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GABAergic and non-GABAergic projections to the superior colliculus from the auditory brainstem

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Abstract

The superior colliculus (SC) contains an auditory space map that is shaped by projections from several subcortical auditory nuclei. Both GABAergic (inhibitory) and excitatory cells contribute to these inputs, but there are contradictory reports regarding the sources of these inputs. We used retrograde tracing techniques in guinea pigs to identify cells in the auditory brainstem that project to the SC. We combined retrograde tracing with immunohistochemistry for glutamic acid decarboxylase (GAD) to identify putative GABAergic cells that participate in this pathway. Following a tracer injection in the SC, the nucleus of the brachium of the inferior colliculus (NBIC) contained the most labeled cells, followed by the inferior colliculus (IC). Smaller populations were observed in the sagulum, paralemniscal area, periolivary nuclei and ventrolateral tegmental nucleus. Overall, only 10% of the retrogradely labeled cells were GAD-immunopositive. The presumptive inhibitory cells were observed in the NBIC, IC, superior paraolivary nucleus, sagulum and paralemniscal area. We conclude that the guinea pig SC receives input from a diverse set of auditory brainstem nuclei, some of which provide GABAergic input. These diverse origins of input to the SC likely represent a variety of functions. Inputs from the NBIC and IC likely provide spatial information for guiding orienting behaviors. Inputs from subcollicular nuclei are less likely to provide spatial information; rather, they may provide a shorter route for auditory information to reach the SC, and could generate avoidance or escape responses to an external threat.

Keywords

inferior colliculus; nucleus of the brachium of the inferior colliculus; inhibition; orienting; avoidance behavior; attention; escape

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COMPLIANCE WITH ETHICAL STANDARDS

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INTRODUCTION

The mammalian superior colliculus (SC) integrates sensory information to produce meaningful motor responses (reviewed by Huerta and Harting 1984; Sparks 1988; Stein and Meredith 1993; Comoli et al. 2012; Costa et al. 2016; Savage et al. 2017). While much research has focused on the reflex nature of orienting responses, views of SC function have broadened to include approach or avoidance behaviors and a role in attention (Dean et al. 1989; Mysore and Knudsen 2011; Comoli et al. 2012). These responses can be triggered by several sensory modalities, including vision, hearing and touch. During development, interactions between inputs from different sensory systems help align the sensory receptive fields with the motor map established by outputs from the intermediate and deep layers of the SC (Doubell et al. 2000). In adults, integration between the sensory systems is revealed by multimodal neurons that respond to multiple sensory modalities (Meredith and Stein 1986; Doubell et al. 2000; Populin and Yin 2002). The circuits and mechanisms that underlie these multimodal responses and their transformation to meaningful motor outputs remain an area of intense study. The SC receives input directly from the retina and from several dozen subcortical nuclei, including nuclei associated with several sensory systems (Edwards et al. 1979; May 2006). Auditory inputs originate from numerous brainstem auditory nuclei, but understanding how these inputs are integrated has been hampered by inconsistencies between studies (reviewed by Huerta and Harting 1984; Oliver and Huerta 1992).

There is general agreement that the lateral cortex of the inferior colliculus (IClc) is a major source of input to the SC (Binns et al. 1992, 1995; Jiang et al. 1997; King et al. 1998). Beyond this, descriptions of auditory inputs to the SC have been contradictory on several points. Most strikingly, some studies have identified the nucleus of the brachium of the inferior colliculus (NBIC) as the largest source of inputs (van Buskirk 1983; Kudo et al. 1984; Schnupp and King 1997; King et al. 1998; Doubell et al. 2000; Skaliara et al. 2004; Nodal et al. 2005) whereas other studies have not included this nucleus in their analyses. Descriptions of input from the dorsal nucleus of the lateral lemniscus (DLL) range from very little (ferret: King et al. 1998) to light (cat: Kudo 1981; rat: Cadusseau and Roger 1985; Bajo et al. 1993; big brown bat: Zhang et al. 1987) to moderate (tree shrew: Casseday et al. 1976; rat: Tanaka et al. 1985; mustached bat: Covey et al. 1987) to the largest (rat: Druga and Syka 1984). The rostral pole of the IC (ICrp) has been identified as a major source of input in cats (Harting and van Lieshout 2000), but this IC subdivision is rarely distinguished in other species. Finally, there is wide variation in the reports of projections from other subcollicular areas, mostly in or around the superior olivary complex (Grofova et al. 1978; Edwards et al. 1979; Huerta and Harting 1984; Appell and Behan 1990). It is difficult to determine how many of the apparent differences represent true differences between species versus differences due to tracer or analytical approach.

A second question arises with regard to the excitatory or inhibitory nature of the auditory projections to the SC. Both glutamatergic and GABAergic inputs have been implicated (Ingham et al. 1998; King et al. 1998). King et al. (1998) hypothesized that GABAergic inputs could sharpen the spatial selectivity of SC cells or contribute to the suppression of surrounds that characterize some auditory receptive fields. Appell and Behan (1990)

assessed GABAergic input to the SC in a broad survey (i.e., not limited to auditory nuclei). Among auditory regions, they identified the IC external cortex (which includes the IC_{lc} and, possibly, the IC_{crp}), the DLL and the ventral nucleus of the lateral lemniscus as sources of GABAergic input to the SC. They did not analyze the NBIC or superior olivary nuclei, which project to the SC and which also contain GABAergic cells (Helfert et al. 1989; Kulesza and Berrebi 2000). The limited data on inhibitory inputs combined with the variability regarding the overall sources of auditory input to the SC make it difficult to assess the GABAergic inputs to the SC within any functional context.

In the current study we used sensitive fluorescent retrograde tracers to identify brainstem auditory nuclei that project to the SC in guinea pigs, a species widely used in auditory research. We combined our tracing results with immunohistochemistry to identify GABAergic cells that contribute to these projections. Our results suggest that 1) the NBIC provides the largest input to the SC from the auditory brainstem, 2) only ~10% of the auditory brainstem input to the SC is GABAergic; 3) the GABAergic inputs arise primarily from the NBIC, the IC, the sagulum, the paralemniscal area, and the superior paraolivary nucleus.

MATERIALS AND METHODS

All procedures were conducted in accordance with the Northeast Ohio Medical University Institutional Animal Care and Use Committee and NIH guidelines. Results are described from six adult pigmented guinea pigs (Elm Hill Labs; Chelmsford, MA, USA) of both genders weighing 314–801 g and ranging in age from five weeks to ~1 year. Efforts were made to minimize the number of animals and their suffering.

Surgery

Each animal was anesthetized with isoflurane (4–5% for induction, 1.75–3% for maintenance) in oxygen. The head was shaved and disinfected with Betadine (Purdue Products L.P., Stamford, CT, USA). Atropine sulfate (0.08 mg/kg i.m.) was given to minimize respiratory secretions and Ketofen (ketoprofen, 3 mg/kg i.m.; Henry Schein, Melville, NY 11747, USA) was given for post-operative pain management. Moisture Eyes PM ophthalmic ointment (Bausch, Lomb, Rochester, NY, USA) was applied to each eye to protect the cornea. The animal's head was positioned in a stereotaxic frame with a mouth bar positioned 5.0 mm ventral to the horizontal plane through interaural zero. Body temperature was maintained with a feedback-controlled heating pad. Sterile instruments and aseptic techniques 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, USA), a long-lasting local anesthetic. A craniotomy was made over the desired target coordinates using a dental drill. Following the tracer injection, Gelfoam (Harvard Apparatus, Holliston, MA, USA) was placed in the craniotomy 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.

