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Analysis of excitatory synapses in the guinea pig inferior colliculus: a study using electron microscopy and GABA immunocytochemistry

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Abstract

The inferior colliculus (IC) integrates ascending auditory input from the lower brainstem and descending input from auditory cortex. Understanding how IC cells integrate these inputs requires identification of their synaptic arrangements. We describe excitatory synapses in the dorsal cortex, central nucleus, and lateral cortex of the IC in guinea pigs. We used electron microscopy (EM) and post-embedding anti-GABA immunogold histochemistry on aldehyde-fixed tissue from pigmented adult guinea pigs. Excitatory synapses were identified by round vesicles, asymmetric synaptic junctions, and GABA-immunonegative presynaptic boutons.

Excitatory synapses constitute ~ 60% of the synapses in each IC subdivision. Three types can be distinguished by presynaptic profile area and number of mitochondrial profiles. Large excitatory (LE) boutons are more than 2 μm^2 in area and usually contain 5 or more mitochondrial profiles. Small excitatory (SE) boutons are usually less than 0.7 μm^2 in area and usually contain 0 or 1 mitochondria. Medium excitatory (ME) boutons are intermediate in size and usually contain 2 to 4 mitochondria. LE boutons are mostly confined to the ICc, while the other two types are present throughout the IC. Dendritic spines are the most common target of excitatory boutons in the IC dorsal cortex, whereas dendritic shafts are the most common target in other IC subdivisions. Finally, each bouton type terminates on both GABA+ and GABA-negative (i.e., glutamatergic) targets, with terminations on GABA-negative profiles being much more frequent.

The ultrastructural differences between the 3 types of boutons presumably reflect different origins and may indicate differences in postsynaptic effect. Despite such differences in origins, each of the bouton types contact both GABAergic and non-GABAergic IC cells, and could be expected to activate both excitatory and inhibitory IC circuits.

Keywords

auditory; dendritic spines; gamma-aminobutyric acid; ultrastructure; inhibition; circuit

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The inferior colliculus (IC) processes the majority of ascending auditory information and thus is associated with most aspects of auditory perception. The IC also connects with motor centers such as the superior colliculus and pontine nuclei, and thus contributes to orienting behavior and acoustic guidance of movement (Huffman and Henson, 1990; Thompson, 2005). Finally, the IC is both a target and a source of descending auditory pathways. Auditory feedback to the IC may play a role in detection of signals in noise, sound localization, and auditory attention (reviewed in Schofield, 2011a; Suga, 2012).

Neurons in the IC show a diverse range of responses to sounds. The diversity arises from variation in intrinsic properties across different IC cell types and from convergence of inputs from different origins (Sivaramakrishnan and Oliver, 2001; Kelly and Caspary, 2005; Oliver, 2005; Saldaña and Merchán, 2005; Schofield, 2005; Wu, 2005; Cant and Benson, 2006). Despite the large number of inputs to the IC, both anatomical and physiological studies suggest that the responses of many IC cells depend on a relatively small subset of the ascending auditory inputs (Brunso-Bechtold et al., 1981; Davis, 2002; Pollak et al., 2003; Oliver, 2005; Cant and Benson, 2006; Loftus et al., 2010). However, there remain many questions about the exact inputs that converge onto specific types of IC cells. Solving this puzzle – identifying the circuits of IC cells – is fundamental to understanding how IC cells integrate auditory information and serve the multitude of functions attributed to the IC.

An important step in understanding circuits is identifying their synaptic connections. Ultrastructural studies with the electron microscope provide the necessary resolution to identify synaptic relationships. A common starting point is identifying synaptic features that distinguish potentially excitatory or inhibitory synapses. Details of the presynaptic profiles may allow particular bouton types to be related to their origins. In addition, identifying the postsynaptic target (dendritic spine, dendritic shaft, soma) of a synapse provides insight into integration of inputs by the postsynaptic cell. While the resolution of electron microscopy provides detailed information about synapses, its high demands in labor and generally small sample sizes have limited the available information. Much of the information on ultrastructure of the IC is from the central nucleus of the IC (ICc), the major target of ascending lemniscal afferents. Much less information is available for the dorsal cortex (ICd) and the lateral cortex (ICl). Given that the three IC subdivisions are functionally distinct, there will be a need to understand the circuitry of each area (Aitkin, 1986; Irvine, 1986; Huffman and Henson, 1990; Rouiller, 1997; Malmierca and Hackett, 2010).

The available information on the synaptic organization of the IC is also limited with respect to species; most studies have been in cats, rats or mice (Rockel and Jones, 1973; Granstrem, 1984; Roberts and Ribak, 1987; Paloff and Usunoff, 1992; Oliver et al., 1995; Saldaña et al., 1996; Gaza and Ribak, 1997; Kazee and West, 1999; Liu et al., 1999; Mei et al., 1999). We have begun a series of studies to examine the synaptic organization of the IC in guinea pigs. This species is widely used in auditory research but, to our knowledge, there are no descriptions of the guinea pig IC ultrastructure.

This report focuses on presumptive excitatory synapses. We identified ultrastructural features that distinguish 3 types of excitatory synapse. Two types are present throughout the IC, whereas the third type is largely confined to the ICc. The three types probably arise from different sources and also differ in the relative targeting of spines versus dendritic shafts, implying that they may have different postsynaptic effects. All 3 types terminate on both GABAergic and non-GABAergic (i.e., glutamatergic) targets, suggesting that inputs associated with each of the excitatory synapse types are likely to activate both excitatory and inhibitory IC cells.

EXPERIMENTAL PROCEDURES

Experiments were performed on 6 adult pigmented guinea pigs of either gender weighing 400 to 900 g (Elm Hill Breeding Laboratories, Chelmsford, MA, USA). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) 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. Five of the six animals used for this study underwent survival surgery for injection of anatomical tracers into the auditory cortex prior to perfusion. The procedures for the tracer injections were similar to previous descriptions from this laboratory (e.g., Schofield, 2011b) and were approved by the IACUC. The use of tissue from these animals allowed us to minimize the number of animals needed for the present study. The results of the tracer injections will be described in a separate report.

Perfusion and sectioning

Each animal was sacrificed by overdose with sodium pentobarbital (440 mg/kg; i.p., Euthasol, Virbac Inc., Fort Worth, TX, USA) or isoflurane (inhalation until cessation of breathing; Aerrane, Baxter, Deerfield, IL, USA). The animal was perfused through the aorta with Tyrode's solution, followed by 2% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate buffer (PB, pH 7.4). The brain was then removed and stored overnight at 4°C in 2% paraformaldehyde and 2% glutaraldehyde in PB. The following day, tissue blocks containing the IC were cut into 50 µm parasagittal sections with a Vibratome. Sections were collected in six series and processed as described below or placed in freezing buffer and stored for future processing.

Identification of IC subdivisions

One series of sections from each animal was stained for NADPH-diaphorase activity (Dawson et al., 1991). The stained sections were mounted on slides, dried overnight and then coverslipped with DPX (Aldrich Chemical Company, Inc., Milwaukee, WI, USA). The NADPH stain reflects the distribution of neuronal nitric oxide synthase and can be used to distinguish IC subdivisions in guinea pigs (Coote and Rees, 2008). In general, the ICc stains very lightly for NADPH whereas the other IC areas are more darkly stained. The borders between the ICc and the surrounding regions can be difficult to specify, particularly in some areas (e.g., the border between the ICc and the dorsomedially adjacent ICd). The ICc appears lightly stained overall because of very limited neuropil staining. The ICd has darker neuropil, but the transition is gradual. The uncertainty can be reduced by looking at the sections under high magnification to allow identification of individually stained cells, which appear in the ICd and also in part of the ICc. In some cases, it is possible to identify cells with dendritic trees that are elongated along a dorso-medial to ventro-lateral axis, consistent with the description of so-called "flat" cells that characterize the ICc. We used such staining to refine our estimation of the borders. As discussed by Coote and Rees (2008), this approach leads to a slightly larger ICc than would otherwise be drawn.

