THE JOURNAL OF COMPARATIVE NEUROLOGY 348:111–120 (1994)

Effect of Altered Neuronal Activity on Cell Size in the Medial Nucleus of the Trapezoid Body and Ventral Cochlear Nucleus of the Gerbil

THOMAS R. PASIC, DAVID R. MOORE, AND EDWIN W RUBEL
Virginia Merrill Bloedel Hearing Research Center and Department of Otolaryngology-HNS, University of Washington School of Medicine, Seattle, Washington 98195

ABSTRACT

Activity-dependent transneuronal regulation of neuronal soma size has been studied in the medial nucleus of the trapezoid body and ventral cochlear nucleus of adolescent gerbils. Cochlear ablation or tetrodotoxin has been used to eliminate afferent electrical activity in auditory nerve fibers permanently or for 24 or 48 hours. Previous studies have shown that the cross-sectional area of spherical cell somata in the ipsilateral anteroventral cochlear nucleus decreases within 24 hours of electrical activity blockade with tetrodotoxin, which is fully reversible when activity is restored. The present findings extend this work by directly comparing the results of unilateral blockade of auditory nerve action potentials or unilateral cochlear ablation on the size of spherical and globular cell bodies in the ventral cochlear nucleus with changes produced by the same manipulations in third-order cells, principal neurons in the medial nucleus of the trapezoid body. Soma size in both ventral cochlear nucleus cell types decreases reliably by 24 hours after cochlear removal or eighth nerve activity blockade by tetrodotoxin. Soma size of neurons in the contralateral medial nucleus of the trapezoid body decreases 48 hours, but not 24 hours, after either manipulation. When activity in auditory nerve fibers is allowed to resume for 7 days following a 48-hour activity blockade, soma size fully recovers in the medial nucleus of the trapezoid body as well as in ventral cochlear nucleus neurons. We also report that the cross-sectional area of neuronal soma in the medial nucleus of the trapezoid body is larger in lateral regions of medial nucleus of the trapezoid body (low-frequency representation) than in the medial regions of the nucleus (high-frequency representation). We conclude that cell body size changes in brainstem auditory neurons are reversible and that the signals associated with the loss and subsequent recovery of soma size are activity related. However, the delayed effect of activity deprivation in the medial nucleus of the trapezoid body suggests that trophic substances released by afferent axons may contribute to the maintenance of anatomical characteristics.

Key words: auditory nuclei, tetrodotoxin, deafferentation, tonotopic organization, morphometry

Manipulation of afferent input in visual, auditory, olfactory, and somatosensory systems has consistently revealed an important role for ongoing stimulation in the development and maintenance of postsynaptic cell structure and function (Cowan, 1970; Globus, 1975; Constantine-Paton et al., 1990; Shatz, 1990). A change in the level of presynaptic electrical activity has been found to be a signal that is associated with changes in a variety of postsynaptic properties. Tetrodotoxin (TTX) blockade of action potentials in the visual or auditory system is associated with similar changes as environmental or surgical manipulations that are intended to reduce presynaptic activity (Dubin et al., 1986; Born and Rubel, 1988). Further evidence for activity dependence is provided by previous demonstrations that temporary auditory nerve activity blockade, followed by a return of activity, is associated with a reduction and then recovery of at least one postsynaptic cell property, neuronal soma size in the gerbil cochlear nucleus (Pasic and Rubel, 1989, 1991). If activity, per se, is a relevant presynaptic regulatory signal, it should continue to affect postsynaptic cells along a neural pathway to the extent that activity in the target cell is affected. Thus, the effect of presynaptic

Accepted March 29, 1994.

Address reprint requests to Edwin W Rubel, Hearing Development Laboratories, RL-30, University of Washington, Seattle, WA 98195.

activity on postsynaptic cells should persist even if the target cell is several synapses distant from the site of manipulation. Moreover, the postsynaptic effect of the manipulation should be virtually simultaneous at each level of the pathway because the removal or modification of the input signal should produce very rapid changes in presynaptic activity at each level. These hypotheses are tested in the present study.

In the mammalian auditory system, second-order neurons in the ventral cochlear nucleus (VCN) receive large excitatory endings (end-bulbs of Held) on spherical bushy cells and modified end-bulbs on globular bushy cells (Cant and Morest, 1979; Rouiller et al., 1986) from ipsilateral spiral ganglion afferent fibers (see Fig. 1). Third-order principal cells in the medial nucleus of the trapezoid body (MNTB) receive similarly large excitatory endings (calyces of Held) from globular bushy cells located in the contralateral VCN (Warr, 1972). Jean-Baptiste and Morest (1975) have previously shown filamentous hyperplasia and reductions in the size and number of vesicles in these terminals as well as reduced size of principal cell bodies, which was apparent several weeks following cochlear damage in adult cats.

In the present report, we further describe the results of two manipulations in separate groups of young adult gerbils: unilateral cochlear ablation and unilateral application of TTX to the cochlea. These procedures result, respectively, in a permanent and a temporary cessation of activity in the spiral ganglion neurons. We then examined soma cross-sectional area (cell size) in second-order globular cells in the VCN and third-order principal cells in the MNTB after 24 or 48 hours of activity blockade, or 7 days following recovery of normal auditory responses when the blockade has been withdrawn. These results are compared with previously published results on spherical cells in VCN. Both cochlear ablation and pharmacological action potential blockade of auditory nerve axons result in a reduction of postsynaptic soma size in both VCN neuron populations and MNTB neurons. The reduction in soma size in the VCN precedes that in the MNTB. Restoration of electrical activity results in a return to previous soma size at both levels of the system.

MATERIALS AND METHODS

Subjects

Mongolian gerbils (*Meriones unguiculatus*) between 4 and 6 weeks of age were obtained from a commercial supplier (Tumblebrook Farms, West Brookfield, MA) or the University of Washington breeding colony that was established from this supplier. All manipulations were carried out within established guidelines and approvals of the University of Washington Animal Care Committee. Animals were anesthetized prior to surgery or physiological monitoring with ketamine (75 mg/kg) and xylazine (5 mg/kg). Supplemental doses were given as required to maintain anesthesia. Body temperature was maintained at 38°C by a heating pad during physiological testing. A total of 25 animals in six groups was studied. Animals undergoing unilateral cochlear ablation were allowed to survive for 24 (n = 8) or 48 (n = 3) hours. Animals undergoing unilateral TTX blockade of auditory nerve action potentials were also allowed to survive for 24 (n = 3) or 48 (n = 4) hours. Other animals were exposed to TTX for 24 (n = 4) or 48 (n = 3) hours and then allowed to survive for an additional 7 days without activity blockade. In each case, the nonmanipulated, contralateral, auditory pathway served as a within-animal control. Many of the animals used in this study were the same as those used in our previous paper on spherical cells on the anteroventral cochlear nucleus (AVCN; Pasic and Rubel, 1991). To avoid repetition, methods are briefly discussed here. More detailed descriptions can be found elsewhere (Pasic and Rubel, 1989, 1991).

TTX crystals (Sigma Chemicals, St. Louis, MO) were dissolved in water and added to ethylene vinyl-acetate copolymer (Elvax: DuPont Co., Wilmington, DE) that had been dissolved in dichloromethane. The suspension was thoroughly mixed and frozen in a Petri dish cooled on dry ice. Dichloromethane was allowed to evaporate by placing the disk at −20°C for 48 hours. Water was removed by placing the disk in a lyophilizer at less than 40 milliTorr and less than −80°C for 5 days. Disks of TTX/polymer were stored at −80°C until needed and then thawed for 2 hours at room temperature before use. Small pieces of polymer (0.1 g) containing TTX (approximately 500 ng) were cut from the larger disk with a 17-gauge stub adapter for placement in the round window niche. Previous results have indicated that cell size in the cochlear nucleus (CN) is not affected by placing polymer without TTX in the round window niche (Pasic and Rubel, 1989).

