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Projections from auditory cortex to midbrain cholinergic neurons that project to the inferior colliculus

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Abstract

We have shown that auditory cortex projects to cholinergic cells in the pedunculo pontine tegmental nucleus (PPT) and laterodorsal tegmental nucleus (LDT). PPT and LDT are the sources of cholinergic projections to the inferior colliculus, but it is not known if the cortical inputs contact the cholinergic cells that project to the inferior colliculus. We injected FluoroRuby into auditory cortex in pigmented guinea pigs to label cortical projections to PPT and LDT. In the same animals, we injected Fast Blue into the left or right inferior colliculus to label PPT and LDT cells that project to the inferior colliculus. We processed the brain to identify cholinergic cells with an antibody to choline acetyltransferase, which was visualized with a green fluorescent marker distinguishable from both FluoroRuby and Fast Blue. We then examined the PPT and LDT to determine whether boutons of FluoroRuby-labeled cortical axons were in close contact with cells that were double-labeled with the retrograde tracer and the immunolabel. Apparent contacts were observed ipsilateral and, less often, contralateral to the injected cortex. On both sides, the contacts were more numerous in PPT than in LDT. The results indicate that auditory cortex projects directly to brainstem cholinergic cells that innervate the ipsilateral or contralateral inferior colliculus. This suggests that cortical projections could elicit cholinergic effects on both sides of the auditory midbrain.

Keywords

arousal; pedunculo pontine nucleus; laterodorsal nucleus; sensory gating; acoustic startle; prepulse inhibition

The inferior colliculus (IC) is a large midbrain nucleus that integrates auditory and other information from many brainstem nuclei and numerous cortical regions and serves as the primary source of auditory projections to the thalamus (Winer and Schreiner, 2005). Several types of data suggest that most or all of the IC cells could be affected by inputs from the brainstem cholinergic system. Acetylcholinesterase, the degradative enzyme for acetylcholine, as well as nicotinic and muscarinic receptors, the two major classes of cholinergic receptors, are distributed throughout the IC (Shute and Lewis, 1967; Schwartz, 1986; Glendenning and Baker, 1988; Henderson and Sherriff, 1991; Morley and Happe, 2000). Physiological studies have confirmed that most IC cells are affected by acetylcholine (Watanabe and Simada,

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1973; Farley et al. 1983; Habbicht and Vater, 1996). These effects are considered modulatory in the sense that application of acetylcholine to IC cells has little effect on their firing at rest but can dramatically alter their responses to sounds. The effects vary across cells and include either enhancement or suppression of evoked responses. Finally, acetylcholine has been implicated in plasticity in the IC induced by fear conditioning (Ji et al., 2001). In this situation, pairing a leg shock with a tone can lead to changes in the frequency tuning of IC cells. This plasticity is blocked if the muscarinic antagonist atropine is applied to the IC prior to conditioning. It seems likely that acetylcholine plays multiple roles in the IC, but identifying these roles has been hindered by lack of information about the underlying circuitry.

The cholinergic inputs to the IC originate from two large tegmental nuclei – the pedunculo pontine and laterodorsal tegmental nuclei (PPT and LDT; Motts and Schofield, 2009). These nuclei are well known as the primary sources of cholinergic projections to much of the brainstem and spinal cord as well as to the thalamus (e.g., Rye et al., 1987; Hallanger et al., 1987; Hallanger and Wainer, 1988; Woolf and Butcher, 1989). Their widespread projections are associated with a wide range of functions, including arousal, the sleep-wake cycle, motor control and sensorimotor gating (e.g., Diederich and Koch, 2005; Mena-Segovia et al., 2005; Winn, 2006; Jones, 2008; Takakusaki, 2008; Jenkinson et al., 2009).

Recently, we identified direct projections from primary auditory cortex to the PPT and LDT (Schofield and Motts, 2009). At least some of the cortical axons appear to terminate on the cholinergic cells. This finding was unexpected in that there are no other reports of auditory cortex, or other primary sensory cortical areas, projecting to the PPT or LDT. The functions of these projections would presumably be reflected in the projections of the target cells. We speculated that the auditory cortical projections contact PPT and LDT cells that project to other auditory nuclei. However, the PPT and LDT project to a number of auditory nuclei, including the medial geniculate body, IC, and cochlear nucleus, raising the question of which (if any) of these output pathways may be the targets of the AC projections (Hallanger et al., 1987; Shute and Lewis, 1967; Steriade et al., 1988; Tebectis, 1972; Woolf and Butcher, 1986; Motts and Schofield, 2005, 2009). For the present study, we combined anterograde and retrograde fluorescent tracers with fluorescent immunohistochemistry to determine whether AC axons are likely to contact cholinergic cells in the PPT and LDT that project to the inferior colliculus. The purpose of the present report was to determine whether the targets of the auditory cortical axons include cells that project to the inferior colliculus.

