# Afferent Influences on Brain Stem Auditory Nuclei of the Chicken: Changes in Succinate Dehydrogenase Activity Following Cochlea Removal

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## ABSTRACT

We have examined one of the metabolic consequences of unilateral cochlea (basilar papilla) removal in the chick brain stem auditory system. We assessed changes in succinate dehydrogenase (SDH), a mitochondrial enzyme involved in energy metabolism, in neurons of second-order n. magnocellularis (NM) and third-order n. laminaris (NL). Chickens undergoing surgery at 10 days of age were perfused 4 hours to 35 days postlesion. Chickens 6 or 66 weeks of age at cochlea removal were examined 1 or 8 days after surgery. In all groups, cryostat sections were prepared for SDH histochemistry or Nissl staining.

In normal chickens, NM cell bodies and NL neuropil contain SDH reaction product. In young birds, the density of SDH reaction product in NM shows a rapid biphasic response to cochlea removal. From 8 to 60 hours postlesion, density *increases* ipsilateral to cochlea removal; for survival times of 3–35 days, SDH density *decreases* in ipsilateral NM. In NL, no changes were observed until 3 days after cochlea removal. Then we observed a long-lasting decrease in density of SDH reaction product in the neuropil regions receiving input from the deafferented NM. All of these changes are age-dependent in that they were observed only following cochlea removal on or before 6 weeks of age.

Key words: metabolism, histochemistry, deafferentation, microdensitometry

The importance of intact, functional afferents for the maintenance and development of central nervous system (CNS) neurons has been well established (see Born and Rubel, '85a; and reviews by Cowan, '70; Guillery, '74; Globus, '75; and Smith, '77). The most frequently reported postsynaptic changes following afferent manipulations involve anatomical or physiological characteristics. Cell loss or cell body shrinkage (e.g., Guillery, '73; Kalil, '80; Levi-Montalcini, '49; Powell and Erulkar, '62; Trune, '82a), rearrangement of dendritic morphology (Harris and Woolsey, '81; Deitch and Rubel, '84), alterations in cytoarchitectonics or connectivity (Woolsey and Wann, '76; Kelly and Cowan,'72; Hubel et al, '77; Rakic, '77), sprouting (Cotman and Nadler, '78), and changes in physiological organization (Shatz and Stryker, '78; LeVay et al., '80, Kaas et al., '83) all have been investigated intensely. Biochemical responses to afferent manipulation have been less well studied although they must underlie these structural and functional

changes. The ability to measure easily macroscopic or microscopic levels of metabolic intermediates (e.g., Nachlas et al., '57; Wong-Riley, '79; Lowry and Passonneau, '72), glucose metabolism (Sokoloff et al., '77), and protein metabolism (Droz and LeBlond, '63) has yielded some clues as to the general metabolic consequences of afferent manipulations (Walker et al., '75). Further investigation of changes in both specific proteins and general metabolic intermediates will yield a greater understanding of the cellular chain of events responsible for afferent regulation of neuronal activity and integrity.

We have been examining the effects of afferent manipulations on the chick auditory system, specifically second-

and third-order n. magnocellularis (NM) and n. laminaris (NL). The major excitatory input to various levels of the system can be manipulated at both embryonic and post-hatch ages (e.g., Levi-Montalcini, '49; Parks, '79; Benes et al., '77; Jackson and Rubel, '76; Parks and Rubel, '78). Some of the postsynaptic morphological (Jackson and Rubel, '76; Parks and Rubel, '78; Parks, '79, '81; Rubel et al., '81; Born and Rubel, '85a) and metabolic (Lippe et al., '80; Durham, et al. '85; Steward and Rubel, '85) consequences of one such manipulation, that of basilar papilla (cochlea) removal, have been described.

This paper presents evidence for changes in another aspect of the postsynaptic response to cochlea removal, changes in the levels of oxidative intermediates. Succinate dehydrogenase (SDH) is a mitochondrial enzyme considered to be one of many control points in oxidative glucose metabolism (Lowry, '75). Thus, levels of SDH within nervous tissue should reflect overall metabolic activity (Padykula, '52; Friede, '66; Carroll and Wong-Riley, '84; Marshall et al., '81). We present a description of both the normal distribution of SDH in chick brain stem and rapid changes that occur following cochlea removal.

## MATERIALS AND METHODS
### Animal preparation

Ross Arbor Acres chickens (Heatwole Hatcheries, Harrisonburg, Virginia) either 10 days, or 6 weeks, or 66 weeks of age were the subjects of these experiments. Chickens in the two younger age groups were obtained by incubating and hatching eggs in a forced draft incubator and were housed in a communal brooder with ad libitum water and feed after hatching. Sixty-six-week-old birds were obtained from a commercial supplier at 65 weeks of age and housed singly before and after surgery.

The basilar papilla (cochlea) was removed unilaterally under ketamine (80 mg/kg i.m.) and Chloropent (1.5 ml/kg i.p.) anesthesia as described in the preceding paper (Born and Rubel, '85a). Briefly, the right tympanic membrane and columella were removed and the cochlea pulled out through the oval window with jeweler's forceps. Cochlea removal severs the peripheral processes of eighth nerve ganglion cells but leaves their cell bodies intact. The cochlea was floated out in water and examined under a dissecting microscope to evaluate the completeness of the operation. The ear cavity was packed with Gelfoam and the wound sutured.

Ten-day-old birds were perfused at each of the following times after cochlea removal: 4, 8, 12, 24, or 36 hours and 2, 3, 4, 8, 15, or 35 days (three to seven subjects at each time). Six- or 66-week-old birds were perfused either 1 or 8 days after surgery (seven to nine subjects in each group). All birds were deeply anesthetized with ketamine and Chloropent and perfused transcardially with 10% glycerin in 0.5% formaldehyde. Brains were removed quickly from the heads, blocked, and frozen in heptane cooled with liquid nitrogen. Brains not sectioned immediately were stored at $-80°C$.

### Tissue preparation

Brain stem blocks were sectioned in the coronal plane at 30 $\mu$m in a cryostat at $-15°C$. Two alternate series of sections were thaw-mounted onto chrome-alum subbed slides. One series was stained with 0.12% thionin, dehydrated in graded alcohols, and coverslipped with DPX. The other series was processed for SDH histochemistry (Nachlas et al., '57; Killackey and Belford, '79). Slides bearing sec-

tions for SDH were dried at least 30 minutes on a 37°C hotplate before the stain solution, consisting of 0.55 mM Nitroblue tetrazolium and 0.05 M sodium succinate in 0.05 M phosphate buffer (pH 7.2), was puddled onto the sections. After a 30–90 minute incubation, slides were transferred in slide racks to 10% formalin in 0.1 M phosphate buffer (pH 7.6) for at least 12 hours. Sections then were rapidly dehydrated in graded alcohols and coverslipped with DPX.

