*Brain Research*, 122 (1977) 1–13



## Research Reports

# RAPID DENDRITIC ATROPHY FOLLOWING DEAFFERENTATION: AN EM MORPHOMETRIC ANALYSIS*

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(Accepted June 8th, 1976)

SUMMARY

The nucleus laminaris (NL), a third-order brain stem auditory nucleus in birds, receives afferents to its dorsal dendrites from the ipsilateral nucleus magnocellularis (NM), while the ventral dendrites of NL neurons are innervated by axons from the contralateral NM via the crossed dorsal cochlear tract (CTrX). The CTrX was transected in young chickens and, 96 h later, NL was examined for cytological changes. A morphometric analysis of electron micrographs from lesioned and sham-operated animals revealed the following.

(1) Degenerating axons and axon terminals were localized almost entirely to the ventral neuropil region of NL.

(2) The volume density of dendrite in the ventral region of NL of lesioned animals was reduced by 85% compared to both the dorsal dendritic region of the same animals and the ventral dendritic region of sham-operated control animals.

(3) The frequency with which primary dendrites were encountered in the ventral neuropil of lesioned animals was 81% lower than in sham-operated controls.

(4) Frequently, there was an apparent reduction in the amount of rough endoplasmic reticulum, Golgi apparatus and cytoplasmic granularity in the deafferented part of the NL neurons.

(5) The data indicate that the cytological integrity of dendritic processes can be specifically, profoundly and very rapidly compromised by removing a significant proportion of their afferents, suggesting that a tonic influence may be exerted by the presynaptic terminals.

* A preliminary account of this research was presented at the Fifth Annual Meeting of the Society for Neuroscience, New York, November, 1975.

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## INTRODUCTION

A variety of morphological changes in vertebrate central neurons are reported to follow direct or indirect deafferentation. These have included changes in cell size[9,23,24,29], distribution of nuclear chromatin[11], integrity of dendritic spines[8,23,31], and other aspects of dendritic morphology[6,12,13,28,33]. Although studies on the deafferentation of invertebrate neurons have yielded somewhat different results[20,30,34], the data on adult vertebrate deafferentation, in conjunction with an extensive literature on muscle denervation[5], is often interpreted as supporting the notion of a trophic or inductive function of nerve terminals[7,10].

These and other studies on 'transneuronal effects' (see refs. 4 and 7) have succeeded in demonstrating that the integrity and/or activity of presynaptic elements influences the metabolism of postsynaptic neurons. However, the heterogeneity of cell types and the diversity of inputs to a single cell, e.g. in the olfactory bulb[23], have previously made it difficult to determine the extent to which specific postsynaptic structures are modified by changes in their innervation. That is, does deafferentation of a neuron lead to an overall change in metabolic processes with concomitant changes in cell size and dendritic surface area or are alterations specific to the cell processes to which innervation has been interrupted? Although some support for the latter possibility can be derived from earlier investigations[14,20], a thorough examination of this question in the vertebrate nervous system has not yet been made.

To properly determine the specificity of changes in postsynaptic elements as a function of presynaptic integrity it is necessary to (1) use a neural system in which the innervation to one known portion of a cell can be disrupted while leaving intact the innervation of a similar but non-overlapping region of the same cell; and (2) incorporate adequate sampling and measurement methods for independent analyses of each postsynaptic element.

In birds, the third-order auditory neurons in nucleus laminaris are aligned in a striking monolayer in the dorsal medulla. These neurons, considered to be homologous to those of the mammalian medial superior olivary nucleus[3,26], receive strictly segregated, topographically organized innervation; dorsally from second-order neurons of the *ipsilateral* nucleus (n.) magnocellularis, and ventrally, via the crossed dorsal cochlear tract, from the *contralateral* n. magnocellularis. Midline transection of the crossed dorsal cochlear tract produces degenerative changes largely confined to the ventral neuropil of n. laminaris[21]. Several characteristics of n. laminaris make it a suitable structure in which to study the effects of partial deafferentation. First, there is only one homogenous population of principal neurons, and no interneurons, in this nucleus. Second, these cells have two distinct dendritic fields, each of which receives its input principally or entirely from one source. Finally, since the dorsal and ventral dendrites are innervated by different sources, it is possible to independently manipulate the innervation to either postsynaptic surface.

Most of the studies on the postsynaptic effects of deafferentation have used

the Golgi methods to examine changes in dendritic structure and Nissl methods to measure changes in the cell body. Given the continuing questions about the selectivity and quantifiability of the former methods[2,25] and the accuracy of the latter for sizing cells[16], more generally applicable and precise methods would be desirable. The morphometric methods described by Weibel and Bolender[32] seem most appropriate.