Processing for Electron Microscopy

One or more additional series of sections (not stained for NADPH) were post-fixed for 1 hour in 2% osmium tetroxide in PB, dehydrated in an alcohol series, embedded in Durcupan resin (Electron Microscopy Sciences, Fort Washington, PA, USA) and flat-mounted between sheets of Aclar Embedding Film (Ted Pella, Inc., Redding, CA, USA). The sections were then examined in a light microscope and compared to NADPH-stained sections in order to determine the boundaries of the IC subdivisions. An area up to 1 mm on a side and located completely within a particular IC subdivision was trimmed from the section with a scalpel and glued onto a resin base with cyanoacrylate (KrazyGlue, Columbus, OH, USA).

The positions of the tissue blocks were plotted and superimposed on a series of NADPH-stained sections (Fig. 1), using a Zeiss Axioplan 2 microscope with a Neurolucida system (MBF Bioscience, Williston, VT). For samples from the ICc, we selected regions with low NADPH staining, well within the borders of the ICc as described in the paragraph above. The locations of the samples are shown in Figure 1 (sections 1300 and 1600). For analysis of the IClc, we took 3 samples from regions associated with strong NADPH staining and located in very lateral sections in the IC (Fig. 1). Finally, for analysis of dorsal cortex, we took 3 samples from areas associated with strong NADPH staining and located in the dorsomedial part of the IC (Fig. 1).

Ultrathin sections (100 nm, gold-silver interference color) were cut from each tissue block using an ultramicrotome (UC6 Ultramicrotome, Leica Microsystems, Buffalo Grove, IL, USA) and every seventh section was collected. Based upon our measurements of the maximum size of the synaptic densities in the guinea pig IC we determined that a spacing of 600 nm was sufficient to avoid taking multiple sections of the same synapse. Each section was collected on a 300-mesh nickel grid and immunostained for GABA as described previously (Coomes et al., 2002). Briefly, grids with ultrathin sections were incubated overnight in anti-GABA antibody (rabbit anti-GABA, Sigma, St. Louis, MO) diluted 1:500 or 1:1000 in 0.05 M Tris-buffered saline with 0.1% Triton X-100, pH 7.6 (TBST), washed in TBST pH 7.6, then TBST pH 8.2, and placed into a secondary antibody conjugated to 15 nm gold particles (goat anti-rabbit, diluted 1:25 in TBST pH 8.2; Ted Pella Inc., Redding, CA). The sections were washed in TBST pH 7.6, washed in water, stained with uranyl acetate (1% aqueous) or uranyl acetate and Reynolds's lead citrate (Reynolds, 1963), and allowed to dry.

Electron microscopy and image preparation

Ultrastructure was observed with a transmission electron microscope (JEM-100S; JEOL, Peabody, MA, USA) at 60kV and 15 000 – 40 000 magnification. Images were recorded on Kodak SO-163 film (Kodak, Rochester, NY, USA). Calibration was done with a parallel line replica grid (Electron Microscopy Sciences, Hatfield, PA, USA) photographed at the same voltage and magnifications as were used for the samples. The negatives were scanned at a resolution of 1200–2000 pixels/inch using a large-format backlit scanner (ScanMaker 800, Microtek, Santa Fe Springs, CA, USA) to produce digital images for analysis. The images were analyzed with ImageJ (Abramoff et al., 2004) for measurements of profile area and vesicle size. Adobe Photoshop and Adobe Illustrator (Adobe, San Jose, CA, USA) were used to adjust brightness and contrast levels, to add colors to facilitate descriptions, and to arrange and label photographs. Bar graphs and pie charts were generated with Excel (Microsoft Corporation, Redmond, WA, USA).

Analysis of excitatory synapses

One hundred fifty synapses (50 from each subdivision of the IC) were collected for initial analysis. For each IC subdivision, a section was examined with a systematic scan across the section, taking care to photograph every potential synapse. Potential synapses were identified by the presence of pre- and post-synaptic densities, a synaptic cleft, and a collection of vesicles in the presynaptic profile. Images were collected in multiple sessions until the sample reached 50 synapses from each IC subdivision.

An initial examination of the data led to the conclusion that excitatory synapses could be divided into three categories that could be differentiated by the size and mitochondrial content of the presynaptic profile. To address this issue images of an additional 300 excitatory synapses (100 in each subdivision of the IC) were collected.

RESULTS

The IC subdivisions differ in ultrastructural features

All IC subdivisions contain neuronal cell bodies, dendrites and spines as well as myelinated and unmyelinated axons. The arrangement of these components varies such that the subdivisions appear quite distinct at low magnification, with the most striking differences in the appearance of the neuropil (Fig. 2). The ICc is characterized by an abundance of large profiles ($>2\ \mu\text{m}$ in diameter) (Fig. 2A, yellow asterisks), many of which contain a large number of mitochondria (Fig. 2, mitochondria are highlighted in purple). Many of these profiles are dendrites, although some particularly large axonal boutons containing many mitochondria are also present in the ICc (described below). Large myelinated axons are prominent in the ICc, although their distribution is not uniform. Many such axons are visible in some fields of view (e.g., Fig. 2A), whereas nearby areas may contain fewer large myelinated axons (not shown). This organization probably reflects the fibrodendritic laminae formed by axons from lemniscal inputs and axons of IC cells (Malmierca et al., 1995; Oliver, 2005; Saldaña and Merchán, 2005). The ICd appears different because of its abundance of small and medium profiles ($<2\ \mu\text{m}$ in diameter) (Fig. 2B). The neuropil in the IClc is similar to that in the ICd. However, myelinated axons more often occupy a substantial part of the field of view in the IClc, producing a distinct appearance (Fig. 2C). The myelinated axons in the IClc vary in size ($0.25\ \mu\text{m}$ to $>5\ \mu\text{m}$ in short diameter) and amount of myelination (2 to >20 myelin lamellae). Interspersed between the myelinated axons are small to medium profiles ($<2\ \mu\text{m}$ in diameter) that contain few mitochondria. These observations do not address variations that occur within a subdivision. For example, the number and arrangement of myelinated axons vary depending on proximity to the various fiber tracts such as the lateral lemniscus, commissure of the IC and brachium of the IC. The recognition of differences between the subdivisions supports the notion of functional differences and provides preliminary data for future, more detailed analyses.

Differentiation of excitatory and inhibitory synapses

We identified synapses by the presence of pre- and post-synaptic densities, a synaptic cleft, and a collection of vesicles in the presynaptic profile. As in most brain areas (Peters et al., 1991), the degree of symmetry of the synaptic densities and the shapes of the synaptic vesicles allow a distinction between putative excitatory and inhibitory synapses (Fig. 3). Thus, excitatory synapses in the IC have presynaptic profiles with round vesicles and form asymmetric densities (Fig. 3A–C). The presynaptic profiles are GABA-negative. Inhibitory synapses have less prominent postsynaptic densities, producing a symmetric synapse (Fig. 3D–F). The synaptic vesicles are pleomorphic or flat. In many instances, the presynaptic boutons are GABA-immunopositive (GABA+; Figs. 3D, E). Other inhibitory presynaptic profiles are GABA-negative and contain flat vesicles (Fig. 3F); the majority of these synapses are probably glycinergic (Liu et al., 1999).

