THE JOURNAL OF COMPARATIVE NEUROLOGY 231:385-395 (1985)

Afferent Influences on Brain Stem Auditory Nuclei of the Chicken: Cessation of Amino Acid Incorporation as an Antecedent to Age-Dependent Transneuronal Degeneration

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ABSTRACT

Previous studies of the avian auditory system have revealed that removal of the peripheral receptor (the cochlea) leads to a transneuronal degeneration of auditory relay neurons in nucleus magnocellularis (NM) of the brain stem. An early manifestation of the degeneration which can be observed within 12 hours is a decrease of histochemical staining for RNA (Nissl staining); such a decrease could relect an alteration in protein synthetic activity within the NM neurons. The present study evaluates this possibility by determining whether the cochlea removal led to an alteration incorporation of protein precursors in the target neurons which exhibit transneuronal degeneration and if so, how early the changes appeared. The cochlea was removed unilaterally in seventeen 10-day-old chicks and two 66-week-old mature chickens, and incorporation of protein precursors was evaluated in the neurons of NM at 0.5, 1.5, 3, 6, 12, and 24 hours following the cochlea removal. Each chick received an intravenous injection of 3H leucine, and was allowed to survive for 30 minutes after the injection of precursor. The brains were then prepared for autoradiography. The extent of incorporation by neurons in NM was determined by counting grains overlying each cell body and determining grain density/μm^2 of neuron cross-sectional area.

We found that auditory relay neurons whose synaptic inputs have been silenced exhibit dramatic decreases in protein synthesis within 30 minutes after removal of the cochlea; leucine incorporation was reduced by about 50%. In chicks sacrificed 3 to 24 hours after removal of the cochlea, some neurons (about 1/3) were entirely unlabeled despite heavy labeling of their neighbors and heavy labeling of all NM neurons on the opposite side of the brain. The remaining neurons exhibited about a 15% reduction in incorporation in comparison with the cells in the contralateral (control) NM. While the decreases in incorporation were apparent at all survival intervals, there was no consistent decrease in Nissl staining until 6 hours after cochlea removal. There were no changes in protein precursor incorporation following removal of the cochlea in adult birds, a result which is in keeping with the relative absence of transneuronal degeneration following removal of the cochlea at maturity. The results suggest a very rapid transneuronal regulation of protein metabolism within target neurons in young animals, perhaps by activity-related events.

Key words: deafferentation, transneuronal degeneration, protein synthesis, nucleus magnocellularis, development, critical periods

Accepted September 13, 1984.

Synaptic interconnections between neurons and their targets not only transmit activation from one cell to another, they also play a role in regulating the metabolism, the structure, and the very existence of the interconnected partners. For example, the targets of synapses, whether they be muscles, glands, or other neurons, depend critically on the integrity of their synaptic inputs (Levi-Montalcini, '49; Globus, '75; Sharpless, '75). The removal of synaptic inputs often leads to disruption of cellular metabolism, deterioration of structural specializations, and sometimes the death of the deafferented target cell. Similarly, increased input brought about experimentally often leads to the growth of the portion of the postsynaptic cell associated with the increased input (for example, see Sotelo and Arsenio-Nunes, '76; Angaut et al., '82). Thus, the regulation of target cells by their inputs is thought to play a pivotal role in the sculpting of neuronal connections during normal development (Cowan, '70; Changeux, '83; Hamburger, '80), in the response of the nervous system to injury or abnormal experience (Globus, '75; Thoenen et al., '79), and perhaps in information storage in the mature nervous system (Stent, '73).

The wealth of experimental evidence for changes in targets following manipulation of inputs leaves no doubt about the interdependency of connected partners; the crucial questions today relate to the cellular mechanisms and include 1) what is the nature of the signaling between cells (activity, exchange of trophic substances, or simple physical contact), and 2) what cellular processes are actually being regulated? The study of the first question has been hampered by the delay which is typically observed between the manipulation of the input and structural changes in the target; most deafferentation-related changes take days or weeks to become evident (for reviews, see, Globus, '75; and Rubel et al., '84). Indeed, changes which are observed within 1 week are considered rapid (Kupperman and Kasamatsu, '84). Thus, it is difficult to independently manipulate activity or the exchange of substances in a way that does not also cause degeneration of the input. For this reason, a system which exhibited rapid transneuronal changes would be of special interest for evaluating the signaling process.

In addressing the second question (the cellular processes actually being regulated), it is necessary to distinguish between primary and secondary effects. Obviously, as cells begin to die, one can expect changes in virtually every aspect of the metabolic economy of the cell. The simple cataloguing of these changes is not likely to lead to insights into the sequence of events which leads to the death. An alternative strategy is to seek the earliest cellular events which can be defined, particularly those which seem likely to be related to cell viability.

Previous studies of neuronal death in the auditory pathways of the chicken following cochlea removal suggest that this example of transneuronal degeneration might provide an opportunity to address both of the questions posed above (Rubel et al., '81). First, the death of neurons of the second order auditory nuclei (nucleus magnocellularis, NM) is quite rapid; cell loss is evident two days following cochlea removal (Born and Rubel, '85). Second, changes can be seen within the nucleus within hours of the cochlea removal (Durham and Rubel, '85). One such change is a decrease in Nissl substance within NM neurons. By 12 hours after removal of the cochlea, about 30% of the neurons exhibit essentially no Nissl staining, appearing as "ghost cells" (Born and Rubel, '85). Since the Nissl stain is at least a rough indicator of the integrity of the protein synthetic machinery of the neuron (RNA and ribosomes), the results suggested that an early consequence of cochlea removal might be some sort of disruption of the protein synthetic activity of the NM neurons. Such a change seems likely to be related to cell viability, and it is perhaps not coincidental that about the same proportion of cells exhibit changes in Nissl staining that eventually die (about 30%, see Born and Rubel, '85). To examine the possibility that protein metabolism is under dynamic regulation by the inputs to those neurons, the present study evaluated the incorporation of protein precursors after cochlea removal by using the autoradiographic technique initially developed by Droz and Leblond ('63). This technique has been used extensively to study changes in incorporation of protein precursors in a variety of preparations and is particularly useful in evaluating incorporation in single neurons (Barron et al., '76; Engh et al., '71; Kung, '71; Sinatra et al., '79; Fass and Steward, '83; Meyer and Edwards, '82). We report herein that following cochlea removal in young animals, neurons of nucleus magnocellularis exhibit dramatic decreases in incorporation of protein precursors within 30 minutes after removal of the receptor. Some neurons appear to cease incorporating precursors entirely by 3 hours. The results suggest 1) that the death of neurons in nucleus magnocellularis may be a direct result of the early, and in some cells complete, disruption of protein synthesis, and 2) that these effects, occurring so early after cochlea removal, seem quite likely to be related to activity rather than changes related to the degeneration of the afferents. By virtue of the rapidity of the changes, an opportunity is provided in which to further explore the role of afferents in regulating postsynaptic metabolism.