Retrograde tracers

Fluorescent tracers (red fluorescent RetroBeads [“red beads”; RB, undiluted] or green fluorescent RetroBeads [“green beads”; GB, undiluted], Luma-Fluor, Inc., Naples, FL, USA; FluoroGold [FG; 4% in water], FluoroChrome, Inc., Englewood, CO, USA) were deposited into the SC via stereotaxic coordinates (2.1–2.6 mm rostral; 1.6–2.2 mm lateral; 6.4–6.6 mm dorsal, all relative to interaural zero). Four of the six animals received deposits of tracer into just one SC (Table 1). In the other two animals, the left SC received a deposit of FG while the right SC was injected with both RB and GB (Table 1). In the SCs that received both a RB and a GB injection, the tracers were deposited at different mediolateral locations. For all experiments, a Hamilton microsyringe (1 μ l; Hamilton, Reno, NV, USA) was used to deposit tracer. Each syringe was dedicated to a single tracer. The volume of tracer (0.05 μ l – 0.2 μ l) injected at each site was designed to account for the diffusibility of each tracer (Schofield 2008; Table 1).

Perfusion and tissue processing

Four to five days after surgery, the animal was deeply anesthetized with isoflurane and perfused transcardially with Tyrode’s solution, followed by 250 ml of 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4 and then by 250 ml of the same fixative with 10% sucrose. The brain was removed and stored at 4°C in fixative with 25–30% sucrose for cryoprotection. The following day the brain was prepared for processing by removing the cerebellum and cortex and blocking the remaining piece with transverse cuts posterior to the cochlear nucleus and anterior to the thalamus. The tissue was frozen and cut on a sliding microtome into 40 or 50 μ m thick transverse or sagittal sections that were collected serially in six sets.

Putative GABAergic cells were stained with immunochemistry for glutamic acid decarboxylase (GAD; Nakamoto et al. 2013). Briefly, the sections were pretreated with 0.2% Triton X-100, normal goat serum to limit non-specific labeling, and then exposed (1–2 days at 4°C) to mouse anti-GAD monoclonal antibody (GAD67; #MAB5406 Millipore, diluted 1:1000 to 1:100). The sections were treated with 1% biotinylated goat anti-mouse antibody (Vector Laboratories, Burlingame, CA, USA: BA-9200) and labeled with streptavidin conjugated to a fluorescent marker (AlexaFluor 488 [green] or AlexaFluor 647 [near-infrared], Invitrogen, Carlsbad, CA, USA). An adjacent series of sections was stained with antibodies to brain nitric oxide synthase (bNOS; #N2280 Sigma, diluted 1:1000) to identify IC subdivisions. Stained sections were mounted on gelatin-coated slides, allowed to dry and coverslipped with DPX (Sigma).

Identification of nuclei

Nuclei were identified according to standard cytoarchitectural criteria, supplemented with the staining patterns evident in the immunostains employed here. Specifically, the major subdivisions of the IC (dorsal cortex, lateral cortex, central nucleus and intercollicular tegmentum) were distinguished with immunochemistry for brain nitric oxide synthase (bNOS; Coote and Rees, 2008). Coote and Rees (2008) did not distinguish a rostral pole, but this region can be distinguished based on criteria described in cats (Harting and van Lishout 2000) as well as with the pattern of GAD staining (Foster et al. 2015). Many studies

(including numerous ones cited in the Discussion) have used the term IC external cortex, or ICx, to refer to the region of the IC largely lateral and rostral to the central nucleus. The terminology of Coote and Rees distinguishes the lateral part of this region as the IC lateral cortex, and the rostral region as “intercollicular tegmentum” (ICT). This terminology follows classic distinctions in the IC (Morest and Oliver 1984), and reflects more recent trends toward distinguishing these regions (Loftus et al. 2008).

Most cases were processed in the parasagittal plane to facilitate distinguishing several of the midbrain nuclei. In particular, the central mesencephalic reticular formation (cMRF), a major source of input to the SC, lies rostral to the DLL and ventral to the ICrp. In the parasagittal plane the borders of the ICrp and the DLL are very conspicuous when reacted for GAD and/or BNOS, and distinctions between the ICrp, cMRF and DLL are readily determined. Identification of subcollicular nuclei followed previous descriptions in guinea pigs (NBIC: Mellott et al. 2014a; lemniscal nuclei and adjacent regions: Schofield and Cant 1997; Schofield et al. 2014a; superior olivary complex: Schofield and Cant 1991, 1999; cochlear nucleus: Schofield et al. 2014b).

Data analysis

The layers of the SC are conspicuous in sections stained for GAD or bNOS so it was straightforward to determine the location and extent of each injection site in the SC. Further analysis was limited to those experiments in which the injections were confined to the SC. Immunostaining revealed GAD-immunoreactive (GAD⁺) cells and boutons throughout the auditory brainstem. Immunopositive cells were labeled intensely and were readily distinguished from immunonegative cells. The GAD immunostain was readily visible in tracer-labeled cells, making it straightforward to distinguish GAD⁺ versus GAD⁻ staining in the retrogradely-labeled cells. Photomicrographs were captured with a Zeiss AxioImager Z1 fluorescence microscope and AxioCam HRm or HRc cameras (Zeiss). Adobe Photoshop (Adobe Systems) was used to add scale bars, crop images, erase background around tissue sections, adjust intensity levels and colorize monochrome images.

Data quantification

Four experiments were chosen for quantification because 1) the deposit sites were restricted to the SC, 2) there were many retrogradely labeled cells, 3) there was robust immunostaining, 4) the deposits included the caudal SC, which is reported to receive denser auditory inputs than the rostral SC (Edwards et al. 1979; Kudo and Niimi 1980; Kudo 1981; Henkel 1983; Cadusseau and Roger 1985; King et al. 1998). Labeled cells in auditory nuclei were plotted with a Neurolucida reconstruction system (MBF Bioscience, Williston, VT, USA) attached to a Zeiss Axioplan II microscope (Carl Zeiss MicroImaging, Inc., Thornwood, NY, USA) or a Zeiss AxioImager Z2. For each case, every labeled cell in each auditory brainstem nucleus was plotted across a series of transverse or sagittal sections (every sixth section). Each combination of tracer and immunolabel was plotted with a unique marker. The results of these plots were used for a quantitative summary of the distributions of the labeled cells. Final images of the plots were refined with Adobe Illustrator (Adobe Systems, Inc., San Jose, CA, USA) for preparation of figures.

The GAD immunostaining sometimes failed to penetrate the full thickness of the section, leaving a central slice of the section unstained. By analyzing the data with a 63× objective (NA = 1.4), it was possible to restrict the quantitative analyses to parts of the section that had successful immunostaining. Sections cut at 40–50 µm typically shrink during dehydration such that the final thickness on slides is 20–30 µm. The GAD staining in these sections usually extended 5–10 µm from each surface. Thus, a central layer up to 20 µm thick could be left unstained. By paying special attention to focusing on the center of the soma when plotting the symbol for a particular cell, data points were obtained with sufficient resolution in the z plane (section depth) to allow subsequent filtering of the data by depth. This yielded two zones of data from each section (one associated with each surface), and a central zone that was not stained with GAD. After the data were plotted, the X, Y, and Z coordinates of all markers were exported from Neurolucida to Microsoft Excel and sorted based on the Z coordinate. Tracer-labeled cells in the unstained or poorly stained central layer were excluded from further analyses. Such depth filtering necessarily reduces the sample size for quantitative comparisons, but a sufficient number of sections were analyzed to yield a substantial number of cells (GP673: 1,349 FG-labeled cells and 1,011 GB-labeled cells; GP 674: 965 GB-labeled cells; GP 738: 935 FG-labeled cells). The relative contributions of each nucleus with input to the SC were assessed by calculating the percentage of retrogradely labeled cells located in that nucleus compared to the total number of retrogradely labeled cells. Calculations were made for GAD⁻ and GAD⁺ cells separately (%GAD⁺ cells in nucleus = (number of GAD⁺ tracer-labeled cells in a nucleus)/(number of all tracer-labeled cells)*100).