The IC subdivisions are remarkably similar in their complement of excitatory vs. inhibitory synapses. Excitatory synapses make up 60% of the contacts in the ICc, 60% in the ICd and 56% in the IClc. Most of the remaining synapses (38–42%) are inhibitory, whereas about 2% were unclassifiable with the present criteria. The remainder of this report focuses on the excitatory synapses. The following description is based on 300 excitatory synapses, collected as detailed in the methods.

Excitatory synapses can be divided into 3 types based on bouton ultrastructure

Variation in size of the presynaptic bouton is the most striking feature of the excitatory synapses. Across the IC, the boutons vary from less than $0.25\ \mu\text{m}^2$ to over $4\ \mu\text{m}^2$ in cross-sectional area. The largest boutons, which we refer to as large excitatory (LE), contain a

large number of mitochondria (Fig. 4). LE boutons are similar to previous descriptions of boutons arising from lemniscal inputs to the IC (Oliver, 1984, 1987; Oliver et al., 1995) and, like lemniscal inputs, are concentrated in the ICc. Small excitatory (SE) boutons contain few or no mitochondria (Fig. 5). SE boutons are most prevalent in the ICd and IClc and match descriptions of boutons arising from cortical inputs to these areas (unpublished observations; Jones and Rockel, 1973; Granstrem, 1984; Saldaña et al., 1996). Several observations suggest the existence of a third group of boutons. These medium excitatory (ME) boutons have intermediate values of cross-sectional area and mitochondrial count (Fig. 6). ME boutons differ from the other two groups in their distribution across the IC subdivisions. As shown in Figure 7, LE boutons are confined almost exclusively (98%) to the ICc; they are extremely rare in the ICd and do not appear in the IClc. SE boutons are present in the ICc, but the majority (>80%) of them are in the ICd and IClc. ME boutons are most common (57%) in the ICc, contrasting with SE boutons, which have their smallest component in the ICc. At the same time, ME boutons differ from LE boutons in that a substantial portion of the ME population is in ICd and IClc.

Other characteristics of the presynaptic boutons vary and may provide a basis for further classification. The distribution of synaptic vesicles varies from relatively sparse throughout some boutons (Fig. 6C) to densely packed in others (Fig. 6D). The size of the synaptic vesicles also varies. Some boutons have a uniform collection of small vesicles (Fig. 6D) whereas other boutons have larger synaptic vesicles (Fig. 6F). Finally, dense core vesicles are present in some profiles (Figs. 4C, 5F, 6E). While the variations suggest subtypes, the number of subtypes and criteria for distinguishing them could not be determined based on the present samples.

Excitatory synapses contact GABA-negative and GABA-positive profiles, including dendritic spines, dendritic shafts, and somas

The targets of excitatory synapses include spines, dendritic shafts and, much less often, somas. While large dendrites are easy to identify, the distinction between small dendrites and spines can be more difficult. Three features are particularly useful in distinguishing spines from dendrites in the guinea pig IC (Fig. 8). Spines contain an electron dense “fluffy material” that is absent in dendrites. Dendrites routinely contain microtubules (Fig. 8, yellow arrowheads), whereas spines do not (Fig. 8). These features characterize spines and dendrites in many brain areas (Peters et al., 1991). The third feature, present in some but not all profiles of spines in the IC, is a thin-walled sac (Fig. 8, arrows). The sacs are similar to those described by Rockel and Jones (1973), who considered these structures as diagnostic for spines in the IC of cats. Mitochondria, commonly present in dendrites, are also clearly present in some spines (e.g., Fig. 8A).

We observed GABA+ and GABA-negative somas, dendrites and spines. Three profile types – GABA-negative dendritic shafts, GABA-negative spines and GABA+ dendritic shafts – form the majority of targets for excitatory synapses. Figure 9 shows the distribution of target profile types for each type of excitatory bouton in each IC subdivision. In the ICc, each type of bouton contacts GABA-negative profiles much more often than GABA+ profiles. Also, each type of bouton contacts dendritic shafts more often than spines, and this holds true for both GABA-negative and GABA+ targets. The main difference between the ICc and the other 2 subdivisions is the near absence of the LE boutons in the ICd and IClc. The ICd is dominated by the SE boutons, with almost all the remainder being ME boutons. The ICd is unique in that the excitatory synapses (both SE and ME types) terminate more frequently on spines than on dendritic shafts. The IClc is similar to the ICd in having many SE boutons and fewer ME boutons, but in the IClc the ME and SE boutons terminate preferentially on dendritic shafts.

Percentage of excitatory synapse types relative to inhibitory synapses

Figure 10 summarizes the relative proportions of the inhibitory and the three types of excitatory boutons. The major points are the relative constancy of excitatory to inhibitory ratios (roughly 60% to 40%); the near total confinement of LE boutons to the ICc, and the dominance of SE boutons in both the ICd and the IClc.

DISCUSSION

The present study describes the ultrastructure and synaptic organization of synapses in several subdivisions of the IC in guinea pigs. We focused on excitatory synapses, which make up about 60% of the synapses in the IC. We identified three types of excitatory synapses that differ in size and mitochondrial content of the presynaptic profile, identity of postsynaptic targets, and distribution across IC subdivisions. LE boutons are concentrated in the ICc whereas SE boutons are concentrated in the ICd and IClc. ME boutons are more evenly distributed, being most numerous in the ICc but also constituting substantial populations in the ICd and IClc. The differences between the bouton types presumably reflect differences in origins: LE boutons, and probably many ME boutons, probably arise from lemniscal axons originating in subcollicular regions. SE boutons likely arise in large part from auditory cortex. Some of the morphological features may also reflect differences in firing properties of the presynaptic fibers. All three bouton types contact non-GABAergic cells much more often than GABAergic cells. We discuss these and other functional implications after a brief consideration of technical issues related to our data collection and analysis.

Technical considerations

We classified synapses as excitatory or inhibitory based upon synaptic ultrastructure (Peters et al., 1991). This approach has proven robust in many brain areas, and is supported in the present study by the results of the GABA immunohistochemistry. A few of the synapses (~2%) did not fit into the classic categories (e.g., an asymmetric synapse with pleomorphic vesicles). Such exceptions were also noted in the original electron microscopic studies (Gray, 1959; Eccles, 1964; Colonnier, 1968). Proper classification of these rare synapses will require study with additional techniques.

The GABA antibody used for this report has been used in numerous brain areas and numerous species, including guinea pigs, rats, rabbits, cats and mice (Coomes et al., 2002; Ruiz et al., 2004; Ponti et al., 2008; Luzzati et al., 2009; Brown et al., 2012; Smith et al., 2012). The ultrastructural features of GABA+ profiles in the present study (boutons, cell bodies, dendrites, spines and axon trunks) are similar to other descriptions of GABAergic structures in the IC, including studies that used antibodies to glutamic acid decarboxylase (GAD), a different marker for GABAergic neurons (Roberts and Ribak, 1987; Oliver and Beckius, 1992). With any antibody, there is a concern about over- or under-staining. We would expect over-staining to label a substantial number of boutons with excitatory ultrastructural features, but we saw few examples of such staining. Under-staining is more difficult to rule out, but we did observe occasional GABA+ spines, which are fairly rare in the IC and, in our experience, are among the more difficult profiles to stain with anti-GABA antibodies. The staining of GABA+ spines suggests that the majority of GABA+ profiles were labeled. Further support comes from the similarities in the percentage of GABA-positive and symmetric synapses across species: 40% in guinea pigs (present study) and 40–50% in the ICc of cats (Oliver et al., 1995).