### Data analysis

Nissl- and SDH-stained sections were examined under the microscope with brightfield illumination. Initially, intra-animal comparisons of the appearance of Nissl substance and the density of SDH reaction product were made between "control" and "affected" portions of NM and NL. In NM, neurons ipsilateral and contralateral to the side of the cochlea removal were compared, with the neurons contralateral to the surgery serving as the "control." In NL, dendrites are segregated into domains dorsal and ventral to the cell body. SDH comparisons were made between dorsal and ventral NL neuropil regions on both sides of the brain. The ventral ipsilateral neuropil and dorsal contralateral neuropil, which receive input from the unaffected NM, served as the "control." In both NM and NL, the changes observed were analyzed as a function of the age at the time of surgery.

Changes in the density of SDH reaction product in NM and NL were quantified using a Leitz Ergolux microdensitometer. At least two brains in which sections were well stained and the plane of section was optimal were chosen for analysis from each survival time and age group. In each brain, a section from each side of the brain located approximately 65% of the distance from the most anterior to the most posterior limit of NM was examined. The optical density of reaction product in NM was estimated by averaging the optical densities of 20 individual NM neurons. Measurements were made with a ×40 dry objective with the measuring aperture positioned over the cytoplasm of the cells, avoiding the unstained cell nucleus. The cells were located in the center of the medial to lateral extent of the nucleus and were chosen at random. To verify that this method would yield an accurate estimate of the average density of the nucleus, we remeasured every NM neuron in the chosen sections from six representative brains and obtained averages within one standard deviation of those calculated from measurement of only 20 neurons. To correct for possible differences in overall staining density among different sections or sides of the brain, the density of an unaffected structure, the medial vestibular nucleus, was measured in each NM section.[1] A "corrected" average NM density then was calculated by dividing the average density in NM by the density in the vestibular nucleus. The data from each animal were expressed as the ratio of corrected average density in NM ipsilateral to cochlea removal to that in NM contralateral to cochlea removal. Ratios were averaged for

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[1]Although it could be argued that the medial vestibular nucleus might be affected by the manipulation, its proximity to NM as well as its uniform and relatively high density made it an optimal choice for use as a standard. All sections were examined specifically for evidence of changes in the density of SDH reaction product in medial vestibular nucleus as a function of cochlea removal and none were found. In addition, material prepared for degeneration from animals sustaining cochlea removal also was examined (Parks and Rubel, '78; Rubel et al., '81). While both NM and NL show degeneration, none was observed in the medial vestibular nucleus.

all birds within a given treatment group and plotted as a function of survival time.

In NL, sections located approximately in 65% of the anterior to posterior extent of the nucleus were examined on both sides of the brain. The density of reaction product in dorsal and ventral neuropil regions was estimated from a single measurement in the selected sections. The ratio of density in the dorsal neuropil to that in ventral neuropil was calculated for sides ipsilateral and contralateral to cochlea removal. Average ratios from several animals were plotted as a function of survival time for each age group.

## RESULTS
### Normal brain stem staining pattern

Figure 1 shows two adjacent sections stained to reveal Nissl substance or SDH in the normal chick brain stem. The SDH histochemical stain is often used to demonstrate the pattern of nerve terminals (e.g., Killackey et al., '76; Durham and Woolsey, '84). As can be seen in Figure 1b and d, predominantly neuropil is stained, although some cell somata also contain reaction product. NM cell bodies are among those that contain reaction product (Fig. 1d). At

higher magnification (not shown), the reaction product appears evenly distributed within the cytoplasm and the cell nucleus is unstained. All neurons in NM appear to be stained to a similar degree, suggesting that they are a homogeneous population with respect to SDH activity.

Unlike the results in NM, a comparison between sections stained for Nissl substance and SDH shows very different staining patterns in NL (Fig 1c,d). In NL, cell somata do not contain SDH reaction product. Rather, two densely stained bands that correspond to the dorsal and ventral neuropil regions can be seen separated by an unstained region. This unstained region corresponds to the region of cell somata. The neuropil staining pattern may be attributable to reaction product in presynaptic NM axons, postsynaptic NL, dendrites or both. The zone of glial cell bodies that surrounds NL is largely unstained by SDH histochemistry.

### Effects of cochlea removal in NM

In 10-day- or 6-week-old birds, cochlea removal produces a rapid, biphasic change in the density of the SDH histochemical reaction product in NM neurons (Fig. 2). Four
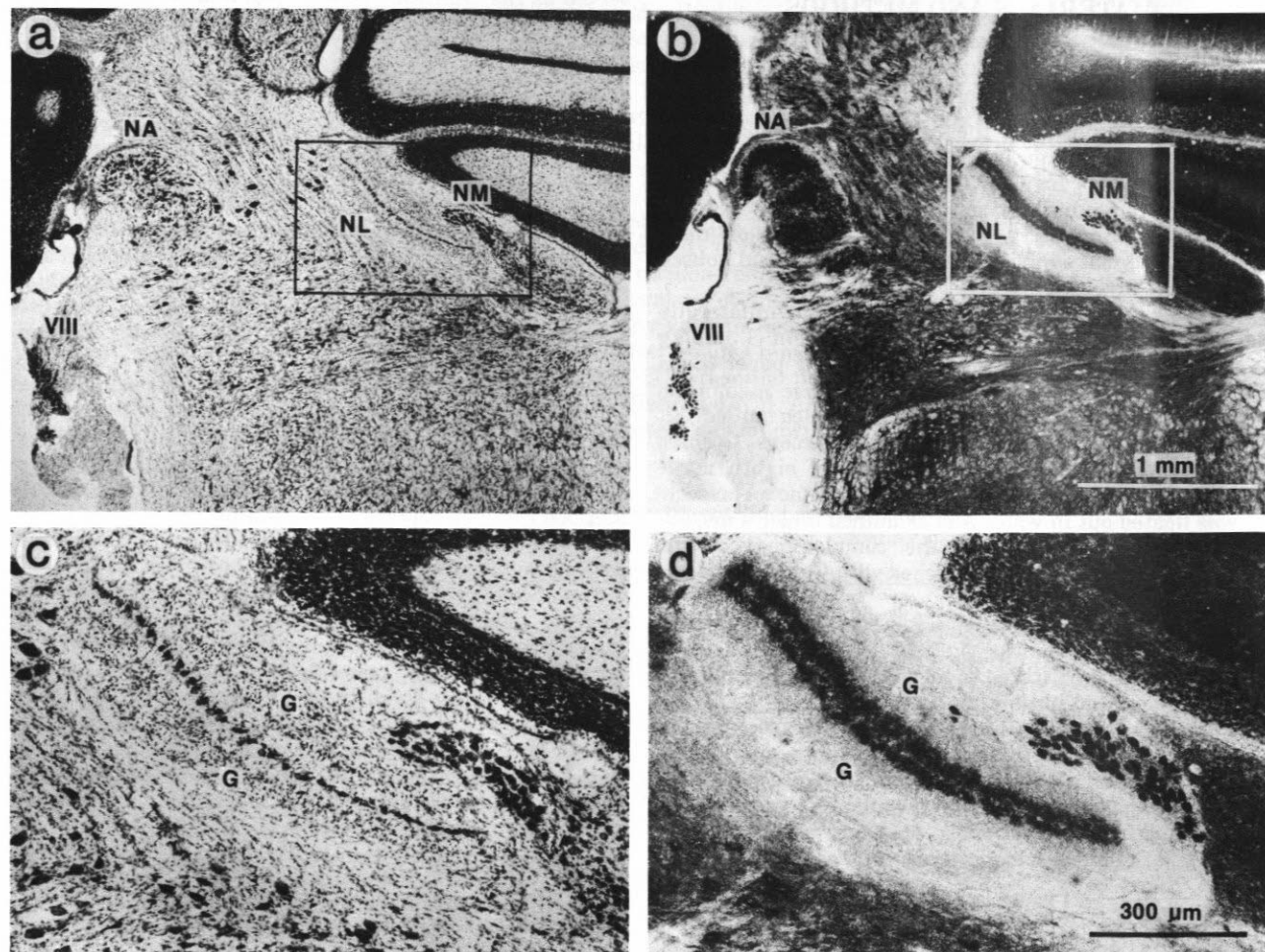