MATERIALS AND METHODS

Experimental animals were Ross Arbor Acres chickens (Heatwole Hatcheries, Harrisonburg, Virginia). The right basilar papilla (cochlea) was removed in 19 10-day-old chickens and two mature birds (66 weeks of age). The 10-day-old chicks were hatched in the laboratory. The mature birds were purchased directly from the commercial supplier. All animals had free access to food and water.

Unilateral basilar papilla removal was performed according to procedures which have been described previously (see Born and Rubel, '85). The animals were anesthetized with a combination of ketamine (80 mg/kg i.m.) and chloropent (1.5 ml/kg i.p.). The cochlea (basilar papilla) was removed using a trans-tympanic surgical approach, removing the columella to reveal the oval window, and then pulling out the basilar papilla intact. The basilar papilla was floated on water and viewed through a dissecting microscope to ensure complete removal. This procedure removes the receptors and severs the peripheral processes of the eighth nerve ganglion cells, but leaves the ganglion cell bodies and their central projections intact. For information on the consequences of this operation for neuronal activity in the nucleus and 2-deoxyglucose uptake, see Lippe et al. ('80), and Born and Rubel, ('85). The 10-day-old chicks survived following the cochlear removal for 0.5 (n = 2), 1.5 (n = 3), 3 (n = 3), 6 (n = 5), 12 (n = 3), or 24 (n = 3) hours. The mature birds (n = 2) survived for 12 hours. Two additional 10-day-old chicks served as unoperated controls. Thirty minutes prior to perfusion, the animals received an intracardiac injection of ^{3}H leucine (0.5 cc of a solution of 1 mCi/ml of ^{3}H leucine for a total injection of 0.5 mCi). For most

birds, the isotope was reconstituted in sterile saline prior to injection. For some birds, however, the isotope was injected into the heart in the 0.01 N HCl vehicle in which it was shipped. Birds were not anesthetized for this procedure, although in the cases prepared 30 minutes after cochlear removal the effects of the initial anesthetic were still apparent. Chicks were placed on their backs, where they remained without restraint for several moments (tonic immobility response, see Ratner, '67). The intracardiac injection was very rapid (~5 sec) and did not cause distress when the birds were maintained on their backs during the procedure. Immediately after the injection they were righted and exhibited no sign of pain.

All the birds survived for 30 minutes postinjection to allow for incorporation of the precursor into protein. This postinjection survival time is adequate to permit substantial incorporation of ^{3}H leucine (Banker and Cotman, '71) but is short enough that there should be little if any transport of labeled proteins away from their sites of synthesis. The birds were then perfused with 10% buffered formalin while deeply anesthetized with sodium pentobarbital. After removal from the skull, the brains were immersed overnight in Bouin's solution. They were then returned to 10% formalin for one week. The formalin perfusions and subsequent histological processing wash out of all the ^{3}H leucine precursor which has not been incorporated into protein. After dehydration and embedding in paraffin, the brain stems were sectioned coronally at 8 μm. A one-in-four series of sections were mounted on acid-cleaned slides and prepared for autoradiography utilizing routine methods (see Fass and Steward, '83). After exposure for 2 to 6 weeks, the autoradiograms were developed in D19, fixed, and counterstained with thionin. When prepared in this manner, the labeling is though to reflect the extent of protein synthesis and the sites at which this synthesis occurs. For more details on this method and the underlying assumptions, see Droz and Leblond ('63). The extent of labeling of individual neurons in n. magnocellularis was evaluated with quantitative autoradiographic methods, as described in more detail in Results. In all cases, comparisons were made on the same tissue section between NM cells on the side of the brain ipsilateral to the cochlea removal and the NM cells on the contralateral side.

RESULTS

As noted in previous studies (Born and Rubel, '85), cochlea removal in posthatch chicks leads to the death of about 30% of the neurons in NM, and a 15% size reduction of the remaining neurons. A similar operation in mature birds leads to little cell death or atropy. Thus, the consequences of cochlea removal on the incorporation of protein precursors by NM neurons will be considered separately for the two ages.

As illustrated in Figure 1A and C, autoradiograms from control material (contralateral to a cochlear removal) reveal that cells in NM are quite active in synthesizing protein, as are most neurons. In all cases, every cell in the section was covered with silver grains over cytoplasmic regions. The extent of labeling varied somewhat from animal to animal, probably as a result of differences in the delivery of the isotope, emulsion thickness, etc.

Figure 1B and D illustrates the changes in incorporation in NM neurons on the side of the cochlea removal at 30 minutes and 6 hours after the operation. As is evident from comparisons with the contralateral control (in Fig. 1A,C respectively), neurons deprived of cochlear input exhibited dramatic decreases in labeling, even as early as 30 minutes after the operation. At the earliest interval, the decreases in grain density appeared to be generalized across all neurons in the nucleus (for quantitative considerations, see below). At 3 hours and thereafter it was possible to divide the NM neurons into two populations, a clearly labeled group and a clearly unlabeled population. The distinction between labeled and unlabeled NM neurons became striking and unambiguous at 6, 12, and 24 hours after the cochlea removal (see for example a 6-hour case in Fig. 1D).