In the present study, therefore, the influence of presynaptic integrity on the morphology of dendritic structure in n. laminaris was investigated. After sectioning the crossed dorsal cochlear tract in hatchling chickens, the volume density of dendritic material and the frequency of primary dendrites on the dorsal and ventral sides of n. laminaris cells were compared. Similar analyses were performed on sham-operated animals.

## METHODS

### *Subjects and surgical procedures*

Five- to seven-day-old Red Cornish chicks, hatched in our laboratory and maintained after hatching in a communal brooder, were used in all experiments. Prior to surgery, each animal was given a 1.0 ml subcutaneous injection of 5% dextrose in 0.9% NaCl. One hour later, the animals were anesthetized with a 20 mg/kg body weight intraperitoneal injection of sodium pentobarbitol.

Under aseptic conditions, skin and muscle overlying the supraoccipital area were incised and retracted. A 27-gauge needle was inserted through the foramen magnum and manipulated in such a way that the axons from n. magnocellularis, which form the crossed dorsal cochlear tract, were severed. Three animals received this treatment and three others served as sham operates, having had the needle inserted into the foramen magnum but not so as to cut the tract. Following surgery, the incision was closed with alpha-cyanoacrylate adhesive (Aron Alpha, Vigor Corp.).

Immediately following surgery, both experimental and sham-operated birds were placed in individual compartments of a temperature-controlled brooder (32 $\pm$ 2 °C) until they were sacrificed. Every 18 h, beginning 18 h after surgery, they received a 2.0 ml subcutaneous injection of 5% dextrose in 0.9% NaCl. This was necessary since the experimental animals were often unable to eat or drink.

Ninety-six hours after surgery, the birds were anesthetized with a 0.2 ml intraperitoneal injection of sodium pentobarbitol, and then perfused transcardially with a fixative (at 4 °C) of the following composition: 3% glutaraldehyde, 1.5% paraformaldehyde, 0.6% acrolein, 0.6% dimethyl sulfoxide and 0.001% calcium chloride in 84 m*M* sodium cacodylate buffer at pH 7.2[27]. Following 10 min of perfusion, the animal was decapitated, bone overlying the cerebellum and forebrain was removed and the head was placed overnight in fresh fixative at 4 °C. The brain was removed from the skull and a 2 mm thick coronal slab containing the auditory nuclei was prepared. Under a binocular microscope, small tissue blocks containing n. magnocellularis and n. laminaris were dissected out and placed in fresh fixative for 2–5 h before postfixation.

*Electron microscopy*

Pieces of aldehyde-fixed medulla were postfixed with 2% osmium tetroxide in 80 m*M* cacodylate buffer at pH 7.2 for 3–5 h, washed with 0.85% NaCl and stained in block overnight with 2% uranium–magnesium acetate. The tissue was then dehydrated in a graded series of ethanols and propylene oxide and embedded in Epon. N. laminaris and n. magnocellularis were localized by light microscopic observation of thick sections stained with 0.1% toluidine blue in 1% sodium borate. Once the nuclei had been found, the blocks were trimmed, leaving only n. laminaris in the block face; in the case of sham-operated preparations, part of n. magnocellularis (which lies immediately dorsal to n. laminaris) and/or the ventricular surface were included in the block face to distinguish the dorsal from ventral regions of n. laminaris. Blocks were sectioned with a Porter–Blume MT-1 ultramicrotome, and silver-gray sections placed on copper grids. All specimens were counterstained with lead citrate and uranyl acetate.

Electron microscopic observations were made using an Hitachi HU-11B microscope with lens currents set to yield a magnification in negatives of × 3500. All micrograph samples were printed with a final magnification of × 14,600.

*Morphometric analysis*

The method for obtaining samples was standardized, so that all cells observed had an adjacent area of neuropil which was clearly identifiable as dorsal or ventral. The samples from each specimen were photographed and printed as described above. All dendritic profiles in each micrograph were outlined with a marking pen. Profiles were defined as dendritic when they contained ultrastructural elements generally associated with dendrites: i.e. rough endoplasmic reticulum, microtubules, irregular contours and numerous mitochondria. Using a 'cut-out-and-weigh' method for quantitation[32], tracing paper was placed over the micrograph, the dendritic profiles were traced onto the paper and these tracings were carefully cut out. A second tracing of each micrograph was made for the entire area of neuropil bounded by the soma and edges of the micrograph. Care was taken to keep the sample size as constant as possible among the various samples within each experimental group; the area of neuropil was approximately 160 sq. $\mu$m in each print. The respective dendrite and neuropil material for each sample was weighed with a Mettler balance, and expressed as a ratio, defined as the volume density of dendrite (dendrite/neuropil = 'volume density'). Data collected for each animal specimen were pooled and expressed as a mean and standard error for the animal. The individual means and standard errors for single animals were later combined for the various experimental groups described in the Results section. The statistical significance of the data was determined by a two-tailed *t*-test.

