Afferent Influences on Brain Stem Auditory Nuclei of the Chicken: Neuron Number and Size Following Cochlea Removal

DONALD E. BORN AND EDWIN W RUBEL
Departments of Otolaryngology and Physiology and Neuroscience Program, University
of Virginia School of Medicine, Charlottesville, Virginia 22908

ABSTRACT

The consequences of cochlea removal on neuron number and soma cross-sectional area were examined in the second order auditory nucleus (n. magnocellularis) of chickens. Both the age of the subjects at the time of cochlea (basilar papilla) removal (1–66 weeks) and the survival period (1–45 days) were varied. Neuron number and soma cross-sectional area were determined from Nissl stained sections. Additional material was processed to examine the relationship of ganglion cell loss to changes in n. magnocellularis.

Neuron number decreased by 25–30% and soma cross-sectional area decreased by 10–20% ipsilateral to the cochlea removal in chickens operated on during the first 6 weeks after hatching. In contrast, in chickens operated on at 66 weeks posthatch neuron number decreased less than 10% and there was no change in soma area. The changes were rapid, being nearly complete 2 days after cochlea removal. An initial change (1 and 2 days after surgery) observed in animals operated on up to 6 weeks posthatch was the presence of a large number of neurons in which no Nissl substance could be detected. These results demonstrate an age-dependent change in the susceptibility of NM neurons to deafferentation. This change is not temporally related to other measures of functional maturation of the auditory system.

Key words: deafferentation, development, n. magnocellularis

The consequences of changes in presynaptic inputs on the normal development and maintenance of central nervous system neurons have been studied extensively (for reviews, see Cowan, '70; Guillery, '74; Globus, '75; Smith, '77). Although relatively little is known about the factors that control the extent and rate of transneuronal degeneration, more dramatic changes often are observed following afferent manipulation in young animals than in adults of the same species (Tsang, '37; Cook et al., '51; Trumpy, '71; Kelly and Cowan, '72; Guillery, '73; Heumann and Rabinowicz, '80; Kalil, '80; Trune, '82a,b; Nordeen et al., '83; Peduzzi and Crossland, '83). It is commonly concluded that this period of greatest susceptibility to manipulation of afferents corresponds to the time when most synaptic connections are being established, during the time of major functional and morphological change (Globus, '75; Heumann and Rabinowicz, '80; Trune, '82a, Peduzzi and Crossland, '83; Nordeen et al., '83).

The auditory system provides a convenient location to manipulate and examine afferent regulation of the central nervous system. Following cochlea destruction in mature cats, Powell and Erulkar ('62) found neuron loss and shrinkage in brain stem auditory areas that receive afferents from the eighth nerve. Other investigators have examined morphological and biochemical changes in auditory neurons following removal of their input but have not carefully

documented differences in response throughout development (Powell and Erulkar, '62; Kane, '74; Jean-Baptiste and Morest, '75; Benes et al., '77; Parks, '79, '81; Webster and Webster, '78; Rubel et al., '81; Trune, '82a,b; Nordeen et al., '83).

In the present study, transneuronal changes following cochlea (basilar papilla) removal were examined in the second order auditory neurons of nucleus magnocellularis (NM) in the chicken brain stem. These neurons form a homogeneous population and are considered to be homologous to the spherical (bushy) cells of the anteroventral cochlear nucleus in mammals (Held, 1893; Ramón y Cajal, '09; Boord, '69; Osen, '69; Rubel and Parks, '75; Parks and Rubel, '78; Jhaveri and Morest, '82a; Hackett et al., '82). The size, distinctiveness, and homogeneity of NM neurons serve to separate them from other cell types (Levi-Montalcini, '49; Knowlton, '67; Parks, '79; Jhaveri and Morest, '82a). In addition, the ontogeny of the chicken auditory system has been well studied (Rubel et al., '76; Rubel, '78; Jackson and Rubel, '78; Gray and Rubel, '81, '84; Jhaveri and Morest, '82b; Jackson et al., 82; Rubel and Ryals, '83; Lippe and Rubel, '83; Rubel et al., '84); thus periods of change in transneuronal effects can be measured against known ontological landmarks.

Transneuronal degeneration in the ipsilateral NM can be produced embryonically by ablation of the primary otocyst, and after hatching by removal of the cochlea. Following otocyst ablation decreased neuron number and soma area in the primary auditory nuclei have been reported (Levi-Montalcini, '49; Parks, '79). Preliminary experiments on posthatch chickens suggested that these effects extended well into the postnatal period (Jackson and Rubel, '76). In the present study, the age of the subject at the time of surgery and the survival period were systematically varied to determine the pattern of transneuronal degeneration throughout development.

MATERIALS AND METHODS

Chickens (Hubbard × Hubbard) were obtained as adults or hatched from eggs incubated in our laboratory (supplier, Heatwole Hatchery, Harrisonburg, VA). All chickens were housed with access to food and water—hatchlings in communal brooders and adults in individual cages.

Surgery and Histology

For the majority of cases the cochlea was removed unilaterally at 2, 6, or 66 weeks after hatching (designated cochlea removal groups). These subjects were anesthetized using ketamine hydrochloride (80 mg/kg i.m.) and Chloropent (1.5 ml/kg i.p., Fort Dodge Laboratories, Inc.). Atropine (.01 mg/kg i.m.) was given to inhibit tracheal secretions. A small incision was made in one external ear canal. The tympanic membrane and columella were removed to expose the oval window. The basilar papilla, including the lagena macula, was removed via the oval window and floated on water to verify complete removal. Removal of the cochlea in this manner caused little trauma to surrounding tissue. It severs the distal processes of the ganglion cells but there appears to be no direct damage to the ganglion cell bodies (Fig. 1).

After survival periods of 2, 7, or 26 days the subjects were deeply anesthetized with Nembutal and perfused transcardially with Heidenhain's fixative without mercuric chloride (Lillie and Fullmer, '76). Three unoperated chickens at each of the ages above also were perfused to serve as controls.

Brains were postfixed in Heidenhain's fix for 12 hours, transferred to 10% buffered formalin for 2–10 days, dehydrated, and embedded in paraffin. Coronal sections were cut at 10 μm and a series of one in every four sections through NM was mounted and stained with thionin. An additional one-in-eight series was stained by a modification of Bodian's protargol method (Bodian, '36).

Additional Material

Additional material was processed to investigate the relationship between loss of cochlear ganglion cells and changes in n. magnocellularis. To determine if the time course or degree of atrophy in NM was influenced by the initial sparing of ganglion cells an additional group of twelve 1- to 2-week-old chickens were operated on to remove unilaterally both the cochlea and ganglion cells (designated cochlea and ganglion cell removal groups). The procedure was identical to that described above except that following removal of the cochlea the ganglion cells were aspirated with a fine pipette. Complete removal of the ganglion cells was verified by examination of the internal auditory meatus at the time of the operation.

To determine ganglion cell survival following removal of the cochlea, counts of ganglion cells were carried out on six chickens. The cochlea was removed at 1 week posthatching. Two animals were perfused with 10% formalin 1, 4, or 21 days later. The skull was decalcified in EGTA; the entire head was embedded in paraffin and sectioned at 15 μm.

Finally, ten chickens were operated on to examine NM at survival periods shorter than 2 days and to insure that the results described below were independent of tissue processing methods. These subjects underwent unilateral cochlea removal at 2 weeks posthatch and were allowed to survive for 1 or 2 days. They were perfused with one of a variety of fixatives (including Heidenhain's, acid alcohol, Carnoy's, glutaraldehyde-paraformaldehyde, or Bouin's) and processed as described for the initial group.

Quantitative analysis

Neuron counts. Neuron number was determined from the one-in-four series of thionin stained sections with the aid of a ×40 planapochromatic objective (N.A. 1.0). Neurons were counted that had a stained cytoplasm surrounding a clear nuclear area with a nucleolus present.[1] Total neuron number was estimated from these counts multiplied by four. No correction factor was employed because of the small diameter of the nucleolus (used in the counting criteria), relative to section thickness (Konigsmark, '70). In each brain, NM neuron counts were made on the side of the brain ipsilateral to the cochlea removal and on the contralateral side, which served as the control.

