '74). The brains and spinal cords down to the mid-thoracic region were placed in fixative for eight hours and then left overnight in a solution of 5% sucrose and 0.05 M tris buffer (pH 7.6) (Nauta et al., '74). Sections 40 μm thick were cut on a freezing microtome. The sections were collected serially in a specially constructed lucite disc equipped with 24 small wells. The bottom of each well was covered with a fine nylon mesh which allowed free communication between the tissue sections and the sucrose-tris buffer in which the lucite dish was immersed. In this way, all serial sections could easily be processed at the same time. The procedure described by Nauta et al. ('74) for demonstration of the HRP reaction product was used. Every fourth section per 200 μm was mounted in alcohol-gelatin and then lightly counterstained with cresyl violet 0.5% for 1–2 minutes. Each section was examined with high magnification lenses using brightfield illumination. In addition, selected slides were also examined with darkfield illumination and Nomarski interference optics. The location of cell bodies labelled with HRP was carefully mapped onto large photographs of Nissl-stained reference sections at appropriate levels of the brainstem.

RESULTS

HRP injections were judged successful when they were located within the cellular boundaries of the lateral nucleus and encompassed most of the nucleus. These were achieved with 0.1 μl to 0.2 μl of a 7% HRP solution (fig. 1). Restriction of the HRP to the lateral nucleus was aided by the heavy encapsulation by myelinated fibers in which the nucleus is embedded. In the examination of the neuron groups containing HRP granules, attention was paid to the total number of labelled cells in that population, the amount of HRP granules contained within each cell, and their ipsilateral or contralateral location with respect to the injection. Figures 2–5 are four selected levels of coronal sections through the rat brainstem stained for cell somata with the Nissl method. On these reference plates are plotted the sites of cell groups with HRP-labelled cells. The right dentate nucleus was injected and this side is indicated by *arrows* at each level. An attempt has been made to keep the number of cells in each site (indicated by *asterisks*) on the reference plates approximately proportional to the actual number of cells seen in the sections. The results are summarized in table 2.

The pontine nuclei had numerous small and medium sized cells labelled with HRP granules both ipsilateral and contralateral to the injection site, with a larger number of cells on the contralateral side (fig. 2). These cells occupy the medial pontine nuclei, and there is a wide range in their size and density of HRP granules, ranging from densely packed and large to a fine stippling. The contralateral inferior olive (principal nucleus) included numerous cells with HRP, particularly in its lateral and rostral aspects (fig. 5). These cells were remarkable in that adjacent labelled cells differed in the amount of HRP granules they contained. Some contained numerous large HRP granules, others only a fine stippling. These are all readily distinguished when viewed with interference

TABLE 2

Afferents to lateral nucleus, HRP (rat)

Nucleus		Ipsi	Midline	Contra
1. Pontine nuclei		+ +		+ + +
2. Inferior olive		+		+ + +
3. Mesencephalic nucleus V		+		
Principal sensory nucleus V		+		
Nucleus of spinal tract V		+		
Motor nucleus V		+		
4. Reticulotegmental nucleus pons		+ + +		+ + +
5. Lateral reticular nucleus		+		+
6. Perihypoglossal nuclei		+		+
7. Dorsal parabrachial body				+
8. Locus coeruleus		+		
9. Raphe nuclei	Dorsalis	+	+	
	Superior centralis	+	+	+
	Pontis	+		+
	Obscurus		+	
	Magnus	+		+
Pertinent negative results				
Commissural fibers, contralateral cerebellar N.				
Paramedian reticular N.				
Dorsal N. of Clarke				
N. gracilis, N. cuneatus				
Lateral cuneate N.				

Location of nerve cells containing HRP granules after an injection into the lateral cerebellar nucleus. Pertinent negative results include those regions not found to contain HRP-labelled cells but which have been reported in the literature as sources of afferent fibers to the cerebellum.

