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Norepinephrine Innervation of the Cochlear Nuclei by Locus Coeruleus Neurons in the Rat

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Summary. The cochlear nuclei (CN) contain a moderate concentration of norepinephrine (445 ± 20 ng/g tissue) with dopamine levels (46 ± 14 ng/g) that are low and within the precursor range expected for a norepinephrine (NE) terminal system. Lesion and horseradish peroxidase (HRP) experiments indicate that this innervation is bilateral and arises from fusiform and multipolar neurons in the locus coeruleus.

Autoradiographic and fluorescence histochemical experiments demonstrate that locus coeruleus fibers reach the ipsilateral ventral cochlear nuclei via a rostral pathway that projects from the rostral locus coeruleus laterally through the brain stem to the rostral tip of the ventral nuclei. This pathway is located dorsal to the motor and spinal trigeminal nuclei and ventral to the middle cerebellar peduncle. Descending coeruleo-cochlear fibers travel between the fourth ventricle and the vestibular nuclei to enter the acoustic striae. These fibers innervate both the dorsal and ventral nuclei. Contralateral locus fibers reach the CN by crossing in the pontine central gray at the rostral border of the fourth ventricle and by decussating with the fibers of the mesencephalic trigeminal nucleus ventral to the medial longitudinal fasciculus. The bilateral locus coeruleus innervation of the cochlear nuclei comprises a highly collateralized network of varicose axons which are not topographically organized. Unlike the cochlear nerve fibers in the CN which form specific projections, the locus coeruleus afferents to these sensory nuclei are diffuse and non-specific.

Key words: Locus Coeruleus – Norepinephrine – Cochlear Nuclei – Coeruleo-Cochlear Bundles.

Introduction

Norepinephrine (NE) neurons which are located in the nucleus locus coeruleus (LC) and the lateral tegmentum of the medulla and caudal pons are known

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to project over a large component of the neuraxis (Dahlström and Fuxe, 1965; Ungerstedt, 1971; Olson and Fuxe, 1971; Lindvall and Björklund, 1974b; Lindvall et al., 1974; Swanson and Hartman, 1975; Jones and Moore, 1977). Although a number of studies describe the ascending and spinal projections of the various NE cell groups little has been done to characterize their projections to the sensory nuclei of the brain stem. A few studies suggest that the NE neurons innervate some sensory nuclei of the cranial nerves (Fuxe, 1965; Swanson and Hartman, 1975; Lindvall et al., 1974; Kromer and Moore, 1976, 1980) but the nucleus of origin and the exact organization of the terminal field has not been characterized for many of these nuclei.

As a continuation of our previous study (Kromer and Moore, 1976), we have attempted to characterize more completely the NE projection to the cochlear nuclei (CN). In the present study a combination of techniques was employed, including fluorescence histochemistry, catecholamine (CA) biochemical assay, mechanical and chemical lesions, autoradiography and the HRP retrograde transport method, in order to identify the neurons of origin of this projection and to describe the terminal distribution of NE fibers within the CN. Throughout this study we have adopted the nomenclature of Dahlström and Fuxe (1964) and Moore and Kromer (1978) in describing the NE cell groups and that of Harrison and Warr (1962) and Harrison and Irving (1965, 1966) for the subdivisions of the rat cochlear nuclei.

Materials and Methods

Experimental Animals

Adult female albino rats (n=150) of the Sprague-Dawley strain (120–250 g) were used in these studies. For each experiment the animal population was kept as homogeneous as possible.

Lesion Procedures

All radiofrequency, knife and chemical lesions were made with a small animal stereotaxic instrument (Kopf Instruments, Tujunga, CA.) as described previously (Kromer and Moore, 1980). The radiofrequency lesions (n=38) were generated with low power, continuous wave radiofrequency current which gave a temperature of 55° C for 1 min. Stereotaxically placed knife lesions (n=52) were made with a simple guillotine-type knife. Brain stem hemisections were produced by making overlapping knife cuts in the same transverse plane. Selective chemical lesions (n=10) of the various CA projection systems were obtained with injections of 6-hydroxydopamine (6-OHDA-HBr; Sigma Chemical Co.).

Catecholamine Assay

The assay procedure for norepinephrine (NE) and dopamine (DA) was adapted from the enzymatic-isotopic assay to Coyle and Henry (1973). All animals (n=46) were sacrificed by decapitation and the cochlear nuclei dissected and frozen on dry ice until the assay could be performed. Histological identification of the dissected region from randomly chosen brains indicated that the region usually contained the entire CN with only minor contamination from the surrounding brain stem.

Histological Methods

In the fluorescence histochemical studies (n = 30) either the Vibratome-formaldehyde methods (Hökfelt and Ljungdahl, 1972; Lorén et al., 1977), the Vibratome-glyoxylic acid method (Lindvall et al., 1973; Lindvall and Björklund, 1974a), or the Aluminium-Freeze Dry procedure (Lorén et al., 1979) was employed.

The autoradiographic technique used for tracing axonal connections was adapted from that of Cowan et al. (1972). For this study four adult female rats received a stereotaxic injection of 10 μCi (^{3}H)-Proline in 0.05 μl saline into the right locus coeruleus. Two days after injection, the animals were perfused with 10% formalin, embedded in paraffin, and processed for autoradiography.

The retrograde transport of horseradish peroxidase (HRP) was used for the further identification of the neurons of origin of the catecholamine innervation to the cochlear nuclei. In these studies the right cochlear nuclei of 14 adult female rats were stereotaxically injected with 0.1–0.2 μl of 30% HRP (Sigma type VI) over a 20–30 min time period. Following a one to two day survival time peroxidase was demonstrated in frozen sections (40 μm) by the 3,3-diaminobenzidine (DAB) procedure (Graham and Karnovsky, 1966; Ralston and Sharp, 1973).

Results

Catecholamine Content of the Cochlear Nuclei

Twenty-two adult female rats were used for the determination of the NE content of the CN. The mean weight for the CN is 3.2 ± 0.1 mg and the mean value for NE is 445 ± 20 ng/g and for DA is 46 ± 14 ng/g tissue. As suggested in a preliminary report (Kromer and Moore, 1976), we propose that the NE and DA concentrations in the CN indicate that this region receives a monoamine innervation entirely from NE neurons. This is further substantiated by some of our additional studies.