Surgical manipulations

Seven of the animals that underwent unilateral cochlear ablation had the pinna removed before subsequent physiological testing. For these and all other animals scheduled for cochlear removal, the tympanic membrane of one ear was incised, and the malleus was removed. The projection of the cochlea into the middle ear was identified, and the bony walls of all three turns of the cochlea were opened. The cochlear contents were then crushed and aspirated, and the modiolus was fractured. Animals undergoing unilateral action potential blockade also had the pinna removed. Then, an incision was made posterior to the ear canal, and the mastoid bulla was identified and opened. TTX/polymer was placed in the round window niche of the middle ear resting against the round window membrane. TTX/polymer was replaced after 24 hours to ensure an adequate duration of activity blockade. In animals surviving for 7 days after activity blockade, TTX/polymer was removed after a total of 44 hours. Previous experiments in our laboratory have shown that the blockade reliably lasts for 4 hours following TTX/polymer removal, with a return of auditory evoked potentials in the ensuing 8 hours (Pasic and Rubel, 1991).

Physiological monitoring

Auditory brainstem response recordings were used to document the efficacy of cochlear ablation (in 7 cases), TTX exposure, and recovery from TTX exposure (Pasic and Rubel, 1989, 1991). Unfiltered clicks of alternating polarity were used as the stimuli, and a 10-msec duration averaged far-field electrical response was measured differentially between the vertex and mastoid using subdermal electrodes. The stimuli were delivered at 10 dB decrements from 80 dB peak equivalent sound pressure level to the auditory threshold. Threshold was defined as the lowest signal intensity that resulted in a reproducible short latency waveform. The auditory threshold and wave I latency-intensity functions were calculated before the manipulation in all cases tested. Testing was repeated following unilateral

cochlear ablation or TTX exposure. Neural responses evoked by stimulation of the manipulated ear were not identifiable in any of the animals that underwent cochlear ablation or TTX placement. Animals allowed to survive for 7 days following TTX blockade showed full recovery of auditory threshold and wave I latency-intensity functions (Pasic and Rubel, 1989, 1991). Thus, both cochlear removal and TTX treatment appear to fully block auditory nerve fiber action potential conduction, and TTX blockade is fully reversible.

Measurements of soma size

After the appropriate survival time and physiological testing, animals were deeply anesthetized and transcardially perfused with 10% buffered formalin or 4% buffered paraformaldehyde. Brains were postfixed in the same solution for 3 days, blocked, and embedded in paraffin. A one-in-four series of 10-μm-thick transverse sections was mounted on chrome alum-treated slides and stained with thionin. Great care was taken to ensure that the sections were bilaterally symmetrical. The cross-sectional area of large spherical and globular cell bodies in the VCN, and of principal cell bodies in the MNTB, was measured on both sides of the brain with the aid of a Zeiss Videoplan interactive image analysis system (Carl Zeiss, Inc., Thornwood, NY), Zeiss microscope, and ×100 or ×64 planapochromatic, oil-immersion objectives. For large spherical cells and principal cells of MNTB, cross-sectional area was determined by outlining the circumference of neurons satisfying previously determined criteria (Morest, 1968; Osen, 1969; Brawer et al., 1974). Large spherical cell data were collected at the rostral-most pole of the AVCN, where large spherical cells are the only neuron type found. These neurons are easily distinguished on the basis of cytoarchitectural characteristics. In the section(s) chosen for analysis, every AVCN neuron meeting the criteria described below was measured in the tissue section. Principal cell data were collected half of the distance between the rostral and the caudal poles of MNTB. The sections were first examined to determine the rostral and caudal boundaries of MNTB. The section at the midpoint was then chosen for analysis, and all principal cell soma were measured on both sides of the brain. Principal cell bodies were distinguished by their relatively large size and ovoid or round shape. If the criterion number of cells was not obtained in a single section, the next most rostral and caudal sections in the one-in-four series were alternately analyzed in the same way. For globular bushy cells, data were obtained from sections that included the most ventral cells in the VCN. The measured cells were adjacent to, or in between, fascicles of cochlear nerve fibers. Their position was identified on the basis of careful examination of sections from normal adult gerbils containing VCN neurons that had been retrogradely labeled with horseradish peroxidase (HRP) following injections into the contralateral MNTB. These sections were kindly provided by Dr. N.B. Cant and revealed the position of cells most consistently retrogradely labeled from MNTB. Again, adjacent sections were analyzed until the criterion number of cells was obtained. Thus, it should be noted that no attempt was made to systematically sample all regions of AVCN or MNTB. Instead, we chose to restrict our sample to the *same* region on both sides of each brain and approximately the same regions across all animals. Previous data by Moore (1990) suggest that this approach should yield data representative of all regions of the affected nuclei. Additional criteria for all cells included clearly identifiable cytoplasmic, nuclear, and nucleolar borders. All cells meeting these criteria were measured in adjacent sections until approximately 50 cells of each type were measured on each side of the brainstem. Measurements were made on each side of the brain in each animal.

The reliability of measurements was assessed in two ways. First, cell size measurements were calculated in two animals by an author and a technician blinded to the side of the brain and the manipulation. No reliable difference was found between measurements by the technician and the author. Second, cell size was measured on both sides of the brain by one of the authors on two separate occasions in two animals. Again, no reliable difference was found between measurement means.

Data analysis

In each brain, the mean difference in cell size between the two sides of the brain was expressed as a percentage change from the mean cell size on the nonmanipulated side using the formula

$$\frac{100 \times (\text{Nonmanipulated} - \text{Manipulated})}{\text{Nonmanipulated}}$$

Because MNTB neurons receive input from the contralateral VCN, the contralateral side of the brain was considered the manipulated side, and the ipsilateral side of the brain was considered the nonmanipulated side. The VCN ipsilateral to the cochlear manipulation was considered the manipulated side. A two-tailed Student's t test was used to compare the absolute cross-sectional areas between the manipulated and nonmanipulated side of each brain. In addition, the manipulated and nonmanipulated sides in each group were compared by paired-comparison t tests, using the means derived from each animal as the samples. Finally, a one-way ANOVA was used to compare percentage change in soma size across experimental groups. This analysis was carried out separately for each nuclear region.

RESULTS

Changes in postsynaptic cell size

The principal findings from this study are as follows: 1) cochlear removal or blockade of eighth nerve action potentials for either 24 or 48 hours results in a reliable decrease in soma size of VCN globular bushy cells; 2) the same manipulations do not result in MNTB cell body atrophy until 48 hours; and, 3) when eighth nerve activity is allowed to resume for 7 days after 48 hours of TTX blockade, the atrophy is reversed.

