THE JOURNAL OF COMPARATIVE NEUROLOGY 202:397-414 (1981)

Sprouting in the Avian Brainstem Auditory Pathway: Dependence on Dendritic Integrity

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ABSTRACT The brainstem auditory pathways of the chicken were used to examine the relationship between the maintenance of dendrites following denervation and the successful reinnervation (sprouting) by surviving afferents. In the system the third-order cells in n. laminaris receive spatially segregated binaural innervation from n. magnocellularis. Afferents from the ipsilateral n. magnocellularis innervate the dendrites on the dorsal aspect of n. laminaris cells, while afferents from contralateral magnocellular neurons innervate ventral dendrites via the crossed dorsal cochlear tract. Denervation of the ventral dendrites of n. laminaris cells by transection at the midline results in rapid and severe atrophy of the denervated dendrite. Unilateral cochlea removal induces transneuronal degeneration of 30–45% of the ipsilateral magnocellular cells, thereby partially denervating one dendrite of the n. laminaris cells on each side of the brain.

In animals with long-standing transections of the crossed dorsal cochlear tract there is no evidence of sprouting of fibers from the ipsilateral n. magnocellularis when the projections of the surviving magnocellular neurons are traced with degeneration methods after a secondary cochlea removal. However, when dendrites of n. laminaris are *partially* denervated dendrites do not disappear. Furthermore, secondary lesions of the crossed dorsal cochlear tract or secondary cochlea removal reveal that these denervated dendrites are reinnervated by the afferents from the opposite n. magnocellularis which are normally restricted to the opposite dendrite of the n. laminaris cells.

The phenomenon of sprouting following deafferenting lesions has generated widespread interest, in part because it may provide a means of exploring growth and structural modifications of neurons in the central nervous system. A number of issues remain to be resolved, however, about the principles governing the afferent rearrangements and underlying mechanisms. Among these issues is the role of the postsynaptic cell's receptive surface. For example, it has been proposed that the extent of sprouting is regulated in part by the availability of denervated sites which can be reinnervated. This proposal arises in part from the observation that the *extent of sprouting* is usually correlated with the *extent of the denervation* produced. Yet in most cases greater denervation (more available sites) is accompanied by more extensive degeneration of presynaptic elements, along with more dramatic glial and other responses to the denervation, any or all of which may be a regulative factor. The role of the postsynaptic cell in regulating sprouting might be readily resolved if there were a preparation in which the amount of denervated membrane available for reinnervation could be manipulated independently of the extent of degeneration of presynaptic elements and of other responses to deafferentation. While the ability to directly manipulate the number of sites available for reinnervation without varying the other deafferentation responses seems difficult to conceive, we feel that an initial approximation is provided by the auditory pathways of the chicken. In this preparation the number of available postsynaptic sites can be varied to some extent independently of the extent of presynaptic degeneration and of the glial responses to the denervation.

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0021-9967/81/2023-0397$05.00

The possibility of manipulating sites available for reinnervation comes about because the maintenance of dendrites in the third-order auditory nucleus, n. laminaris (NL), depends on the integrity of its afferent input. In birds the cells of this nucleus possess bipolar dendrites and receive spatially segregated binaural innervation. The dorsal dendrites of n. laminaris receive input from the ipsilateral second-order auditory relay nucleus (n. magnocellularis) while dendrites on the other side of the NL cells receive matching inputs from the contralateral n. magnocellularis (Rubel and Parks, '75; Parks and Rubel, '75). When the inputs from n. magnocellularis are destroyed, the denervated dendrites in n. laminaris undergo rapid and extensive atrophy; in this situation, there has been no evidence of reinnervation of the dendrites through sprouting of the inputs terminating on the opposite side of the cell (Benes et al., '77). Similar findings have been reported for the presumed mammalian homologs of these nuclei, the anteroventral cochlear nucleus (AVCN) and the medial superior olive (MSO) by Liu and Liu ('71) and White and Nolan ('74).

It is possible however, to partially denervate the dendrites of n. laminaris by removing the cochlea, and consequently induce transneuronal degeneration of some of the cells of n. magnocellularis (Jackson and Rubel, '76). A similar phenomenon occurs following cochlea removal in mammals (Trune, '79). In this situation, the dendrites appear to be preserved for the most part, thus presumably preserving sites available for reinnervation. These findings present an interesting situation in which there is massive presynaptic degeneration after nearly complete destruction of the inputs to one set of dendrites, but few or no available sites. Partial denervation, on the other hand, results in less presynaptic degeneration but presumably a greater number of denervated sites available for reinnervation as a result of dendritic preservation. In the present study, we investigate whether sprouting of axons from the opposite nucleus magnocellularis occurs when the dendrites of nucleus laminaris cells are partially denervated. In this way we hope to begin defining the roles of presynaptic and postsynaptic elements in the regulation of sprouting.

METHODS

Subjects

Over 40 domestic chickens (Hubbard × Hubbard) served as subjects. Eggs obtained from a commercial hatchery were incubated and hatched in the laboratory; the chicks were maintained in brooding cages until 25–35 days of age, and then housed in pairs.

Experimental groups

Five experimental treatment groups were used in this investigation.

Tract cut (TC). Contralateral innervation reaches the ventral dendrites and ventral half of the nucleus laminaris cell bodies via the crossed dorsal cochlear tract. In subjects of this group, the crossed dorsal cochlear tract was transected at the midline in order to assess the effects on the dendrites of n. laminaris cells using the Golgi method. Chicks survived 8 or 16 days postsurgery, and the brainstems were processed by the Stensaas modification of the Golgi-Kopsch method (Stensaas, '67).

Cochlea removal (CR). The projections from nucleus magnocellularis were traced by taking advantage of the partial degeneration of these cells following cochlea removal (Jackson and Rubel, '76; Parks and Rubel, '78). Both the optimal and survival time for Fink-Heimer staining the time course of removal of degeneration debris were determined by removing one cochlea in 12 chicks 10–15 days post-hatch. Following survival intervals of 7, 14, 21, or 45 days, the animals were deeply anesthetized and perfused with 10% formalin. The brains were sectioned at 30-μm on a freezing microtome. Sections were processed to show terminal degeneration by several modifications of the Fink-Heimer (Fink and Heimer, '67) method, and alternate sections were stained with cresyl violet or thionin. An additional four chicks survived 28 days and the brains were impregnated by the Stensaas ('67) modification of the Golgi-Kopsch method to assess the dendrites in n. laminaris.

Tract cut-cochlea removal (TC-CR). To determine whether sprouting of the magnocellular axons which normally innervate the dorsal dendrites of n. laminaris occurs following complete denervation of ventral dendrites, the crossed dorsal cochlear tract was sectioned at the midline in 10-day post-hatch chicks. After 3 months, a unilateral cochlea removal was performed, and the subjects were allowed to survive for 14 days. The brains were then processed by several modifications of the Fink-Heimer method. Alternate sections were stained with cresyl violet. Comparisons were made with tissue from control animals which sus-

tained cochlea removal at 3 months of age and survived for 14 days postlesion.

Cochlea removal-tract cut (CR-TC). This group represented the primary experimental group used to determine if the axons which normally innervate only the ventral side of the n. laminaris cell lamina can be induced to reinnervate the dorsal dendritic region which is normally innervated by the ipsilateral n. magnocellularis. At 10 days post-hatch one cochlea was removed, resulting in transneuronal degeneration of ipsilateral n. magnocellularis cells. This results in partial deafferentation of the dorsal n. laminaris dendrites on the ipsilateral side and of the ventral n. laminaris dendrites on the contralateral side of the brain. The animals survived for 60–75 days to allow for clearing of degeneration from the primary lesion. The crossed dorsal cochlear tract was then transected at the midline. The chicks survived 2–8 days following the secondary lesion and were perfused with 10% formalin. The brainstems were processed to observe degenerating axons and terminals by several variants of the Fink-Heimer method. Alternate sections were stained with cresyl violet.

Cochlea removal-cochlea removal (CR-CR). This series of manipulations takes advantage of the transneuronal terminal degeneration in n. laminaris produced by cochlea removal in order to examine the possibility of ectopic innervation of n. laminaris on both sides of the brain. An initial cochlea removal was performed at 10 days of age, and 70–80 days later (to allow for clearing of the degeneration from the primary lesion) the remaining cochlea was removed. Fourteen days after the secondary lesion, animals were perfused with 10% formalin or with a 2% glutaraldehyde-2% paraformaldehyde mixture. Formalin-fixed tissue was processed for staining by variants of the Fink-Heimer procedure. Glutaraldehyde-fixed tissue was osmicated, embedded in plastic, and 1 μm semithin sections were cut and stained with toluidine blue.