An additional technical issue is related to axosomatic synapses, which are of interest because, 1) their relative proximity to the spike trigger zone provides the potential for

substantial effect on postsynaptic spiking; and, 2) a subset of IC cells receive many axosomatic contacts (Ribak and Roberts, 1986; Ito et al., 2011). Axosomatic synapses made up only 2.3% of our sample. This relative rarity of synapses on somas has been described for both excitatory and inhibitory synapses in the ICc of cats (Oliver and Beckius, 1992; Oliver et al., 1995). Consequently, a thorough characterization of axosomatic synapses will require additional study.

We describe results from several subdivisions of the IC but, as with most studies using electron microscopy, there are concerns about the small regions of analysis versus the desire to generalize the findings. It is possible that there are ultrastructural differences between the specific regions analyzed and other parts of the IC. Our samples from the ICd were focused on the dorsomedial part of this subdivision. The caudal portions of the ICd (caudal to the ICc, and sometimes called caudal cortex) could have ultrastructural characteristics that differ from our current sample. Our samples from the ICc did not include its most ventral regions, so we cannot comment on possible variation there. The rostral cortex of the IC (anterior to the ICc and bordering the superior colliculus) has often been grouped with the lateral cortex under the broad term “external cortex” of the IC (see discussion in Malmierca and Hackett, 2010). As Malmierca and colleagues have suggested (e.g., Malmierca et al., 2011), the rostral cortex may differ from the lateral cortex in numerous ways. Information on the synaptic organization of the rostral cortex will also require further study.

Finally, the use of ultrathin sections, without 3-dimensional reconstruction of serial sections, carries inherent limitations. A section through one SE bouton will presumably be small regardless of the location of the section through the bouton. On the other hand, a section through one LE bouton could range from small to large. We analyzed only those profiles that contained a clear synaptic zone, which should eliminate many of the smaller, “tangential” sections. However, it remains possible (perhaps likely) that some profiles through large boutons could appear small, particularly if the LE boutons are flattened in one or more dimensions (i.e., if they are pancake-shaped or cylindrical, rather than, say, spherical). We cannot rule out inclusion of profiles that would be “deceptively” small in area; in fact, this issue may explain why some of the LE boutons exhibit a relatively small profile area. This issue could be addressed in the future with reconstruction of boutons from serial sections.

Three major types of excitatory synapses

We distinguished 3 types of excitatory synapses based on ultrastructural characteristics of the presynaptic profiles. LE boutons have large profile areas and numerous mitochondria (generally 4 or more). ME boutons are intermediate in size and number of mitochondria. SE boutons are the smallest and generally have one or no mitochondria. Note that “zero” mitochondria does not indicate that the bouton is completely devoid of mitochondria, just that the observed section did not cut through a mitochondrion. Similarly, “many” mitochondrial profiles in a LE bouton may indicate multiple profiles through a smaller number of complex mitochondria. Three-dimensional reconstruction through serial sections would be required to determine an accurate count of mitochondria within any one bouton. Our measure of the number of profiles has the advantage of being applicable to standard ultrathin sections. Both of these criteria have been used previously to distinguish synaptic bouton types (Pierce and Lewin, 1994; Hupé-Gourgues et al., 2006), and have been used qualitatively for classification of boutons in the IC (Rockel and Jones, 1973; Paloff and Usunoff, 1992). Both features have potential functional relevance that we discuss here.

Large boutons have been associated with larger post-synaptic effects, which can be due to larger synaptic zones or to release of larger numbers of vesicles (Pierce and Lewin, 1994). Our data did not indicate larger synaptic zones in LE compared to other boutons (compare

Figs. 4–6). However, the LE boutons occasionally had multiple synaptic zones formed with a single postsynaptic target. Whether these short zones are different portions of a single, irregular zone or form two distinct zones will have to be assessed by serial section EM to reconstruct the full synapse. Regardless of synaptic zone size, LE boutons were characterized by a very large number of vesicles. This feature may reflect a larger postsynaptic effect or an ability to sustain a higher rate or longer duration of firing by LE boutons compared to ME or SE boutons.

The number of mitochondrial profiles in a single section through the bouton was our second criterion for distinguishing excitatory bouton types. Previous authors commented on varying numbers of mitochondria in synapses in the cat ICc, but the present study is the first to quantify such differences (Rockel and Jones, 1973; Granstrem, 1984; Paloff and Usunoff, 1992). A large number of mitochondria has been associated with high metabolic activity and the ability of a synapse to sustain high rates of firing and use reserve vesicles pools (Nguyen et al., 1997; Ly and Verstreken, 2006). Our data suggest that these properties would be associated most closely with the LE boutons. Further, because the LE boutons are restricted almost exclusively to the ICc, high firing rates and or high metabolic activity would be most likely associated with the ICc. This conclusion agrees well with studies showing high levels of cytochrome oxidase activity (a metabolic marker) in the ICc (Gonzalez-Lima and Jones, 1994; Cant and Benson, 2006).

It is quite possible that the excitatory boutons comprise more than three types. We noted a number of ultrastructural characteristics, such as vesicle size, vesicle packing density, and the presence of dense-core vesicles, that may prove useful in distinguishing subtypes within the groups we defined. Other researchers have reached similar conclusions in analyzing other species, and have in fact distinguished as many as 9 types of boutons in the cat IC (Paloff and Usunoff, 1992). We predict that such criteria will prove useful in identifying meaningful distinctions, perhaps across species, but reserve such judgments in guinea pigs pending more data (e.g., correlation of ultrastructural characteristics with information about the source of the axons or possible use of other neurotransmitters).

The differences in bouton types likely reflect different origins of inputs

Excitatory inputs to the IC arise from a large number of extrinsic sources, including lower auditory nuclei (cochlear nucleus, nuclei of the superior olivary complex and lateral lemniscus), auditory cortex and somatosensory regions (Cant, 2005; Schofield, 2005; Winer, 2005; Schofield, 2010) as well as commissural and intrinsic connections (Saldaña and Merchán, 2005). Jones and Rockel (1973)¹ studied degenerating boutons in the IC of cats after lesioning the lateral lemniscus or the auditory cortex. They concluded that large boutons, similar to our LE type, originate from lemniscal sources and terminate in the ICc on dendritic shafts, less often on spines and least often on cell bodies. In the cat, axons from individual regions have been labeled and their boutons in the IC analyzed with the electron microscope (Oliver, 1984, 1985, 1987; Oliver et al., 1995). Oliver described large boutons that originated from a variety of lemniscal sources. Many of these boutons were quite large and contained many mitochondria, similar to our LE boutons. Such endings were labeled from the contralateral dorsal cochlear nuclei, the ipsilateral medial superior olivary nucleus, and the contralateral and ipsilateral anteroventral cochlear nucleus and lateral superior olivary nucleus. While Oliver did not distinguish bouton types based on size, he demonstrated a wide range of bouton profile sizes, ranging from <1 μm to >3 μm in

¹Jones and Rockel (1973) examined boutons originating in auditory cortex and terminating in an area they called the dorsomedial part of the ICc. This area differed considerably from their ventrolateral part of ICc, which contained few or no cortical boutons. Based on comparison with subsequent parcellation of the cat IC, it appears that the dorsomedial ICc of Jones and Rockel includes at least part of the ICd (as defined in the present study), whereas their ventrolateral subdivision would fall within current definitions of the ICc.

diameter (e.g., see Oliver, 1987, Figs. 12–13; Oliver 1985, Fig. 2). The smaller boutons may correspond to our ME type. Further evidence for a lemniscal origin of LE and ME boutons comes from a light microscopic study in rats and cats showing that projections from the cochlear nucleus provide two sizes of axon terminals in the ICc (Malmierca et al., 2005). The profile areas of boutons in this study ranged from $0.85 \mu\text{m}^2$ to nearly $20 \mu\text{m}^2$, covering a range consistent with our ME and LE boutons. Significantly, Malmierca et al. (2005) described differences between the two sizes of boutons in their termination patterns within the ICc, suggesting distinct functions for the types. While further work is needed, lemniscal sources are probably the major contributors of LE boutons and, perhaps, ME boutons as well.

