THE EFFECT OF UNILATERAL BASILAR PAPILLA REMOVAL UPON NUCLEI LAMINARIS AND MAGNOCELLULARIS OF THE CHICK EXAMINED WITH [3H]2-DEOXY-D-GLUCOSE AUTORADIOGRAPHY*

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SUMMARY

The effect of unilateral basilar papilla removal on glucose uptake in the 2nd and 3rd order auditory nuclei in the chick's brain stem, nucleus magnocellularis and nucleus laminaris, respectively, was examined with [3H]2-deoxy-D-glucose (2-DG) autoradiography. The tissue was processed according to a thaw-mount technique, and the number of grains in the resulting autoradiographs was counted to assess changes in glucose uptake. It was observed that there is a greater density of grains over the neuropil regions of nucleus laminaris which receive input from the normal ear than over the corresponding regions which receive input from the operated ear. Similarly, differences in grain density are found between the normally innervated and deafferented magnocellular nuclei although these differences are not as great as those in nucleus laminaris. Differences in grain density were also apparent between the glial/fiber regions which bound the neuropil areas of nucleus laminaris; there is a greater density of grains overlying those glial/fiber regions through which fibers receiving input from the normal ear course than over those regions through which fibers which normally carry input from the operated ear travel. It is likely that this difference mainly reflects glucose uptake in the fibers although a possible contribution of glial tissue cannot be excluded. All these effects of basilar papilla removal are seen with survival times as short as 70 min and thus likely reflect the reduction of neural activity rather than the degeneration of pre- or postsynaptic elements. Finally,

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the same pattern of results as described above was found when using the more common [^{14}C]2-DG procedure or when using [^{3}H]2-DG but processing the tissue using the freeze-dried technique.

The present results thus show the neuropil regions of nucleus laminaris and the adjacent glial/fiber areas to be areas of high glucose utilization. Unilateral basilar papilla removal results in the removal of an excitatory input to these regions, and this results in a reduction of glucose utilization that is specific to those neuropil regions and glial/fiber areas that receive input from the operated ear. These findings are contrasted with another study in which removal of a major excitatory input to the dentate gyrus of the rat results in a reduced glucose utilization which is not specific to the deafferented region and which largely reflects post- rather than presynaptic events.

INTRODUCTION

The development of the 2-deoxy-D-glucose (2-DG) technique for the in vivo determination of glucose consumption by Sokoloff and his associates has provided a non-invasive method to study cerebral patterns of glucose utilization during different functional states[37]. Using this technique, investigators have obtained pictorial representations of regional cerebral glucose utilization resulting from various behaviorally[30,31], physiologically[9,12,32,33], pharmacologically[40], and pathologically[5] induced changes in cerebral functional activity.

Despite these observed changes in glucose consumption, a number of fundamental questions still remain unanswered. For example, it is not known which aspect or aspects of functional activity–synaptic transmission, action potentials, slow wave activity, etc. — are reflected by changes in glucose consumption. In addition, it is not known which cellular component or components: axons, dendrites, neuron cell bodies, presynaptic terminals, glial cells, etc. — are responsible for the changes in glucose consumption which have been observed. This latter question has only been directly addressed by a few studies (e.g. see the recent study of Schwartz et al.[28]), and the complexity of most vertebrate neural systems will make it a difficult question to answer. The relative simplicity of the avian brain stem auditory system, however, makes it well suited for addressing some of these issues. The avian brain stem auditory nuclei, nucleus magnocellularis (NM) and nucleus laminaris (NL), possess a well-defined morphology and functional organization, a homogeneity of cell types and a limited number of inputs which are known to be excitatory in function[3,14,18,22,23,25,26,35]. Most importantly, the highly laminated structure of NL with its spatial segregation of different cellular components and afferent inputs makes it well suited for determining which cellular components are affected by manipulations of afferent input (e.g. see ref. 2).

In the present study, the question of how the elimination of auditory input affects the pattern of glucose consumption in the brain stem auditory nuclei of the chick was examined. The afferent input to NM and NL was manipulated by unilateral removal of the chick's basilar papilla, and the effect of this removal upon glucose

consumption in these nuclei was examined at different postoperative times. In order to assess changes in glucose utilization, we have employed a 2-DG method which uses tritiated-2-DG rather than [14C]2-DG as a tracer[31]. Because of the lower emission energies of ^3H versus ^{14}C, this method can provide greater resolution of glucose consumption than is possible with the commonly used [14C]2-DG procedure.

MATERIALS AND METHODS

The present experiment is based upon 10 experimental and two normal control White Leghorn chicks which were incubated and hatched in the laboratory. Between 6 and 16 days of age posthatch, each experimental bird sustained a unilateral removal of the left basilar papilla. The bird was anesthetized with a combination of Ketalar (8 mg/100 g body weight, i.m.) and EquiThesin (0.15 ml/100 g body weight, i.p.), and under a dissecting scope the tympanic membrane and colummella of the left ear were removed. The basilar papilla was then easily removed using a fine forceps inserted through the exposed oval window. While this procedure severs the distal processes of both the spiral and lagena ganglion cell bodies, previous observations in our laboratory indicate that it does not directly damage the ganglion cell bodies themselves. The middle ear cavity was then packed with Gelfoam, and the skin surrounding the outer ear opening was sealed with alpha Cyanoacrylate adhesive.

Following postoperative survival periods of 8–9 days (long term: n = 2), 9–12 h (medium term: n = 3), or 70–85 min (short term: n = 3), the experimental chicks were again anesthetized and injected intravenously with 170 μCi/100 g body weight of [1,2-^{3}H]2-deoxy-D-glucose (New England Nuclear; 39 mCi/mmol) which had been dessicated to dryness and reconstituted in sterile saline to a final concentration of 2 μCi/ml. They were then exposed to broadband auditory stimulation of tape-recorded music at 80–85 dB (SPL) inside a sound attenuated room (IAC No. 1204). Following 45–60 min of sound stimulation, the chicks were decapitated and their brains quickly removed. Brains were mounted on a cryostat stage covered with minced liver, and the two-thirds of the brain that included the brain stem auditory nuclei was immersed into methylbutane cooled to approximately —150 °C with liquid nitrogen. The tissue was held in this position until a slowly advancing freezing line had covered the remaining one-third of tissue, and the entire brain was then immersed in the methylbutane and transferred to an American Optical Cryo-Cut II cryostat at —20 to —25 °C or stored at —70 °C for later sectioning. The advantage of this two-step freezing procedure is that the brain tissue of interest is immediately frozen while the tissue extending above the methylbutane undergoes a slower freezing that allows for tissue expansion and thereby minimizes tissue fracturing.