Tables 1 and 2 provide the mean cross-sectional area of MNTB principal cells and of VCN globular bushy cells ipsilateral and contralateral to the manipulated ear in each of the experimental animals. Figure 2 shows the distributions of MNTB and globular cell body areas in representative animals from the groups treated with TTX for 24 hours and of MNTB cells from the other TTX treated groups. Finally, the mean change in soma size is presented in Figure 3 for each of the groups. In this figure, a positive-going bar denotes smaller soma sizes on the side of the brain receiving input from the manipulated ear, whereas a negative-going bar indicates that the cell bodies of neurons receiving input from the nonmanipulated ear are smaller. The data in Figure 3C (MNTB) and B (VCN globular cells)

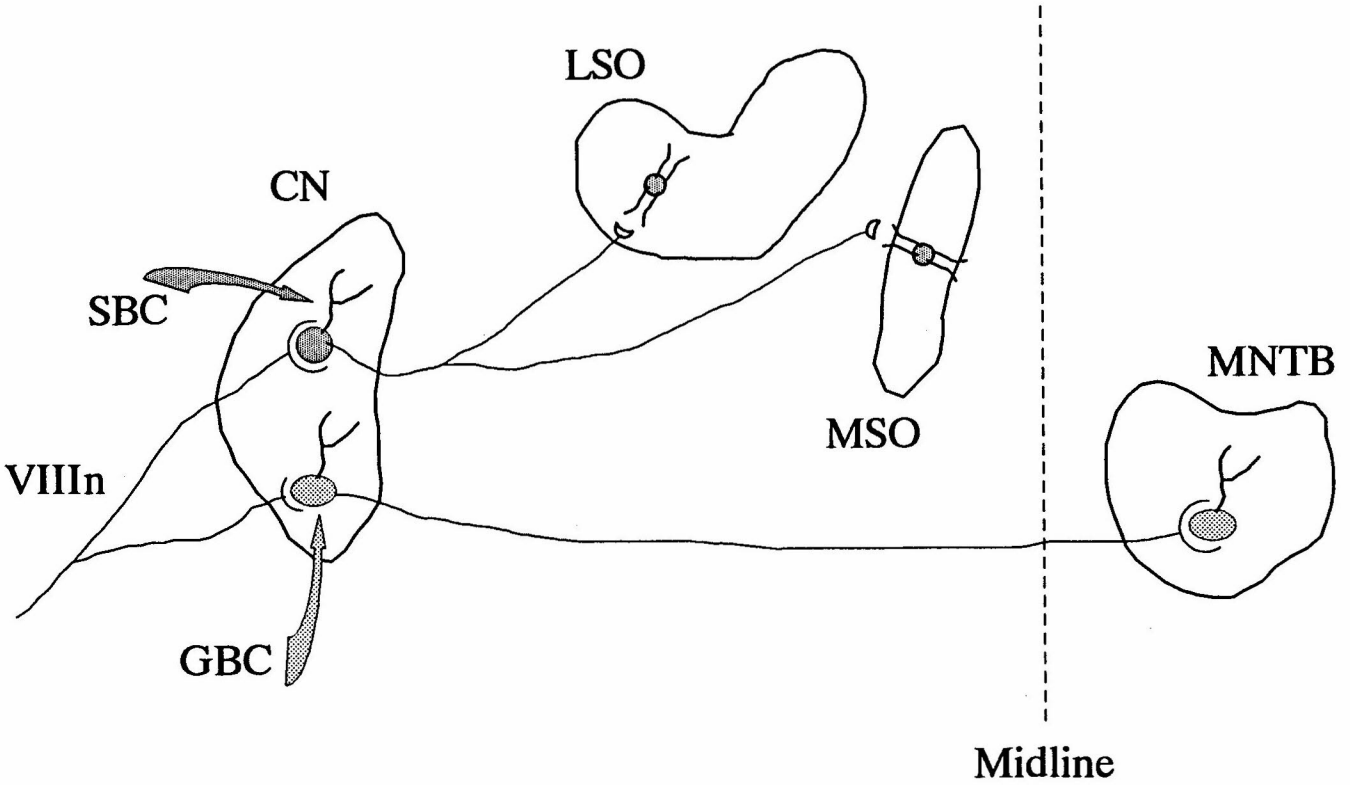

Fig. 1. Schematic representation of afferent pathways from the ventral cochlear nucleus (CN) to the principal nuclei of the superior olivary complex (SOC). Eighth nerve fibers make end-bulb of Held synapses with spherical bushy cells (SBC) and modified end-bulb synapses with globular bushy cells (GBC). SBC axons innervate neurons in the ipsilateral lateral (LSO) and medial (MSO) superior olivary nuclei. GBC axons innervate neurons in the contralateral medial nucleus of the trapezoid body (MNTB) via calyx of Held synapses.

TABLE 1. Mean Soma Cross-Sectional Area (SEM) in μm^2 in MNTB Principal Cells Ipsilateral and Contralateral to Cochlear Ablation or TTX Blockade of Auditory Nerve Electrical Activity

	Ipsilateral	Contralateral	Percentage decrease[1]
Cochlear ablation 24 hours	176.0 (3.95)	176.7 (3.80)	−0.40
	193.3 (5.47)	179.1 (5.20)	7.35
	201.2 (5.32)	206.7 (4.93)	−2.73
	194.7 (4.99)	206.2 (5.25)	−5.91
Cochlear ablation 48 hours	135.1 (2.83)	111.3 (2.38)	17.62
	167.4 (4.20)	153.6 (2.90)	8.24
	210.6 (5.89)	177.4 (5.39)	15.76
TTX 24 hours	186.8 (5.66)	181.4 (5.29)	2.89
	141.2 (3.39)	141.6 (2.72)	−0.28
	196.5 (6.36)	193.5 (6.41)	1.53
TTX 48 hours	173.4 (5.35)	124.2 (5.35)	28.37
	136.4 (3.47)	100.5 (3.09)	26.32
	164.8 (3.89)	146.4 (3.47)	11.17
	187.5 (6.87)	180.7 (6.77)	3.36
TTX 48 hours/7 days	203.4 (5.24)	193.0 (4.25)	5.11
	196.1 (4.39)	200.6 (4.17)	−2.29
	148.4 (3.45)	147.5 (3.89)	0.61

[1]A negative value indicates that the mean cross-sectional area on the experimental side (contralateral to cochlear removal or TTX injection) is larger than on the control (ipsilateral) side of the brain.

TABLE 2. Mean Soma Cross-Sectional Area (SEM) in μm^2 of VCN Globular Bushy Cells Ipsilateral and Contralateral to Cochlear Ablation or TTX Blockade of Auditory Nerve Electrical Activity

	Ipsilateral	Contralateral	Percentage decrease[1]
Cochlear ablation 24 hours	194.7 (6.83)	215.4 (5.69)	9.61
	228.3 (7.09)	283.0 (10.49)	19.33
	271.5 (8.93)	311.8 (15.49)	12.92
	280.7 (6.74)	290.9 (10.21)	3.55
Cochlear ablation 48 hours	183.2 (5.40)	217.1 (7.29)	15.61
	171.7 (5.91)	194.3 (8.08)	11.63
	175.6 (4.07)	224.9 (5.84)	21.92
TTX 24 hours	159.6 (4.43)	181.4 (4.45)	12.02
	144.6 (4.63)	168.3 (3.67)	14.08
	192.2 (5.45)	231.8 (5.34)	17.08
	239.2 (6.76)	275.4 (9.45)	13.14
TTX 48 hours	160.7 (4.07)	178.4 (4.82)	9.92
	170.0 (4.25)	205.5 (5.22)	17.27
	138.6 (3.98)	184.4 (5.27)	24.84
	202.5 (7.25)	228.1 (6.60)	11.22
TTX 48 hours/7 days	206.4 (4.71)	212.2 (4.67)	2.73
	200.0 (5.62)	187.5 (4.72)	−6.67
	259.2 (6.70)	265.7 (7.28)	2.45
	269.8 (7.76)	228.9 (5.50)	−17.87

[1]A negative value reflects an increase in soma cross-sectional (on the side of the brain ipsilateral to the cochlear removal or TTX injection) area with respect to the control (ipsilateral) measurements.

are compared to soma size data collected under identical conditions from the large spherical cell region of AVCN and published previously (Pasic and Rubel, 1991; Fig. 3A).