Those dendritic profiles in continuity with the cell which extended two or more micra beyond the radius of curvature of the cell were designated as primary dendrites. Their frequency was expressed as the number of primary dendrites observed relative to the total number of cells in the sample population and was computed for both dorsal and ventral regions of cells in lesioned and sham-operated groups.

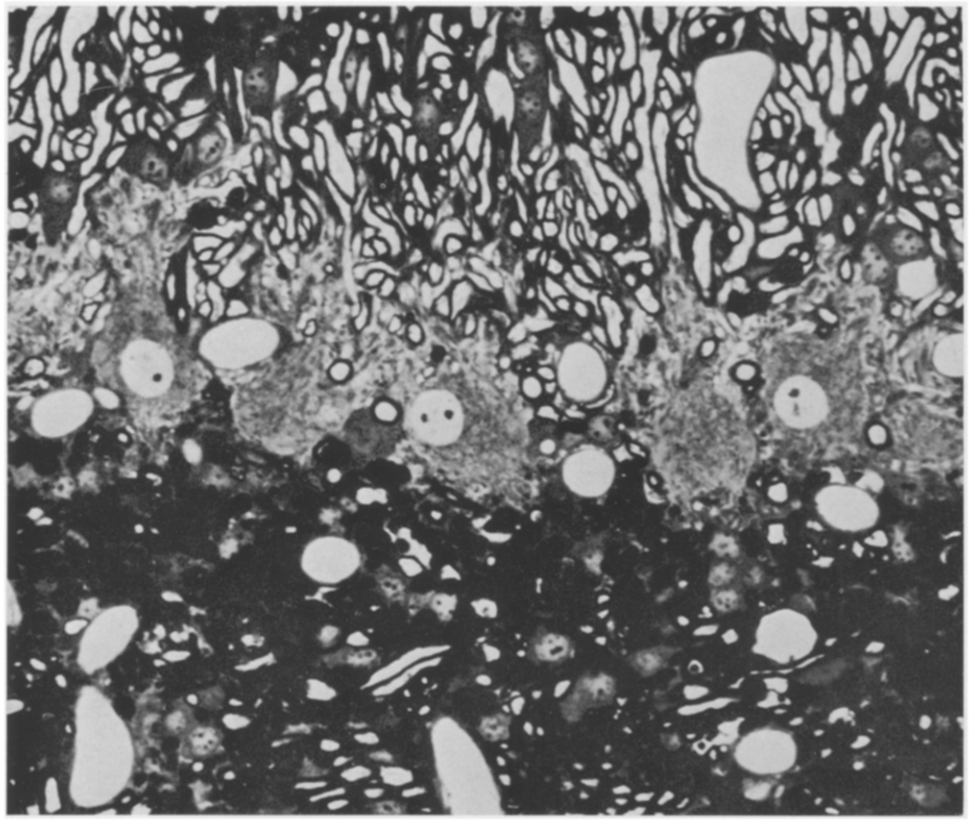

Fig. 1. A coronal section through n. laminaris from an animal lesioned 96 h before sacrifice. The tissue was postfixed in osmium tetroxide, embedded in plastic, sectioned at 1 $\mu$m and stained with toluidine blue. Above the line of n. laminaris neurons, the undisturbed dorsal neuropil region is apparent. Dorsal dendrites of n. laminaris neurons can be seen protruding deep within the neuropil and glial cells are sparsely distributed near the cell body. Below the cell body lamina, however, in the deafferented ventral neuropil region, the intense osmiophilia characteristic of degenerating axons and axon terminals is apparent, as is the larger number of glial cells near the cell bodies. × 730.

## RESULTS

The results of a midline transection of the crossed dorsal cochlear tract are severe degenerative changes largely confined to the ventral region of n. laminaris. Thick sections from electron microscopic preparations which were stained and viewed under the light microscope showed large amounts of osmiophilic material in the ventral portion of the nucleus and an increased number of glial cells near the cell soma (Fig. 1). When these specimens were viewed under the electron microscope, similar observations were made. At these higher magnifications a very few degenerate terminal boutons could also be seen in the dorsal neuropil of the nucleus. The cytological integrity of the ventral region was severely disrupted in the lesioned animals; in addition to degenerate boutons, myelinated axons showed a typical Wallerian pattern of breakdown, and in some cases these axons were apparently being phagocytized by glial cells.