Surgical procedures

All surgical manipulations were performed under aseptic conditions. Chicks were anesthetized with 1.5 cc/kg of body weight (I.P.) of Chloropent (Fort Dodge Labs.) potentiated with 80 mg/kg of body weight (I.M.) Ketamine hydrochloride (Ketalar, Park Davis). Cochlea removals were performed under an operating microscope. The tympanic membrane was reflected and the columella extracted. Through the oval window, the membranous cochlea was then grasped with fine forceps and extracted intact. The duct and middle ear were packed with gelfoam and the meatus was closed with cyanoacrylic adhesive. This operation is rapid, results in little or no bleeding, and leaves the vestibular labyrinth intact. To be assured that the entire basilar papilla was removed it was floated on water and examined under the operating microscope.

To transect the crossed dorsal cochlear tract the anesthetized subject was mounted in stereotaxic ear bars, and an incision was made above the juncture of the skull and first cervical vertebra. The neck muscles were retracted down to the foramen magnum and a microscalpel was inserted through the foramen magnum into the fourth ventricle and then rotated to make a 1.0–1.5 mm transection at the floor of the ventricle along the midline. After withdrawing the microscalpel, the posterior sinus was packed with gelfoam and the wound was closed with cyanoacrylic adhesive.

Golgi analysis

The analytic methods used for measuring dendrites have been previously described (Smith and Rubel, '79; Smith, '81). Briefly, fully impregnated cells from throughout n. laminaris were traced as "stick figures" under a camera lucida at a total magnification of × 1,325 or × 3,330. The total length of the dorsal and ventral dendritic arbors were *each* measured using an electronic planimeter; these length measurements are highly correlated with dendritic surface area ($r = 0.96$; Smith and Rubel, '79). Dendritic size data from the experimental chicks were compared with data from normal 25-day post-hatch animals prepared in our laboratories using the same methods (Smith, '81).

RESULTS

Figure 1 illustrates the brainstem auditory pathways of the chick. The focus of the present study (nucleus laminaris) is comprised of a sheet of neurons with dendrites radiating bidirectionally from the soma (dorsally and ventrally). The dendritic ramifications and associated afferents form a neuropil on each side of the line of cell bodies which may be clearly seen in Nissl-stained sections (Fig. 1B). This neuropil is relatively free of glial or other cell bodies, but is bounded by a glia rich zone on each side just distal to the tips of the dendrites. The configuration of the dendrites of NL neu-

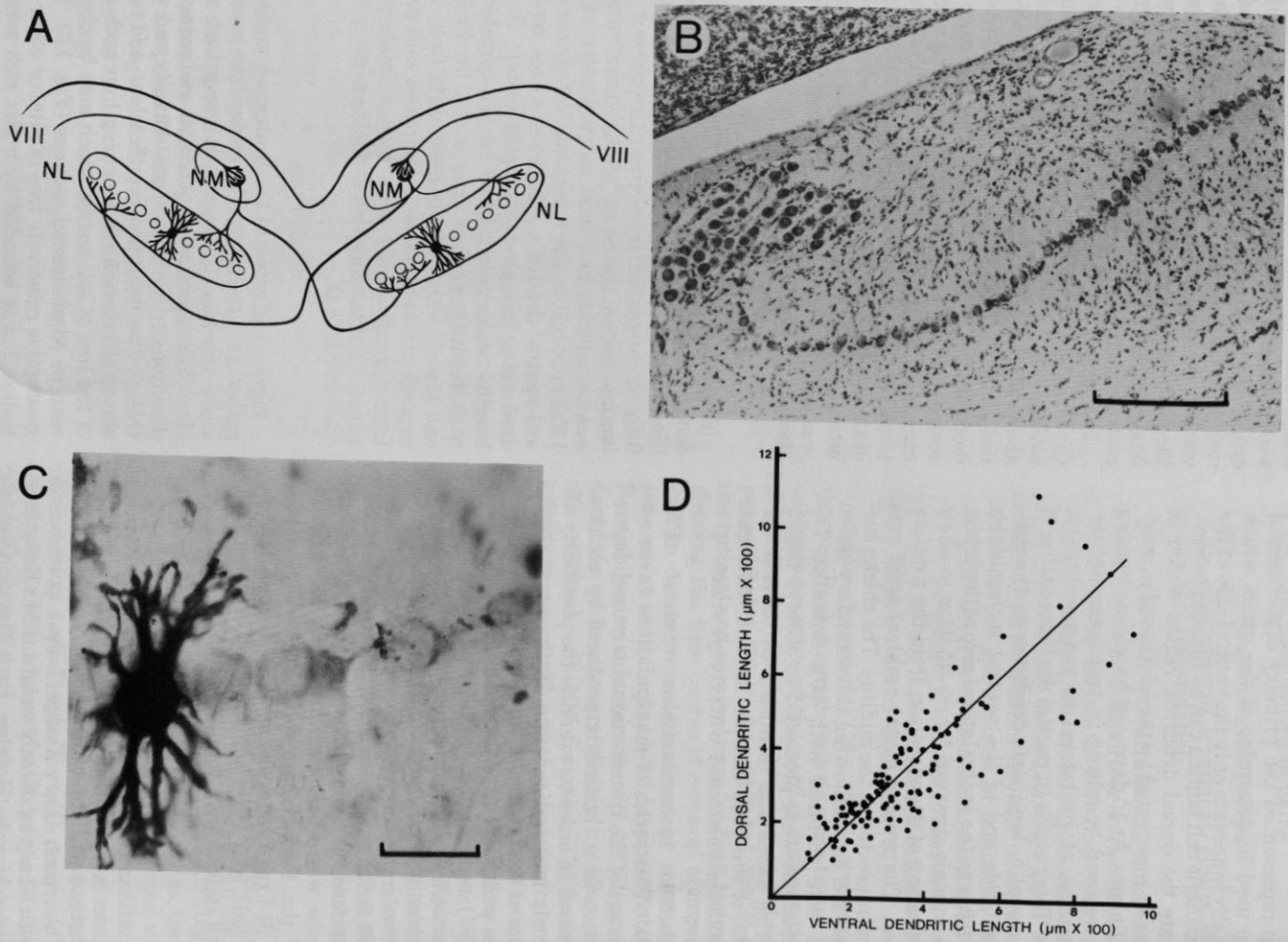
A
VIII
NL
NM
NM
VIII
NL
B
C
D
DORSAL DENDRITIC LENGTH (μm X 100)
VENTRAL DENDRITIC LENGTH (μm X 100)
0
2
4
6
8
10
12

Figure 1

rons varies as a function of the cell's position in the nucleus, with cells in the anterolateral regions possessing longer dendrites (Smith and Rubel, '79). Throughout the nucleus, the lengths of the dorsal and ventral dendrites are highly correlated, with the line of best fit being essentially unity (Fig. 1D). As illustrated diagrammatically in Figure 1A, the two sets of dendrites receive binaurally segregated inputs form the two magnocellular nuclei (NM); the input from the ipsilateral n. magnocellularis terminates in the dorsal neuropil, while the inputs from the contralateral n. magnocellularis terminate in the ventral neuropil after crossing the midline in the crossed dorsal cochlear tract.

The projections of n. magnocellularis onto n. laminaris can be traced in two ways. First, the distribution of the crossing pathways can be traced following transection of the crossed dorsal cochlear tract at the midline (group TC) by staining the tissue with the Fink-Heimer method (see Fig. 2). In this situation, selective degeneration of the crossed pathways to the ventral neuropil is induced, as illustrated in Figure 2B and C. In normal animals, this degeneration is symmetrical on the two sides of the brain, and is virtually completely restricted to the ventral dendritic surface of the n. laminaris cells (see Fig. 2C; Parks and Rubel, '75).

The second way of tracing these projections to n. laminaris is by transneuronal degeneration of n. magnocellularis neurons induced by cochlea removal. After removal of the cochlea, the cells of n. magnocellularis undergo a severe transneuronal degeneration, the severity of which depends on the nature of the cochlea removal. Although the lesions produced in n. magnocellularis by this manipulation are partial, resulting in the degeneration of 30–45% of magnocellular neurons (Jackson and Rubel, '76), the degeneration of axons and terminals in n. laminaris can be easily traced with the Fink-Heimer method. Figure 3 illustrates the crossing projections to the ventral neuropil of the contralateral n. laminaris (Fig. 3C,E) and the projections to the dorsal neuropil of n. laminaris ipsilateral to the cochlea removal (Fig. 3B,D). We should point out that an underlying assumption of this analysis is that the cells which undergo transneuronal degeneration are representative of the entire population of cells in n. magnocellularis. Because the pattern of termination of the *crossed* projections revealed by transneuronal degeneration following cochlear removal is indistinguishable from the pattern revealed by section of the crossed dorsal cochlear tract (compare Fig. 2C and 3E), this assumption is probably reasonable (see also Parks and Rubel, '75).