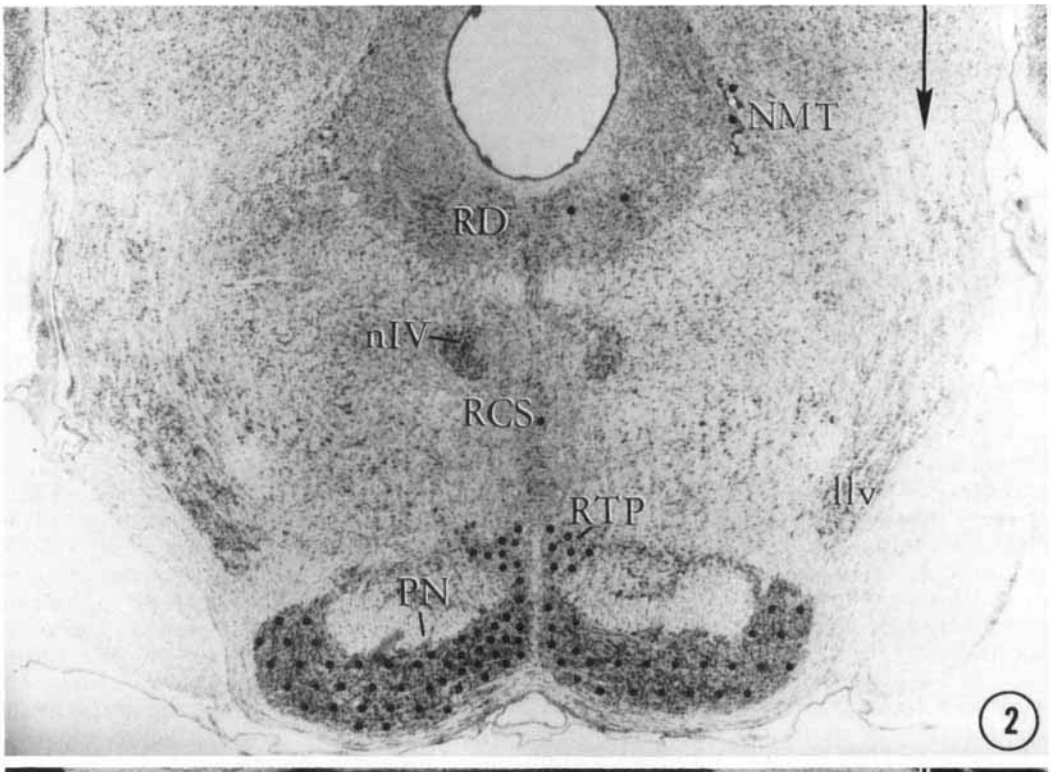
contrast or Nomarski optics (fig. 6). In comparison, an injection into the interpositus nucleus revealed only a very few cells with light stippling of HRP granules in the rostral pole of the contralateral inferior olive. A small number of cells consistently contained HRP granules in the ipsilateral mesencephalic nucleus of V. The ipsilateral principal sensory nucleus of V and the ipsilateral nucleus of the spinal tract of V both contained several cells with moderate HRP granulation (figs. 4, 5). Similarly, one or two cells were also found in the ipsilateral motor nucleus of V (fig. 4).

In the reticular formation, two cell groups were clearly labelled. Throughout its entire extent bilaterally the reticulotegmental nucleus of the pons contained numerous labelled neurons, almost every one densely packed with large HRP granules (figs. 2–4). One such cell is shown in fig. ure 7. The lateral reticular nucleus was labelled bilaterally, primarily the rostral regions where the nucleus extends dorsally into the reticular formation. Here a small number of labelled neurons showed a wide variation in number and size of HRP granules. The ipsilateral perihypoglossal nucleus which receives proprioceptive information from the tongue contained a few cells with a moderate number of HRP granules. The contralateral dorsal parabrachial body had an occasional HRP-containing cell (fig. 3).

The ipsilateral locus coeruleus consistently showed one or two medium sized neurons with remarkably large and dense HRP granules (fig. 8). Similarly, single cells heavily labelled with large HRP granules were found in the midline of the raphe obscurus. The dorsal raphe had two cells

Fig. 2 Section through lower midbrain. There are a few HRP-labelled cells in the raphe dorsalis (RD) and the raphe centralis superior (RCS). The reticulotegmental nucleus (RTP) and the pontine nuclei (PN) are heavily labelled bilaterally. (n IV) trochlear nucleus; (llv) ventral nucleus of lateral lemniscus. Arrow indicates side of injection; cells containing HRP granules are indicated by black stars or white dots. × 25.

Fig. 3 Section through upper pons. There are a few labelled cells in the ipsilateral mesencephalic nucleus of V (NMT), and contralateral dorsal parabrachial body (DPB). There are several labelled cells bilaterally in the raphe pontis (RP). (RTP) reticulotegmental nucleus. Arrow indicates side of injection; cells containing HRP granules are indicated by black stars or white dots. × 25.


NMT
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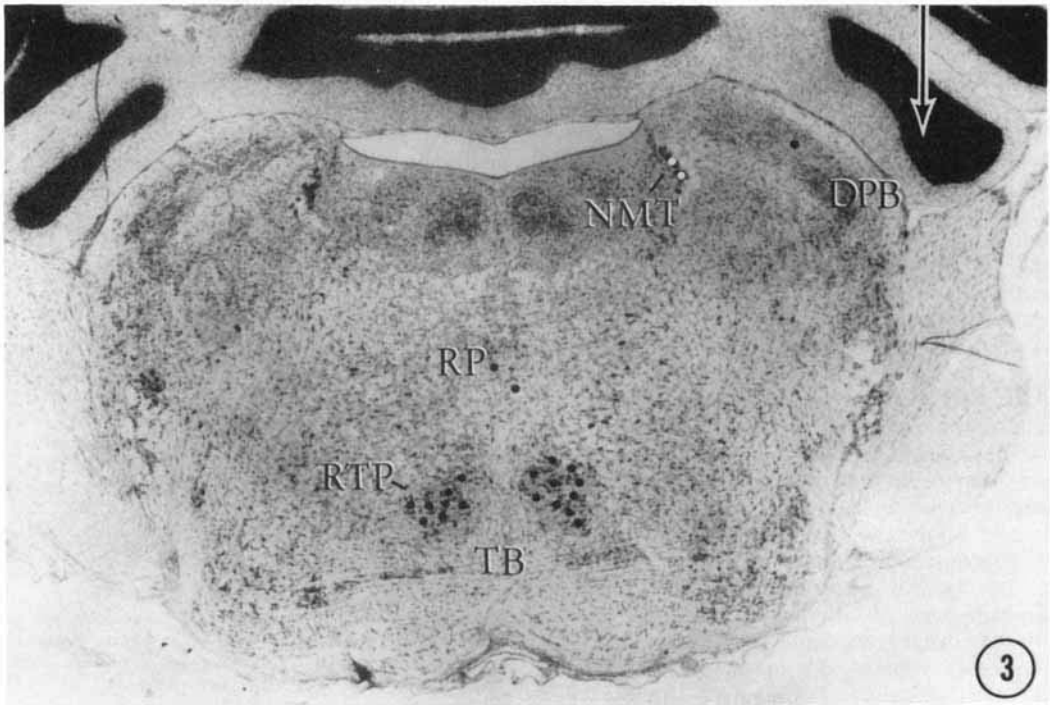