Fig. 1. Photomicrographs of two adjacent 30 μm coronal sections through the chick brain stem auditory nuclei. Section shown in a and c is stained to reveal Nissl substance, and that in b and d is stained with SDH histochemistry. In a and b, second- (NA, NM) and third- (NL) order auditory neurons are visible. Areas enclosed by boxes in each view are shown at higher magnification below (c,d). In c and d, note the similarity between Nissl and SDH staining in NM (cell somata), while in NL, cell bodies are stained with thionin and neuropil by the SDH method. Scale bar for a and b is at right in panel b, for c and d at right in panel d. G, glial zone; NA, n. angularis; NL, n. laminaris; NM, n. magnocellularis; VIII, eighth nerve.
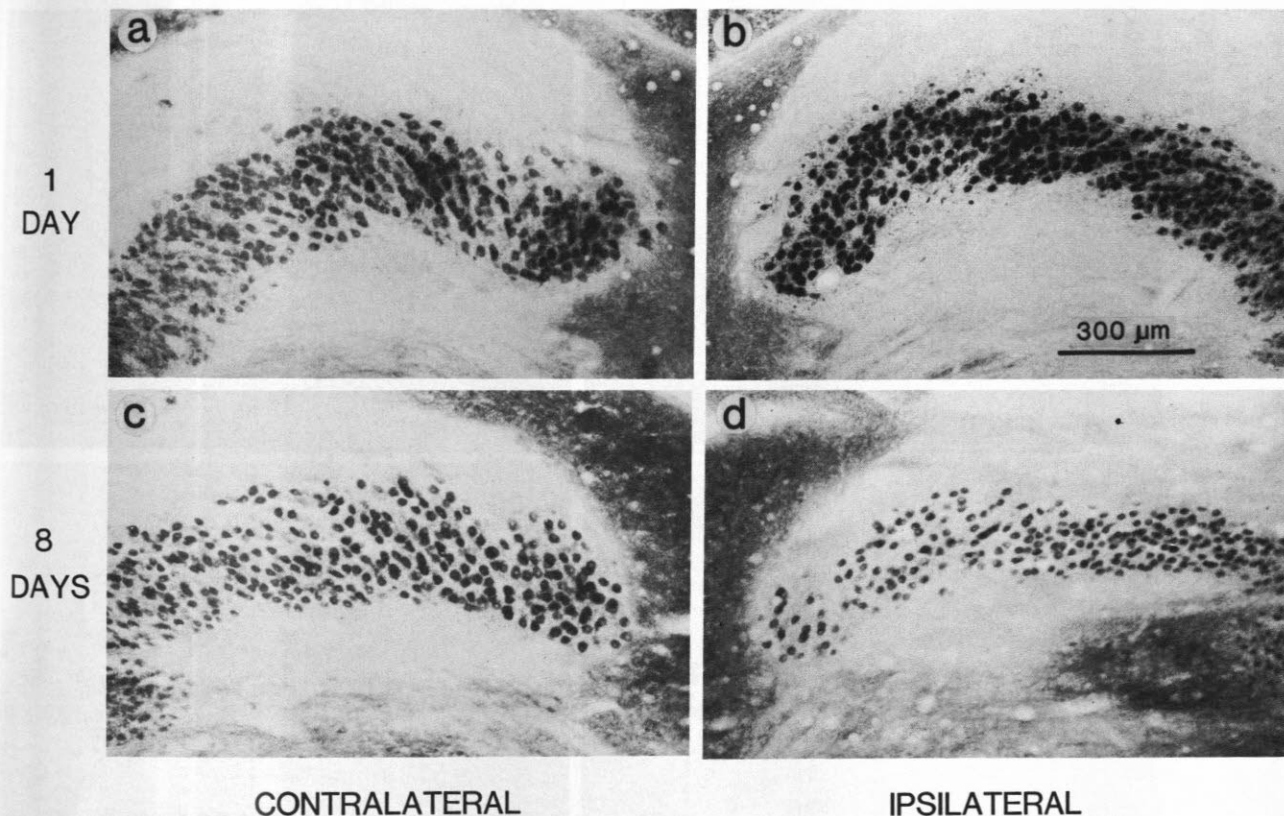
Fig. 2. Photomicrographs of 30 μm coronal sections through NM stained for SDH from a chicken sustaining cochlea removal at 10 days of age. Sections are located in 65% of the anterior to posterior extent of NM. a, b: One-day survival. c, d: Eight-day survival. a and c are from the control side, contralateral to cochlea removal, b and d from the ipsilateral "affected" side. In these and subsequent photomicrographs from SDH-stained tissue, sections from a given survival time are from the same animal. Photomicrographs from each animal are printed so that the density of the reaction product in the medial vestibular nucleus is identical. Scale for all panels is shown in b; medial is toward the middle of the figure and dorsal toward the top. In NM ipsilateral to the lesion, note the darker SDH reaction product 1 day after the lesion but lighter density with longer survivals.

hours after cochlea removal, a comparison of neurons in NM ipsilateral and contralateral to the lesion reveals few differences. However, in animals perfused 8–60 hours after surgery, NM neurons ipsilateral to cochlea removal show a dramatic *increase* in the density of cytoplasmic SDH reaction product compared to neurons on the opposite side of the brain. As is evident in Figure 2a and b, this increase occurs in virtually all of the neurons (verified with quantitative densitometry as described below). In animals perfused 3 or more days after cochlea removal, the opposite result is found; NM neurons ipsilateral to surgery display *decreased* density of SDH reaction product compared to the contralateral NM (Fig. 2c,d). As with shorter survival intervals, this change was observed in all NM cells and persists at least up to 35 days after surgery. There also appear to be fewer neurons on the ipsilateral side, as would be expected on the basis of cell counts in Nissl material (Jackson and Rubel, '76; Born and Rubel, '85a).