Complete denervation of nucleus laminaris dendrites

As indicated above, virtually complete denervation of the ventral dendrites of n. laminaris results from cutting the decussating fibers in the crossed dorsal cochlear tract. In this situation, we have previously shown that the ventral dendrites undergo a rapid and extensive atrophy, with the ventral surface of n. laminaris neurons rounding up to essentially an ovoid shape when viewed in plastic sections or electron microscopically (Benes et al., '77). Quantitative EM morphometrics indicated an 80% reduction in the ventral dendrites. As illustrated in Figure 4 (A and B), this effect is reflected in Nissl sections by the elimination of the glial-free zone in the ventral neuropil of n. laminaris which is replaced by a rich glial bed.

The shrinkage of the dendrites can also be revealed utilizing the same quantitative Golgi procedures as have previously been used to establish the correlation between dorsal and ventral dendritic length in normal animals (Smith and Rubel, '79; Smith, '81). As illustrated in Figure 4C, there was an evident decrease in the dendritic arborization of n. laminaris cells in the ventral neuropil revealed in the quantitative measurements by a dramatic shift in the slope of the dorsal vs. ventral dendritic length regression. The dorsal dendritic lengths remained normal throughout the nucleus, whereas the ventral dendritic lengths were reduced by an average of 75%. This mas-

Fig. 1. Normal morphology of chick brainstem auditory nuclei. A. Schematic representation showing VIIIth nerve (VIII) axons innervating n. magnocellularis (NM) and the bilateral spatially segregated innervation of n. laminaris (NL). B. Low-power photomicrograph of Nissl-stained section of the right medulla from a hatchling chick, showing NM and NL. C. Counterstained Golgi-Kopsch section of NL, showing typical NL cell with its bipolar symmetrical dendrites, 25-day-old hatchling chick. D. Scatterplot showing bipolar symmetry of dorsal and ventral dendritic lengths measured from 128 normal NL cells from 25 day post-hatch chicks. Correlation is 0.71 and best fit line is unity (solid line). Calibration bars: B, 0.2 mm; C, 20 μm.