NMT
DPB
RP
RTP
TB
3

with heavy HRP granules in the midline portion and two in its contralateral wing. The raphe superior centralis had several small cells bilaterally with many HRP granules as well as two cells situated clearly in the midline. The raphe magnus contained numerous large neurons with moderate HRP granulation bilaterally. Bilaterally, the raphe pontis had several medium sized cells heavily labelled. Thus at least five brainstem raphe sub-groups of neurons are involved in afferent projections to the lateral nucleus.

The Purkinje cells of the cortex provide the major afferent contingent to the deep nuclei. In this material, the paraflocculus and lateral cerebellar hemispheres contained numerous Purkinje cells with HRP granules; however, a precise determination of corticonuclear projections could not be made. Numerous neuroglial cells with HRP granules, macrophages, and Purkinje cells with diffuse staining from artifactual uptake by injured axons in the adjacent cortex indicated that this would be a perilous task. Occasional diffusely labelled fibers were seen radiating from the injection site. These represent injured fibers which have taken up the HRP by passive diffusion and do not represent retrograde transport (Nauta et al., '74; LaVail and LaVail, '74). Similarly, a few groups of Purkinje cells were seen which were diffusely labelled with brown reaction product (not granules). These also represent non-specific or artifactual uptake. The cells which were diffusely stained with HRP were easily identified as such. The cells with physiological uptake of HRP contained distinct small granules of the brown reaction product within the cytoplasm. Though searched for intensively, no neurons other than the few Purkinje cells described above were seen with diffuse (artifactual) staining in the cytoplasm.

The usefulness or necessity of a "control" injection into the parafloccular cortex should be considered. Such an injection might allow the identification of afferents to the cortex which give rise to collaterals to the lateral nucleus. In order to obtain an injection of all of the appropriate cortex necessary to trace corticonuclear projections, several large injections would have to be made. Because of the complex folding of the cerebellar cortex it is technically very difficult to make uniform injections of selected cortical regions (unpublished results). Hoddevik ('75) has also noted similar unpredictable distribution of HRP after cerebellar cortical injections.

The question of uptake of HRP by fibers of passage has been investigated by others (LaVail et al., '73; Nauta et al., '74). They found no light microscopic evidence for accumulation of HRP by known fibers of passage in their injection sites. Ultrastructural studies have shown that HRP is taken up predominantly by the axon terminal or preterminal segment and not by the axonal shaft (Turner and Harris, '74; LaVail and LaVail, '74).