EXPERIMENTAL PROCEDURES

All procedures were performed in accordance with the Institutional Animal Care and Use Committee and NIH guidelines. Six adult pigmented guinea pigs (Elm Hill; Chelmsford, MA) of either gender weighing 400–900 grams were used. During all experiments, efforts were made to minimize suffering and the number of animals used.

Surgery

Each guinea pig was anesthetized with isoflurane (4–5% for induction, 1.75–2.25% for maintenance) in oxygen. The animal was given atropine sulfate (0.05 mg/kg, i.m.) to minimize respiratory secretions during anesthesia and Ketofen (ketoprofen, 3 mg/kg, i.m.; Henry Schein, Melville, NY 11747) for post-operative pain control. The animal's head was shaved and disinfected. Moisture Eyes PM ophthalmic ointment (Bausch & Lomb, Rochester, NY) was applied to each eye. The animal's head was positioned in a stereotaxic frame. Body temperature was maintained with a feedback-controlled heating pad. Sterile instruments and aseptic technique were used for all surgical procedures. An incision was made in the scalp and the surrounding skin was injected with Marcaine (0.25% bupivacaine with epinephrine 1:200,000; Hospira, Inc., Lake Forest, IL), a long-lasting local anesthetic. A small hole was drilled in the

skull using a dental drill. Following the tracer injection, Gelfoam (Harvard Apparatus, Holliston, MA) was placed in the craniotomy site and the scalp was sutured. The animal was then removed from the stereotaxic frame and placed in a clean cage. The animal was monitored until it could walk, eat and drink without difficulty.

Injections into the auditory cortex were made as described previously (Schofield and Motts, 2009). Briefly, FluoroRuby (tetramethylrhodamine dextran amine, 10,000 MW, D-1817 Invitrogen, Carlsbad, CA; 10% in saline) was injected into the left temporal cortex in each animal. Injections were made with a 10 μ l Hamilton microsyringe, angled approximately perpendicular to the cortical surface. The syringe was inserted approximately 1 mm into cortex, 0.1–0.2 μ l was injected as a single bolus, and then the syringe was removed. Injections were made at 5 – 18 sites in order to label projections from a wide area of auditory cortex. In all cases, the array of injections was centered on primary auditory cortex (A1), localized approximately according to surface landmarks (Bregma; pseudosylvian sulcus; cf. Wallace et al., 2000, 2002).

Stereotaxic coordinates were used to guide injections of Fast Blue (EMS-Chemie GmbH, Gross Umstadt, Germany; 5% in water) into one IC. Fast Blue was injected with a 1 μ l Hamilton microsyringe. The tracer was injected at 2–4 sites within the IC (total volume = 0.3–0.8 μ l).

Perfusion and sectioning

After 7–14 days, the animal was perfused with Tyrode's solution followed by 250 ml of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 followed by 250 ml of the same fixative with 10% sucrose added. The brain was removed and stored at 4°C in fixative with 25% sucrose. The following day the cerebellum was removed and the brain was frozen and cut in the transverse plane on a sliding microtome into 50 μ m thick sections, which were collected serially in 6 sets. For identification of cytoarchitectural borders and landmarks, one series was stained with thionin. The remaining series were used for immunohistochemistry.

Immunohistochemistry

Putative cholinergic cells were stained with immunohistochemistry for choline acetyltransferase (ChAT). Details of the procedure are described in Motts et al. (2008) Briefly, the sections were exposed (1–3 days at 4°C) to goat anti-ChAT polyclonal antibody (Chemicon AB 144P, diluted 1:25 to 1:100). The sections were treated with 1% biotinylated rabbit anti-goat antibody (Vector BA-5000) and labeled with streptavidin conjugated to AlexaFluor 488 (green; Invitrogen; Carlsbad, CA). The sections were mounted on gelatin-coated slides, allowed to dry and coverslipped with DPX (Sigma). We have previously reported the results of control experiments for the ChAT antibody, as used in the present experiments (Motts et al. 2008). Control studies included Western blot analysis of guinea pig brain tissue as well as staining in tissue sections that was eliminated by pre-adsorption of the primary antibody with ChAT or by omission of primary or secondary antibody from the solutions.