For MNTB, we did not find a reliable difference in the distributions of soma size 24 hours after a unilateral cochlear ablation in any animal ($P > 0.05$ for each animal; Table 1), and the overall t test did not approach statistical significance ($t = 0.22$, $P < 0.5$). Similarly, unilateral activity blockade for 24 hours with TTX was not associated with a reliable difference in mean principal cell cross-sectional area in any animal or across the group ($t = 1.31$, $P < 0.30$). A histogram showing the distributions of MNTB neuron cell size in a representative animal that underwent

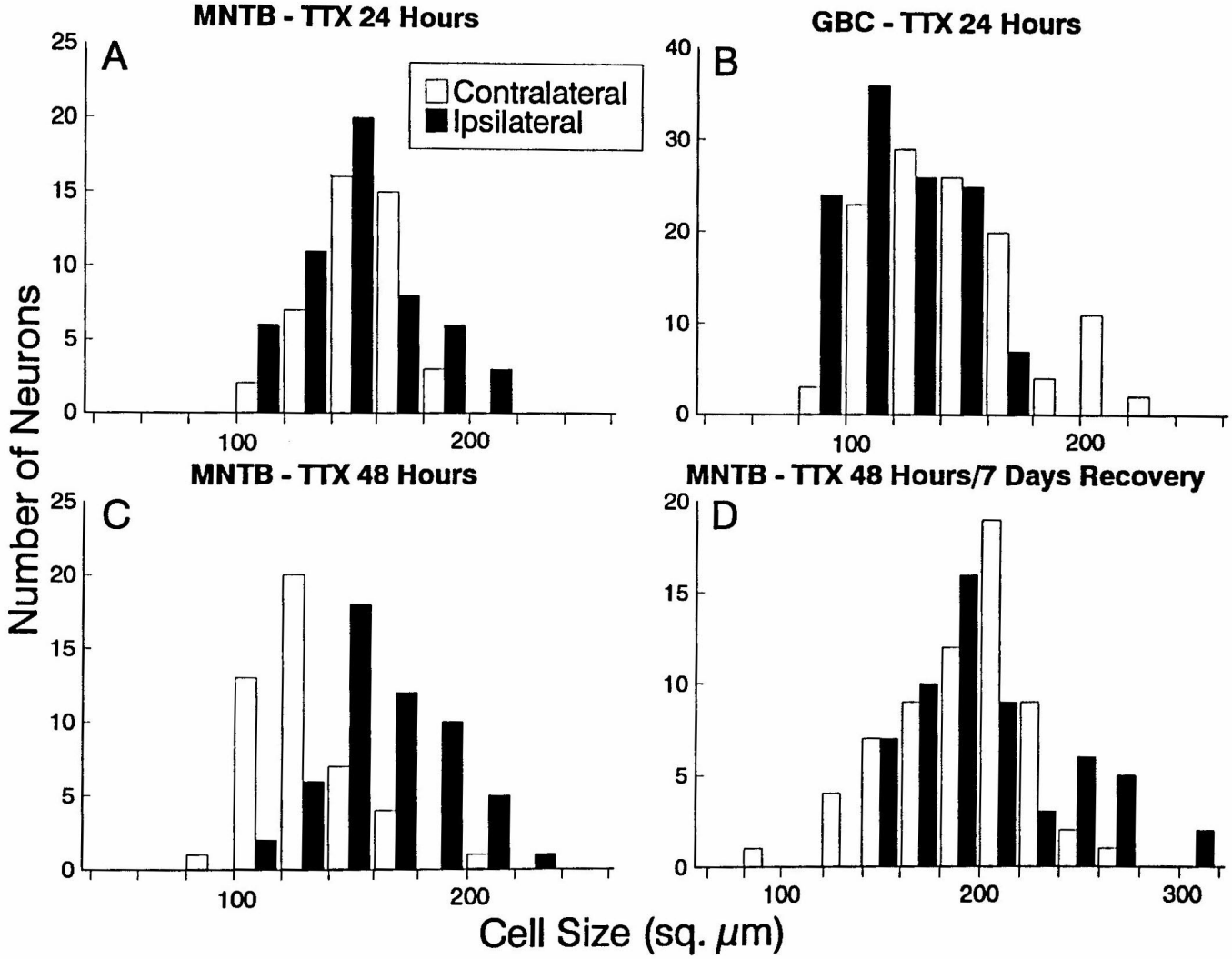

Fig. 2. Distribution of soma cross-sectional area among MNTB principal cells and VCN globular cells in individual animals after unilateral, eighth nerve activity blockade. **A:** After 24 hours of blockade, there is no difference in the distributions of MNTB cell sizes ipsilateral (n = 54) and contralateral (n = 48) to manipulation. **B:** After 24 hours, globular cell sizes differ markedly between the VCN ipsilateral (n = 118) and contralateral (n = 165) to the blockade. **C:** After 48 hours of blockade, a difference in the distributions of cell sizes in the MNTB ipsilateral (n = 54) and contralateral (n = 46) to manipulation is evident. **D:** A 48-hour activity blockade followed by return of activity for 7 days no longer shows a difference in the distributions of MNTB cell size ipsilateral (n = 58) and contralateral (n = 64) to the manipulation.

auditory nerve blockade for 24 hours is shown in Figure 2A. The distributions of MNTB cell sizes on the two sides of the brainstem are normally distributed and nearly identical.

In contrast to the MNTB principal cells, VCN globular bushy cell soma area was reliably larger on nonmanipulated than on the manipulated side 24 hours after either cochlear removal or TTX. These differences were significant ($P < 0.05$) in seven of the eight individual animals examined (Table 2), and, in spite of the small sample sizes, comparisons of the group means yielded reliable differences between the activity-deprived and control sides in both groups ($t = 3.16$, $P < 0.05$; $t = 6.79$, $P < 0.01$). Data from one animal that underwent auditory nerve blockade for 24 hours are presented in Figure 2B, showing a clear difference between the two sides. Similarly, a significant difference in soma size was found in VCN large spherical cells at 24 hours after cochlear ablation and 24 hours following TTX blockade (Pasic and Rubel, 1989, 1991). These data are summarized in Figure 3A and show a mean spherical soma area decrease of approximately 15% within 24 hours of manipulation. This is approximately the same decrease as that seen in the globular bushy cells 24 hours after either manipulation (Fig. 3B).

A significant difference in MNTB soma area was found 48 hours after cochlear ablation ($P < 0.01$ in each animal; $t = 4.31$, $P < 0.05$ for group) or after 48 hours of activity blockade ($P < 0.01$ in three of four animals; $t = 3.18$, $P < 0.05$ for group). Figure 2C shows distributions of MNTB cell sizes in an animal that was sacrificed after 48 hours of afferent activity blockade; a marked difference in the distributions is evident. The smaller cell size contralateral to the manipulation is consistent with the innervation of MNTB cells by contralateral AVCN neurons. The change in cell size averaged 17% after 48 hours of activity blockade and 13% at 48 hours after cochlear ablation (Fig. 3). As expected, globular bushy cells were also reliably reduced in size after 48 hours of activity blockade or cochlear removal ($t = 4.36$, $P < 0.05$; $t = 5.12$, $P < 0.02$). As indicated in