SE boutons, on the other hand, are likely to arise at least in part from cells in auditory cortex. Projections from auditory cortex terminate in the IC as small boutons containing round vesicles and forming asymmetric synapses (Rockel and Jones, 1973; Granstrem, 1984; Saldaña et al., 1996) and are believed to be excitatory (Mitani et al., 1983; Feliciano and Potashner, 1995). Cortical boutons terminate on spines (in cats; Rockel and Jones, 1973; Granstrem, 1984) or spines and dendrites (in rats; Saldaña et al., 1996). Of particular note is the distribution of cortical terminals within the IC. Cortical axons terminate densely in the ICd and IClc as well as in the rostral cortex of the IC, and less densely in the ICc (Feliciano and Potashner, 1995; Winer et al., 1998; Bajo and Moore, 2005; Bajo et al., 2007). This distribution is similar to that of the SE (and ME) boutons described in the present study.

Finally, there are extensive connections within the IC, including connections within a subdivision, connections between subdivisions of a single IC, and commissural connections between the 2 ICs (reviewed by Saldaña and Merchán, 2005). There is heterogeneity in both the cells of origin of these connections and the axons and their terminals (González-Hernández et al., 1986, 1996; Malmierca et al. 1995, 2009; Okayama et al., 2006). Furthermore, both intrinsic and commissural projections appear to include glutamatergic (as well as GABAergic) projections, suggesting that some of the axons would have excitatory boutons included in our analysis (e.g., González-Hernández et al., 1996; Saint Marie, 1996; Hernandez et al., 2006). The identification of these boutons must await direct study, but we would predict that they would include both SE and ME terminals; the presence of LE boutons cannot be ruled out, but does not appear to have direct support at this time.

Targets of excitatory boutons

All 3 excitatory bouton types contact GABA-negative targets much more frequently than GABA-positive targets. GABAergic cells are believed to make up about 20–40% of IC neurons with glutamatergic cells making up nearly all the rest (Oliver et al., 1994). It follows that nearly all of the GABA-negative synaptic targets are from glutamatergic IC cells. If, as we suggest above, different bouton types arise from different sources, then the association of bouton type with GABAergic targets may provide insight into which IC inputs could directly activate IC GABAergic circuits. The present data indicate that all three types of excitatory bouton contact IC GABAergic cells. Lemniscal inputs, representing the ascending auditory inputs to the IC, are known from physiological studies to activate both glutamatergic and GABAergic circuits in the IC (reviewed by Kelly and Caspary, 2005; Pollak et al., 2003, 2011). As we suggested above, lemniscal inputs probably include both LE and ME boutons. The fact that both LE and ME boutons contact GABAergic IC cells is consistent with the physiological observations.

Many physiological studies have demonstrated that activation (or deactivation) of the AC can lead to both increased and decreased activity in IC cells (e.g., Nakamoto et al, 2010; Suga 2012; Anderson and Malmierca, 2013), leading many to suggest that the AC directly activates GABAergic circuitry in the IC. However, direct evidence for AC activation of IC

GABAergic cells is not available. Mitani et al., (1983) showed cortically-evoked IPSPs in colliculothalamic cells in cat IC, and the evidence (including relative latencies of IPSPs and EPSPs) strongly supports a conclusion of AC activation of inhibitory cells in the IC. Jen et al. (2001) provided further evidence from big brown bats that stimulation of the AC activates GABAergic cells in the ICx. In their experiments, cortically-driven excitation was partially blocked by NMDA blockers, consistent with release of glutamate from AC axons. In addition, the cortically-driven inhibition could be blocked by bicuculline, a GABA-A antagonist, showing that GABAergic cells mediate at least some of the inhibition in the IC. These results are consistent with excitatory contacts onto GABAergic profiles. However, the vast majority of SE and ME boutons contact GABA-negative targets. Whether cortical axons are the source of some of the excitatory inputs to IC GABAergic cells will have to be examined with selective labeling of the cortical axons.

The ICd is the only subdivision in which spines were contacted more frequently than dendritic shafts or somas. The topic is of interest here given the common association of spines with synaptic plasticity (Nimchinsky et al., 2002; Harris, 2003; Roberts et al., 2010). In many ways, the ICd is less well understood than the other IC subdivisions. One prominent feature of the ICd is its close association with projections from the auditory cortex (Winer, 2005). From this perspective, it is interesting that cortical inputs to the IC have been associated with various forms of plasticity in adults or during development (Bajo et al., 2010; Suga, 2012). The present data highlight the dominance of spines as targets of excitatory inputs to the ICd.

Acknowledgments

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List of Abbreviations

D	dorsal
GABA-negative	gamma-aminobutyric acid immunonegative
GABA+	gamma-aminobutyric acid immunopositive
IC	inferior colliculus
ICc	central nucleus of the inferior colliculus
ICd	dorsal cortex of the inferior colliculus
IClc	lateral cortex of the inferior colliculus
LE	large excitatory bouton type
LSO	lateral superior olive
M	mitochondria
ME	medium excitatory bouton type
Mo5	motor trigeminal nucleus
pa	punctum adherens
PB	phosphate buffer
PN	pontine nuclei
R	rostral

SC	superior colliculus
scp	superior cerebellar peduncle
SE	small excitatory bouton type
SN	substantia nigra
TBST	tris-buffered saline with 0.1% triton X-100
VLL	ventral nucleus of the lateral lemniscus
VNTB	ventral nucleus of the trapezoid body

References

- Abramoff MD, Magalhaes PJ, Ram SJ. Image processing with ImageJ. *Biophotonics International*. 2004; 11:36–42.
- Aitkin, LM. The auditory midbrain. Clifton: Humana Press; 1986.
- Anderson LA, Malmierca MS. The effect of auditory cortex deactivation on stimulus-specific adaptation in the inferior colliculus of the rat. *Eur J Neurosci*. 2013; 37:52–62. [PubMed: 23121128]
- Bajo VM, Moore DR. Descending projections from the auditory cortex to the inferior colliculus in the gerbil, *Meriones unguiculatus*. *J Comp Neurol*. 2005; 486:101–116. [PubMed: 15844210]
- Bajo VM, Nodal FR, Bizley JK, Moore DR, King AJ. The ferret auditory cortex: descending projections to the inferior colliculus. *Cereb Cortex*. 2007; 17:475–491. [PubMed: 16581982]
- Bajo VM, Nodal FR, Moore DR, King AJ. The descending corticocollicular pathway mediates learning-induced auditory plasticity. *Nat Neurosci*. 2010; 13:253–260. [PubMed: 20037578]
- Brown KN, Chen S, Han Z, Lu CH, Tan X, Zhang XJ, Ding L, Lopez-Cruz A, Saur D, Anderson SA, Huang K, Shi SH. Clonal production and organization of inhibitory interneurons in the neocortex. *Science*. 2012; 334:480–486. [PubMed: 22034427]
- Brunso-Bechtold JK, Thompson GC, Masterton RB. HRP study of the organization of auditory afferents ascending to central nucleus of inferior colliculus in cat. *J Comp Neurol*. 1981; 197:705–722. [PubMed: 7229134]
- Cant, NB. Projections from the cochlear nuclear complex to the inferior colliculus. In: Winer, JA.; Schreiner, CE., editors. *The inferior colliculus*. New York: Springer; 2005. p. 115–131.
- Cant NB, Benson CG. Organization of the inferior colliculus of the gerbil (*Meriones unguiculatus*): differences in distribution of projections from the cochlear nuclei and the superior olivary complex. *J Comp Neurol*. 2006; 495:511–528. [PubMed: 16498677]
- Colonnier M. Synaptic patterns on different cell types in the different laminae of the cat visual cortex. An electron microscope study. *Brain Res*. 1968; 9:268–287. [PubMed: 4175993]
- Coomes DL, Bickford ME, Schofield BR. GABAergic circuitry in the dorsal division of the cat medial geniculate nucleus. *J Comp Neurol*. 2002; 453:45–56. [PubMed: 12357431]
- Coote EJ, Rees A. The distribution of nitric oxide synthase in the inferior colliculus of guinea pig. *Neuroscience*. 2008; 154:218–225. [PubMed: 18400412]
- Davis KA. Evidence of a functionally segregated pathway from dorsal cochlear nucleus to inferior colliculus. *J Neurophysiol*. 2002; 87:1824–1835. [PubMed: 11929904]
- Dawson TM, Bredt DS, Fotuhi M, Hwang PM, Snyder SH. Nitric oxide synthase and neuronal NADPH diaphorase are identical in brain and peripheral tissues. *Proc Natl Acad Sci U S A*. 1991; 88:7797–7801. [PubMed: 1715581]
- de Souto MC, Costa IG, de Araujo DS, Ludermit TB, Schliep A. Clustering cancer gene expression data: a comparative study. *BMC Bioinformatics*. 2008; 9:497. [PubMed: 19038021]
- Eccles, JC. The physiology of synapses. Berlin: Springer-Verlag; 1964.
- Feliciano M, Potashner SJ. Evidence for a glutamatergic pathway from the guinea pig auditory cortex to the inferior colliculus. *J Neurochem*. 1995; 65:1348–1357. [PubMed: 7643112]

