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Rapid Changes in Protein Synthesis and Cell Size in the Cochlear Nucleus Following Eighth Nerve Activity Blockade or Cochlea Ablation

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ABSTRACT

Destruction of the cochlea causes secondary changes in the central auditory pathway through transynaptic regulation. These changes appear to be mediated by an activity-dependent process and can be detected in the avian auditory system as early as 30 minutes after deafferentation. We compared the early changes in cochlear nucleus neurons following deafferentation by cochlea ablation with those seen following activity deprivation by perilymphatic tetrodotoxin (TTX) exposure. Protein synthesis and size of large spherical cells in the anteroventral cochlear nucleus (AVCN) of 14-day-old gerbils were measured during the first 48 hours after the manipulations.

Both cochlea ablation and TTX produced a reliable decrease in protein synthesis by AVCN neurons (30–40%) by 1 hour. The magnitude of change in tritiated leucine incorporation was similar at all survival times, in both experimental groups. In contrast to the rapid changes in protein synthesis, the decrease in cell size was first evident 18 hours after TTX exposure and 48 hours after cochlea ablation. There was no significant change in protein synthesis or cell size in control groups at any of the survival times. These findings are consistent with changes in the avian auditory system in response to deafferentation and TTX exposure.

Cochlea ablation and TTX exposure induced similar transneuronal changes, supporting the hypotheses that activity of auditory afferents in young mammals plays a regulatory role in the metabolism and morphology of their target neurons in the central auditory pathway, and that early changes following destruction of the peripheral receptor are due to reduction of activity-dependent interactions of presynaptic and postsynaptic cells.

Key words: deafferentation, transynaptic regulation, tetrodotoxin, gerbil

The ontogeny of neural circuits is dependent upon interactions of developing neurons with both presynaptic and postsynaptic structures. Deafferentation causes changes related to alterations in the integrity or number of presynaptic elements. Interruption of presynaptic elements may induce transynaptic changes through a variety of mechanisms, including release of unidentified toxic substances, cessation of release of trophic substances, and cessation of activity-dependent release of neurotransmitters or other proteins.

It is also possible to change the amount or pattern of activity of a neuron by modifying its input or by using an exogenous substance, without physically destroying the presynaptic neuron. These manipulations are usually referred to as deprivation or enrichment. The subsequent postsynaptic changes depend exclusively on the presynaptic and postsynaptic events related to voltage-dependent activity at the presynaptic terminal. The distinction between physical elimination of afferent input, deafferentation, and alteration of the character of afferent input, deprivation, may be useful in studying the nature of transynaptic regulation and degeneration.

The auditory system lends itself well to examination of this issue. The cochlear nucleus receives its primary excitatory input from the spiral ganglion, whose dendrites innervate hair cells in the cochlea. The predominant excitatory input to the large spherical cells (LSC) of the anteroventral cochlear nucleus (AVCN) is from eighth nerve fibers, which terminate as end bulbs of Held (Lorente de Nó, '33; Harrison and Irving, '68). These mammalian neurons appear homologous to the cells of the avian nucleus magnocellularis (NM). In the avian auditory system, deafferenta-

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tion by cochlea ablation affects morphologic parameters in NM such as dendritic density (Conlee and Parks, '83), cell size, cell number, and Nissl staining (Born and Rubel, '85), as well as metabolic parameters such as protein synthesis (Steward and Rubel, '85), 2-deoxyglucose uptake, succinate dehydrogenase, and cytochrome oxidase activity (Lippe et al., '80; Durham and Rubel, '85; Hyde and Durham, '90). The changes in protein synthesis (Steward and Rubel, '85) in NM neurons occur as early as 30 minutes after deafferentation. This rapid effect suggests that at least one regulatory signal affecting the postsynaptic neuron is the activity of presynaptic elements.

Similar changes in the second order auditory neurons are seen after pharmacological blockade of eighth nerve activity by using tetrodotoxin (TTX). Born and Rubel ('88) injected TTX into the perilymph of young chicks and studied morphological and physiological parameters in the NM. The changes elicited by TTX exposure are similar to those elicited by cochlea ablation, except that the effects of TTX injection are temporary and fully reversible.

Cell viability in the avian auditory system is also affected by deafferentation (Levi-Montalcini, '49; Parks, '79; Born and Rubel, '85). After unilateral cochlea ablation in young chicks, there is considerable cell loss in the ipsilateral NM within 2–3 days. Furthermore, about one-third of cells ipsilateral to deafferentation lose their Nissl staining (Born and Rubel, '85) by 2 days. This proportion of "ghost" neurons correlates with the proportion of cells that eventually die. Using autoradiographic techniques after unilateral deafferentation in chicks, Steward and Rubel ('85) showed that there are two distinct populations of grain densities by 6 hours after cochlea ablation. The population of cells with markedly decreased tritiated leucine incorporation is thought to have lost the ability to synthesize protein. Recently, ultrastructural analysis of the "ghost" neurons has shown that the ribosomes are completely degraded within 6 hours of cochlea ablation or activity blockade (Rubel et al., '91). These cells have lost the ability to produce proteins and will rapidly die.