Data analysis

Injection sites were plotted with a Neurolucida reconstruction system (MBF Bioscience, Williston, VT) attached to a Zeiss Axioplan II microscope. Borders of nuclei containing labeled cells were identified by comparison with adjacent thionin-stained sections or by removing the coverslips from plotted sections, staining the sections with thionin and re-applying coverslips. Cholinergic cell groups were identified as described previously (Motts et al. 2008).

Photomicrographs were obtained with a Zeiss Axioskop fluorescence microscope and Magnafire camera (Optronics) or a Zeiss Imager Z1 fluorescence microscope and AxioCam HRm camera (Zeiss). Adobe Photoshop CS3 was used to add scale bars, to adjust the size and

cropping of images, to erase background around tissue sections, and to adjust brightness and contrast. Adobe Illustrator CS3 was used to add lines to the images in Figure 1.

RESULTS

We combined anterograde and retrograde tracer injections with immunolabeling for ChAT to identify auditory cortical inputs to brainstem cholinergic cells that project to the IC. Details of the cortical injections and the distribution of labeled axons were described previously (Schofield and Motts, 2009). Briefly, the injections were centered in the primary auditory cortex (A1) and, in some cases, extended into surrounding areas (e.g., the dorsocaudal field, ventrostral and dorsostral belt areas and most likely area S; Wallace et al., 2000). Figure 1 shows several FR deposit sites in the AC (Fig. 1A) and labeled axons in the ipsilateral PPT (Fig. 1B). Labeled axons were present bilaterally in PPT and LDT, with more axons on the ipsilateral side and, on each side, more axons and boutons in the PPT than in the LDT.

For retrograde tracing, injections of Fast Blue (FB) were centered in the IC (Fig. 1C). They frequently extended to the dorsal or caudal IC borders. Some of the injections extended ventrally or rostrally to approach the borders. None of the injections spread ventrally into the tegmentum, rostrally into the superior colliculus or medially into the periaqueductal gray or across the midline into the contralateral IC. Retrogradely labeled cells were present in many areas, including the cochlear nuclei, superior olivary complex, sagulum and nuclei of the lateral lemniscus as well as the superior colliculus and thalamus. In most cases, the dorsal column nuclei and spinal trigeminal nuclei also contained retrogradely labeled cells, indicating that the injection sites included the ICx. The damage caused by making multiple injections made it impossible to distinguish IC subdivisions with certainty. However, given the large size of injections and the patterns of retrograde labeling, we conclude that the injections always involved the central nucleus and usually included part of external and/or dorsal cortical areas. Across cases, the injection sites included all major parts of the IC except the most dorsomedial regions.

ChAT-immunolabeled cells were present in many brainstem areas, as described in detail previously (Motts et al. 2008). Immunolabeled cells that were also labeled with Fast Blue were located in the PPT and LDT, consistent with previous reports that these nuclei are the primary source of cholinergic projections to the IC (Motts and Schofield, 2009). The remainder of this report will focus on the labeled cells and axons in the PPT and LDT. Given that the cortical projections terminate bilaterally in PPT and LDT, and that PPT and LDT project bilaterally to the IC, there are several possible patterns of projections. We describe results from two types of experiments. In the first experiment, we made injections into the AC and IC on the same side of the brain. In the second experiment, we made injections into the AC on one side of the brain and the IC on the other side.

Results of injections into left AC and left IC

We examined both left and right PPT and LDT for cortical contacts onto retrogradely-labeled, immunopositive cells. Such contacts were numerous on the left side of the brain, ipsilateral to the cortical injection. The vast majority of contacts were in the PPT. Figure 2 shows examples of retrogradely labeled cells (Fig. 2, first column) that were also immunopositive (Fig. 2, second column) and appeared to be contacted by cortical boutons that were labeled with FluoroRuby (Fig. 2, third column). The boutons appeared to contact either dendrites (e.g., Figs. 2A) or cell bodies (Figs. 2B–D). Most often the contacts were associated with cell bodies, but it is hard to determine whether this is representative because the dendrites of many cells were not labeled extensively.