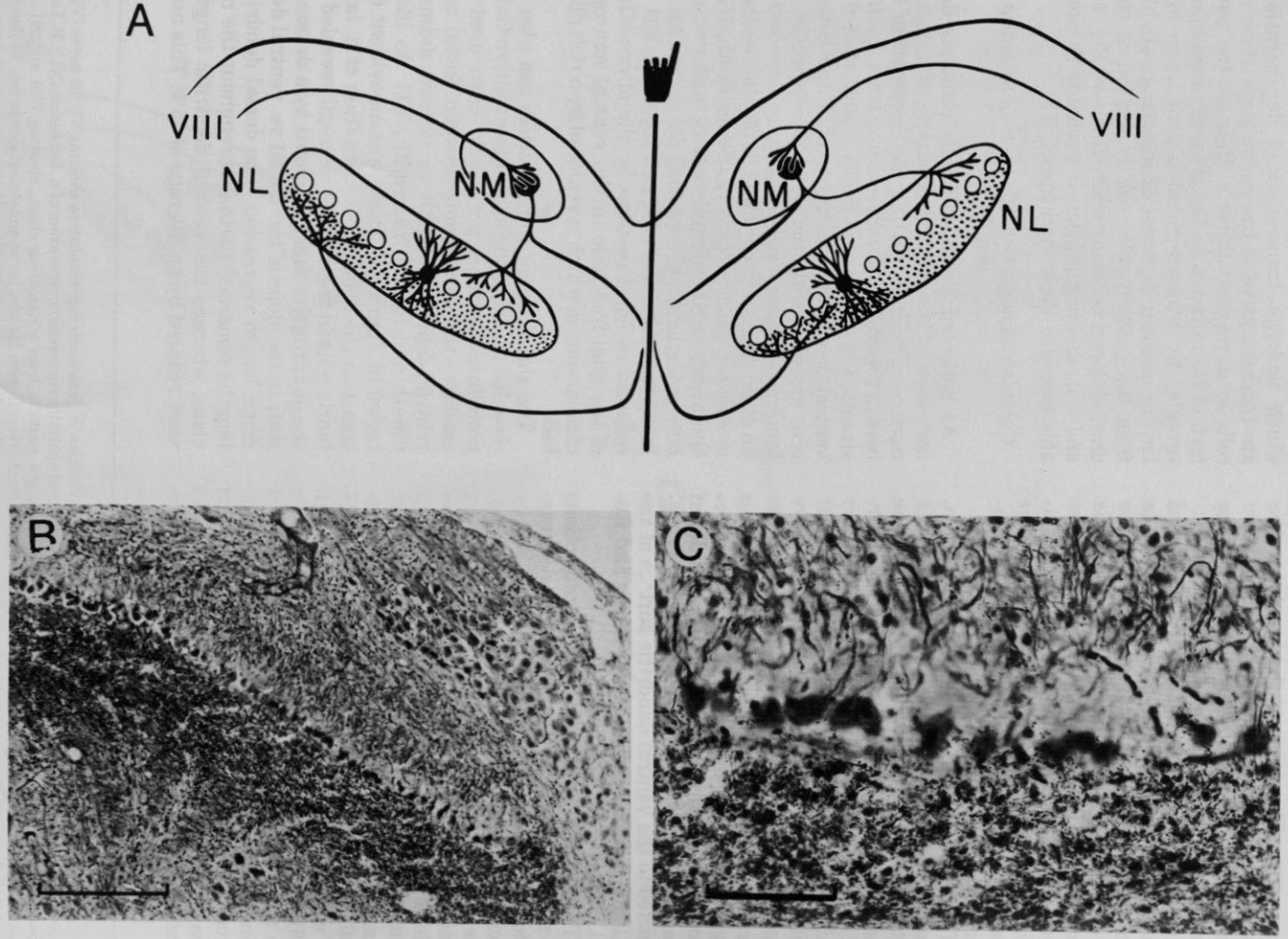

Figure 2

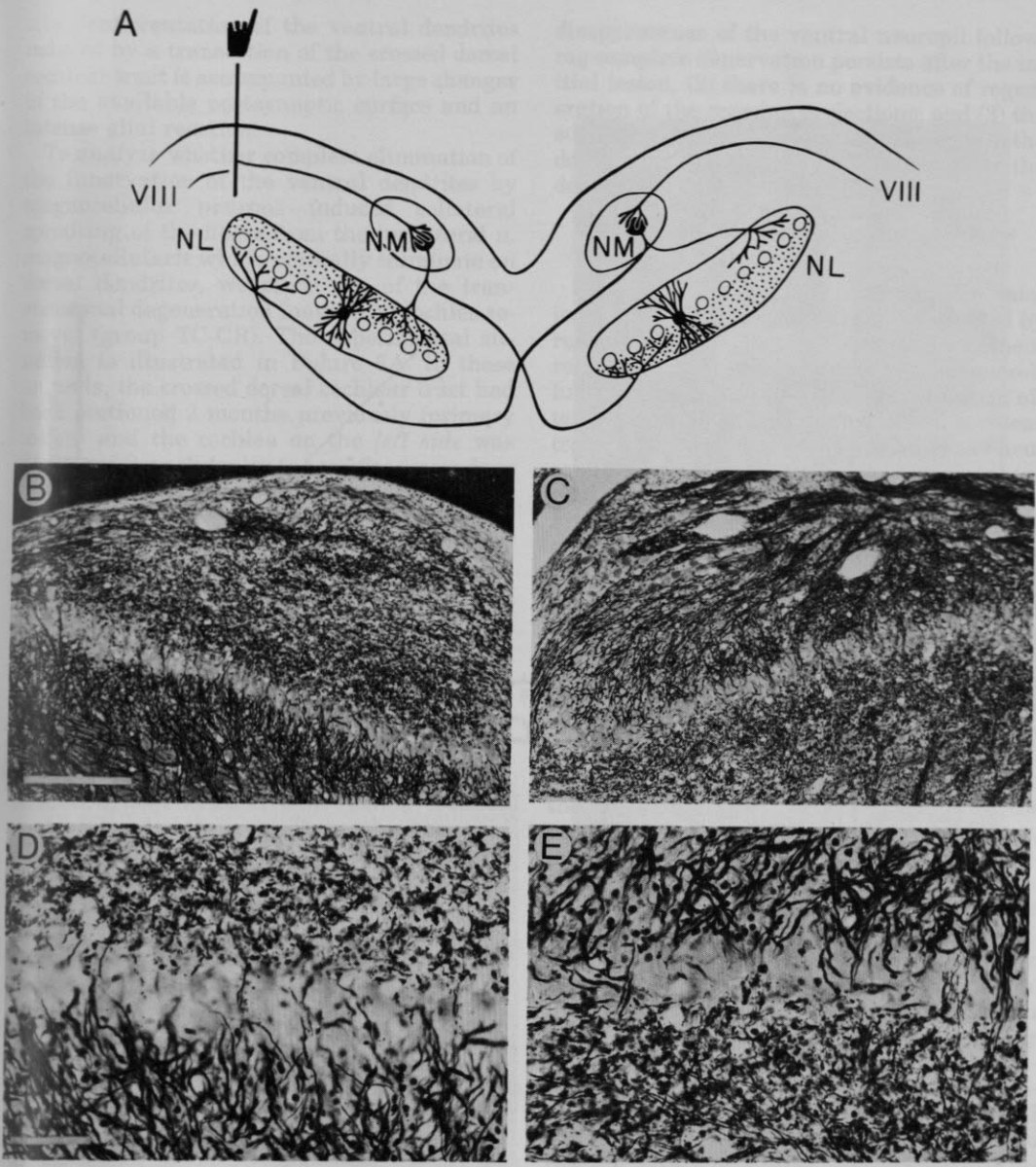

Fig. 3. Axonal degeneration in NL resulting from cochlea removal or VIIIth nerve transection. A. Schematic showing manipulation, indicated by the pointer on left VIIIth nerve, and resulting degeneration in NL (stippling). B and C. Low-power photomicrographs of Fink-Heimer-stained sections ipsilateral and contralateral to the cochlea removal, respectively. D and E. Higher-power photomicrographs taken ipsilateral and contralateral to the cochlea removal. Fourteen-day survival. Calibration bars: B and C, 0.2 mm; D and E, 50 μm.

Fig. 2. Acute effects of transecting the crossed dorsal cochlear tract (XDCT) at the midline. A. Schematic showing transection and areas of degeneration below NL on each side of the brain (stippling). B and C. Low- and high-power photomicrographs of Fink-Heimer-stained sections showing degeneration limited to below the NL cell body later. Four-day survival. Calibration bars: B, 0.2 mm; C, 50 μm.

A
B
C
D
DORSAL DENDRITIC LENGTH (μm X 100)
VENTRAL DENDRITIC LENGTH (μm X 100)
0
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Figure 4

sive deafferentation of the ventral dendrites induced by a transection of the crossed dorsal cochlear tract is accompanied by large changes in the available postsynaptic surface and an intense glial reaction.

To analyze whether complete elimination of the innervation of the ventral dendrites by magnocellular neurons induced collateral sprouting of the fibers from the ipsilateral n. magnocellularis which normally terminate on dorsal dendrites, we made use of the transneuronal degeneration induced by cochlea removal (group TC-CR). The experimental situation is illustrated in Figure 5A. In these animals, the crossed dorsal cochlear tract had been sectioned 2 months previously (primary lesion) and the cochlea on the *left side* was destroyed 2 weeks prior to sacrifice (secondary lesion). If sprouting of the magnocellular projections into the ventral neuropil had occurred, one would expect to see degeneration not only in the normally innervated dorsal dendritic regions, but also in the ventral neuropil of n. laminaris on the left side of the brain. As illustrated in Figure 5B and D, degeneration in the dorsal neuropil was prominent; however, there was no evidence of degeneration ventral to the cell body lamina of n. laminaris. The success of the primary lesion in transecting the crossing fibers is indicated by the complete absence of degeneration in the laminar nucleus contralateral to the *secondary* cochlea removal (Fig. 5C). In addition, an interesting feature of the completely denervated neuropil is illustrated in this "bleached" Fink-Heimer preparation, namely an obvious increase in the vascular bed in the former location of the neuropil on the ventral side of n. laminaris (e.g., Fig. 5C). Finally, in a Nissl preparation (Fig. 5E) it is apparent that the glial bed which was prominent at the early postlesion intervals is somewhat less rich in glial cells, as if some of the glia which invaded this zone early after the initial lesion had disappeared. From these observations, it can be concluded that (1) the disappearance of the ventral neuropil following complete denervation persists after the initial lesion; (2) there is no evidence of regeneration of the crossing projections; and (3) the surviving n. magnocellularis projections to the dorsal neuropil do not sprout to innervate the denervated ventral neuropil.

Partial denervation of nucleus laminaris dendrites

As indicated above, the dendrites of n. laminaris neurons can be partially denervated by removing the cochlea and inducing transneuronal degeneration of neurons in n. magnocellularis (Fig. 3). In contrast to the situation after transection of the crossed dorsal cochlear tract, there is partial preservation of the neuropil following unilateral cochlea removal (as reflected by the remaining glial-free zone, see Fig. 6B), and substantial preservation of the denervated dendrites. This is illustrated by the sample Golgi-impregnated cells of Figure 6C, and the plot of dorsal vs. ventral dendritic lengths in an animal with a longstanding unilateral cochlea removal in Figure 6D. While there is some shrinkage of the dendrites and of the denervated neuropil the shrinkage is not nearly as extensive as that observed following midline transection.

The potential sprouting of projections from the nondenervated n. magnocellularis to the partially denervated dendritic zones of n. laminaris was analyzed in animals with longstanding unilateral cochlea destruction in two ways. First, the crossed dorsal cochlear tract was sectioned (group CR-TC). As has been illustrated (Fig. 2), the degeneration induced in n. laminaris by such a lesion is restricted to the ventral neuropil. If sprouting does occur into the region which had been chronically partially denervated, then this midline transection should result in degenerating terminals in *both* the ventral and the dorsal neuropil regions on the side of the brain ipsilateral to the initial cochlea removal. This is diagra-

Fig. 4. Chronic effects of transecting the XDCT at the midline. The manipulation is diagrammatically shown in Figure 2A. A and B. Low- and high-power photomicrographs taken 8 days following XDCT transection. Note obliteration of normally glia-free neuropil zone below NL. C. Golgi-Kopsch-impregnated NL cells 8 days following transection; note asymmetry of dendrites. D. Scatterplot showing dorsal and ventral dendritic lengths of NL neurons from normal animals (dots) and from 19 cells of an animal 8 days following XDCT transection (open hexagons). Note that the range of dorsal dendritic lengths is normal while ventral dendritic lengths are markedly decreased. Dotted line is least squares linear fit. Calibration bars: A, 0.2 mm; B and C, 50 μm.

