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Rapid Growth of Astrocytic Processes in N. Magnocellularis Following Cochlea Removal

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ABSTRACT

Removal of the cochlea or pharmacological blockade of eighth nerve activity in young postnatal chickens results in rapid transneuronal cell death and atrophy in neurons of n. magnocellularis. The present experiments were designed to examine the influence of afferent input on astrocyte structure in n. magnocellularis. Young chickens were subjected to unilateral cochlea removal. At times ranging from 5 minutes to 72 hours later, the brainstems were histologically processed with a polyclonal antibody against glial fibrillary acidic protein (GFAP). A second group of chick brainstems was impregnated by a Golgi method 6 hours after unilateral cochlea removal and impregnated three-dimensional reconstructions were made of glial cells in n. magnocellularis (NM).

Analyses of GFAP positive processes in NM revealed an observable increase in the number of astrocytic processes at the borders of the nucleus within 30 minutes of cochlea removal and a twofold increase in GFAP+ glial processes by 6 hours. A secondary increase in the number and density of GFAP+ processes occurred between 24 and 72 hours following cochlea removal, during the period of axonal degeneration, and transneuronal cell atrophy and death.

Analyses of astrocytes impregnated by the Golgi method revealed that individual glial cells had increased their total process length and the number of processes by approximately twofold by 6 hours after cochlea removal.

These results suggest that the structure of astrocytes is rapidly and dramatically influenced by the level of excitatory activity in a neuronal system. Furthermore, the similarity of results obtained with GFAP immunohistochemistry and three-dimensional reconstruction of astrocytes provides evidence that the short-term changes observed following cochlea removal represent the actual growth of glial processes. We speculate that modulations in glial processes as a function of afferent activity may act to influence synaptic efficacy.

Key words: glia, deafferentation, auditory, activity

Astrocytes are the most abundant cell type in the mature nervous system. Many reports document the reactions of astrocytes to injury of the central or peripheral nervous system (Lindsay, '86; Malhotra et al., '90). These reactions include extension of new astrocytic processes (Wong-Riley, '72), increases in immunoreactivity for the intermediate filament proteins such as glial fibrillary acidic protein (GFAP) and vimentin (Dahl et al., '81; Eng and DeArmand, '82; Pixley and de Vellis, '84), increases in GFAP mRNA (Steward et al., '90), and proliferation and migration of microglia (Graeber et al., '88). In most cases, these changes are reported days or weeks following the insult and are considered to be permanent.

In addition, there is a plethora of information describing neuronal changes that occur with variations in hormonal or environmental conditions. But little is known about the involvement of glial cells in these forms of plasticity. The few reports that have examined glial cells distant from the site of injury or as a result of variations in extrinsic events have evaluated changes days or weeks following such manipulations, and thereby, cannot elucidate the cellular interactions responsible for changes in glial cell structure or function (Anders and Johnson, '90; Cass and Goshgarian, '90; Hajos et al., '90).

The avian brainstem auditory pathways have provided a useful preparation in which to study the influence of afferent integrity and afferent activity on the maturation

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and maintenance of postsynaptic neurons (Levi-Montalcini, '49; Rubel et al., '90). Axons of the auditory portion of the avian eighth cranial nerve enter the dorsolateral brainstem and then bifurcate, providing excitatory afferents to the ipsilateral cochlear nucleus, which is composed of two major subnuclei, n. magnocellularis (NM) and n. angularis. While n. angularis receives a variety of other inputs (both excitatory and inhibitory), auditory nerve axons provide the sole excitatory input to NM (Hackett et al., '82; Rubel and Parks, '88). Surgical elimination of the cochlea (basilar papilla) or blocking eighth nerve activity by intraperilymphatic injection of tetrodotoxin (TTX) completely eliminates action potentials in the ipsilateral eighth nerve and NM. The contralateral nerve and NM maintain normal levels of spontaneous and acoustically evoked neuronal activity. The unilateral nature of these anatomical and physiological features has allowed us to document a series of rapid cellular events in the postsynaptic NM neurons that result from alterations in afferent electrical activity (Rubel et al., '90); NM cells ipsilateral to peripheral manipulations are compared to contralateral NM cells within the same tissue sections. We now use this strategy to examine structural changes in astrocytes following interruption of the excitatory input to n. magnocellularis.

Following removal of the cochlea in neonatal chicks, there was a rapid increase in glial processes which stained positively for glial fibrillary acidic protein (GFAP) in n. magnocellularis. The increase was apparent within ½ hour of cochlea removal and statistically reliable by 1–3 hours. In a second series of animals, astrocytes were stained by the Golgi method and three-dimensional reconstructions were made. Measurements of the number and total length of astrocytic processes in NM revealed an increase within 6 hours after cochlea removal.

Preliminary results of these studies have been presented in abstract form (Rubel and MacDonald, '87; MacDonald and Rubel, '89).

MATERIALS AND METHODS

Subjects

Post-hatch chickens (10–14 days old) were used for both the GFAP and the Golgi impregnation studies. White leghorn eggs were obtained from a local hatchery (H&N Farms, Redmond, WA), and were incubated and hatched in the University of Washington vivarium. The post-hatch animals were maintained in warmed brooders with free access to food and water after hatching, until the time of surgery. For surgical procedures, the chicks were anesthetized with sodium pentobarbital (18.75 mg/kg, i.p.) and ketamine hydrochloride (80 mg/kg, i.m.). Prior to sacrifice by transcardiac perfusion, chicks were given a lethal dose of sodium pentobarbital.

Surgical procedures

The procedure for removal of the cochlea (basilar papilla) has been described in several previous publications (c.f. Born and Rubel, '85; Durham and Rubel, '85). Briefly, after induction of anesthesia and removal of feathers surrounding the ear, the tympanic membrane is reflected and the columella removed from the middle ear. A pair of fine forceps is inserted through the oval window to grasp and extract the cochlea. The extracted cochlea is then examined under an operating microscope to ensure complete receptor removal. The oval window cavity is filled with gelfoam to prevent bleeding and the incision is closed with cyanoacrylate glue. All procedures are carried out under aseptic conditions. The animals recover from anesthesia in a warmed incubator and subsequently have free access to food and water. Following recovery from anesthesia, the animals are fully mobile and usually show no overt signs of vestibular dysfunction. This procedure immediately eliminates all action potentials in the auditory nerve and NM, but leaves the cochlear ganglion cell bodies and eighth nerve axons intact (Born and Rubel, '85; Born et al., '91). The ganglion cells and their central projections begin to degenerate after 18–24 hours (Parks and Rubel, '78; Born and Rubel, '85).