On the right side of the brain, small numbers of labeled axons and labeled cells were intermingled in the PPT and LDT. However, we did not observe any evidence for contacts between the labeled axons and labeled cells.

Results of injections into the left AC and right IC

These experiments yielded contacts between labeled axons and labeled cells on both sides of the brain. Figure 3 shows representative contacts on the left side of the brain. Once again, the majority of contacts were in the PPT. Contacts appeared to be equally common on cell bodies and dendrites in the PPT.

On the right side of the brain, contacts were relatively uncommon. Most were in the PPT, where they were associated with cell bodies (Fig. 4). A single contact was observed in the LDT; it was associated with a dendrite.

Cortical contacts on non-cholinergic cells

The PPT and LDT contain non-cholinergic as well as cholinergic cells, and members of both groups project to the IC (Motts and Schofield, 2009). While the cholinergic (i.e., ChAT-immunopositive) cells were the focus of the present study, we observed cortical boutons in close contact with retrogradely-labeled cells that were ChAT-immunonegative. In some cases, these cells were near immunopositive cells, suggesting that the lack of immunolabel was not due to technical limitations (e.g., incomplete penetration of the antibodies). The results suggest that AC axons also contact non-cholinergic cells in the “cholinergic nuclei” that project to the IC. Finally, all the experiments contained many cortical boutons that were located at some distance from labeled cells, presumably associated with unlabeled cell bodies or dendrites.

DISCUSSION

The present study identifies projections of PPT and LDT cholinergic cells that receive inputs from auditory cortex. The results identify several pathways by which auditory cortex could activate cholinergic cells that project to one or the other IC (Fig. 5). AC projections to the ipsilateral tegmentum appear to contact IC-projecting cholinergic cells in the PPT and, much less often, in the LDT. In both nuclei, some of the target cells project to the ipsilateral IC. The ipsilateral AC projections also contact cholinergic cells that project to the *contralateral* IC. These contacts are also more common in the PPT than LDT. Projections from the AC to the contralateral tegmentum contact cholinergic cells that project to the IC. These contacts were much less common than those on the side ipsilateral to the injected AC, possibly related to the much smaller cortical projection to the contralateral tegmentum than the ipsilateral tegmentum. In the contralateral tegmentum, the contacted cells were located almost exclusively in the PPT, and all the cells projected to the IC on the same side as the contacted cell (contralateral to the injected cortex). The AC projections originate from layer V cells and are presumed to be excitatory (Schofield and Motts, 2009). It is likely, then, that cortical projections activate PPT and LDT cells, leading to release of acetylcholine bilaterally in the IC.

Technical Considerations

We used methods that are well established for identifying cholinergic cells (Levey and Wainer, 1982; Armstrong et al. 1983; Maley et al. 1988). The specific antibody as well as the fixation and histological procedures used in the present study have been validated with Western blot analysis as well as pre-adsorption and antibody omission controls in guinea pigs (Motts et al. 2008). We conclude that the immunolabeled cells are likely to be cholinergic. It can be difficult to interpret a lack of immunolabel, but we believe that some of the immunonegative cells are indeed non-cholinergic (particularly in cases when the cells in question were located in close proximity to immunopositive cells). Both GABA and glutamate as well as neuropeptides such

as substance P and corticotropin-releasing factor have been identified in PPT or LDT (Lavoie and Parent, 1994; Ford et al. 1995; Leonard et al. 1995; Vincent et al. 1986; Jia et al. 2003). It seems likely that one or more of these substances are associated with the ChAT-negative cells that we observed.

An important limitation in the present study concerns the attempt to draw conclusions about synaptic circuitry on the basis of light microscopic data. Notably, our previous conclusion of AC projections to the cholinergic cells was bolstered by showing cortical boutons that appeared to contact immunolabeled cells and were themselves immunopositive for the synaptic marker SV2 (Schofield and Motts, 2009). Nonetheless, contacts such as we observed will have to be analyzed with electron microscopy to confirm the presence of synapses.