Figures 2 and 3 provide an illustration of the progression of the change in incorporation and its relationship to changes in Nissl staining. The changes in protein precursor incorporation at 30 minutes and 1.5 hours occurred over neurons which were not distinguishable from their neighbors on the basis of Nissl staining. Some cells with apparently normal Nissl staining exhibited little or no incorporation. At 3 hours, some neurons began to exhibit slight changes in Nissl staining, in that some of the unlabeled cells stained more lightly, and the staining appeared more diffuse than in normal neurons which are darkly stained and have obvious clumps of Nissl substance. Even at 3 hours, though, some cells with apparently normal Nissl staining were unlabeled. This was also true at 6 hours. By 12 and 24 hours, the unlabeled neurons exhibited a dramatically different pattern of Nissl staining than their labeled neighbors, appearing as "ghost cells" (see Fig. 2G,H) which have been previously described (Born and Rubel, '85). At these intervals, we did not observe cells with normal appearing Nissl substance which were unlabeled, nor did we observe "ghost cells" with labeling. The ghost cells are virtually invisible in Nissl stained preparations, and appear as open spaces with no labeling (Fig. 2G). They can be visualized by phase-contrast microsopy, however, and in this case the extent of their cytoplasm can be defined (see Fig. 3). This figure illustrates the almost complete lack of labeling over the cytoplasm of the ghost cells. An interesting feature of this figure is the heavily labeled small cells (presumably glia) amongst the unlabeled neurons. These are as heavily labeled as similar cells on the control side, indicating that the changes in incorporation by neurons are not likely to be due to general effects such as changes in blood flow, capillary permeability, or other variables which would effect precursor availability. The results illustrated in Figures 2 and 3 indicate that changes in incorporation are evident before changes in Nissl staining, but both seem to involve the same population of cells at longer survival intervals. A quantitative analysis of the relationship between Nissl staining and incorporation was not possible in this material because overlying silver grains interfere with densitometric measurements of Nissl staining.

The qualitative observations suggested that at the shortest survival time there was a dramatic decrease in labeling involving the entire population of NM neurons, while at 3 hours and beyond, the labeled and unlabeled cells were distributed into two discontinuous populations. Nevertheless, it is possible that these qualitative impressions did not accurately reflect the actual distributions; the distribution at the earliest intervals may be bimodal but less striking than at later intervals, or the distribution may not be bimodal at the later intervals, but simply appear so because of the dramatic absence of labeling over some neurons. To address this question, grains were counted over a random sample of 50 neurons from experimental and control sides