Histological procedures

GFAP Immunohistochemistry. Several fixatives, tissue processing methods, and antibodies were used; all yielded similar experimental results. Animals were perfused with either 4% paraformaldehyde in 0.1 M phosphate buffer (pH = 7.4) or 2% paraformaldehyde, 0.2% lysine, 0.05% sodium periodate (PLP). The brains were then postfixed for 12–18 hours at 4°C. Some tissue was fixed by immersion for 12–24 hours in Bouin's, Zamboni's or methacarn (methanolic Carnoy's fluid). The tissue was embedded in paraffin and sectioned at 6 μm or 10 μm, or sectioned on a vibratome (30 μm), or cryoprotected with 30% sucrose and cut on a cryostat (20 μm). While each of the tissue processing methods has particular advantages, all were effective in staining astrocytic processes, and the comparisons reported below were independent of tissue processing methods. Standard immunocytochemical procedures were used. The polyclonal antisera to GFAP (DAKO, Santa Barbara, CA; or East Acres Biologicals, Inc., Southbridge, MA) were diluted 1/600 to 1/4,800 and applied to the tissue for 16–40 hours. Immunolabeling was visualized by the avidin-biotin-peroxidase method (ABC) using Vectastain reagents (Vector Labs, Burlingame, CA) and diaminobenzidine (DAB) as the chromagen. Non-immune binding was blocked with 4% normal goat serum. Control sections were incubated with non-immune rabbit immunoglobulin at concentrations such that the IgG content approximated that of the primary antiserum. Control sections showed no labeling.

In all cases, the entire rostral-caudal extent of NM was serially sectioned in the coronal plane and great care was taken to orient tissue blocks so that the sections were bilaterally symmetrical. Alternate sections were processed for GFAP immunohistochemistry or for Nissl with thionin. All together, approximately 80 chicks were processed by these methods. The best tissue for the quantitative comparisons reported below came from 30 μm vibratome sections labeled with the DAKO antibody at dilutions of 1:600 to 1:2,400.

Golgi impregnation. Anesthetized chicks were perfused with 0.9% saline containing 5% chloral hydrate, followed by an aqueous solution of 5% potassium dichromate, 5% chloral hydrate, 2% paraformaldehyde, 2% dimethyl sulfoxide, and 1.25% glutaraldehyde (Stensaas, '67). Brains were then immersed in fixative for 80 hours. Brainstems were impregnated in aqueous 1% silver nitrate for 36 hours, embedded in celloidin, sectioned at 120 μm, and mounted with DPX.

Quantitative analyses

GFAP stained tissue. Our objective for analysis of the tissue stained for GFAP positive processes was to compare objectively the *relative* density of GFAP positive glial processes in NM on the two sides of the brain at various

times following unilateral cochlea removal. The methods employed are not intended to provide absolute measurements of the amount of protein or even interval scale data. The immunolabeling method employed is sensitive to low antigen concentrations, allowing detection of slight changes in antigen presence or exposure. However, it saturates quickly and becomes non-linear with rising antigen concentration. Furthermore, GFAP positive processes occupy a relatively small amount of the total volume of NM (4–20%). Therefore, low magnification measurements of average optical density will be insensitive to changes in the number of stained processes. Thus, our measurements are based on the area-density of GFAP positive processes. That is, stained processes were detected on the basis of an optical density above a visually determined threshold. By computing the thresholded area relative to the total area of NM on each side of the brain in a single section, and by maintaining a constant threshold value on the two sides, we could determine the relative area-density of GFAP positive elements between the two sides of any tissue section. It is important to note that this algorithm does not take into account any changes in the staining intensity of GFAP processes once they were above the detection threshold. Thus, the method we employed allows a relatively conservative, but unbiased, comparison of immunostaining within a single tissue section.

The difference in density of GFAP positive processes between NM ipsilateral and contralateral to cochlea removal was quantified in 25 experimental and 8 control brains chosen on the basis of staining quality and bilateral symmetry of the transverse sections. A Bioquant IV Imaging System (R&M Biometrics, Nashville, TN) coupled to a Leitz Aristoplan microscope and a DAGE video camera (model 68) was used to compare the relative density of GFAP+ processes on the two sides of the brain. Sections were chosen at 25%, 50%, and 75% of the anterior-posterior level of NM. Using a 40× oil immersion objective (N.A. 1.0), NM on the control side was viewed and a threshold level for GFAP+ processes was established. The entire NM region on that side was then carefully outlined with a cursor; blood vessels were excluded. The area-density of thresholded pixels was then computed, giving a measure of the density of GFAP+ processes. *Without changing the threshold detection level,* the same procedure was carried out on NM from the experimental side of the brain and the ratio of labeling density on the two sides of the section was computed. The ratios from the three sections from each brain were then averaged, yielding a single number for each brain to be used in the statistical analysis. Statistical analyses were performed by using a single factor ANOVA. Individual comparisons between treatment groups used the Fisher PSLD method (Stat View 512™, Brain Power, Inc., Clabasas, CA).

Golgi impregnated processes. Silver impregnated glial cells were traced by means of the Neuron Tracing System (NTS) computer hardware and software package (Eutectics Electronics, Inc., Raleigh, NC) controlling a Zeiss WL microscope. Sections were selected at points 30% and 60% along the rostral-caudal axis of NM. Fully impregnated glial cells which fell within or immediately adjacent to NM, and which could be completely resolved, were selected. All glial cells in the tissue sections meeting these criteria were analyzed. Glial cells were traced under oil immersion (100×, NA = 1.3). Glial process length and branching were compared between the ipsilateral and contralateral sides of each animal. Comparisons were also made between grouped data, including all glial cells traced in the contralateral NM region of experimental animals and both sides of unoperated control animals versus all traced glia in the experimental NM region. These data were subjected to a single factor ANOVA.

