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Hearing Research 206 (2005) 3–11
HEARING RESEARCH
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Projections from auditory cortex contact cells in the cochlear nucleus that project to the inferior colliculus

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Received 24 September 2004; accepted 7 March 2005
Available online 12 April 2005

Abstract

Anterograde and retrograde tracing techniques were combined to determine whether auditory cortical axons contact cells in the cochlear nucleus that project to the inferior colliculus. FluoroRuby or fluorescein dextran was injected into auditory cortex to label cortical axons by anterograde transport. Different fluorescent tracers (Fast Blue, FluoroGold, FluoroRuby or fluorescein dextran) were injected into one or both inferior colliculi to label cells in the cochlear nucleus. After 12–15 days, the brain was processed for fluorescence microscopy and the cochlear nuclei were examined for apparent contacts between cortical axons and retrogradely labeled cochlear nucleus cells. The results suggest that axons from the ipsilateral or contralateral cortex contact fusiform and giant cells in the dorsal cochlear nucleus and multipolar cells in the ventral cochlear nucleus that project directly to the inferior colliculus. The contacts occur on cell bodies and dendrites. The target cells in the cochlear nucleus include cells that project ipsilaterally, contralaterally or bilaterally to the inferior colliculus. The results suggest that auditory cortex is in a position to exert direct effects on the monaural pathways that ascend from the cochlear nucleus.

Keywords: Auditory system; Descending pathways; Efferent

1. Introduction

The cochlear nucleus (CN) receives all acoustic information from the inner ear, processes this information to various degrees and sends it to a wide variety of targets by means of multiple ascending pathways (reviewed by Cant and Benson, 2003). Several studies have now documented direct projections from the auditory cortex to the CN, suggesting that cortex could modify processing at very early stages of the ascending auditory pathways (Feliciano et al., 1995; Weedman and Ryugo, 1996a,b; Doucet et al., 2003; Jacomme et al., 2003; Schofield and Coomes, 2005). An important clue to functions of the cortical projections is the identity of the target cells in the CN.

Studies in rats show that cortical axons terminate on granule cells in the CN (Weedman and Ryugo, 1996b). Granule cells project in turn to cells in the dorsal cochlear nucleus (DCN), where they can modify the responses of fusiform cells (Oertel and Young, 2004). The fusiform cells are the major source of projections from the DCN; along with giant cells, the fusiform cells project to the inferior colliculus (IC). Most of the cells of

Abbreviations: A1, primary auditory cortex*; CN, cochlear nucleus; DCB, dorsocaudal belt of auditory cortex*; DCN, dorsal cochlear nucleus; DC, dorsal caudal field of auditory cortex*; DRB, dorsorostral belt of auditory cortex*; FB, Fast Blue; FD, fluorescein dextran; FG, FluoroGold; FR, FluoroRuby; IC, inferior colliculus; I–VI, cortical layers; ps, pseudosylvian sulcus; rs, rhinal sulcus; Som, somatosensory cortex*; S, small field of auditory cortex*; T, transition zone of auditory cortex*; VCB, ventrocaudal belt of auditory cortex*; VCN, ventral cochlear nucleus; Vis, visual cortex*; VRB, ventrorostral belt of auditory cortex*; WM, white matter; *, as defined by Wallace et al., 2000, 2002

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doi:10.1016/j.heares.2005.03.005

this pathway project to the contralateral IC. A much smaller number project to the ipsilateral IC, and a subset of these cells project bilaterally to the IC (Schofield and Cant, 1996). Taken together, these studies suggest that the cortical projections to the CN probably modify the activity in intrinsic CN circuits that, in turn, modify the outputs of the DCN to the ipsilateral and contralateral IC.

Studies in guinea pigs revealed a similar projection from auditory cortex to the granule cell areas of the CN (Jacomme et al., 2003; Schofield and Coomes, 2005). Additional cortical projections terminate in regions of the CN outside the granule cell areas, including all three layers of the DCN as well as portions of the ventral cochlear nucleus (VCN). These areas contain cells that project outside the CN, and raise the question of whether cortical axons might make direct contacts with these cells. Such contacts would suggest that cortical projections modify CN output both indirectly, via intrinsic granule cell circuits, and directly via contacts on projection cells. The present experiments were designed to reveal potential contacts between cortical axons and CN cells that project to the IC.