Origin of the Norepinephrine Projection

The location of the NE neurons which project to the CN was determined by combining lesions of various brain stem NE cell groups and their ascending pathways with biochemical determination of the NE concentration and fluorescence histochemical observations of the density and distribution of NE fibers within the CN after the lesions. To supplement the above studies small amounts of HRP were injected into the CN and the catecholamine (CA) cell groups were analyzed for reaction product indicating retrograde transport.

Lesion Studies. The analysis of the biochemical results from the seven experimental lesion groups is given in Table 1. Of the various lesions, only those that completely destroy the locus coeruleus either unilaterally or bilaterally result in a significant decrease in the NE content of the CN from control values. In addition, a unilateral lateral tegmental CA lesion produces a significant difference between sides but not from control values.

No significant changes in the NE content of the ipsilateral or contralateral CN are observed following: 1) unilateral transections of either the ascending

Table 1. Norepinephrine content-cochlear nuclei

Experiment	Side (*n*)	NE (ng/g) ±S.E.M.	Percentage of control	P Values	
				Difference from control	Difference between sides
Controls	both (22)	445±25			
Unilateral dorsal and ventral tegmental bundle transection	right (5)	438±98	98	NS	NS
	left (5)	503±80	113	NS	
Unilateral central tegmental tract transection	right (10)	430±52	97	NS	NS
	left (10)	489±66	110	NS	
Unilateral lateral tegmental CA lesion	right (5)	377±35	85	NS	
	left (5)	495±65	111	NS	<0.05
Unilateral lesion lateral to the locus coeruleus	right (7)	353±39	79	NS	NS
	left (7)	414±59	93	NS	
Unilateral locus coeruleus lesion	right (7)	214±18	48	<0.001	<0.001
	left (7)	327±19	73	<0.05	
Bilateral lesion of the rostral locus coeruleus	right (4)	369±81	83	NS	NS
	left (4)	395±59	89	NS	
Bilateral locus coeruleus lesion	right (5)	93±11	21	<0.001	NS
	left (5)	95± 9	21	<0.001	

All unilateral lesions were placed on the right side. P values obtained using a two-tailed *t*-test. NS refers to $p > 0.05$

catecholamine pathways (located in the dorsal and ventral tegmental bundles rostral to the locus) or the descending central tegmental tract (caudal to the locus), 2) unilateral lesions located lateral to the locus coeruleus, and 3) bilateral lesions of the rostral locus coeruleus. Observations from fluorescence histochemical material indicate that all regions of the CN still contain an apparently normal density and distribution of the NE fibers following the first two types of lesions. Animals with bilateral lesions of the rostral half of the locus were not studied in fluorescence histochemical preparations.

Unilateral lesions of the lateral tegmental CA neurons produce no significant change in the NE content of the CN compared with controls. However, there is a significant difference between sides due to an increased content in the contralateral nucleus and a decrease ipsilateral to the lesion. Fluorescence histochemical material indicates that the large lateral tegmental CA lesions produce considerable chromatolysis and an accumulation of autofluorescent debris within both CN. (This debris partially interferes with the interpretation of the CA fluorescence.) Moreover, the ipsilateral anteroventral cochlear nucleus (AVCN) has a decrease in NE fiber density, due to the destruction of fibers which enter this region.

Following unilateral locus coeruleus (LC) lesions there is a significant difference in the NE concentrations of the CN between the two sides and between

control values and both sides. In fluorescence material both the ipsilateral and contralateral CN appear to contain fewer NE fibers than in controls. On both sides some preterminal NE fibers still enter the dorsal cochlear nucleus (DCN) via the acoustic striae, but there is a decrease in the density of NE fibers in all areas of the nuclei. In horizontal sections of the DCN, which also contain the LC lesion, NE fibers from the contralateral intact locus are observed to curve around the rostral tip of the fourth ventricle and travel caudally within the central gray between the lesion site and the ventricle. Some of these NE fibers appear to enter the acoustic striae and DCN ipsilateral to the lesion. Both the left and right AVCN and posteroventral cochlear nucleus (PVCN) contain fewer NE fibers than are present in control animals. In addition, all divisions of the ipsilateral CN have sparser densities of NE fibers than in corresponding regions in the contralateral CN.

Although the bilateral radiofrequency lesions appear to completely destroy the entire compact portion of the locus coeruleus, a few fluorescent neurons are sometimes observed immediately rostral to the lesion. Therefore, some of these NE neurons, which cannot be identified in cresyl violet stained material, are probably present in the animals used for the CA assay. With the bilateral LC lesion there is a pronounced decrease in the NE content of both CN (79%). These values do not differ from each other but represent a highly significant difference from controls. Fluorescence histochemical experiments show no difference in the NE innervation between the left and right CN both of which contain very few NE fibers. Bilaterally, the acoustic striae contain no visible NE axons whereas a few NE fibers enter the rostral tip of the AVCN. Only an occasional fluorescent axon is visible within the DCN or PVCN bilaterally, however both AVCN contain very few scattered NE fibers.

Horseradish Peroxidase Experiments. Only two animals with HRP injections into the CN (R7667A and D) will be discussed here. Both animals contain dense reactive zones which are confined primarily to the ventral cochlear nuclei (VCN) and ventral DCN but with minor spread into the trapezoid body and spinal trigeminal tract (Fig. 1). The peripheral zone of reactive cells extends into the lateral regions of the reticular formation, facial nucleus, superior olivary complex, and vestibular nuclei.