The tissue was allowed to equilibrate to cutting temperature for approximately 1 h in the cryostat, and then 5–30 μm coronal sections were cut at —20 to —25 °C through the brain stem auditory nuclei. Sections were mounted under a Thomas Duplex Super Safelight (vanes down) onto slides which had been previously coated with Kodak NTB2 nuclear emulsion, thoroughly air-dried and stored in light-tight boxes with Drierite present. Two methods of mounting sections were used: (1) the cold

mounting of frozen sections onto cold emulsion-coated slides[24]; and (2) the thaw-mounting of thin (5 μm) frozen sections onto room temperature emulsion-coated slides[39]. The thaw-mounting procedure was used in most of the experiments since it generally resulted in more even contact between the tissue and emulsion and less diffusion of the label as judged by the presence of grains around the tissue edges.

Mounted slides were placed in light-tight boxes with Drierite and exposed in a freezer at −30 °C. After 1–4 weeks they were removed in the dark and developed at approximately 17 °C according to the following protocol: chloroform, 1 min; 95% ethanol, 1 min; 70% ethanol, 1 min; 3 changes of distilled water, 1 min each; D19, 3 min; rinse in distilled water; Kodak Rapid Fix A diluted 1-to-1 with distilled water, 5 min; wash in cold-running tap water for 1 h. The slides were then counterstained with cresyl violet and examined in the microscope under both bright- and dark-field illumination. Grain counts were made directly from dark-field photomicrographs (×575) using an acetate overlay with lined grids.

In order to provide a comparison with the more commonly employed [14C]2-DG method[37], one medium term survival experimental chick was injected with 15 μCi/100 g body weight of [14C(U)]2-DG (New England Nuclear; 316 mCi/mmol) which had been desiccated to dryness and reconstituted with sterile saline to a concentration of 100 μCi/ml. Cryostat-cut frozen sections were processed according to the protocol of Sokoloff et al.[37], autoradiographs were prepared using Kodak SB-5 X-ray film, and the tissue sections from which the autoradiographs were prepared were stained with cresyl violet for comparison. In addition, alternate sections from one medium term survival experimental chick injected with [3H]2-DG were processed by the freeze-dried technique to serve as controls against possible chemographic artifacts or translocation of the label which might occur during tissue processing with the thaw-mount procedure[39]. Finally, two normal control birds were injected with [3H]2-DG, exposed to the same broadband auditory stimulation as the experimental chicks and the tissue was processed according to the thaw-mount procedure described above.