Auditory deafferentation in mammals has similar effects on ventral cochlear nucleus neuron cell size, cell number, and Nissl staining (Trune, '82a; Hashisaki and Rubel, '89; Pasic and Rubel, '89). In order to study the effects of presynaptic activity deprivation, Pasic and Rubel ('89) developed a sustained-release preparation of TTX by using ethylene-vinyl acetate (Elvax; Dupont Chemicals) as a copolymer carrier. An Elvax disk can be placed securely in the round window (RW) niche without violating the integrity of the inner ear. A single application of this sustained-release form of TTX reliably blocks auditory evoked responses for approximately 40 hours. After this period of time, the auditory brainstem response (ABR) thresholds and latency-intensity functions return to normal. Tetrodotoxin exposure and cochlea ablation cause similar changes in the cross-sectional area of AVCN neurons (Pasic and Rubel, '89). Furthermore, exposure to TTX for up to 48 hours followed by a 7-day recovery period results in reversal of the decrease in cell size of AVCN neurons (Pasic and Rubel, '90). "Ghost" cells, similar to those seen in the avian NM, have not been identified in the mammalian auditory system. Unilateral deafferentation and deprivation also cause reversible changes in the size of neurons in the contralateral medial nucleus of the trapezoid body, the third order auditory neurons (Pasic and Rubel, submitted). These findings support the hypothesis that the transynaptic message is activity dependent and bidirectional. Indirect evidence leading to the same conclusion can be found in the correlation between AVCN cell size and spontaneous discharge rate of afferent axons in the cat (Sento and Ryugo, '89).

TABLE 1. Number of Subjects for Each Experimental Group

	Survival (hrs after surgery)			
Experimental group	1	6	18	48
Unilateral TTX[1]	4	3	3	2
Unilateral cochlear ablation	3	3	2	2
Unilateral Elvax	2	1	1	2
Unilateral sham operation	2	2	2	2

[1]TTX, tetrodotoxin.

The goal of the present study was to compare directly the early transynaptic changes in mammalian cochlear nucleus neurons when the same presynaptic elements were either physically destroyed by cochlea ablation or electrically silenced by TTX exposure. We examined changes in protein synthesis and cell size of the large spherical cells in the AVCN during the first 48 hours after unilateral cochlea ablation or eighth nerve activity blockade in 14-day-old gerbils. At this age, gerbils have reliable brainstem responses to acoustic stimuli, yet their hearing is quite immature (Woolf and Ryan, '84, '85; Sanes and Rubel, '88). Therefore, we were able to measure brainstem responses to confirm the effects of the treatments at an immature developmental stage. From previous studies, we expected changes by 48 hours, and therefore studied time intervals within the first 48 hours after manipulation.

MATERIALS AND METHODS

Breeding pairs of Mongolian gerbils, *Meriones unguiculatus,* (Tumblebrook Farms, West Brookfield, MA) were used to establish our colony. The cages were checked each morning for new litters; new pups were considered 1 day of age. Each litter was culled to 6 pups, and all animals had free access to food and water. At 14 days of age, each animal was randomly assigned to one of the treatment groups. Each animal weighed approximately 10 g at the time of manipulation, and all had normal external and middle ears on microscopic examination.

The gerbils were divided into four groups. In each animal, one ear was manipulated so that the normal ear and its central connections would serve as an intra-animal control. In one group, TTX was placed in the RW niche to silence eighth nerve activity. This effect was confirmed by ABR analysis. Animals in the second group underwent unilateral transtympanic cochlea ablation. Two groups served as controls; animals in one control group received an Elvax plug without TTX in the RW niche, and the other group of subjects underwent sham operation. Each of the four groups was subdivided into four survival groups: 1, 6, 18, and 48 hours. There were one to four animals in each of the 16 subgroups (Table 1).

TTX and Elvax preparation

A sustained-release preparation of tetrodotoxin (Sigma Chemicals) was made by using Elvax as the carrier. The procedure is described in detail by Pasic and Rubel ('89). TTX disks were stored at −80°C. Just prior to use, they were brought to room temperature and single aliquots of ~250 ng were made using a 17-gauge stub adaptor. Each disk was soaked in distilled water for 2 hours, and then

placed in the round window niche of the appropriate animal. Elvax disks without toxin were made and stored in a similar manner.

Surgical procedures

Ketamine (75 mg/kg) and xylazine (5 mg/kg) (IM) were used for surgical procedures and acquisition of ABRs. Halothane vapor anesthesia was used for the intracardiac injections and deep halothane anesthesia was used for transcardiac perfusion and sacrifice. Body temperature was maintained at approximately 38°C with an electric heating pad for the duration of anesthesia.

An Elvax or TTX/Elvax disk was placed in the round window niche by using a transmastoid approach. Through an incision caudal to the pinna, the mastoid cortex was removed, exposing the oval window, basal turn of the cochlea, and RW niche. Under an operating microscope, a disk was placed in the RW niche without disrupting the RW membrane, thereby maintaining the integrity of the inner ear. The size of the disk allowed secure placement within the niche, minimizing the possibility of dislodgement. The skin incision was closed with cyanoacrylic glue. For cochlea ablation, the animal was anesthetized and the pinna removed. The tympanic membrane and ossicles were removed, exposing the middle and apical turns of the cochlea. The bony cochlea was perforated with a curved pick and care was taken to destroy the entire length of the modiolus.

Measurement of ABR

Click-evoked ABRs were used to demonstrate the physiological effect of each of the four manipulations. The animals were anesthetized with ketamine and xylazine, and body temperatures were maintained at 38°C by using an electric heating pad.