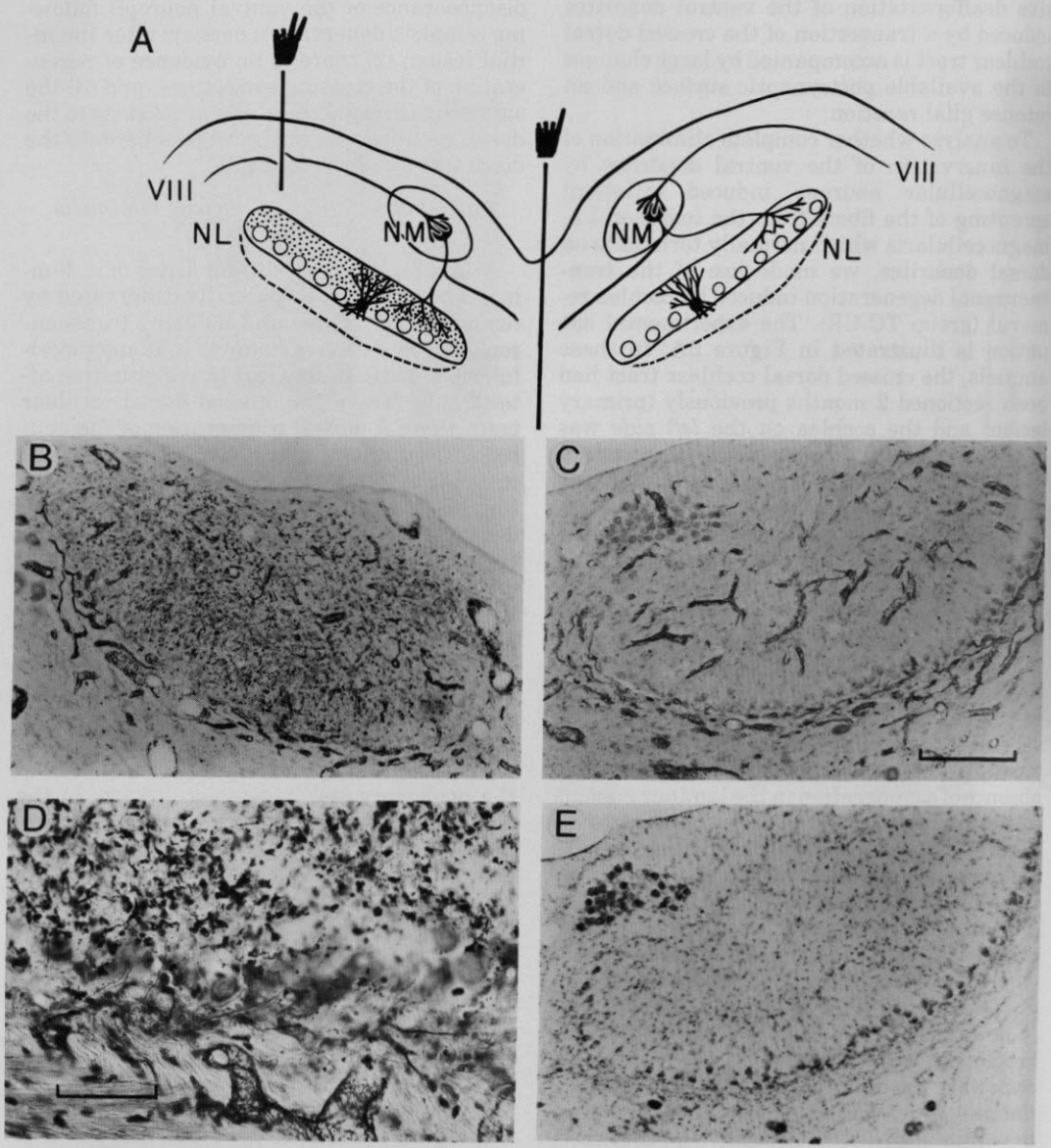

Fig. 5. Transection of the XDCT (primary lesion) followed several weeks later by cochlea removal (group TC-CR). A. Diagrammatic representation of the primary (first) and secondary lesions. Results of the first lesion are indicated by the absence of the contralateral projection to NL and the absence of ventral dendrites in NL. Results of the secondary cochlea removal are indicated by the stippling. B, C, D, and E show results of this experiment in a representative subject. B and C. Low-power photomicrographs bleached and then counterstained. Fink-Heimer-stained section ipsilateral and contralateral to the secondary cochlea removal, respectively. Note degeneration products dorsal to NL in B and their absence in C. Also note the hypertrophy of the vascular bed ventral to NL on both sides of the brain. D. High-power photomicrograph of Fink-Heimer-stained section ipsilateral to the secondary cochlea removal, showing degeneration above the NL cell body lamina but not extending below it. E. Low-power Nissl-stained section contralateral to the secondary cochlea removal showing glia obliteration of the ventral neuropil zone. XDCT transection at 10 days post-hatch, left cochlea removed at 100 days of age, sacrificed 14 days later. Calibration bars: C (for B, C, and E), 0.2 mm; D, 50 μm.

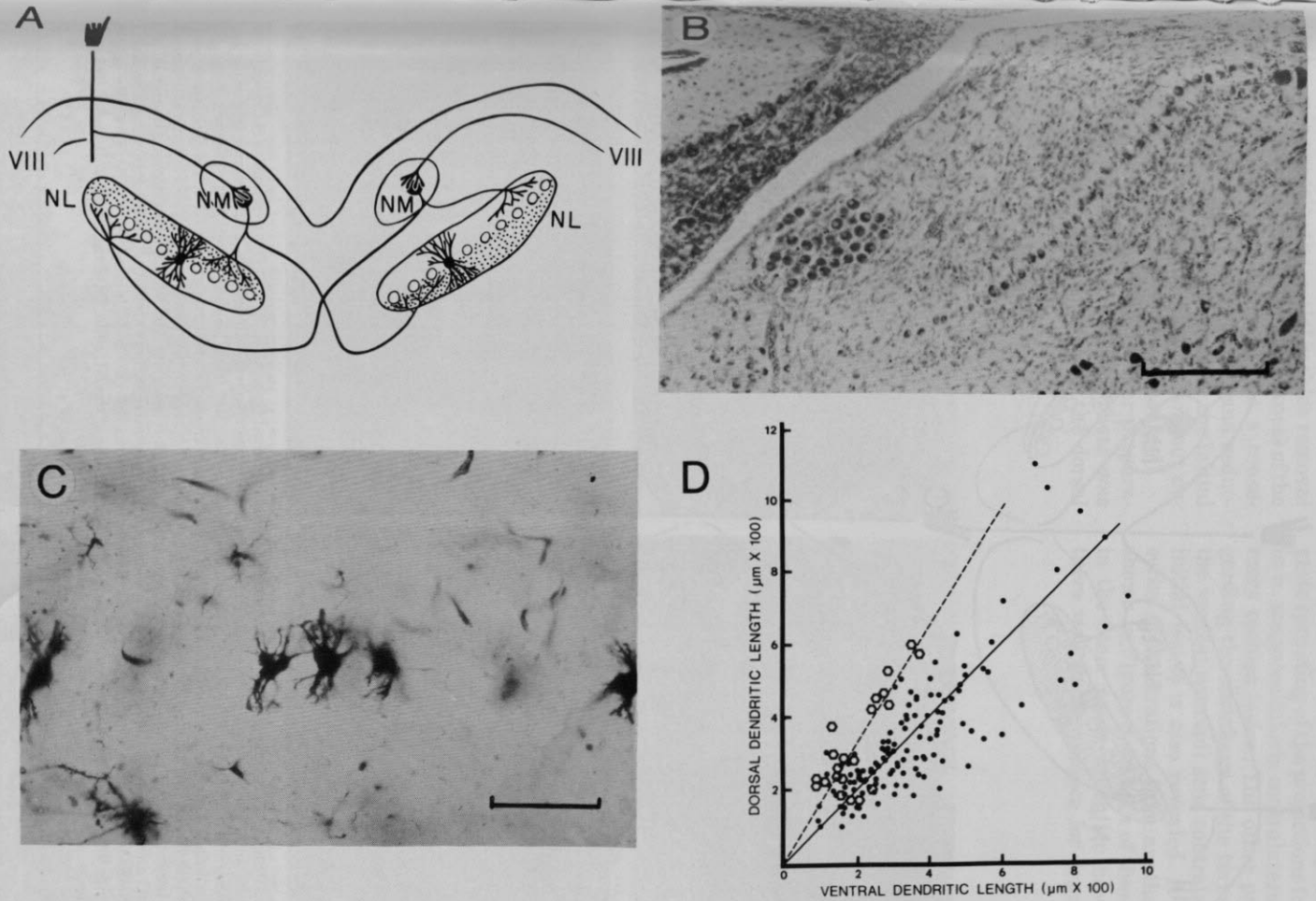

Fig. 6. Chronic effects of unilateral cochlea removal (group CR). A. Diagrammatic representation showing the manipulation. B. Low-power photomicrograph of Nissl-stained section of right medulla 28 days following cochlea removal; note intact neuropil region ventral to NL. C. Golgi-Kopsch-impregnated NL cells contralateral to cochlea removal, 28 days survival. Note that the ventral dendrites are only slightly smaller than dorsal dendrites. D. Scatterplot showing normal dorsal and ventral dendritic lengths of NL cells (dots) and the lengths of dendrites contralateral to a cochlea removal from the subject shown in C (open hexagons). Note that there is a slight reduction of ventral dendrites as compared to dorsal dendritic lengths; dashed line is least squares fit. Calibration bars: B, 0.2 mm; C, 50 μm.