We obtained strikingly different results when we removed one cochlea in 66-week-old chickens. NM neurons ipsilateral and contralateral to the lesion are virtually identical when examined 1 (Fig. 3a,b) or 8 (Fig. 3c,d) days after cochlea removal. There also are no *apparent* changes in cell number (but see Fig. 4 in Born and Rubel, '85a).

We examined thionin-stained sections from the brains described above for changes in the density of Nissl substance following cochlea removal. In young birds, beginning 12 hours after the lesion, ipsilateral NM neurons displayed less dense Nissl stain as compared to NM neurons contralateral to the site of cochlea removal. By 6 days following the lesion this difference is less pronounced, but it persists in birds examined after a 35-day survival time. "Ghost" neurons (neurons containing little or no stained Nissl substance, see Born and Rubel, '85a) also were observed in NM ipsilateral to the lesion in birds perfused 12 hours to 4 days after surgery.

In chickens with cochlea removal at 10 days of age, the difference in appearance between Nissl- and SDH-stained alternate sections often is quite dramatic. When stained for Nissl substance, NM neurons can be divided into two populations: those that apparently contain Nissl substance (albeit in reduced amounts compared to controls) and those which do not ("ghost" neurons). When examined with the SDH stain, however, the NM neurons appeared as a homogeneous population, in which all neurons showed increased SDH reaction product (e.g., Fig. 2). Quantitative densitometry confirms this impression. Figure 4 shows data from two alternate sections from a young bird perfused 24 hours after cochlea removal. Based on measurements of density of Nissl substance, two populations of neurons are evident on the side of the brain ipsilateral to cochlea removal. The mean density of the larger of these groups is significantly lower than that for neurons on the contralateral control side ($t_{137} = 14.32$; P < .002). In contrast, only one popula-
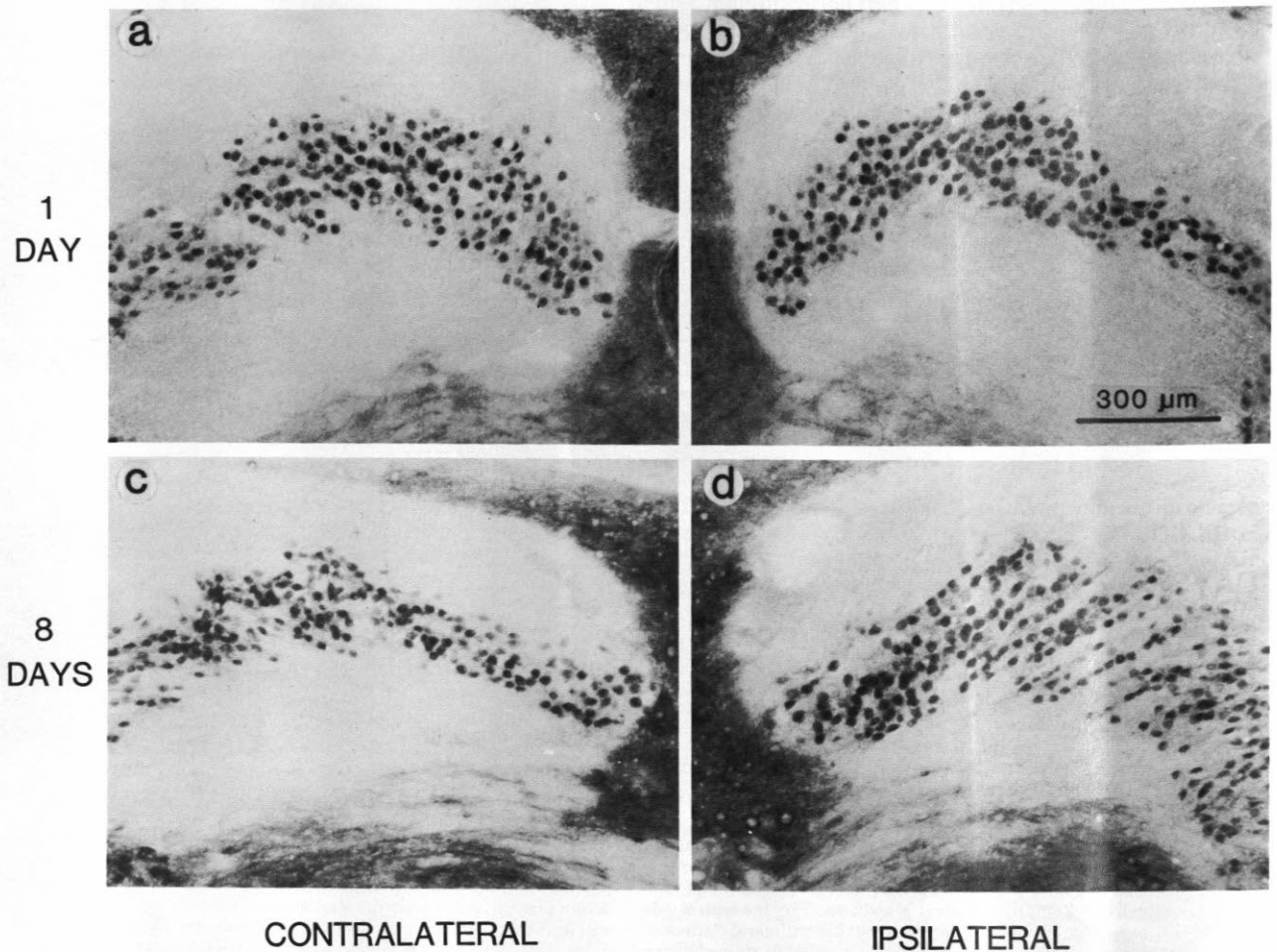
Fig. 3. Photomicrographs of 30 $\mu$m coronal sections through NM stained for SDH from chickens sustaining cochlea removal at 66 weeks of age. Birds were perfused 1 day (a,b) or 8 days (c,d) after the lesion. All other details as in Figure 2. Note that the density of SDH reaction product is similar in "control" and "affected" sides of the brain for both survival times.

tion of neurons was observed on either side of the brain in SDH sections, with the mean density of reaction product significantly higher on the ipsilateral side ($t_{163} = 16.52$; $P < .002$).

Again, in contrast to hatchlings, chickens 66 weeks old at the time of cochlea removal show only a very slight and inconsistent decrease in Nissl staining density at any of the survival times examined, and no ghost neurons were observed. These Nissl results in young and old chickens are entirely consistent with those reported in the preceding paper (Born and Rubel, '85a) and confirm that neither the decrease in Nissl staining density nor the presence of "ghost" neurons is dependent on methods of tissue fixation or processing.

