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PROJECTIONS TO THE INFERIOR COLLICULUS FROM LAYER VI CELLS OF AUDITORY CORTEX

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Abstract—A large injection of a retrograde tracer into the inferior colliculus of guinea pigs labeled two bands of cells in the ipsilateral auditory cortex: a dense band of cells in layer V and a second band of cells in layer VI. On the contralateral side, labeled cells were restricted to layer V. The ipsilateral layer VI cells were distributed throughout temporal cortex, suggesting projections from multiple auditory areas. The layer VI cells included pyramidal cells as well as several varieties of non-pyramidal cells. Small tracer injections restricted to the dorsal cortex or external cortex of the inferior colliculus consistently labeled cells in layer VI. Injections restricted to the central nucleus of the inferior colliculus labeled layer VI cells only rarely. Overall, 10% of the cells in temporal cortex that project to the ipsilateral inferior colliculus were located in layer VI, suggesting that layer VI cells make a significant contribution to the corticocollicular pathway.

Key words: auditory system, efferent, descending pathways, corticofugal, pyramidal cell, non-pyramidal cell.

A projection from cerebral cortex to the inferior colliculus (IC) has been known for many years, but interest in this pathway has grown as recent physiological studies have revealed a remarkably wide range of effects associated with it (e.g. Popelár et al., 2003; Suga and Ma, 2003; Yan et al., 2005; Sun et al., 2007; Wu and Yan, 2007; Nakamoto et al., 2008; Suga, 2008). Most of these studies have recorded the responses of IC cells to acoustic stimuli before, during and after various manipulations of the cortical inputs (e.g. stimulation or inactivation of auditory cortex). The results show that auditory cortex can affect the selectivity of IC cells for the frequency or intensity of a sound, the relative timing of two sounds, or binaural cues associated with the location of a sound.

Almost all of the studies cited above have recorded from cells in the central nucleus of the inferior colliculus (ICc). While there is evidence in some species for a direct projection from auditory cortex to the ICc (e.g. guinea pig: Feliciano and Potashner, 1995; rat: Saldaña et al., 1996), this pathway may not be present across species and, even when present, can be dwarfed by a much denser projection from auditory cortex to the dorsal cortex of the inferior colliculus (ICd) and external cortex of the inferior colliculus (ICx). These latter subdivisions project to the ICc, and could provide a disynaptic route through which cortical projections affect the ICc cells (Jen et al., 2001). Thus there may be several pathways by which cortex could affect ICc cells. The situation is further complicated by the discovery that auditory cortex also projects directly to brainstem auditory nuclei below the IC (Feliciano et al., 1995). Recent studies suggest that these cortical projections could directly affect the ascending inputs to the IC (Schofield and Coomes, 2005; Peterson and Schofield, 2007). It seems likely that numerous corticofugal pathways could play an important role in IC function; understanding these roles will require more detailed understanding of the circuitry, including the origins and termination patterns of the direct pathway from cortex to the IC.

A hallmark of projections from auditory cortex, like those from other neocortical areas, is the discrete laminar organization of their cells of origin (reviewed by Winer, 1992). Projections to other cortical areas arise from all the cellular layers, but projections to subcortical targets originate from layers V and VI. Two of the largest projections, in terms of the number of cortical cells of origin, are to the IC and to the thalamus. Kelly and Wong (1981) demonstrated clear differences in the laminar distributions of auditory cortical projection cells, with corticocollicular cells located in a band in layer V and corticothalamic cells distributed in two bands, including a wide band in layer VI and a narrower band in layer V. The layers differ in their inputs and in the morphology and physiology of their cells, suggesting that projections from the two layers would serve distinct functions (Winer, 1992). This hypothesis has been explored in greatest detail for the corticothalamic projection. A bi-laminar origin of corticothalamic cells has been demonstrated in many cortical areas across many systems. Most importantly, the projections from the two layers have been distinguished not only anatomically but also physiologically; they have different effects on thalamic cells and are considered to serve different functions (e.g. Ojima, 1994; see reviews by Sherman and Guillery, 2002; Sherman, 2007).

