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Research report

Pyramidal cells in primary auditory cortex project to cochlear nucleus in rat

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Abstract

Recent work has demonstrated that the auditory cortex in rat sends direct projections to the auditory nuclei of the brainstem, including the cochlear nucleus and superior olive. To determine the cortical origin of the projections to cochlear nucleus, Fast Blue, a retrograde fluorescent tracer, was injected into the cochlear nucleus. Labeled cells in the forebrain were then studied with light microscopy and mapped. The projection was found to originate from large pyramidal neurons in layer V of primary auditory cortex. The projection was predominantly ipsilateral, and no labeled neurons were found in other cortical areas. These data imply that primary auditory cortex exerts influence over ascending auditory information at the earliest stages of the central auditory system.

Keywords: Auditory cortex; Cochlear nucleus; Corticofugal; Efferent; Fast blue; Layer V

1. Introduction

The auditory cortex, which may be considered the primary recipient of all ascending auditory information, is also the origin of descending pathways which innervate the lower structures of the auditory pathway. Well-characterized descending projections from auditory cortex innervate the medial geniculate nucleus [6,15] and the inferior colliculus [2,5,6,10]. This efferent pathway is robust and topographic, but as is the case with other descending sensory systems, little is known about its function. The inferior colliculus, originally thought to be an obligate synapse in the descending system, projects topographically to lower auditory nuclei of the brainstem, including the nucleus of the lateral lemniscus, the superior olive and periolivary nuclei, and the cochlear nucleus [3,17]. Although it has long been hypothesized that the auditory cortex could influence signalling in the auditory brainstem via the inferior colliculus, the anatomy of this multisynaptic pathway has been difficult to demonstrate. Recently, through the use of more sensitive anterograde tracers such as biotinylated dextran amine (BDA) and *Phaseolus vulgaris* leucoagglutinin (PHA-L), it has been shown that there are monosynaptic projections from the auditory cortex directly to the auditory brainstem nuclei [8]. These projections bypass the inferior colliculus and synapse on target neurons in the cochlear nucleus and superior olivary nuclei [19,20], indicating that the cortex may have a direct effect on the processing of auditory information at the earliest levels of the auditory system.

In the present study, the fluorescent retrograde tracer Fast Blue was injected into the cochlear nucleus of the rat. Tissue was analyzed to determine which cortical areas and cell populations gave rise to the descending corticobulbar fibers. The descending projections were found to originate solely from layer V pyramidal neurons of primary auditory cortex.

2. Materials and methods

Five adult male Sprague–Dawley rats were used in this study. Rats were anesthetized with pentobarbital at a dosage of 45 mg/kg. The dorsal cochlear nucleus was exposed unilaterally by a posterodorsal approach through the cerebellum. Fast Blue (Sigma) was injected into the cochlear nucleus midway between the ventral and dorsal cochlear nucleus by direct visual control using an operating microscope. An oocyte injector (Drummond) and glass micropipette (20 μm inner tip diameter) were used to inject 0.25 μl of an aqueous 4% Fast Blue solution over a period of 5 min. Immediately after the injection, the animal was sutured and allowed to recover. Three to 4 days later, the

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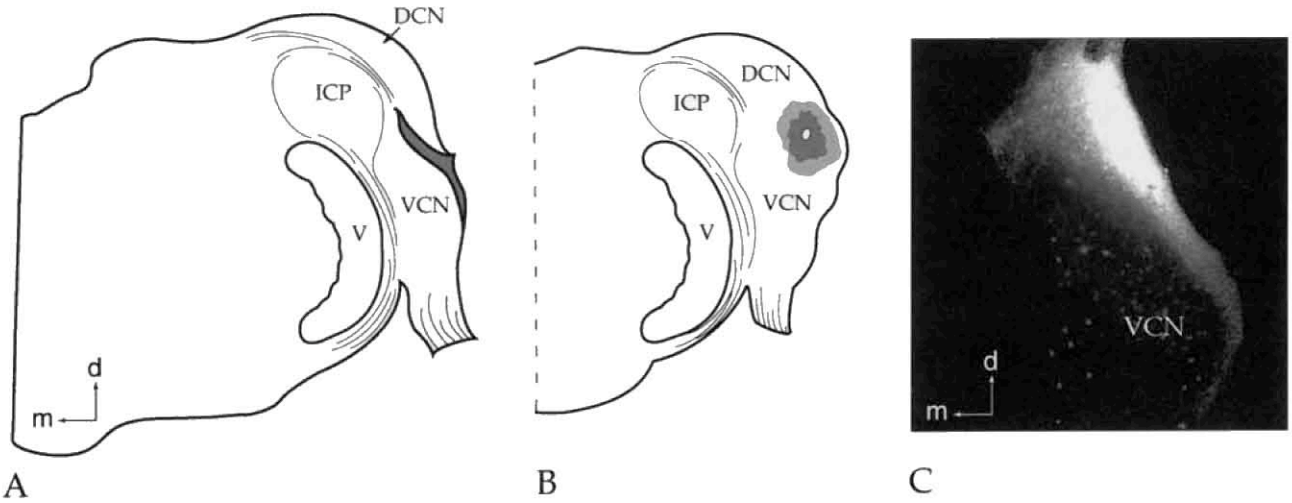