RESULTS

Organization of NM and NL

The present report focuses mainly upon NL, a third order auditory nucleus considered to be homologous to the mammalian medial superior olivary nucleus[3,25]. As shown in Fig. 1A, NL receives a binaural, spatially segregated input from NM: NM sends an ipsilateral projection to the dorsal neuropil region of NL and a crossed collateral projection to the ventral neuropil region of contralateral NL. Throughout most of its extent, NL consists of a monolayer cell body lamina which is surrounded dorsally and ventrally by glial-free neuropil regions (Fig. 1B). Glial-rich areas through which the afferent fibers to NL course bound these neuropil regions: afferent fibers from ipsi- and contralateral NM course through the dorsal and ventral glial-rich areas, respectively, but synapses are restricted to the associated neuropil regions and the laminar neuron cell bodies[2].
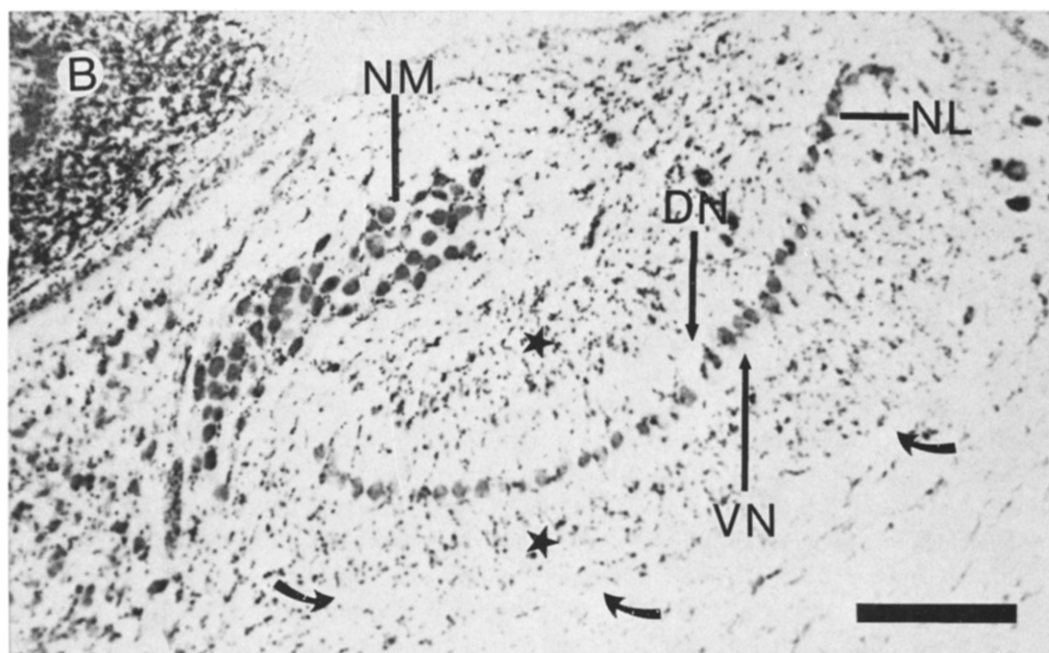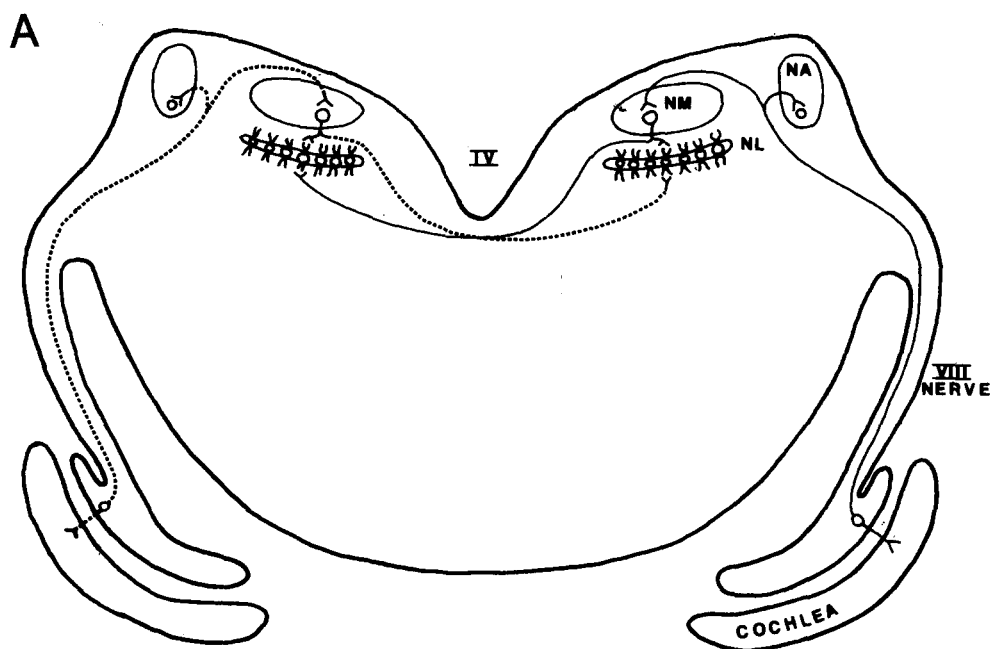
Fig. 1. A: schematic drawing of the chick's brain stem auditory system. Note that nucleus laminaris (NL) receives a binaural, spatially segregated input from nucleus magnocellularis (NM). B: coronal section through NL and NM. The dorsal (DN) and ventral (VN) neuropil regions of NL are bounded by dense glial cell body areas (★) through which afferent fibers innervating NL pass. Arrows indicate the region where axons arising from the contralateral NM course before giving off collaterals which pass through the overlying glial region and innervate the ventral neuropil and laminar neuron cell bodies. Abbreviations for this and following figures: Ax, axonal region; C, cerebellum; CBL, cell body lamina; DN, dorsal neuropil; GF, glial/fiber region; NA, nucleus angularis; NL, nucleus laminaris; NM, nucleus magnocellularis; VN, ventral neuropil; IV, 4th ventricle. Bar = 185 μm.
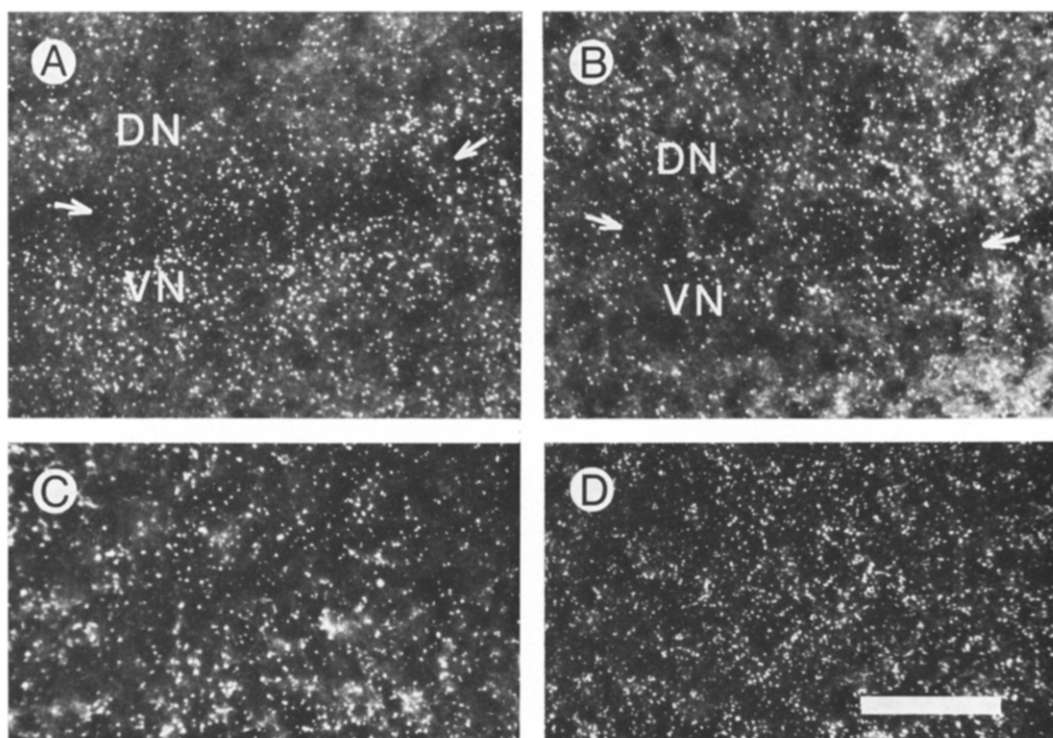
Fig. 2. Dark-field photomicrographs of the distribution of grains across NL (A and B) and NM (C and D) both ipsilateral (A and C) and contralateral (B and D) to a long-term (survival time — 9 days), unilateral basilar papilla removal. Arrows indicate the cell body lamina. The magnocellular nucleus and those neuropil regions of NL receiving input from the operated ear have fewer grains than the corresponding regions which receive input from the unoperated ear. The photomicrographs of NL were made from tissue sections which were exposed for a shorter period of time than those showing NM. Tissue sections were prepared by mounting frozen sections onto cold emulsion-coated slides. Abbreviations as in Fig. 1. Bar — 50 μm.