The study by Kelly and Wong (1981) was done in cats and suggested that corticocollicular cells originate exclusively from layer V. Other studies have demonstrated a projection from layer V cells to the IC in many species (reviewed in Winer, 1992). However, there are also reports that a portion of the corticocollicular pathway arises from

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Abbreviations: CTB, cholera toxin B subunit; FB, Fast Blue; GB, green beads; IC, inferior colliculus; ICc, central nucleus of inferior colliculus; ICd, dorsal cortex of inferior colliculus; ICx, external cortex of inferior colliculus; nNOS, neuronal nitric oxide synthase; PB, phosphate buffer; RB, red beads.

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layer VI cells. Injection of a retrograde tracer into the IC has labeled layer VI cells in rats (Games and Winer, 1988; Doucet et al., 2003), hedgehog tenrecs (Künzle, 1995) and gerbils (Bajo and Moore, 2005). We described the presence of layer VI corticocollicular cells in guinea pigs (Haas et al., 2003; Coomes et al., 2005), but these results conflicted with earlier reports (Druga et al., 1988).

The purpose of the present study was to address several questions about the layer VI corticocollicular cells in guinea pigs. How many layer VI corticocollicular cells are there (in comparison to layer V cells)? What cortical areas contain the layer VI cells? Previous studies describe the layer V corticocollicular projection as originating from a wide area of temporal cortex including primary and secondary (or core and belt) auditory areas; however, the distribution of the layer VI cells has not been described in any detail. What is the morphology of the layer VI cells? Layer V corticocollicular cells are described as medium and large pyramids (Winer and Prieto, 2001) with tufted or non-tufted apical dendrites (Bajo and Moore, 2005). Layer VI contains a variety of cell types. Both pyramidal and non-pyramidal layer VI cells project to the thalamus (Kelly and Wong, 1981). The types of layer VI cells that project to the IC are unknown. Finally, which IC subdivisions are targets of layer VI cells? The corticocollicular projection in guinea pigs terminates densely in the ICd and ICx (Druga et al., 1988); a less dense projection to the ICc has been documented with both anatomical and physiological techniques (Feliciano and Potashner, 1995; Lim and Anderson, 2007). Identifying the subdivisions that are targets of layer VI cells will be essential for understanding the functional role of the layer VI projections.

EXPERIMENTAL PROCEDURES

Experiments were performed on adult guinea pigs (13 albino animals obtained from Charles River Laboratories, Wilmington, MA, USA and two pigmented animals obtained from Elm Hill Breeding Laboratories, Chelmsford, MA, USA). All procedures were approved by the Institutional Animal Care and Use Committee and administered following the National Institutes of Health guidelines for the care and use of laboratory animals. In accordance with these guidelines, all efforts were made to minimize the number of animals used and their suffering.

Surgery and perfusion

Prior to surgery, each guinea pig was anesthetized with halothane (3.5% for induction, 2.5–2.75% for maintenance) in oxygen and nitrous oxide or with isoflurane (4–5% for induction, 1.75–3% for maintenance) in oxygen. The guinea pig was given atropine sulfate (0.08 mg/kg, i.m.) to reduce bronchial secretions. The eyes were kept moist with a coating of antibiotic ointment (Neosporin Ophthalmic) or moisturizer. The animal was placed in a stereotaxic frame on a feedback-controlled heating pad to maintain body temperature.

During surgery, an incision was made in the scalp and the margins of the incision were injected with a long-lasting local anesthetic (0.25% bupivacaine; Sensorcaine; Astra USA, Inc., Westborough, MA, USA). Stereotaxic coordinates were used to guide all injections. A dental drill was used to open the skull at appropriate locations.