The distribution of HRP-positive neurons is nearly identical in both animals. Reactive neurons are present in many noncatecholamine-containing cell regions. However, no regions containing DA cells and only two NE cell regions have labeled neurons. The ipsilateral A5 region contains some HRP-positive cells. Most of these cells are located lateral to the preolivary and lateral nuclei of the superior olivary complex in the region which also contains the cells of origin of the olivo-cochlear bundle. This area is also along the edge of the peripheral zone of the injection. The locus coeruleus contains HRP-positive cells bilaterally which possess varying densities of reaction product and are located throughout the dorsoventral and rostrocaudal levels of the compact portion of the locus (Fig. 1). Moreover, both fusiform and multipolar neurons (described in the Golgi studies of Swanson, 1976 and Shimizu et al., 1978) contain reaction product. Comparison of the number of labeled cells in both

nuclei indicates that there are 2–3 times more HRP-positive neurons in the ipsilateral than in the contralateral nucleus (17 vs 9 for R7667A and 29 vs 9 for R7667D).

Afferent Norepinephrine Pathways to the Cochlear Nuclei

To better identify the NE pathways to the CN, NE fibers entering either the rostral or caudal regions of the CN were transected and the animals prepared for observation with the fluorescence histochemical technique. In a second set of experiments tritiated proline was injected into the right locus coeruleus and the CN were analyzed by the autoradiographic procedure.

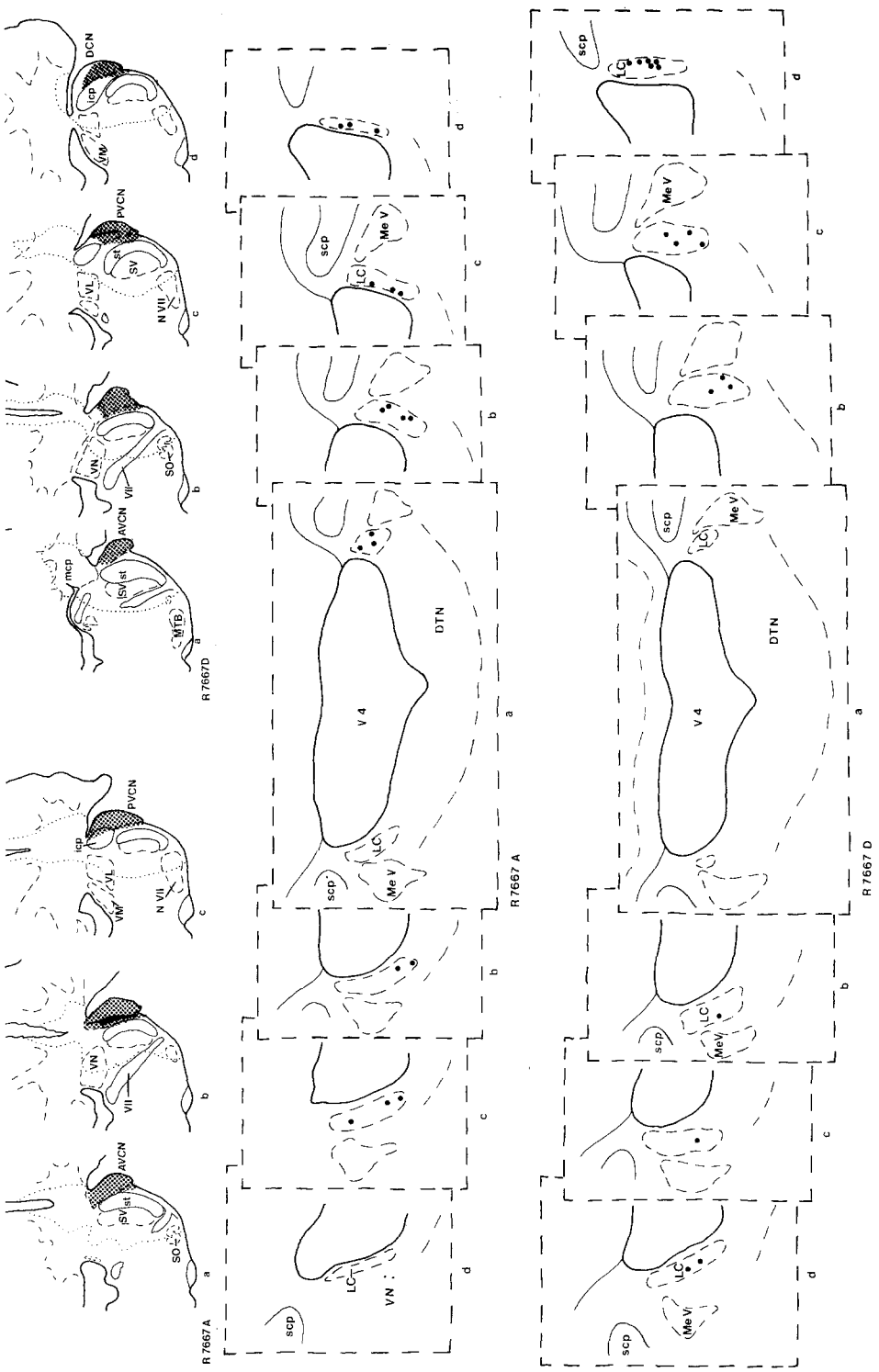
Histochemical Analysis of Lesion Experiments. Normal fluorescence histochemical material indicates that preterminal NE fibers enter the CN via two separate pathways. A rostral bundle enters the AVCN and a caudal bundle enters the PVCN and DCN. To further identify these pathways knife lesions were made through either of the NE bundles and after 2–13-day survival periods the region was prepared for fluorescence histochemical analysis.

Both transverse and sagittal cuts made through the caudal bundle, at a level which was immediately rostral to the DCN but caudal to the locus, produce an accumulation of catecholamines in the proximal portions of the CA axons located between the lesion site and the LC. Sagittal cuts through the rostral bundle along the medial aspect of the AVCN produce swollen CA fibers which are oriented in a medial to lateral direction. These fibers are located between the middle cerebellar peduncle and the spinal trigeminal tract and along the dorsal and caudal borders of the motor trigeminal nucleus. The general trajectory of the fibers suggests that they arise from the rostral region of the locus coeruleus.