- Gaza WC, Ribak CE. Immunocytochemical localization of AMPA receptors in the rat inferior colliculus. *Brain Res.* 1997; 774:175–183. [PubMed: 9452206]
- González-Hernández TH, Meyer G, Ferres-Torres R. The commissural interconnections of the inferior colliculus in the albino mouse. *Brain Res.* 1986; 368:268–276. [PubMed: 2421840]
- González-Hernández T, Mantolan-Sarmiento B, Gonzalez-Gonzalez B, Perez-Gonzalez H. Sources of GABAergic input to the inferior colliculus of the rat. *J Comp Neurol.* 1996; 372:309–326. [PubMed: 8863133]
- Gonzalez-Lima F, Jones D. Quantitative mapping of cytochrome oxidase activity in the central auditory system of the gerbil: a study with calibrated activity standards and metal-intensified histochemistry. *Brain Res.* 1994; 660:34–49. [PubMed: 7828000]
- Granstrem EE. Ultrastructural organization of AI and AIV projection zones of the cat auditory cortex in the inferior colliculi of the lamina tecti. *Neurosci Behav Physiol.* 1984; 14:296–302. [PubMed: 6472612]
- Gray EG. Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscope study. *J Anat.* 1959; 93:420–433. [PubMed: 13829103]
- Harris, KM. Dendritic spines. In: Adelman, G.; Smith, BH., editors. *Encyclopedia of Neuroscience*. New York: Elsevier Science; 2003.
- Hernandez O, Rees A, Malmierca MS. A GABAergic component in the commissure of the inferior colliculus in rat. *NeuroReport.* 2006; 17:1611–1614. [PubMed: 17001278]
- Huffman RF, Henson OW Jr. The descending auditory pathway and acousticomotor systems: connections with the inferior colliculus. *Brain Res Brain Res Rev.* 1990; 15:295–323. [PubMed: 2289088]
- Huppe-Gourgues F, Bickford ME, Boire D, Ptito M, Casanova C. Distribution, morphology, and synaptic targets of corticothalamic terminals in the cat lateral posterior-pulvinar complex that originate from the posteromedial lateral suprasylvian cortex. *J Comp Neurol.* 2006; 497:847–863. [PubMed: 16802329]
- Irvine, DRF. The auditory brainstem: A review of structure and function of auditory brainstem processing mechanisms. In: Ottoson, D., editor. *Progress in sensory physiology*. Vol. 7. Berlin: Springer-Verlag; 1986. p. 1-279.
- Ito T, Bishop DC, Oliver DL. Expression of glutamate and inhibitory amino acid vesicular transporters in the rodent auditory brainstem. *J Comp Neurol.* 2011; 519:316–340. [PubMed: 21165977]
- Jen PH, Sun X, Chen QC. An electrophysiological study of neural pathways for corticofugally inhibited neurons in the central nucleus of the inferior colliculus of the big brown bat, *Eptesicus fuscus*. *Exp Brain Res.* 2001; 137:292–302. [PubMed: 11355376]
- Jones EG, Rockel AJ. Observations on complex vesicles, neurofilamentous hyperplasia and increased electron density during terminal degeneration in the inferior colliculus. *J Comp Neurol.* 1973; 147:93–118. [PubMed: 4682185]
- Kazee AM, West NR. Preservation of synapses on principal cells of the central nucleus of the inferior colliculus with aging in the CBA mouse. *Hear Res.* 1999; 133:98–106. [PubMed: 10416868]
- Kelly, JB.; Caspary, DM. Pharmacology of the inferior colliculus. In: Winer, JA.; Schreiner, CE., editors. *The inferior colliculus*. New York: Springer; 2005. p. 248-281.
- Liu Y, Sakurai H, Kurokawa K, Yamada H, Kudo M. Glycine-immunoreactive synapses in the central nucleus of the inferior colliculus. An electron microscopic study in the cat. *Neurosci Lett.* 1999; 269:183–185. [PubMed: 10454162]
- 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]
- Loftus WC, Bishop DC, Oliver DL. Differential patterns of inputs create functional zones in central nucleus of inferior colliculus. *J Neurosci.* 2010; 30:13396–13408. [PubMed: 20926666]
- Luzzati F, Bonfanti L, Fasolo A, Peretto P. DCX and PSA-NCAM expression identifies a population of neurons preferentially distributed in associative areas of different pallial derivatives and vertebrate species. *Cereb Cortex.* 2009; 19:1028–1041. [PubMed: 18832334]
- Ly CV, Verstreken P. Mitochondria at the synapse. *Neuroscientist.* 2006; 12:291–299. [PubMed: 16840705]