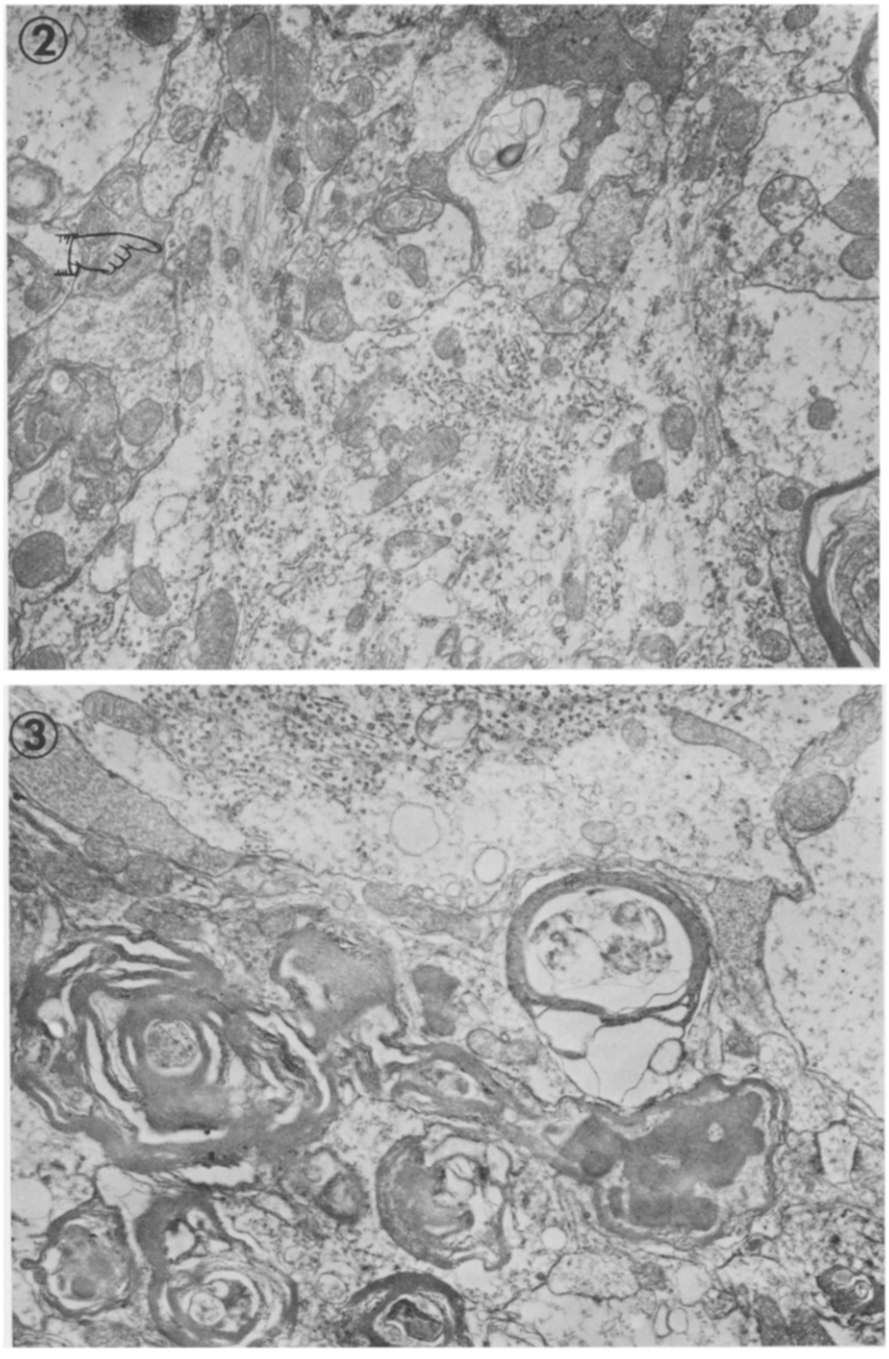


Fig. 2. The dorsal side of a n. laminaris neuron from a lesioned preparation. Two shafts of primary dendrite in continuity with the soma are evident (pointer); each has several presynaptic endings in contact and the overall cytological appearance of both terminals and dendrites is normal. The organelles in the soma appear normal and are present in the usual density, though some breakdown of the mitochondrial matrix and swelling of Golgi cisternae is seen. × 8800.