Recounts on a sample of ten brains yielded less than 10% difference between the counts. To check the possibility that some aspect of the changes in neuron number may be the result of changes in cell form (such as dispersion of the nucleus or nucleolus) counts were made on a sample of brains in which all NM neurons were counted irrespective

---

[1]Neurons of the lagenar area (Rubel and Parks, '75) were counted separately and were not included in the total counts for NM. Lagenar neurons were identified by their posterolateral position, relatively smaller size, increased clumping of Nissl substance, and less columnar organization.
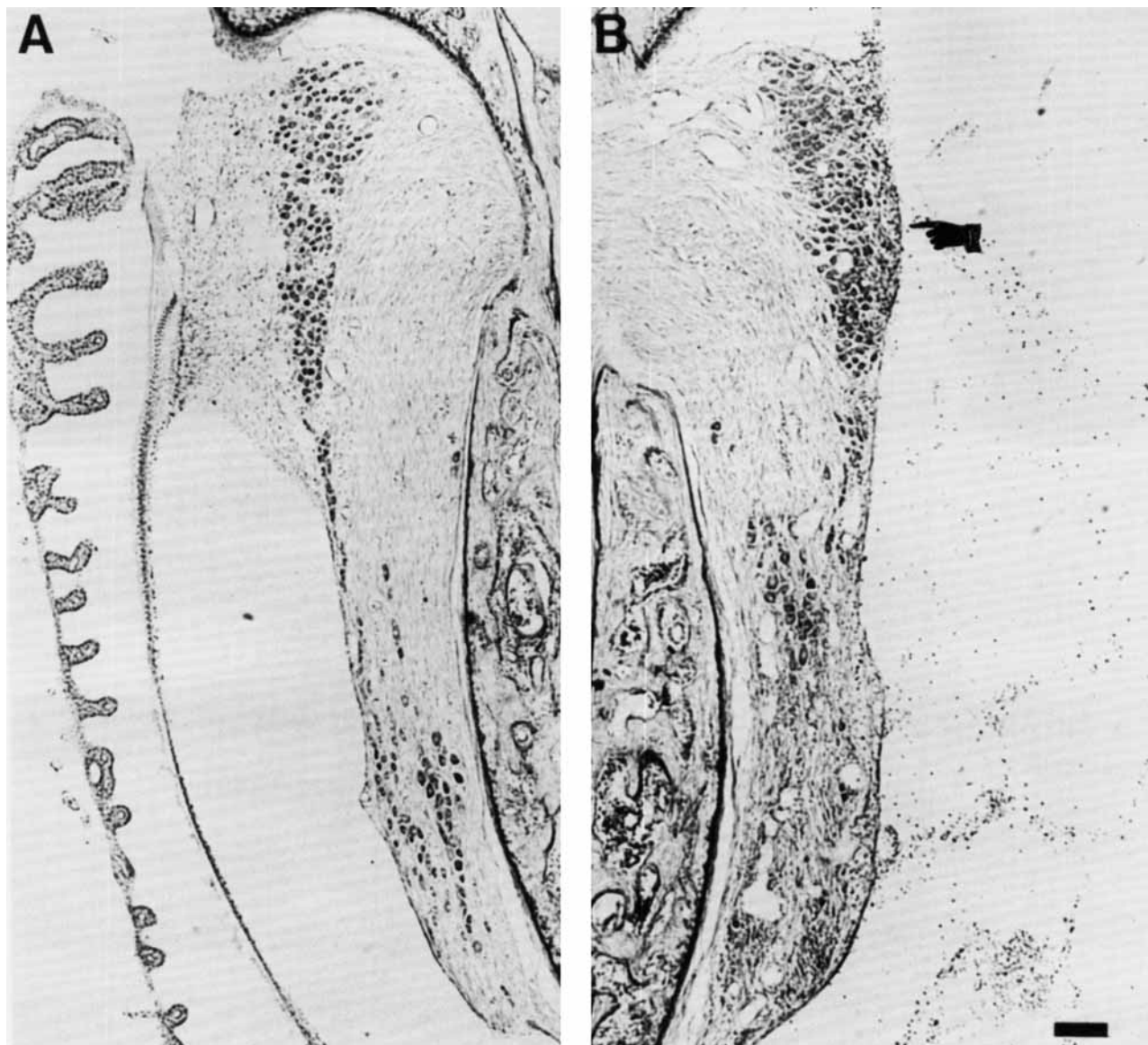
Fig. 1. Photomicrographs of ganglion cells in the cochlear ducts 1 day following unilateral cochlea removal in a 1 week old chicken. On the unoperated side (A) the intact membraneous labyrinth is attached. On the operated side (B) note the ganglion cells (pointer) and the complete absence of the membranous labyrinth. Scale bar = 100 μm.

of the presence of a nucleolus. Neuron numbers obtained by using these two methods were compared by examining the difference between the two sides of the brain expressed as a percent of the unoperated side. The results for counting all neurons were within 10% of the routine method.

Cochlear ganglion cells were counted on each side of the brain from the whole head preparations. Cells were counted that had cytoplasmic staining for Nissl substance, with a distinct nucleus and nucleolus. Each section from a one-in-eight series through the entire cochlea was analyzed and the total was calculated by multiplying by eight. Percent decrease was calculated from the difference between the

two sides relative to the contralateral side. The mean for each of the three survival times was then calculated.