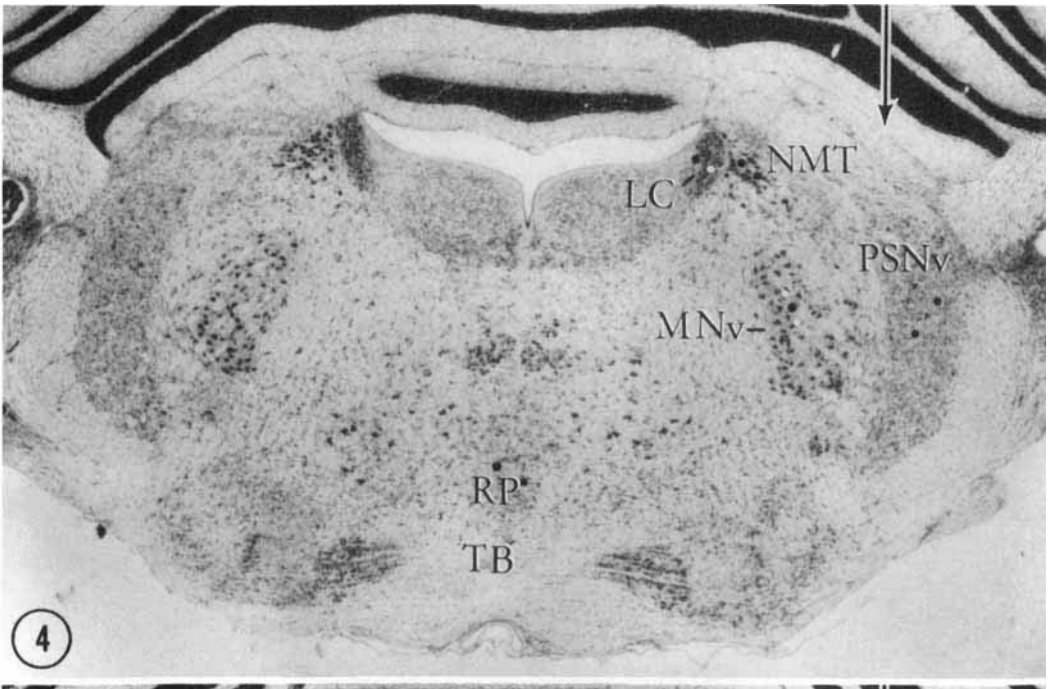
Thus far, neuron groups with HRP-labelled cells have been described. There are, however, several key locations in the brainstem and spinal cord which have been implicated in cerebellar afferent connections and in which no HRP-containing cells were found. These include the paramedian reticular nucleus, the dorsal nucleus of Clarke, the external cuneate nucleus, and the nuclei cuneatus and gracilis. These negative results suggest that the spinocerebellar afferents are not connected with the lateral nucleus. None of the cells in the ipsilateral cerebellar medial and interpositus nuclei and the contralateral medial, interpositus and lateral nuclei contained any HRP granules, evidence that ipsilateral and commissural fibers connecting the cerebellar nuclei do not exist.

DISCUSSION

The writings of Herrick ('24) and

Fig. 4 Section through mid-pons. There are a few HRP-labelled cells in the ipsilateral mesencephalic nucleus of V (NMT) and locus coeruleus (LC). The ipsilateral motor nucleus of V (MN v) and principal sensory nuclus of V (PSN v) also contain scattered labelled cells. There are a few cells bilaterally in the raphe pontis (RP). (TB) trapezoid body. Arrow indicates side of injection; cells containing HRP granules are indicated by black stars or white dots. × 25.

Fig. 5 Section through mid-medulla. There are a few labelled cells in the ipsilateral nucleus of the spinal tract of V (NST v). The rostral lateral reticular nucleus (LRN) is labelled bilaterally, while the inferior olive (IO) is labelled primarily contralaterally. (n XII) hypoglossal nucleus. Arrow indicates side of injection; cells containing HRP granules are indicated by black stars or white dots. × 25.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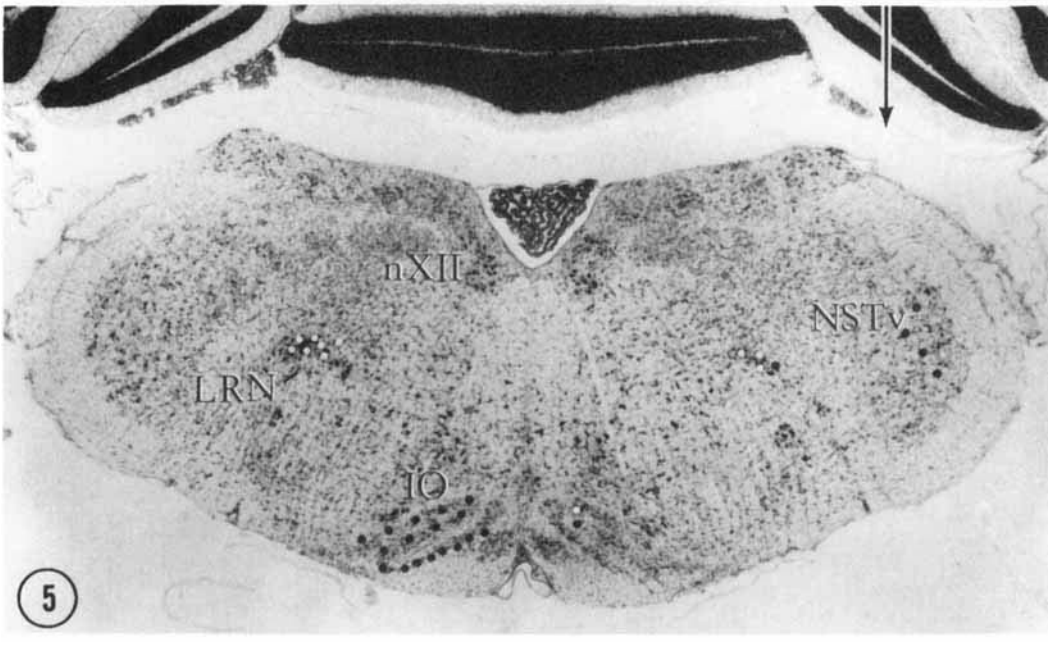
nXII
NSTv
LRN
IO
5