Experimental design

The goal of the GFAP experiments was to determine the time course of changes in GFAP reactive astrocytic processes in NM following unilateral removal of the cochlea, and to relate any short term changes to the classical astrogliosis reaction expected to occur following degeneration of the eighth nerve. We also sought to relate any changes in GFAP expression to the rapid changes we have observed in NM neurons following cochlea removal. Thus, following cochlea removal, animals were killed at the following intervals (the number of animals used for quantitative analyses): 0.5 hour (4); 1–3 hours (7); 6 hours (8); 12–24 hours (4); 72 hours (2). Eight animals served as controls; 4 were allowed to survive 6 hours after brief induction of anesthesia and 4 were subjected to unilateral cochlea removal, and then sacrificed 5 minutes later. These two groups were combined for statistical analyses.

Our objective for the experiments using Golgi impregnated tissue was to evaluate whether the rapid changes we observed in GFAP immunoreactive processes were best interpreted as a change in the expression of, or binding to, intermediate filaments in established glial processes, or could reflect the actual growth (or retraction) of new processes. Ten experimental animals underwent unilateral cochlea removal and were allowed to survive 6 hours; ten unoperated control animals of the same age were sacrificed at the same time.

RESULTS

Time course of changes in GFAP immunoreactivity

The normal NM typically displays relatively few GFAP positive astrocytic somata within the nucleus. Most immunolabeling is associated with processes reaching into NM from astrocytic somata situated outside the nucleus, residing at the dorsal and ventral margins. These immunoreactive processes tend to course between the columns of NM neurons for a distance before branching into perineuronal endfeet. As in other parts of the brain, GFAP positive processes are abundant in and around blood vessels. Astrocytes located at the medial and lateral edges of NM appear to circumscribe the nucleus a short distance before sending their processes laterally into NM.

Increases in GFAP immunoreactive processes in NM on the side of the brain ipsilateral to the cochlea removal could be seen in all experimental animals. At ½ hour after cochlea removal, the difference was subtle and only apparent at the dorsal and ventral edges of the nucleus. Figure 1 shows a representative section from an animal in this group. At the ventral edge of NM on the side ipsilateral to the cochlea removal (Fig. 1A), an increase in immunostained processes can be seen as compared with the same position on the contralateral side (Fig. 1B). Careful examination of the staining pattern suggests that this increase is due to processes from cell bodies in the glia-rich zone surrounding NM, which grow into the nucleus and ramify around the NM neurons. By 1–3 hours, small but obvious increases in the density of immunoreactive processes can be seen throughout NM on the side ipsilateral to receptor removal. By 6 hours, the increase is pronounced and readily apparent throughout the nucleus (Fig. 2). As exemplified in Figure 3,

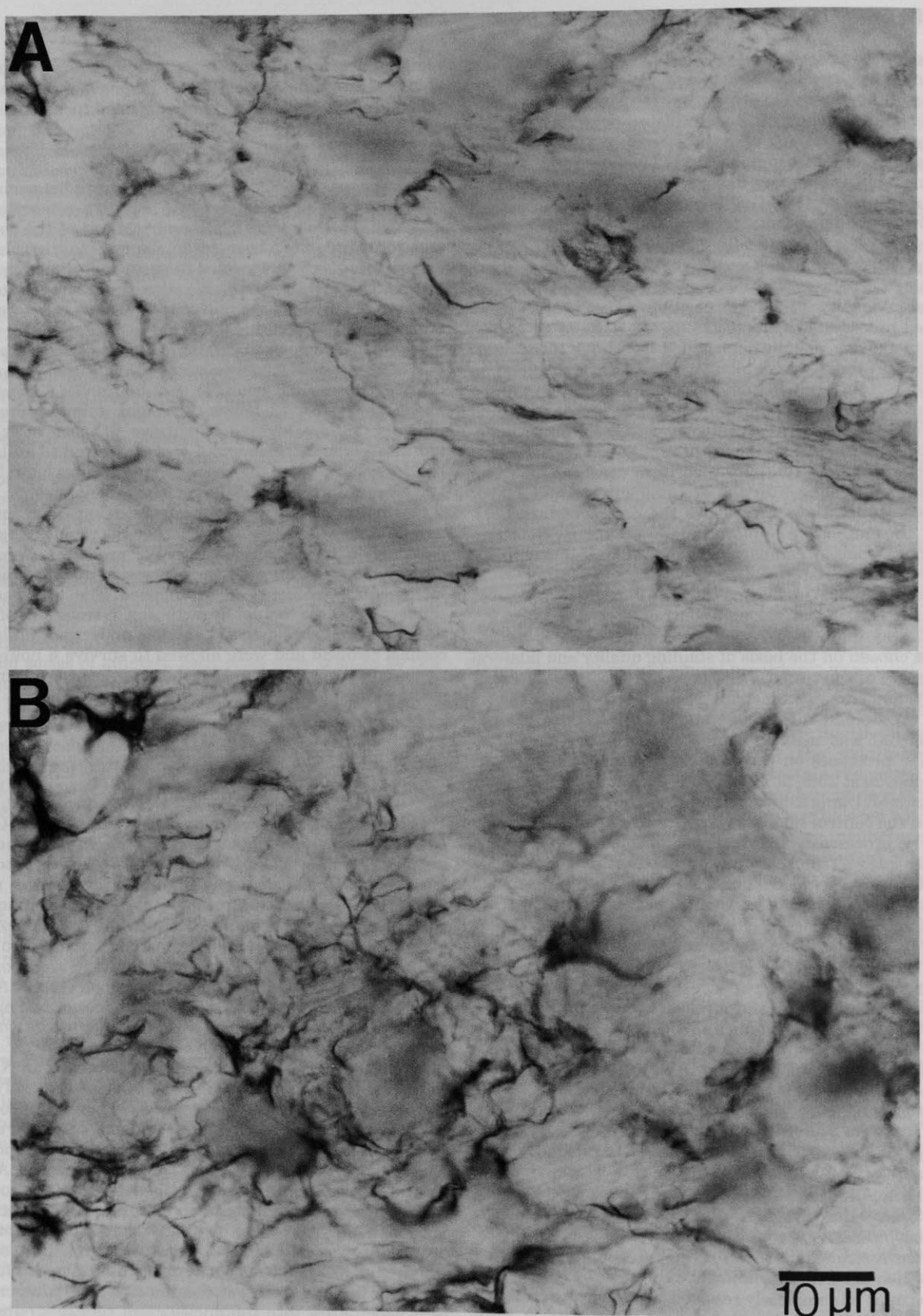

Fig. 1. Photomicrographs of n. magnocellularis (NM) from a single tissue section stained for GFAP immunoreactive processes. The chick was sacrificed 30 minutes following unilateral cochlea removal. These micrographs are taken at the ventral border of NM. **A:** Contralateral to cochlea removal. **B:** Ipsilateral to cochlea removal. Note that in NM on the ipsilateral side of the brain, glial fibrillary acidic protein (GFAP) immunoreactive processes appear to have grown into the nucleus; thin GFAP+ processes are beginning to encircle the cell bodies.