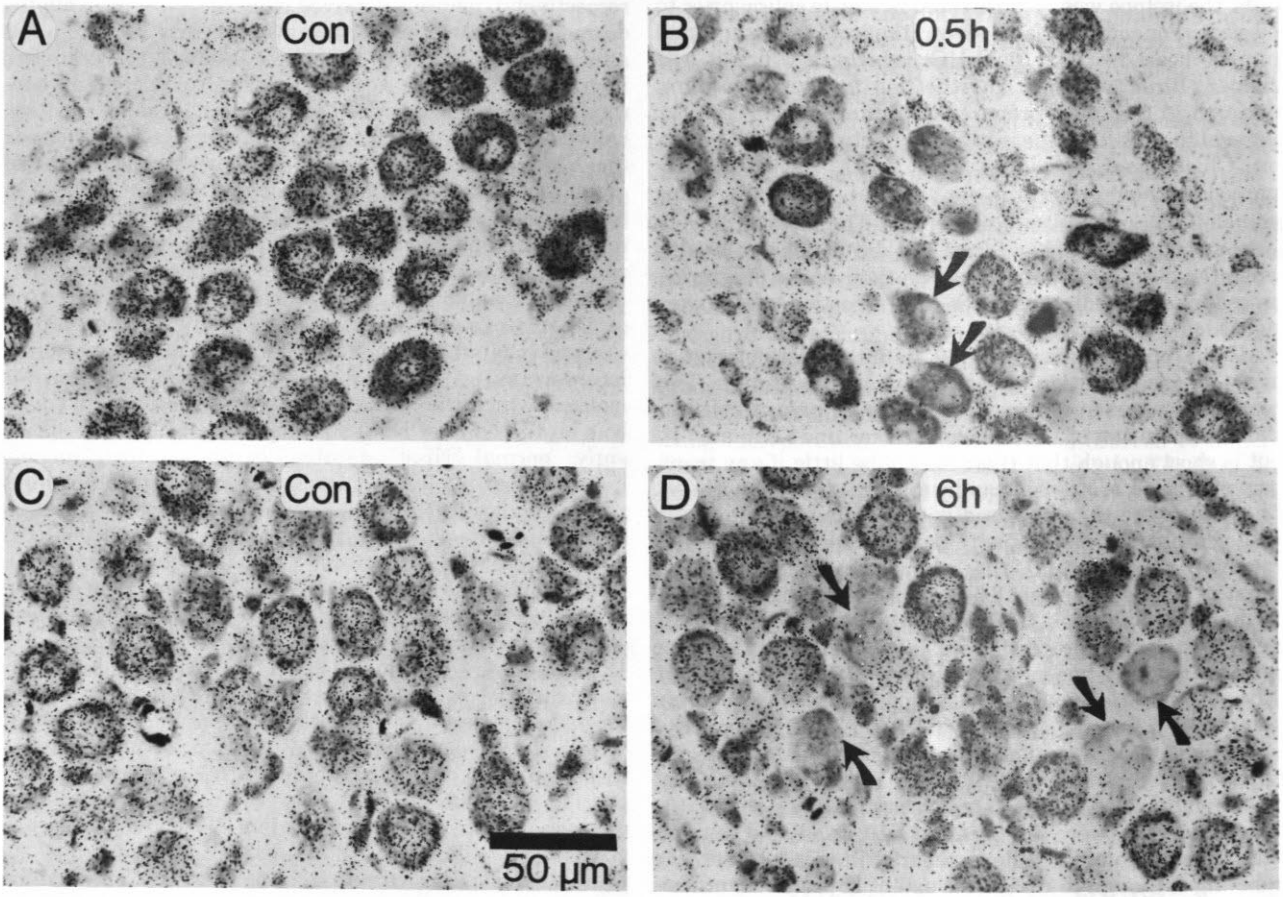


Fig. 1. Pattern of labeling of cells in n. magnocellularis on both sides of the brain (same section) 30 minutes following intraveous injection of ^{3}H leucine. A and C illustrate the normal pattern of labeling over the neurons in n. magnocellularis contralateral to a cochlea removal (con) in a 10-day-old chick. B and D indicate the pattern of labeling in the n. magnocellularis deprived of peripheral input 0.5 hours (B) and 6 hours (D) following cochlea removal. Note the general decrease in labeling across the entire population of cells in B in comparison to the contralateral control in A. Some cells also appear unlabeled (arrows). In D, there are separate populations of labeled and unlabeled cells (arrows). Note also in D the lack of Nissl staining of the cells without overlying silver grains (which suggests some change in neuronal RNA in the cells which are not producing protein).

of the brain in animals sacrificed at 0.5 and 6 hours after cochlea removal, without regard to whether the cells appeared "labeled" or "unlabeled." This was done by selecting a section midway through NM and counting grains over all NM neurons with an identifiable nucleus encountered in that section beginning at the medial edge and moving laterally. An adjacent section was then sampled until a criterion number of cells was obtained (50 cells per side). A frequency distribution of grain densities over neurons was then generated for each side of the brain. As illustrated in Figure 4A, at 0.5 hours, there was no evidence of bimodality in the distribution of grain densities on either side of the brain. In fact, grain densities appeared quite normally distributed. The entire population of NM neurons ipsilateral to the receptor removal was simply much less heavily labeled than the cells on the control side. However, by 6 hours after cochlea removal, these analyses revealed clearly bimodal distributions of grain densities on the experimental side (Fig. 4B). Some neurons were clearly unlabeled, while grain densities over the remaining cells were at least twice as high and usually 5 to 10 times higher. The labeled cells appeared less heavily labeled than the cells on the control side.

The fact that the "labeled" neurons on the side ipsilateral to the cochlea removal appeared less heavily labeled than control cells on the opposite side of the brain was of interest. It could be that the cells which survive are also affected by the loss of input, but to a lesser extent than the cells which die. The quantitative evaluation above could not answer this question, since the random sample included the popu-

Fig. 2. The relationship between changes in incorporation of protein precursors (as revealed by grain density over cells) and changes in Nissl staining 0.5 hours (A,B), 1.5 hours (C,D), and 3.0 hours (E,F) and 12 hours (G,H) after cochlea removal in chicks. The right hand micrograph for each case focuses on the silver grains; the left hand micrograph is focused on the Nissl substance. The cells with little or no labeling in the autoradiograms are indicated by the arrows in the right hand panels. The position of a ghost cell (revealed by a lightly stained nucleus) is indicated by the pointer. These cells are essentially invisible in bright field photomicrographs of Nissl preparations but can be seen using phase contrast (see Fig. 3).