These lesions also provide information about the distribution of the fibers within the various regions of the CN. With survival times of over ten days

Abbreviations. as acoustic stria; *AVCN* anteroventral cochlear nucleus; *CA* catecholamine; *CCB* caudal coeruleo-cochlear bundle; *cn* cochlear nerve; *CN* cochlear nucleus; *DA* dopamine; *DCN* dorsal cochlear nucleus; *DTN* dorsal tegmental nucleus; *FCL* fusiform cell layer; *HRP* horseradish peroxidase; *icp* inferior cerebellar peduncle *I–V* divisions of the ventral cochlear nucleus according to Harrison and Irving (1965, 1966); *LC* locus coeruleus; *mcp* middle cerebellar peduncle; *MeV* mesencephalic nucleus of the trigeminal nerve; *ML* molecular layer; *MTB* medial nucleus of the trapezoid body; *N VII* nucleus of the seventh cranial nerve; *NE* norepinephrine; *PCL* polymorphic cell layer; *PVCN* posteroventral cochlear nucleus; *RCB* rostral coeruleo-cochlear bundle; *scp* superior cerebellar peduncle; *SO* superior olive; *st* spinal trigeminal tract; *SV* spinal nucleus of the trigeminal nerve; *tb* trapezoid body; *VII* facial nerve; *V4* fourth ventricle; *VCN* ventral cochlear nucleus; *VL* lateral vestibular nucleus; *VM* medial vestibular nucleus; *vn* vestibular nerve; *VN* vestibular nuclei; *6-OHDA* 6-hydroxydopamine

Fig. 1a–d. HRP injections for animals R7667A and D. *Top:* Location of injection site for each animal. The dense zone of the injection is indicated by the shaded region, whereas the border of the peripheral zone is demonstrated by dotted lines. *Bottom:* Coronal sections (**a–d** rostral-caudal) through the region of the locus coeruleus for each animal. The locations of HRP-positive neurons are indicated by filled circles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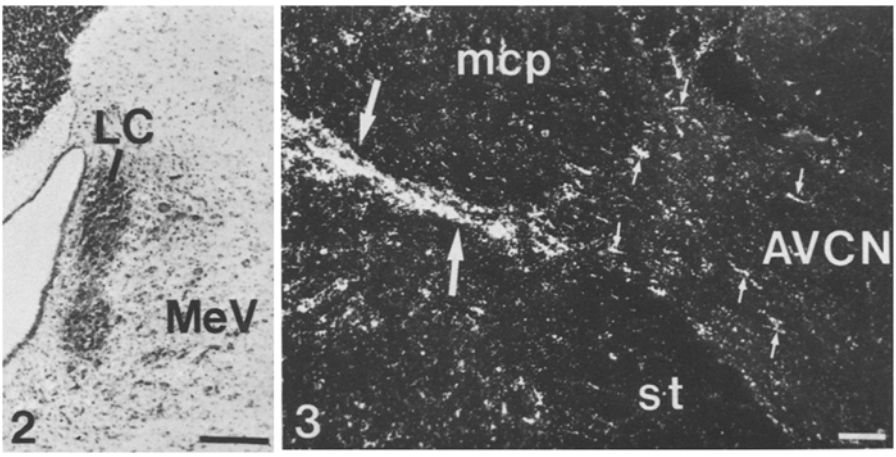


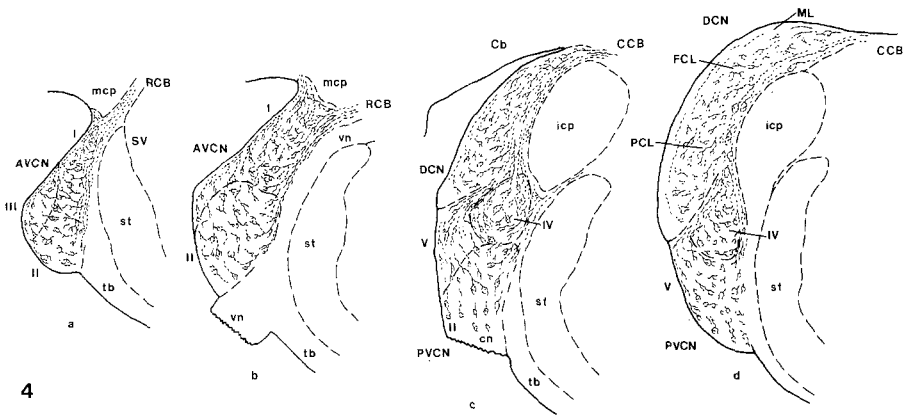
Fig. 2. Locus coeruleus injection site for animal 76-78D. Densely labeled neurons are located primarily within the locus (*LC*) although some neurons in the mesencephalic trigeminal nucleus (*MeV*) also contain isotope. One week exposure time. Coronal section, scale equals 100 μm

Fig. 3. Dark field autoradiograph of a coronal section through the rostral AVCN of animal 76–78D. A dense accumulation of silver grains is present in the region of the rostral coeruleo-cochlear bundle (*large arrows*) which is located ventral to the middle cerebellar peduncle (*mcp*) and dorsal to the spinal trigeminal tract (*st*). Short labeled axonal segments (*small arrows*) are also present within the *AVCN*. One month exposure, scale equals 50 μm

there is a differential decrease in the density of CA fibers in the AVCN, PVCN and DCN dependent upon whether the rostral or caudal bundle is transected. These experiments demonstrate that the DCN receives its NE innervation via the caudal bundle. Regions IV of V of the PVCN also appear to receive their innervation predominantly via the caudal bundle. In contrast, regions I and III of the AVCN are innervated almost solely from the fibers in the rostral NE bundle. Since no single lesion removes all the fibers in region II of the AVCN and PVCN it seems likely that this area contains NE fibers which arise from both the rostral and caudal bundles.

Autoradiography – Locus Coeruleus Injections. Localized labeling of the right locus coeruleus was obtained in two injections which produced similar distributions of silver grains within the brain stem. Only animal 76–78D will be described here.