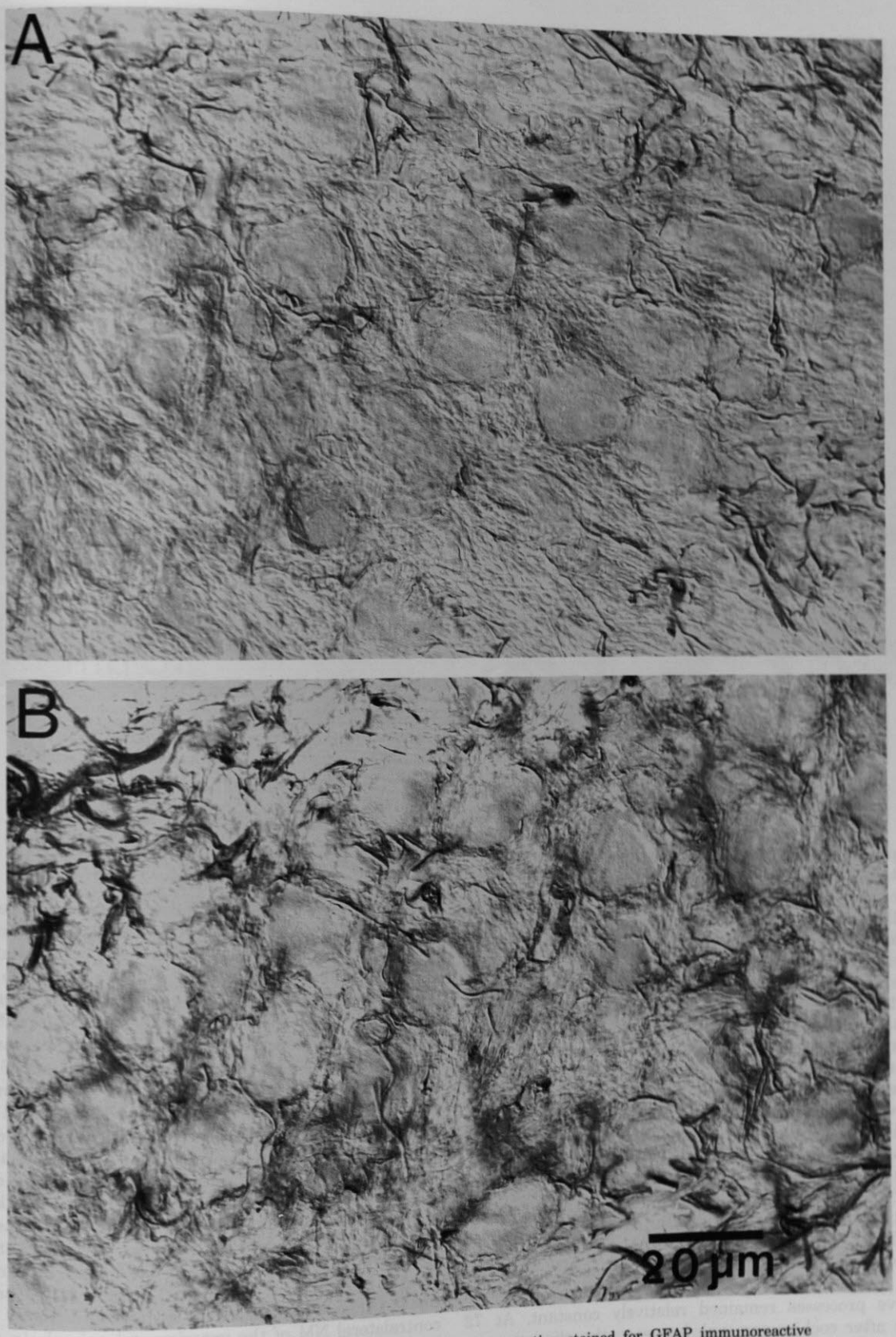

Fig. 2. Photomicrographs of NM from a single tissue section stained for GFAP immunoreactive processes. This animal was sacrificed 6 hours after cochlea removal. **A:** Contralateral (control) side of the brain. **B:** Ipsilateral side of the brain. Note that there are an abundance of GFAP+ processes in B, compared to A. In addition, processes in the ipsilateral NM are seen extending in all directions among the NM cell bodies.