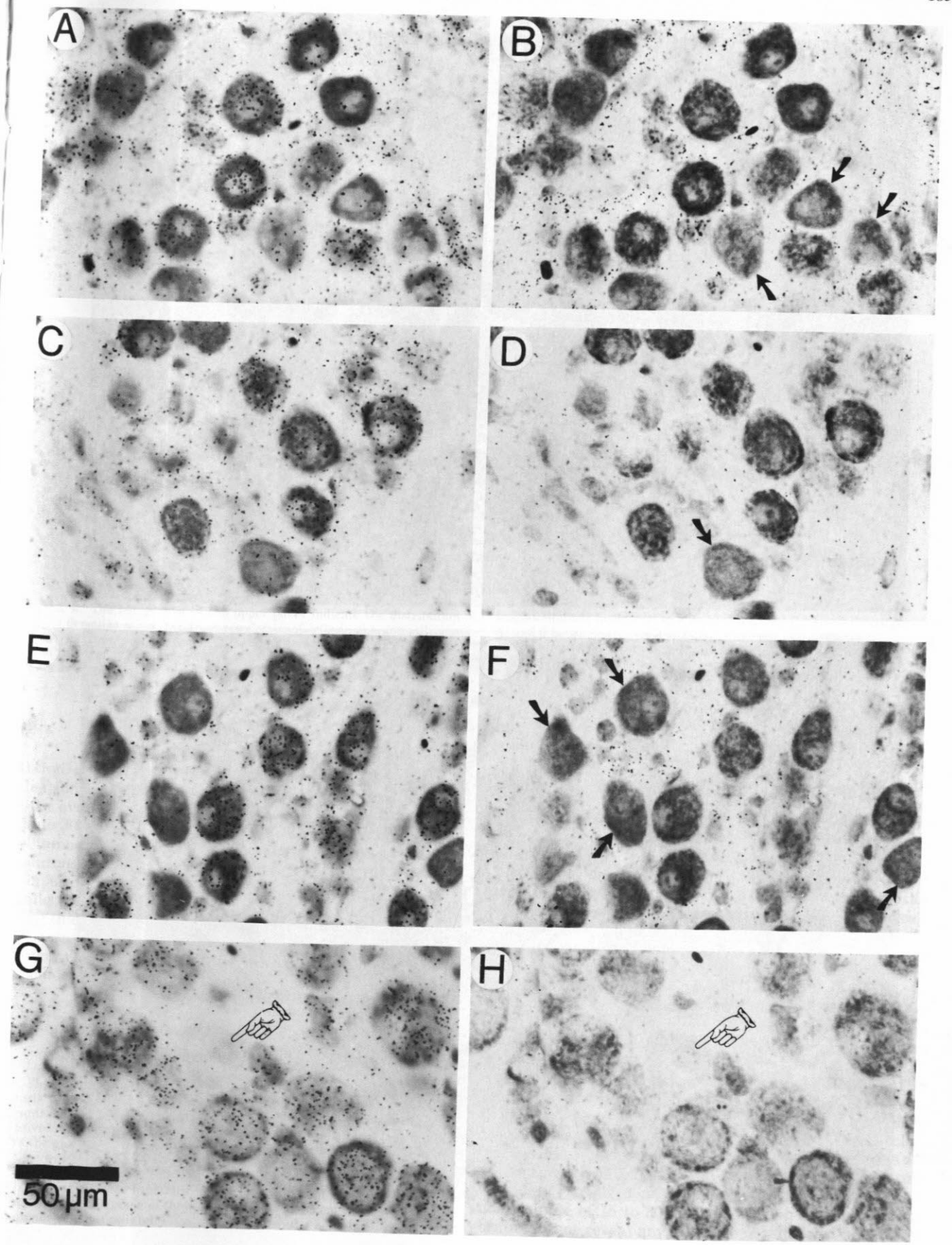


Figure 2

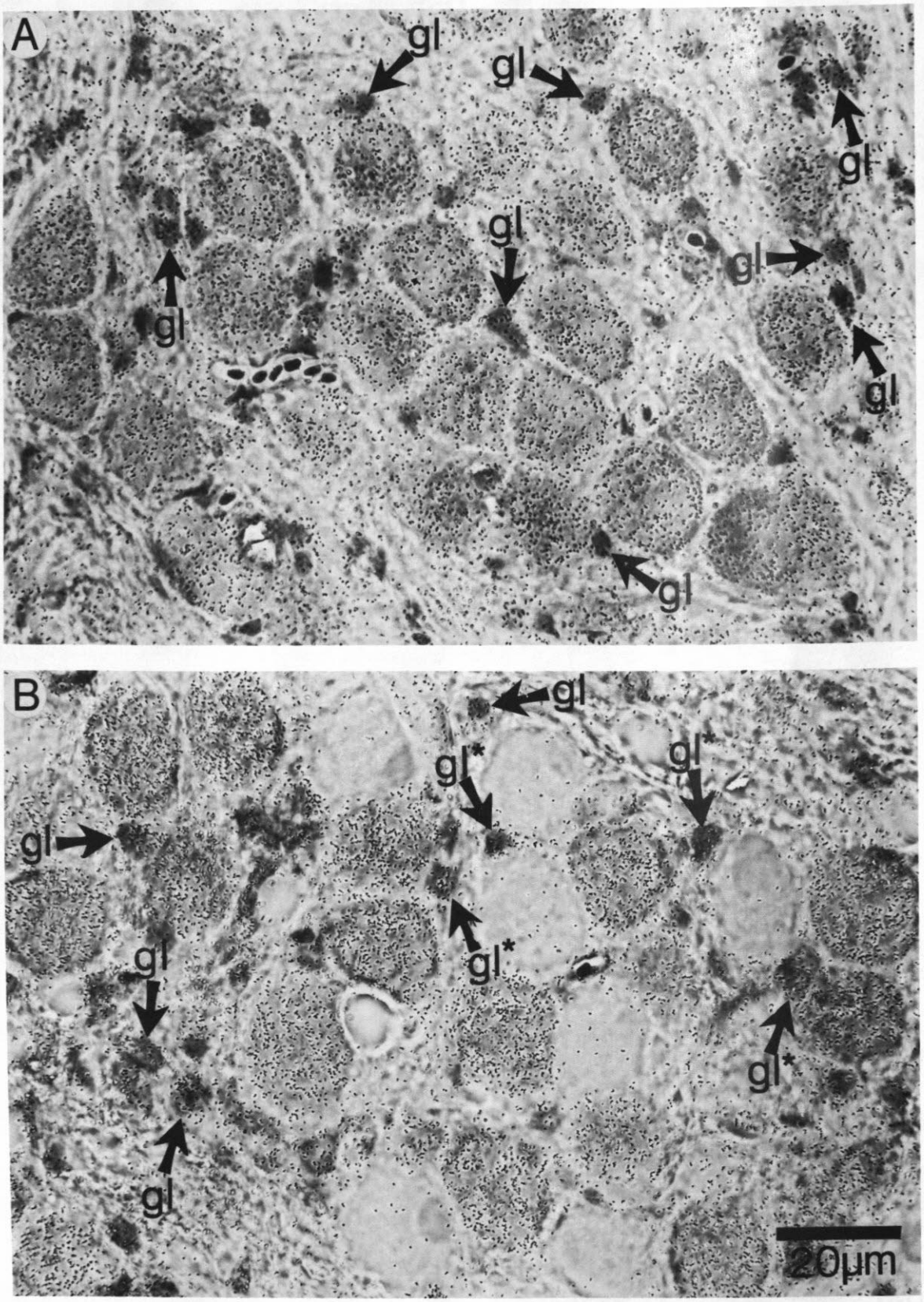


Figure 3

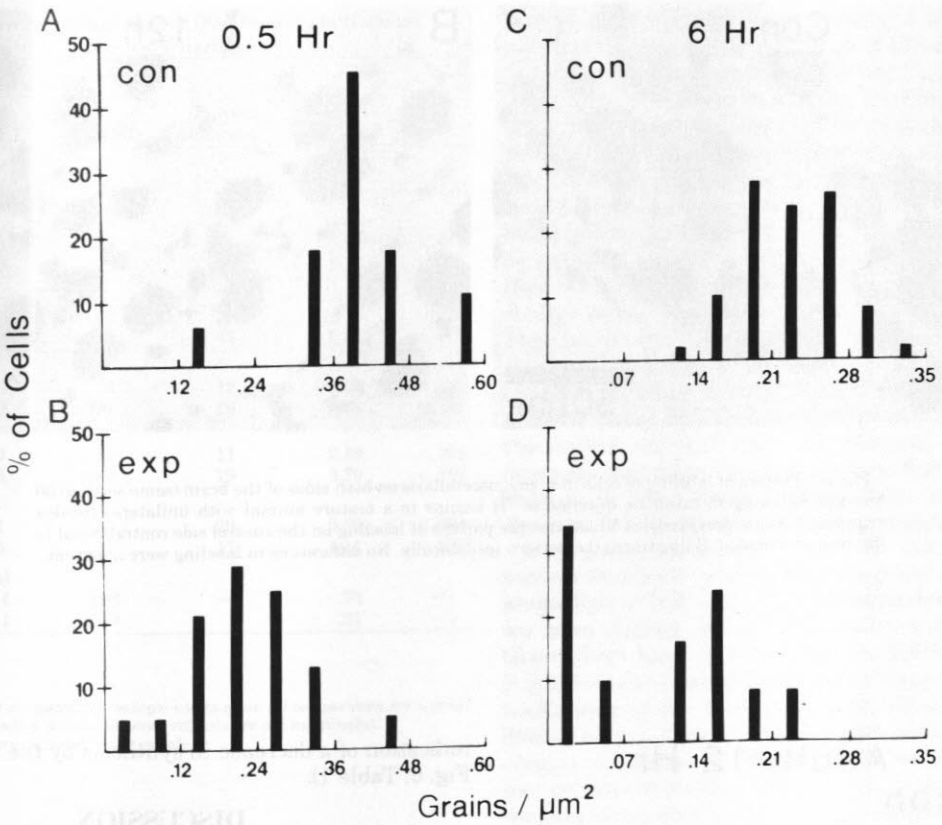


Fig. 4. The bar graphs in the upper panel indicate the distribution of grain densities across the total population of cells in the normally innervated n. magnocellularis *contralateral* to a cochlea removal in chicks. Note that the distribution of grain densities appears to be unimodal. The lower panels illustrate the distribution of grain densities over neurons of n. magnocellularis ipsilateral to a cochlea removal 0.5 hours (lower panel of A) and 6 hours (lower panel of B) following the operation. Note the apparently unimodel distribution of grain densities at 0.5 hours, and the bimodality of the distribution at 6 hours.

lation of entirely unlabeled cells which would shift the distribution of grain densities for the labeled cells. To more fully evaluate this question we compared grain counts over 15 to 30 "labeled neurons" on the affected side with counts obtained from a randomly selected population of 15 to 30 neurons on the control side (all of which were invariably "labeled"). The comparisons were made between samples collected in single sections to control for variations in section thickness, autoradiographic procedures, etc. This analysis was carried out on two of the animals at each survival interval except for 0.5 hours, where only one subject was included.

Fig. 3. Absence of incorporation by "ghost cells" in nucleus magnocellularis 12 hours after removal of the cochlea. A illustrates the control side contralateral to the operation; B illustrates the side which had been deprived of cochlear input. In the phase-contrast photomicrographs, the extent of the ghost cells' cytoplasm can be clearly defined (clear oval areas). Note the