Changes in SDH staining density in NM were quantified using a microdensitometer. Our initial observations suggested that on both control and experimental sides of the brain all neurons were stained to a similar degree. This observation was verified in those cases in which all neurons in one section through NM were measured; cell densities appear normally distributed on both sides of the brain. Figure 5 summarizes the results of microdensitometric measurements of NM neurons from birds sustaining cochlea removal at 10 days, 6 weeks, or 66 weeks of age. For the two younger age groups, there is a rapid *increase* in the density of SDH reaction product followed by a decrease in density among NM neurons ipsilateral to cochlea removal. The decrease in density observed more than 5 days after the lesion is consistent and lasts for at least 35 days after surgery. Measurements of NM density in 66-week-old birds revealed little change following cochlea removal.

### Effects of cochlea removal in NL

Changes in the density of SDH reaction product occur less rapidly in the third-order neurons of NL than in NM. Figure 6 depicts the qualitative observations from birds sustaining lesions at 10 days or 6 weeks (Fig. 6a,b) or 66 weeks of age (Fig. 6c,d). In the younger birds, cochlea removal results in a monophasic decrease in SDH density in the dorsal neuropil ipsilateral and the ventral neuropil contralateral to the lesion. These changes first were observed at 3 days postlesion ipsilaterally and occasionally at 3, but reliably at 4 days postlesion contralaterally. The neuropil regions that exhibited these decreases are those
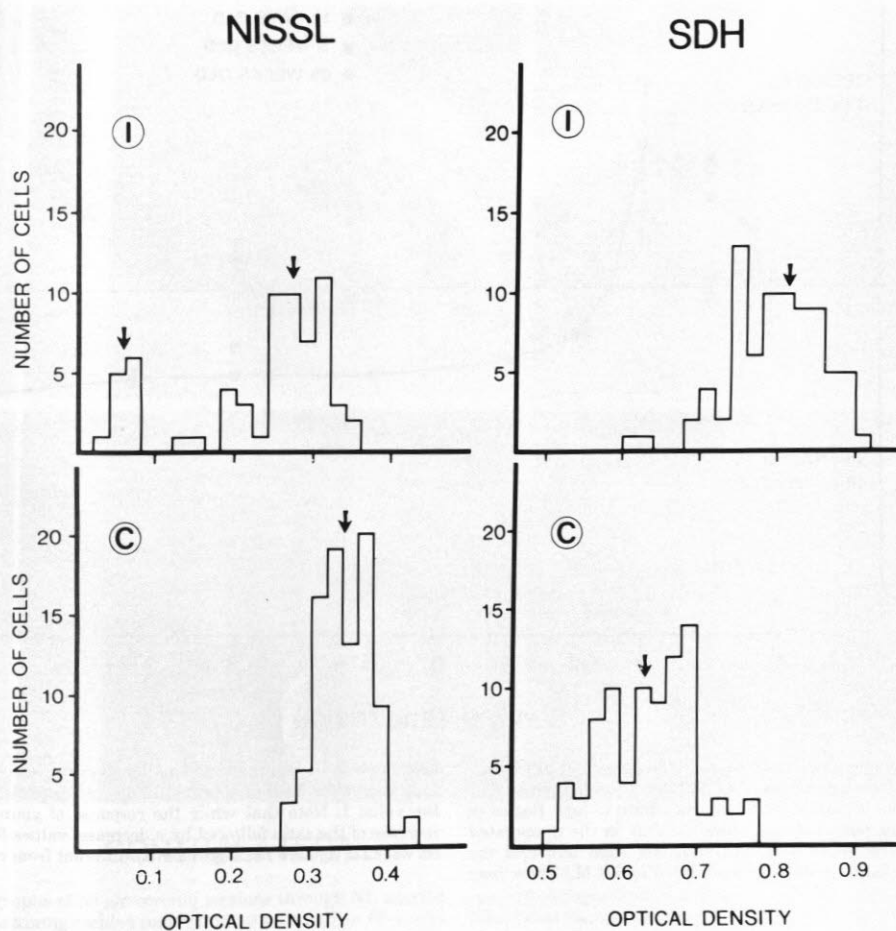
Fig. 4. A comparison of SDH and Nissl content of NM neurons 24 hours after cochlea removal in 10-day-old chickens. Shown are histograms of optical density (OD) measurements of all NM neurons in a section stained for Nissl (left) or SDH (right). Top graphs are for the side ipsilateral to cochlea removal (I), bottom for the contralateral control side (C). Arrows denote mean density for each population of neurons. Note that on the ipsilateral side, NM neurons fall into two populations on the basis of their Nissl substance but a single population with respect to SDH levels. When compared to the contralateral control side, the average SDH OD is higher and the Nissl ODs lower than control.

that receive their major excitatory input from those NM cells deafferented by cochlea removal. At the light microscopic level, it is not possible to determine whether the changes are confined to NM axons, NL dendrites, or occurred in both.

In contrast, chickens undergoing cochlea removal at 66 weeks of age showed no qualitative differences in dorsal and ventral neuropil staining densities at any survival time examined (Fig. 6c,d). These results in NL are consistent with the failure to observe any changes in NM.

The time course and magnitude of the NL density changes as determined by microdensitometry are shown in Figure 7. In young birds, note the longer latency of change in NL as compared to NM and the symmetry of ipsilateral and contralateral responses. No differences in NL density were revealed by microdensitometric measurements in adult birds.

## DISCUSSION

We have described rapid and long-lasting alterations in the distribution of the mitochondrial enzyme SDH in second- and third-order auditory neurons in the chick brain stem following cochlea removal. We observed a very rapid and surprising increase, followed by a consistent and long-lasting decrease in the density of SDH reaction product in NM neuronal somata. In NL, neuropil regions receiving input from the "affected" NM neurons showed only a persistent decrease in density of SDH reaction product, beginning 3 days after the lesion. Changes were observed only in birds sustaining cochlea removal on or before 6 weeks posthatch; auditory neurons in 66-week-old chickens were seemingly immune to the effects of cochlea removal. In the discussion below, we will consider first some of the methodological constraints of our histochemical stain, including its relationship to other measures of neuronal metabolism. We then will relate the salient features of the SDH response to the broader question of the means by which afferents influence their targets.