Fig. 3. The ventral side of a laminaris neuron from a lesioned preparation. Though one synapse is evident laterally, there are fewer synapses than normally encountered. The underlying neuropil has undergone a dramatic change in appearance, with few pre- or postsynaptic structures present. Many degenerate myelinated axons can be observed. × 8800.

There was a striking difference between the laminaris neuron dendrites in the ventral and the dorsal neuropil regions (Figs. 2–4). Qualitatively, it appeared that the overall amount of dendrite profiles was reduced in the ventral area and the cell bodies themselves seemed to have fewer primary dendrites visibly continuous with the cell soma. Morphometric analyses of the volume density of dendritic structure and the frequency with which primary dendrites in continuity with the soma could be observed in dorsal and ventral neuropil of both lesioned and sham-operated animals supported the gross morphological observations (Table I). Little difference in the volume density of dendritic structure between the two regions of the nucleus was found in sham-operated preparations. In lesioned preparations, however, there was an 85% reduction in this measure in the ventral area relative to the dorsal. A similar per cent reduction is apparent when the volume density of dendrite in the lesioned ventral neuropil is related to the same area of sham-operated controls.

The frequency of primary dendrites in the ventral neuropil of lesioned animals showed an 81% reduction relative to control ventral regions. Interestingly, there appeared to be a slight, statistically insignificant, lowering of this parameter in the dorsal area of lesioned animals, possibly reflecting the rare occurrence of degenerate boutons in this region. The frequency data could reflect a reduction in the diameter of ventral dendrites, since this would also reduce the probability of a profile appearing in continuity with the soma at one level of section. In order to rule out this possibility, the cross-sectional diameter of dendrites observed in ventral sham-operated and lesioned samples were measured and compared. The results showed that the dimensions found in both cases were almost identical (1.88 $\mu$m versus 1.80 $\mu$m, respectively). It should be noted, however, that the number of primary dendrites continuous with the cell body in the ventral neuropil of lesioned samples was very small, so that this measurement is not necessarily a reliable one.

Cytological disturbances of the cell soma were also found in most lesioned specimens. Such changes were primarily confined to the ventral region of the cytoplasm and included disturbances of mitochondria and swelling of cisternae in both Golgi apparatus and endoplasmic reticulum (ER) (Figs. 3 and 4A and B). In some instances

TABLE I

*Morphometric analysis of dendrites in nucleus laminaris 96 h after deafferentation of the ventral region*

The volume density of dendrite and the frequency of primary dendrites in continuity with the cell soma were determined as described in Methods. Significance levels were obtained for the differences between dorsal and ventral data within the sham-operated and tract cut groups using a two-tailed *t*-test.

| *Group* | *Region of nucleus* | *Number of animals* | *Number of samples* | *Volume density (± S.E.M.)* | *P* | *Frequency of primary dendrites* |
|---|---|---|---|---|---|---|
| Sham-operated | Dorsal | 3 | 15 | 0.15 ± (0.02) | > 0.10 | 1.06 |
| | Ventral | 3 | 18 | 0.12 ± (0.02) | | 1.00 |
| Lesioned | Dorsal | 3 | 17 | 0.14 ± (0.02) | < 0.002 | 0.88 |
| | Ventral | 3 | 16 | 0.02 ± (0.01) | | 0.19 |