Cross-sectional area. The cross-sectional areas of NM somata were measured on both sides of the brain in thionin stained sections. Measurements were performed in two regions of the nucleus, at 33% and 67% of the total posterior-to-anterior (PA) length of NM on each side of the brain. Soma area was determined by outlining the stained portion of the soma at the largest diameter of neurons which contained a recognizable nucleolus using a ×100 planapochromatic objective (N.A. 1.3) and a Zeiss Videoplan morphometry system. At least 50 neurons were measured
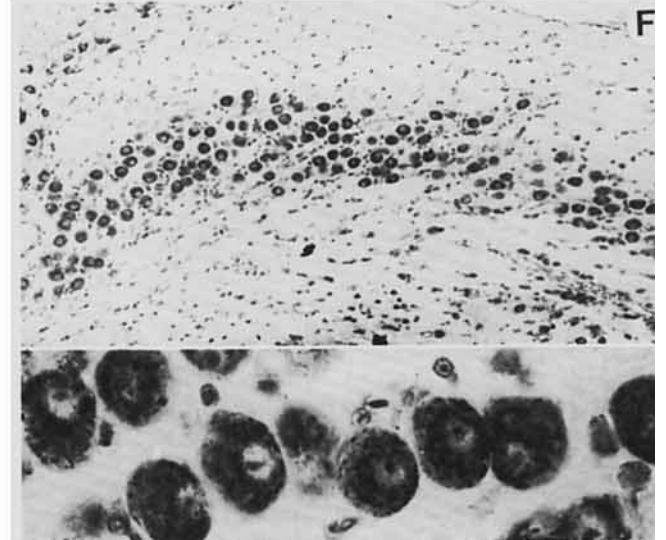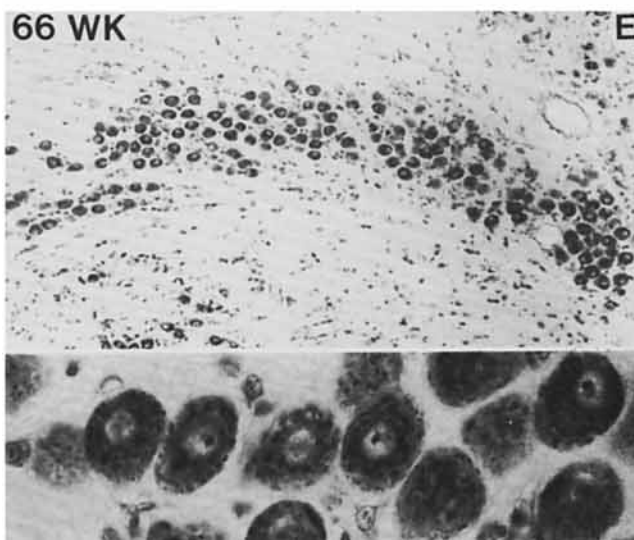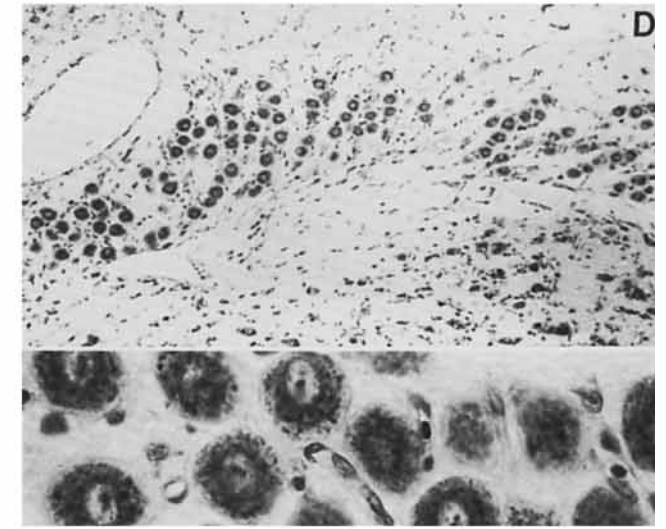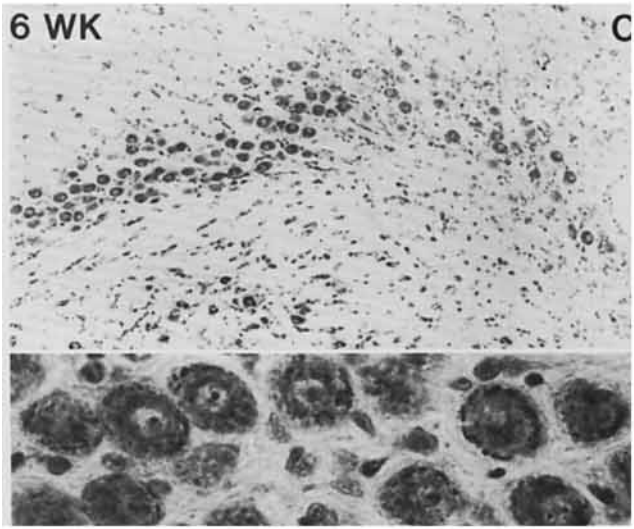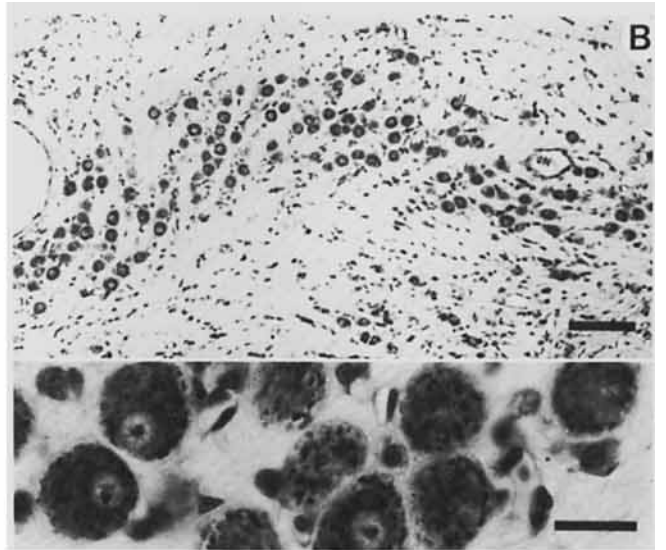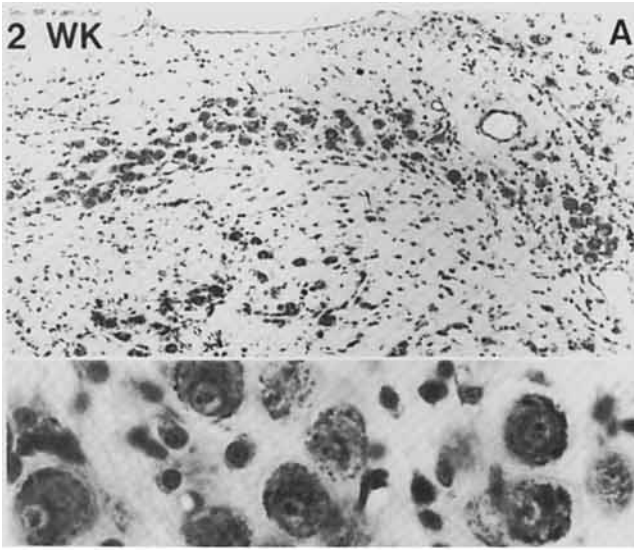
IPSILATERAL   CONTRALATERAL

Figure 2

at each position in the nucleus on both sides of each brain. This yielded at least 200 neurons measured per brain and a total of 14,241 measured neurons.

Statistics. Means were calculated for neuron number and soma area, and for the difference in number and area between the two sides of the brain. The data for chickens with only the cochlea removed were analyzed by a two way analysis of variance (ANOVA) (age × survival period) for neuron number on both sides of the brain and a three way ANOVA (age × survival period × PA position) for soma cross-sectional area on both sides of the brain. Similar analyses were performed on the difference scores between the two sides of the brains. All analyses were carried out on a Control Data Corporation Cyber 730 computer using SPSS routines (Nie et al., '75).

RESULTS

The thionin stained sections through nucleus magnocellularis showed differences in reaction to cochlea removal among the age groups. Neuron loss, shrinkage, and decreased staining on the side of the brain ipsilateral to the cochlea removal was apparent in chickens operated on at 2 or 6 weeks after hatching. Figure 2 shows representative results from chickens perfused 26 days after cochlea removal. When the cochlea was removed at 2 or 6 weeks posthatch there is a noticeable decrease in the number of neurons. In addition, neurons remaining appear shrunken and lighter staining, and the columnar organization is disrupted on the side of the brain ipsilateral to surgery (panels A and C) when compared to the contralateral side of the brain (panels B and D). In chickens operated on at 66 weeks posthatch (panels E and F), NM neurons on the two sides of the brain were comparable in estimated number, size, and staining characteristics. The columnar organization of NM neurons on the side of the brain ipsilateral to the cochlea removal was disrupted, which is probably due to the degeneration of the eighth nerve fibers which separate the columns (Rubel and Parks, '75). Material stained by Bodian's method revealed massive fiber loss in the eighth nerve and loss of terminal end bulbs in NM. These effects appeared equivalent in all age groups.

In chickens operated on 2 or 6 weeks posthatch the effect of cochlea removal was rapid. Fewer neurons were apparent in NM ipsilateral to the cochlea removal by 2 days after surgery (compare A and B in Fig. 3). Additional chickens operated on at 2 weeks posthatch and allowed to survive for *one* day showed a similar reduction in neuron number. As illustrated in Figure 3C, this rapid decrease in the apparent number of neurons was caused by the presence of neurons that did not stain for Nissl substance. These "ghost" neurons were detectable by the presence of cytoplasmic and nuclear membranes and staining of the nucleolus and nuclear chromatin. Neurons lacking stained Nissl

substance were reliably found on the operated side of 2 and 6 week posthatch chickens at 1 or 2 day survival periods, but not at longer survival periods. This reaction was never seen in unoperated chickens or on the unoperated side of 2 or 6 week old chickens. Interestingly, "ghost" neurons also were never seen in chickens operated on at 66 weeks after hatching. Finally, "ghost" neurons were found in 1 to 2 week posthatch chickens perfused 2 days following cochlea removal regardless of the fixative employed and regardless of whether the ganglion cells were removed or left intact at the time of the operation.

Quantitative analysis

Neuron number. There was an overall mean of 4,834 neurons in NM from unoperated chickens and from the side contralateral to cochlea removal of operated chickens. While there was some variation in the absolute number of neurons (Table 1), there were no consistent differences between groups. An analysis of variance supported this conclusion; there were no main effects of age or survival period ($F_{2,49}$ = 0.25, $F_{3,49}$ = 0.37) and the interaction was not significant ($F_{6,49}$ = 1.1). Thus, we find no evidence of a change in NM neuron number contralateral to cochlea removal.