The stimulus was a series of 500 alternating half-cycle sine waves producing a broad-band click with its peak around 5 kHz. This signal was delivered at a rate of 10/sec through an ear piece sealed to the external auditory meatus, creating a closed system. The maximum output of this system was 97 dBp.e. SPL. Brainstem evoked responses were measured by averaging differential response signals from the subdermal Grass pin electrodes placed anterior to the external auditory canal, and at the base of skull, with a ground in the left leg. The signals were led to a Grass P15 preamplifier (×100), filtered to pass 30 Hz to 3 kHz, viewed on an oscilloscope, and further amplified for input to a 12-bit A to D converter for computer averaging.

The responses during the first 10 msec following the stimulus were averaged over 1,000 stimulus repetitions by a PDP 11-73 computer. Input-output functions were generated and responses near threshold were repeated to ensure replicability. Thresholds were determined by the lowest stimulus intensity at which a repeatable response could be visually detected on the computer output.

Measurement of protein synthesis

The relative rate of protein synthesis was assessed using tritiated leucine incorporation and standard emulsion autoradiographic techniques (Droz and LeBlond, '63; Steward and Rubel, '85). After the appropriate survival time had elapsed, the animal was lightly anesthetized and given an intracardiac injection of 0.25 mCi tritiated leucine in 0.25 cc sterile water. Thirty minutes later, the subject was deeply anesthetized and transcardially perfused with 4% paraformaldehyde. The heads were removed and postfixed for 3 days. The brains were removed, blocked, and placed in fresh fix for another 2–3 days, dehydrated, and embedded in paraffin.

Serial sections (6 μm) of the brainstems were taken in the coronal plane and a 1:8 series was mounted onto acid-washed, chrome-alum subbed slides. Care was taken to orient the tissue symmetrically so that measurements of AVCN neurons on one side could be compared to the other side in the same tissue section. The sections were deparaffinized, hydrated, dried, and coated with Kodak NTB-2 emulsion diluted 1:1 with distilled water. The slides were stored in light-proof containers at 2°C, generally requiring 2–3 weeks for adequate development. Slides were developed in D19, washed in distilled water, fixed in Kodak Rapid Fix, and then lightly counterstained with thionin.

The slides for each brainstem were reviewed to determine the rostral and caudal limits of the LSC region of AVCN. A single section from each brainstem was selected for analysis based on its location midway between the limits of the LSC region. At the level of AVCN analyzed, large spherical cells accounted for 80–90% of the neurons. The LSCs were easily recognized by their larger diameter (even in experimental animals), more uniform staining pattern, and more uniformly circular shape. The smaller, presumably stellate, neurons were not included in the analysis. Because there is no absolute way to discriminate between the cell types (e.g., antigenic markers), it is possible that a few of the cells measured were stellate neurons. Since they represent a minority of neurons in this region, it would not materially change the results. Approximately 40 large spherical cells from AVCN on each side of the brain in a single tissue section were examined for grain density and cell area measurements. Only those cells with distinct cell membranes, nuclei, and nucleoli were included. Cell selection started at the lateral aspect of the nucleus and proceeded medially until up to 40 cells on each side had been analyzed.

A Leitz microscope equipped with a Bioquant Image Analysis System (R&M Systems, Nashville, TN) was used to measure both grain density and cell area. Measurements were taken using a 100× planachromatic objective (N.A. = 1.3). For the grain density measurements, a density detection threshold of a silver grain was set on the Bioquant using cells from the normal side of the brainstem. This threshold setting remained fixed for all measurements within that section. This procedure was the same as that described by Hyson and Rubel ('89) in which we presented validation functions for these procedures. Once the LSC region of AVCN was identified, the image from the microscope was transferred to the video display terminal where a cursor was used to outline the cells. The computer calculated the density of the thresholded pixels. This density was proportionate to grain density and independent of overlap of two or more silver grains at the magnification and image size (512 × 512) used. The computer used the same cell outlines to calculate cross-sectional cell areas.

Data analysis

Absolute grain density measurements using emulsion autoradiographic techniques vary considerably with precise amount of tracer incorporated, tissue processing techniques, exposure time, etc. Therefore, comparisons of absolute grain densities of cells from one side of the brain to cells of the other side can only be made within a single tissue section. Therefore, for each animal, the grain density measurements were plotted as density distribution histograms (Fig. 2).

Two techniques were used to group data across animals. A grouped density distribution graph was plotted after the

grain density measurements from each side of all animals were converted to standard Z-scores [$Z = (X_i - \mu)/Sd$; μ and Sd were based on the control side of the tissue section]. The second technique allowed statistical analysis of the difference between the two sides of the brainstems across animals and groups. The mean percent decrease was calculated as $[(\text{mean}_{\text{contralateral grain density}} - \text{mean}_{\text{ipsilateral grain density}})/\text{mean}_{\text{contralateral grain density}}] \times 100$, comparing the ipsilateral, manipulated grain density to the contralateral control side of the same tissue section. Using this formula, a positive value indicates a decrease in grain density and a negative value indicates an increase in grain density. These data from individual animals within a group were combined and expressed as mean percent decrease, and compared to the other groups by ANOVA (Winer, '71). These same techniques, i.e., individual distribution graphs, grouped distribution graphs using Z-scores, and ANOVA comparing the percent differences, were used for cell size comparisons.

RESULTS

Auditory brainstem responses were measured on one to two animals from each group to demonstrate the physiological effects of each manipulation. Placement of a single TTX disk in the round window or cochlea ablation reliably blocked ipsilateral evoked responses to unfiltered clicks at 97 dBp.e. SPL for at least 48 hours. Elvax disks without TTX and sham operation had no effect on threshold or latency of the ABR. Pasic and Rubel ('89, '90) showed that the activity blockade produced by TTX, at the dosage used here, is fully reversible, indicating that TTX does not permanently damage the cochlea or the auditory nerve.