Functional Implications

The PPT and the LDT are associated with a wide range of functions, including arousal, control of the sleep/wake cycle, various aspects of motor control and possibly attention (e.g. Jackson and Crossman, 1983; Scarnati et al. 1987; Garcia-Rill, 1991; Steckler et al. 1994; Reese et al. 1995a, b; Lee et al. 2000; Vincent, 2000; Inglis et al. 2001; Mena-Segovia et al. 2005; Steriade, 2004; Miller and O'Callaghan, 2006; Jones, 2008; Winn, 2006). These functions are associated with projections to many targets, including the thalamus, ventral tegmental area, various brainstem motor and sensory nuclei and the spinal cord. It is possible that the AC projections to the PMT have very widespread effects through the multiple output pathways from the PPT and LDT (discussed in Schofield and Motts, 2009). The present discussion focuses on the cholinergic projections to the IC.

Arousal and control of the sleep-wake cycle are among the functions most prominently associated with the PPT and LDT cholinergic cells (Garcia-Rill, 1991; Jones, 1993; Vincent, 2000; Steriade, 2004). Velluti and colleagues (Peña et al. 1992, 1999; Pedemonte et al. 1994; Goldstein-Daruech et al. 2002) have shown that the responses of IC cells are modulated by the sleep-wake cycle. The present results raise the possibility that AC projections could have an impact on this effect. Of particular interest is that acoustic stimuli that carry special significance, such as one's own name, are more likely than neutral stimuli to wake an individual from sleep (Oswald, et al., 1960; Bastuji et al., 2002). The cortical projections to the brainstem arousal system, which includes the tegmental cholinergic cells, may contribute to arousal that depends on analysis of sound content.

Sensory gating is another function closely associated with the PPT and LDT (Swerdlow et al., 2001; Yeomans et al., 2006). Prepulse inhibition of the acoustic startle response has been used as a model for studying sensory-motor gating (Koch et al., 1993; Braff et al., 2001; Li et al., 2009). In this case, an acoustic stimulus that is sub-threshold for inducing startle can suppress the response to a startling stimulus presented soon after the prepulse. Abnormalities in sensory gating, including specific deficits in prepulse inhibition, are associated with a number of psychiatric disorders, including schizophrenia and some anxiety-related disorders (e.g., Braff et al., 2001; Geyer et al., 2001; Weiss and Feldon, 2001; Li et al., 2009). The PPT is part of the circuit proposed to underlie prepulse inhibition (Yeomans et al., 2006; Li et al., 2009). This circuit includes projections from the IC to the superior colliculus to the PPT. Cholinergic cells in the PPT inhibit the startle response via projections to the caudal pontine reticular nucleus, which contains the premotor cells for the startle response (Bosch and Schmid, 2008). This circuit does not rely on cholinergic projections to the IC; it is not clear whether these projections play a role in prepulse inhibition. However, a proposed role of prepulse inhibition is to allow the auditory system to continue to analyze sounds (i.e., the prepulse) despite the subsequent startling stimulus (Graham, 1975; Fendt et al., 2001). The cholinergic projections to the premotor cells inhibit the motor reflex. Perhaps the cholinergic projections to the IC adjust the sensitivity of the IC cells so they are not overwhelmed by the signal from the startling stimulus.

Higher cognitive functions, such as attention, have been shown to affect prepulse inhibition (reviewed by Li et al., 2009). It is possible that projections from the AC to the cholinergic cells can affect sensory gating in part through the PPT and LDT projections to the IC.

PPT and LDT cells habituate rapidly to sensory stimuli (Koyama et al., 1994; Reese et al., 1995a, b). Descending projections from the forebrain apparently contribute to the habituation (Reese et al., 1995a, b). We speculated in a previous study that the cholinergic projections to the IC may contribute to novelty detection by IC cells (Schofield and Motts, 2009). These “novelty” units in the IC habituate rapidly to a repeated stimulus, but resume responding with even small changes in the stimulus (Pérez-González et al., 2005; Malmierca et al., 2009). The present data raise the possibility that projections from auditory cortex to brainstem cholinergic cells that project to the IC could also be involved. It would be interesting to determine the effect of cholinergic antagonists on the responses of IC novelty units.

Finally, acetylcholine is implicated in plasticity in the IC associated with fear conditioning and activation of the corticofugal system (Ji et al., 2001; Suga, 2008; Xiong et al., 2009). We speculate that cortically-evoked release of acetylcholine could be temporally linked to the effects of the direct AC projection to the IC, and thus may be particularly important in synaptic plasticity associated with the corticocollicular system.

List of Abbreviations

AC	auditory cortex
ACh	acetylcholine
Aq	cerebral aqueduct
ChAT	choline acetyltransferase
IC	inferior colliculus
LDT	laterodorsal tegmental nucleus
PPT	pedunclopontine tegmental nucleus

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Figure 1.