virtually complete absence of silver grains overlying these cellls. Note also that small cells (presumably glia, gl) appear to be at least as heavily labeled on the side ipsilateral to the removal of the cochlea (B) as on the control side (A). Some of these small cells lie adjacent to or even between unlabeled ghost cells (gl*).

As noted above, it was not possible to make a clear distinction between labeled and unlabeled cells at the short survival times (0.5 to 3 hours). Thus, in the analysis of the 0.5-hour case, no attempt was made to exclude unlabeled cells from the sample. Furthermore, some very lightly labeled cells were included in the sample of the 1.5- and 3-hour cases. This inclusion may have biased the distribution somewhat, leading to a slight overestimate of the percent decreases at 1.5 and 3 hours, compared to the longer survival intervals. Nevertheless, the counts revealed that in every brain from the 10-day-old chicks, the labeled cells on the experimental side were significantly less heavily labeled than their counterparts on the control side. The mean grain density on each side, the percent difference, and the t and P values for the individual comparisons are illustrated in Table 1.

As illustrated in Figures 5 and 6, cochlea removal in mature birds did not result in the same pattern of changes in protein precursor incorporation. Twelve hours after cochlea removal, there was no indication of any "ghost cells" (see also Born and Rubel, '85). Qualitative observations did not reveal any differences between experimental and control sides. Furthermore, counts of grain density over a random sample of 15 to 30 NM neurons on each side of the brain did not reveal any evidence of unlabeled neurons or

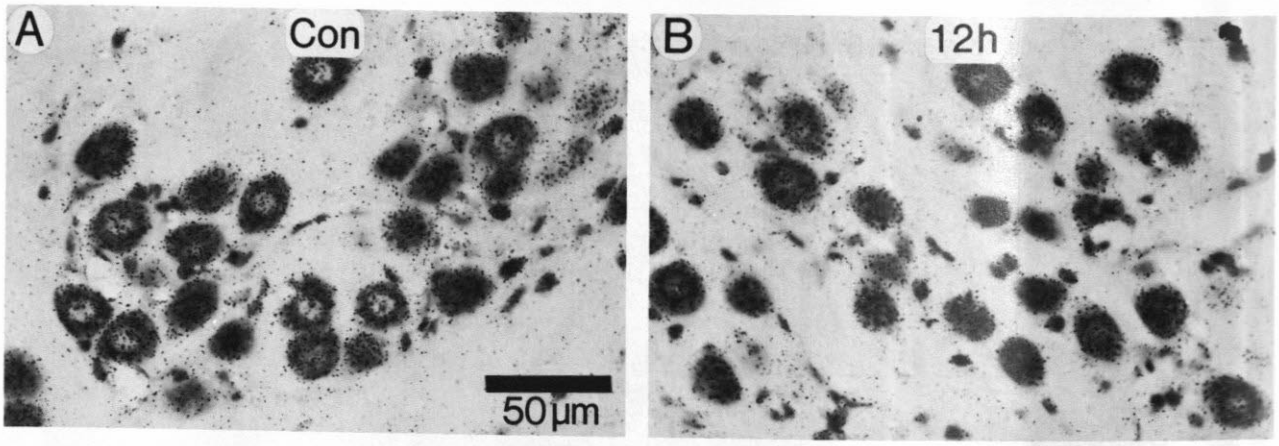
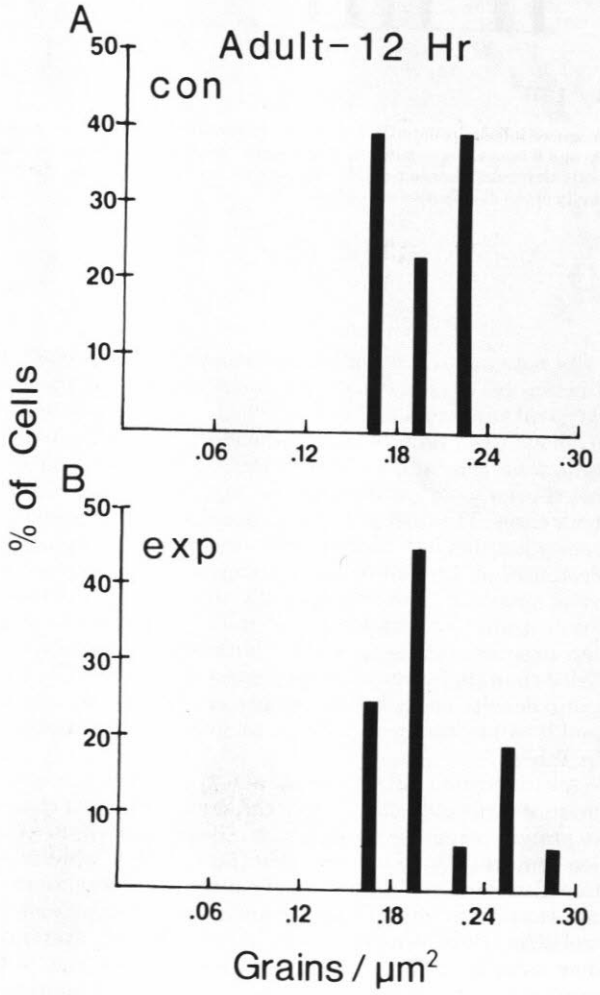