Figs. 4–6. Schematic representation of the NE innervation in the *AVCN, PVCN* and *DCN*. **4a–d.** In coronal sections NE fibers enter the *AVCN* via the rostral coeruleo-cochlear bundle (*RCB*) and the *PVCN* and *DCN* via the caudal coeruleo-cochlear bundle (*CCB*). **5a–c.** Sagittal sections of the cochlear nuclei (**a–c** lateral-medial). Within the ventral regions of the VCN most NE fibers are oriented parallel to either the ascending and descending branches of the cochlear nerve (*cn*) or to the fibers of the trapezoid body (*tb*). Some locus fibers in the *DCN* are continuous with those in the dorsomedial *AVCN* and caudal *PVCN*. $R \rightarrow C$ indicates the rostral to caudal axis. **6a–d.** In horizontal sections NE fibers from the locus coeruleus (*LC*) can be traced directly into the acoustic striae and *DCN*. At a more ventral level (**b**) the *RCB* appears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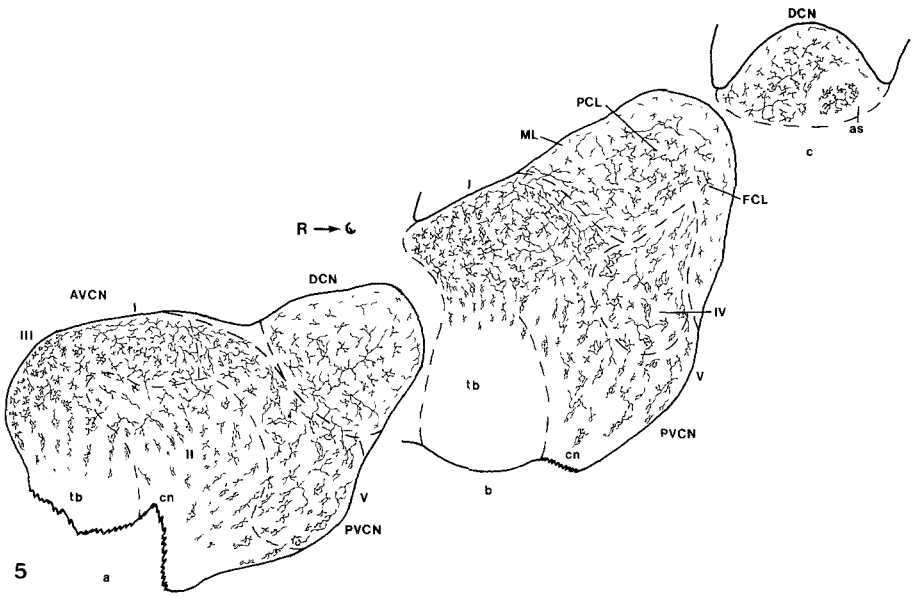
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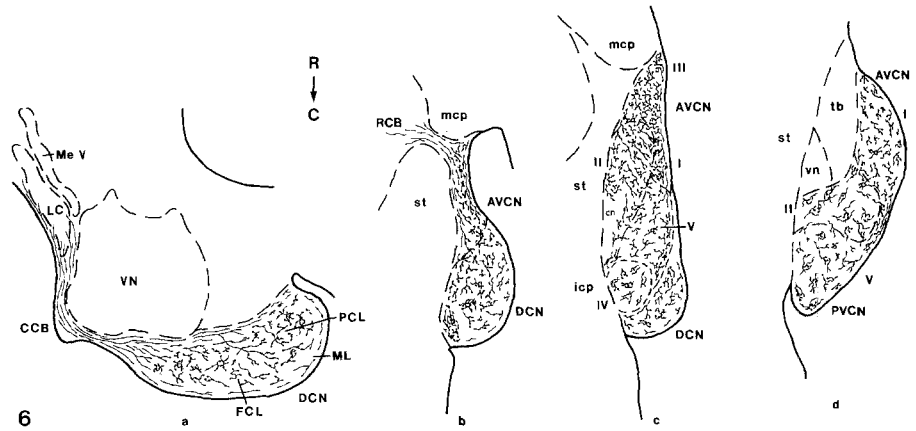
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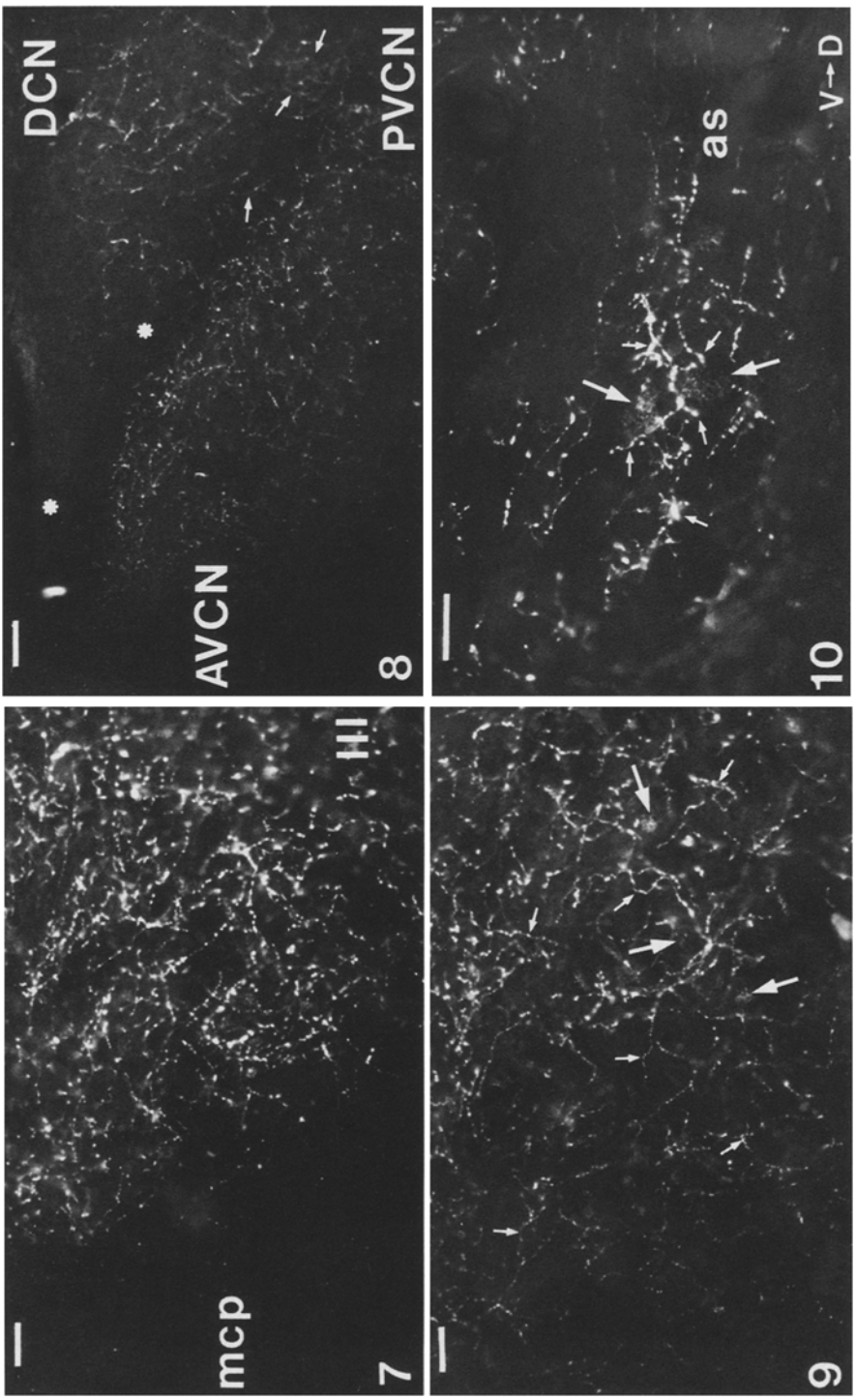
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The densest labeling of neurons for injection 76–78D occurs in the caudal to mid-levels of the compact portion of the right locus coeruleus and within some neurons located in the mesencephalic trigeminal nucleus (Fig. 2). Several heavily labeled fiber bundles are identified in the vicinity of the right locus coeruleus and at more caudal brain stem levels. In addition to the fibers which arise from the mesencephalic nucleus and project within the tract of this nucleus, a heavily labeled descending projection from the LC is apparent in the region of the right central tegmental tract. Some labeled axons, at the level of the rostral LC, decussate in the pontine gray ventral to the medial longitudinal fasiculus. These fibers project laterally to the dorsal region of the spinal trigeminal nucleus and into the AVCN.