RESULTS

We combined retrograde tracing and immunolabeling for GAD to identify GABAergic and non-GABAergic cells in the auditory brainstem that project to the SC. By injecting different tracers into both the left and right SC in three of the animals, we maximized the data from those animals and also identified individual cells that send collateral projections to both SCs (the collateral projections will be described in a future report). We first describe the injection sites and cellular distribution and then describe the results of the GAD immunostaining.

Injection sites

The results are based on tracer deposits in 8 SCs (Table 1). All tracer deposits included intermediate and deep layers of the SC (Fig. 1). The injections did not spread rostrally into the medial geniculate body, ventrally into the NBIC or midbrain reticular formation, caudally into the IC, medially into the periaqueductal gray or across the midline into the contralateral SC. The total number of labeled cells varied between cases, but the overall patterns and percentages of cells in the NBIC, the IC and the auditory subcollicular nuclei were consistent between animals and between different tracers. This was true despite variations in the medial/lateral position of the deposit sites within the SC.

Identification and distribution of labeled cells

Retrogradely labeled cells were readily identified in many brainstem regions. The following description is limited to the numerous auditory regions that contained labeled cells. About

10% of the tracer-labeled cells were also labeled with the GAD antibody (GAD⁺), suggesting these cells are GABAergic and project to the SC (Fig. 2). The remaining 90% of retrogradely-labeled cells were GAD⁻ (Fig. 2A,C, arrowheads). These tracer-labeled cells were often intermingled with GAD⁺ cells that were not tracer labeled (Fig. 2). Close proximity of the GAD⁺ cells (with or without tracer label) to GAD⁻ tracer-labeled cells suggest that the lack of immunolabel in the latter cells cannot be attributed to failure of antibody penetrance or other vagaries of the immunohistochemical processing. In other words, the majority of the retrogradely labeled cells are likely non-GABAergic.

Figures 3 and 4 show the distribution of GAD⁺ and GAD⁻ cells in the brainstem auditory nuclei in a representative experiment. Labeled cells were concentrated in the NBIC and in the IC. In the NBIC, labeled cells were present bilaterally, with the majority located ipsilaterally. In the IC, labeled cells were found almost exclusively on the ipsilateral side, where most were located in the IClc and ICrp. A few cells were observed in the dorsal cortex and central nucleus of the IC; none were identified in the intercollicular tegmentum. Additional retrogradely labeled cells were present consistently in numerous subcollicular auditory nuclei, including the sagulum (Sag), the paralemniscal area (PL), the superior paraolivary nucleus (SPN) and the ventrolateral tegmental nucleus (VLTg). In some cases, a few labeled cells were observed in the dorsal periolivary nucleus and among the fibers of the trapezoid body (medial to the medial nucleus of the trapezoid body).

As described above (Introduction), the dorsal nucleus of the lateral lemniscus (DLL) has been identified as a major source of input to the SC in some studies. We found a few labeled cells around the margins of the DLL. These cells appeared to belong to surrounding structures, a conclusion consistent with their lack of GAD immunostaining (the DLL is primarily or exclusively composed of GABAergic cells; Adams and Mugnaini 1984). Notably, in every case a large cluster of labeled cells was observed rostral to the DLL in the cMRf. This is a tegmental nucleus well known for its projections to the SC; it is not associated with auditory pathways and so was not included in the present analysis (May 2006).

Quantitative analyses of GAD⁻ and GAD⁺ cell distributions

Four experiments were chosen for quantitative analysis (Table 2). For this table, GAD⁻ and GAD⁺ retrogradely labeled cells were totaled across all nuclei, then percentages of each type, GAD⁻ or GAD⁺, were calculated for individual nuclei. In all four experiments, the ipsilateral NBIC contained the largest group of GAD⁻ cells (average: $29.3 \pm 4.3\%$ of all retrograde cells; see “Ipsi GAD⁻” in Table 2). Tracer-labeled GAD⁺ cells were also most abundant in the ipsilateral NBIC, although they totaled only $4.3 \pm 1.0\%$ of the labeled cells (Table 2, “Ipsi GAD⁺”). On the contralateral side of the brain, the NBIC again dominated, with the largest values for both GAD⁻ and GAD⁺ cells. On both sides of the brain, additional retrograde cells were found in smaller numbers in a variety of nuclei, listed from left to right in the table in the order of decreasing percentage of retrograde cells in that nucleus. Figure 5 presents these results graphically, highlighting several points. First, the majority of retrograde cells are located ipsilaterally. Second, NBIC is the predominant source of inputs, with the IC, sagulum and paralemniscal regions secondary. Additional

contributions arise from numerous subcollicular nuclei. Finally, on both sides, and within each nucleus, GAD⁻ retrograde cells outnumbered GAD⁺ retrograde cells.

DISCUSSION

The current study examines the projections from auditory brainstem nuclei to the SC. Our first finding, similar to what has been found in ferret (King et al. 1998), is that the largest projection originates from the NBIC (Fig. 6). In the current study, 33.6% of the tracer labeled cells quantified across four cases were in the ipsilateral NBIC. The IC is the second largest source of inputs, with projections arising mainly from the IC_{lc} and the IC_{rp}. The remaining inputs are located in numerous subcollicular nuclei, including the Sag, the PL, the VLTg, and the SPN. Our second finding is that only a small part of this projection, about 10%, arises from GABAergic cells. Most of these cells are located in the NBIC. In the following sections, we discuss technical aspects of our analysis, compare our findings to previous studies, and then consider some functional implications of these pathways.

Technical Considerations

The tracer deposits covered substantial portions of the SC without encroaching on surrounding nuclei. Thus, it is likely that all the labeled cells project to the SC. None of the injections completely filled the deeper layers of the SC, so there is a risk that projections that terminate in only a portion of the SC could go undetected. Smaller injection volumes may also risk incomplete labeling because of limits to the sensitivity of tracers. We minimized these limitations by using multiple tracers (green beads, red beads and FluoroGold). The beads are particularly valuable in this regard because of their high sensitivity and limited diffusibility in the tissue (Schofield 2008). The fact that we obtained similar results across animals and across tracers, and that results from larger deposits were consistent with those from the smaller deposits, suggests that our results are generally valid.

The GAD antibody used here has been validated in previous studies in guinea pigs (Xiong et al. 2008; Nakamoto et al. 2013; Mellott et al. 2014b) and we believe that our tissue contained few false positive cells. Incomplete penetration of immunoreagents can lead to false negative staining, which could substantially affect quantitative analyses. We systematically limited our analysis such that, for each tissue section, labeled structures were analyzed only at tissue depths that included robust immunostaining. We conclude that GAD⁺ cells are GABAergic and that the GAD⁻ cells are almost certainly non-GABAergic.