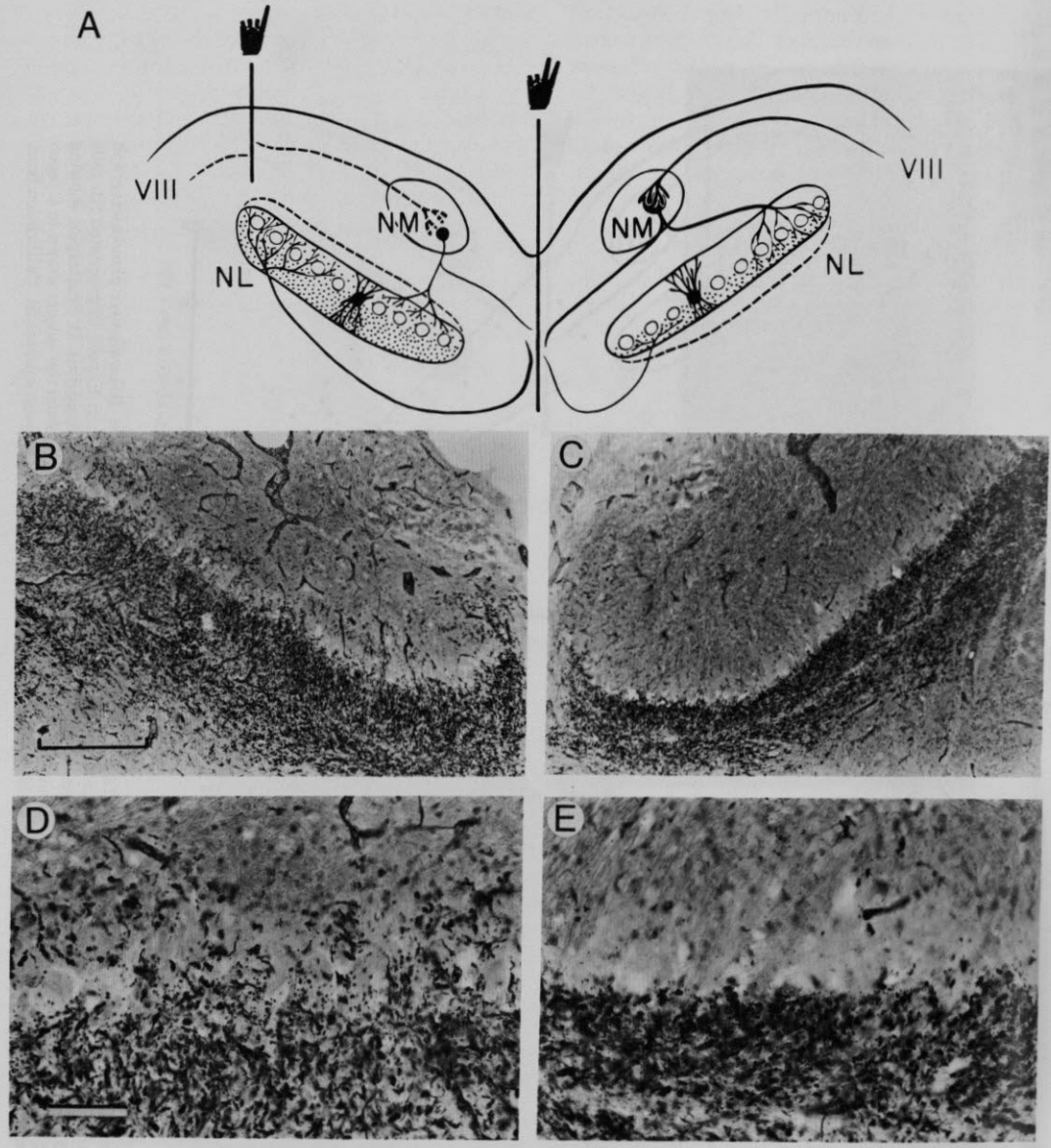

Fig. 7. Sprouting in n. laminaris following unilateral cochlea removal (group CR-TC). A. Diagrammatic representation of manipulations and results. Primary manipulation was a left cochlea removal, eliminating VIIIth nerve fibers (dashed line), and causing a reduction of afferents to NL from the deafferented NM (thin lines from left NM). After 2–3 months the secondary manipulation, transection of the XDCT, was performed. The degeneration resulting from the cochlea removal is shown by stippling and the hypothesized sprouting from the intact NM is shown by the extension of terminals through the cell body lamina. B, C, D, and E show photomicrographs of Fink-Heimer-stained sections ipsilateral (B and D) and contralateral (C and E) to the initial cochlea removal from a representative subject sacrificed 4 days following the XDCT transection. Note degeneration products dorsal as well as ventral to the cell body lamina in B and D while on the contralateral side degeneration products are limited to the ventral side of the NL cells. Calibration bars: B and C, 0.2 mm; D and E, 50 μm.

Fig. 8. Shows another example of sprouting in NL induced by unilateral cochlea removal (group CR-TC). For complete description see Figure 7A. Diagrammatic representation of manipulations and results. B, C, D, and E. Fink-Heimer-stained sections ipsilateral and contralateral to the initial cochlea removal. Calibration bars: B and C, 0.2 mm; D and E, 50 μm.

matically shown in Figure 7A. In this situation an intra-animal control is also available since on the side opposite the initial cochlea removal one would anticipate a reduced thickness of the zone of degeneration ventrally (as a consequence of the partial denervation and reduction of the terminal field induced by the initial transneuronal degeneration), but without degeneration products in the dorsal neuropil.

As is evident in the Fink-Heimer preparations illustrated in Figure 7B–E, there was clear evidence for degeneration in the dorsal neuropil on the side ipsilateral to the primary cochlea removal. This finding strongly suggests sprouting of the projections from the cells in n. magnocellularis contralateral to the primary cochlea lesion. The other prediction regarding the shrinkage of the terminal field in the ventral neuropil zone contralateral to the initial lesion is also revealed. A comparable series of illustrations from another subject is shown in Figure 8. In both of these cases, and in the other cases which we have prepared, there was clear evidence for degeneration in

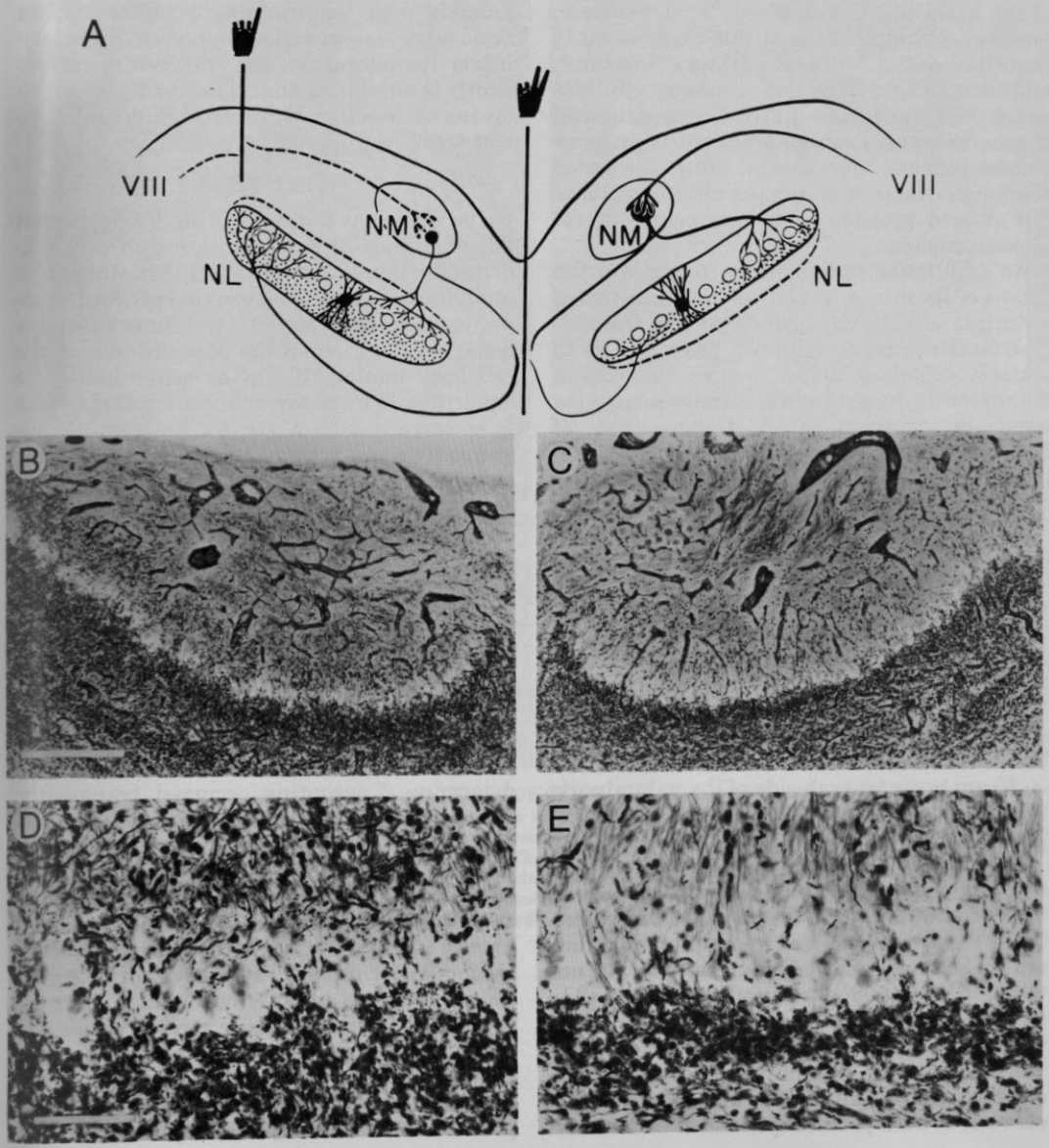

Figure 8

the dorsal neuropil region of n. laminaris ipsilateral to the primary cochlea removal. The degeneration was not uniformly distributed, however, tending to be more pronounced laterally and rostrally in the nucleus (see Figs. 7B and 8B).

The experimental situation in which a primary lesion of the cochlea is followed by a secondary (acute) transection of the crossed dorsal cochlear tract provides a situation with a convenient *intra*-animal control, where the presence of sprouting is demonstrated by degeneration in the dorsal neuropil on one side of the brain but not on the other. Obviously, however, a complication of this experiment is that the "control," crossed pathway is not normal; it is in fact from the n. magnocellularis which had undergone partial transneuronal degeneration as a consequence of the primary cochlea removal. In addition, using this series of manipulations, it is only possible to evaluate the crossed projections to the contralateral laminar nucleus.