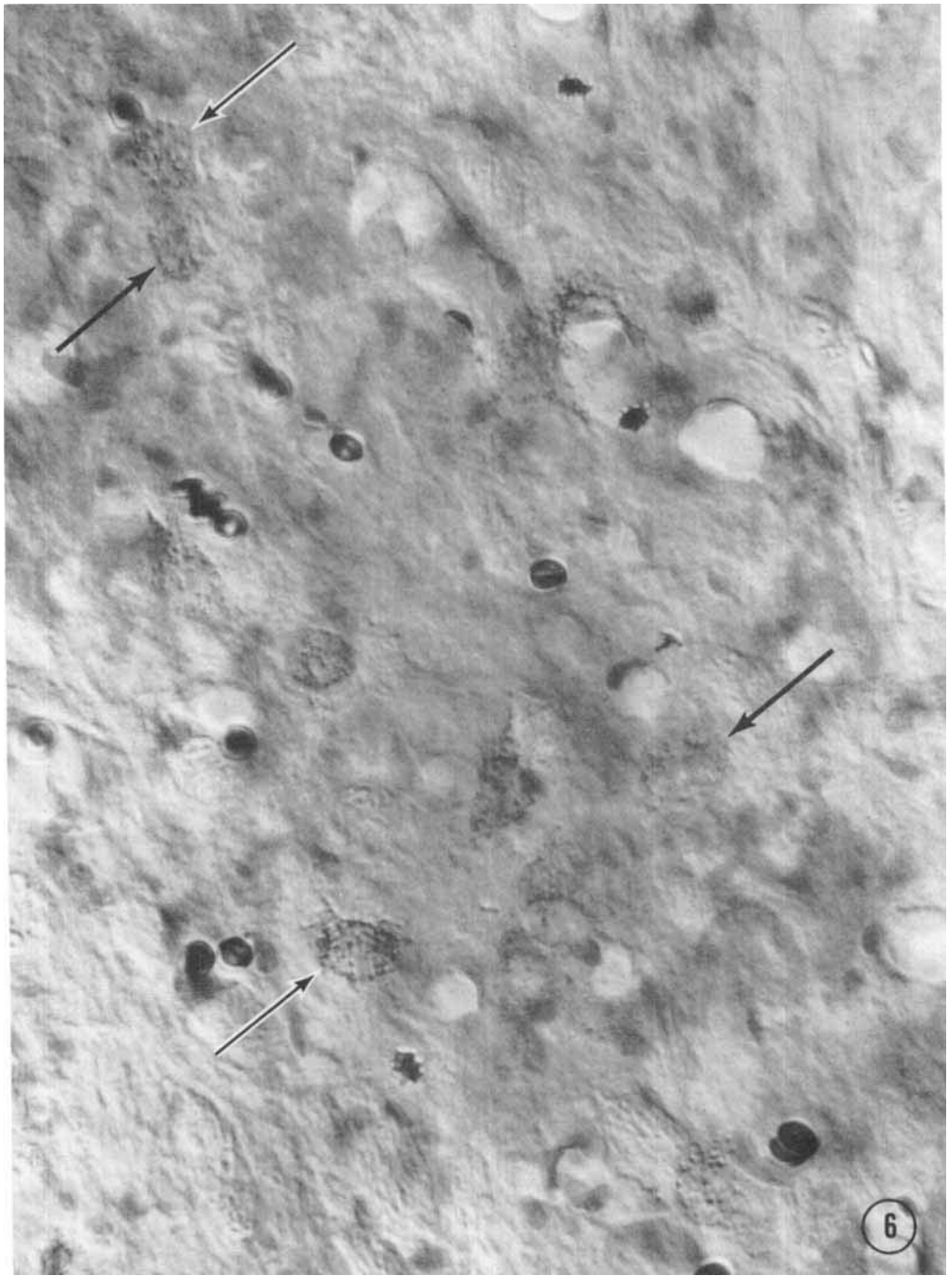

Fig. 6 Nomarski interference photomicrograph of the contralateral inferior olive. Of the many neurons labelled with HRP, some are densely packed with large granules (white arrows) while others have only a few fine granules (black arrow). × 200.