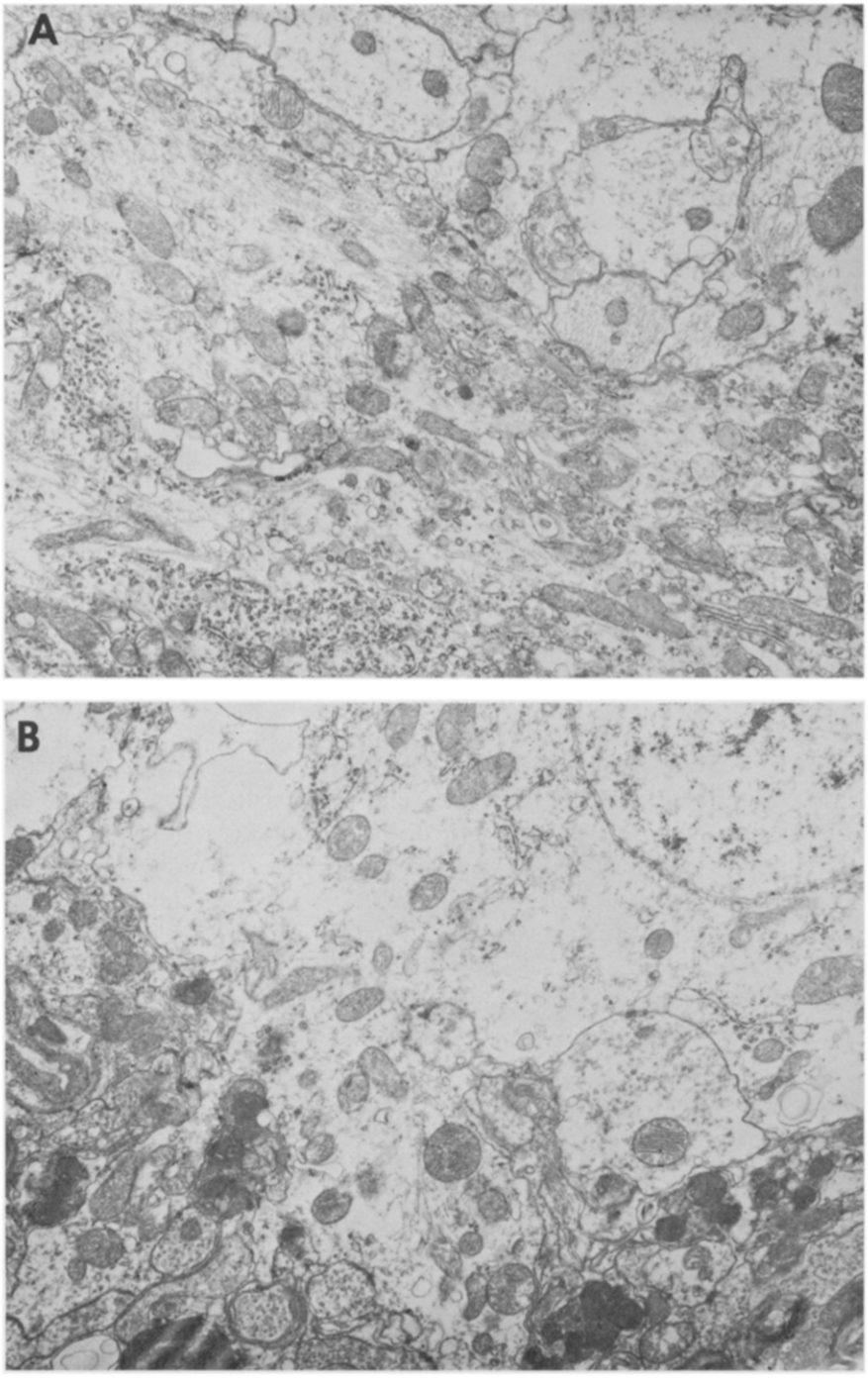


Fig. 4. A: the dorsal side of a laminaris neuron from a lesioned preparation. The overlying neuropil appears relatively normal and presynaptic endings can be seen in contact with the neurolemma. The most proximal part of a primary shaft can be seen to the left and its morphological appearance is normal, with filaments and tubules evident. The cytoplasm also appears undisturbed, with a full complement of rough ER, mitochondria and Golgi apparatus. × 8800. B: the ventral side of the same neuron as in A. In sharp contrast, the neuropil shows several degenerate boutons, and the density of organelles is severely reduced. There are two profiles of primary dendrite continuous with the cell body and the shape and cytological integrity of each is not normal. A few presynaptic endings remain in contact with these dendrites. × 8800.