Ipsilateral to the cochlea removal there were reduced numbers of NM neurons in chickens operated on at 2 or 6 weeks posthatch at all survival times. Chickens operated on at 66 weeks posthatch have relatively normal numbers of neurons in NM on both sides of the brain (Table 1). An analysis of variance again confirmed these conclusions. There was a highly significant main effect of age ($F_{2,40}$ = 19.69, P < .001), while there was no reliable effect of survival period ($F_{2,40}$ = 0.16), and the interaction was not significant ($F_{4,40}$ = 1.35). It is clear from Table 1, and was confirmed by post hoc statistical comparisons, that the differences between groups resulted from the reduction in neuron number in the chickens operated on at 2 or 6 weeks posthatch.

Since the contralateral NM neuron number was not affected by cochlea removal, neuron loss can be expressed as the percent difference in neuron number between the two sides of each brain. As depicted in Figure 4, there was a 25–30% reduction in neuron number at each survival time for chickens operated on at 2 or 6 weeks posthatch while

Fig. 2. Low and high power photomicrographs of nucleus magnocellularis in chickens with cochlea removal at different ages. All sections are from chickens 26 days after unilateral cochlea removal. The age of the chickens at the time of receptor removal is indicated for each pair of photomicrographs; NM ipsilateral to the cochlea removal (A, C, E) and contralateral to the cochlea removal (B, D, F). In chickens operated on at 2 and 6 weeks posthatch there are fewer neurons and the remaining neurons appear shrunken in NM on the side ipsilateral (A, C) compared to the contralateral side (B, D). In contrast, chickens operated on at 66 weeks posthatch show no obvious differences between the two sides of the brain (E, F). Thionin stain. Scale bars: low power = 100 μm; high power = 20 μm.

TABLE 1. Mean Neuron Number (±Standard Deviation) Ipsilateral and Contralateral to Cochlea Removal[1]

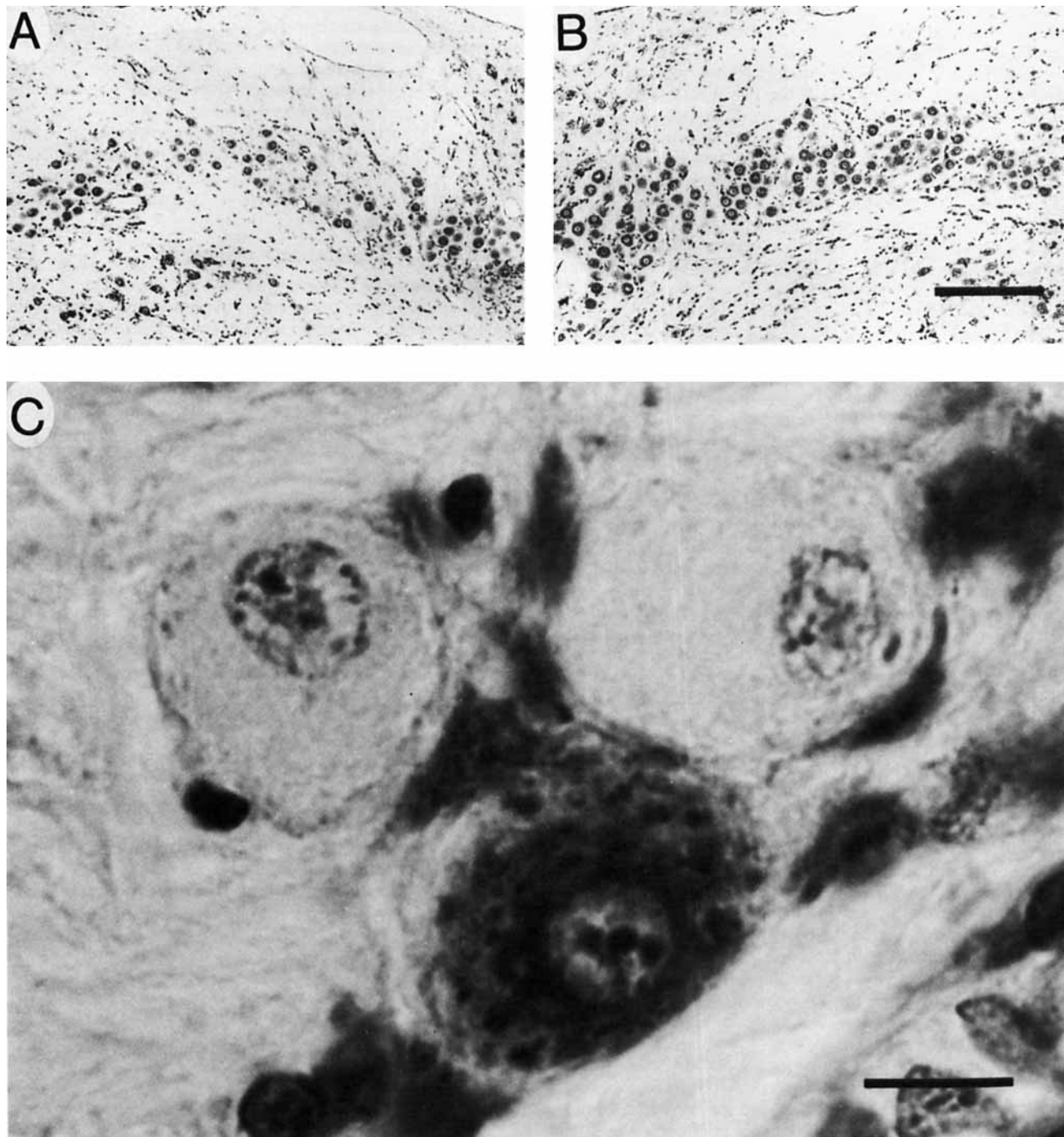| Survival	Age at surgery		
	2 Week	6 Week	66 Week
Unoperated	(3)[1]	(3)	(3)
Left	4,536 (± 577)	4,868 (± 509)	4,721 (± 246)
Right	4,584 (± 645)	4,785 (± 527)	4,650 (± 253)
2 Days	(5)	(6)	(5)
Contra	4,614 (± 445)	4,867 (± 675)	5,186 (± 151)
Ipsi	3,339 (± 929)	3,482 (± 283)	4,989 (± 211)
7 Days	(4)	(4)	(4)
Contra	4,874 (± 330)	4,907 (± 521)	4,735 (± 401)
Ipsi	3,428 (± 470)	3,616 (± 803)	4,413 (± 446)
26 Days	(4)	(4)	(5)
Contra	5,008 (± 97)	4,797 (± 319)	4,769 (± 244)
Ipsi	3,688 (±450)	3,605 (± 359)	4,344 (± 365)

[1]No. of animals in each group.

Fig. 3. Low and high power photomicrographs of nucleus magnocellularis 2 days following receptor removal in a 6-week-old chicken. There are noticeably fewer neurons in NM on the side of the brain ipsilateral to the cochlea removal (A) when compared to the contralateral side (B). The high power photomicrograph (C) shows that the apparent reduction in neuron number at this survival period is due to neurons whose cytoplasm is not stained for Nissl substance. These neurons are detectable due to their membranes and stained nuclear material. These "ghost" neurons are seen in chickens operated on from 5 days to 6 weeks posthatch at 1 and 2 day survival periods but are not seen at longer survival periods. "Ghost" neurons were never seen in chickens operated on at 66 weeks posthatch. Scale bars: A and B = 200 μm C = 10 μm.
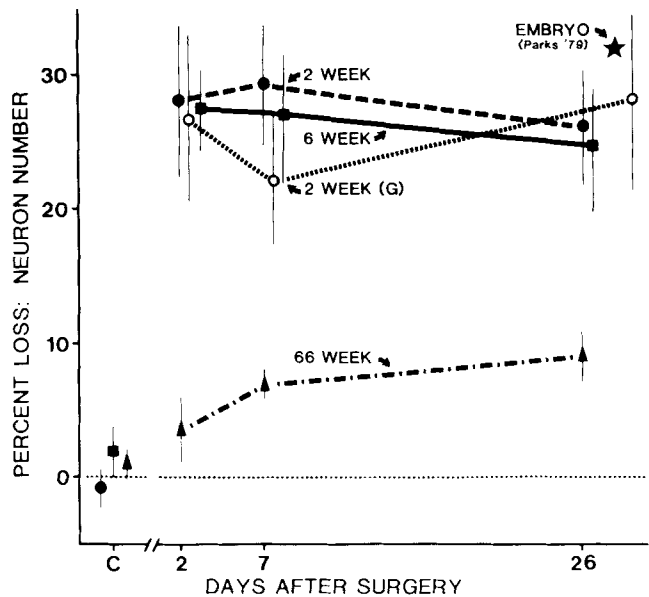
Fig. 4. Percent neuron loss as a function of survival period for chickens with cochlea removal at different ages. Percent loss is the mean of (# contralateral − # ipsilateral)/# contralateral × 100. Bars indicate standard error of the mean. Results from unoperated controls of each age (C) and from survival periods of 2, 7, and 26 days are plotted for chickens operated on at 2 weeks, 6 weeks, or 66 weeks posthatch. Neuron losses for chickens with the cochlea and ganglion cells removed at 1–2 weeks after hatching (G) are plotted in the same manner. Also plotted is the data point from two chickens that sustained unilateral otocyst removal on embryonic day 2 and survived until 28 days after hatching (Parks, '79).