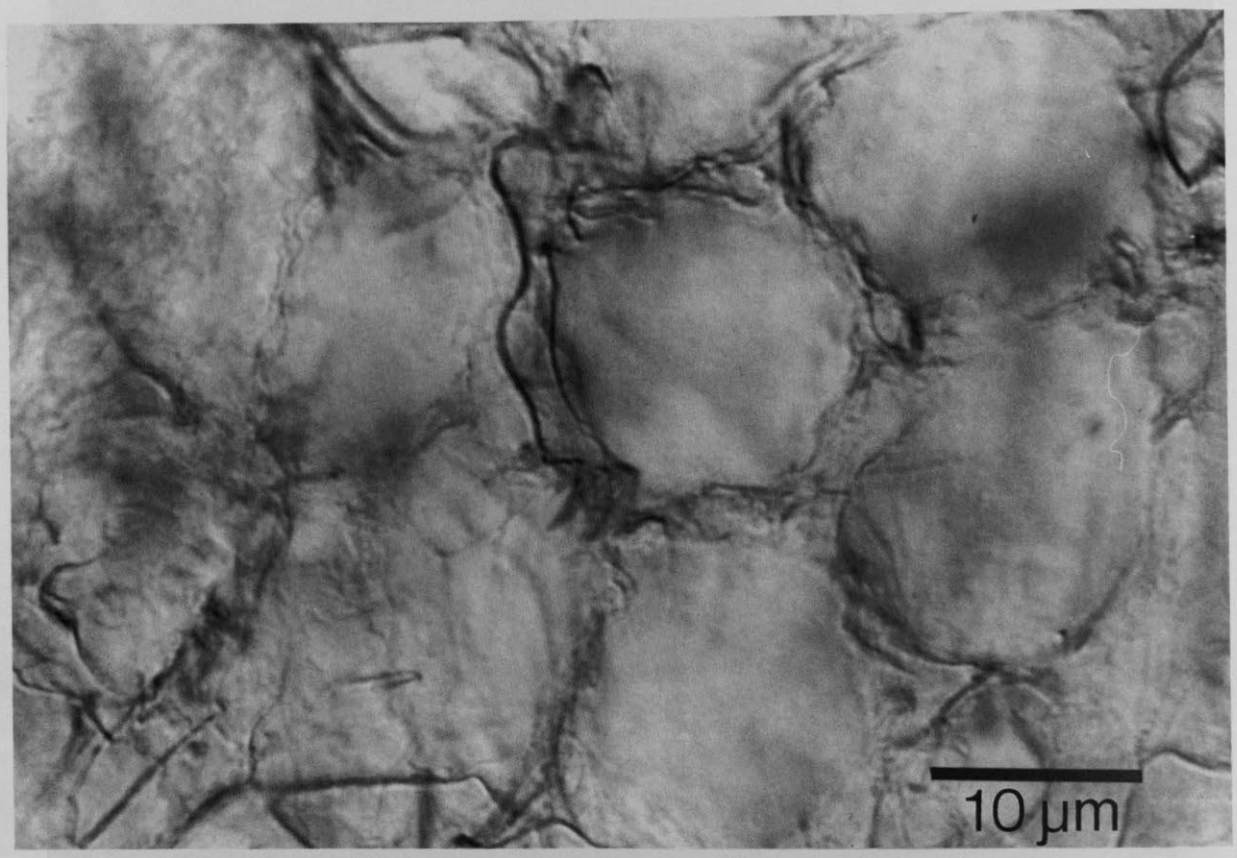

Fig. 3. High power photomicrograph of NM ipsilateral to cochlea removal from an animal sacrificed 6 hours after the surgery. Note GFAP immunoreactive processes encircling NM neuron. This is routinely seen in the ipsilateral NM of animals sacrificed at 6 hours or later but rarely in tissue from the contralateral side or in control animals.

glial processes envelop some NM neurons and course in all directions through the neuropil. On the contralateral side of the brain, the immunoreactive glial elements are sparse, usually coursing down the columns of neurons without enveloping the cell bodies to the extent seen on the ipsilateral side. By 72 hours after receptor removal, eighth nerve axons are degenerating, and a dramatic reactive gliosis is found in NM (Fig. 4). This later-appearing gliosis is typical of that observed during axonal degeneration in other systems.

Quantitative comparisons of the density of GFAP immunoreactive processes ipsilateral and contralateral to cochlea removal are shown in Figure 5. Although the increase in GFAP positive elements observed 0.5 hours following receptor removal is apparent at the borders of NM, it is not seen throughout the nucleus and there is no statistically significant difference between experimental and control brains at this time. On the other hand, by 1–3 hours, there is a significant 40–50% increase in the density of GFAP processes ($p < .05$), and by 6 hours this increase averages 84% ($p < .01$). Between 6 and 24 hours, the increase in GFAP positive processes remained relatively constant. At 72 hours after cochlea removal, however, the glia astrocyte reaction in the deafferented NM yields an average increase of 517% over the control side of the brain.

Golgi impregnation

The rapid increase in GFAP immunoreactive processes in NM following cochlea removal might be due to an increase in the amount of immunoreactivity of intermediate filaments in existing glial processes, or to an increase in the number and length of processes on glial cells. To address these possibilities, we reconstructed individual NM glial cells from unoperated chicks and from both experimental and control sides of NM from animals sacrificed 6 hours after receptor removal. Figure 6A shows representative glial cells from NM of control chicks and the control side of operated chicks. Astrocytes from NM ipsilateral to cochlea removal are shown in Figure 6B. These tracings and the quantitative data presented in Figures 6C and D indicate that by 6 hours after elimination of the receptor, glial cells in NM have reliably greater total process length and more branches extending from each process. Statistical analyses revealed that astrocytes in the ipsilateral NM have reliably greater total process length and significantly more processes per cell than in either control animals or in the contralateral NM of the experimental animals ($p < .01$). Values from the contralateral side and from control animals were not significantly different. Thus, the rapid increase in the density of GFAP+ processes following receptor elimina-