Effect of basilar papilla removal on NM and NL

Following long-term (i.e. 8–9 days) removal of the basilar papilla and injection with [3H]2-DG, there is a conspicuous asymmetry in the density of grains overlying the neuropil regions of NL (Fig. 2): the regions receiving input from the decochleated ear (the dorsal neuropil ipsilateral and the ventral neuropil contralateral to the decochleated ear) have fewer grains than the neuropil regions receiving input from the normal ear (the ventral neuropil ipsilateral and the dorsal neuropil contralateral to the decochleated ear). Similarly, fewer grains are present over the magnocellular nucleus which receives input from the decochleated versus normal ear although this difference does not appear to be as great as that seen between the neuropil regions of NL.

It has been previously reported that cell atrophy and loss are present in NM as soon as two days following basilar papilla removal while axonal degeneration and glial infiltration are present in NL 5 days postoperatively[15,22]. Thus, it is possible that the lower grain densities over the deafferented regions of NM and NL merely reflect the degeneration of presynaptic terminals and/or postsynaptic sites. That this is not the
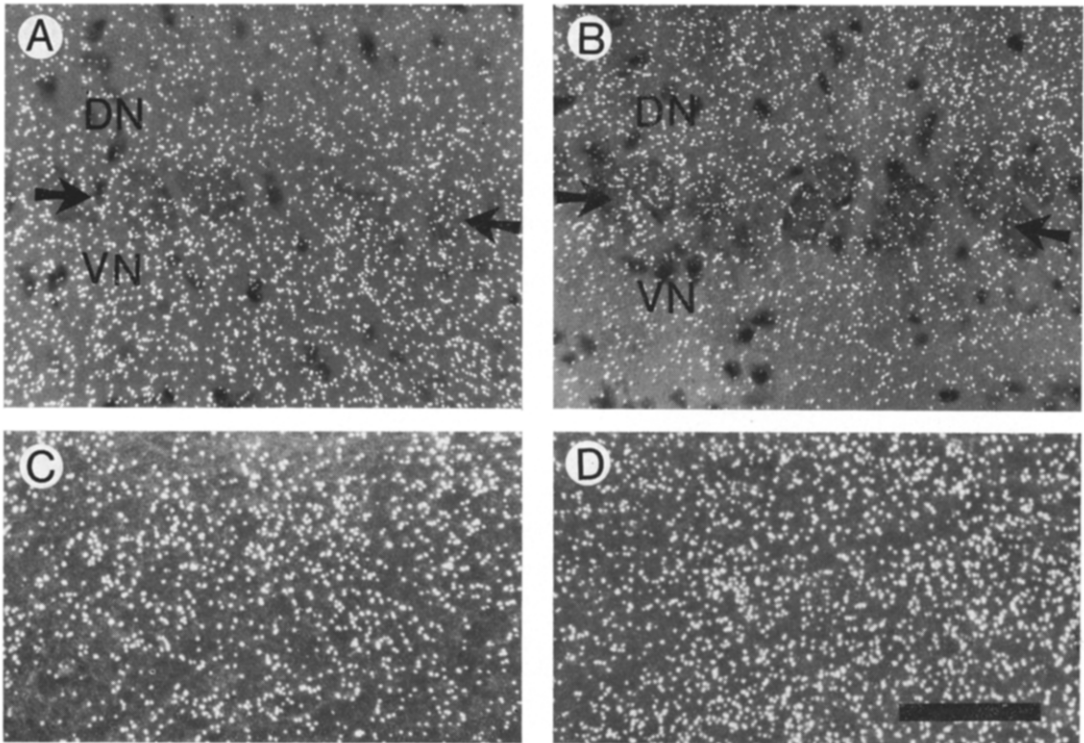
Fig. 3. Dark-field photomicrographs of the distribution of grains across NL (A and B) and NM (C and D) both ipsilateral (A and C) and contralateral (B and D) to a short-term (survival time = 85 min), unilateral basilar papilla removal. Arrows indicate the cell body lamina. The distribution of grains is similar to that observed following a long-term removal. Abbreviations as in Fig. 1. Tissue sections prepared by the thaw-mount procedure. Bar = 50 μm.

case is shown by the observation that in medium-term (i.e. 9–12 h) and, especially, short-term (i.e. 70–85 min) survival birds where these degenerative changes are not yet present, lower grain densities are also present over the neuropil regions of NL which receive input from the decochleated ear (Fig. 3). Similar observations were made for NM although as was found with long-term removals, the differences in grain density between deafferented and normally innervated magnocellular nuclei appeared to be less than the differences observed between the neuropil regions of NL.

In order to both confirm the impression of lower grain densities over the deafferented neuropil regions of NL, and to establish the reliability of this effect, grain counts were done on 1–3 tissue sections/chick in one long-term, two medium-term, and two short-term survival chicks. The ratios of

$$\frac{\text{grain density in the normally innervated neuropil region}}{\text{grain density in the deafferented neuropil region}}$$

were calculated independently for the two sides of the brain in individual tissue sections (Table I). In those brains where the sectioning angle was such that individual tissue sections did not show comparable regions from the left and right laminar nuclei,

TABLE I

Effect of unilateral basilar papilla removal upon glucose consumption in the neuropil and glial/fiber regions of NL

The number of grains in 400 sq.μm areas (40 μm × 10 μm) was counted in the dorsal and ventral neuropil and glial/fiber regions in individual tissue sections. The values shown represent:

$$\frac{\text{average number of grains/400 sq.}\mu\text{m over regions receiving input from the normal ear}}{\text{average number of grains/400 sq.}\mu\text{m over regions receiving input from the operated ear}}$$

and were calculated separately for the sides of the brain ipsilateral (Ipsi) and contralateral (Contra) to the operated ear. Counts corrected for background. LT, long-term survival; MT, medium-term survival; ST, short-term survival.