Both CN have grain densities which are above background although this difference is small for the contralateral nuclei. Within both the ipsilateral and contralateral CN the majority of silver grains are evenly dispersed throughout the nuclei. A consistent feature between sides is the accumulation of grains in the area between the middle cerebellar peduncle and the spinal trigeminal nucleus and tract in the region of the rostral coeruleo-cochlear bundle (Fig. 3). The density of grains in the ipsilateral and contralateral PVCN is less than that observed in the AVCN. At the level of the rostral DCN, labeled fibers are very prominent within the dorsal acoustic stria as it curves around the inferior cerebellar peduncle. These fibers are most numerous in the ipsilateral DCN both within the acoustic stria and the deeper regions of the nucleus. Some fibers continue within the acoustic stria to reach the caudal PVCN. In the ipsilateral DCN labeled axons can be traced to the region of the fourth ventricle and probably represent locus coeruleus fibers of the caudal coeruleo-cochlear bundle.

Morphology of the Norepinephrine Innervation

In an earlier fluorescence histochemical study (Kromer and Moore, 1976), we reported that the CN: 1) possess no NE perikarya, 2) contain fluorescent fibers

Fig. 7. Dense distribution of NE fibers within region *III* of the AVCN along its border with the middle cerebellar peduncle (*mcp*). Sagittal section through approximately the same level as 5b, scale equals 30 μm. The left side of Figs. 7, 8, and 9 is rostral and the right is caudal

Fig. 8. Sagittal section illustrating the lack of NE fibers along the dorsolateral border (*asterisk*) of the *AVCN* and *DCN* (same level as Fig. 5a). However, some fibers (*arrows*) enter the caudal *PVCN* from the *DCN*, scale equals 50 μm

Fig. 9. Branching NE fibers in the caudal portion of the PVCN (region V in Fig. 5a). Most of the varicose fibers (*small arrows*) are located in the neuropil and not directly adjacent to neuronal perikarya (*large arrows*). Sagittal section, scale equals 30 μm

Fig. 10. Pericellular plexuses of NE fibers (*small arrows*) located among the large neurons (*large arrows*) situated within the intermediate acoustic stria (*as*). Sagittal section, $V \rightarrow D$ indicates the ventral to dorsal axis, scale equals 30 μm

in all subdivisions of the nuclei, and 3) receive NE-containing axons via a rostral bundle to the AVCN and a caudal bundle to the DCN and PVCN. Since our present results indicate that the NE projection to the CN originates in the LC, these two pathways will be termed the rostral coeruleo-cochlear bundle (RCB) and the caudal coeruleo-cochlear bundle (CCB).

The present section is designed to further clarify our earlier descriptions of the NE projection to the CN by combining the information obtained from observations of fluorescence histochemical material sectioned in the coronal, sagittal and horizontal planes (Figs. 4, 5 and 6, respectively). All subdivisions of the CN (as described by Harrison and Irving, 1965, 1966) receive locus coeruleus fibers; however, the different subdivisions do not contain the same density of fluorescent fibers. Regions III and I of the AVCN receive the densest NE innervation followed by regions IV and V of the PVCN and the polymorphic and fusiform cell layers of the DCN. Region II of the VCN has a sparser innervation and the molecular layer of the DCN contains even fewer NE axons. No fluorescent fibers are evident within the densely myelinated portions of the cochlear and vestibular nerves or the trapezoid body.