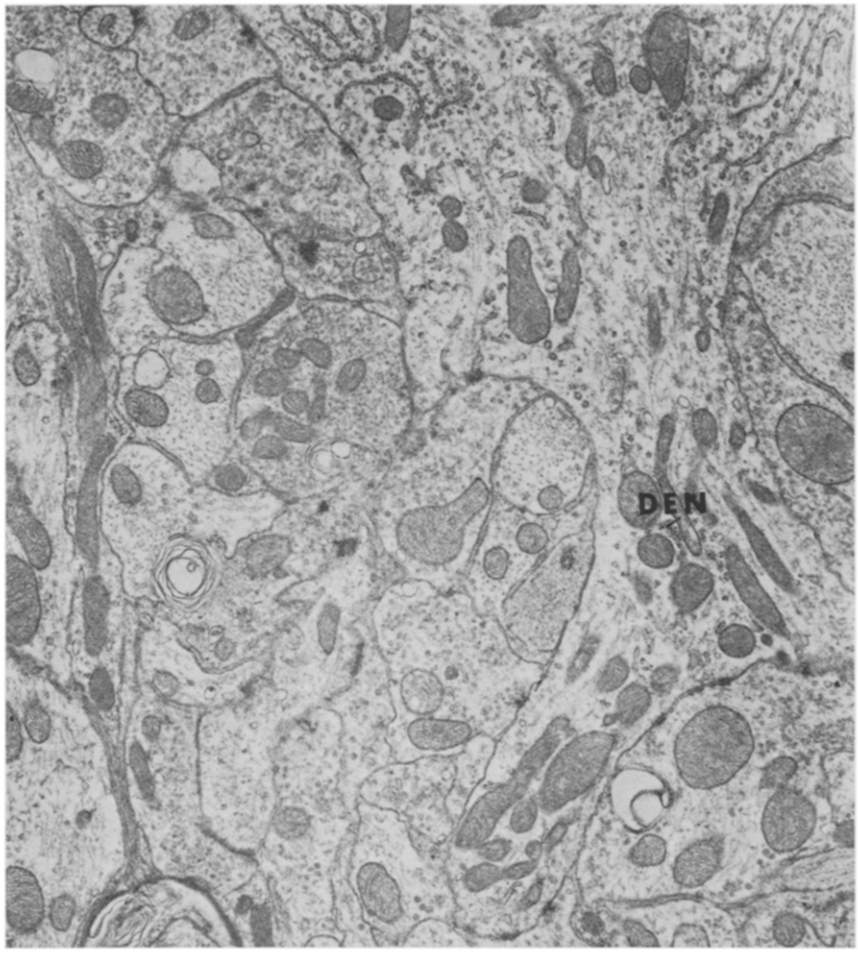


Fig. 5. The ventral side of a n. laminaris neuron from a sham-operated control specimen. A large primary branch of a dendrite (DEN) is bifurcating into two secondary branches, and synaptic endings in contact with the dendrite are evident. The overall appearance of the surrounding neuropil is normal. × 8800.

there was also an apparent reduction in the amount of rough ER, Golgi apparatus, mitochondria and cytoplasmic granularity localized to the ventral part of the cell (Fig. 4B). In general, these changes were seen in cells in which the degeneration of the presynaptic boutons was in an intermediate stage, i.e., some terminals were not as yet disrupted and degenerating myelinated axons were seen less frequently. Primary dendrites could often be seen in such cells; however, they were quite abnormal in appearance, reflecting a general cytoplasmic perturbation similar to that described for the soma. The normally well-defined longitudinal profile of the primary dendrite in these cases also seemed disturbed (cf. Figs. 2 and 5 with Fig. 4B).

## DISCUSSION

The results of the present study support the concept that the presence of pre-

synaptic elements is important in maintaining the overall integrity of the postsynaptic structures. The most striking and consistent observation has been that within 96 h following deafferentation of the ventral portion of n. laminaris there is a reduction in the amount and, in many cases, the morphological integrity of dendrites normally innervated by the interrupted axons. Although the rapid change in dendritic volume density could be secondary to the swelling of degenerating axons and changes in surrounding glia cells, two factors argue against such an interpretation. First, the parallel decreases in both volume density of dendrite and the frequency with which primary dendrites in continuity with the cell soma were encountered suggests that the dendrites were not merely being displaced by other structures. Second, after 16 days, when axonal debris is essentially gone, the dendritic changes persist (Rubel and Brandow, unpublished observations).

Previous studies have shown that, following deafferentation, reduction in the number of dendritic spines occurs in the cat lateral geniculate and visual cortex[8], mouse visual cortex[31], the olfactory bulb and pyriform cortex of both rat and rabbit[23], and the Purkinje cells of the rat[19]. Atrophy of dendritic shafts in the olfactory bulb[18,23] and lateral geniculate nucleus[13] has also been noted. The dendrites of n. laminaris neurons differ markedly from those of the systems cited above in that (1) spines are not present at all, (2) primary shafts constitute the major portion of the dendritic sprout with second- and third-order branches occurring less frequently, and (3) the afferent projections come almost entirely from the ipsilateral n. magnocellularis dorsally and from the contralateral side ventrally. Thus, in the present case, a midline transection of the crossed dorsal cochlear tract results in a nearly complete deafferentation of the ventral region of the n. laminaris neurons.

Although complete morphometric analyses were only undertaken at the one postoperative interval of 96 h, observations of similar preparations indicate that at 48 h and 60 h postoperatively the dendritic changes in n. laminaris are well advanced. Over a postoperative period of several weeks there is little change from the 96 h condition, and we have observed no evidence of collateral sprouting. (White and Nolan[33] have also reported an absence of collateral reinnervation in the mammalian medial superior olive following a unilateral deafferentation similar to that produced in the present study.) It should also be noted that transneuronal degenerative changes are generally thought to occur more readily in younger animals than in adults[4,29] and more rapidly in some species than in others[23]. Since the present study has been concerned only with 5–7-day-old chicks, the extent and rapidity of the changes may, at least in part, be age- or species-related phenomena.