Bird/Section	Condition	Neuropil		Glial/Fiber	
		Ipsi	Contra	Ipsi	Contra
		Ratio of $\dfrac{Grain\ density\ normally\ innervated\ region}{Grain\ density\ deafferented\ region}$			
78-1101	LT	1.67	1.84		
78-1114*	MT				
5C		1.74	2.16	5.84	5.02
79-1119	MT				
87; 83B		2.21	2.72		
24; 22B		1.53	1.62		
30; 28B		1.51	1.60	2.60	2.47
79-1120	ST				
63D		1.49	1.75	2.46	2.53
67D		1.44	1.54		
79-1123	ST				
24B		1.33	1.67	2.72	2.18
27B		1.50	1.66		

* Values for the neuropil and glial/fiber regions are based upon counts from different tissue sections which were exposed for different time periods.

comparisons of grain ratios across NL ipsi- and contralateral to the operated ear were made from two tissue sections which included matching regions. These ratios show that the grain densities over the normally innervated neuropil regions are 35–170% (median 72%) greater than the densities over the deafferented regions.

It is also interesting to note that the difference in grain densities between the normally innervated and deafferented neuropil regions are not equivalent for the two sides of the brain (e.g. see Fig. 3): the difference between the dorsal and ventral neuropil regions contralateral to the operated ear is 10–50% greater than the difference between the neuropil regions ipsilateral to the operated ear. One of the factors which might contribute to this difference between the two sides of the brain is a greater amount of glucose utilization in the dorsal versus ventral neuropil regions of normal chicks. To test this possibility, the ratio of

$$\frac{\text{grain density in the dorsal neuropil}}{\text{grain density in the ventral neuropil}}$$
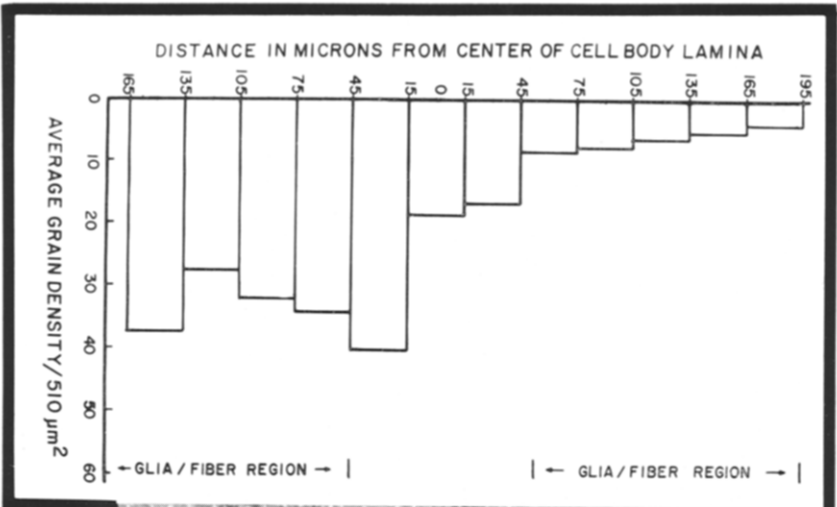
was calculated in three tissues from the two normal control birds which had been injected with [³H]2-DG and exposed to broadband auditory stimulation. The resulting ratios of 1.02, 1.07, 1.11, 1.15, 1.24 and 1.29 indicate that the uptake of 2-DG is greater in the dorsal versus ventral neuropil regions of normal birds. Thus, the difference between the two sides of the brain following a unilateral basilar papilla removal reflects, in large part, this normally occurring asymmetry in glucose utilization. On the basis of the present data it is not possible to determine whether or not a differential effect of basilar papilla removal on glucose uptake in the dorsal and ventral neuropil regions also contributes to this difference between the two sides of the brain.

Table I fails to reveal any clear relationship between the length of survival following basilar papilla removal and the relative number of grains over the normally innervated versus deafferented neuropil regions. Ipsilateral to the operated ear, the ratios were slightly smaller for the short-term survival birds than for the other groups. On the other hand, the largest ratios were found for the medium- and not the long-term survival birds. Contralateral to the operated ear, no trend was apparent.
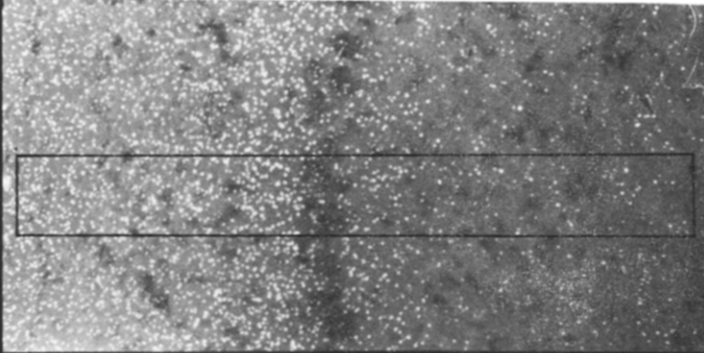
Effect of basilar papilla removal on glial/fiber regions bounding NL