### Interpretation of SDH histochemistry

Use of a light microscopic histochemical stain for SDH places some constraints on interpretation of the results. At the light level, it is not possible to determine the ultrastructural compartment in which the SDH reaction product is located, e.g., in axonal or dendritic processes, or the subcellular compartment in which changes occur in response to
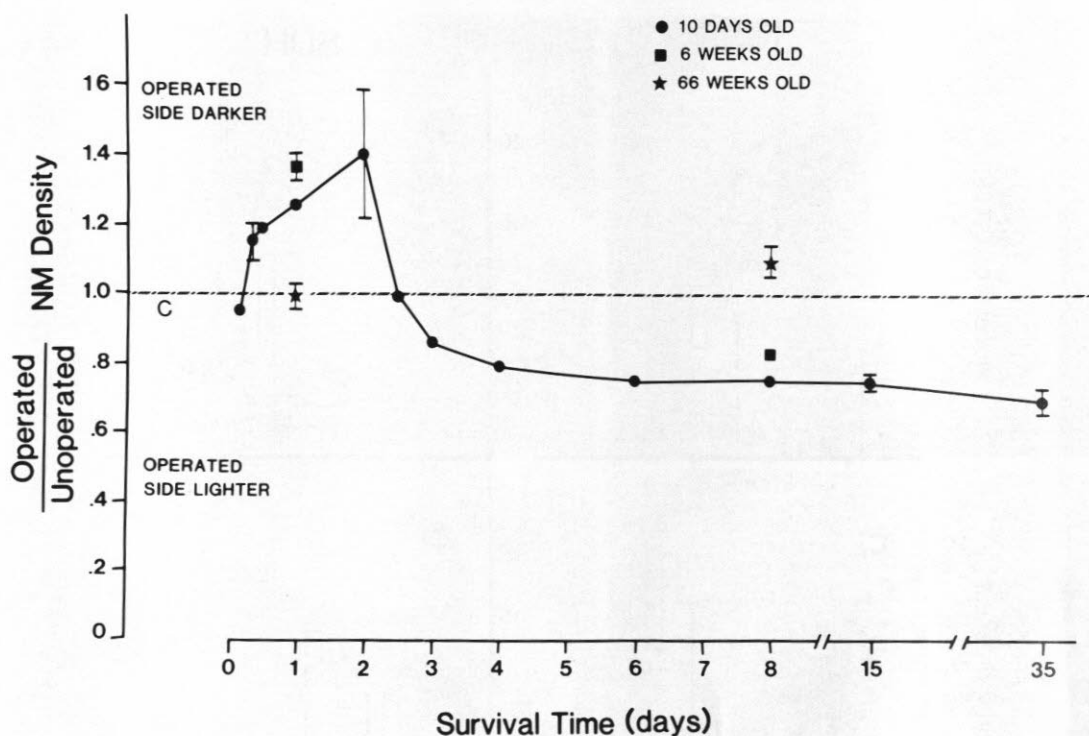
Fig. 5. Graph showing quantitative changes in the density of SDH reaction product in NM as a function of time after unilateral cochlea removal at 10 days (circles), 6 weeks (squares), or 66 weeks (stars) of age. Ratios of mean SDH density on the operated (ipsilateral) to that on the unoperated (contralateral) sides of the brain were calculated for each bird and the averages for two to five birds in each group plotted (±S.E.M.) Error bars are not shown when they are smaller than the size of the symbol. C at left indicates ratios from unoperated 10-day-old controls. The dotted line shows the value 1. Note that while the response of young birds shows a rapid increase of the ratio followed by a decrease, values for birds operated on at 66 weeks of age are not significantly different from control.

cochlea removal. Recent investigations with the metabolic marker cytochrome oxidase, for which the histochemical reaction product is electron-dense, suggest that in some systems dendritic processes contain a high proportion (but not all) of the reactive mitochondria (Kageyama and Wong-Riley, '82; Carroll and Wong-Riley, '84, Wong-Riley and Carroll, '84). Investigation of cytochrome oxidase changes in chick brain stem might prove useful.

A number of cellular mechanisms might account for the changes in density of SDH reaction product observed with the histochemical stain. Changes could reflect alterations in the number of mitochondria, the amount of SDH per mitochondrion, the activity of SDH, or some combination of these possibilities. In addition, increases in the density of reaction product could be due to an increased availability of SDH to the stain solution which might occur with a general increase in cell membrane permeability. It is not possible to distinguish between these speculations at the present time. One way in which some of these issues could be addressed would be the application of quantitative microhistochemical assays (Lowry and Passonneau, '72; Godfrey and Matschinsky, '76, '83). By systematically examining a number of related enzymes, one could hope to eliminate or confirm some of the possible explanations. The histochemical data presented here provide information with which to guide the application of more difficult and time-consuming biochemical techniques.

By examining changes in the density of histochemical SDH reaction product, one would like to add more details to our

knowledge of how afferent manipulations alter target cell metabolism. In this regard, it would be useful to relate changes in SDH histochemistry to other measures of neuronal metabolism such as glucose utilization measured with 2-deoxyglucose (2-DG) autoradiography (Sokoloff et al., '77), amino acid incorporation measured with $^3H$ leucine uptake (Droz and LeBlond, '63), or changes in RNA levels measured by examining alterations in Nissl staining. Levels of oxidative enzymes such as SDH or cytochrome oxidase are thought to reflect the general level of metabolic activity within nervous tissue (Padykula, '52; Pope et al., '56; Friede, '66; Lowry, '75; Walker et al., '75; Wong-Riley et al., '78, '81; Marshall et al., '81; Carroll and Wong-Riley, '84; Wong-Riley and Carroll, '84) and might be expected to change in the same manner as glucose utilization or overall protein synthesis. However, the bulk of the evidence suggests that SDH histochemistry is measuring some aspect of cellular metabolism different from that shown with 2-DG autoradiography, amino acid incorporation, or the pattern of Nissl staining. For example, in the chick brain stem, SDH density rises initially after cochlea removal, while 2-DG labeling and $^3H$ leucine incorporation fall (Lippe et al., '80; Steward and Rubel, '85). Also, neither of the latter measures demonstrated a biphasic response of NM to cochlea removal. In addition, as seen in Figure 4, measurements of SDH staining density following cochlea removal in young birds indicate that *all* NM neurons show increases or decreases in levels of reaction product. This homogeneity is in marked contrast to short-term changes in Nissl staining (Born and Rubel, '85a) or amino acid uptake