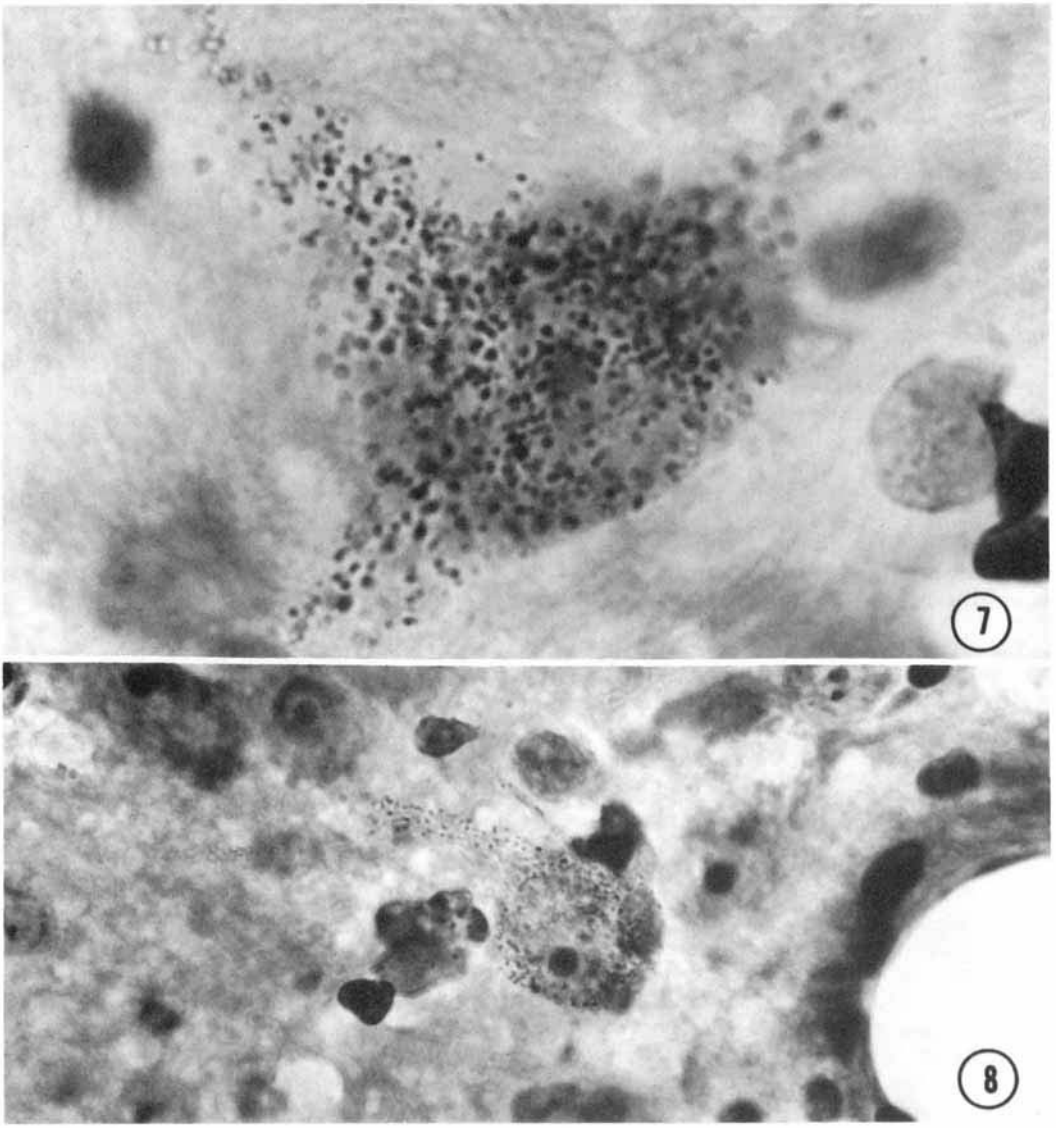

Fig. 7 HRP-labelled neuron in the reticulotegmental nucleus of pons. The neuron is densely packed with large and small granules of HRP, which extend out into the proximal dendrites. × 2,200.

Fig. 8 HRP-labelled neuron in the locus coeruleus. The cell contains many homogeneously fine granules of HRP. × 900.

Holmes ('39) suggest three hypotheses for cerebellar participation in movement: the initiation of corticospinal output; regulation of motoneuronal responses to this corticospinal discharge; and adjustment and correction of the motor output following its initiation. The lateral hemispheres and the dentate (lateral) nucleus with their close association with the motor cortex have also been implicated in the direct initiation of movement (Evarts and Thach, '69).

Direct evidence that cerebellar activity precedes movement has been obtained from recordings of cerebellar Purkinje and nuclear cells in association with the perform-

ance of learned arm movements (Thach, '70a,b, '75). These ideas are also being explored by Allen and Tsukahara ('74). The argument inherent in this hypothesis is that the association cortex rather than the motor cortex provides the major input to the lateral cerebellum and dentate (lateral nucleus) via the cortico-pontine and ponto-cerebellar systems.

Furthermore, the current hypotheses suggest that the intermediate cerebellar cortex and the interpositus nuclei are responsible for the regulation, adjustment and correction of motor movement following initiation. Evarts and Thach ('69) suggest that the sensorimotor cortex, mediated via corticopontine and corticoreticular pathways, and spinal inputs are a major source of afferents to these parts of the cerebellum.

How do the results of the present investigation amplify or clarify the bases for these functional separations of the cerebellum?

This study demonstrates that the reticulotegmental nucleus of the pons provides a major crossed and uncrossed source of afferents to the lateral nucleus, comparable to or greater in number than the crossed and uncrossed inputs of the pontine nuclei. The crossed input from the inferior olive is another major afferent source, and a small contingent of uncrossed olivary fibers also exists. The lateral reticular nucleus and the trigeminal nuclei provide a modest number of fibers. The locus coeruleus provides afferents via one or two cells; the raphe cells, from at least five subnuclei, provide a considerable input. The perihypoglossal nucleus and dorsal parabrachial body provide a minor source of afferents as well.