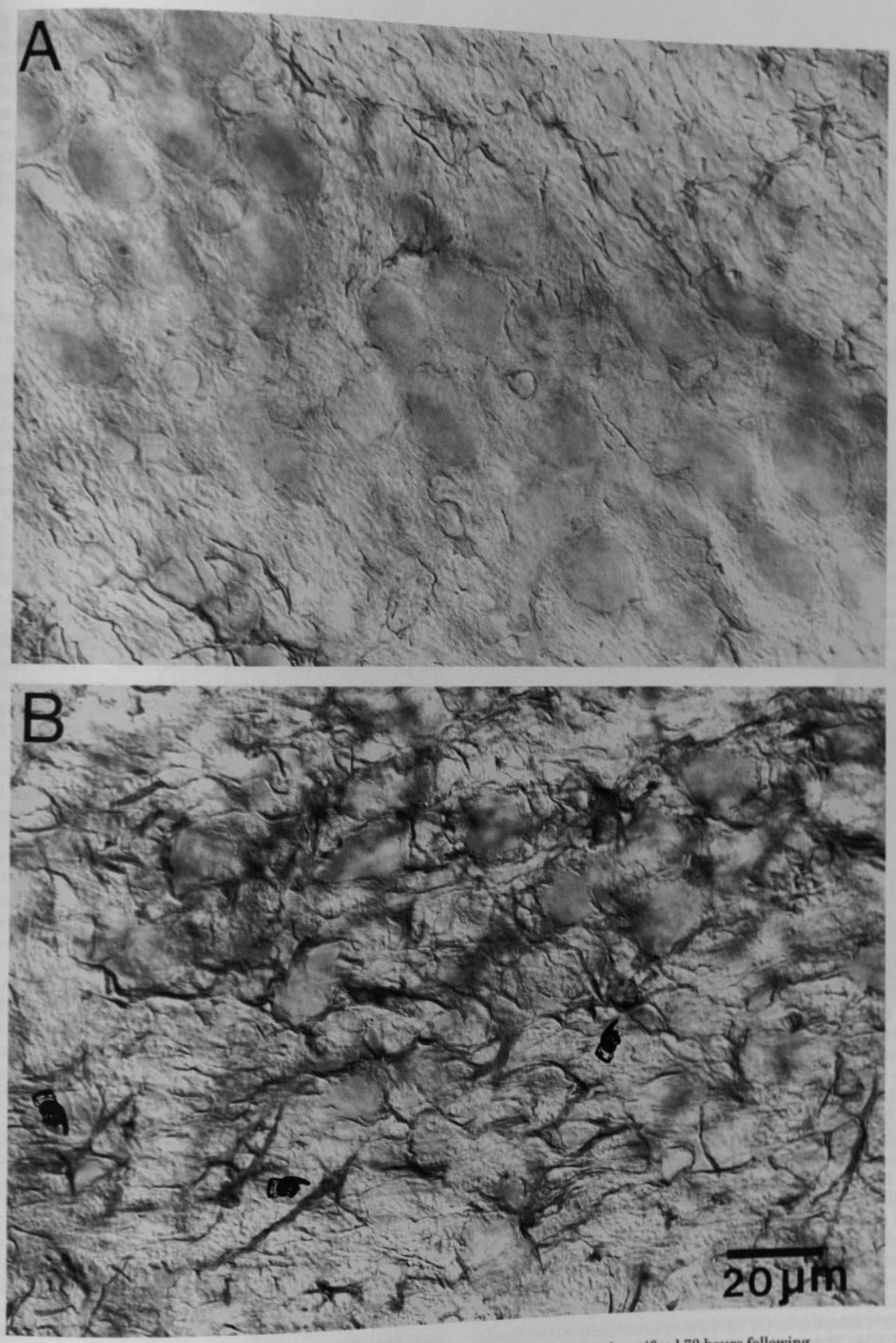

Fig. 4. Photomicrographs of NM from a single tissue section in an animal sacrificed 72 hours following cochlea removal. On the contralateral side (**A**) few GFAP processes are seen. In the ipsilateral NM (**B**), classical astrogliosis is evident with an increase in GFAP+ cell bodies as well as marked proliferation of immunoreactive processes. Pointers indicate some of the GFAP+ glial somata.

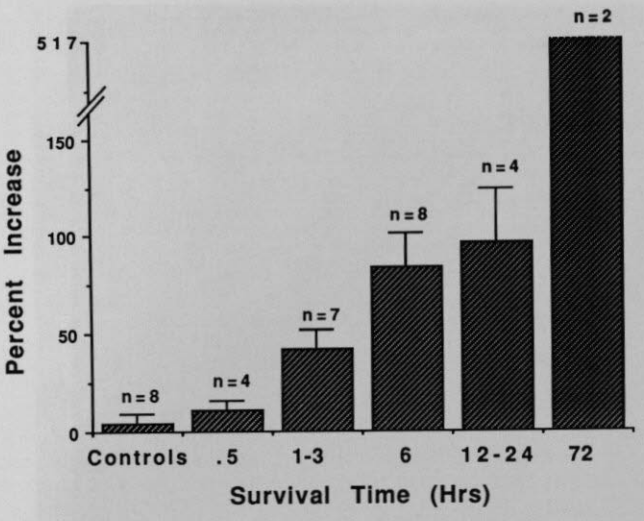

Fig. 5. Results of quantitative analysis of GFAP+ processes on the ipsilateral vs. contralateral NM. Each bar represents the average (+S.E.M.) percent increase in the density of GFAP+ processes on the ipsilateral vs. contralateral NM. This average (and S.E.M.) was computed by obtaining the average from the three sections analyzed for each animal (see Materials and Methods) and treating this as a single score for that subject. Scores for the subjects (n) in each group were then averaged to provide the values shown. Statistical analyses (ANOVA) revealed .5 hour survival values were not reliably different from controls. Values at all other survival times were significantly greater than controls ($p < .05$ for 1–3 hours; $p < .01$ for 6, 12–24, and 72 hour groups). Error bar is not evident in 72 hour group because both values were within 4%.

tion probably reflects the growth of processes rather than exclusively an increase in the number of intermediate filaments or their affinity for the antibody.

DISCUSSION

The observations reported in this paper suggest that structural changes in astrocytes may begin within the first few minutes after altering afferent input, and may involve the actual growth (or retraction) of glial processes. The tissues stained for GFAP immunoreactive processes 30 minutes after receptor removal show increases in the number and staining density of processes near the edges of NM, suggesting that processes are invading the nucleus from the glia rich zones which surround it. By 6 hours following cochlea removal, well before any signs of degenerating axons or terminals can be seen in NM (Parks and Rubel, '78), immunoreactive processes can be seen throughout the nucleus. At this time, there is approximately a twofold increase in the density of immunoreactive processes over that seen on the side of the brain receiving input from the unmanipulated ear, and a similar increase in the overall length of processes on glial cells impregnated by the Golgi method. At these times, there does not appear to be an increase in the total number of immunoreactive glial cell bodies. Between 24 and 72 hours following cochlea removal, there appears to be another large increase in the astrocytic reaction; both animals allowed to survive for 3 days showed a fivefold increase in GFAP immunoreactivity in the deafferented NM. This period is marked by massive degeneration of cochlear nerve terminals and a noticeable increase in the number of GFAP immunoreactive cell bodies in NM and in the eighth nerve axonal bundle approaching NM. In the discussion which follows, we will consider three topics: (1) the relationships of astrocytic changes to neuronal changes in NM following cochlea removal and eighth nerve activity blockade; (2) the time course of astrocytic changes; and (3) speculations about functional significance of the astrocytic changes we have observed. We will not consider in detail the possible cellular or molecular signals initiating the growth of astrocytic processes. That topic is discussed in another manuscript (Canady and Rubel, '91).