- Malmierca MS, Blackstad TW, Osen KK. Computer-assisted 3-d reconstructions of Golgi-impregnated neurons in the cortical regions of the inferior colliculus of rat. *Hear Res.* 2011; 274:13–26. [PubMed: 20600744]
- Malmierca, E.; Hackett, TA. Structural organization of the ascending auditory pathway. In: Rees, A.; Palmer, AR., editors. *The oxford handbook of auditory neuroscience vol 2: The auditory brain.* Oxford: Oxford University Press; 2010. p. 9-41.
- Malmierca MS, Hernandez O, Antunes FM, Rees A. Divergent and point-to-point connections in the commissural pathway between the inferior colliculi. *J Comp Neurol.* 2009; 514:226–239. [PubMed: 19296464]
- Malmierca MS, Rees A, Le Beau FE, Bjaalie JG. Laminar organization of frequency-defined local axons within and between the inferior colliculi of the guinea pig. *J Comp Neurol.* 1995; 357:124–144. [PubMed: 7673462]
- Malmierca MS, Saint Marie RL, Merchán MA, Oliver DL. Laminar inputs from dorsal cochlear nucleus and ventral cochlear nucleus to the central nucleus of the inferior colliculus: Two patterns of convergence. *Neuroscience.* 2005; 136:883–894. [PubMed: 16344158]
- Mei Y, Gawai KR, Nie Z, Ramkumar V, Helfert RH. Age-related reductions in the activities of antioxidant enzymes in the rat inferior colliculus. *Hear Res.* 1999; 135:169–180. [PubMed: 10491965]
- Mitani A, Shimokouchi M, Nomura S. Effects of stimulation of the primary auditory cortex upon colliculogeniculate neurons in the inferior colliculus of the cat. *Neurosci Lett.* 1983; 42:185–189. [PubMed: 6664630]
- Nakamoto KT, Shackleton TM, Palmer AR. Responses in the inferior colliculus of the guinea pig to concurrent harmonic series and the effect of inactivation of descending controls. *J Neurophysiol.* 2010; 103:2050–2061. [PubMed: 20147418]
- Nguyen PV, Marin L, Atwood HL. Synaptic physiology and mitochondrial function in crayfish tonic and phasic motor neurons. *J Neurophysiol.* 1997; 78:281–294. [PubMed: 9242280]
- Nimchinsky EA, Sabatini BL, Svoboda K. Structure and function of dendritic spines. *Annu Rev Physiol.* 2002; 64:313–353. [PubMed: 11826272]
- Okoyama S, Ohbayashi M, Ito M, Harada S. Neuronal organization of the rat inferior colliculus participating in four major auditory pathways. *Hear Res.* 2006; 218:72–80. [PubMed: 16814970]
- Oliver DL. Dorsal cochlear nucleus projections to the inferior colliculus in the cat: a light and electron microscopic study. *J Comp Neurol.* 1984; 224:155–172. [PubMed: 19180810]
- Oliver DL. Quantitative analyses of axonal endings in the central nucleus of the inferior colliculus and distribution of 3H-labeling after injections in the dorsal cochlear nucleus. *J Comp Neurol.* 1985; 237:343–359. [PubMed: 4044892]
- Oliver DL. Projections to the inferior colliculus from the anteroventral cochlear nucleus in the cat: possible substrates for binaural interaction. *J Comp Neurol.* 1987; 264:24–46. [PubMed: 2445792]
- Oliver, D. Neuronal organization in the inferior colliculus. In: Winer, JA.; Schreiner, A., editors. *The inferior colliculus.* New York: Springer; 2005. p. 69-114.
- Oliver DL, Beckius GE. Fine structure of GABA-labeled axonal endings in the inferior colliculus of the cat: immunocytochemistry on deplasticized ultrathin sections. *Neuroscience.* 1992; 46:455–463. [PubMed: 1542418]
- Oliver DL, Beckius GE, Shneiderman A. Axonal projections from the lateral and medial superior olive to the inferior colliculus of the cat: a study using electron microscopic autoradiography. *J Comp Neurol.* 1995; 360:17–32. [PubMed: 7499562]
- Oliver DL, Winer JA, Beckius GE, Saint Marie RL. Morphology of GABAergic neurons in the inferior colliculus of the cat. *J Comp Neurol.* 1994; 340:27–42. [PubMed: 7909821]
- Paloff AM, Usunoff KG. The fine structure of the inferior colliculus in the cat. II. Synaptic organization. *J Hirnforsch.* 1992; 33:77–106. [PubMed: 1447517]
- Peters, A.; Palay, SL.; Webster, Hd. *The fine structure of the nervous system.* New York: Oxford University Press; 1991.
- Pierce JP, Lewin GR. An ultrastructural size principle. *Neuroscience.* 1994; 58:441–446. [PubMed: 8170532]

- Pollak GD, Burger RM, Klug A. Dissecting the circuitry of the auditory system. *Trends in neurosciences*. 2003; 26:33–39. [PubMed: 12495861]
- Pollak GD, Gittelman JX, Li N, Xie R. Inhibitory projections from the ventral nucleus of the lateral lemniscus and superior paraolivary nucleus create directional selectivity of frequency modulations in the inferior colliculus: A comparison of bats with other mammals. *Hear Res*. 2011; 273:134–144. [PubMed: 20451594]
- Ponti G, Peretto P, Bonfanti L. Genesis of neuronal and glial progenitors in the cerebellar cortex of peripuberal and adult rabbits. *PLoS One*. 2008; 3:e2366. [PubMed: 18523645]
- Reynolds ES. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J Cell Biol*. 1963; 17:208–212. [PubMed: 13986422]
- Ribak CE, Roberts RC. The ultrastructure of the central nucleus of the inferior colliculus of the Sprague-Dawley rat. *J Neurocytol*. 1986; 15:421–438. [PubMed: 3746353]
- Roberts RC, Ribak CE. An electron microscopic study of GABAergic neurons and terminals in the central nucleus of the inferior colliculus of the rat. *J Neurocytol*. 1987; 16:333–345. [PubMed: 3302119]
- Roberts TF, Tschida KA, Klein ME, Mooney R. Rapid spine stabilization and synaptic enhancement at the onset of behavioural learning. *Nature*. 2010; 463:948–952. [PubMed: 20164928]
- Rockel AJ, Jones EG. Observations on the fine structure of the central nucleus of the inferior colliculus of the cat. *J Comp Neurol*. 1973; 147:61–92. [PubMed: 4682184]
- Rouiller, EM. Functional organization of the auditory pathways. In: Ehret, G.; Romand, R., editors. *The central auditory system*. New York: Oxford University Press; 1997. p. 3-96.
- Ruiz A, Walker MC, Fabian-Fine R, Kullmann DM. Endogenous zinc inhibits GABA(A) receptors in a hippocampal pathway. *J Neurophysiol*. 2004; 91:1091–1096. [PubMed: 14561688]
- Saint Marie RL. Glutamatergic connections of the auditory midbrain: Selective uptake and axonal transport of d-[3h]aspartate. *J Comp Neurol*. 1996; 373:255–270. [PubMed: 8889926]
- Saldaña E, Feliciano M, Mugnaini E. Distribution of descending projections from primary auditory neocortex to inferior colliculus mimics the topography of intracollicular projections. *J Comp Neurol*. 1996; 371:15–40. [PubMed: 8835717]
- Saldaña, E.; Merchán, MA. Intrinsic and commissural connections of the inferior colliculus. In: Winer, JA.; Schreiner, CE., editors. *The Inferior Colliculus*. New York: Springer; 2005. p. 155-181.
- Schofield, BR. Superior olivary complex and lateral lemniscal connections of the auditory midbrain. In: Winer, JA.; Schreiner, CE., editors. *The inferior colliculus*. New York: Springer; 2005. p. 132-154.
- Schofield, BR. Structural organization of the descending auditory pathway. In: Rees, A.; Palmer, AR., editors. *The oxford handbook of auditory science: The auditory brain*. Oxford: Oxford University Press; 2010. p. 44-64.
- Schofield, BR. Central descending auditory pathways. In: Ryugo, DK., et al., editors. *Auditory and vestibular efferents*. New York: Springer; 2011a.
- Schofield BR. Projections from auditory cortex to midbrain cholinergic neurons that project to the inferior colliculus. *Neuroscience*. 2011b; 166:231–240. [PubMed: 20005923]
- Sivaramakrishnan S, Oliver DL. Distinct k currents result in physiologically distinct cell types in the inferior colliculus of the rat. *J Neurosci*. 2001; 21:2861–2877. [PubMed: 11306638]
- Smith PH, Uhlrich DJ, Manning KA, Banks MI. Thalamocortical projections to rat auditory cortex from the ventral and dorsal divisions of the medial geniculate nucleus. *J Comp Neurol*. 2012; 520:34–51. [PubMed: 21618239]
- Suga N. Tuning shifts of the auditory system by corticocortical and corticofugal projections and conditioning. *Neurosci Biobehav Rev*. 2012; 36:969–988. [PubMed: 22155273]
- Thompson, AM. Descending connections of the auditory midbrain. In: Winer, JA.; Schreiner, CE., editors. *The inferior colliculus*. New York: Springer; 2005. p. 182-199.
- Winer, JA. Three systems of descending projections to the inferior colliculus. In: Winer, JA.; Schreiner, CE., editors. *The inferior colliculus*. New York: Springer; 2005. p. 231-247.
- Winer JA, Larue DT, Diehl JJ, Hefti BJ. Auditory cortical projections to the cat inferior colliculus. *J Comp Neurol*. 1998; 400:147–174. [PubMed: 9766397]

Wu, SH. Biophysical properties of inferior colliculus neurons. In: Winer, JA.; Schreiner, CE., editors. The inferior colliculus. New York: Springer; 2005. p. 282-311.