Fig. 1. Coronal sections through the rat brainstem. (A) Schematic illustrating the position of the granule cell lamina, shown in dark grey. (B) Camera lucida drawing of a typical injection site in the cochlear nucleus. Although some swelling and distortion has occurred due to surgery, the injection is clearly in the lamina. (C) Photomicrograph of a rostral section through the cochlear nucleus. At this level, the DCN is not present. The Fast Blue injection has labeled the entire granule cell region overlying the VCN. Few cells in VCN are labeled. Abbreviations: DCN, dorsal cochlear nucleus; ICP, inferior cerebellar peduncle; V, spinal tract of the trigeminal; VCN, ventral cochlear nucleus.

animal was perfused transcardially with 0.1 M phosphate buffered saline (pH 7.3) followed by 10% formalin in phosphate buffer. After perfusion, the brain was removed from the skull and allowed to postfix for 2 h. The cortex and brainstem were sectioned on a Vibratome at 50–75 μm. The sections were collected in serial order and

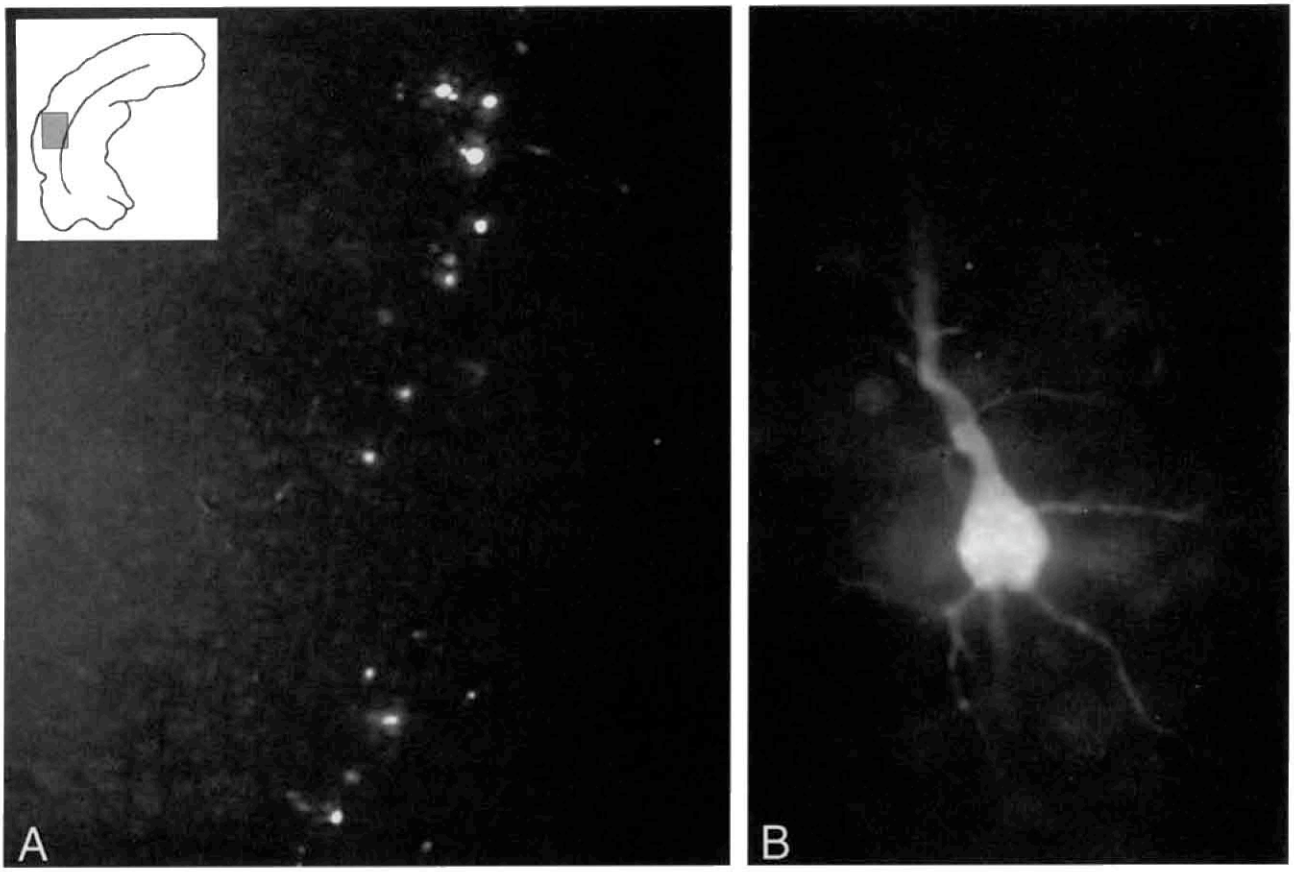

Fig. 2. Fast Blue-labeled cells in auditory cortex. (A) A low magnification fluorescence micrograph shows a band of labeled cells in auditory cortex. The inset shows the location of the photograph. (B) A higher magnification micrograph of a typical labeled pyramidal cell. Pial surface is toward the top.