Comparisons with previous studies

Projections from the IC_{lc} to the SC are thought to play a role in triggering orienting movements and have been associated directly with the auditory space map that characterizes the deep SC (Binns et al. 1992, 1995; Jiang et al. 1997; King et al. 1998). Of particular interest in this respect has been the recognition of multimodal integration in the IC_{lc}. Somatosensory inputs to the IC_{lc} have long been known and interactions between auditory and somatic inputs have been demonstrated physiologically (Aitkin et al. 1978, 1981; Jain and Shore, 2006). Additional inputs related to visual stimuli, eye position and behavioral state have also been shown to modulate IC neuronal responses (reviewed in Gruters and

Groh, 2012). These multimodal interactions may play a role in aligning space maps according to different reference frames (e.g., head-centered or eye-centered, accounting for changes in eye position). The extent to which these properties are associated with projections to the SC are unclear. In addition to its projections to the SC, the IC_{lc} is a major source of projections to other brain regions, including multiple subdivisions of the medial geniculate nucleus (MG; Mellott et al. 2014c). Parts of the MG also show multimodal responses, to which the IC_{lc} projections could contribute (Anderson and Linden, 2011). Preliminary studies suggest that the IC_{lc} projections to the MG and to the SC arise from different populations of IC_{lc} cells (Mellott and Schofield, 2015). Whether multimodal interactions are associated with the projections to the SC or the MG, or to both, will need to be determined for a full understanding of the functions of these circuits.

The IC_{rp} is less studied than other IC subdivisions. It has been identified in morphological and connectional studies (Morest and Oliver 1984; Meininger et al. 1986; Martin et al. 1988); its greatest distinction appears to be its prominence in projections to the SC (Harting and van Lieshout 2000). The present study shows a similarly strong projection from this area to the SC. Similar to the descriptions in cat (Harting and van Lieshout 2000), the density of SC-projecting cells in guinea pig sets the IC_{rp} apart from the caudally adjacent IC_c, and also from the IC_t, which surrounds the remaining borders of the IC_{rp}. The IC_d, spatially separated from the IC_{rp}, differs from the IC_{rp} in having only minimal projections to the SC. The IC_{lc} is the only other IC subdivision with substantial projections to the SC. Distinguishing the IC_{rp} from the IC_{lc} is justified by a consideration of their ascending inputs. Briefly, the IC_{rp} receives many of the same inputs as the IC_c, including direct projections from the medial and lateral superior olivary nuclei, that do not reach the IC_{lc} (reviewed in Harting and van Lieshout 2000; Oliver, 2005). In contrast, the IC_{lc} receives direct projections from somatosensory structures that do not project to the IC_{rp} (reviewed in Oliver, 2005). These differences in inputs undoubtedly confer different physiology and functions to the IC_{rp} and IC_{lc}, but further insight awaits more detailed characterization of IC_{rp} neuronal responses.

Projections from the NBIC to the SC are also demonstrated in a variety of species, including hamster (van Buskirk 1983), cat (Kudo et al. 1984), mouse (Wallace and Fredens 1989), ferret (King et al. 1998) and guinea pig (current study). Anatomical studies show that the projection from the NBIC to the SC has a rostrocaudal organization and is broadly topographic (King et al., 1998). Physiological studies show that many NBIC cells are tuned for sound source location, and the projections to the SC are generally thought to contribute to the auditory space map and orienting behavior (Schnupp and King 1997; Nodal et al. 2005).

The DLL input to the SC has been described as very little (ferret: King et al. 1998) to light (cat: Kudo 1981; rat: Cadusseau and Roger 1985 Bajo et al. 1993; big brown bat: Zhang 1987) to moderate (tree shrew: Casseday et al. 1976; rat: Tanaka et al. 1985; mustached bat: Covey et al. 1987) to the largest (rat: Druga and Syka 1984). Species differences could exist, and technical differences (especially differences in anatomical tracers) could also explain some of the discrepancies. Another factor may be inconsistency in identifying the DLL borders, especially its rostral border where it abuts the cMRF. The cMRF is a non-auditory

nucleus of the tegmentum that provides a substantial projection to the SC (Chen and May 2000; May 2006; Wang et al. 2010). In guinea pigs, it is located rostral to the DLL. Its caudal end is ventral to the ICrp and its rostral end is ventral to the SC. These relationships, especially the distinction between the cMRF and the DLL, are most easily seen in sagittal sections, which we used for many of our analyses. The cMRF in our experiments consistently contained many retrogradely labeled cells. A large majority of these cells were GAD⁺, providing an additional distinction compared to DLL cells, most or all of which are GABAergic (Adams and Mugnaini 1984). We conclude that few to no DLL cells project to the SC in guinea pigs.

Several studies describe subcollicular projections to the SC, often originating in superior olivary regions or areas in or around the lemniscal nuclei (Grofova et al. 1978; Edwards et al. 1979; Huerta and Harting 1984; Appell and Behan 1990). However, the number of cells is often small and the descriptions too brief to allow detailed comparisons. An anterograde tracer study identified a small projection from cochlear root neurons to the deep layer of the SC in rats (López et al. 1999). This pathway has not yet been confirmed with retrograde studies (present or previous studies). Our tracer deposits extended into the deep layer, but may have been inadequate to identify a small projection. Another possibility is that guinea pigs, which may lack cochlear root neurons, may not have a direct projection from the cochlear nucleus to the SC.

GABAergic vs. non-GABAergic projections

Appell and Behan (1990) identified GABAergic projections to the SC in cats from many areas, including numerous auditory nuclei. The present results are generally consistent with those in cats, but detailed comparisons are difficult because of differences in which nuclei were examined in the two studies. One of the main conclusions of the present study is that GABAergic projections to the SC originate from several nuclei but, overall, represent a small minority (10%) of the auditory inputs. This suggests that GABAergic inhibition may play a relatively small role in auditory projections to the SC. The transmitter phenotype(s) of the remaining projections are unknown. Glutamate is a likely candidate for the majority of the cells in the NBIC and the IC. However, some neurons in the nuclei giving rise to these projections, such as the sagulum and periolivary nuclei, also contain glycine or other transmitters. Very little is known about the transmitter phenotype(s) of the PL and the VLTg. The specific functions of the GABAergic or the non-GABAergic components of these projections remain to be determined.

Functional implications: projections from the IC and the NBIC vs. projections from subcollicular nuclei

Orienting behavior is a well-known function of the SC and it is well established that animals will orient to an acoustic stimulus. This perspective has provided the functional framework for many previous interpretations of the role of auditory projections to the SC, especially those from the IC and the NBIC (van Buskirk 1983; Kudo et al. 1984; Binns et al. 1991, 1992; Jiang et al. 1997; Schnupp and King 1997; King et al. 1998; Doubell et al. 2000; Skaliora et al. 2004; Nodal et al. 2005). Strong support for such a role comes from studies showing that auditory inputs play an important role during development in aligning the

spatial maps for acoustic stimuli with motor orientation maps (Meredith and Stein 1983; Groh and Sparks 1992; Doubell et al. 2000). Our results identifying the NBIC and IC_{lc} as major sources of input to the SC are consistent with a role of the SC in generating orienting responses to acoustic stimuli.