Conspicuous differences in grain density are also present over the glial/fiber areas which bound the neuropil regions in NL. The most dramatic difference in grain density we observed between the glial/fiber regions is shown in Fig. 4, but all autoradiographs examined showed the same trend. There are fewer grains present over the glial/fiber regions through which axons activated by the decochleated ear normally travel (the dorsal glial/fiber region ipsilateral and the ventral glial/fiber region contralateral to the decochleated ear) than over the glial/fiber regions through which projections from the normal ear travel (the ventral glial/fiber region ipsilateral and the dorsal glial/fiber region contralateral to the decochleated ear). In fact, examination of the autoradiographs suggests that the difference in grain density between the glial/fiber regions is actually greater than the difference between the neuropil regions. This impression was confirmed by grain counts from single tissue sections in four different chicks where the differences in grain density between glial/fiber areas were found to be 30–100% greater than the differences between the associated neuropil regions (Table I). As shown in Fig. 4, the greater difference in grain density between the glial/fiber regions is due to the fact that there are fewer grains over the glial/fiber region which bounds the deafferented neuropil than over the deafferented neuropil itself.
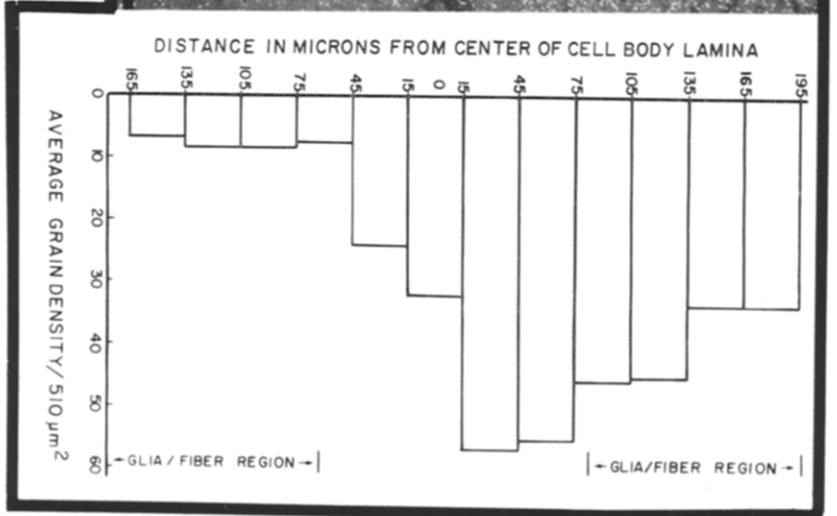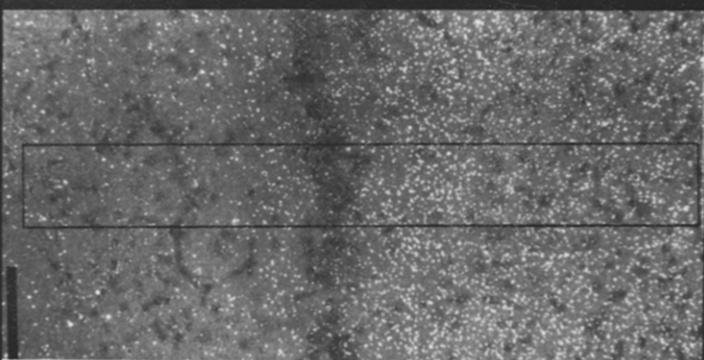
Differences in grain density are also present over the axonal areas which lie just ventral to the ventral glial/fiber regions. As previously noted (Fig. 1B), the axons in this region arise from the contralateral NM and give off collaterals which course through the ventral glial/fiber region before synapsing in the ventral neuropil and on the laminar neuron cell bodies. Fig. 5 illustrates that the grain density over these axonal areas appears comparable to the density over the adjacent glial/fiber regions. Thus, a low density of grains is present over the axonal and ventral glial/fiber regions contralateral to the operated ear while ipsilaterally there is a high grain density over these regions.

IPSI

CONTRA

DISTANCE IN MICRONS FROM CENTER OF CELL BODY LAMINA

AVERAGE GRAIN DENSITY/510 μm²

← GLIA / FIBER REGION →
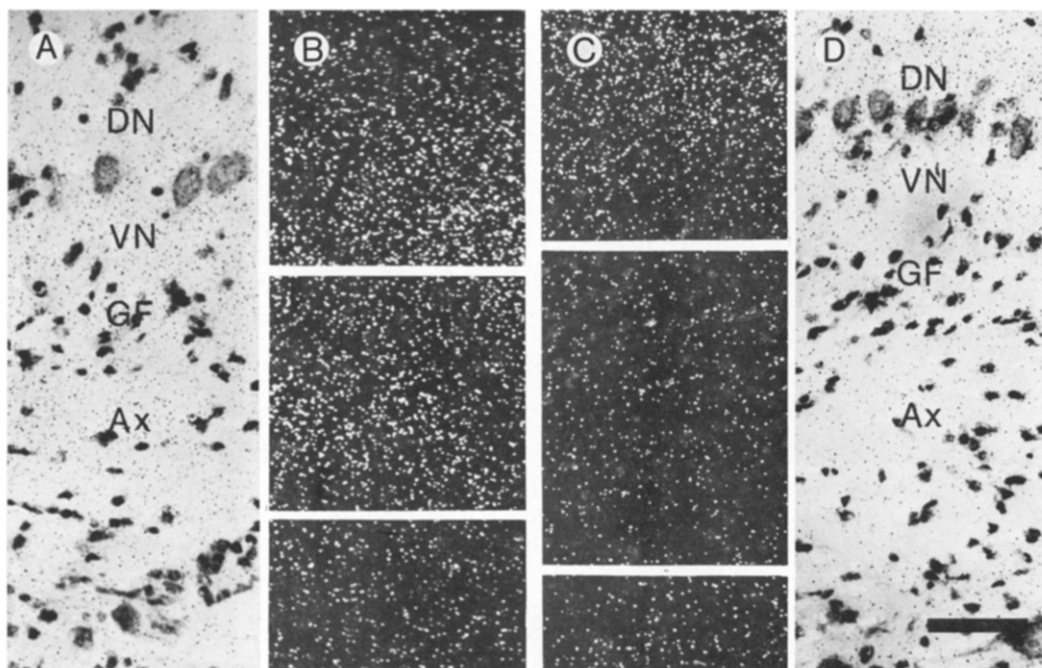
← GLIA / FIBER REGION →

Fig. 5. Corresponding dark- and bright-field photomicrographs of both NL and the ventrally located glial/fiber (GF) and axonal (Ax) regions ipsilateral (A and B) and contralateral (C and D) to a unilateral basilar papilla removal. Note that the grain density in each glial/fiber region appears comparable to that in the adjacent axonal region. Tissue sections prepared by the thaw-mount procedure. Survival time = 11 h. Abbreviations as in Fig. 1. Bar = 60 μm.