That the pontine nuclei are a major source of inputs to the lateral nucleus comes with little surprise. These were postulated by Szentágothai (in Eccles et al., '67), and Ito et al. ('70) and Tsukahara et al. ('68) in their physiological studies. Brodal ('54) showed a crossed pontocerebellar pathway to the cerebellar hemispheres and crossed and uncrossed projection to the vermal regions. Furthermore, these pontine areas also receive precise reciprocal connections or "feedback loops" from the dentate and interpositus nuclei (Voogd, '64; Brodal et al., '72a). These results support the thesis that the major descending pathways from the cerebral cortex to the cerebellum, mediated via the pontine nuclei, do terminate in the lateral nucleus. The failure to discover HRP granule-laden cells in nuclear groups mediating the spinocerebellar input indicates no direct ascending afferents from the spinal cord. This is in agreement with previous studies by Grant ('62), Voogd et al. ('69), and Matsushita and Ikeda ('70b).

Two major sets of inputs of integrated information from ascending and descending sources are represented in the afferents from the inferior olive and the reticular formation. The inferior olive is a major source of climbing fibers to the cerebellum and the details of its morphology and afferent and efferent connections have been reviewed recently by Armstrong ('74). The present study shows a large crossed pathway from the principal olive to lateral nucleus and a small ipsilateral projection. Because of the nature of the present experiments, it is not possible to know whether this set of fibers remains uncrossed in the cerebellum. Furthermore, it is impossible to know if the olivary inputs are collaterals of fibers that continue to the overlying cortex or the primary axons of olivary cells that do not project to the cortex. In a recent exhaustive electron microscopic study of the lateral nucleus in the rat, Chan-Palay ('73f) identified a class of axon terminals bearing a remarkable resemblance in fine structure to climbing fiber terminals of the cerebellar cortex. These fibers were seen to come off as collaterals from parent axons that were enroute to the cerebellar cortex, and they were tentatively identified as collaterals of the cortical climbing fibers. The evidence from these HRP studies — that a major olivary climbing fiber afferent system to the lateral nucleus exists — gives strong support to the earlier interpretation. The present studies also provide evidence that whereas the olivary input to the lateral nucleus is considerable, that to the interpositus is limited to a much smaller, crossed input. This supports the postulate based upon physiological studies by Armstrong ('74) that the interpositus receives few afferents from the inferior olive. In another study in which the interpositus

was injected with HRP (Hoddevik, '75) only a few scattered labelled cells were seen in the pontine nuclei.

Of the three precerebellar reticular nuclei defined by Brodal ('57), two, the reticulotegmental nucleus of the pons and the lateral reticular nucleus, provide inputs to the lateral nucleus. The third, the paramedian reticular nucleus, does not, which supports the findings of Brodal and Torvik ('54). The reticulotegmental nucleus of the pons (sometimes referred to as the nucleus reticularis tegmenti pontis of Bechterew or the nucleus papilloformis) is a dorsal extension of the pontine gray matter (Jansen and Brodal, '54). In 1946, Brodal and Jansen concluded that this nucleus sends most of its output to the cerebellar cortex, to the vermis as well as the hemispheres. More recently, Brodal and Szikla ('72) and Brodal et al. ('72b) showed that the interpositus and dentate nuclei send massive crossed reciprocal projections to the reticulotegmental nucleus. These authors suggested that it must be the most important nucleus involved in cerebelloreticular feedback systems. The present study indeed confirms the importance of this nucleus in the afferent pathways as well; it is probably the major contributor of afferents to the lateral cerebellar nucleus in the rat.

The projections of the lateral reticular nucleus (LRN) to the cerebellar cortex have been known for nearly a century. Brodal ('43) using retrograde cell degeneration, demonstrated global projections to the ipsilateral cortex whereas Voogd ('64), using anterograde degeneration methods, reported a bilateral projection to specific lobules, which has been confirmed by Clendenin et al. ('74) and Künzle ('75). The bilateral projection encompasses the pars intermedia and vermis of the anterior lobe while the ipsilateral projection goes to the paramedian lobule. These are all the terminal fields of spinocerebellar pathways.

Does the LRN project to the cerebellar nuclei? Recent physiological studies in cats show LRN collaterals to the dentate nucleus (Ito et al., '70), the interpositus nucleus (Eccles et al., '72), and the fastigial nucleus (Eccles et al., '74). Thus, until now, there has been physiological evidence for collateral innervation of the deep nuclei but not anatomical demonstration of it. In fact, Künzle ('75), in an autoradiographic study of anterograde transport after incorporation of tritiated leucine, showed very few fibers from the LRN in the cerebellar nuclei. In the present investigation, HRP granules were found only in cells of the rostralmost lateral reticular nucleus. This region of the LRN was not included in Künzle's ('75) injection and thus may account for the deficiency in his findings.