It is more difficult to speculate on functions of the projections from subcollicular nuclei. These nuclei accounted for about one third of the auditory projections to the SC in the present study, suggesting a potentially significant functional role. The small projection from the paralemniscal area may contribute to orienting responses given its potential association with pinna movements (Henkel and Edwards 1978; May et al. 1990), but to our knowledge none of the other nuclei have been particularly associated with sound localization or orienting behavior. Additional perspectives include attention, fear, and avoidance or escape responses (Dean et al. 1989; Mysore and Knudsen 2011; Comoli et al. 2012; Wei et al. 2015; Savage et al. 2017). A great deal of recent work focuses on the SC as part of a midbrain defense circuit that includes connections with the periaqueductal gray, parabigeminal nucleus and lateral posterior nucleus/pulvinar complex of the dorsal thalamus (e.g., Brandão, 2003; Schenberg et al. 2005; Wei et al. 2015; Xiong et al. 2015; Pereira and Moita, 2016; Savage et al. 2017). In this context, projections from the IC to the SC are thought to contribute to acoustically-driven defensive behaviors. We suggest that projections to the SC from subcollicular auditory nuclei could also contribute to this behavior. Most of these subcollicular projections originate from areas that receive direct projections from the cochlear nucleus (Warr, 1982; Kandler and Herbert, 1991; Herbert et al. 1997), and thus could serve as a relatively fast conduit for auditory stimuli to activate the SC. Such a pathway might provide an alerting signal relevant to avoidance or attention (perhaps including orienting movements). In presumed contrast with the NBIC and IC projections, these subcollicular projections would provide little or no information about sound location. Whether these functions or others are associated with the projections to the SC await more information, particularly regarding the response properties of the projecting cells.

Conclusions

Auditory inputs to the SC originate from numerous brainstem nuclei. A small proportion of the projections are GABAergic, consistent with physiological evidence for GABAergic inhibition in the SC. Major inputs arise from the IC and the NBIC. These projections likely play a substantial role in providing spatial information about a sound, and thus direct orienting behaviors. Additional inputs arise from an array of subcollicular auditory nuclei. These inputs are probably less closely associated with sound localization, but may provide an alerting signal important for avoidance and escape behaviors and attention.

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List of Abbreviations

AQ cerebral aqueduct

cMRF	central mesencephalic reticular formation
D	dorsal periolivary nucleus
DCN	dorsal cochlear nucleus
DL	dorsolateral periolivary nucleus
DLL	dorsal nucleus of the lateral lemniscus
DpG/W	deep gray/white layers of the superior colliculus
FG	FluoroGold
GAD	glutamic acid decarboxylase
GAD⁺	GAD-immunopositive
GAD⁻	GAD-immunonegative
GB	Green Beads
grca	granule cell area of cochlear nucleus
IC	inferior colliculus
ICc	central nucleus of the inferior colliculus
ICd	dorsal cortex of the inferior colliculus
IClc	lateral cortex of the inferior colliculus
ICrp	rostral pole of the inferior colliculus
ICT	intercollicular tegmentum
ILL	intermediate nucleus of the lateral lemniscus
InG/W	intermediate gray/white layers of the superior colliculus
LSO	lateral superior olive
LTB	lateral nucleus of the trapezoid body
MSO	medial superior olivary nucleus
MTB	medial nucleus of the trapezoid body
NBIC	nucleus of the brachium of the inferior colliculus
op	optic layer of the superior colliculus
PAG	periaqueductal gray
PL	paralemniscal area
PN	pontine nuclei

RB	Red Beads
Sag	Sagulum
SC	superior colliculus
scp	superior cerebellar peduncle
SOC	superior olivary complex
SPN	superior paraolivary nucleus
SuG	superficial gray layer of the superior colliculus
VCN	ventral cochlear nucleus
VLL	ventral nucleus of the lateral lemniscus
VLtg	ventrolateral tegmental nucleus
VTB	ventral nucleus of the trapezoid body

LITERATURE CITED

- Adams JC, Mugnaini E. Dorsal nucleus of the lateral lemniscus: a nucleus of GABAergic projection neurons. *Brain Res Bull.* 1984; 13:585–590. [PubMed: 6098348]
- Aitkin LM, Dickhaus H, Schult W, Zimmermann M. External nucleus of the inferior colliculus: auditory and spinal somatosensory afferents and their interactions. *J Neurophysiol.* 1978; 41:837–847. [PubMed: 681989]
- Aitkin LM, Kenyon CE, Philpott P. The representation of the auditory and somatosensory systems in the external nucleus of the inferior colliculus. *J Comp Neurol.* 1981; 196:25–40. [PubMed: 7204665]
- Anderson LA, Linden JF. Physiological differences between histologically defined subdivisions in the mouse auditory thalamus. *Hear Res.* 2011; 274:48–60. [PubMed: 21185928]
- Appell PP, Behan M. Sources of GABAergic projections to the superior colliculus in the cat. *J Comp Neurol.* 1990; 302:143–158. [PubMed: 2086611]
- Bajo VM, Merchán MA, López DE, Rouiller EM. Neuronal morphology and efferent projections of the dorsal nucleus of the lateral lemniscus in the rat. *J Comp Neurol.* 1993; 334:241–262. [PubMed: 8366195]
- Binns KE, Grant S, Withington DJ, Keating MJ. A topographic representation of auditory space in the external nucleus of the inferior colliculus of the guinea-pig. *Brain Res.* 1992; 589:231–242. [PubMed: 1393591]
- Binns KE, Withington DJ, Keating MJ. The developmental emergence of the representation of auditory azimuth in the external nucleus of the inferior colliculus of the guinea-pig: the effects of visual and auditory deprivation. *Brain Res Dev Brain Res.* 1995; 85:14–24. [PubMed: 7781161]
- Brandão ML, Troncoso AC, de Souza Silva MA, Huston JP. The relevance of neuronal substrates of defense in the midbrain tectum to anxiety and stress: empirical and conceptual considerations. *Eur J Pharmacol.* 2003; 463:225–233. [PubMed: 12600713]
- Cadusseau J, Roger M. Afferent projections to the superior colliculus in the rat, with special attention to the deep layers. *J Hirnforsch.* 1985; 26:667–681. [PubMed: 4093595]
- Casseday HJ, Diamond IT, Harting JK. Auditory pathways to the cortex in *Tupaia glis*. *J Comp Neurol.* 1976; 166:303–340. [PubMed: 816825]
- Chen B, May PJ. The feedback circuit connecting the superior colliculus and central mesencephalic reticular formation: a direct morphological demonstration. *Exp Brain Res.* 2000; 131:10–21. [PubMed: 10759167]