Protein synthesis

Figure 1 shows representative photomicrographs from the ipsilateral and contralateral LSC region of AVCN in a single tissue section from an animal sacrificed after 1 hour of TTX exposure. The grain density over neurons ipsilateral to TTX (Fig. 1B) is less than that over the neurons in the contralateral side of the brainstem (Fig. 1A). The grain density over neuropil regions seems relatively high as compared to that seen in n. magnocellularis of the chick. This increased activity is not due merely to background labeling. The area of the slide without tissue was free of grains and areas without extensive neuropil were relatively unlabeled. Instead, this activity may be related to the higher dendritic density of the LSC area of AVCN in mammals compared with NM in the chick. Steward and colleagues (e.g., Rao and Steward, '91) have shown that RNA message is transported into dendrites where considerable protein synthesis occurs.

Distribution histograms for the grain density measurements for each animal were plotted. Representative histograms from individual animals 1 hour after TTX, ablation, and placement of Elvax without TTX are shown in Figure 2. In each histogram, the distribution of grain density measurements from the LSC region ipsilateral to the manipulation is compared with the distribution of grain densities in the contralateral, nonmanipulated side of the brain. The absolute grain density measurements for each animal are not important due to the variability associated with emulsion autoradiography. As seen in Figure 2, at 1 hour there is a clear shift in the distribution of grain densities on the manipulated (ipsi) side of the brain in the animals that underwent TTX exposure or cochlea ablation, but no difference between the two sides in the control animal. In

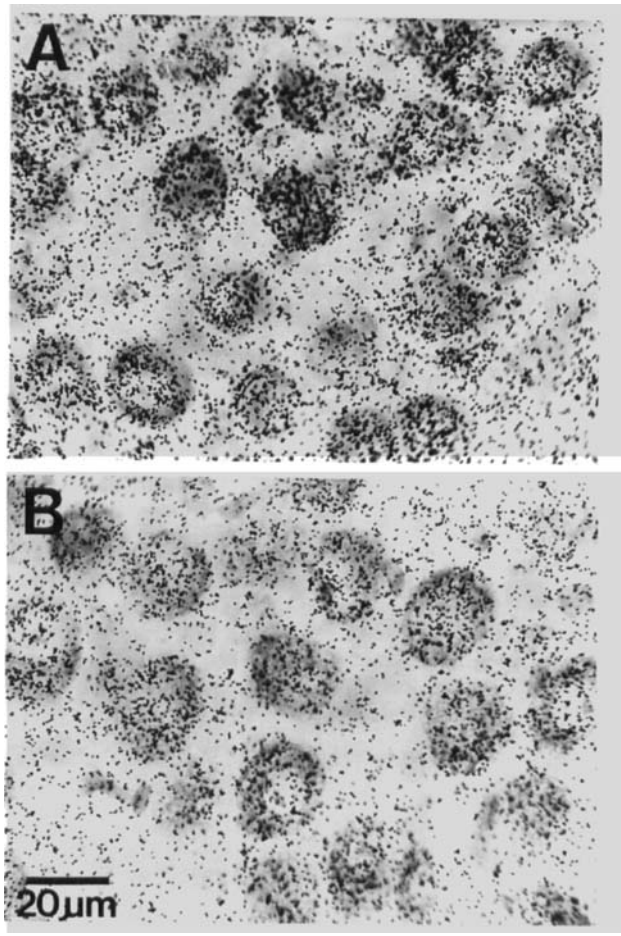

Fig. 1. Representative photomicrographs taken from the large spherical cell region of the anteroventral cochlear nucleus (AVCN) contralateral (**A**) and ipsilateral (**B**) to tetrodoxin (TTX) exposure after 1 hour. Photographs are taken from opposite sides of a single tissue section. The grain density overlying the ipsilateral large spherical cells (LSCs) is less than the grain density overlying the contralateral cells, reflecting a decrease in amino acid incorporation ipsilateral to TTX deprivation.

each animal that received TTX or cochlea ablation, regardless of survival time, the difference in mean grain densities between the two sides of the brain was statistically reliable ($P < 0.01$, t-tests). The histograms from individual animals were also used to determine if a bimodal distribution of grain densities ipsilateral to the manipulation was apparent. A bimodal distribution can indicate the presence of "ghost" cells (Born and Rubel, '88). However, there was no evidence of a population of totally unlabeled cells in any of the animals.

In order to pool the data according to treatment and survival groups, the grain density measurements for each animal were normalized using the Z-scores. Figure 3 shows the distribution graphs of the normalized grain density measurements from the experimental and control sides for the ablated and TTX groups at 1 hour. These data, and similar distribution graphs at each of the other survival times (6, 18, and 48 hours), clearly show that there was reliably less amino acid incorporation following TTX and cochlea ablation, at all survival times.