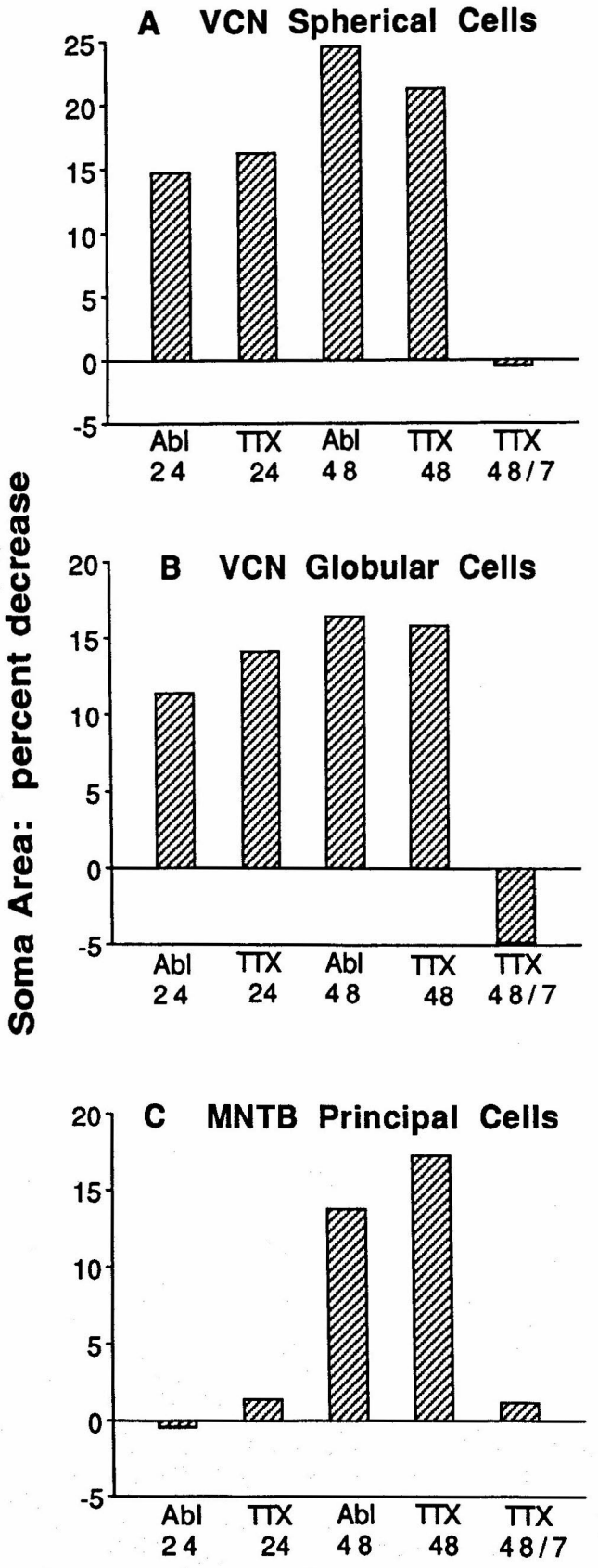

Figure 3, a similar, although slightly larger, change is seen after 48 hours in large spherical cells of VCN. Although the average reductions in both globular and spherical soma areas were slightly greater at 48 than at 24 hours, this difference was not statistically significant (see also Sie and Rubel, 1992).

Seven days after a 48-hour blockade of eighth nerve activity, a significant difference was no longer evident in either MNTB or VCN globular cells. Recall that, by 1 week after activity blockade, auditory thresholds have returned to normal values (Pasic and Rubel, 1989, 1991). Especially in light of the large difference in cell size after 48 hours of activity blockade, the similarity of the distributions of cell size on the two sides of the brain after this recovery period is striking (example in Fig. 2D). The mean difference between MNTB neuronal cross-sectional area on the two sides of the brain after 7 days recovery was 0.5% ($t = 0.57$, $P > 0.5$). In MNTB, none of the individual animals showed a reliable difference between sides (Table 1). In one of four animals in which globular cells were measured, a reliable difference between sides was observed, but, in this case (Table 2, last animal), the contralateral neurons were reliably *smaller* than neurons ipsilateral to the formerly deprived side of the VCN. Because the overall t tests on the group means failed to approach statistical significance ($t = 0.91$, $P > 0.4$), we attribute this one animal's results to sampling variation.

Examination of Figure 3 also suggests that the pattern of change in soma size in VCN and MNTB is strikingly different. There is a 10–15% decrease in globular cell soma area on the experimental side of the brain in all groups except animals allowed to recover for 7 days following the 48 hour TTX blockade. In MNTB principal cells, on the other hand, only groups deprived of activity for 48 hours show the decrease in soma area on the experimental (contralateral in this case) side of the brain. Single-factor ANOVAs confirmed these conclusions. Analysis of the globular cell percent difference scores yielded a reliable main effect of groups ($F_{4,14} = 6.71$, $P < 0.01$) and reliable differences between group TTX48/7 and each of the other groups, which did not reliably differ among themselves (Fisher's PLSD). In MNTB, this analysis also yielded a significant main effect $F_{4,12} = 4.90$, $P = 0.01$), and the 48-hour groups (Abl48, TTX48) were each reliably greater than each of the other groups. The 24-hour groups (Abl24, TTX24) did not differ and were not reliably different than the 7-day recovery group (TTX48/7).

Fig. 3. Mean percentage change in soma cross-sectional area is shown following each manipulation. A positive bar indicates a *decrease* in soma size on the side of the brain, which receives monosynaptic (VCN) or disynaptic (MNTB) excitatory input from the auditory nerve. Spherical (**A**) and globular (**B**) VCN neurons show a decrease in soma size as early as 24 hours of activity blockade. The spherical and globular cell atrophy is reversible within 7 days of return of activity. **C:** MNTB neurons show a significant decrease in soma size after 48 hours of auditory nerve activity blockade or cochlear ablation (Abl). This loss also is reversible within 7 days of return of activity. Statistical comparisons were made between mean soma sizes on opposite sides of the brainstem within each animal (n = 3 or 4 in each group). Abl 24 or 48, unilateral cochlear ablation with 24 or 48 hour survival time; TTX 24 or 48, unilateral auditory nerve electrical activity blockade for 24 or 48 hours; TTX 48/7, unilateral electrical activity blockade for 48 hours followed by 7 days without activity blockade (data presented in A are from Pasic and Rubel, 1989, 1991).

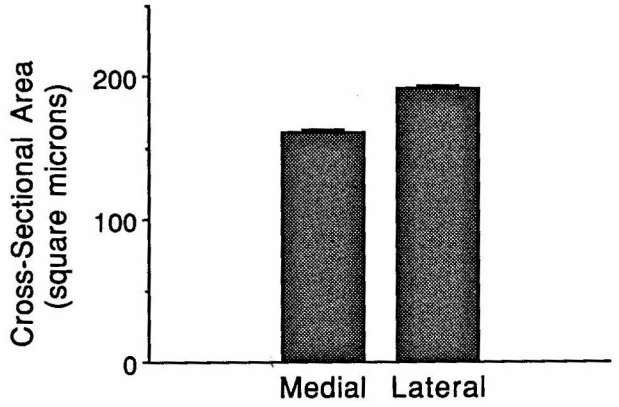

Fig. 4. Mean neuronal cross-sectional area (±SEM) is shown in MNTB neurons in medial (n = 447) and lateral (n = 403) regions of the nucleus on the nonmanipulated side. Neurons in the medial regions of the nucleus are responsive to high-frequency sound stimuli and are smaller than cells in the lateral regions of the nucleus.

Medial-lateral difference in MNTB cell size

The tonotopic organization of MNTB is such that neurons most sensitive to low-frequency auditory stimuli are located in the lateral aspect of the nucleus, and progressively higher frequencies are found at successively more medial locations. Our measurements revealed MNTB neurons show a difference in soma cross-sectional area that parallels the tonotopic organization. Cell bodies in the lateral region of the nucleus are significantly larger than cell bodies in medial regions ($t = 4.8, P < 0.01$; Fig. 4).

Independent analyses of the changes in MNTB cell size following afferent activity manipulation revealed an equivalent effect on both lateral and medial MNTB neurons. In other words, there was not a significant difference in the changes in cell size produced by unilateral ablation or TTX between the lateral and medial regions of MNTB in any of the groups ($P > 0.10$). Additionally, the recovery of cell size following reversal of activity blockade occurs in both regions of the nucleus.