mounted directly onto clean glass slides and air dried, after which they were coverslipped in Vectashield mounting medium (Vector). Cells labeled with Fast Blue were visualized, counted, and photographed with fluorescence microscopy at a wavelength of 360 nm. Every fourth forebrain section was drawn in brightfield with a projector, including landmarks such as neural structures, fissures, and blood vessels. The labeled cells were then mapped according to landmarks.

3. Results

An injection of anterograde tracer into the auditory cortex of rat labels corticobulbar fibers in the cochlear nucleus [8,19]. Labeled fibers are distributed throughout the granule cell domain but are concentrated in the granule cell lamina (Fig. 1A), located at the border between ventral cochlear nucleus (VCN) and dorsal cochlear nucleus (DCN). In order to identify the cells and cortical field(s) of origin of this projection, Fast Blue was injected directly into the granule cell lamina. The injection sites were typically centered over the lamina itself, and the injections did not extend beyond the cochlear nucleus (Fig. 1B). The injection labeled the entire granule cell domain of the injected nucleus, but there was little label in the magnocellular VCN or DCN (Fig. 1C).

Other brainstem auditory nuclei were labeled by the injection, and served as a control for the specificity of the label. Labeled cell bodies were visible bilaterally in the ventral nucleus of the trapezoid body (VNTB). These VNTB cells were more numerous contralateral to the injection, and were presumed to be medial olivocochlear neurons. Large multipolar cells were labeled in the contralateral cochlear nucleus, which is consistent with prior