Effect of basilar papilla removal demonstrated with freeze-dried tissue and [^{14}C]*2-DG*

It should finally be noted that the same pattern of results obtained with [^{3}H]2-DG and the thaw-mount procedure is obtained when the tissue is freeze-dried (Fig. 6), or when the more conventional [^{14}C]2-DG method is used (Fig. 7). Since the freeze-dried procedure virtually eliminates problems of chemography and translocation of label during tissue processing, it would appear that the thaw-mount procedure, routinely used for the autoradiography of other water-soluble compounds[39], can be successfully used with 2-DG without the results being confounded by these possible artifacts.

Fig. 4. Dark-field photomicrographs of NL showing that the difference in grain density between the dorsal and ventral glial/fiber regions both ipsilateral (ipsi) and contralateral (contra) to a unilateral basilar papilla removal is similar to the difference in grain density between neuropil regions which they bound. Graphs show the results of grain counts made in the rectangular sectors outlined in the photomicrographs. Tissue sections prepared by the thaw-mount procedure. Survival time = 10.5 h. Bar = 50 μm.
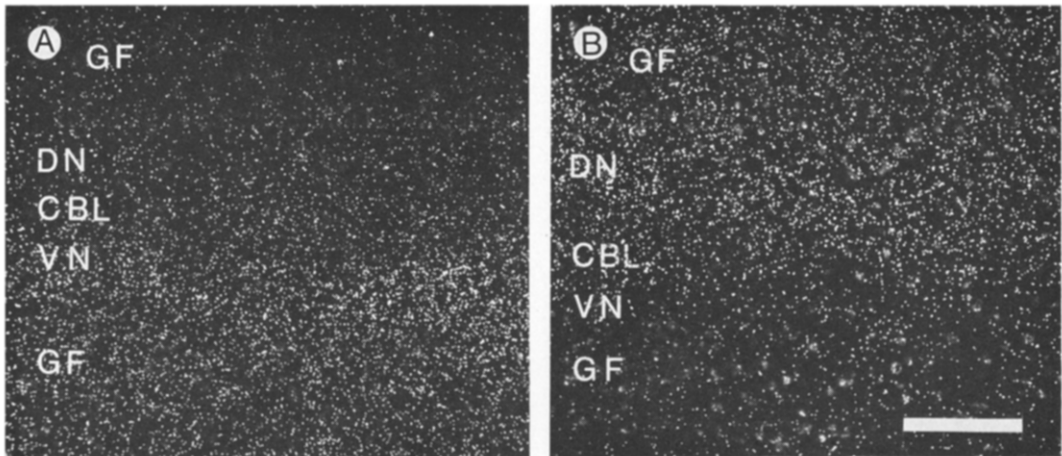
Fig. 6. Dark-field photomicrographs of the distribution of grains across NL and the adjacent glial/fiber regions both ipsilateral (A) and contralateral (B) to a unilateral, basilar papilla removal. The results seen in this tissue prepared by the freeze-dried technique are comparable to those observed with the thaw-mount procedure. Survival time = 11 h. Abbreviations as in Fig. 1. Bar = 75 μm.
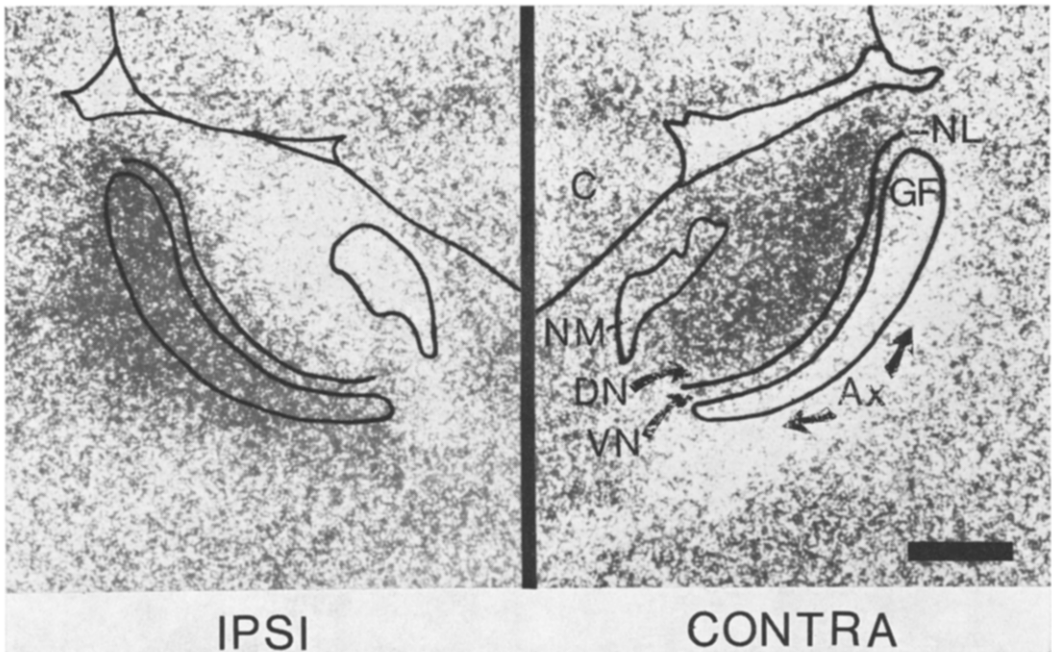

Fig. 7. The effect of a unilateral basilar papilla removal upon glucose utilization in a bird which had been injected with [14C]2-DG and processed by the standard [14C]2-DG procedure of Sokoloff et al.[37]. Regions of high glucose utilization are seen as darkenings in these autoradiographs. The lines labeled NL pass through the center of the cell body lamina, and the glial/fiber (GF) and axonal regions (Ax) which lie ventral to NL are clearly indicated. The results appear comparable to those seen with the [3H]2-DG procedure. (1) The magnocellular nucleus and those neuropil regions of NL receiving input from the operated ear show less glucose utilization than the corresponding regions which receive input from the normal ear. (2) The glial/fiber regions show differences in glucose utilization which are qualitatively similar but more striking than the differences between the neuropil regions they bound. (3) The greater difference in glucose utilization between the glial/fiber regions is due to a striking depression of glucose utilization in the glial/fiber regions through which input from the operated ear to NL travels. (4) The glial/fiber and axonal regions which lie ventral to NL appear to show comparable degrees of glucose utilization. Survival time = 12 h. Abbreviations as in Fig. 1. Bar = 300 μm.