Three bouton types differ in size and mitochondrial content.
Large excitatory boutons are confined to the inferior colliculus (IC) central nucleus.
Medium and small excitatory boutons occur throughout the IC.
All 3 bouton types contact GABAergic and non-GABAergic IC cells.

Figure 1.

Line drawings of parasagittal sections through the IC showing the relative locations of the 9 tissue blocks (black trapezoids) collected for analysis. The blocks were collected from different cases and, for simplicity of presentation, were drawn on a series of sections from a single case by placing each block in its appropriate location in the most similar section of the representative case. Three blocks were collected from each subdivision of the IC. Sections are arranged from lateral to medial; the numbers at lower left of each section indicate the approximate distance of the section, in μm , from the first (lateral-most) section. Abbreviations: bic, brachium of the inferior colliculus; D, dorsal; ICc, central nucleus of the inferior colliculus; ICd, dorsal cortex of the inferior colliculus; IClc, lateral cortex of the inferior colliculus; LSO, lateral superior olive; Mo5, motor trigeminal nucleus; Pn, pontine nuclei; R, rostral; SC, superior colliculus; scp, superior cerebellar peduncle; SN, substantia nigra; VLL, ventral nucleus of the lateral lemniscus; VNTB, ventral nucleus of the trapezoid body.

Figure 2.

Low magnification (3 000x) electron micrographs showing general differences in the ultrastructure of the subdivisions of the IC. Myelinated axons are highlighted in orange and mitochondria are highlighted in purple. (A) Micrograph of the ICc. Note the large profiles with many mitochondria (yellow asterisks). (B) Micrograph of the ICd. Note the lack of large profiles, low number of mitochondria, and few myelinated axons. (C) Micrograph of the IClc. Note the high proportion of area taken up by myelinated axons and the scarcity of mitochondria. Images are taken from the same blocks as the bouton analyses (locations shown in Fig. 1). Scale bar = 5 μ m.

Figure 3.

Electron micrographs showing examples of excitatory and inhibitory synapses in the IC. Presynaptic (Pre) and postsynaptic (Post) profiles are indicated, and synaptic densities are bracketed by arrows placed in the postsynaptic profiles. (A–C) Images of excitatory synapses with asymmetric synaptic densities and round synaptic vesicles. GABA immunoreactivity is demonstrated by the increased density of gold particles (black dots) relative to surrounding tissue. The presynaptic profiles are GABA-negative. The postsynaptic profiles are GABA-negative in A and B, and GABA+ in C. (D–E) Images of inhibitory synapses with symmetric synaptic densities, pleomorphic vesicles, and GABA+ presynaptic profiles. (F) Image of an inhibitory synapse with a GABA-negative presynaptic profile, symmetric synaptic densities and flat vesicles. The postsynaptic profile is GABA+. Scale bar = 0.5 μ m.

Figure 4.

Electron micrographs showing examples of large excitatory (LE) synapses in the ICc. Presynaptic profiles are shown in green and postsynaptic profiles are shown in blue. The presynaptic profiles of these excitatory synapses have many mitochondria and a large cross-sectional area. Arrows placed on the postsynaptic profile bracket the synaptic densities. The arrowhead indicates a dense core vesicle. The pre- and post-synaptic profiles in these three examples are GABA-negative (compare with GABA-positive (G+) profiles in panels A and C). Panel A also includes an example of a punctum adherens (pa) between the pre- and postsynaptic profiles. Scale bar = 1 μ m.

Figure 5.

Electron micrographs showing examples of the small excitatory (SE) synapses in the ICc (A, B), in the ICd (C, D) and in the IClc (E–F). The presynaptic profiles of these excitatory synapses have one or no mitochondria and a small cross-sectional area. G+, GABA-positive profiles. Conventions same as Figure 4. Scale bar = 1 μ m.

Figure 6.

Electron micrographs showing examples of medium excitatory (ME) synapses. (A–B) Examples from the ICc. (C–D) Examples from ICd. (E–F) examples from IClc. The presynaptic profiles of these excitatory synapses have an intermediate number of mitochondria and a medium sized cross-sectional area. Abbreviations: G+, GABA-positive profiles; pa, punctum adherens. Conventions same as Figure 4. Scale bar = 1 μ m.

Figure 7.

Bar graphs of the distribution of the excitatory types across the subdivisions IC. The percentage of the excitatory types in each subdivision is plotted. (A) The LE type occurred mainly in the ICc. (B) The ME type occurred in all subdivisions, though the largest percentage was in the ICc. (C) The SE type occurred mostly in the ICd and the IClc.

Figure 8.

Electron micrographs showing examples of synapses (*) onto dendritic spines (A–C) and dendritic shafts (D, E). (A–B) Dendritic spines with parent dendrites visible. Dashed line indicates the junction of the spine neck and dendritic shaft. Note the electron-dense “fluffy material” indicated by the diffuse net-like staining in the spines (and absent in the parent dendrites). Microtubules (yellow arrowheads) are present in dendrites, but not in spines. Smooth walled sacs (white arrows) are present in some spines. (C) Synapses from a single excitatory bouton onto two spines without visible parent dendrites. Note the lack of microtubules and presence of smooth walled sacs in the spines. (D–E) Synapses onto dendritic shafts, identified by microtubules that extend into the postsynaptic region. Asterisks indicate synapses and are placed on the pre-synaptic profile. Scale bar = 1 μ m. Abbreviations: G+, GABA-positive profiles; m, mitochondria.

Figure 9.

Bar graphs showing the frequency with which the different excitatory bouton types contact GABA+ or GABA-negative spines, dendritic shafts or somas in each subdivision of the IC. The number of synapses (n) for each graph is shown for each graph. In each subdivision, the identity of the post-synaptic targets in a small number of synapses (<10) could not be determined with certainty and were excluded from the graph. Abbreviations: GABA+, GABA-immunopositive; GABA-neg, GABA-immunonegative; ICc, central nucleus of the inferior colliculus; ICd, dorsal cortex of the inferior colliculus; IClc, lateral cortex of the inferior colliculus; LE, large excitatory synapse; ME, medium excitatory synapse; SE, small excitatory synapse.

Figure 10.

Pie charts showing the distribution of synapse types within each IC subdivision. The percentage of excitatory and inhibitory synapses was similar across subdivisions. The percentages of the excitatory subtypes differed considerably by subdivision. A few synapses could not be identified as excitatory or inhibitory and are shown with a question mark. Abbreviations: ICc, central nucleus of the inferior colliculus; ICd, dorsal cortex of the inferior colliculus; IClc, lateral cortex of the inferior colliculus; LE, large excitatory synapse; ME, medium excitatory synapse; SE, small excitatory synapse.