Four different fluorescent retrograde tracers were used: Fast Blue (FB, 5% aqueous solution; EMS-Chemie GmbH, Gross-Umstadt, Germany), red and green fluorescent microspheres ("RetroBeads," both from Lumafluor, Naples, FL, USA) and FluoroRuby (FR, 10% solution in saline; tetramethylrhodamine dextran, 10,000 molecular weight, Invitrogen). Large injections were made with a 10 μl Hamilton microsyringe. The tracer was injected at multiple sites (two to six) in one IC. Each site received an injection of 0.1–0.2 μl. Each tracer was injected with a microsyringe used only for that tracer. Small injections were made with a micropipette (25–28 μm inside diameter) attached to a Nanoliter Injector (World Precision Instruments, Sarasota, FL, USA). Up to 138 nl was injected at a single site by injecting smaller amounts (9.2 nl, 13.8 nl or 23.0 nl) at 1-min intervals until the desired total was reached. In some animals, different tracers were injected at different locations within a single IC (Table 1). Data from some animals were used in a previous study identifying cortical cells that project bilaterally to the IC (Coomes et al., 2005). In two animals, cholera toxin B subunit ("CTB," 1% in saline; List Biological Laboratories, Campbell, CA, USA) was injected with a 1 μl Hamilton microsyringe (Table 1).

In most cases, the microsyringe or micropipette ("needle") was inserted into the IC using a vertical approach. In these cases, the needle traversed the visual cortex on the way to the IC (Spatz et al., 1991). This approach sometimes left a small amount of tracer in visual cortex along the insertion track. To make certain that this deposit was not responsible for the label in temporal cortex, we also made injections using two other approaches (Table 1). For two injections (in GP374), the needle was mounted in a horizontal plane and entered the IC from the caudal surface. For this approach, the needle traversed the cerebellum. In a third approach, the needle was mounted at an angle, rotated in the parasagittal plane caudally 40 or 50° from vertical (Table 1). This angle approximates the angle of the caudal surface of the IC as well as the border between the caudal part of ICd and the ICc. This approach was the most effective for producing injections confined to the caudal ICd. This approach also traversed the cerebellum, albeit a different part than was traversed by horizontally-oriented needles.

Following the injections, the exposed brain was covered with Gelfoam and the scalp was sutured. Ketoprofen (3 mg/kg, i.p.) was injected to provide post-operative analgesia. After surgery the animal was placed in a clean cage and monitored until it regained the ability to stand. At that point, it was transported in its cage to the animal facility.

After an appropriate interval for the transport of the injected tracers (4–12 days for fluorescent tracers; 7–8 days for CTB), the animal was sacrificed with CO_2 gas (inhalation, 10 min) or with an overdose of pentobarbital (440 mg/kg; i.p.) and the animal was perfused through the aorta with approximately 100 ml of Tyrode's solution (pH 7.4), 350 ml of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (PB), and 350 ml of 4% paraformaldehyde with 10% sucrose in PB. The brain was then removed and stored at 4 °C in 4% paraformaldehyde with 25–30% sucrose in PB. The following day, the brain was cut on a sliding microtome into 40, 50, or 80 μm thick sections. In some cases, the entire brain was cut in the transverse plane. In other cases, the cortex was separated from the brainstem and thalamus. The cortex was frozen and cut in the transverse plane on a sliding microtome. The brainstem and thalamus were split at the midline and each piece was frozen and cut in the parasagittal plane. Sections were collected in six series. For cases with fluorescent tracers, at least four series of sections were mounted on gelatin-coated slides and allowed to dry. For cases with CTB injections, at least two series of sections were stained for CTB. These sections were treated with 3% normal rabbit serum with 0.2% Triton-X 100 followed by goat anti-CTB (diluted 1:12,000; List Biological), biotinylated rabbit–anti-goat secondary antibody (Vector) and avidin–biotin–peroxidase (ABC Elite kit, Vector Laboratories). The label was visualized with nickel-enhanced diaminobenzidine.

In all cases, at least one series of sections was stained with thionin for cytoarchitecture. In most cases with small injections, one