Temporal relationship between astrocytic and neuronal changes in NM

The time course of a wide variety of structural and metabolic changes in NM neurons and eighth nerve axons following cochlea removal has been described in detail (reviewed in Rubel et al., '90). Immediately following removal of the cochlea, there is a complete cessation of extracellularly recorded action potentials in the ipsilateral eighth nerve and NM (Born et al., '91). Within the first 90 minutes, a variety of metabolic and structural changes in NM neurons is observed. These include a decrease in glucose uptake throughout NM which has not been localized to specific subcellular elements (Lippe et al., '80), a 50% reduction in protein synthesis within the soma (Steward and Rubel, '85), disruption of ribosomes within the endoplasmic reticulum (Rubel et al., '91), reduced immunostaining for a variety of cytoskeletal elements (Seftel et al., '86), and reduced immunostaining for ribosomal RNA components (Canady and Rubel, '91). These neuronal changes are correlated with mild but significant increases in GFAP immunoreactive processes in NM, which appear to begin at the edges of the nucleus. It is somewhat surprising that the increases in GFAP are initially restricted to the edge of NM since the neuronal changes are observed throughout the nucleus. This difference may be an indication that the signals initiating the neuronal and glial changes are independent. Alternatively, it may be a detection problem. That is, the distribution of immunoreactive processes in the normally active NM is relatively sparse and highly variable, while in the fiber bundle surrounding NM, it is more dense and homogeneous. This would allow easy detection of small increases in processes growing into the nucleus from the periphery.

Between 3 and 6 hours after cochlea removal, the neuronal responses become more complex. They include a subset (~30%) of cells which entirely cease protein synthesis, show complete ribosome degradation (Steward and Rubel, '85; Rubel et al., '91), and an overall increase in enzymatic activity associated with mitochondria (Durham and Rubel, '85; Hyde and Durham, '90a). Electron microscopic examination of the tissue does not reveal degenerative changes in the central processes of eighth nerve axons or terminals in NM at this time. Both GFAP immunoreactivity and the number of processes observed on silver impregnated astrocytes are increased approximately twofold at this time, and GFAP positive processes appear to be encircling neuronal somata. This temporal relationship suggests, but by no means proves, that signals from affected neurons are influencing the growth of glial processes. It will be of interest to correlate the changes in individual neurons with the glial structures surrounding them, or to manipulate the extent of neuronal changes by blocking the mitochondrial response (Hyde and Durham, '90b) and observing the glial reactions.

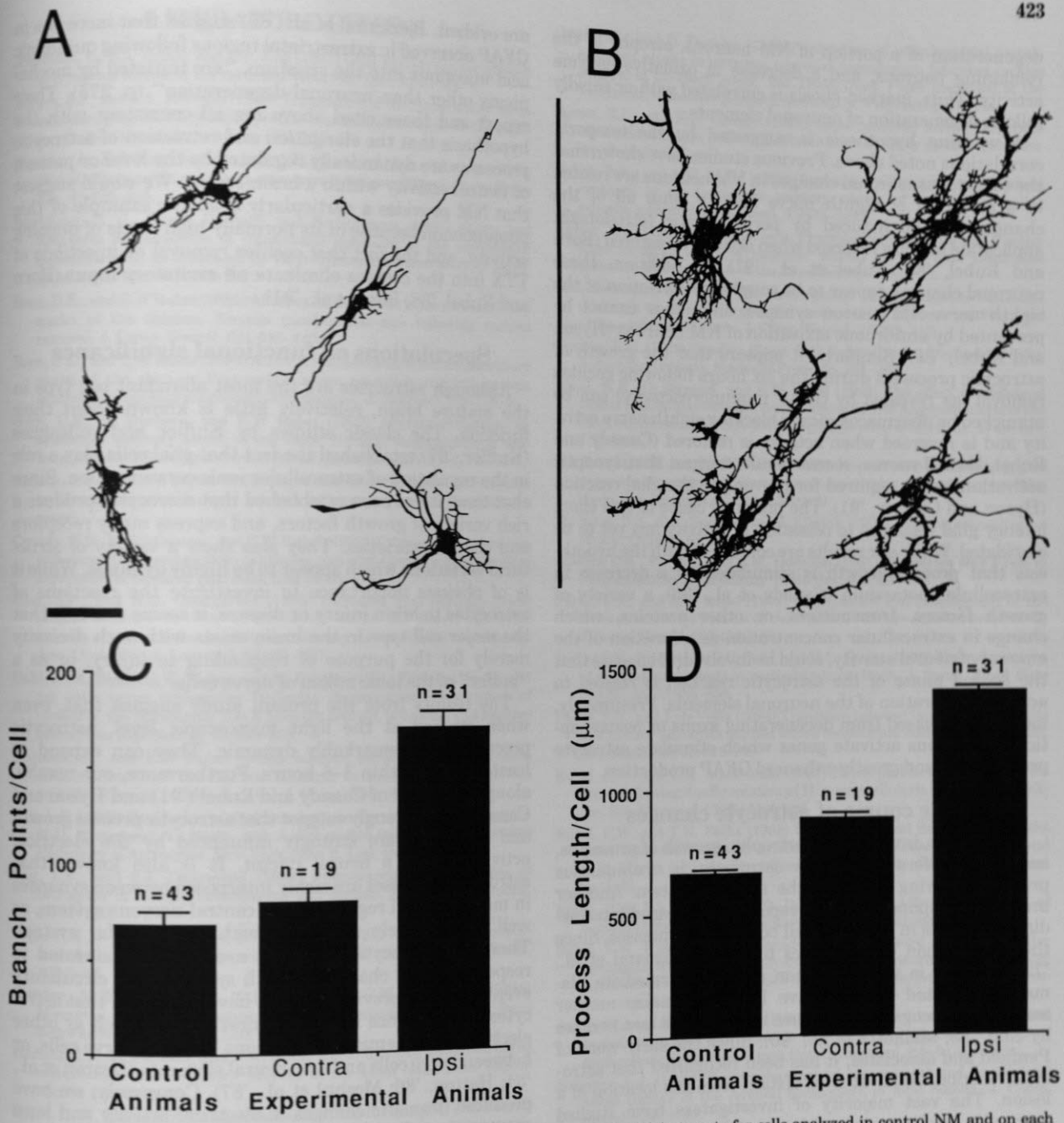

Fig. 6. Analysis of Golgi impregnated astrocytes from control animals and animals sacrificed 6 hours after cochlea removal. **A:** Representative camera lucida tracings of astrocytes from control animals and the contralateral side of experimental animals. **B:** Representative camera lucida tracings of astrocytes from NM ipsilateral to cochlea removal. Bar in A represents 10 μm and also applies to B. Panels **C** and **D:** Data on average branch points/astrocyte and average total process length/astrocyte for cells analyzed in control NM and on each side of NM from experimental animals. Because the number of cells impregnated in each animal was quite variable, all cells from each condition were pooled to determine means and S.E.M. (error bars). As evident from comparing A and B, both the number of branch points and the total process lengths of astrocytes from the ipsilateral side of experimental animals are reliably greater than the other groups ($p < .01$).