- Comoli E, Das Neves Favaro P, Vautrelle N, Leriche M, Overton PG, Redgrave P. Segregated anatomical input to sub-regions of the rodent superior colliculus associated with approach and defense. *Front Neuroanat.* 2012; 6:9.doi: 10.3389/fnana.2012.00009 [PubMed: 22514521]
- Coote EJ, Rees A. The distribution of nitric oxide synthase in the inferior colliculus of guinea pig. *Neuroscience.* 2008; 154:218–225. [PubMed: 18400412]
- Covey E, Hall WC, Kobler JB. Subcortical connections of the superior colliculus in the mustache bat, *Pteronotus parnellii*. *J Comp Neurol.* 1987; 263:179–197. [PubMed: 3667975]
- Dean P, Redgrave P, Westby GW. Event or emergency? Two response systems in the mammalian superior colliculus. *Trends Neurosci.* 1989; 12:137–147. [PubMed: 2470171]
- Doubell TP, Baron J, Skaliara I, King AJ. Topographical projection from the superior colliculus to the nucleus of the brachium of the inferior colliculus in the ferret: convergence of visual and auditory information. *Eur J Neurosci.* 2000; 12:4290–4308. [PubMed: 11122340]
- Druga R, Syka J. Projections from auditory structures to the superior colliculus in the rat. *Neurosci Lett.* 1984; 45:247–252. [PubMed: 6728318]
- Edwards SB, Ginsburgh CL, Henkel CK, Stein BE. Sources of subcortical projections to the superior colliculus in the cat. *J Comp Neurol.* 1979; 184:309–329. [PubMed: 762286]
- Foster, NL., Noftz, WA., Schofield, BR. Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience; 2015. Differences in GABAergic cell types distinguish the rostral pole of the inferior colliculus and the medial and lateral intercollicular areas. Program no. 507.11.
- Grofová I, Otterson OP, Rinvik E. Mesencephalic and diencephalic afferents to the superior colliculus and periaqueductal gray substance demonstrated by retrograde axonal transport of horseradish peroxidase in the cat. *Brain Res.* 1978; 146:205–220. [PubMed: 77176]
- Groh JM, Sparks DL. Two models of transforming auditory signals from head-centered to eye-centered coordinates. *Biol Cybern.* 1992; 67:291–302. [PubMed: 1515508]
- Gruters KG, Groh JM. Distribution of visual and saccade related information in the monkey inferior colliculus. *Front Neural Circuits.* 2012; 6:96. [PubMed: 23248584]
- Harting JK, Van Lieshout DP. Projections from the rostral pole of the inferior colliculus to the cat superior colliculus. *Brain Res.* 2000; 881:244–247. [PubMed: 11036169]
- Helfert RH, Bonneau JM, Wenthold RJ, Altschuler RA. GABA and glycine immunoreactivity in the guinea pig superior olivary complex. *Brain Res.* 1989; 501:269–286. [PubMed: 2819441]
- Herbert H, Klepper A, Ostwald J. Afferent and efferent connections of the ventrolateral tegmental area in the rat. *Anat Embryol.* 1997; 196:235–59. [PubMed: 9310315]
- Henkel CK, Edwards SB. The superior colliculus control of pinna movements in the cat: possible anatomical connections. *J Comp Neurol.* 1978; 182:763–767. [PubMed: 730846]
- Henkel CK. Evidence of sub-collicular auditory projections to the medial geniculate nucleus in the cat: An autoradiographic and horseradish peroxidase study. *Brain Res.* 1983; 259:21–30. [PubMed: 6824933]
- Huerta MF, Harting JK. Connectional organization of the superior colliculus. *Trends Neurosci.* 1984; 7:286–289.
- Ingham NJ, Thornton SK, McCrossan D, Withington DJ. Neurotransmitter involvement in the development and maintenance of the auditory space map in the guinea pig superior colliculus. *J Neurophysiol.* 1998; 80:2941–2953. [PubMed: 9862897]
- Jain R, Shore S. External inferior colliculus integrates trigeminal and acoustic information: unit responses to trigeminal nucleus and acoustic stimulation in the guinea pig. *Neurosci Lett.* 2006; 395:71–75. [PubMed: 16298057]
- Jiang ZD, Moore DR, King AJ. Source of subcortical projections to the superior colliculus in the ferret. *Brain Res.* 1997; 755:279–292. [PubMed: 9175895]
- Kandler K, Herbert H. Auditory projections from the cochlear nucleus to pontine and mesencephalic reticular nuclei in the rat. *Brain Res.* 1991; 562:230–242. [PubMed: 1773339]
- King AJ, Jiang ZD, Moore DR. Auditory brainstem projections to the ferret superior colliculus: anatomical contribution to the neural coding of sound azimuth. *J Comp Neurol.* 1998; 390:342–365. [PubMed: 9455897]

- Kudo M. Projections of the nuclei of the lateral lemniscus in the cat: and autoradiographic study. *Brain Res.* 1981; 221:57–69. [PubMed: 6168337]
- Kudo M, Niimi K. Ascending projection of the inferior colliculus in the cat: an autoradiographic study. *J Comp Neurol.* 1980; 191:545–556. [PubMed: 7419733]
- Kudo M, Tashiro T, Higo S, Matsuyama T, Kawamura S. Ascending projections from the nucleus of the brachium of the inferior colliculus in the cat. *Exp Brain Res.* 1984; 54:203–211. [PubMed: 6327347]
- Kulesza RJ Jr, Berrebi AS. Superior paraolivary nucleus of the rat is a GABAergic nucleus. *J Assoc Res Otolaryngol.* 2000; 1:255–269. [PubMed: 11547806]
- Loftus WC, Malmierca MS, Bishop DC, Oliver DL. The cytoarchitecture of the inferior colliculus revisited: a common organization of the lateral cortex in rat and cat. *Neuroscience.* 2008; 154:196–205. [PubMed: 18313229]
- López DE, Saldaña E, Nodal FR, Merchán MA, Warr WB. Projections of the cochlear root neurons, sentinels of the rat auditory pathway. *J Comp Neurol.* 1999; 415:160–174. [PubMed: 10545157]
- Martin RL, Webster WR, Servièrre J. The frequency organization of the inferior colliculus of the guinea pig: A [14C]-2-deoxyglucose study. *Hear Res.* 1988; 33:245–255. [PubMed: 3384759]
- May PJ, Vidal PP, Baker R. Synaptic organization of tectal-facial pathways in cat. II. Synaptic potentials following midbrain tegmentum stimulation. *J Neurophysiol.* 1990; 64:381–402. [PubMed: 1698936]
- May PJ. The mammalian superior colliculus: laminar structure and connections. *Prog Brain Res.* 2006; 151:321–378. [PubMed: 16221594]
- Meininger V, Pol D, Derer P. The inferior colliculus of the mouse. A Nissl and Golgi study. *Neuroscience.* 1986; 17:1159–79. [PubMed: 2423921]
- Mellott JG, Bickford ME, Schofield BR. Descending projections from auditory cortex to excitatory and inhibitory cells in the nucleus of the brachium of the inferior colliculus. *Front Syst Neurosci.* 2014a; 8:188. [PubMed: 25339870]
- Mellott JG, Foster NL, Nakamoto KT, Motts SD, Schofield BR. Distribution of GABAergic cells in the inferior colliculus that project to the thalamus. *Front Neuroanat.* 2014b; doi: 10.3389/fnana.2014.00017
- Mellott JG, Foster NL, Ohl AP, Schofield BR. Excitatory and inhibitory projections in parallel pathways from the inferior colliculus to the auditory thalamus. *Front Neuroanat.* 2014c; doi: 10.1523/fnana.2014.00124
- Mellott, JG., Schofield, BR. Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience; 2015. Projections from the auditory midbrain to the superior colliculus and the thalamus have separate origins. Program No. 507.13 2015. 2015. Online
- Meredith MA, Stein BE. Interactions among converging sensory inputs in the superior colliculus. *Science.* 1983; 221:389–391. [PubMed: 6867718]
- Meredith MA, Stein BE. Visual, auditory, and somatosensory convergence on cells in superior colliculus results in multisensory integration. *J Neurophysiol.* 1986; 56:640–662. [PubMed: 3537225]
- Morest DK, Oliver DL. The neuronal architecture of the inferior colliculus in the cat: defining the functional anatomy of the auditory midbrain. *J Comp Neurol.* 1984; 222:209–36. [PubMed: 6699208]
- Mysore SP, Knudsen EL. The role of a midbrain network in competitive stimulus selection. *Curr Opin Neurobiol.* 2011; 21:653–660. [PubMed: 21696945]
- Nakamoto KT, Sowick CS, Schofield BR. Auditory cortical axons contact commissural cells throughout the guinea pig inferior colliculus. *Hear Res.* 2013; 306:131–144. [PubMed: 24140579]
- Nodal FR, Doubell TP, Jiang ZD, Thompson ID, King AJ. Development of the projection from the nucleus of the brachium of the inferior colliculus to the superior colliculus in the ferret. *J Comp Neurol.* 2005; 485:202–217. [PubMed: 15791643]
- Oliver, DL., Huerta, MF. Inferior and superior colliculi. In: Webster, DB, Popper, AN., Fay, RR., editors. *Springer Handbook of Auditory Research, Vol. 1, The Mammalian Auditory Pathway: Neuroanatomy.* New York: Springer-Verlag; 1992. p. 168–221.

