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Research report

Projections from auditory cortex to cholinergic cells in the midbrain tegmentum of guinea pigs

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ABSTRACT

Anterograde and retrograde tracing techniques were used to characterize projections from the auditory cortex to the pedunculopontine and laterodorsal tegmental nuclei (PPT and LDT, respectively) in the midbrain tegmentum in guinea pigs. For anterograde tracing, tetramethylrhodamine dextran (FluoroRuby) was injected at several sites within auditory cortex. After sufficient time for transport, the brain was processed for immunohistochemistry with anti-choline acetyltransferase to reveal presumptive cholinergic cells. Anterogradely labeled axons were observed ipsilaterally and, in smaller numbers, contralaterally, in both the pedunculopontine and laterodorsal tegmental nuclei. In all four nuclei, tracer-labeled boutons appeared to contact immunolabeled (i.e., cholinergic) cells. The contacts occurred on cell bodies and dendrites. The results were similar following injections that spread across multiple auditory cortical areas or injections that were within primary auditory cortex. In order to confirm the anterograde results, in a second series of experiments, retrograde tracers were deposited in the pedunculopontine tegmental nucleus. These injections labeled layer V pyramidal cells in the auditory cortex. The results suggest an excitatory projection from primary auditory cortex bilaterally to cholinergic cells in the midbrain tegmentum. Such a pathway could allow auditory cortex to activate brainstem cholinergic circuits, possibly including the cholinergic pathways associated with arousal and gating of acoustic stimuli.

1. Introduction

Two midbrain cholinergic nuclei – the pedunculopontine tegmental nucleus (PPT) and the laterodorsal tegmental nucleus (LDT) – have been associated with the ascending arousal system (reticular activating system) [13,19,56,61]. The cholinergic cells in PPT and LDT project to many brainstem nuclei. They are also the source of cholinergic projections to the thalamus. Indeed, it is their projection to the thalamus that has been studied in most detail and has been most closely related to arousal. The cholinergic cells show different levels of activity during different stages of the sleep–wake cycle, with cellular spiking activity higher during waking and during paradoxical sleep than during slow-wave sleep.

The PPT and LDT are in a position to have a major influence on auditory processing via projections to nuclei at numerous levels of the ascending auditory pathway. Targets of the cholinergic projections to the thalamus include the multiple subdivisions of the medial geniculate nucleus [15,32,52,55,59,65]. Recent studies have also identified cholinergic projections from the PPT and LDT to the cochlear nucleus [36] and to the inferior colliculus [38].

Physiological recordings suggest that a large percentage of the cells in the PPT and LDT respond to acoustic stimuli [24,43]. The exact route by which acoustic information reaches the midbrain cholinergic cells is unknown but may include direct projections from the superior colliculus and possibly from the inferior colliculus as well [11,51,66]. It is possible that other auditory structures project to the cholinergic nuclei, but the proximity of these nuclei to auditory pathways has made it difficult to identify other inputs. The lateral part of the PPT overlaps with the medial portion of the lateral lemniscus such that the cholinergic cells are intermingled with axons of this large auditory pathway. In addition, the dorsal nucleus of the lateral lemniscus is also nearby. This large auditory nucleus gives rise to a commissural pathway (the commissure of Probst) with fibers that directly traverse the caudal PPT and travel near the LDT. Injections of retrograde tracers into the LDT (or PPT) label cells in the DNLL but it has been difficult to conclude whether this label represents a direct projection or labeling of fibers of passage [51]. The issue is further complicated if one is interested in identifying auditory inputs that are related specifically to cholinergic cells because both the PPT and the LDT contain significant numbers of cells that use neurotransmitters other than acetylcholine (e.g., glutamate, GABA) [5,18,19,26,27,29,53,58,64].

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The present study stems from an earlier series of experiments in which we made injections of anterograde tracer into the auditory cortex to examine projections to brainstem auditory nuclei in guinea pigs [6,41,48,49]. Subsequent analysis has revealed labeled axons in the area of both the PPT and LDT (unpublished observations). This was surprising because, to the best of our knowledge, there are no reports of such projections. The present study examines these projections directly, and includes staining with a cholinergic marker to relate the cortical axons to the cholinergic brainstem cells. We also use retrograde tracers injected into the PPT to confirm the projections from auditory cortex.

2. Materials and methods

2.1. Surgery and perfusion

Experiments were performed on eight adult pigmented guinea pigs (450–900 g, either gender) obtained from Elm Hill Laboratories (Chelmsford, MA, USA). Appropriate measures were taken to minimize pain and suffering. Sterile instruments and aseptic technique were used for all surgical procedures. All procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee. Surgical methods have been described in detail previously (e.g., [6]). Briefly, the animal was anesthetized with isoflurane in oxygen (4% for induction; 1.5–2.5% for maintenance). Atropine (0.05 mg/kg, i.m.) was administered to reduce bronchial secretions. Body temperature was maintained with a feedback-controlled heating pad. Once anesthetized, the animal's scalp was prepared for surgery and the animal was mounted in a stereotaxic frame. The skin was incised and the margins were injected with a long-acting local anesthetic (0.25% bupivacaine with epinephrine 1:200,000; Hospira, Inc., Lake Forest, IL). The skin was retracted and a dental drill was used to open the skull at an appropriate location. For cortical injections (six animals), FluoroRuby (FR, tetramethylrhodamine dextran, 10,000 MW, dissolved as 10% in saline, Invitrogen, Eugene, OR) was injected at 5–13 sites in temporal cortex. The injections were placed across an area spanning 2–3 mm caudal to Bregma in the rostrocaudal axis. In the mediolateral axis, the injections spread 2–3 mm ventrolateral to the pseudosylvian sulcus. The injections were thus aimed at primary auditory cortex (A1; as mapped and defined by Wallace et al. [62,63]). Injections of 0.15 μl were made with a 10 μl Hamilton microsyringe angled 50° laterally in the transverse plane (approximately perpendicular to the cortex). Deposit sites were separated by about 0.5 mm and formed a grid within the dimensions described above. After completion of injections, Gelfoam (Harvard Apparatus, Holliston, MA, USA) was placed over the skull opening and the skin was sutured. Ketofen (ketoprofen 3 mg/kg, i.m.; Henry Schein, Melville, NY) was given to provide postoperative analgesia. The animal was returned to its cage and monitored until it was returned to the animal facility.