Regions I and III of the AVCN and the dorsal region of the entire VCN appear to have the greatest density of NE fibers. Many fluorescent fibers are organized into stellate or bush-like clusters scattered throughout the neuropil close to neuronal perikarya. Along the medial edge of the VCN, faintly fluorescent preterminal fibers of the RCB enter the rostral tip of the VCN. Upon entering the AVCN the RCB fibers lose their preterminal appearance and project in a rostrocaudal direction along either the medial or lateral edge of the nucleus. No clear demarcation between the distribution of NE fibers in the dorsomedial VCN and the anteroventral DCN is evident since some fluorescent fibers from the DCN enter the dorsomedial edge of the caudal VCN.

Throughout the lateral region of the VCN the densest distribution of NE fibers is present immediately below the dorsal surface of the nucleus. Within the rostrodorsal region of the AVCN (regions I and III), dense clusters of NE fibers are scattered throughout the neuropil (Fig. 7). The longer fibers in this network often have rostrocaudal orientations and appear to traverse the AVCN-PVCN border.

In the ventral regions of the AVCN the appearance of the NE fibers differs from the rostral to the caudal extent of the nucleus. In the anteroventral areas (region III), the myelinated fibers of the trapezoid body are organized into fascicles which are separated by groups of neurons that receive dense pericellular plexuses of varicose fibers. In slightly more caudal levels (region II), some of the fluorescent fibers are oriented parallel to the trapezoid body whereas other fibers are oriented parallel to the ascending and descending branches of the cochlear nerve. Region II of the PVCN appears continuous with region II of the AVCN with respect to both cell types and the distribution and morphology of its NE innervation. A variety of neuronal types are present within both areas (Harrison and Irving, 1965, 1966) and all autofluorescent neurons appear to receive some perisomatic and peridendritic plexuses of NE fibers.

Within the dorsal PVCN, a dense plexus of fibers is located along the dorsal border above the bifurcation of the cochlear nerve, which contains no

fluorescent axons. Further caudally in region V, the densest accumulation of fibers is located beneath the granule cell cap which contains few NE fibers and separates the lateral PVCN from the DCN (Fig. 8). In contrast, the caudal most border of these two nuclei is traversed by many NE fibers which are located within the intermediate acoustic stria. These fibers have many collaterals, which are distributed among the neurons situated between the descending branches of the cochlear nerve (Fig. 9). The preterminal NE fibers in the intermediate acoustic stria also give rise to a dense pericellular plexus of fibers that possess large varicosities which are closely apposed to both the perikarya and proximal dendrites of the large multipolar neurons located within the stria and region IV of the PVCN (Fig. 10).

The DCN and caudal PVCN receive a NE innervation from the LC via the CCB. Occasionally a displaced LC neuron can be observed along this path before it reaches the DCN. At the level of the DCN and PVCN, locus fibers in the CCB which project to the PVCN travel in the intermediate acoustic stria (stria of Held) whereas those to the DCN are located in the dorsal acoustic stria. As the NE fibers leave the acoustic stria and travel laterally through the DCN they branch repeatedly, sending collaterals into all areas of the DCN. The deep or polymorphic cell layer of the DCN contains the richest distribution of NE fibers, followed by the fusiform cell layer. The majority of fluorescent fibers in both cell layers do not appear to have any particular orientation. However, some axons are arranged in ringlets, or have stellate and fork-like branching patterns. The few, faintly fluorescent axons that are located within the molecular layer are frequently oriented parallel to the surface of the nucleus and to the fusiform cell layer.

Discussion

Identification of the Catecholamine Neurons which Project to the Cochlear Nuclei

Although there are several groups of CA-containing neurons within the brain stem of the rat, it is our conclusion that only the NE neurons of the locus coeruleus project to the cochlear nuclei. Several different experimental results support this. The catecholamine assay demonstrates that the CN contain a moderate concentration of NE with a very low DA content which probably represents a precursor pool within the NE terminals. Both our lesion and HRP experiments indicate that the LC is the source of this NE projection. This is further supported by the autoradiographic experiments.

All lesion experiments which do not involve the destruction of the locus coeruleus result in no significant change in the NE content of the cochlear nuclei. However, some non-locus lesions do produce measurable decreases in NE levels. Both the unilateral lateral tegmental CA lesion and the lesion lateral to the locus coeruleus produce some decreases in the NE content of the ipsilateral CN. Fluorescent observations of both lesions indicate that there is some injury to the NE fibers located in the rostral coeruleo-cochlear bundle. This could account for the slight decreases in NE that are observed.

Bilateral lesions of the rostral portion of the locus do not produce a significant change in the NE concentration of the CN although there is a slight decrease in NE in these nuclei. In contrast, the fluorescence histochemical experiments indicate that the cochlear nuclei are almost totally devoid of NE fibers following a complete bilateral locus lesion, although the biochemical experiments indicate that 21% of the NE content is still present.

The cochlear nuclei present a special problem when analyzing the lesion experiments, since they receive NE fibers via two pathways. Fibers in the rostral NE bundle arise as collaterals of the ascending axons from the locus neurons, whereas the fibers in the caudal NE bundle are part of the descending axonal system. If a single neuron possesses axon collaterals in both pathways, then destruction of only one pathway could result in an increased transport of NE and its synthesizing enzymes in the remaining axonal projection. Therefore, a decrease in the number of NE fibers in the CN caused by a partial lesion of the coeruleo-cochlear fibers would be masked in the biochemical experiments because of an increased NE concentration in the remaining fibers. This would be particularly true for a partial lesion of the rostral LC where only the ascending projections are destroyed.

The HRP experiments (which labeled predominantly the VCN) indicate that there is a bilateral projection from the locus coeruleus to the CN with two to three times more neurons located in the ipsilateral nucleus. Labeled neurons are identified throughout the dorsoventral and rostrocaudal axes of the compact portion of the locus coeruleus and do not have any specific topographical distribution. However, the morphology of reactive cells suggests that fusiform cells provide the main projection to the CN although some multipolar neurons are also labeled.

Some HRP-positive neurons are also present in the vicinity of the ipsilateral A5 cell group. These cells lie immediately adjacent to the superior olivary complex and probably represent cholinergic periolivary cells giving rise to the olivo-cochlear bundle, since lesions in this region do not produce a significant change in the NE content of either the ipsilateral or contralateral CN.