An additional manipulation to explore the extent of sprouting, which permits analysis of sprouting of both the ipsilateral and the contralateral n. magnocellularis projection is to perform secondary (acute) cochlea removals in animals with longstanding unilateral cochlea removals (group CR-CR). Comparisons of Fink-Heimer-stained tissue or osmicated, toluidin blue-stained material from animals which had sustained a secondary cochlea removal with animals which had only a single cochlea removal revealed excess degeneration products in the neuropil regions of n. laminaris normally innervated by the opposite n. magnocellularis. To quantitatively assess this difference we counted degenerating (dark) profiles in 1-μm sections on both sides of the n. laminaris cell body lamina from group CR-CR and from control subjects (-CR). Degenerating profiles were counted in a sample of ten 50 μm × 20 μm areas on each side of the n. laminaris cell bodies, from several sections from experimental and control subjects.

As illustrated in Figure 9, a primary lesion in an otherwise intact animal (Fig. 9A) results in degeneration in the dorsal neuropil ipsilaterally and the ventral neuropil contralaterally. Focusing on the ipsilateral laminar nucleus, the counts of dark profiles (confirmed to be degeneration by electron microscopy) revealed few, if any, degenerating profiles in the ventral neuropil, but numerous profiles dorsally in control animals (the mean ventral/dorsal ratio of dark profiles ipsilateral to the cochlea removal in the control animal was 0.14). In contrast, in the experimental animal which had sustained a primary lesion of the contralateral cochlea, thus denervating the ventral neuropil of the left side (see Fig. 9B for a diagrammatic illustration of the situation), numerous degenerating profiles were evident not only in their normal location in the dorsal neuropil, but also ventrally. Indeed, the mean ventral/dorsal ratio of degenerating profiles was 0.42 in this case. This difference in V/D ratios was highly significant ($P < 0.01$). Thus, the secondary lesions of the surviving cochlea in animals with longstanding primary cochlea removal reveal sprouting in the ventral n. laminaris ipsilateral to the surviving cochlea which is similar to that described above, following transection of the crossed dorsal cochlear tract.

DISCUSSION

The principal finding of this study is that following partial deafferentation of one dendrite of n. laminaris neurons, fibers which normally terminate almost exclusively on the opposite dendrite sprout to innervate the denervated region on the opposite side of the cell body lamina. If, on the other hand, the dendrites are more severely denervated (which is accompanied by a greater preterminal degeneration and accompanying glial reaction), there is a rapid atrophy of the affected dendrite, and no evidence of "ectopic" connections at long survival intervals. It is our interpretation that the rapid and extensive atrophy of the denervated dendrites is responsible for the lack of evidence of lesion-induced sprouting in animals with longstanding lesions.

At this point, it is important to indicate that our results only indicate that ectopic synapses are not present at long survival intervals, and this *persistent* reinnervation has not occurred. The present observations do not allow us to determine if sprouting occurred transiently. This is quite important since if sprouting occurred, and subsequently disappeared, one would conclude that the presence of available postsynaptic territory was necessary for the culmination or maintenance of lesion-induced growth (synapse formation) but not for its initiation. Alternatively, if presynaptic growth never occurred, one might conclude that the availability of postsynaptic territory was necessary for the initiation of the growth response.

Perhaps the most interesting aspect of the present results is that the results begin to explore the role of the postsynaptic cell in the

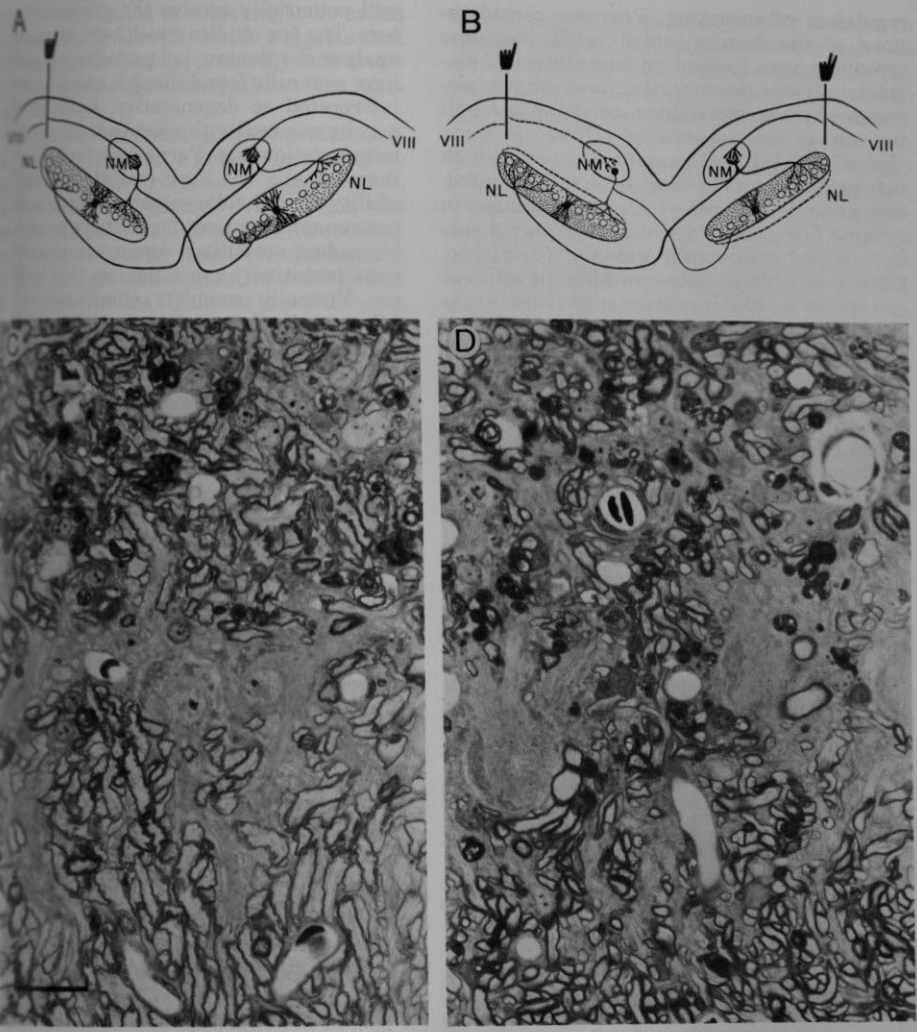

Fig. 9. Results of secondary cochlea removal (group CR-CR). In A and C (control animal) the effects of removing one cochlea in a 3-month-old subject are shown. Survival time is 14 days. C is a high-power photomicrograph of the ipsilateral NL showing many degenerating profiles dorsal but not ventral to the cell bodies of NL. B. Diagrammatic representation of manipulations and results in experimental subjects. The primary manipulation was to remove one cochlea (left side); after 70 to 80 days the other cochlea was removed (secondary manipulation) and 14 days later subjects were sacrificed. D. Representative section from right NL. (ipsilateral to secondary manipulation) of an experimental subject showing degenerating profiles below as well as above NL cell bodies. Toluidine blue stain of osmicated, plastic-embedded tissue. Calibration bar: for C and D, 20 μm.

regulation of sprouting. Previous considerations of the factors which might regulate sprouting have focused on four classes of elements; (1) the degenerating presynaptic elements which might induce sprouting through the release (or cessation of release) of some "factor"; (2) the postsynaptic element, which may passively *provide sites* to be reinnervated, may undergo changes which are *permissive* to synapse formation, or which may play a role in *inducing* presynaptic growth; (3) interactions (competition) between different afferent systems for available postsynaptic sites; (4) the elements extrinsic to the neuronal participants, including glia, hormones, and the vasculature. In general, attempts to evaluate any one of these have been complicated by the fact that any lesion initiates changes in all of the above elements, only some of which may be relevant to the induction and/or maintenance of the sprouting response. The potential contribution of the present observations is to begin to dissociate some of these processes.

Degenerating presynaptic elements

In every demonstration of sprouting, the most obvious aftermath of the primary insult is massive presynaptic degeneration. From observations in the peripheral nervous system, it has been suggested that the degenerating axons or terminals or their breakdown products stimulate or induce sprouting in nearby intact fibers (e.g., Hoffman, '50; Williams et al., '73). If this hypothesis is correct, then one would expect that the amount of sprouting should be monotonically related to the amount of degeneration produced by the primary lesion. In the present study quite the opposite results were found. Following midline transection which results in the degenerating of virtually all of the axons on one dendrite, no sprouting was detected. Partial denervation of the same region, however (probably resulting in the removal of about half of the normal projections), did result in ectopic projections. Again, because of the caveat that we do not know about the early postlesion responses of the afferents, we cannot say that sprouting was not induced to a greater extent by the complete denervation. We can say, however, that massive presynaptic degeneration is not sufficient for the induction of persistent ectopic projections.