An additional observation in this study has been the disruption of the normal morphological integrity of the soma at its ventral interface with the neuropil. In these cases, there was a severe reduction in the amount of rough ER, Golgi apparatus, mitochondria and overall granularity of the cytoplasm, while those organelles remaining in the ventral region of the perikaryon all showed signs of structural disruption. Other studies have reported decreased basophilia[29], increased condensation of nuclear chromatin[11] and the appearance of a 'watery' cytoplasm[1,6,23] in neurons which have been deafferented. The possibility cannot be ruled out that the cytoplasmic

disturbances reported here are due to inadequate fixation. It is commonly known that with experimental tissue it is often difficult to obtain satisfactory fixation and 'injured' cells do not always show the same reaction to changes in aldehyde or buffer concentrations as normal cells[22]. On the other hand, all sides of the n. laminaris cells were presumably exposed equally to the fixatives and adjacent cells were probably similarly exposed. However, within any one row of n. laminaris cells some neurons showed cytoplasmic disturbances and some did not; where these changes occurred, organelle loss was much more severe in the ventral than in the dorsal regions. Thus, it appears that there may be a differential response to the fixation procedures which is associated with deafferentation. At this time the exact nature of this 'differential response' is not clear, but a number of possibilities should be considered: (1) osmotic instability of the soma membrane may be associated with degenerating presynaptic elements; (2) insufficient cross-linking of remaining structural elements may occur; (3) cytoplasmic structures may be shifted dorsally; or (4) there may be a gradual dissolution of organelles which proceeds from the deafferented to 'normally innervated' regions. Further insight into these possibilities will require additional morphometric analyses of the regional distribution of volume densities of the various organellar compartments within such cells. In any case, the observations do suggest that the entire cell body does not uniformly change in association with local disruption of afferent innervation.

In the light of the present findings, it is of interest to consider the fate of the dendrites which disappear after removal of their presynaptic input. One possibility is that the dendrites are pinched off and later phagocytized by glial elements in a manner similar to that of the degenerate boutons and axons. On the basis of the present observations, this does not seem likely since no indications of this were seen in any of the samples examined. Alternatively, the shaft of the dendrite may be resorbed into the somal region, and excess plasma membrane could then be eliminated by the cell through normal channels for the degradative turnover of structural material. It is not likely that the soma would maintain an increase in diameter as a result of such a process since shrinkage following deafferentation has been reported in pontine nuclei[29], the olfactory system[18,23], and the lateral geniculate[9,17].

The results of the present study support the notion that the dendrite, rather than being a rigid structural element, is maintained through a tonic influence exerted by its presynaptic input. Deafferentation appears to severely compromise the cytological integrity of the dendrite in proportion to the amount of input removed. Moreover, these effects are largely confined to the specific postsynaptic surface from which innervation has been removed. This result may be of general theoretical interest, as it provokes at least two questions for future studies of the relationships between afferent stimulation and the efficacy of neural networks. First, is the dendritic atrophy due to (1) disruption of the physical contact between presynaptic and postsynaptic elements; (2) the elimination of a specific 'trophic substance' released from the presynaptic element; or (3) the elimination of local depolarizations of the postsynaptic membrane by afferent activity? If the latter possibility is tenable[15], then the neural system used in the present experiment will have advantages for future study

of the influence of an organism's environment on neural structure and function.

A second question concerns the mechanisms by which the size and shape of cell processes are regulated. Both the atrophy of ventral dendrites and the observations of local cytoplasmic changes adjacent to denervated regions of the soma suggest that synthesis and/or transport of cell constituents may be regulated as a function of input to specific membrane surfaces. Identification of the mechanisms responsible for such regulation may aid in understanding how neurons assume and maintain their characteristic shapes as well as how they are modified by changes in synaptic function.

ACKNOWLEDGEMENTS

We are grateful to Prof. Russell J. Barnett for advice and material support, to Mr. John Brandow for valuable assistance, to Mr. J. R. H. Jackson for comments on the manuscript and to Ms. Joan MacDonald for typing the manuscript. Raoul Garcia y Vega's contributions were not insignificant.

This research was supported by USPHS Training Grant 5-T01-CA5055-15, NSF Grant GB 31934 and funds from the Deafness Research Foundation.

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