Pathways of the Coeruleo-Cochlear Projections

Two ipsilateral coeruleo-cochlear pathways have been identified. The rostral coeruleo-cochlear pathway to the AVCN originates at the rostral tip of the locus and projects laterally through the brain stem dorsal to the motor trigeminal nucleus. The locus fibers then coalesce into a bundle that projects between the spinal trigeminal nucleus and tract and the middle cerebellar peduncle to enter the anterodorsal AVCN. Descending fibers of the caudal coeruleo-cochlear bundle leave the caudal end of the LC and travel between the ependymal covering of the fourth ventricle and the vestibular nuclei. At the level of the dorsal cochlear nucleus, these fibers curve laterally and enter the acoustic striae.

Contralateral locus fibers reach the CN via two additional pathways. In the autoradiographic studies some labeled axons decussate ventral to the medial longitudinal fasciculus at the level of the rostral locus coeruleus and join the

contralateral rostral coeruleo-cochlear bundle. A second group of decussating locus fibers is observed in fluorescence material. These preterminal axons project from the locus through the pontine central grey along the rostral tip of the fourth ventricle. After decussating, the fibers turn caudally and pass between the wall of the fourth ventricle and the contralateral locus coeruleus to enter the descending axonal system and the acoustic striae.

The only other study dealing with the projections of CA fibers to the cochlear nuclei is the work of Swanson and Hartman (1975). These authors reported that dopamine-β-hydroxylase (DBH)-positive fibers course through the principal sensory trigeminal nucleus to reach the rostral VCN. The present study also identified a similar pathway of NE fibers into the rostral AVCN. However, no such efferent pathway to the VCN has been reported by other investigators (Held, 1893; Ramón y Cajal, 1909; Lorente de No. 1933; Rasmussen, 1960, 1967; Cant & Morest, 1978). In addition to the rostral bundle, a caudal group of NE fibers leaves the locus coeruleus and enters the acoustic striae. These fibers travel within the dorsal acoustic stria to innervate the DCN and within the intermediate stria to reach the PVCN. Although Swanson and Hartman (1975) do not specifically identify this pathway, their diagrams indicate that DBH-positive fibers are located within the striae. The only reference in the classical literature to efferent fibers within the acoustic striae is the study of Held (1893) in which an efferent projection to the bipolar neurons in the DCN was reported. However, Held's Golgi-stained fibers most probably are not NE axons since they lack the latter's widespread distribution throughout the DCN.

Distribution of Locus Coeruleus Fibers within the Cochlear Nuclei

The DCN receives the majority of its NE input via the caudal coeruleo-cochlear bundle which enters the dorsal acoustic stria. When the stria is transected there is a depletion of all fluorescent fibers within the dorsal and medial regions of the DCN and a nearly complete loss of fibers in the anteroventral tip of the nucleus. The few fibers which remain in the polymorphic cell layer appear continuous with fibers in the dorsal VCN. The coeruleo-cochlear fibers which project to the DCN arise from both the ipsilateral and contralateral nuclei with the majority being ipsilateral. There is no specific regional distribution of either the ipsilateral or contralateral projections, instead, both nuclei contribute fibers to all regions of the DCN. All morphological types of neurons could receive a NE input since fluorescent varicosities are observed juxtaposed to somatic and dendritic profiles of most neurons. The morphology of NE fibers within the DCN of the rat suggests that these axons may correspond, at least in part, to the type 5 and 6 axons described in a Golgi study of the DCN of the cat (Kane, 1974).

The NE innervation of the PVCN arises bilaterally in the locus coeruleus with both nuclei projecting to all regions of the PVCN. Most of these fibers reach the nucleus via the intermediate acoustic stria since a transection of the stria produces a loss of NE fibers within the region IV and the caudal area of region V. A majority of fluorescent fibers in the remaining areas of

region V and the caudal area of region II are also destroyed. The NE fibers within the PVCN form both peridendritic and perisomatic plexuses. The latter are most prevalent on the multipolar neurons of region II and V, and on the large cells of region IV. The NE varicosities may correspond to some of the small ring-like endings of Harrison and Irving (1966) which remain after destruction of the cochlear nerve.

All subdivisions of the AVCN also receive a bilateral projection from the locus coeruleus. LC fibers reach region III and the rostral area of region I via the rostral coeruleo-cochlear bundle since a transection of this bundle produces a nearly total depletion of fluorescent fibers within these areas. Many NE axons originating in the rostral bundle may correspond to a portion of the thin (0.1–1.0 μm) multiply branched group III axons described in the Golgi study of the feline cochlear nucleus by Cant and Morest (1978). Region II and the caudal aspect of region I also receive a significant projection of NE fibers from the caudal coeruleo-cochlear bundle since many fluorescent fibers remain after a transection of the rostral bundle. These NE fibers may correspond to some of the "endogenous" fibers of Lorente de No (1976) and to the group IV non-cochlear axons described by Cant and Morest (1978). The thin varicose group IV axons (diameter 1.0 μm) of the latter study also emerge from the DCN to form perisomatic terminals on neurons in the AVCN.

Unlike the cochlear nerve fibers which form a specific topographic organization within the cochlear nuclei, the coeruleo-cochlear afferents project to diffuse areas of the CN. Therefore, a single NE axon probably contacts neurons with a wide range of characteristic frequencies. This non-specific type of projection is quite similar to the diffuse coeruleo-geniculate projection described by Kromer and Moore (1979). Both the lateral geniculate and cochlear nuclei, which are primary centers for the integration of sensory input to the central nervous system, receive diffuse and overlapping bilateral projections from the locus coeruleus. Moreover, the morphology and locations of labeled LC neurons are similar following HRP injections into the lateral geniculate and cochlear nuclei, suggesting that the same or neighboring neurons may project to both areas. Although the function of the locus coeruleus innervation of these two sensory nuclei is not well understood, the projection to the lateral geniculate nuclei is implicated in the generation of ponto-geniculo-occipital activity during sleep (Laurent et al., 1974). Likewise, the coeruleo-cochlear projection may also be involved in sleep related alterations of the spontaneous or evoked activity of neurons in the cochlear nuclei.

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