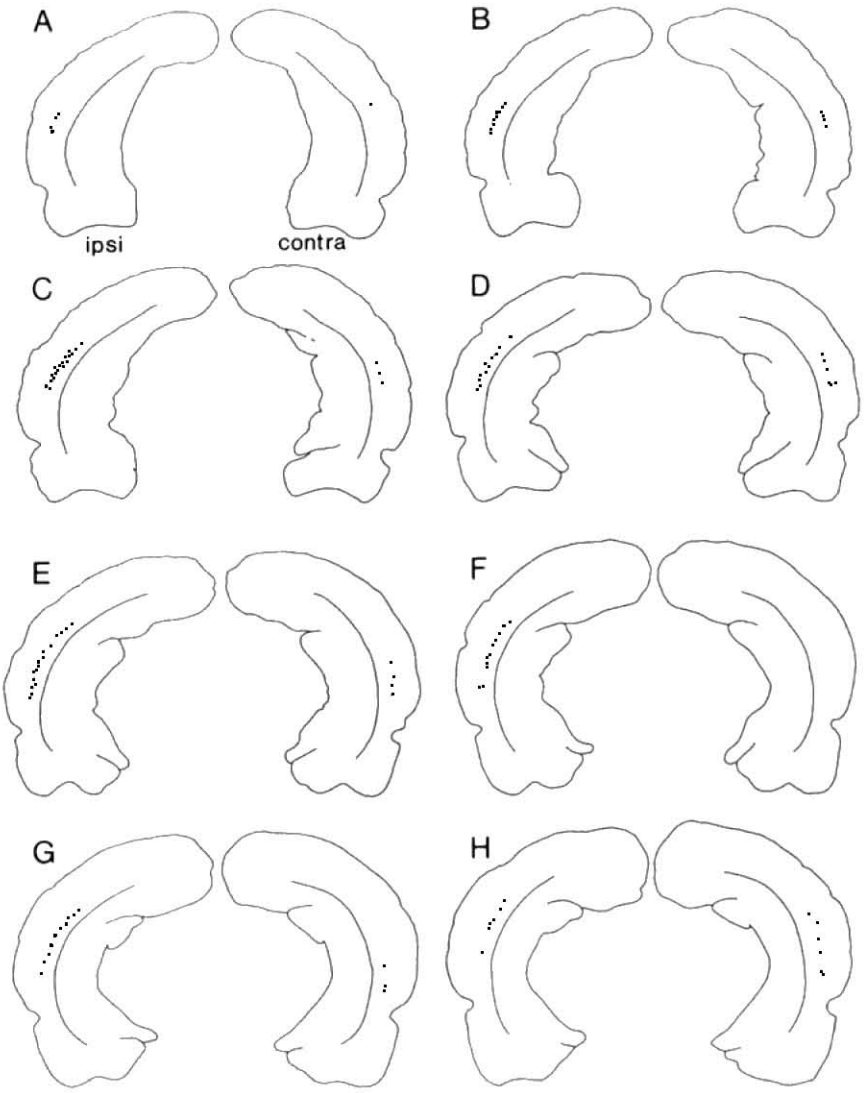

Fig. 3. Camera lucida drawings of eight coronal sections through auditory cortex. To minimize the possibility of contamination, the data shown is from the smallest injection. Sections are arranged from caudal (A) to rostral (H), and each dot represents one labeled cell. Labeled cells are consistently more numerous on the ipsilateral (left) side.

reports [4]. Medium-sized multipolar cells were labeled in both the central and external nuclei of the inferior colliculus. As previously described [7], the labeled neurons of the inferior colliculus were bilaterally distributed, but more numerous on the side ipsilateral to the injected cochlear nucleus compared to the contralateral side (data not shown).

The focus of this report is the population of labeled cells in the temporal cortex. The neurons were distributed in a band within layer V (Fig. 2A). All labeled cells exhibited the characteristic morphology of pyramidal cells, including a pyramidal-shaped cell body, a clearly-defined apical dendrite, and several prominent basal dendrites (Fig. 2B). There was no obvious difference in size, shape, or distribution between labeled cells ipsilateral to or contralateral to the injection site.

The number of labeled cells was, on average, two to three times greater in the cortex ipsilateral to the injection site (Fig. 3). The total number of labeled cells from the most specific injection site was approximately 500 ipsilateral and 200 contralateral to the injection site. This ratio of ipsilateral to contralateral label was similar across animals. The absolute number of labeled cells was directly correlated to the location of the injection site; injections which labeled the largest fraction of the granule cell lamina produced the greatest number of labeled pyramidal neurons.

The distribution of cells in representative sections of auditory cortex were plotted and compared to similar control sections that had been stained for myelin or Nissl substance (Fig. 4). Primary auditory cortex (Te1), which is located on the posteriolateral temporal surface just above the rhinal fissure, can be distinguished by several features. The Nissl stain (Fig. 4A) reveals a prominent layer IV in Te1, marked by the presence of an increased density of cells, comprised mostly of small (granule) cells. Layer V is characterized by a decreased cell density but the presence of many obvious pyramidal cells. Adjacent to Te1 is tissue with less distinct layering, a feature which is characteristic of the separation between primary and secondary sensory cortex. The Weil stain for myelin (Fig. 4B) reveals dark, columnar striations in layers V and IV of primary auditory cortex. The density of this staining tapers off within layer IV. This pattern of myelin staining is consistent with the known distribution of myelinated thalamocortical afferents to primary sensory areas [16]. Cortical areas adjacent to this dark myelin staining exhibited reduced levels of staining. The region of retrogradely labeled cells (Fig. 4C) corresponded to the cyto- and fibroarchitectonic boundaries of primary auditory cortex. When injection sites were entirely confined to the cochlear nucleus, no labeled cells were found in the cortical areas surrounding primary auditory cortex, nor were any found in visual, parietal, or somatosensory cortex, as defined by an atlas of the rat brain [18].