In the lagenar area, mean neuron numbers on the two sides of the brain were very similar and there were no apparent differences between any groups. Statistical analysis on the neuron number in the lagenar area revealed no reliable differences on the contralateral ($F_{5,49} = 0.54$) or ipsilateral ($F_{5,49} = 1.7$) sides.

Examination of the standard errors in Figure 4 reveals an interesting pattern in the variability of transneuronal cell loss. The standard errors are 5–7% of the mean percent difference for chickens operated on at 2 weeks after hatching, 3–5% for chickens operated on at 6 weeks after hatching, and 1–2% for chickens operated on at 66 weeks after hatching. The standard errors for 66 week old chickens are similar for operated and unoperated chickens. Extensive variation in neuron loss in young animals has been a consistent finding in each of our experiments.

Cross-sectional area. Table 2 presents mean NM soma areas at the two rostro-caudal levels measured in unoperated animals and on the side of the brain contralateral to cochlea removal in operated animals. There is an obvious and statistically significant difference between the two levels examined ($F_{1,99} = 21.85$, P < .001). The more posterior location consistently has smaller soma areas. Other apparent differences on the control side do not show any consistent trends. The large absolute differences in soma areas tended to occur between groups that were not perfused and processed together. Similar differences were also observed throughout the brain stem of these groups. Soma areas are susceptible to small differences in standard protocols for tissue fixation and processing (Kalil, '80). Many factors contribute to the variability, in particular the duration of perfusion and post-fixation, the precise duration of dehydration, and the duration, temperature, and pressure of embedding, as well as the interaction of age with these variables. Thus, direct comparisons of soma area across animals, particularly at different ages, are probably subject to many qualifications and may not be a robust measure of cellular differences. Soma area in NM on the side of the brain contralateral to cochlea removal was not reliably different from that in unoperated chickens of the same age ($F_{3,99} = 2.18$). Therefore the differences between the two sides of the brain at the same level serve as the best indicator of the effect of the cochlea removal.

there was less than 10% decrease in cell number for chickens operated on at 66 weeks. The results were similar whether or not the ganglion cells were removed with the cochlea (compare 2 week to 2 week (G) inf Fig. 4). Also included in Figure 4 are data from Parks ('79) in which the otocyst was ablated during embryonic day 2 and the chickens were perfused after hatching. Neuron loss following embryonic otocyst ablation was comparable to that following cochlea removal at 2 or 6 weeks posthatch.

TABLE 2. Mean Nucleus Magnocellularis Soma Cross-Sectional Area Contralateral to Receptor Removal and the Mean Difference (Diff) Between the Two Sides of the Brain in μm^2 (Standard Deviation)

| | Age at surgery | | | | | |
| | 2 Week | | 6 Week | | 66 Week | |
Survival	67%[1]	33%	67%	33%	67%	33%
Unoperated	(3)[2]		(3)		(3)	
Right	387.5 (54.5)	313.5 (28.9)	351.2 (68.9)	313.4 (34.6)	258.8 (41.2)	229.2 (20.1)
Diff	−1.1 (9.9)	+5.8 (9.1)	+6.9 (32.9)	+5.3 (4.3)	+5.5 (19.4)	+7.0 (17.8)
2 Days	(5)		(6)		(5)	
Contra	269.5 (33.0)	229.7 (11.8)	417.6 (49.4)	367.8 (36.9)	332.7 (54.7)	308.4 (50.2)
Diff	−39.2 (10.7)	−21.3 (10.1)	−72.1 (22.5)	−62.2 (22.7)	+7.6 (21.4)	−0.5 (16.0)
7 Days	(4)		(4)		(4)	
Contra	234.3 (49.2)	224.4 (42.6)	379.9 (38.1)	317.2 (25.0)	408.0 (73.2)	353.4 (58.4)
Diff	−32.4 (46.3)	−27.9 (27.9)	−34.5 (29.0)	−34.1 (10.7)	−4.7 (8.3)	+6.8 (21.3)
26 Days	(4)		(4)		(5)	
Contra	368.3 (37.2)	303.4 (35.1)	412.7 (44.2)	333.9 (38.3)	349.0 (86.2)	310.5 (77.9)
Diff	−65.2 (27.7)	−38.9 (22.5)	−82.5 (27.6)	−56.2 (21.3)	−6.5 (28.1)	−5.8 (18.6)

[1]Level in the nucleus as a percentage of the total posterior-anterior distance.
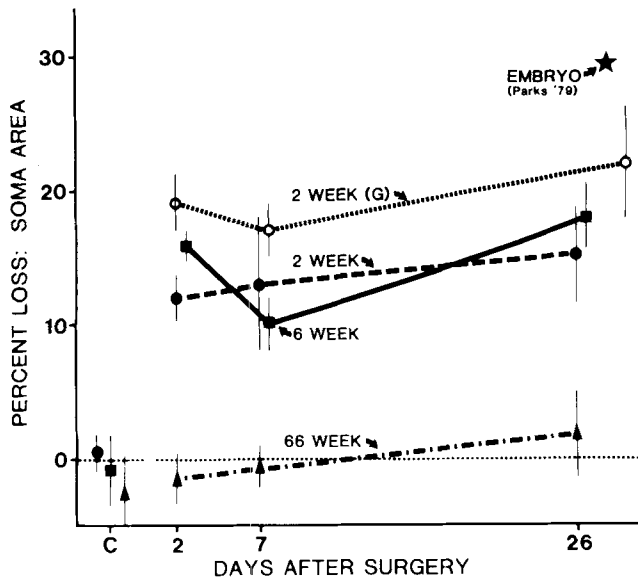[2]No. of animals in each group.

Fig. 5. Percent change in soma cross-sectional area as a function of survival period for chickens operated on at 2 weeks, 6 weeks, and 66 weeks after hatching. The percent change was determined at two anterior-posterior levels in nucleus magnocellularis and averaged for this figure. Percent loss of soma cross-sectional area is calculated for each position from (mean area contralateral − mean area ipsilateral)/mean area contralateral ×100. Bars indicate standard error of the mean. Means from unoperated controls of each age (C) and from survival periods of 2, 7, and 26 days following cochlea removal are shown. Also plotted are the results following cochlea and ganglion cell removal (G) and the data point from Parks' ('79) study as described in Figure 4.

TABLE 3. Mean Cochlear Ganglion Cell Number Contralateral to Cochlea Removal and Percent Decrease on the Ipsilateral Side

Survival period (days)	Mean No.	Percent decrease
1	16,679	16
4	17,420	31
21	14,416	74

Examination of the differences in soma area between the operated and unoperated sides of each brain (Table 2) revealed that in all groups operated on at 2 and 6 weeks posthatch the neurons in NM on the deafferented side of the brain were markedly reduced in size. At 66 weeks, however, this operation produced no observable effect on soma area. The differences were of similar magnitude at the two rostrocaudal levels of the nucleus. These differences were therefore combined to yield a single score for each animal. The amount of change in soma area is reliably different among age groups ($F_{2,40} = 30.19$, P < .001). In addition, there was a small (and marginally significant) effect of survival time ($F_{2,40} = 3.39$, P <.05) due to the slight increase in soma atrophy. As can be seen from Table 2 and as was confirmed by additional analyses, soma area differences between the two sides of the brain for 2 and 6 week old groups are reliably greater than in unoperated chickens or in chickens operated on at 66 weeks posthatch.