The afferents to the cerebellum that remain to be discussed fall under the class of "other specific afferents," which include the trigeminal and perihypoglossal complexes and the locus coeruleus and the raphe, sources of monoamine terminals. The various nuclei of the trigeminal complex and the perihypoglossal nucleus have previously demonstrated afferent inputs to the cerebellar cortex (Woodburne, '36; Larsell, '47; Carpenter and Hanna, '61; Brodal, '52). Pearson ('49) demonstrated an uncrossed system from the mesencephalic nucleus of V to the dentate nucleus, which has been confirmed in the present study. In addition, we have provided new evidence for afferent sources to the dentate from the principal sensory nucleus, the nucleus of the spinal tract and the motor nucleus of V.

In a study of catecholamine fluorescence in brain slices, Fuxe ('65) discovered terminals in the cerebellar cortex which were later shown to contain norepinephrine (Andén et al., '66; Hökfelt and Fuxe, '69; Iversen and Glowinski, '66; Bloom et al., '71). These cortical fibers originate from the locus coeruleus (Olsen and Fuxe, '71; Ungerstedt, '71; Segal et al., '73; Pickel et al., '74; Kobayashi et al., '75) and enter the cerebellum via the superior cerebellar peduncle (Pickel et al., '73; Krebs, '76; Mugnaini and Dahl, '75). The existence of a norepinephrine input to the deep cerebellar nuclei of the rat was first suggested by Hökfelt and Fuxe ('69), and confirmed in the dentate nucleus by Chan-Palay ('73h). In the present study, an HRP injection into the dentate results in one or two heavily labelled cells in the locus coeruleus, confirming the presence of an uncrossed afferent group of fibers from that nucleus.

The original demonstration of a projec-

tion from the raphe nuclei to the deep cerebellar nuclei was given by Brodal et al. ('60). These authors showed that a few cells in the raphe pontis provided afferents to the dentate nucleus in cats, and similarly from the raphe pontis, obscurus, and pallidus to the interpositus and fastigial nuclei. No innervation of the cerebellar cortex was discovered. Bloom et al. ('72), in an autoradiographic study with tritiated serotonin, showed a serotonin innervation from some pontine raphe neurons to the granular layer of the cerebellar cortex. Hökfelt and Fuxe ('69), in a fluorescence study, found serotonin terminals mainly in the molecular layer of the cerebellar cortex and questioned the presence of rare examples of similar fibers in the cerebellar nuclei. Chan-Palay ('73h), in an electron microscope and fluorescence study of the lateral nucleus, suggested that certain fluorescent fibers containing dense core vesicles might be serotonin axons from the raphe nuclei. The present investigation confirms the existence of extensive crossed and uncrossed afferent fibers from at least five of the raphe nuclei to the cerebellum: dorsalis, superior centralis, pontis, magnus and obscurus. Except for the raphe magnus in which many cells are labelled, the other raphe neurons are usually single and very heavily labelled. Indeed, these raphe neurons constitute several sources for the extensive network of serotonin-containing unmyelinated axons in the cortex and all the cerebellar nuclei. These vast and separate serotonin systems from studies with labelled transmitters for light and electron microscopy have been the subject of other discussions (Chan-Palay, '75a,c,d) to which the reader is referred.

Comments on the Method

Pressure injections of HRP through micropipettes provide a simple means of delivering very small amounts of the tracer into deep central nervous system structures. In dealing with the delivery of HRP into a small, circumscribed region such as the lateral nucleus, the following aspects must be considered. If the injection is precisely confined to the nucleus but occupies only part of the total nuclear territory, then the number of cells with HRP granules and probably the amount of granules per cell, will be smaller than if the entire nucleus were filled with HRP up to its boundaries. In the latter situation, HRP-labelled cells will be more readily found. When the entire lateral nucleus is injected, the results obtained represent the large majority of the total afferent sources present. One cannot derive any information about the differential distribution of afferent sources to the different parts of the lateral nucleus. Since it has been demonstrated that this nucleus consists of several different regions, for example, a region with columnar neurons or with small interneurons (Chan-Palay, '73a,d), it would be important to know whether there is a differential distribution of afferents inputs as well. For example, are the climbing fiber collaterals from the inferior olive confined to part of the lateral nucleus? Partial injections into specific subdivisions of the nucleus are more promising for this type of study.

Secondly, consideration needs to be given to the quantity of HRP granules present in positive cells from experimental animals with an established post-injection survival time and tissue processed in a technically consistent manner. For example, the locus coeruleus and some of the raphe inputs in the lateral nucleus are represented by single neurons with a dense accumulation of large HRP granules, whereas the two or three cells in the inferior olive which give rise to the uncrossed olivary-lateral nucleus input have only a light stippling of small HRP granules. The terminal arborizations of the norepinephrine, locus coeruleus, and the serotonin raphe systems are extensive Chan-Palay, '75c,d) and correlate well with the heavy HRP granulations seen. Can one infer that the uncrossed olivary projections have a sparse terminal arborization in the lateral nucleus? Does the amount of HRP collected in a cell indicate the density of its axonal field? Jones ('75) has suggested that it does, in his studies of thalamic connections using retrograde and anterograde transport methods, and we would support his opinion. If individual cells in the raphe and locus coeruleus can each encompass considerable territory by their axonal arborizations, then one might understand how these small numbers of neurons in the brainstem can give rise to large

systems that supply axons to such numerous sites all over the brain.

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