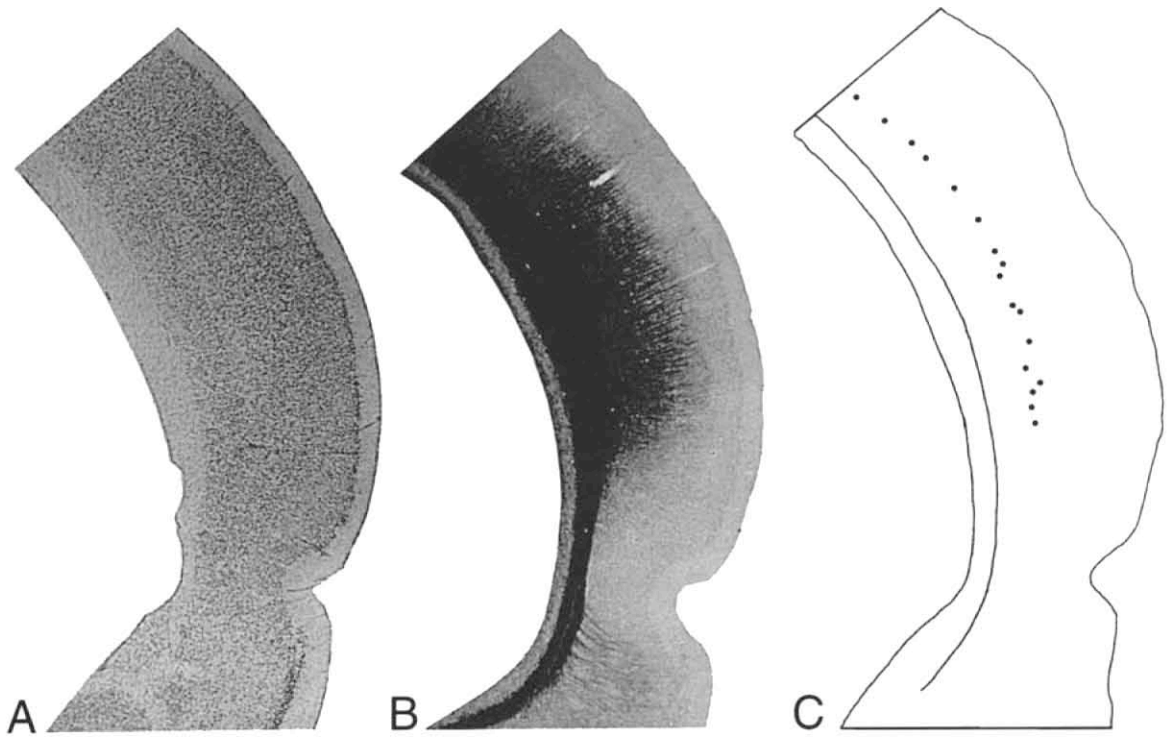

Fig. 4. Comparison of labeled cell distribution with Nissl and Weil stains. (A) Nissl stain of a control section through auditory cortex. Primary auditory cortex is marked by a decreased cell density in layer V. (B) Weil stain for myelin of a control section through auditory cortex. Primary cortex is characterized by dense labeling of myelinated afferents through layers IV and V. (C) Camera lucida drawing of one section through labeled auditory cortex. Fast Blue-labeled cells are shown by dots. There were no other labeled cells above the border of the drawing. The distribution of labeled cells matches the extent of primary auditory cortex, illustrated in (A) and (B).

4. Discussion

The injection of a retrograde marker into the cochlear nucleus labeled pyramidal neurons in layer V of primary auditory cortex. This result confirms anterograde labeling studies where tracers injected into auditory cortex were found to mark corticobulbar axons in the cochlear nucleus [8,19]. Moreover, it greatly expands our knowledge by revealing primary auditory cortex as the sole source of this descending projection, and identifies the projection neurons as layer V pyramidal cells.