Figure 5 shows the average percent difference in soma cross-sectional area between ipsilateral and contralateral NM neurons. Again similar decreases were found in animals with cochlea removal or cochlea and ganglion cells removed (compare 2 week with 2 week (G)). Along with the

data of the present study are those from Parks ('79). The soma shrinkage following embryonic otocyst ablation and cochlear removal at 2 or 6 weeks posthatch was 10–30% while there was no shrinkage in chickens operated on at 66 weeks posthatch. In contrast to the variability of neuron loss, there were no consistent patterns in the variability of soma shrinkage.

Ganglion cells. The number of cochlear ganglion cells appeared to be relatively constant on the side of the brain contralateral to cochlea removal. Decreases in the number of cochlear ganglion cells in the ipsilateral ganglion were observed following cochlea removal. As seen in Table 3, the decrease appeared progressive over the survival times examined.

DISCUSSION

The neuron loss and decrease in soma area following cochlea removal depends on the age at surgery. This was evident at all survival times in both qualitative and quantitative evaluations. In NM of hatchling and young chickens (2 and 6 weeks old at the time of surgery) there was decreased neuron number, shrinkage of the soma, decreased cytoplasmic staining, and disruption of normal columnar organization. Similar changes were reported in posthatch chickens that underwent embryonic otocyst ablation (Parks, '79). In 66 week posthatch chickens there was only disruption of the columnar organization, probably reflecting the loss of eight nerve fibers. Transneuronal cell degeneration and atrophy were rapid in chickens operated on between hatching and 6 weeks posthatch. The most significant decrease in neuron number and soma area occurred during the first 2 days following cochlea removal; there was little subsequent decrease. While we did not quantitatively examine NM neurons at shorter survival periods it is clear that the effects of cochlea removal begin almost immediately after surgery.

While the transneuronal effects of cochlea removal depend on the age at the time of surgery, they appear largely independent of most other factors. There was very little effect of survival time; by 2 days the atrophy and cell loss are almost maximum. They also appear relatively independent of the presence of the ganglion cells. Although this conclusion can only be proven by finding a way to sustain the ganglion cells following cochlea removal, two findings provide evidence in favor of this interpretation. First, the time course of ganglion cell loss is markedly different than NM neuron loss and atrophy (compare Figs. 4, 5 with Table 3). Second, direct removal of the ganglion cells at the time of the cochlea removal did not seem to alter the time course or amount of NM neuron atrophy. The results also appear independent of methodological concerns such as fixation or counting methods. We found similar changes in NM neuron number regardless of the fixative used and whether or not the counts were limited to neurons with a nucleolus. Finally, it is unlikely that our results were influenced by glial changes, since neurons and glia are distinct in this brain region.

Age-related changes

Deafferentation causes greater transneuronal degeneration in young animals as compared to adults (Tsang, '37; Cook et al., '51; Trumpy, '71; Loewy, '72; Guillery, '73; Heumann and Rabinowicz, '80; Kalil, '80; Peduzzi and Crossland, '83; Trune, '82a,b; Nordeen et al., '83; Cowan, '70; Globus, '75). This age-related decline in susceptibility

is usually thought to be limited to periods of morphological and functional maturation, particularly the period when synaptic connectivity is achieved (Globus, '75; Heumann and Rabinowicz, '80; Trune, '82a; Nordeen, et al., '83; Peduzzi and Crossland, '83). All available measures indicate that the major developmental changes in morphology and function of the chicken auditory system are complete by 2–4 weeks posthatch: evoked potential thresholds across frequency are adultlike by 1 week posthatch (Saunders et al., '73; Rebillard and Rubel, '81); behavioral thresholds and frequency discrimination are mature at 10 days after hatching (Rubel and Rosenthal, '75; Kerr et al., '79; Gray and Rubel, '81, '84); the size and morphology of NM neurons appear adultlike about 3 weeks posthatch (Parks and Rubel, '78; Jhaveri and Morest, '82b); reduction of eighth nerve synapses is probably complete by 1 week posthatch (Jackson and Parks, '82); and electrophysiological maps from 2–3 week old chickens are similar to those of adults (Rubel and Parks, '75; Lippe and Rubel, '83). If the period of susceptibility to transneuronal influences is determined by the period of morphological or functional maturation then a decrease in susceptibility to transneuronal influences should take place either before hatching or during the first weeks after hatching. Paradoxically, chickens with otocyst ablation as embryos (Parks, '79), and chickens with cochlea removal at 2 or 6 weeks posthatch all reacted similarly; the decrease in susceptibility occurred between 6 weeks and 66 weeks after hatching. Thus our findings contradict the assumption that susceptibility to afferent manipulation is limited to the period of functional maturation.

There are several studies of degeneration in mammalian auditory pathways following deafferentation (Powell and Erulkar, '62; Trune, '82a,b; Nordeen et al., '83). However, it is difficult to draw any conclusion concerning the precise period of susceptibility to cochlea removal in mammals. It has been demonstrated that neuron loss in the cochlear nucleus of rodents following neonatal cochlea destruction (Trune, '82a; Nordeen et al., '83) was greater than in other mammals lesioned as adults (Powell and Erulkar, '62; Kane, '74; Webster and Webster, '78), but there is no study that systematically examines the consequences of cochlea destruction in a single species throughout development and maturity. There are studies that have examined the development of the rodent auditory system (Alford and Reuben, '63; Mikaelian and Ruben, '65; Ehret, '76; Shnerson and Willott, '80; Webster and Webster, '80; Ryan et al., 82), but there are not sufficient data to permit a conclusion about the relationship of periods of susceptibility to functional or structural development.

Studies of transneuronal degeneration in other sensory relay nuclei are consistent with our findings. Decreases in lateral geniculate nucleus (LGN) neuron number or soma area were similar in cats enucleated at 1 or 4 weeks of age (Kalil, '80) and 1, 16, or 32 weeks of age (Guillery, '73), while there was less degeneration in cats enucleated as adults (Cook et al., '51). Connections between LGN neurons and their afferents undergo major changes during the first 4 weeks after birth (Kalil, '78; Daniels et al., '78). If functional maturation determines the degree of susceptibility to deafferentation, then cell loss in animals operated on at 1 and 4 weeks of age and older should not be similar (Kalil, '80) and soma shrinkage in 1, 16, and 32 week old animals should not be similar. These results following enucleation indicate the period of susceptibility remained constant over a period extending beyond the time of major maturational changes. Results in the somatosensory system follow a similar pattern. For example, Clarke's nucleus neurons underwent approximately a 15% shrinkage following dorsal root lesion in 2, 4, and 6 week old kittens, whereas relatively little shrinkage occurred in cats lesioned as adults (Loewy, '72). Neurons of Clarke's nucleus receive synaptic contacts and undergo major morphological changes between 1 to 3 weeks postnatal (Smith, '69, '73). Again, if periods of susceptibility to dorsal root lesion are limited to the major developmental period, then differences should be observed between the three groups of young animals. These results suggest that the maturity of synaptic connections does not always determine the amount of transneuronal degeneration. Similar reactions in animals at different maturational stages suggest that the outcome is determined by some factor other than functional maturity. Considering the age of the animals at the time when the change in susceptibility to deafferentation occurs, hormonal influences associated with sexual maturity and the cessation of general body growth must be considered.

Rate and form of transneuronal degeneration

Neuronal changes were already present in chickens perfused 1–2 days following cochlea removal. Other studies of transneuronal degeneration following cochlea destruction did not include the period immediately following surgery and do not permit conclusions about the rapidity of the degeneration (Powell and Erulkar, '62; Trune, '82a,b; Jean-Baptiste and Morest, '75; Rubel et al., '81; Nordeen et al., '83). Other sensory systems show transneuronal degeneration when examined at short periods following receptor removal (Guillery, '73; Peduzzi and Crossland, '82); this also may be the case in the auditory system of mammals and needs to be addressed.