After 7–14 days, the animals were given an overdose of anesthesia (inhalation of 5% isoflurane in oxygen until cessation of breathing and absence of withdrawal reflex). The animal was then perfused through the aorta with Tyrode's solution (similar to artificial cerebrospinal fluid) followed by approximately 300 ml of fixative (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4) and then by a similar volume of fixative with 10% sucrose added. The brain was removed and stored overnight at 4 °C in fixative with 25% sucrose.

A retrograde tracer was injected into the PPT in two animals. In one animal, 23 nl of FluoroGold (FG, FluoroChrome, Inc., Englewood, CO; 4% in water) was injected into the PPT through a micropipette (tip inside diameter = 30 μm) attached to a Nanoliter injector (World Precision Instruments, Sarasota, FL, USA). The micropipette was left in place for 5 min after injection and then withdrawn. In a second animal, 23 nl of red beads (RB; Lumafluor, Naples, FL, USA) was injected through a micropipette (tip

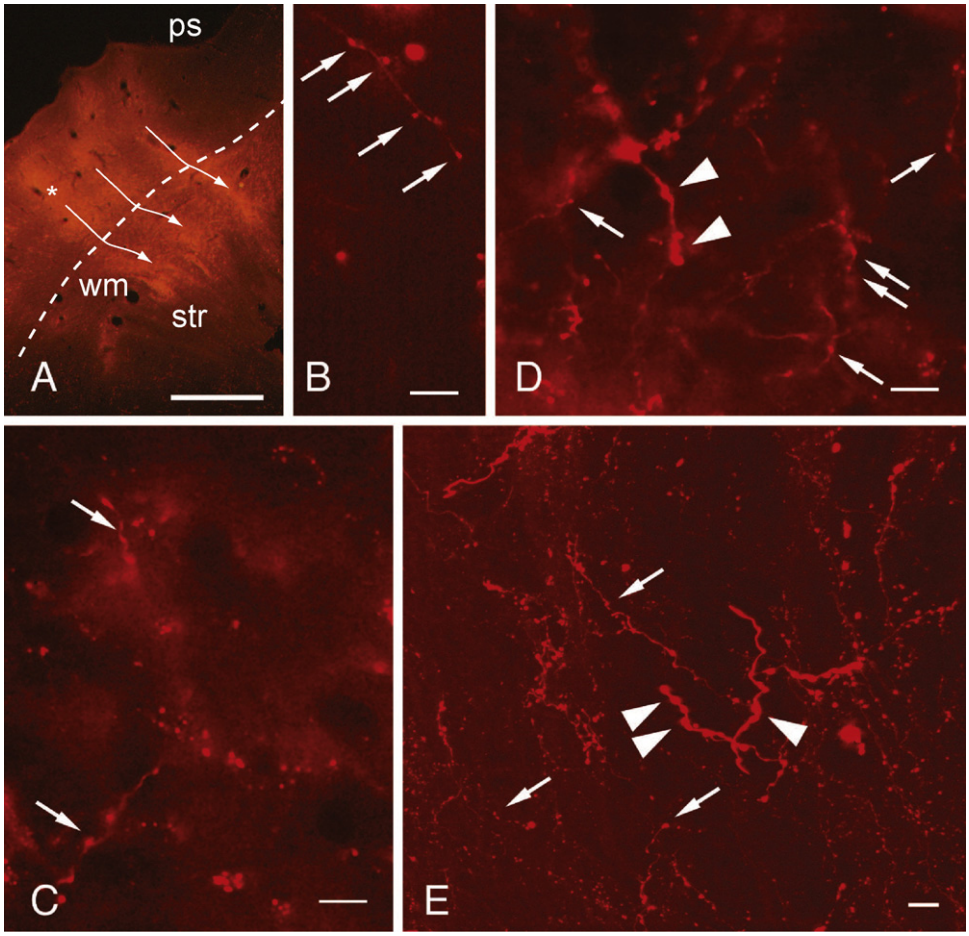

Fig. 1. Fluorescence photomicrographs showing a typical FluoroRuby (FR) injection into auditory cortex and representative labeled axons in the brainstem. (A) Transverse section showing one of 12 FR deposit sites (*). The injections are in auditory cortex, just ventrolateral to the pseudosylvian sulcus (ps). Bundles of labeled axons (white arrows) traverse the white matter (wm) and continue into the striatum (str) from the indicated injection site (*) as well as from two more dorsal sites (centered in nearby sections). Dashed line: border between cortex and white matter. Dorsal: up; medial: right. GP553. Scale bar = 1 mm. (B–E) FR-labeled axons in the laterodorsal tegmental nucleus (B and C) and the pedunculopontine tegmental nucleus (D and E) ipsilateral to the injected cortex. Thin axons exhibit many boutons (arrows) in the LDT and the PPT. Thicker axons with boutons (arrowheads) are visible in the PPT. The image in (E) is a z-stack (through-focal series) containing 24 optical sections. GP553 (C and D); GP556 (B and E). Scale bars = 10 μm. Adobe Photoshop CS3 was used to adjust brightness and contrast and Adobe Illustrator CS3 was used to add labels.