Fig. 5. Pattern of labeling of cells in n. magnocellularis on both sides of the brain (same section) 30 minutes following intravenous injection of ^{3}H leucine in a mature animal with unilateral cochlea removal 12 hours previously. A illustrates the pattern of labeling on the control side contralateral to the cochlea removal; B illustrates the pattern ipsilaterally. No differences in labeling were apparent.



indication of a decrease in synthesis by the population (see Fig. 6, Table 1).

DISCUSSION

The present observations may be of considerable relevance for understanding 1) the mechanisms of signaling between a neuron and its target which result in transneuronal regulation, 2) the sequence of events whereby elimination of a major afferent input leads to the death of the target neuron.

In the effort to evaluate the signaling mechanisms by which neurons regulate the metabolism of their targets, the rapid effects described herein provide an ideal opportunity to selectively alter many of the relevant variables such as activity or axonal transport without inducing physical degeneration of the afferents. Previous efforts to define the presynaptic signal(s) regulating the metabolic and structural parameters of postsynaptic cells have been seriously hampered by the apparently long latency of denervation-induced effects; the longer a treatment must be maintained, the more likely that direct damage of afferents will occur. In the present case, a treatment would presumably have to be maintained for only 30 minutes before an effect would be expected. This should have few undesirable side effects. It seems quite unlikely that degenerative changes would be set in motion in the ganglion cell axons within 30 minutes after cochlea removal, although the possibility of very rapid degenerative changes in the NM terminals cannot be

Fig. 6. The bar graphs indicate the distribution of grain densities across a sample of cells in the control n. magnocellularis and in the magnocellularis ipsilateral to the cochlea removal in the mature birds (from the case illustrated in Fig. 4). Note the lack of any difference in the distribution of grain densities on the two sides.

TABLE 1. Average Grain Density Over Neurons in Nucleus Magnocellularis

Survival time	Grains/μm^2 Right (exper.)	Grains/μm^2 Left (control)	Percent Difference[1]	t^2	P
0.5 hr					
831114	.22	.40	45	6.38	<.001
1.5 hr[3]					
831111	.13	.19	38	5.08	<.001
831110	.40	.78	49	8.10	<.001
3.0 hr[3]					
831109	.41	.63	35	4.16	<.01
831106	.30	.53	43	5.20	<.001
6 hr[3]					
821106	.48	.59	18	3.24	<.01
821108	.80	.97	18	2.31	<.02
12 hr[3]					
821113	.39	.44	11	2.19	<.025
821114	1.13	1.40	19	3.79	<.001
24 hr[3]					
821110	1.11	1.55	29	5.39	<.001
821111	1.05	1.27	17	4.41	<.001
Adult 12 hr survival					
831103	.204	.196	−4	.74	>.1
831191	.161	.159	1	.31	>.1

[1] $\left(1-\frac{Rt}{L}\right)\times 100$

[2] Grain densities were compared by multiple t-tests since all comparisons are a priori and comparisons of labeling density between animals are not meaningful.

[3] Only labeled cells were included in the sample.

excluded entirely. The most obvious and probably most profound consequence of cochlea removal, particularly 30 minutes to 6 hours postoperative, is the silencing of the eighth nerve afferents. Both 2-deoxyglucose and electrophysiological measurements show immediate and maximum decreases in activity in NM and NL (Lippe et al., '80; Born and Rubel, '85) whereas obvious anatomical changes in the ganglion cell population or in the proximal portion eighth nerve axons have not been seen (for a more complete discussion, see Born and Rubel, '85). These facts and the finding of extremely large changes already present at the shortest survival time (30 minutes) leads us to favor the hypothesis that it is activity *per se*, or some activity-related event, which influences protein precursor incorporation in the postsynaptic magnocellular neurons. Whatever the final answer regarding the mechanisms of communication between pre- and postsynaptic elements, it is clear that this system provides an ideal opportunity to evaluate the nature of these signaling mechanisms.