DISCUSSION

The influences of afferent input and auditory experience on development and maintenance of auditory system structures and function have been a topic of considerable interest. For example, Levi-Montalcini (1949) found a decrease in the number of second-order auditory neurons following unilateral otocyst ablation in chicks. Cochlear ablation in this study and subsequent studies has been used to alter the integrity and activity of excitatory input to the cochlear nucleus. It is associated with complete loss of spontaneous and evoked activity in neurons of the ipsilateral ventral cochlear nucleus in mammals and in its homolog, n. magnocellularis, in birds (Koerber et al., 1966; Born et al., 1991).

Decrease in VCN soma size

A decrease in cochlear nucleus neuronal soma area following cochlear ablation has been found in gerbils, mice, rats, cats, ferrets, and chicks (Powell and Erulkar, 1962; Coleman and O'Connor, 1979; Trune, 1982; Born and Rubel, 1985; Hashisaki and Rubel, 1989; Moore, 1990). The present findings, together with those of other recent studies from this laboratory (Pasic and Rubel, 1989, 1991; Sie and Rubel, 1992), indicate that the time course of these changes in mammals is much shorter than was previously appreciated. The decrease in either spherical or globular soma size can be reliably detected as soon as 24 hours after cochlear ablation. In addition, these studies have indicated that electrical activity blockade of auditory nerve axons with TTX results in changes in postsynaptic cells that are similar in time course and magnitude to changes following cochlear ablation. Figure 3 reviews data that show soma size changes in cochlear nucleus neurons following cochlear ablation or activity blockade: Within the resolution of our techniques, unilateral blockade and ablation are associated with a similar magnitude of neuronal atrophy and a similar time course for both the spherical and globular cells. Additionally, the decrease in cochlear nucleus neuron protein synthesis following blockade of auditory nerve action potentials is similar in magnitude to that seen after cochlear ablation in chicks and gerbils (Steward and Rubel, 1985; Born and Rubel, 1988; Sie and Rubel, 1992). Changes in protein synthesis have been noted as soon as 1 hour after cochlear ablation in gerbils and as soon as 30 minutes after cochlear removal in chicks. The maintenance of cochlear nucleus protein synthesis requires orthodromic stimulation of cochlear nucleus neurons by auditory nerve axons (Hyson and Rubel, 1989). We have inferred from these studies that the relevant signal that has been altered by cochlear removal and results in changes in cochlear nucleus structure and function is the electrical activity of auditory nerve fibers (Rubel et al., 1990).

Decrease in MNTB soma size

Other investigators have found a decrease in contralateral MNTB soma size 30 days after cochlear removal in adult cats (Powell and Erulkar, 1962; Jean-Baptiste and Morest, 1975) and after 42 days of acoustic deprivation in neonatal mice (Webster and Webster, 1977). The relatively short survival periods used in the present study indicate that there is a different time course of cellular atrophy in VCN (spherical and globular cells—less than 24 hours) than in MNTB principal cells (more than 24 hours). Several possible mechanisms may be invoked to explain this difference. These include intrinsic or extrinsic differences in the metabolic plasticity of MNTB and VCN neurons, differences in the *relative* amount of change in activity in VCN vs. MNTB, or a delay induced by the fact that MNTB is one additional synapse removed from the site of the manipulation.

At present, there are no independent criteria by which to evaluate possible differences in the intrinsic properties of VCN and MNTB neurons that might render the two populations differentially responsive to manipulations of afferent activity. However, direct manipulations of the globular cell area of VCN may be a means to evaluate this possibility. If the delayed reaction in MNTB results from differences in the intrinsic nature of these neuronal phenotypes, lesions of VCN might be expected to result in a delay in MNTB neuronal atrophy, which is similar to the results presented here. An extrinsic difference at the level of MNTB may relate to nonmanipulated excitatory afferents. Beginning with the report of Levi-Montalcini (1949), several investigators have suggested that the degree of transneuronal cell death and atrophy are related to the relative proportion of afferents removed from a target structure.

If cell size changes are related to the relative amount of afferent electrical activity that is altered, one might predict that cochlear ablation affects net electrical activity to a lesser extent in MNTB principal cells than in VCN bushy cells. Decreases in radiolabeled 2-deoxyglucose (2-DG) uptake in brainstem auditory nuclei following cochlear removal, but in the absence of auditory stimulation, have been used as a measure of spontaneous electrical activity of cochlear origin in mice (Durham et al., 1989). Results indicate that spontaneous electrical activity is affected to a lesser extent in the contralateral MNTB than in the ipsilateral VCN. To the extent that 2-DG uptake is related to net electrical activity, these data are consistent with the present results. It is well known that both VCN neurons and principal cells of MNTB receive one or more sources of extrinsic input in addition to afferents from the cochlear nerve and VCN, respectively (Irvine, 1986; Kuwabara et al., 1991; Ostapoff and Morest, 1991). Unfortunately, the sources of many of these inputs have not been confirmed, and their physiological properties are relatively unknown. One possibility that would be consistent with the 2-DG data reported above and the results of the present study is that MNTB neurons receive more sources of excitatory innervation that is not affected by interruption of eighth nerve activity than VCN neurons. In this case, the *proportion* of excitatory input that is interrupted may be less for MNTB neurons than VCN neurons (Levi-Montalcini, 1949). On the other hand, at this time, there is no compelling evidence that either MNTB or globular bushy cells receive other major sources of excitatory innervation, much less a difference in the total amount that could account for the differences noted in this report.

Another possibility is that cochlear removal and eighth nerve activity blockade result in a delayed change in MNTB compared to VCN because of intrinsic differences in the overall rate of activity in the auditory nerve compared to globular bushy cells of VCN. In this case, the *relative change* in excitatory input might be less in MNTB than in globular cells. Some support for this hypothesis can also be gleaned from the literature. For example, extracellular single unit recordings in AVCN of cats indicate that spontaneous activity is higher in spherical bushy cells with characteristic frequencies less than 2 kHz than in globular bushy cells with similar characteristic frequencies (Blackburn and Sachs, 1989). Recordings from trapezoid body axons emanating from each of these regions show similar findings (Smith et al., 1991). Others have found a low spontaneous rate across all characteristic frequencies in globular bushy cells in posterior AVCN of the rat (Friauf and Ostwald, 1988). Thus the AVCN globular bushy cell to MNTB principal cell pathway may be characterized by a lower spontaneous rate than the spherical cell region of AVCN.

Finally, the delayed response of MNTB neurons may result from the fact that, although trophic regulation of postsynaptic neurons is activity-dependent, it also relies on cellular events other than activity-dependent release from presynaptic terminals. There are precedents for this view as well. For example, it is well known that the metabolic integrity of muscles depends on electrical activity that is mediated by the motor nerves (Jacobson, 1991). A persistent finding, however, is that, after nerve section, the rate at which deafferentation-induced changes begin is inversely proportional to the length of the nerve stump left attached to the muscle. This result is usually interpreted to mean that depletion of a trophic substance within the axons is also involved in denervation-induced atrophy of muscles (Fernandez and Donoso, 1988). Within the central nervous system, there is evidence that blockade of axoplasmic transport alone, or in combination with TTX blockade of action potentials, can potentiate cell death in the embryonic chick optic tectum (Catsicas et al., 1992). Furthermore, some reports suggest a relationship between electrical activity and the amount of specific materials transported by fast axoplasmic transport (Edwards and Grafstein, 1984; Hammerschlag and Bobinski, 1992). Thus, in the present case, it is possible that the relative delay of MNTB neuronal atrophy is due to activity-independent trophic molecules released from the VCN-MNTB nerve terminals that are eventually exhausted because of decreases in synthesis or transport by the VCN globular cells.