The first sign of nucleus magnocellularis neuron degeneration was decreased neuron number and absence of stained Nissl substance in about one third of the neurons ("ghost" neurons). This group of unstained neurons probably accounts for the neuron loss found at the 1–2 day survival period and possibly represents the population that is not present at longer survival periods. The evidence for this hypothesis presented in a later paper (Steward and Rubel, '84). "Ghost" neurons were not observed at survival periods of 7 days and longer. This phenomenon has not been described previously, perhaps due to its transient occurrence at short times after receptor removal. Another possibility is that the auditory system is unique in that cochlea removal essentially eliminates all post-synaptic activity in NM neurons and in the homologous mammalian ventral cochlear nucleus neurons (Lippe et al., '80; Durham et al., '84; Koerber et al., '66; Born and Rubel, '84), thus producing greater changes than in other systems. In this regard it is perhaps surprising that only about one third of the neurons become "ghosts" and subsequently about one third die. The reasons for this differential effect are not understood at present and warrant further attention. The absence of stained Nissl substance probably results from loss of the normal protein synthetic machinery. This possibility is supported by the finding that "ghost" neurons have ceased incorporation of amino acids into protein (Steward and Rubel, '85). Other changes in the metabolic machinery that occur following cochlea removal are addressed in the subsequent paper (Durham and Rubel, '85).

Neurons remaining after 26–45 day survival periods were lightly stained and shrunken. Decreased Nissl staining density is a common finding following deafferentation (Cowan, '70). Soma shrinkage in NM of chickens operated on at or before 6 weeks posthatch was 15–18% after 4 weeks survival and similar at shorter times. This is comparable to the average of 17% (9–24%) atrophy of soma area found in the ventral cochlear nucleus (VCN) of adult mice following neonatal cochlea destruction (Trune, '82a). It is interesting to compare these values with the changes following auditory deprivation procedures which presumably produce only a conductive hearing loss. In chickens that were ear plugged from embryonic day 18 there was 9–14% atrophy at 60 days posthatch (Conlee and Parks, '81). Webster ('83) found 15–18% decreases in soma areas for a variety of VCN neurons following removal of the external auditory meatus and similar changes following rearing in sound proof chambers (Webster and Webster, '79). In rats, Coleman and O'Connor ('79) reported a 10–20% area decrease in VCN neurons when deprivation was begun at 10 or 16 days after birth.

The similarity in the amount of neuron atrophy following deprivation or cochlea destruction is surprising in light of the drastic differences in the procedures. In one case the manipulation is thought to produce only conductive changes while the other involves total elimination of the receptors. Several possible explanations may be advanced. One is that the change in activity produced by cochlea removal or auditory deprivation is similar and it is this change of activity that is regulating cellular atrophy. This seems unlikely. There is a very high amount of ongoing activity in these structures, even in silence or with ear plugs in place, whereas removal of the cochlea effectively eliminates auditory nerve and ventral cochlear nucleus activity (Koerber et al., '66, Born and Rubel, '84). Alternatively, there may be a direct or secondary sensorineural component to these deprivation paridigms. A sensorineural component could result from gradual degeneration of the cochlea or ganglion cells over the course of deprivation or from direct damage, where the deprivation procedure involves surgery or ear plugging that may inadvertantly impinge on the membraneous labyrinth. Some support for this argument is the relative lack of "reversibility" of the shrinkage following auditory deprivation in sound attenuated chambers (Webster and Webster, '79). On the other hand, Conlee and Parks ('83) found a reliable effect of ear plugging on the size of NM dendrites in 10 day old chickens. This duration of ear plugging does not cause permanent threshold changes (Kerr et al., '79). In addition, Evans et al. ('83) found that auditory brain stem responses of deprived mice had normal or shorter latencies than those from normal mice of the same age.

Finally, it is possible that the similarities in soma area shrinkage do not reflect similar injury. If atrophy of 15–20% reflects the maximum change that can occur in these cell types while remaining viable, the amount of neuron loss may be a more accurate reflection of the relative severity of the manipulations. Careful studies of *both* cell size and neuron number in the cochlear nucleus as well as thorough evaluation of peripheral changes following auditory deprivation should help resolve these issues.

ACKNOWLEDGMENTS

LITERATURE CITED

Alford, B.R., and R.J. Ruben (1963) Physiological, behavioral and anatomical correlates of the development of hearing in the mouse. Ann. Otol. Rhinol. Laryngol. 72:237–247.

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

Bodian, D. (1936) A new method for staining nerve fibers and nerve endings in mounted paraffin sections. Anat. Rec. 65:89–97.

Boord, R.L. (1969) The anatomy of the avian auditory system. Ann. N.Y. Acad. Sci. 167:186–198.

Born, D.E., and E.W Rubel (1984) Cochlea removal eliminates physiological activity in brain stem auditory nuclei of the chicken. Soc. Neurosci. Abstr. 10:843.

Coleman, J.R., and P. O'Connor (1979) Effects of monaural and binaural sound deprivation on cell development in the anteroventral cochlear nucleus of rats. Exp. Neurol. 64:553–566.

Conlee, J.W., and T.N. Parks (1981) Age- and position-dependent effects of monaural acoustic deprivation in nucleus magnocellularis of the chicken. J. Comp Neurol. 202:373–384.

Conlee, J.W., and T.N. Parks (1983) Late appearance and deprivation-sensitive growth of permanent dendrites in the avian cochlear nucleus (nuc. magnocellularis). J. Comp. Neurol. 217:216–226.

Cook, W.H., J.H. Walker, and M.L. Barr (1951) A cytological study of transneuronal atrophy in the cat and rabbit. J. Comp. Neurol. 94:267–291.

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. 217–251.

Daniels, J.D., J.D. Pettigrew, and J.L. Norman (1978) Development of single-unit responses in kitten's lateral geniculate nucleus. J. Neurophysiol. 41:1373–1393.

Durham, D., D.E. Born, and E.W Rubel (1984) Effects of cochlea removal in adult chickens on metabolic activity in the brain stem auditory nuclei. Paper presented at the Seventh Midwinter Res. Meet., Assoc. Res. Otolaryngol., Feb. 5–9, St. Petersburg Beach, FL.

Durham, D., and E.W. Rubel (1985) Afferent influences on brain stem auditory nuclei of the chicken: changes in succinate dehydrogenase activity following cochlea removal. J. Comp. Neurol. 231:446–456.

Evans, W.J., D.B. Webster, and J.K. Cullen, Jr. (1983) Auditory brainstem responses in neonatally sound deprived CBA/J mice. Hearing Res. 10:269–277.

Ehret, G. (1976) Development of absolute hearing thresholds in the house mouse (Mus musculus). J. Am. Audiol. Soc. 1:179–184.

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

Gray, L., and E.W Rubel (1981) Development of responsiveness to suprathreshold acoustic stimulation in chickens. J. Comp. Physiol. Psychol. 95:188–198.

Gray, L., and E.W Rubel (1984) Development of absolute thresholds in chickens. J. Acoust. Soc. Am. (in press).

Guillery, R.W. (1973) Quantitative studies of transneuronal atrophy in the dorsal lateral geniculate nucleus of cats and kittens. J. Comp. Neurol. 149:423–438.

Guillery, R.W. (1974) On structural changes that can be produced experimentally in the mammalian visual pathways. In R. Bellairs and E.G. Gray, (eds): Essays on the Nervous System. Oxford: Clarendon Press, pp. 299–326.

Hackett, J.T., H. Jackson, and E.W Rubel (1982) Synaptic excitation of the second and third order auditory neurons in the avian brain stem. Neuroscience 7:1455–1469.

Held, H. (1893) Die centrale Gehorleitung. Arch. Anat. Physiol., Anat. Abt. 201–248.

Heumann, D., and T. Rabinowicz (1980) Postnatal development of the dorsal lateral geniculate nucleus in the normal and enucleated albino mouse. Exp. Brain Res. 38:75–85.

Jackson, H., J.T. Hackett, and E.W Rubel (1982) Organization and development of brain stem auditory nuclei in the chick: Ontogeny of postsynoptic responses. J. Comp. Neurol. 210:80–86.

Jackson, H., J.T. Hackett, and E.W Rubel (1982) Organization and development of brain stem auditory nuclei in the chick: Ontogeny of postsynaptic responses. J. Comp. Neurol. 210:80–86.