Cortical layer V neurons are known to give rise to corticotectal, corticobulbar, and corticospinal pathways in many systems, i.e., [1,11,12,14]. In the auditory system, there is evidence that there are several populations of layer V projection neurons. The corticocollicular projection arises from a population of layer V pyramidal cells that appears distinct from the commissural projection neurons [9]. A double labeling experiment has shown that the auditory corticostriate projection has some overlap with the corticocollicular projection, indicating that some layer V pyramidal neurons send terminals to more than one subcortical area [13]. It is therefore possible that the neurons projecting to the cochlear nucleus are a subset of those that project to inferior colliculus, or to the superior olivary nuclei, or to both. Double and triple labeling experiments, where retrograde tracers are placed into any combination of these three structures will help to determine to what extent individual cells project to one, two, or three independent locations. How we think of cortical organization will be different if, for example, pyramidal cells send collaterals to multiple brainstem structures, or if subpopulations of pyramidal cells project separately to these different sites.

The possibility of contamination must be addressed in any study involving the injection of label. The injection sites for the two experiments from which the displayed data were taken were highly specific. The tracer was injected superficially into the DCN, so that the inferior cerebellar peduncle, which lies medial to the DCN, was not labeled. No tracer was observed in the subdural space around the injection site, so there appeared to be no leakage. Upon examining sections through the brainstem with fluorescence microscopy, it was apparent that the tracer was confined to the cochlear nucleus. Auditory brainstem nuclei were labeled, such as the superior olive and the inferior colliculus, but nuclei that are not known to project to the cochlear nucleus were not labeled.

Another control for the specificity of label was the pattern of labeling in cortex itself. In some preliminary experiments (data not shown), additional label was found outside the cochlear nucleus, contaminating the cerebellar peduncle and spinal tract of the trigeminal nerve. In these cases, the usual pattern of labeling was observed in auditory cortex, but there were also labeled cells in somatosensory cortex. In injection cases which were confined completely to the cochlear nuclei, there was no labeling in somatosensory cortex, indicating that somatosensory label is associated exclusively with contamination. Injections confined to the cochlear nuclei never labeled cells in occipital or parietal cortex, further evidence that the corticobulbar pathway arises solely from auditory cortex.

The distinction between primary and secondary auditory areas by purely anatomical methods is difficult. There has been some disagreement on the exact locations of Te1 (area 41, primary auditory area) and Te2 and Te3 (secondary auditory areas) based on cytoarchitectural, myeloarchitectural, and connectional studies, i.e., [10,16,21]. There are few structural landmarks to use in rat temporal cortex besides the rhinal fissure and the hippocampus, and difficulties in comparison are compounded by differences in the plane of sectioning and collection of tissue across research groups. To circumvent this problem, tissue sections were compared to Nissl and Weil stained material cut in the same plane and mounted at the same angle. Comparison between control and experimental tissue demonstrates that the labeled cells are confined to primary auditory cortex, and do not extend into the secondary areas.

The number of neurons labeled in this study was fairly small, when compared to the number of neurons in the cortico-colliculo-cochlear nucleus pathway. On the basis of rough counts of labeled cells, we estimate that the colliculo-cochlear nucleus projection is at least five times greater than the cortico-cochlear nucleus projection. Nevertheless, the corticobulbar pathway seems sufficiently large to have an effect upon the cochlear nucleus. It may be that both corticobulbar and corticocollicular projections ultimately influence the same neural circuits in the cochlear nucleus, given their axonal convergence to the granule cell lamina [8,17,19]. However, until the specific cellular targets of each projection are described, it is fruitless to speculate on the effects of descending input. The granule cell domains contain both excitatory and inhibitory interneurons, and the connections and influences of these interneurons have not yet been elucidated. The two pathways may also differ in their speed of conduction. The direct corticobulbar projection would be expected to convey messages that will arrive sooner than those with synaptic interruptions, but there may also be differences in axon caliber and myelination which would influence conduction. In summary, until more is known about the descending cortical projections, it is fair to assume that each pathway is unique and necessary.

The function of the efferent pathway between primary auditory cortex and the cochlear nucleus is unknown. The existence of this direct pathway suggests, however, that the cortex may play some role in manipulating or gating ascending information. There are many phenomena of selective listening within the auditory system, and they rely on a wide array of cues, including spatial, spectral, and temporal. A direct communication between cortex and

cochlear nucleus may enable the auditory cortex to select ascending signals based on auditory cues, or to modify basic qualities of the ascending information.

Acknowledgements

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