The degree of glial reaction does not appear to change markedly between 6 and 24 hours after cochlea removal, whereas the degenerative changes in NM neurons and eighth nerve axons are becoming more pronounced. The neurons are beginning to atrophy and a subset has lost all basophilic Nissl substance (Born and Rubel, '85). In addition, eighth nerve axons and terminals in NM are showing marked degeneration by 24 hours (Parks and Rubel, '78).

The increased astrocytic reaction, characteristic of classical gliosis, begins somewhere between 24 and 72 hours after the lesion; by 72 hours it includes a fivefold increase in GFAP immunoreactive processes and an obvious (but not quantified) increase in the number of GFAP positive astrocytic perikarya. In addition, by 3 days, newly proliferated astrocytes are seen in NM (Lurie and Rubel, unpublished observations). This period is associated with transneuronal

degeneration of a portion of NM neurons, atrophy of the remaining neurons, and a decrease in oxidative enzyme activity. Thus, marked gliosis is correlated with or rapidly follows degeneration of neuronal elements.

A working hypothesis is suggested by the temporal correlations noted above. Previous studies have shown that the early transneuronal changes in NM neurons are related to a cessation of eighth nerve activity since all of the changes can be induced by perilymphatic tetrodotoxin application and are reversed when activity is restored (Born and Rubel, '88; Rubel et al., '91). In addition, these neuronal changes appear to be coupled to activation of the eighth nerve-NM neuron synapse, since they cannot be prevented by antidromic activation of NM neurons (Hyson and Rubel, '89). Similarly, it appears that the growth of astrocytic processes during the six hours following cochlea removal (as revealed by GFAP immunoreactivity) can be mimicked by pharmacologically blocking eighth nerve activity and is reversed when activity is restored (Canady and Rubel, '91). However, recent results suggest that synaptic activation is not required for preventing the glial reaction (Hyson and Canady, '91). The proximal cause of this short latency glial response to cessation of activity has yet to be elucidated. While our results are consistent with the hypothesis that process growth is stimulated by a decrease in extracellular potassium (Canady et al., '90), a variety of growth factors, transmitters, or other proteins, which change in extracellular concentration as a function of the amount of neural activity, could be involved. It appears that the second phase of the astrocytic reaction is related to actual degeneration of the neuronal elements. Presumably, molecules released from degenerating axons or postsynaptic NM neurons activate genes which stimulate astrocyte proliferation and greatly enhanced GFAP production.

Time course of astrocyte changes

Astrogliosis, involving hypertrophy, growth of processes, and the proliferation of new astrocytes, is a ubiquitous process following injury to the nervous system. Another universal component of this response is increased intermediate filaments in astrocyte cell bodies and processes. Since the introduction of antibodies to GFAP (Bignami et al., '72), changes in the expression of this intermediate filament associated protein have been the major marker method for recognizing changes in astrocytes (see reviews by Eng, '88; Malhotra et al., '90). Since the early work of Penfield and associates, it has been recognized that astrocytic reactions occur at sites distant from the location of a lesion. The vast majority of investigators have studied astrocytic reactions several days, weeks or months following injury. Some notable exceptions can be found. Adamucci et al. ('81) report increases in GFAP immunoreactivity after 30 minutes in white matter surrounding a cryogenic insult to the rat cerebral cortex. Other investigators have reported increases in GFAP immunoreactivity at the sites of lesions or at sites innervated by the lesioned area within 6–24 hours (Björklund et al., '86; Gage et al., '88; Petito et al., '90; Cass and Goshgarian, '90). In addition, Steward et al. ('90) report a fivefold increase in mRNA for GFAP in the dentate gyrus and hippocampus within 24 hours after entorhinal cortex lesions in rats. Thus, our findings that GFAP immunoreactivity and the lengths of astrocytic processes increase in NM within a few hours following cochlea removal are not entirely novel. The important point is that these events occur well before any necrotic changes are evident. Björklund et al. ('86) suggest that increases in GFAP observed in extrastriatal regions following quinolinic acid injections into the striatum, "are initiated by mechanisms other than neuronal degeneration" (p. 275). Their report and those cited above are all consistent with the hypothesis that the elongation and retraction of astrocytic processes are dynamically regulated by the level or pattern of neural activity within a brain region. We would suggest that NM provides a particularly dramatic example of this phenomenon because of its normally high levels of ongoing activity, and the fact that cochlea removal or injections of TTX into the cochlea eliminate all excitatory input (Born and Rubel, '88; Born et al., '91).

Speculations on functional significance

Although astrocytes are the most abundant cell type in the mature brain, relatively little is known about their function. The classic studies by Kuffler and colleagues (Kuffler, '67) established the fact that glial cells play a role in the regulation of extracellular ionic concentration. Since that time, it has been established that astrocytes produce a rich variety of growth factors, and express many receptors and channel varieties. They also show a variety of structural variations which appear to be highly dynamic. While it is of obvious importance to investigate the reactions of astrocytes to brain injury or disease, it seems unlikely that the major cell type in the brain exists with such diversity merely for the purpose of responding to injury, or as a "buffer" of the ionic milieu of nerve cells.

The results from the present study suggest that, even when studied at the light microscopic level, astrocytic processes are remarkably dynamic. They can expand at least twofold within 3–6 hours. Furthermore, our results, along with those of Canady and Rubel ('91) and Hyson and Canady ('91), strongly suggest that astrocytic process growth and retraction are strongly influenced by the electrical activity within a neural circuit. It is also known that astrocytic processes are richly interposed between synapses in most neuropil regions in the central nervous system, as well as intimately associated with the vascular system. Therefore, astrocytic processes are elegantly situated to respond to local changes in both synaptic and circulatory events. Several previous studies have suggested that astrocytes can influence synaptic connectivity as well as other electrical and chemical interactions between nerve cells, or between nerve cells and non-neural elements (Hatton et al., '84; Hatton, '86; Meshul et al., '87). Conversely, we have produced dramatic changes in electrical activity and have seen marked changes in the elaboration of astrocytic processes. It is intriguing to speculate that subtle and highly localized changes in synaptic activities, such as those produced by chronic changes in the organism's interaction with its environment, can produce localized changes in astrocyte processes, which, in turn, modulate the strength of synaptic connections.

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