Jackson, J.R.H., and E.W Rubel (1976) Rapid transneuronal degeneration following cochlea removal in chickens. Anat. Rec. 184:434–435.

Jackson, H., and E.W Rubel (1978) Ontogeny of behavioral responsiveness to sound in the chick embryo as indicated by electrical recordings of motility. J. Comp. Physiol. Psychol. 92:682–696.

Jhaveri, S., and D.K. Morest (1982a) Neuronal architecture in nucleus magnocellularis of the chicken auditory system with observations on nucleus laminaris: A light and electron microscope study. Neuroscience 7:809–836.

Jhaveri, S., and D.K. Morest (1982b) Sequential alterations of neuronal architecture in nucleus magnocellularis of the developing chicken: An electron microscope study. Neuroscience 7:855–870.

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. (1978) Development of the dorsal lateral geniculate nucleus in the cat. J. Comp. Neurol. 182:265–292.

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.

Kane, E.C. (1974) Patterns of degeneration in the caudal cochlear nucleus of the cat after cochlear ablation. Anat. Rec. 179:67–92.

Kerr, L.M., E.M. Ostapoff, and E.W Rubel (1979) Influence of acoustic experience in the ontogeny of frequency generalization gradients in the chicken. J. Exp. Psychol. 5:97–115.

Kelly, J.P., and W.M. Cowan (1972) Studies on the development of the chick optic tectum. III. Effects of early eye removal. Brain Res. 42:263–288.

Knowlton, V.Y. (1967) Correlation of the development of membranous and bony labyrinths, acoustic ganglia, nerves, and brain centers of the chick embryo. J. Morphol. 121:179–208.

Koerber, C., R. R. 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.

Konigsmark, B.W. (1970) Methods for the counting of neurons. In W.J.H. Nauta and S.O.E. Ebbesson (eds): Contemporary Research Methods in Neuroanatomy. New York: Springer-Verlag, pp. 315–340.

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.

Lillie, R.D., and H.M. Fullmer (1976) Histopathologic Technic and Practical Histochemistry. New York: McGraw-Hill.

Lippe, W., and E.W Rubel (1983) Development of the place principle: Tonotopic organization. Science 219:514–516.

Lippe, W.R., O. Steward, and E.W Rubel (1980) The effect of unilateral basilar papilla removal upon nuclei laminaris and magnocellularis of the chick examined with [³H]2-deoxy-D-glucose autoradiography. Brain Res. 196:43–58.

Loewy, A.D. (1972) The effects of dorsal root lesions on Clarke neurons in cats of different ages. J. Comp. Neurol. 145:141–164.

Mikaelian, D., and R.J. Ruben (1965) Development of hearing in the normal CBA-J mouse: Correlation of physiological observations with behavioral responses and with cochlear anatomy. Acta Otolaryngol. 59:451–461.

Nie, N.H., C.H. Hull, J.G. Jenkins, K. Steinbrenner, and D.H. Bent (1975) Statistical Package for the Social Sciences. New York: McGraw-Hill.

Nordeen, K.W., H.P. Killackey, and L.M. Kitzes (1983) Ascending projections to the inferior colliculus following unilateral cochlear ablation in the neonatal gerbil, Meriones unguiculatus. J. Comp. Neurol. 214:144–153.

Osen, K.K. (1969) The intrinsic organization of the cochlear nuclei in the cat. Acta Otolaryngol. 67:352–359.

Parks, T.N. (1979) Afferent influences on the development of the brain stem auditory nuclei of the chicken: Otocyst ablation. J. Comp. Neurol. 183:665–678.

Parks, T.N. (1981) Morphology of axosomatic endings in an avian cochlear nucleus: Nucleus magnocellularis of the chicken. J. Comp. Neurol. 203:425–440.

Parks, T.N., and E.W Rubel (1978) Organization and development of the brain stem auditory nuclei of the chicken: Primary afferent projections. J. Comp. Neurol. 180:439–448.

Peduzzi, J.D. and W.J. Crossland (1983) Anterograde transneuronal degeneration in the ectomamillary nucleus and ventral lateral geniculate nucleus of the chick. J. Comp. Neurol. 213:287–300.

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

Ramón y Cajal, S. (1909) The acoustic nerve: Its cochlear branch or cochlear nerve. Translated from: Histologie du Système Nerveux de l'Homme et des Vertébrés, Tome I. pp. 774–838, 1952. (National Technical Information Service Publication, 1971).

Rebillard, G., and E.W Rubel (1981) Electrophysiological study of the maturation of auditory responses from the inner ear of the chick. Brain Res. 229:15–23.

Rubel, E.W (1978) Ontogeny of structure and function in the vertebrate auditory system. In M. Jacobson (ed): Handbook of Sensory Physiology, Development of Sensory Systems. New York: Springer-Verlag. Vol. 9, pp. 135–237.

Rubel, E.W, D.E. Born, J.S. Deitch, and D. Durham (1984) Recent advances toward understanding auditory system development. In C. Berlin (ed): Recent Developments in Hearing Science. San Diego: College-Hill Press (in press).

Rubel, E.W, and T.N. Parks (1975) Organization and development of brain stem auditory nuclei of the chicken: Tonotopic organization of n. magnocellularis and n. laminaris. J. Comp. Neurol. 164:411–433.

Rubel, E.W, and M.H. Rosenthal (1975) The ontogeny of auditory frequency generalization in the chicken. J. Exp. Psychol. 1:287–297.

Rubel, E.W, and B.M. Ryals (1983) Development of the place principal: Acoustic trauma. Science 219:512–514.

Rubel, E.W, D.J. Smith, and L.C. Miller (1976) Organization and development of brain stem auditory nuclei of the chicken: Ontogeny of n. magnocellularis and n. laminaris. J. Comp. Neurol. 166:469–490.

Rubel, E.W, Z.D.J. Smith, and O. Steward (1981) Sprouting in the avian brainstem auditory pathway: Dependence on dendritic integrity. J. Comp. Neurol. 202:397–414.

Ryan, A.F., N.K. Woolf, and F.R. Sharp (1982) Functional ontogeny in the central auditory pathway of the mongolian gerbil: A 2-deoxyglucose study. Exp. Brain Res. 47:428–436.

Saunders, J.C., R.B. Coles, and G.R. Gates (1973) The development of auditory responses in the cochlea and cochlear nuclei of the chick. Brain Res. 63:59–74.

Shnerson, A., and J.F. Willott (1980) Ontogeny of the acoustic startle response in C57BL/6J mouse pups. J. Comp. Physiol. Psychol. 94:36–40.

Smith, D.E. (1969) Observations on the postnatal development of Clarke's nucleus in the kitten. J. Comp. Neurol. 135:263–274.

Smith, D.E. (1973) The location of neurofilaments and microtubules during the postnatal development of Clarke's nucleus in the kitten. Brain Res. 55:41–53.

Smith, D.E. (1977) The effect of deafferentation on the development of brain and spinal nuclei. Prog. Neurobiol. 8:349–367.

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

Trumpy, J.H. (1971) Transneuronal degeneration in the pontine nuclei of the cat: Part I. Neuronal changes in animals of varying ages. Ergebn. Anat. Entwick. 44:7–70.

Trune, D.R. (1982a) 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.

Trune, D.R. (1982b) Influence of neonatal cochlear removal on the development of mouse cochlear nucleus: II. Dendritic morphometry of its neurons. J. Comp. Neurol. 209:425–434.

Tsang, Y. (1937) Visual centers in blinded rats. J. Comp. Neurol. 66:211–261.

Webster, D.B. (1983) Auditory neuronal sizes after a unilateral conductive hearing loss. Exp. Neurol. 79:130–140.

Webster, D.B., and M. Webster (1978) Long-term effects of cochlear nerve destruction on the cochlear nuclei. Anat. Rec. 190:578–579.

Webster, D.B., and M. Webster (1979) Effects of neonatal conductive hearing loss on brain stem auditory nuclei. Ann. Otol. Rhinol. Laryngol. 88:684–688.

Webster D.B., and M. Webster (1980) Mouse brainstem auditory nuclei development. Ann. Otol. Rhinol. Laryngol. 89 (Suppl. 68):254–256.