Activity-dependent recovery

The use of tetrodotoxin offers advantages over cochlear ablation as a method to alter electrical activity in the auditory nerve, not the least of which is the reversibility of the manipulation. Investigators using TTX to block afferent activity in the visual system of cats have examined postsynaptic structure and function following restoration of activity: Lateral geniculate nucleus (LGN) soma size partially recovers 3–4 weeks after a 1-week blockade of retinal afferents (Kupperman and Kasamatsu, 1983), LGN neuron cytochrome oxidase activity fully recovers 5–6 weeks after a 4-week blockade (Wong-Riley and Riley, 1983), and the functional segregation of neural input to LGN can recover within 2 days following a 20–23-day blockade (Dubin et al., 1986).

In the auditory system, a variety of insults to the inner ear have been shown to result in decreases in neuron size in the cochlear nucleus of birds and mammals, and some have been shown to be reversible. For example, aminoglycoside-induced cochlear toxicity in the chicken is associated with loss of cell size in ipsilateral cochlear nucleus neurons. The decrease in cell size is partially reversible following regeneration of cochlear hair cells (Lippe, 1991). We have found that both AVCN spherical cells (Pasic and Rubel, 1991) and now MNTB neuron size can fully recover within 1 week following a 24 or 48 hour blockade of afferent activity. The recovery of soma size indicates that the original atrophy is not a strictly pathological reaction to injury but a physiological response to a cascade of extrinsic and intrinsic events altered by afferent activity.

We interpret the inability to detect a difference in soma size between the two sides of the brain 7 days after TTX exposure as an increase (recovery) in cell size on the manipulated side. However, alternate interpretations are possible. For example, the cells in the nonmanipulated auditory pathway may decrease in size, or there may be a combination of the smaller cells enlarging and the larger cells becoming smaller after activity is allowed to resume. Although technical factors make comparisons of absolute soma size between animals difficult (Kalil, 1980), the mean size of contralateral MNTB cell bodies from animals that survived for 7 days after activity blockade is greater than that of the same cells during activity blockade. This suggests that the contralateral neurons increase in size (i.e., return to their previous size) following reversal of activity blockade.

Medial-lateral difference in MNTB cell size

We found a difference in neuronal soma size that appears to correspond with the tonotopic organization of MNTB. Neural cell bodies believed to be in the low-frequency regions of MNTB are significantly larger than cells in the high-frequency regions (Guinan et al., 1972). This finding raises the possibility that there is a difference in afferent activity along the tonotopic axis.

Morphological gradients that parallel tonotopic organization have been previously described in several auditory nuclei (Brawer et al., 1974; Smith and Rubel, 1979; Sanes et al., 1987; Code et al., 1989; Sanes and Siverls, 1991). With regard to tonotopic differences in afferent activity, a greater proportion of high-spontaneous-rate auditory nerve fibers has been found at lower characteristic frequencies than higher characteristic frequencies in the cat and gerbil (Liberman, 1988; Schmiedt, 1989). Sento and Ryugo (1989) have found that individual AVCN spherical cells that are postsynaptic to high-spontaneous-rate auditory nerve fibers are significantly larger than spherical cells that are postsynaptic to low-spontaneous-rate fibers (Sento and Ryugo, 1989). These investigators also reported that the relationship between auditory nerve characteristic frequency and postsynaptic cell size is nonmonotonic. Although data from MNTB on the spontaneous rate distributions and their relationships with characteristic frequency or soma size are not currently available, our cell size data would lead to the prediction that there is a greater amount of activity in low-frequency regions compared to high-frequency regions of MNTB.

The above discussion assumes that the neurons we measured in medial and lateral MNTB are of the same type (i.e., principal cells). Another possible explanation of the difference in soma size is that a larger proportion of medial MNTB neurons are stellate cells and are not distinguishable from principal cells in Nissl-stained material in gerbils, except by size. Anatomical localization of calyces of Held surrounding medial MNTB neurons and/or physiological recordings from medial MNTB cells in the gerbil would be required to dismiss this possibility entirely. However, we believe the distinction between principal and stellate cells in Nissl-stained material was reliably made. Furthermore, if one region included a larger percentage of stellate cells, we would expect greater variability in that region as well as a smaller mean soma size. In our sample, the medial region did not have a larger variance.

ACKNOWLEDGMENTS

We thank Dianne Durham for helpful comments during the study, Shawn Kreig and Barbara Tuschen for valuable technical assistance, and Mark Walter for typing and editorial assistance. Nell Cant kindly provided HRP material that greatly assisted in the identification and localization of globular cells in the gerbil. This work was supported by NIDCD grant DC00520 and by funds from the University of Washington Department of Otolaryngology-Head and Neck Surgery. D.R.M. was supported by a Visiting Professorship from the Virginia Merrill Bloedel Hearing Research Center, University of Washington, and by a grant from the Medical Research Council (U.K.).

LITERATURE CITED

Blackburn, C.C., and M.B. Sachs (1989) Classification of unit types in the anteroventral cochlear nucleus: PST histograms and regularity analysis. J. Neurophysiol. *62:*1303–1329.

Born, D.E., and E.W Rubel (1985) Afferent influences on brainstem auditory nuclei of the chicken: Neuron number and size following cochlea removal. J. Comp. Neurol. *231:*435–445.

Born, D.E., and E.W Rubel (1988) Afferent influences on brainstem auditory nuclei of the chicken: Presynaptic action potentials regulate protein synthesis in nucleus magnocellularis neurons. J. Neurosci. *8:*901–919.

Born, D.E., D. Durham, and E.W Rubel (1991) Afferent influences on brainstem auditory nuclei of the chick: Nucleus magnocellularis neuronal activity following cochlea removal. Brain Res. *577:*37–47.

Brawer, J.R., D.K. Morest, and E. Kane (1974) The neuronal architecture of the cochlear nucleus of the cat. J. Comp. Neurol. *155:*251–300.

Cant, N.B., and D.K. Morest (1979) The bushy cells in the anteroventral cochlear nucleus of the cat. A study with the electron-microscope. Neuroscience *4:*1925–1945.

Catsicas, M., Y. P'equignot, and P.G.H. Clarke (1992) Rapid onset of neuronal death induced by blockade of either axoplasmic transport or action potentials in afferent fibers during brain development. J. Neurosci. *12:*4642–4650.

Code, R.A., G.D. Burd, and E.W Rubel (1989) Development of GABA-immunoreactivity in brainstem auditory nuclei of the chick: Ontogeny of gradients in terminal staining. J. Comp. Neurol. *284:*504–518.

Coleman, J.R., and P.O. O'Connor (1979) Effects of monaural and binaural sound deprivation on cell development in the anteroventral cochlear nucleus of rats. Exp. Neurol. *64:*533–566.

Constantine-Paton, M., H.T. Cline, and E. Debski (1990) Patterned activity, synaptic convergence, and the NMDA receptor in developing visual pathways. Annu. Rev. Neurosci. *13:*129–154.

Cowan, W. M. (1970) Anterograde and retrograde transneuronal degeneration in the central and peripheral nervous system. In W. J. H. Nauta and S. O. E. Ebbesson (eds.): Contemporary Research Methods in Neuroanatomy. New York: Springer-Verlag, pp. 217–249.

Dubin, M.W., L.A. Stark, and S.M. Archer (1986) A role for action-potential activity in the development of neuronal connections in the kitten retinogeniculate pathway. J. Neurosci. *6:*1021–1036.

Durham, D., E.W Rubel, and K. Steel (1989) Cochlear ablation in deafness mutant mice: 2-deoxyglucose analysis suggests no spontaneous activity of cochlear origin. Hearing Res. *43:*39–46.