- Oliver, DL. Neuronal organization in the inferior colliculus. In: Winer, JA., Schreiner, CE., editors. *The Inferior Colliculus*. New York: Springer; 2005. p. 69-114.
- Pereira AG, Moita MA. Is there anybody out there? Neural circuits of threat detection in vertebrates. *Curr Opin Neurobiol*. 2016; 41:179–187. [PubMed: 27750206]
- Populin LC, Yin TC. Bimodal interaction in the superior colliculus of the behaving cat. *J Neurosci*. 2002; 22:2826–2834. [PubMed: 11923447]
- Savage MA, McQuade R, Thiele A. Segregated fronto-cortical and midbrain connections in the mouse and their relation to approach and avoidance orienting behaviors. *J Comp Neurol*. 2017; 525:1980–1999. [PubMed: 28177526]
- Schenberg LC, Pova AL, Costa AL, Caldellas AV, Tufik S, Bittencourt AS. Functional specializations within the tectum defense systems of the rat. *Neurosci Biobehav Rev*. 2005; 29:1279–1298. [PubMed: 16087233]
- Schnupp JW, King AJ. Coding for auditory space in the nucleus of the brachium of the inferior colliculus in the ferret. *J Neurophysiol*. 1997; 78:2717–2731. [PubMed: 9356421]
- Schofield BR. Retrograde axonal tracing with fluorescent markers. *Curr Protoc Neurosci*. 2008; (Unit 1):17. Chapter 1. [PubMed: 18428673]
- Schofield BR, Cant NB. Organization of the superior olivary complex in the guinea pig. I. Cytoarchitecture, cytochrome oxidase histochemistry, and dendritic morphology. *J Comp Neurol*. 1991; 314:645–670. [PubMed: 1726174]
- Schofield BR, Cant NB. Ventral nucleus of the lateral lemniscus in guinea pigs: cytoarchitecture and inputs from the cochlear nucleus. *J Comp Neurol*. 1997; 379:363–385. [PubMed: 9067830]
- Schofield BR, Cant NB. Descending auditory pathways: Projections from the inferior colliculus contact superior olivary cells that project bilaterally to the cochlear nuclei. *J Comp Neurol*. 1999; 409:210–223. [PubMed: 10379915]
- Schofield BR, Mellott JG, Motts SD. Subcollicular projections to the auditory thalamus and collateral projections to the inferior colliculus. *Front Neuroanat*. 2014a; 8:16. [PubMed: 24723858]
- Schofield BR, Motts SD, Mellott JG, Foster NL. Projections from the dorsal and ventral cochlear nuclei to the medial geniculate body. *Front Neuroanat*. 2014b; 8:10. [PubMed: 24634646]
- Skaliora I, Doubell TP, Holmes NP, Nodal FR, King AJ. Functional topography of converging visual and auditory inputs to neurons in the rat superior colliculus. *J Neurophysiol*. 2004; 92:2933–2946. [PubMed: 15229210]
- Sparks DL. Neural cartography: sensory and motor maps in the superior colliculus. *Brain Behav Evol*. 1988; 31:49–56. [PubMed: 3334905]
- Stein, BE., Meredith, MA. *The merging of the senses*. MIT Press; Cambridge: 1993.
- Tanaka K, Otani K, Tokunaga A, Sugita S. The organization of neurons in the nucleus of the lateral lemniscus projecting to the superior and inferior colliculi in the rat. 1985; 341:252–260.
- Van Buskirk RL. Subcortical auditory and somatosensory afferents to hamster superior colliculus. *Brain Res Bull*. 1983; 10:583–587. [PubMed: 6871733]
- Wallace MN, Fredens K. Relationship of afferent inputs to the lattice of high NADPH-diaphorase activity in the mouse superior colliculus. *Exp Brain Res*. 1989; 78:435–445. [PubMed: 2599053]
- Wang N, Warren S, May PJ. The macaque midbrain reticular formation sends side-specific feedback to the superior colliculus. *Exp Brain Res*. 2010; 201:701–717. [PubMed: 19940983]
- Warr, WB. Parallel ascending pathways from the cochlear nucleus: neuroanatomical evidence of functional specialization. In: Neff, WD., editor. *Contributions to Sensory Physiology*. Vol. 7. New York: Academic Press; 1982. p. 1-38.
- Wei P, Liu N, Zhang Z, Liu X, Tang Y, He X, Wu B, Zhou Z, Liu Y, Li J, Zhang Y, Zhou X, Xu L, Chen L, Bi G, Hu X, Xu F, Wang L. Processing of visually evoked innate fear by a non-canonical thalamic pathway. *Nat Commun*. 2015; 6:6756. [PubMed: 25854147]
- Xiong XR, Liang F, Zingg B, Ji XY, Ibrahim LA, Tao HW, Zhang LI. Auditory cortex controls sound-driven innate defense behaviour through corticofugal projections to inferior colliculus. *Nat Commun*. 2015; 6:7224. [PubMed: 26068082]

- Xiong K, Luo DW, Patrylo PR, Luo XG, Struble RG, Clough RW, Yan XX. Doublecortin-expressing cells are present in layer II across the adult guinea pig cerebral cortex: partial colocalization with mature interneuron markers. *Exp Neurol*. 2008; 211:271–282. [PubMed: 18378231]
- Zhang SQ. Anatomical study of neural projections to the superior colliculus of the big brown bat, *Eptesicus fuscus*. *Brain Res*. 1987; 416:375–380. [PubMed: 3620966]

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Fig. 1.
Photomicrograph of a sagittal section showing a representative deposit of Red Beads (RB) throughout the layers of the superior colliculus (SC) in GP674. See list of abbreviations.
Scale bar = 1 mm.

Fig. 2.

Paired photomicrographs showing retrogradely-labeled cells that are GAD⁺ (arrows) or GAD⁻ (arrowheads) and project to the superior colliculus. The top panel in each pair shows cells retrogradely labeled by either Green Beads (green) or Red Beads (red). The bottom panel in each pair shows the same field viewed for immunoreactivity to GAD (cyan). Most panels also demonstrate the presence of GAD⁺ cells that did not contain retrograde tracer. See list of abbreviations. Scale bars = 20 μ m. Cases GP669 (SPN); GP673 (remaining images).

Fig. 3. Distribution of retrogradely labeled cells ipsilateral to an injection of FluoroGold (gray shading) into the left SC. The tissue was immunostained for GAD to distinguish tracer-labeled GABAergic cells (GAD⁺, black stars) from tracer-labeled non-GABAergic cells (GAD⁻, red circles). Each symbol represents one retrogradely-labeled cell. D - dorsal; R - rostral. Sections are arranged from lateral (L1) to medial (L6). See list of abbreviations.

Fig. 4. Distribution of retrogradely labeled cells contralateral to an injection of FluoroGold (shown in Fig. 3) into the left SC. The tissue was immunostained for GAD to distinguish tracer-labeled GABAergic cells (GAD⁺, black stars) from tracer-labeled non-GABAergic cells (GAD⁻, red circles). Each symbol represents one retrogradely-labeled cell. D - dorsal; R - rostral; Sections are arranged from lateral (R1) to medial (R6). See list of abbreviations.

Fig. 5. Histograms summarizing the distribution of retrogradely labeled neurons in the auditory brainstem that project to the SC (n = 5,274 cells; values are mean ± SD; see Table 2). Tracer-labeled neurons were either GAD⁻ (red bars) or GAD⁺ (black bars). The nuclei are grouped according to their location ipsilateral or contralateral to the side of tracer injection. Nuclei are listed in decreasing order of their overall number of retrograde labeled cells (see bottom row of Table 2). See list of abbreviations.