In addition to defining the time course of transneuronal regulation and providing a framework for future studies of the signaling mechanism, the present results demonstrate that an extremely critical aspect of the metabolic economy of the neuron (the incorporation of amino acids into protein) is rapidly and dramatically affected by silencing excitatory inputs. It is difficult to conceive of how removal of the cochlea could result in such a dramatic reduction of incorporation within one-half hour and lead to a complete cessation of incorporation within 3 to 6 hours, yet that is precisely what is observed. It is unlikely that this cessation of incorporation involves some nonspecific effect such as a change in blood flow or a change in uptake of the ^3H precursor. A consistent feature of the complete cessation of synthesis is that it is selective to about 1/3 of the total population of neurons in the nucleus. A change in blood flow is not likely to result in changes in individual neurons when their nearest neighbors are heavily labeled. Furthermore, even in the most dramatic cases there are no apparent differences between the two sides in the labeling over glial cell bodies in NM. A change in uptake is also unlikely since the effect would have to be complete. Even passive exchange of labeled leucine between the blood and neurons in NM would be expected to lead to detectable incorporation. One is left then with the conclusion that the changes indicate some change in the protein synthetic machinery of the neuron. This is certainly a reasonable proposition at the longer survival intervals (6 to 24 hours) when there are clear changes in Nissl staining which presumably reflect the amount and/or distribution of RNA within the neurons. At the earlier survival intervals, however, where there are dramatic decreases in incorporation but with no obvious changes in Nissl staining, the cellular mechanisms of the decrease in incorporation remain to be elucidated.

It is quite reasonable to propose that the cessation of protein synthesis is the ultimate cause of the death of a proportion of NM cells. The correspondence between what we have dubbed "ghost cells" (Born and Rubel, '85) and those which have ceased producing proteins indicates that it is the neurons which cease producing protein which eventually degenerate. Obviously, cells which have ceased producing protein cannot remain viable indefinitely. Since the change in protein synthesis is the earliest change which has yet been detected in these cells, it may actually represent the first event in the cascade that leads to the death of a proportion of the NM neurons. Other rapid changes which have been described in NM following silencing of cochlear afferents such as changes in 2DG uptake (Lippe et al. '80) and changes in staining for succinate dehydrogenase (Durham and Rubel, '85) are less likely candidates since they appear to be generalized to all cells in the nucleus. In contrast, the cessation of protein synthesis involves about 1/3 of the cells in the nucleus, which is the same proportion of cells which eventually die (Born and Rubel, '85). Furthermore, following cochlea removal in adult birds, there are no detectable changes in protein precursor incorporation 12 hours after the cochlea removal, and there is little transneuronal cell death. If the cessation of protein synthesis is not the *first* in the chain of events which leads to cell death, it seems virtually certain that it is in the causitive chain, and relatively early in sequence.

As noted in the introduction, the changes reported herein are considerably more rapid and more pronounced than any previously reported. Other studies of changes in protein synthesis following deafferentation have reported dramatic effects, but only after long intervals. For example, Meyer and Edwards ('82) report 50% decreases in amino acid incorporation in somata and dendrites of neurons of the terminal ganglion of young crickets. These effects were only observed with chronic deafferentation, however. Short term deafferentation (1 to 2 weeks in duration) did not affect incorporation and very rapid effects (0 to 24 hours) were not examined. These authors conclude that the deafferentation affected the target cell only if maintained throughout the critical stages of postembryonic development. Similarly, in vertebrate systems, evidence of transneuronal changes following deafferentation is not usually observed for days or weeks (see Globus, '75) although there are several reports of very rapid changes in neuronal structure or metabolism

following denervation in young animals (see Born and Rubel, '85). For example Deitch and Rubel ('84) have shown 20% changes in the size of dendrites 2 hours after denervation. On the other hand, even massive deafferentation of dendrites of the adult rat's dentate gyrus does not alter protein precursor incorporation in the denervated target cells for several days, (Fass and Steward, '83). Interestingly, when changes do appear, they are increases in incorporation rather than decreases. These have been interpreted as reflecting increased protein synthesis within dendrites which might be related to the reinnervation process which begins at about the same time (Fass and Steward, '83; Steward and Fass, '83). The present results would suggest that more dramatic and rapid transneuronal effects might be observed in developing animals.

There are also scattered reports of changes in dendritic spines following deafferentation which appear within hours (Chen and Hillman, '82), and a growing number of reports of changes in spine size or shape which correlate with changes in synaptic efficacy following specific patterns of stimulation (Fifkova and Van Harreveld, '77; Desmond and Levy, '83). Recent studies have revealed a selective localization of polyribosomes under the base of dendritic spines of CNS neurons; these seem optimally positioned to have their synthetic activity affected by activity over the synapse (Steward and Levy, '82; Steward, '83). These dendritic polyribosomes are much more numerous under spines during synapse growth, either in developing animals (Steward, '83a) or in response to lesions in adult animals (Steward, '83b; Steward and Fass, '83). This prominence in growing synapses would reinforce the implications of the present study that protein metabolism within target cells is much more closely coupled to afferent innervation in developing animals than in mature ones.

In addition to the obvious question of the nature of the signaling between synaptic inputs and their targets, and the sequence of events which leads to the cessation of protein production within some neurons, crucial questions for future investigation will be 1) why do only some neurons in the NM deprived of cochlear input in developing animals cease producing protein, while the majority exhibit only slight decreases in incorporation?; 2) do some neurons show initial drastic reductions in incorporation and then recover?; 3) what renders the adult NM neurons seemingly immune to the transneuronal effects of receptor removal?; and 4) how general are these very rapid transneuronal effects? If it is possible for neurons to recover from initial dramatic reductions in protein metabolism and for the metabolism of older neurons to be uncoupled from their input, it may be possible to determine what variables determine cell survival and cell death in this setting. It may even be possible to devise means to rescue neurons which would otherwise die, and thus manipulate the progression of the lesion which results from the process of transneuronal degeneration. We will then be in a position to develop therapeutic interventions which could greatly improve the clinical outcome of CNS injury in developing nervous systems.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the help of Dr. Dianne Durham and Mr. Donald Born in carrying out these experiments, Ms. Doris Hannum for expert histological assistance, and Dr. T. Norville Parks for comments on the manuscript. We also thank Miss M.P. Janssen for secretarial help. Support was provided by PHS grants NS 12333 to O.S. and NS15395 to E.W.R., The Lions of Virginia Hearing Foundation, and the University of Virginia Pratt Foundation. O.S. was supported by an NIH Research Career Development Award (NS00325).

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