The mean percent decrease in grain density for each group is shown in Figure 4. The two control groups, sham

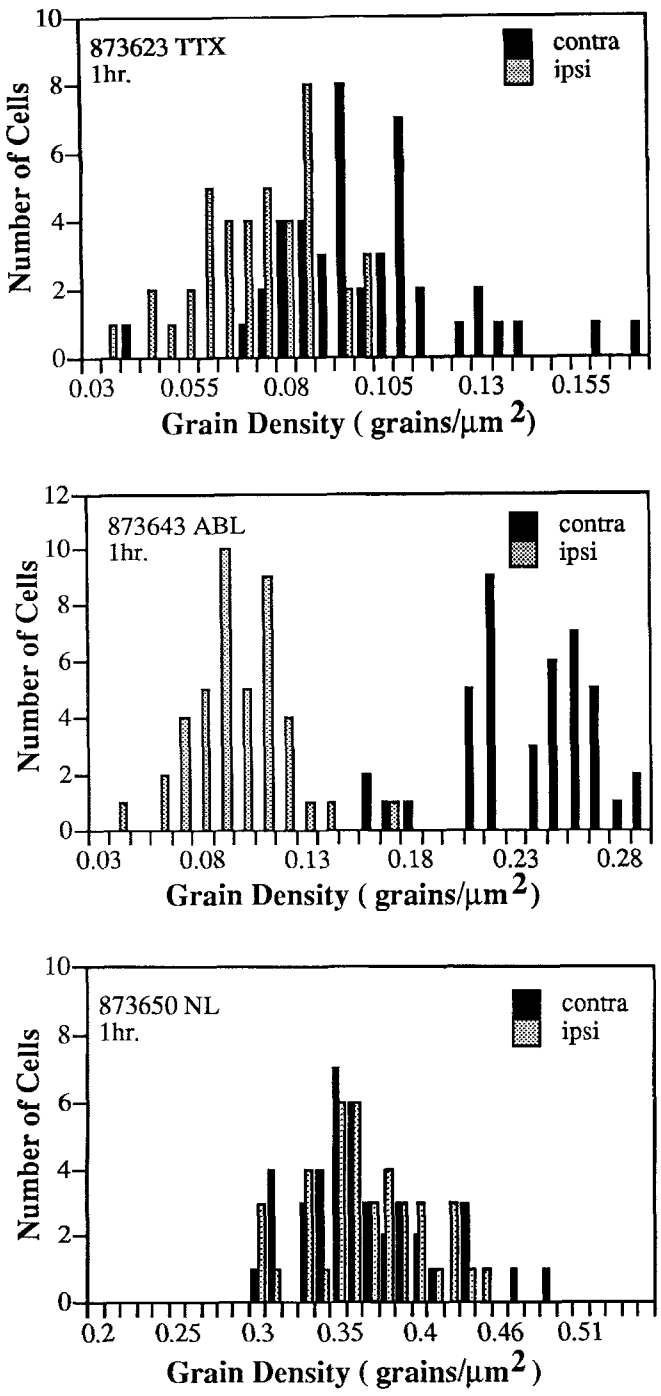

Fig. 2. Individual distribution histograms from three representative animals. The top panel is from an animal 1 hour after tetrodotoxin (TTX) exposure (TTX); the middle panel from an animal 1 hour after cochlea ablation (ABL); and the bottom panel from an animal 1 hour after Elvax without TTX was placed in the RW niche (NL). In each panel, the solid bars represent the distribution of grain density measurements in the cells contralateral (contra) to the manipulation, and the hatched bars represent the distribution of grain density measurements ipsilateral (ipsi) to the manipulation. There was a shift of the distribution of grain densities ipsilateral to TTX and cochlea ablation, but not ipsilateral to Elvax alone. Note that there was no evidence of a bimodal distribution ipsilateral to the manipulation in animals that underwent either TTX exposure or cochlea ablation.

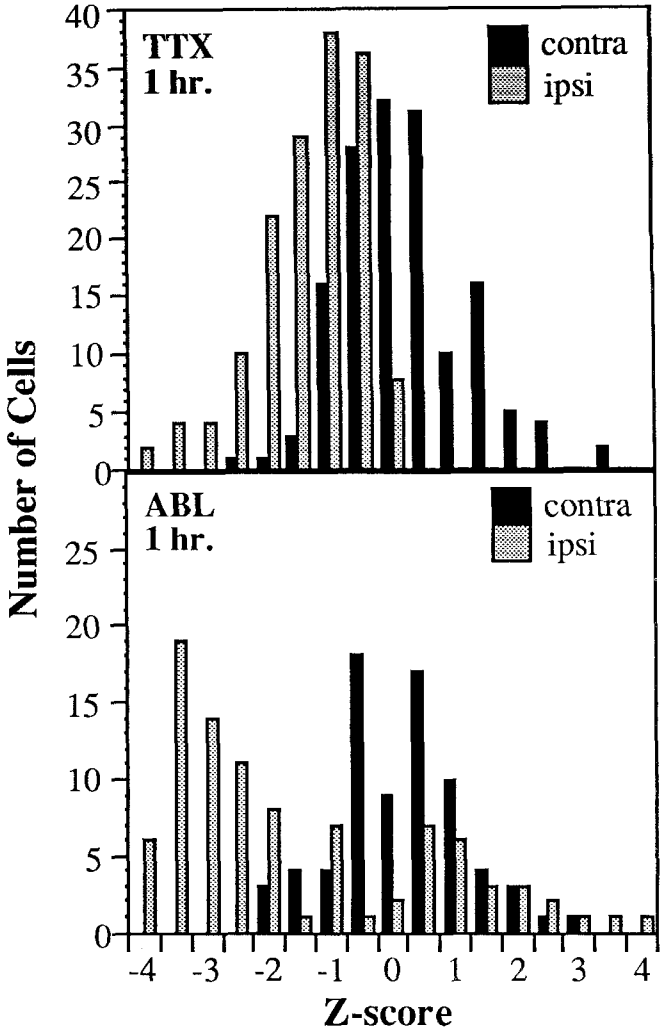

Fig. 3. These histograms represent the grouped data (see text) for the grain density measurements 1 hour after tetrodotoxin (TTX) exposure (top) and cochlea ablation (ABL) (bottom). At 1 hour after cochlea ablation or TTX, there was a shift in grain density ipsilateral to the manipulation.