2. Materials and methods

The experiments were carried out on five Hartley guinea pigs weighing 280–480 g (Charles River Laboratories, Wilmington, MA). The care and use of animals were approved by the University of Louisville Animal Care and Use Committee and conformed to the National Institutes of Health guidelines for the care and use of laboratory animals. A brief description of the experimental methods is provided below; details are described in previous reports from this laboratory (e.g., Schofield and Cant, 1999). Each animal was anesthetized with halothane in a mixture of nitrous oxide and oxygen. Prior to surgery, the animal was premedicated with atropine (0.08 mg/kg, i.m.) and ophthalmic ointment was placed in the eyes to protect the cornea during surgery. The scalp was disinfected and shaved, and the animal was placed in a stereotaxic holder fitted with an anesthesia mask. The scalp was incised and infiltrated with a long-lasting local anesthetic (Sensorcaine with epinephrine 1:200,000; Astra USA, Inc., Westborough, MA). Following exposure of the brain, stereotaxic coordinates were used to guide injections of tracers into the appropriate targets (described in detail below). After completion of tracer injections, the exposed brain was covered with Gelfoam and the scalp was sutured. Ketoprofen (3 mg/kg, i.p.) was administered immediately after surgery and again 24 h later to provide 48 h of post-operative analgesia. After surgery, the animal was monitored carefully until it regained the ability to walk. It was then returned to the animal facility, where it was checked daily until it was sacrificed. Ten days after surgery, the animal was anesthetized as described above and the sutures were removed.

Four different fluorescent tracers were used: FluoroRuby (FR, tetramethyl rhodamine-conjugated dextran, MW = 10,000; 10% in saline, Molecular Probes), fluorescein dextran (FD, MW = 10,000; 10% in saline, Molecular Probes); Fast Blue (Sigma); and FluoroGold (FluoroChrome Inc., Engelwood, CO). Injections were made with 10 μl Hamilton syringes, each dedicated to use with only one of the tracers. One tracer was injected into left temporal cortex to label axons that project to the cochlear nucleus. Injections (0.1–0.2 ml) were made at 22–32 sites within temporal cortex. Different tracer(s) were then injected into one or both IC (Table 1). Injections (0.1–0.2 μl each) were made at four sites within each IC.

Following a survival time of 12–15 days, each animal was sacrificed with an overdose of sodium pentobarbital (440 mg/kg, i.p.) and fixed by perfusion through the heart with Tyrode's solution, followed by 300 ml of 0.1 M phosphate buffer, pH 7.4 (PB) containing 4% paraformaldehyde and then 300 ml of 4% paraformaldehyde and 10% sucrose in PB. The brain was removed and stored at 4 °C in 4% paraformaldehyde and 30% sucrose in PB. Before sectioning, the cortical surface was photographed with a fluorescence microscope to document the injection site. Frozen sections were cut 50 μm thick and collected into six series. Each series was mounted onto slides and allowed to dry. One series was counterstained with thionin; then all series were coverslipped with DPX (Aldrich).

Sections were examined in a Zeiss fluorescence microscope. Areas of the CN were identified according to criteria described by Hackney et al. (1990). Apparent contacts between labeled boutons and labeled cells were examined with a high numerical aperture objective (63× objective with NA = 1.25 or 100× objective with NA = 1.30). Contacts were photographed with a cooled digital camera (Magnafire, Optronics, Inc.). Separate photographs were taken through each fluorescence filter and then the images were overlaid with Adobe Photoshop. Photoshop was used to adjust image contrast, brightness and color balance and to add labels.

Table 1
Locations of tracer injections in each case

Case #	Tracer injected into left AC	Tracer injected into left IC	Tracer injected into right IC
GP367	FR	FD	None
GP369	FD	FG	FR
GP376	FR	FD	FB
GP377	FR	FB	FD
GP380	FR	FD	FB

AC – auditory cortex; FB – Fast Blue; FD – fluorescein dextran; FG – FluoroGold; FR – FluoroRuby; IC – inferior colliculus.