10
DAYS

66
WEEKS

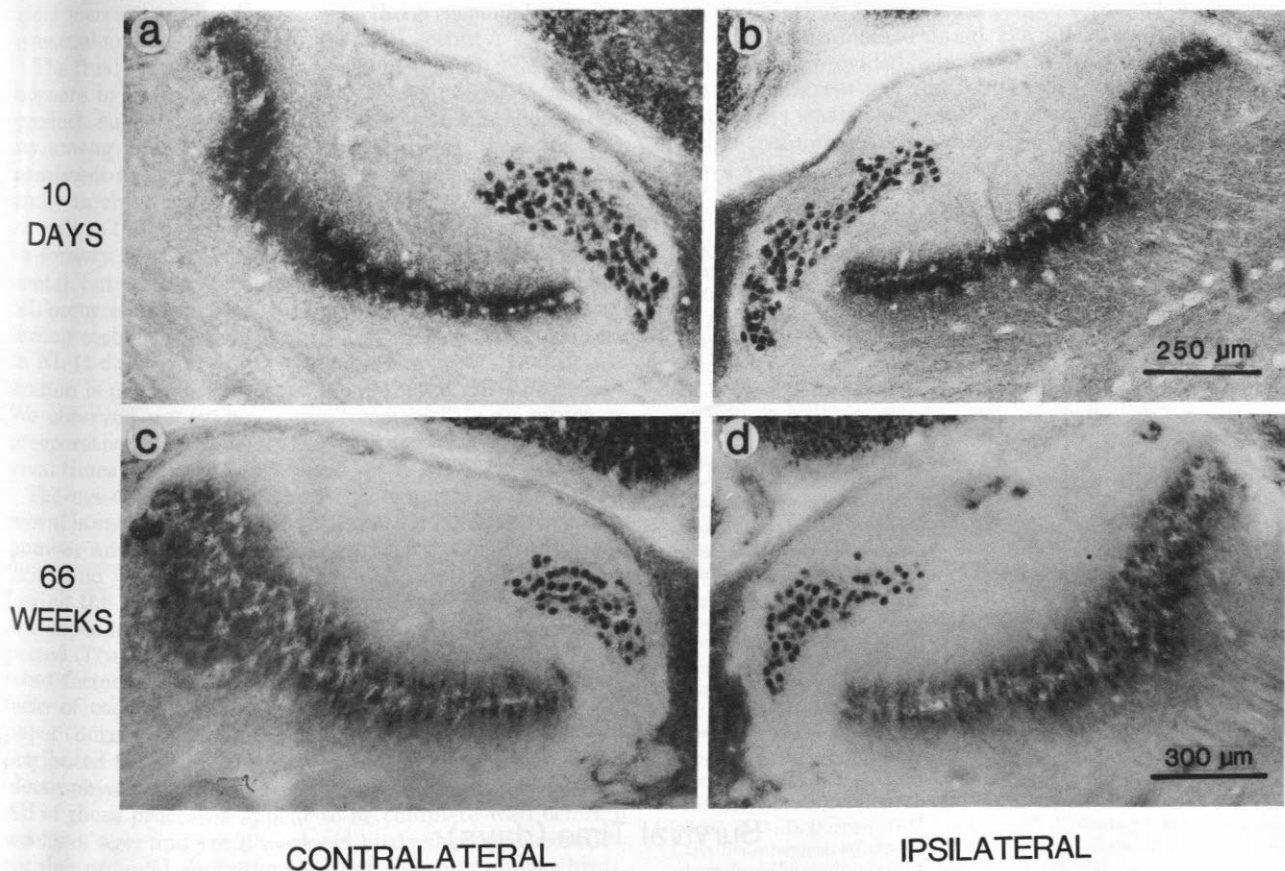CONTRALATERAL                    IPSILATERAL

Fig. 6. Photomicrographs of 30 μm coronal sections through NL stained for SDH from birds sustaining cochlea removal at 10 days (a,b) or 66 weeks (c,d) of age. Scale bars are shown at the right for each age group; all other conventions as in Figure 2. Note the differences in staining density between dorsal and ventral neuropil in young birds. No differences can be seen in old birds.

and incorporation (Steward and Rubel, '85), in which only a subpopulation of NM neurons (30%) showed dramatic decreases in Nissl substance or labeled proteins. Finally, in NM and NL the ultimate decrease in SDH reaction product density that we observed several days after cochlea removal is similar to decreases seen in 2-DG labeling. However, the SDH time course is much slower. A similar delay in the response of cytochrome oxidase or SDH histochemistry compared to 2-DG autoradiography has been suggested in other systems (Marshall et al., '81; Kozlowski, et al., '80; Durham and Woolsey, '78; Wong-Riley and Welt, '80; Dietrich et al., '81, '82) but has not yet been tested directly. The meaning of these dissociations and the exact relationship between SDH histochemistry and cell metabolism is still unknown.

### Afferent influences

Within the limitations inherent in the technique, several aspects of the SDH histochemical data reported here provide insight into the nature of the metabolic and trophic relationships between presynaptic and postsynaptic elements of the central nervous system. These aspects include (1) the initial rapid increase in density of SDH reaction product in NM (and the lack of such a change in NL); (2) long-term SDH changes in NM and NL; and (3) the age-dependence of the SDH changes.

The initial SDH change in NM in response to cochlea removal was unexpected, both for its rapidity (4–8 hours after the lesion) and its direction (an increase). Two other aspects of NM biochemistry, glucose utilization (Lippe et al., '80) and amino acid uptake and incorporation (Steward and Rubel, '84), have been shown to change within 8 hours after cochlea removal. In other sensory systems, changes in levels of metabolic enzymes following afferent manipulations have been reported only after relatively long survival times (1 week–6 months) in directly affected neurons (Kupfer, '63; Kupfer and Palmer, '64; Wong-Riley, '79) as well as at sites several synapses removed from the location of the lesion (Wong-Riley et al., '78, '81; Wong-Riley and Welt, '80; Dietrich et al., '81, '82; Wong-Riley and Riley, '83; Durham and Woolsey, '84). Although cochlea removal deprives NM of its major source of excitatory afferent input, the initial SDH changes suggest a rapid, transient *increase* in some aspect of metabolic activity in NM neurons. The majority of studies employing similar manipulations in other sensory systems report decreases in levels of metabolic enzymes (Kupfer, '63; Kupfer and Palmer, '64; Wong-Riley et al., '78; Wong-Riley, '79; Wong-Riley and Riley, '83). However, Kupfer and Downer ('67) reported transient increases in levels of RNA 24–48 hours after denervation of the lateral geniculate nucleus in adult monkeys. It is possible that similar transient increases in metabolic intermediates
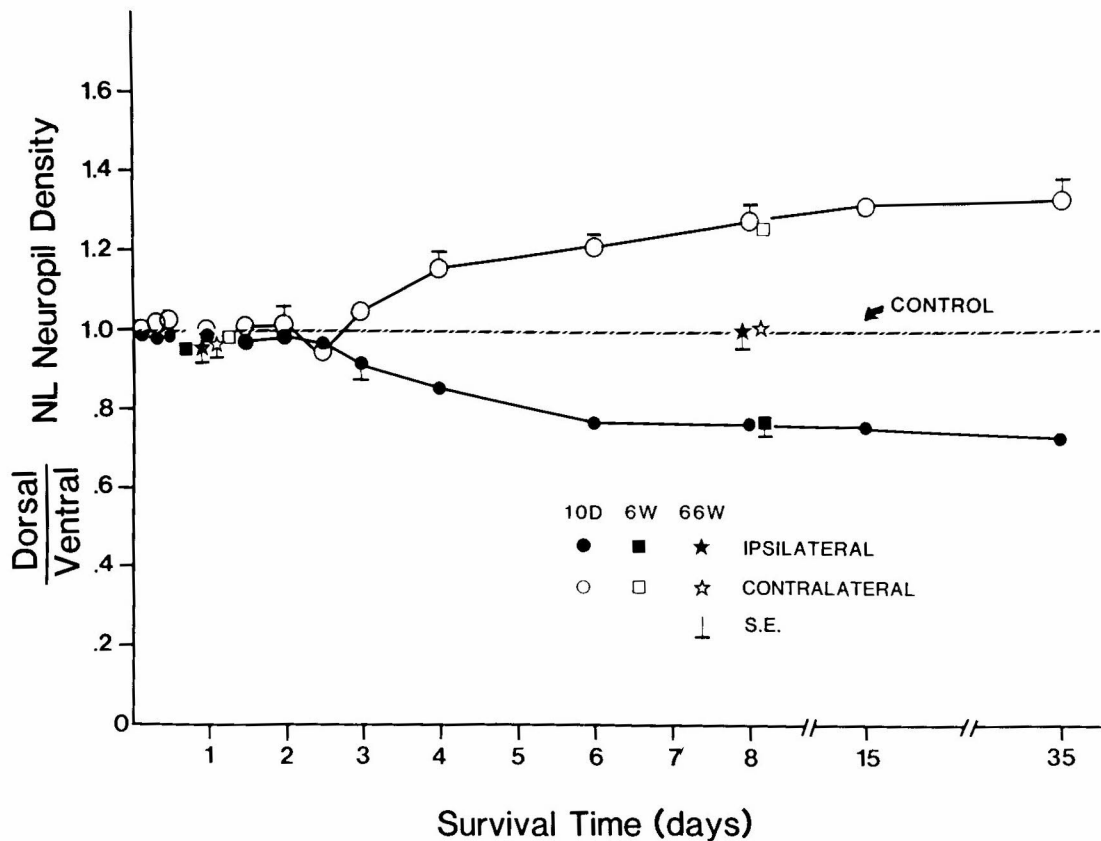
Fig. 7. Graph showing results of quantitative measurement of changes in the density of SDH reaction product in NL as a function of time after cochlea removal at 10 days (circles), 6 weeks (squares), or 66 weeks (stars) of age. The ratio of SDH density in dorsal to that in ventral neuropil was calculated for each bird in NL ipsilateral (filled symbols) and contralateral (open symbols) to cochlea removal. Averages for 2 to 5 birds are plotted (±S.E.M.). Values for 6-week-old birds are slightly offset at 1- and 8-day survivals for clarity. Error bars are not shown when they are smaller than the size of the symbol. Dotted line indicates value of ratio for control 10-day-old birds. Note that all age groups show values similar to control up to 3 days after cochlea removal. For longer survival times, parallel, long-lasting changes in ratios for ipsilateral and contralateral sides of the brain are observed following cochlea removal at 10 days or 6 weeks of age.