Edwards, D.L., and B. Grafstein (1984) Intraocular injection of tetrodotoxin in goldfish decreases fast axonal transport of [^{3}H]glucosamine-labeled materials in optic axons. Brain Res. *299:*190–194.

Fernandez, H.L., and J.A. Donoso (1988) Nerve-Muscle Cell Trophic Communication. Boca Raton, FL: CRC Press, pp. 245.

Friauf, E., and J. Ostwald (1988) Divergent projections of physiologically characterized rat ventral cochlear nucleus neurons as shown by intra-axonal injection of horseradish peroxidase. Exp. Brain Res. *73:*263–284.

Globus, A. (1975) Brain morphology as a function of presynaptic morphology and activity. In A. H. Riesen (ed.): The Developmental Neuropsychology of Sensory Deprivation. New York: Acadamic Press, pp. 9–91.

Guinan, J.J., B.E. Norris, and S.S. Guinan (1972) Single auditory units in the superior olivary complex. II: Locations of unit categories and tonotopic organization. Int. J. Neurosci. *4:*147–166.

Hammerschlag, R., and J. Bobinski (1992) Does nerve impulse activity modulate fast axonal transport? Mol. Neurobiol. *6:*191–201.

Hashisaki, G.T., and E.W Rubel (1989) Effects of unilateral cochlear removal on anteroventral cochlear nucleus neurons in developing gerbils. J. Comp. Neurol. *283:*465–473.

Hyson, R.L., and E.W Rubel (1989) Transneuronal regulation of protein synthesis in the brainstem auditory system of the chick requires synaptic activation. J. Neurosci. *9:*2835–2845.

Irvine, D.R.F. (1986) Progress in Sensory Physiology, Vol. 7: The Auditory Brainstem. New York: Springer-Verlag.

Jacobson, M. (1991) Developmental Neurobiology. New York: Plenum Press.

Jean-Baptiste, M., and D.K. Morest (1975) Transneuronal changes of synaptic endings and nuclear chromatin in the trapezoid body following cochlear ablations in cats. J. Comp. Neurol. *162:*111–134.

Kalil, R. (1980) A quantitative study of the effects of monocular enucleation and deprivation on cell growth in the dorsal lateral geniculate nucleus of the cat. J. Comp. Neurol. *189:*483–524.

Koerber, K.C., W.B. Pfeiffer, W.B. Warr, and N.Y.S. Kiang (1966) Spontaneous spike discharges from single units in the cochlear nucleus after destruction of the cochlea. Exp. Neurol. *16:*119–130.

Kupperman, B.D., and T. Kasamatsu (1983) Changes in geniculate cell size following brief monocular blockade of retinal activity in kittens. Nature *306:*465–468.

Kuwabara, N., R.A. DiCaprio, and J.M. Zook (1991) Afferents to the medial nucleus of the trapezoid body and their collateral projections. J. Comp. Neurol. *314:*684–706.

Levi-Montalcini, R. (1949) The development of the acoustico-vestibular centers in the chick embryo in the absence of the afferent root fibers and of descending fiber tracts. J. Comp. Neurol. *91:*209–242.

Liberman, M.C. (1988) Physiology of cochlear efferent and afferent neurons: Direct comparisons in the same animal. Hearing Res. *34:*179–191.

Lippe, W.R. (1991) Reduction and recovery of neuronal size in the cochlear nucleus of the chicken following aminoglycoside intoxication. Hearing Res. *51:*193–202.

Moore, D.R. (1990) Auditory brainstem of the ferret: Early cessation of developmental sensitivity of neurons in the cochlear nucleus to removal of the cochlea. J. Comp. Neurol. *302:*810–823.

Morest, D.K. (1968) The collateral system of the medial nucleus of the trapezoid body of the cat, its neuronal architecture and relation to the olivo-cochlear bundle. Brain Res. *9:*288–311.

Osen, K.K. (1969) Cytoarchitecture of the cochlear nuclei in the cat. J. Comp. Neurol. *214:*453–484.

Ostapoff, E.M., and D.K. Morest (1991) Synaptic organization of globular bushy cells in the ventral cochlear nucleus of the cat: A quantitative study. J. Comp. Neurol. *314:*598–613.

Pasic, T.R., and E.W Rubel (1989) Rapid changes in cochlear nucleus cell size following blockade of auditory nerve electrical activity in gerbils. J. Comp. Neurol. *283:*474–480.

Pasic, T.R., and E.W Rubel (1991) Cochlear nucleus cell size is regulated by auditory nerve electrical activity. Otolaryngol. Head Neck Surg. *104:* 6–13.

Powell, T.P.S., and S.D. Erulkar (1962) Transneuronal cell degeneration in the auditory relay nuclei of the cat. J. Anat. *96:*249–268.

Rouiller, E.M., R. Cronin-Schreiber, D.M. Fekete, and D.K. Ryugo (1986) The central projections of intracellularly labeled auditory nerve fibers in cats: An analysis of terminal morphology. J. Comp. Neurol. *249:*261–278.

Rubel, E.W., R.L. Hyson, and D. Durham (1990) Afferent regulation of neurons in the brainstem auditory system. J. Neurobiol. *21:*169–196.

Sanes, D.H., and V. Siverls (1991) Development and specificity of inhibitory terminal arborizations in the central nervous system. J. Neurobiol. *22:*837–854.

Sanes, D.H., W.A. Geary, G.F. Wooten, and E.W Rubel (1987) Quantitative distribution of the glycine receptor in the auditory brainstem of the gerbil. J. Neurosci. *7:*3793–3802.

Schmiedt, R.A. (1989) Spontaneous rates, thresholds and tuning of auditory nerve fibers in the gerbil: Comparisons to cat data. Hearing Res. *42:*23–35.

Sento, S., and D.K. Ryugo (1989) Endbulbs of Held and spherical bushy cells in cats: Morphological correlates with physiological properties. J. Comp. Neurol. *280:*553–562.

Shatz, C.J. (1990) Impulse activity and the patterning of connections during CNS development. Neuron *5:*745–756.

Sie, K.C.Y., and E.W Rubel (1992) Rapid changes in protein synthesis and cell size in the cochlear nucleus following eighth nerve activity blockade and cochlea ablation. J. Comp. Neurol. *320:*501–508.

Smith, D.J., and E.W Rubel (1979) Organization and development of brainstem auditory nuclei in the chicken: Dendritic gradients in n. laminaris. J. Comp. Neurol. *186:*213–239.

Smith, P.H., P.X. Joris, L.H. Carney, and T.C.T. Yin (1991) Projections of physiologically characterized globular bushy cell axons from the cochlear nucleus of the cat. J. Comp. Neurol. *304:*387–407.

Steward, O., and E.W Rubel (1985) Afferent influences on brainstem auditory nuclei of the chicken: Cessation of amino acid incorporation as an antecedent to age-dependent transneuronal degeneration. J. Comp. Neurol. *231:*385–395.

Trune, D.R. (1982) Influence of neonatal cochlear removal on the development of mouse cochlear nucleus: I. Number, size and density of its neurons. J. Comp. Neurol. *209:*409–424.

Warr, W.B. (1972) Fiber degeneration following lesions in the multipolar and globular cell areas in the ventral cochlear nucleus of the cat. Brain Res. *40:*247–270.

Webster, D.B., and M. Webster (1977) Neonatal sound deprivation affects brainstem auditory nuclei. Arch. Otolaryngol. *103:*392–396.

Wong-Riley, M., and D.A. Riley (1983) The effect of impulse blockade on cytochrome oxidase activity in the cat visual system. Brain Res. *261:*185–193.