Postsynaptic reactions

To date, relatively little attention has been paid to changes in the postsynaptic cell which will potentially receive the reinnervating fibers. The few studies which have attempted to analyze the denervated postsynaptic element have generally found changes which have been interpreted as degenerative, some of which may be reversed with a time course paralleling reinnervation (e.g., Parnavelas et al., '74; Matthews et al., '76). Such results raise the possibility that the degenerative changes in the postsynaptic element facilitate or even possibly induce sprouting. Again, our present results tentatively argue against this hypothesis. Virtually complete elimination of the afferents to the ventral dendrites (through transection of the crossed dorsal cochlear tract) results in massive though incomplete dendritic atrophy, but no detectable sprouting; partial denervation results in less drastic degenerative changes in the dendrites, but more ectopic projections. Therefore we would propose that the degenerative responses to deafferentation of the dendrites do not facilitate or induce sprouting, and that such events certainly place limitations on the formation of lasting ectopic projections. With regard to the postsynaptic element, our results would suggest that *preservation* rather than *denervation* of the deafferented dendritic membrane is a prerequisite to the establishment of persistent new connections. If the preservation of dendrites in other neurons requires synaptic input, then the conclusion we must draw is that partial denervation, obtained when degenerating afferents are relatively dispersed or surrounded by intact terminals, may be far more conducive to sprouting than situations in which large expanses of dendritic territory are stripped of synaptic connections. An interesting prediction of this hypothesis would be that greater sprouting might be found with equivalent deafferentation of proximal than distal dendrites, since in the former case the denervated regions are surrounded by intact terminals which may serve to preserve the dendrites.

Presynaptic interventions (competition)

One of the strongest and perhaps more important conclusions that can be drawn from the present results has to do with the notion of *presynaptic competition* for available postsynaptic sites. In general, the concept of competition is invoked to explain why in many situations the removal of a potential competitor for a given afferent increases the extent to which another afferent captures available postsynaptic sites (see, for example, Schneider and Jhaveri, '74). The notion of presynaptic

competition is linked to what might be called the *proximity hypothesis,* which suggests that when competition exists, *proximity* will determine the success of the competition (see Raisman, '69).

If competition was acting in the present setting, one would predict a greater sprouting response following complete degeneration, whereas following partial denervation, the presence of a potential competitor (the surviving projections from the magnocellular nucleus without cochlear innervation) should reduce the sprouting. In fact, again the opposite result is obtained. Indeed, since the surviving projection to the neuropil of nucleus laminaris is much closer than the projections to the opposite neuropil, one would expect the n. magnocellularis afferents which survive the denervation by cochlea removal to have a considerably competitive advantage. One way that it might be possible to account for this lack of competition is that the surviving n. magnocellular cells which have been deafferented by cochlea removal are not successful competitors because of a low level of activity. Alternatively, it must be recalled that deafferentation does induce transneuronal degeneration of n. magnocellularis cells, and these which survive may simply be too feeble to effectively compete for available sites in n. laminaris. This may not actually represent an *alternative* to the activity hypothesis, since it may be decreased in activity which results in transneuronal degeneration. In either case, the present results suggest that the presence of a potential presynaptic competitor does not necessarily mean that competition will actually occur.

Nonneural factors

In addition to the neuronal changes associated with deafferentation, a number of nonneural factors have been suggested to play a regulatory role. In this regard, the proliferation, migration, and hypertrophy of glia is particularly interesting, and has led to the speculation that these elements may either release a "factor which actively stimulates or guides axonal growth," or remove obstacles (degeneration debris) which retard growth (Lynch, '76; Lynch and Gall, '79). In the present study, we were again able to partially dissociate glial responses from sprouting; the greatest glial response occurred following complete denervation, while the glial response following the partial denervation was much less extensive. Again, however, we are faced with the fact that we have not measured the possible changes in afferents early in the postlesion interval, and it is possible that glia induce the growth, but that the unavailability of postsynaptic territory results in the "withering of the sprouts."

In conclusion, the experiments reported here have begun to dissociate some of the changes which occur in response to lesions from those which may facilitate or induce the reinnervation of the denervated elements. Certainly these experiments raise more questions than they answer. For example, differentiating between a process being necessary for the *induction* of afferent reorganization and being necessary for the *maintenance* of ectopic synapses was impossible, since we do not yet know what happened in the early postlesion interval. What we have provided is the impetus to ask such questions and, we hope, an experimental preparation in which answers can be obtained. It may then be possible to determine more precisely the contributions of presynaptic, postsynaptic and nonneuronal elements in the growth and establishment of synaptic reorganization in the neural system.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the help of Dr. T. Norville Parks, who collaborated on some of our initial experiments in this series; Ms. Patricia Palmer, Ms. Lodi Smith, and Ms. Sharon Vinsant provided expert histological assistance; Ms. Mary Patton Janssen, Mrs. Marilyn Engelhardt, and Ms. Deborah Stein provided excellent secretarial assistance, and Dr. Barry Fass provided criticisms of the manuscript. Support was provided by NIH RCDA's NS00305 (E.W.R.), NS00325 (O.S.), Predoctoral Fellowship MH0594903 (Z.D.J.S.), Program Project grant NS14620, and research grants NS12333 and NS15395.

LITERATURE CITED

Benes, F.M., T.N. Parks, and E.W Rubel (1977) Rapid dendritic atrophy following deafferentation: An EM morphometric analysis. Brain Res. *122*:1–13.

Fink, R.P., and L. Heimer (1967) Two methods for selective silver impregnation of degenerating axons and their synaptic endings in the central nervous system. Brain Res. *4*:369–374.

Hoffman, H. (1950) Local reinnervation in partially denervated muscle; a histo-physiological study. Aust. J. Exp. Biol. Med. Sci. *28*:383–397.

Jackson, J.R.H., and E.W Rubel (1976) Primary cochlear projections in the chicken. Anat. Rec. *187*:434–435 (Abstract).

Liu, C.N., and C.Y. Liu (1971) Role of afferents in maintenance of dendritic morphology. Anat. Rec. *169*:369 (Abstract).

Lynch, G. (1976) Neuronal and glial responses to the de-

struction of input: The "deafferentation syndrome." In P. Scheinberg (ed): Cerebrovascular Diseases. New York: Raven Press, pp. 209–227.

Lynch, G., and C. Gall (1979) Organization and reorganization in the central nervous system: Evolving concepts of brain plasticity. In F. Falkner and J. M. Tanner (eds): Human Growth, 3. New York: Plenum, pp. 125–144.

Matthews, D.A., C. Cotman, and G. Lynch (1976) An electron microscopic study of lesion-induced synaptogenesis in the dentate gyrus of the adult rat. II. Reappearance of morphologically normal synaptic contacts. Brain Res. *115:*23–41.

Parks, T.N., and E.W Rubel (1975) Organization and development of brain stem auditory nuclei of the chicken: Organization of projections from n. magnocellularis to n. laminaris. J. Comp. Neurol. *164:*435–448.

Parks, T.N., and E.W Rubel (1978) Organization and development of brain stem auditory nuclei of the chicken: Primary afferent projections. J. Comp. Neurol. *189:*439–443.

Parnavelas, J., G. Lynch, N. Brecha, C. Cotman, and A. Globus (1974) Spine loss and regrowth in the hippocampus following deafferentation. Nature *248:*71–73.

Raisman, G. (1969) Neuronal plasticity in the septal nuclei of the adult rat. Brain Res. *14:*25–48.

Rubel, E.W, and T.N. Parks (1975) Organization and development of brain stem auditory nuclei of the chicken: Tonotopic organization of n. magnocellularis and n. laminaris. J. Comp. Neurol. *164:*411–434.

Schneider, G.E., and S.R. Jhaveri (1974) Neuroanatomical correlates of spared or altered function after brain lesions in the newborn hamster. In D.G. Stein, J.J. Rosen, and N. Butters (eds): Plasticity and Recovery of Function in the Central Nervous System. New York: Academic Press, pp. 65–110.

Smith, Zaid D.J. (1981) Organization and development of brain stem auditory nuclei of the chicken: Dendritic development of n. laminaris. J. Comp. Neurol. (in press).

Smith, D.J., and E.W. Rubel (1979) Organization and development of brain stem auditory nuclei of the chicken: Dendritic gradients in n. laminaris. J. Comp. Neurol. *186:*213–240.

Stensaas, L.J. (1967) The development of hippocampus and dorsolateral pallial regions of the cerebral hemisphere in fetal rabbits. I. Fifteen millimeter stage, spangioblast morphology. J. Comp. Neurol. *129:*59–70.

Trune, D.R. (1979) Influence of Neonatal Cochlear Removal on Cochlear Nuclear Development. Unpublished Ph.D dissertation. Dept. of Anatomy, Louisiana State University.

White, E.L., and F.D. Nolan (1974) Absence of reinnervation in the chinchilla medial superior olive. Anat. Rec. *178:*486 (Abstract).

Williams, T.H., J. Jew, and S.L. Palay (1973) Morphological plasticity in the sympathetic chain. Exp. Neurol. *39:*181–203.