operation and placement of Elvax without TTX, were not significantly different from one another when compared with two-way ANOVA (treatment × survival), so they were combined as a single control group for this figure. There was a reliable decrease in grain density with both cochlea ablation and TTX as early as 1 hour. The changes in grain density at the later survival times were not significantly different from the change at 1 hour. There was also no reliable difference between the changes in grain density after TTX exposure versus after cochlea ablation when compared by ANOVA. Using a two-way ANOVA (treatment × survival), there was a main effect of group ($P < 0.001$), but not of survival ($P > 0.5$). In summary, the effects of both TTX and cochlea ablation on protein synthesis in the LSC of the AVCN were similar, were seen at the earliest survival time, 1 hour, and remained similar for up to 48 hours.

Cell area

Grouped histograms were plotted at each of the survival times using the Z-scores (see Materials and Methods). The

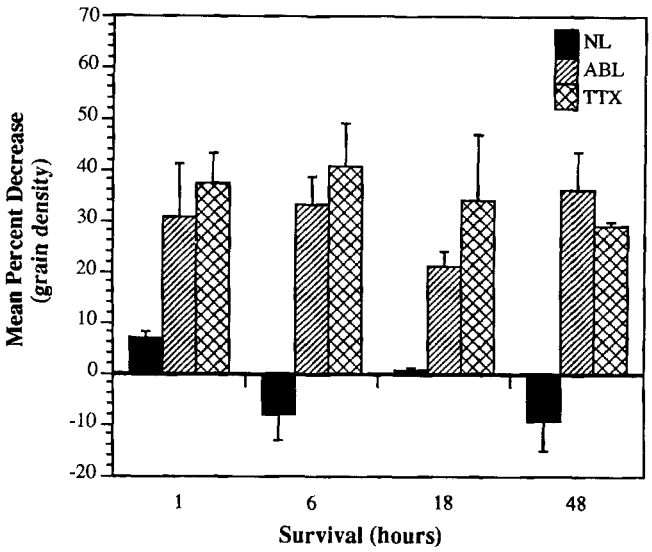

Fig. 4. Mean percent decrease of grain density (±SEM) over large spherical cells in the control group (NL), the cochlea ablation group (ABL), and the tetrodotoxin (TTX) group (TTX), at each survival time. Within each group, the percent decrease in grain density of the ipsilateral side was calculated for individual animals. The mean percent decrease was then determined for the group. A positive value indicates a decrease in grain density. There was a 20–40% decrease in grain density ipsilateral to both cochlea ablation and TTX exposure as early as 1 hour. The changes at the four survival times were not reliably different from each other. There was no significant difference between the manipulated and nonmanipulated sides in the control groups.

histograms for the 1-hour TTX and 1-hour ablation groups showed no reliable change in the distributions of cell size measurements in the ipsilateral (manipulated) side compared to the contralateral side. A decrease in cross-sectional cell area of LSC neurons in AVCN occurred only at the later survival times. By 48 hours after TTX or cochlea ablation, there was a reliable decrease in distributions of cell sizes on the ipsilateral manipulated side relative to the contralateral nonmanipulated side (Fig. 5).

The percent change in cell area for each animal was calculated. These values were grouped, by treatment and survival, to calculate mean percent decrease for each group and are shown in Figure 6. As with the grain density measurements, two-way ANOVA on the control groups alone revealed no significant difference between the two control groups ($P > 0.5$), or as a function of survival time ($P > 0.5$). Therefore, the animals from the two groups were combined as a single control group at each survival time. There was no significant difference between the ablated and TTX groups ($P > 0.50$) at any of the survival times ($P > 0.1$), using two-way ANOVA (treatment group × survival). The changes in cell area were significant in the ablated and TTX groups only at the later survival times, i.e., 18 and 48 hours after TTX and 48 hours after cochlea ablation. The decrease in cross-sectional cell area after 18 hours of deprivation with TTX was 15 ± 3.5% (mean ± SEM), and after 48 hours was essentially the same, 16 ± 5%. When the TTX group was compared to the combined control group using two-way ANOVA (treatment × survival), there was a significant effect of treatment ($P < 0.0001$) but no effect of survival ($P > 0.05$). The decrease in area 48 hours following cochlea ablation, 23 ± 5.8%, was also reliable ($P < 0.001$). Although the effect of survival time was not reliable in either the TTX or cochlea ablation groups, there was a definite trend toward a greater decrease in cell size in the later survival times in both groups, and the interaction terms of the ANOVAs were reliable. The degree of change in cell size is similar to that reported by Hashisaki and Rubel ('89) in gerbils and by Born and Rubel ('85) in chicks 48 hours following receptor destruction.