Representative injection sites and labeled cortical axons. A. Transverse section through two FluoroRuby (FR) deposit sites (*) in the left auditory cortex. GP553. A third deposit site was centered in a nearby section and encroached on the section illustrated. Arrows indicate label associated with axons leaving the three deposit sites and traveling ventromedially to enter the white matter (wm) and continue into the striatum (str). The deposits were centered in primary auditory cortex, and extended dorsally to approach the pseudosylvian sulcus (ps). Dorsal is up, lateral to the left. Scale bar = 1 mm. B. Photomicrograph of FR-labeled axons in the pedunclopontine tegmental nucleus ipsilateral to an injection of FR in the auditory cortex. Many boutons are present, some of which are indicated by arrows. GP566. Transverse section, dorsal is up; lateral left. Scale bar = 10 μ m. C. Photomicrograph of a transverse section through the center of a deposit of Fast Blue (FB) in the left inferior colliculus (IC). The midline is at the right edge of the image. The white line indicates the ventral and medial edge of the IC. Aq: cerebral aqueduct. GP553. Dorsal is up; lateral to the left. Scale bar = 1 mm.

Figure 2.

Auditory cortical axons terminating in the ipsilateral tegmentum on cells that have uncrossed projections to the IC. The first column shows cells retrogradely labeled with Fast Blue. The second column shows the same field of view, filtered for the ChAT immunolabel. Arrowheads indicate several cells that are labeled with both Fast Blue and ChAT immunolabel. The third column shows an overlay of the Fast Blue label and cortical axons labeled with FluoroRuby (FR, red). Arrows indicate apparent contacts between FR-labeled cortical boutons and the FB-labeled cholinergic cells. A–C. contacts in the PPT in 3 different cases (A: GP556; B: GP557; C: GP571). D. Contact in the LDT in GP557. All panels: transverse sections; dorsal is up and lateral to the left; scale bar = 10 μ m.

Figure 3.

Auditory cortical axons terminating in the ipsilateral tegmentum on cells that have crossed projections to the IC. Conventions as in Figure 2. Arrowheads indicate cells that are labeled with both Fast Blue and ChAT immunolabel. The third column shows an overlay of the Fast Blue and immunolabel with cortical axons labeled with FluoroRuby (FR, red). Arrows indicate apparent contacts between FR-labeled cortical boutons and the FB-labeled and immunolabeled cholinergic cells. A, B. Contacts in the PPT. C, D. Contacts in the LDT. GP566. All panels: transverse sections; dorsal is up and lateral to the right; scale bar = 10 μ m.

Figure 4.

Auditory cortical axons terminating in the contralateral PPT on cells that have uncrossed projections to the IC. Conventions as in Figure 2. Arrowheads indicate cells that are labeled with both Fast Blue and ChAT immunolabel. The third column shows an overlay of the Fast Blue label and cortical axons labeled with FluoroRuby (FR, red). Arrows indicate apparent contacts between FR-labeled cortical boutons and the FB-labeled and immunolabeled cholinergic cells. All panels: GP566; transverse sections; dorsal is up and lateral to the left; scale bar = 10 μ m.

Figure 5.

Summary diagram illustrating the pathways observed in the present study. Black lines with round terminals represent projections from auditory cortex to the pedunculopontine and laterodorsal tegmental nuclei (PPT, LDT; the branching of the line indicates the distribution of the pathway overall; it does not indicate branching of individual axons). Black triangles in auditory cortex represent layer V pyramidal cells, which give rise to the descending axons. Projections of cholinergic cells in the PPT and LDT that were contacted by cortical axons are represented by black arrows ending in the inferior colliculus. The thickness of the black arrows reflects the relative frequency with which cholinergic cells of the indicated pathway were contacted by cortical axons (i.e., a thicker arrow means more contacts were associated with that pathway).

Table 1

Summary of injection sites.

Experiment	Tracer in left AC	Tracer in left IC	Tracer in right IC
GP553	FR		FB
GP556	FR	FB	
GP557	FR	FB	
GP558	FR		FB
GP566	FR		FB
GP571	FR	FB	

List of tracers injected into the auditory cortex (AC) and the inferior colliculus (IC) in the experiments used for this study. FB – Fast Blue; FR – FluoroRuby.