Fig. 6.

Quantitative graphical summary of the auditory brainstem projections to the SC. The shading/hatching of the nuclei and the thickness of the arrows indicate the relative proportion of the retrogradely labeled cells that were located in each nucleus after large deposits of retrograde tracer into the SC ($\% \text{ in nucleus} = (\text{number of tracer-labeled cells in a nucleus}) / (\text{number of tracer labeled cells in all auditory nuclei}) * 100$). These values were arbitrarily divided into five categories and shaded or cross-hatched as indicated in the legend. Arrows are drawn from groups of nuclei (superior olivary complex (SOC), lemniscal and adjacent tegmental regions, the inferior colliculus or the nucleus of brachium of the

inferior colliculus), with the thickness of the arrow representing the relative number of labeled cells in each group. Individual nuclei are named in the cross sections or, for the SOC, in the adjacent enlargement. See list of abbreviations.

Table 1

Summary of tracers and volumes injected into left (L) or right (R) superior colliculus.

Case	Side	Tracer	Total Volume	Plane of Cut
GP667	L	FG	0.05 µl	Sagittal
GP668	L	FG	0.05 µl	Sagittal
GP669	L	RB	0.1 µl	Sagittal
GP669	L	GB	0.1 µl	Sagittal
GP673	L	FG	0.05 µl	Transverse
GP673	R	RB	0.2 µl	Transverse
GP673	R	GB	0.2 µl	Transverse
GP674	L	FG	0.05 µl	Sagittal
GP674	R	RB	0.2 µl	Sagittal
GP674	R	GB	0.2 µl	Sagittal
GP738	L	FG	0.05 µl	Sagittal

See list of abbreviations.

Table 2

Percent of retrogradely-labeled cells among brainstem auditory nuclei after injection of retrograde tracer into the superior colliculus.

	Experiment	Tracer	NBIC	IClc	Sag	PL	ICrp	VLtg	SPN	D	VTB	ILL	VLL	MTB	MTB	DLL
Ipsi GAD⁻	GP673	FG	31.7	16.8	6.2	4.5	4.2	1.6	1.0	1.9	2.1	0.9	0.3	0.2	0.2	0.1
	GP673	GB	33.7	12.2	7.0	5.4	3.1	2.9	1.5	1.7	1.2	1.9	1.4	0.2	0.3	0.6
	GP674	GB	23.8	20.4	8.7	4.4	9.3	2.6	1.4	2.3	1.1	1.2	0.8	0.5	0.1	0.1
	GP738	FG	28.0	10.6	7.2	6.6	5.5	2.2	1.1	0.8	1.5	1.0	0.2	0.3	0.3	0.0
Ipsi GAD⁺	Average		29.3	15.0	7.3	5.2	5.5	2.3	1.2	1.7	1.5	1.2	0.7	0.3	0.2	0.2
	SD		4.3	4.4	1.1	1.0	2.7	0.6	0.3	0.6	0.5	0.4	0.6	0.1	0.1	0.3
	GP673	FG	4.7	1.2	0.2	0.4	0.8	0.0	0.6	0.2	0.1	0.0	0.1	0.0	0.0	0.0
	GP673	GB	5.5	1.6	0.3	0.4	0.4	0.1	0.5	0.1	0.2	0.0	0.2	0.1	0.0	0.1
	GP674	GB	3.0	0.9	0.6	0.7	0.6	0.2	0.7	0.1	0.1	0.0	0.2	0.0	0.0	0.0
	GP738	FG	4.0	1.3	0.4	0.6	0.9	0.0	0.4	0.0	0.1	0.0	0.0	0.0	0.0	0.0
	Average		4.3	1.2	0.4	0.5	0.7	0.1	0.5	0.1	0.1	0.0	0.1	0.0	0.0	0.0
Contra GAD⁻	SD		1.0	0.3	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0
	GP673	FG	8.2	2.4	3.2	1.5	0.7	0.6	0.6	0.2	0.2	0.1	0.2	0.0	0.0	0.0
	GP673	GB	6.8	0.6	3.0	2.4	0.2	0.7	0.4	0.4	0.5	0.2	0.5	0.0	0.1	0.0
	GP674	GB	3.7	0.9	5.4	2.1	0.4	0.7	0.4	0.4	0.4	0.2	0.3	0.0	0.1	0.1
	GP738	FG	13.1	1.9	3.7	2.6	0.5	0.9	0.2	0.3	0.3	0.1	0.1	0.1	0.1	0.1
	Average		7.9	1.4	3.8	2.1	0.4	0.7	0.4	0.3	0.3	0.1	0.3	0.0	0.1	0.1
	SD		3.9	0.8	1.1	0.5	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.0	0.0	0.1
Contra GAD⁺	GP673	FG	1.4	0.1	0.2	0.2	0.1	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	GP673	GB	1.0	0.1	0.2	0.3	0.0	0.1	0.1	0.0	0.2	0.0	0.0	0.0	0.0	0.0
	GP674	GB	0.7	0.1	0.4	0.2	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0
	GP738	FG	2.5	0.2	0.3	0.2	0.1	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Average		1.4	0.1	0.3	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Summary (averages)	SD		0.8	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0
	Total															
Ipsi GAD⁻			29.3	15.0	7.3	5.2	5.5	2.3	1.2	1.7	1.5	1.2	0.7	0.3	0.2	0.2
																72

Experiment	Tracer	NBIC	IClc	Sag	PL	ICrp	VLtg	SPN	D	VTB	ILL	VLL	MTB	MTB	DLL
Ipsi GAD ⁺		4.3	1.2	0.4	0.5	0.7	0.1	0.5	0.1	0.1	0.0	0.1	0.0	0.0	8
Contra GAD ⁻		7.9	1.4	3.8	2.1	0.4	0.7	0.4	0.3	0.3	0.1	0.3	0.0	0.1	18
Contra GAD ⁺		1.4	0.1	0.3	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	2
Total		42.9	17.8	11.7	8.1	6.7	3.1	2.3	2.1	2.0	1.4	1.1	0.3	0.3	100

Four experiments with the largest number of retrograde cells were quantified. Each cell was coded for presence or absence of GAD immunoreactivity (GAD⁺ or GAD⁻, respectively). Data rows are grouped according to the GAD immunoreactivity and the location, ipsilateral (“Ipsi”) or contralateral (“Contra”), of the nuclei relative to the injection site. Each number represents the percentage of GAD⁺ or GAD⁻ retrogradely labeled cells in that nucleus (e.g., for the GP 673 experiment with FluoroGold (FG), GAD⁻ retrogradely labeled cells in the ipsilateral NBIC represented 31.7% of all the FG-labeled cells in the auditory nuclei in that experiment. In order to assess the variance across experiments, the average percentage and standard deviation are provide for each data group. The bottom panel lists the averages for each data group. In the summary panel, the total at the end of each row represents the overall percentage of cells averaged over all four experiments; i.e., on average, 72% of the retrogradely labeled cells were GAD⁻ and located in ipsilateral nuclei. Totals at the bottom of each column show the overall average percentage of retrograde cells located in a given nucleus (Ipsi and Contra, GAD⁻ and GAD⁺), e.g., on average, the NBIC contained on average 42.9% of the retrogradely labeled cells. See list of abbreviations. Number of cells analyzed in each experiment: GP673 FG: 1,349; GP673 GB: 1,011; GP 674 GB: 965; GP 738 FG: 935.