DISCUSSION

Most studies of transynaptic influences in the mammalian central auditory system have examined long-term changes in cell morphology or physiology. For example, Trune ('82a,b) performed unilateral cochlea aspiration on neonatal mice at an age prior to the onset of hearing, and evaluated the changes in cochlear nucleus neurons at 45 days of age. He found significant reduction in the cochlear nucleus volume and LSC cell numbers, though the remaining LSCs were of normal size. Nordeen et al. ('83) used horseradish peroxidase and physiological studies to examine the effects of unilateral cochlea aspiration on the inferior colliculus (IC) in neonatal and adult gerbils after 4–12 months. They showed changes in the ratio of neurons projecting to the ipsilateral inferior collicular neurons versus neurons projecting to the contralateral inferior colliculus. Similarly, several investigators (Webster and Webster, '79; Blatchley et al., '83) have studied the long-term effects of acoustic deprivation by surgically closing the external auditory meatus in young animals and examining neurons in the central auditory pathways. They have reported decreases in cell size after 34–60 days of deprivation. These long-term changes may result from a number of processes such as degeneration, accumulation of toxic substances, change in blood supply, and glial responses. Examining earlier changes after deafferentation may allow us to make inferences about the transynaptic messages that contribute to these chronic changes, and may provide information necessary to determine the cellular events underlying these alterations in structure and function.

Grain density

There is abundant evidence from studies done in the avian auditory system that the changes in NM neurons after cochlea removal occur early, within 72 hours after deafferentation; these early changes are essentially identical following blockade of eighth nerve activity with TTX (Rubel et al., '90). The changes we found in ^{3}H-leucine incorporation were consistent with findings in the avian auditory system (Steward and Rubel, '85; Born and Rubel, '88). A reliable decrease in grain density was seen as early as 1 hour and persisted for 48 hours. As in the avian system, the magnitude of the decrease appeared slightly greater at the earlier survival times, and there was no reliable difference between the ablated and TTX-treated groups. This similarity between changes after deafferentation and pharmacologic deprivation supports the hypothesis that both processes are mediated by a common activity-dependent process. Furthermore, the rapid decrease in ^{3}H-leucine incorporation suggests that protein synthesis plays an early role in the sequence of events that result in later morphological changes in the mammalian auditory system.

Although deafferentation and deprivation appear to elicit similar changes in protein synthesis in mammals and birds, there was one significant difference. In several studies, we have described "ghost" cells in NM after deafferentation (Steward and Rubel, '85; Born and Rubel, '85) or after TTX exposure (Born and Rubel, '88). These cells stop synthesiz-