DISCUSSION

The present experiment has shown that unilateral ablation of the basilar papilla results in an immediate and persistent reduction of glucose consumption in the magnocellular nucleus and those portions of NL which receive input from the operated ear. The reduction in glucose consumption cannot be attributed to the degeneration of either presynaptic terminals or postsynaptic sites since reduced glucose consumption occurs as soon as 70 min following basilar papilla removal. These results are thus consistent with previous findings that sensory deprivation or ablation of peripheral sensory receptors results in reduced glucose consumption[9,29,34,36].

The consequences of basilar papilla removal in birds have not been studied electrophysiologically, however, electrophysiological studies of the auditory system of normal birds have shown that NM receives excitatory input from the VIIIth nerve while NL receives excitatory input from NM[14,18,25,26]. This, coupled with the observation that hair cell elimination in mammals results in the elimination of spontaneous and acoustically evoked activity in both the VIIIth nerve and cochlear nuclei[16,17], strongly suggests that the reduced glucose consumption observed here reflects a reduction in impulse conduction and excitatory synaptic transmission.

The laminar organization of NL has made it possible to associate patterns of glucose consumption with specific cellular components, something which is difficult to accomplish in most vertebrate neural systems in which different cellular components do not have a clear spatial segregation. First, the normally innervated neuropil regions of NL showed a high level of glucose consumption, an observation which is consistent with previous studies which have reported both high levels of glucose consumption and metabolic activity in other neuropil regions of high synaptic density[4,18,21,33] and the high affinity transport of deoxyglucose into synaptosomal fractions[7]. Unilateral ablation of the basilar papilla resulted in a decrease in glucose consumption which was specific to the neuropil regions receiving input from the operated ear. This finding extends previous observations in the olfactory bulb of large decreases in glucose utilization in neuropil regions following reductions in sensory input[29,34]. On the basis of the present data it is not possible to associate the changes in glucose utilization observed here with the dendritic and/or nerve terminal compartments of the neuropil. However, the recent finding of a high correlation between neural activity and glucose utilization in the nerve terminals of the hypothalamic-neurohypophysial projections[28] suggests that the changes in glucose utilization observed here are occurring, at least in part, within axon terminal endings.

Secondly, a major reduction in glucose utilization was observed in the glial/fiber regions bounding the deafferented neuropil regions. The present data do not indicate whether the low grain density over these areas is primarily due to the reduction of glucose consumption in the axonal or glial compartments. A reduction of glucose consumption in axons might be expected since the elimination or reduction in impulse conduction which likely results from ablation of the basilar papilla would result in the reduction of such energy dependent processes as the active pumping of ions across the axonal membrane. In fact, examination of the axonal regions which lie

adjacent to the glial-dense areas beneath NL provides some support for this idea. The axons which occupy these regions give off collaterals that turn dorsally and course through the ventral glial-dense areas before synapsing in the ventral neuropil and on the laminar neuron cell bodies. Since the density of grains overlying the axonal and glial-dense regions appears to be comparable, one might infer that the grains overlying the glial-dense regions primarily reflect glucose utilization in the axon collaterals coursing through the regions rather than glucose utilization in the glial cells themselves. On the other hand, a possible contribution of glial tissue should not be excluded. It has recently been reported that following injection of [3H]2-DG, grains over axons are localized primarily over the myelin sheaths and not over the axon proper[8]. In addition, glial cells are known to perform such energy-dependent functions as the synthesis and transport of proteins into axons[10,20], and the accumulation of extracellular potassium that is released during neural activity[11,12], and it has been suggested that these functions are directly linked to or triggered by neural activity. Thus, in the absence of the neural 'trigger', a reduction in the glial uptake and utilization of glucose necessary to drive such functions might occur. In this context, it is interesting to note the recent report that under conditions of low extracellular potassium concentration, a condition which would result from the elimination or reduction of impulse conduction, there is a reduced uptake of glucose by glial cells[6].

It is instructive to compare the present results with those of a recent study which has examined the effects of entorhinal cortical lesions upon glucose uptake in the dentate gyrus of the rat[38]. Thus, in both studies the effects of removal of a major excitatory input upon a laminated structure have been studied. In this latter study, only slight decreases (5–10%) in 2-DG utilization were found, the decreases were not specific to the deafferented portion of the postsynaptic cell but occurred throughout the cell, and it was concluded that the reduced 2-DG uptake did not reflect presynaptic events. In contrast, the present study has found larger decreases (35–170%) in 2-DG uptake, the decreases were specific to the neuropil regions of NL which receive input from the operated ear, and the finding of decreased 2-DG uptake over those regions in which the afferent fibers to NL course indicates that the reduced 2-DG uptake reflects, in part, presynaptic events. While the manipulations in these two studies were different, the results do emphasize that two systems may respond quite differently to removal of a major excitatory input. These results and those of a recent study which examined the effect of lateral hypothalamic lesions upon brain glucose consumption[27] also emphasize the caution one must exercise in making general statements about which cellular compartments are responsible for and which cellular events are reflected by 2-DG uptake.

It should finally be noted that the use of [3H]2-DG offers certain advantages over the more commonly used [14C]2-DG procedure. While the tissue processing is slightly more time-consuming, the use of [3H]2-DG enables the distribution of grains over different neural regions to be both directly observed and easily quantified. Additionally, a higher degree of resolution than is possible using [14C]2-DG can be obtained at a relatively low cost. In fact, the recent demonstration of the labeling of single cells in the retina of goldfish using [3H]2-DG[1] suggests that it may be possible to

resolve differences in 2-DG uptake by single cells using this method. Thus, the use of [^{3}H]2-DG might be profitably used in conjunction with the conventional [^{14}C]2-DG procedure.

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