occur in other systems but that very early time points simply have not been examined.

The speed with which cochlea removal elicits changes in SDH staining density in NM places some constraints on the cellular processes that might mediate these changes. Cochlea removal potentially could alter several aspects of the afferent innervation of NM, including physical continuity (following degeneration of eighth nerve axons), physiological activity, and axonal transport, any or all of which could be involved in the SDH response. Degeneration of eighth nerve afferents (as indicated by the presence of argyrophilic debris at the light microscopic level) has not been reported in NM prior to 2 days after cochlea removal (Parks and Rubel, '78; Rubel et al., '81). Thus, the rapidity with which metabolic changes occur would appear to rule out degeneration as the factor responsible for these alterations. However, all available evidence suggests that physiological activity in the eighth nerve ceases immediately after cochlea removal in young (Lippe et al., '80; Born and Rubel, '84b) and adult (Koerber et al., '66; Sasaki et al., '80; Durham et al., '84) animals. Recent experiments involving reversible blockade of electrical activity in the visual (Wong-Riley and Riley, '83) and auditory (Wong-Riley et al., '81) systems have suggested that decreased physiological activity can mediate postsynaptic changes in cytochrome oxidase in adult animals. Finally, rapid changes may occur in the amount or rate of transport of some chemical "signal" from eighth nerve ganglion cells to postsynaptic NM neurons. The distance from the ganglion cell bodies to NM (1–2 mm) is such that specific substances could be transported (or their absence detected) within 4–8 hours (Brady and Lasek, '82). Experiments designed to alter only activity or axoplasmic transport will be necessary to distinguish between these alternatives.

If changes in physiological activity are responsible for changes in SDH metabolism, it is perhaps surprising that NL neuropil did not show an increase in SDH staining density comparable to that seen in NM, given that NL receives its major afferent input from NM. Since both NM and NL undergo abrupt decreases in presynaptic activity after cochlea removal (based on 2-DG data; Lippe et al., '80), one might argue that activity cannot mediate the initial NM SDH change. It is equally likely, however, that the metabolism of NM is not coupled to activity in the same manner as that of NL, such that only NM shows the initial response. Both NM and NL respond with similar decreases in SDH reaction product after longer survival times, which suggests that the factors responsible for the initial tran-

sient increase are not the same as those responsible for the eventual and lasting decrease in SDH activity.

The final result of cochlea removal in both NM and NL appears to be a decrease in the density of SDH reaction product, suggesting a general decrease in metabolic activity among neurons in the entire pathway. This result is consistent with data from other measures of metabolic activity in chick auditory nuclei following cochlea removal, as well as the majority of studies involving deafferentation in sensory systems referred to above. It is perhaps coincidental, but certainly interesting, that decreases in NM and NL occur simultaneously. The fact that degeneration following cochlea removal occurs first in NM and only later in NL (4 days after the lesion) again suggests that degeneration is not responsible for the observed SDH decreases. We observed no evidence for "recovery" (e.g., return to preoperative SDH levels), although this may require survival times longer than 35 days.

The age-dependence of the SDH response to cochlea removal is entirely consistent with that for changes in neuron number and size, Nissl staining density (Born and Rubel, '85a), and $^3$H leucine uptake (Steward and Rubel, '85) following this manipulation. While the demonstration of the age-dependence of cochlea removal in birds is not unexpected (Trune, '82a,b; Nordeen et al., '83), we do not know what factors make old birds seemingly immune to the effects of cochlea removal. As discussed in the preceding paper (Born and Rubel, '85a) the age-dependence cannot be attributed to the immaturity of anatomical connections or electrophysiological function in the chick auditory system. All of these processes appear to be complete well before 6 weeks of age, and yet 6-week-old birds show a response to cochlea removal equivalent to that seen in younger birds. The possibility that cochlea removal does not alter physiological activity in adult birds as it does in young birds does not seem likely. Preliminary evidence using the 2-DG technique suggests that decreases in functional activity in NM and NL are similar following cochlea removal in young and old birds (Durham et al., '84). One additional interesting finding emerged from this 2-DG experiment. Under the same stimulus conditions, overall levels of glucose utilization were decreased in the auditory system of old birds as compared to young birds. These results suggest either an overall decrease in physiological activity or a change in the relationship between glucose utilization and physiological activity in old birds. Further investigation of these differences, as well as the possibility of hormonal differences or a general uncoupling of the relationship between physiological activity and metabolism in old birds, should yield important information about the mechanism by which afferent manipulations affect their targets.

In summary, we have demonstrated another aspect of the chick brain stem auditory system that changes in response to cochlea removal. The time course of the alterations in density of SDH reaction product suggests that changes in activity are likely to be important for the maintenance of neuronal metabolism. The mechanism most likely also involves regulation of specific proteins, followed by a complicated chain of cellular events. The "global" changes we have described suggest where and when to look for further information about the way in which afferents influence their targets.

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