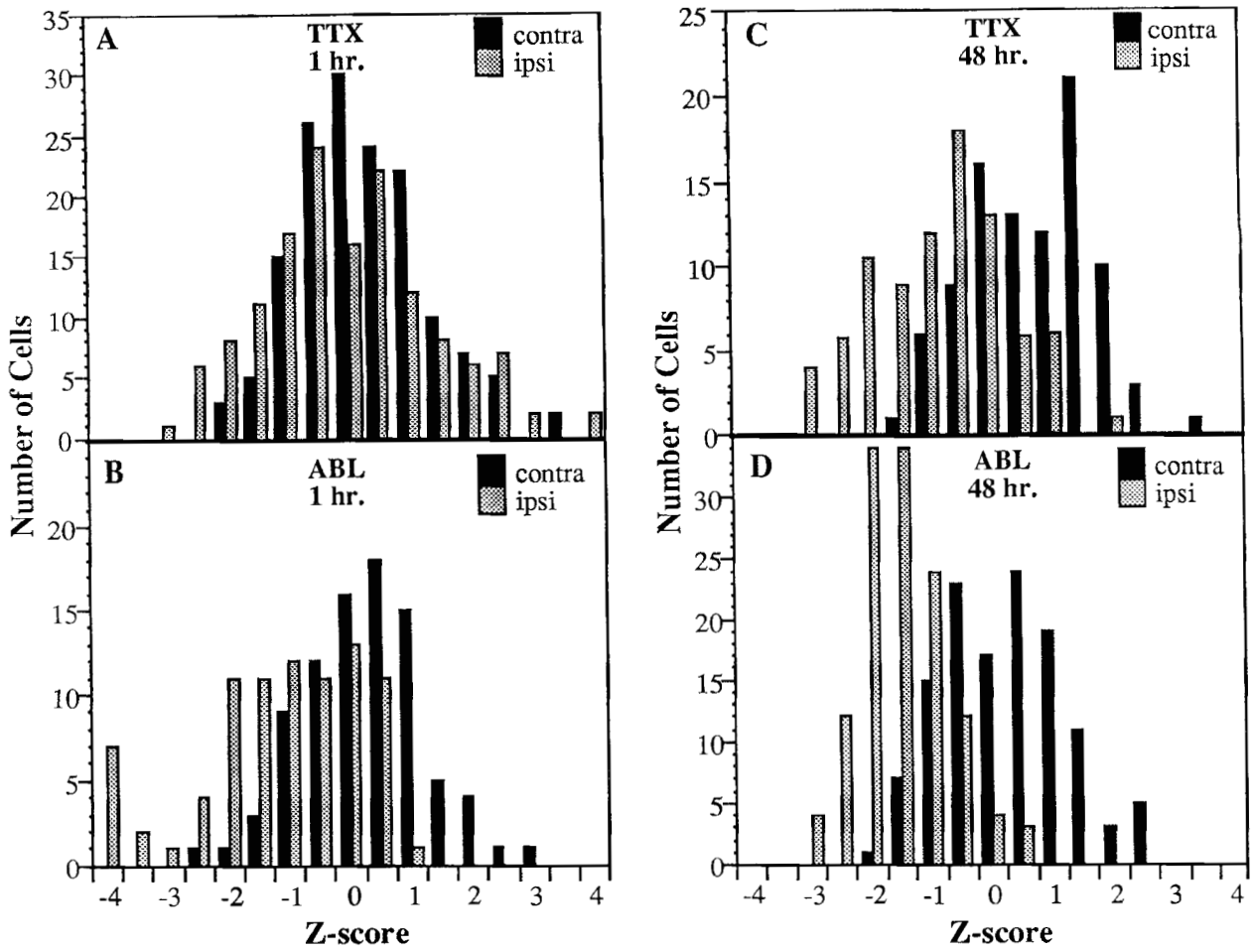

Fig. 5. Distribution histograms for the cross-sectional cell area measurements from the grouped data (see text), 1 hour after tetrodotoxin (TTX) (**A**), 1 hour after cochlea ablation (ABL) (**B**), 48 hours after TTX (**C**), and 48 hours after cochlea ablation (**D**). At 1 hour after either manipulation (A and B), there was no difference between the distribution of the cell area measurements on the two sides of the brainstems. At 48 hours, there was a shift of the distribution of cell area measurements ipsilateral to TTX and cochlea ablation (C and D).

ing protein 3–6 hours after cochlea removal. This subpopulation of cells can be demonstrated quantitatively by plotting histograms for individual animals, showing a bimodal distribution. The proportion of the "ghost" cells is approximately equal to the proportion of cells that eventually die after deafferentation. Observations on the survival of unlabeled cells confirm that these cells constitute the population that will undergo transneuronal degeneration (Rubel and Steward, unpublished observations). We did not see any of these cells in our tissue, nor did we see bimodal distributions in the histograms for individual animals. It is unclear why these "ghost" cells would appear in the avian auditory system and not in the mammalian auditory system. It is possible that the LSCs of AVCN in gerbils receive secondary excitatory afferent input, from sources other than the eighth nerve, which maintains the neurons' metabolic activity. Considering the finding that 7-day-old gerbils undergo rapid AVCN cell loss after deafferentation but 8-week-old and adult animals do not show signs of transneuronal degeneration (Hashisaki and Rubel, '89), the central connections in 2-week-old animals may be sufficiently mature that transneuronal degeneration does not occur. Alternatively, cell loss, if it occurs in this age group, may be less precipitous and/or less synchronized in mammals than in birds.

Cell size

Hashisaki and Rubel ('89) studied changes in size and number of LSCs in AVCN after unilateral cochlear ablation in neonatal, prepubertal, and adult gerbils. We measured these parameters 2 days and 2 weeks after deafferentation. Neonates show a 20–25% decrease in cell area 2 days after the manipulation and a 30–40% decrease 2 weeks after deafferentation. We also studied a group of neonatal animals that was allowed to survive for 70 days after cochlea ablation. The change in cell size at 70 days is similar to the change at 2 weeks. In both older age groups, the decreases in cell size 2 weeks after cochlea removal (15–20%) are similar to the changes seen at 2 days. These findings suggest that, except in neonatal animals, the critical message mediating transneuronal changes in cell size occurs within 48 hours after termination of afferent input. This time course is remarkably similar to the findings in the avian system.

Tetrodotoxin in the mammalian auditory system causes a decrease in cross-sectional cell area in the AVCN on the same order of magnitude (about 20%) as cochlea ablation and over a similar time course, i.e., within 48 hours (Pasic and Rubel, '89). These effects of TTX are fully reversible after a 7-day recovery period (Pasic and Rubel, '90). In light

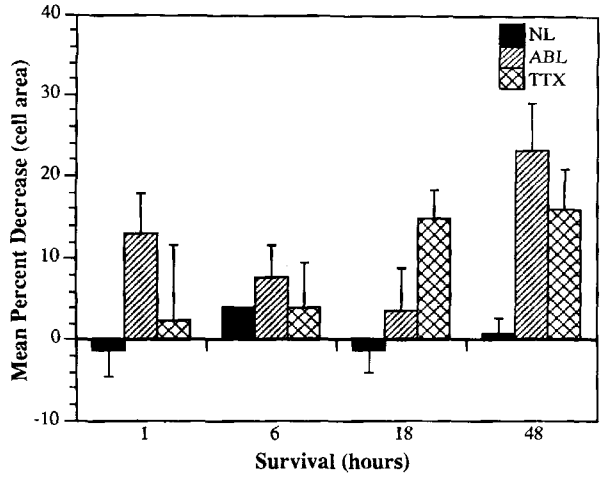

Fig. 6. Mean percent decrease of cross-sectional cell area (±SEM) of large spherical cells in the control group (NL), the cochlea ablation group (ABL), and the tetrodotoxin group (TTX), at each survival time. Within each group, the percent decrease in cell area of the ipsilateral side was calculated for individual animals. The mean percent decrease was then determined for the group. A positive value indicates a decrease in cell area. There was a significant decrease in cell area by 48 hours after cochlea ablation, and at both 18 and 48 hours after TTX. There was no significant change in cell area at any of the survival times in the control groups.

of these findings in gerbils, we looked at the changes in cell size during the first 48 hours after deafferentation and pharmacologic deprivation with TTX. AVCN cell size is reliably decreased by 48 hours after cochlea ablation and by 18 hours after TTX exposure. The changes seen in AVCN after deafferentation and deprivation are similar to those in the avian auditory system. The similarities between the changes after TTX exposure or cochlea ablation again support the hypothesis that they are both mediated by some common activity-dependent mechanism.

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