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Functional organization of the local circuit in the inferior colliculus

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Abstract

The inferior colliculus (IC) is the first integration center of the auditory system. After the transformation of sound to neural signals in the cochlea, the signals are analyzed by brainstem auditory nuclei that, in turn, transmit this information to the IC. However, the neural circuitry that underlies this integration is unclear. This review consists of 2 parts, one is about the cell type which is likely to integrate sound information, and the other part is about a technique which is useful for studying local circuitry.

Large GABAergic (LG) neurons receive dense excitatory axosomatic terminals that originate from the lower brainstem auditory nuclei as well as local IC neurons. Dozens of axons coming from both local and lower brainstem neurons converge on a single LG soma. Excitatory neurons in IC can innervate many nearby LG somata in the same fibrodendritic lamina. The combination of local and ascending inputs is well-suited for auditory integration.

LG neurons are one of the main sources of inhibition in the medial geniculate body (MGB). LG neurons and the tectothalamic inhibitory system are present in a wide variety of mammalian species. This suggests that the circuitry of excitatory and inhibitory tectothalamic projections may have evolved earlier than GABAergic interneurons in the MGB that are found in fewer species.

Cellular-level functional imaging provides both morphological and functional information about local circuitry. In the last part of this review, we will describe an *in vivo* calcium imaging study that sheds light on the functional organization of the IC.

Keywords

tract tracing; functional imaging; synapse; comparative neuroanatomy; hearing

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1. The organization of the central auditory pathways

Sound is very common in our environment and the sound scene is a rich source of information. Consider that an emergency car is coming towards you and passes by your side. You will hear the siren change its location, pitch, and loudness over time, but you recognize that the sound is emitted by a single entity passing by. It is clear that every sound will have several characteristics, such as location and spectrum, and both can change over time. Our brain not only extracts these characteristics but also connects them to create a sound object. Indeed, the lower part of the auditory system excels in the extraction of sound information, while the higher centers integrate this information. In this section, we will review underlying neural circuitry of the extraction and integration of the auditory information.

Sound is transformed into neural signals by the cochlea where the frequency of sound is analyzed. Auditory information such as spectrum, timing, and location of sound is analyzed in parallel by the nuclei in the lower auditory brainstem (Fig. 1), i.e. cochlear nuclei (CN), superior olivary complex (SOC), and nuclei of the lateral lemniscus (NLL). For example, T-stellate neurons in the ventral cochlear nucleus (VCN) can convey sound spectra for a wide range of sound intensity (Young and Oertel, 2004). Fusiform cells in the dorsal cochlear nucleus (DCN) have a complex receptive field for sound intensity and spectrum, and they are thought to analyze spectral cues created by head and pinnae that are used for sound location (Young and Oertel, 2004). Neurons in the lateral superior olive (LSO) code interaural level differences, while those in the medial superior olive (MSO) code interaural time differences, both cues are used to analyze the location of sound (Grothe et al., 2010). Neurons in the intermediate nucleus of the lateral lemniscus (INLL) are suited well for coding timing information (Covey and Casseday, 1991). Each of these brainstem nuclei project to the inferior colliculus (IC, Fig. 1, Adams, 1979; Oliver, 1984; Oliver, 1987; Whitley and Henkel, 1984).

The IC is subdivided into the central nucleus (ICC) and surrounding dorsal and lateral cortices (ICD and ICL), and the ascending fibers from the brainstem auditory nuclei mainly terminate in the ICC. Descending fibers from the cerebral cortex terminate primarily in the IC cortices (rat, Herbert et al., 1991; cat, Winer, 2005). It is thought that the ICC is the first integration center for auditory information from the brainstem that is analyzed in parallel, while the IC cortex receives strong descending inputs from the auditory cortex to produce sound-related behaviors (Xiong et al., 2015). Accordingly, there is accumulating evidence that ICC neurons integrate several features of sound and create specific responses to complex sounds. For example, some IC neurons are sensitive to specific durations of sound (Casseday et al., 1994), the direction of chirps (sweep frequency modulated tones, Kuo and Wu, 2012; Poon et al., 1992). However, little is known about the underlying local circuitry and cell types responsible for this integration.

Although various cell types have been identified in the IC by analysis of different characteristics, such as the electrophysiological properties (Sivaramakrishnan and Oliver, 2001), neurotransmitter expression (Nakagawa et al., 1995), and dendritic arborization (Malmierca et al., 1993; Oliver and Morest, 1984), the roles of these cell types in the auditory integration are largely unknown. In the next section we will focus on a cell type

with unique synaptic organization, which may underlie the integration of auditory information.

2. Synaptic organization of LG neurons in the IC

The convergence of inputs on a single neuron from multiple sources that code different types of information about sound is an ideal substrate for this integration. Such convergence is seen on the large GABAergic (LG) neurons of IC (Fig. 2) that are distributed throughout the IC (Ito et al., 2009; Ito and Oliver, 2012). The LG neurons are distinguished from other IC neurons by the presence of dense axosomatic excitatory synapses and the large cell body size (Altschuler et al., 2008; Ito et al., 2009). In rats, GABAergic cells with diameters larger than 16.5 μm receive dense excitatory axosomatic endings (and classified as LG cells) whereas those with diameters smaller than 10.7 μm do not receive such endings (small GABAergic, SG cells, Ito et al., 2009). GABAergic cells with the intermediate diameter are the mixed population of LG and SG cells. Although the dendritic morphology of LG cells has not been determined in detail, LG cells are assumed to have a stellate dendritic field because largest IC neurons have stellate dendrites ("simple" stellate cells in the cat IC described in Oliver and Morest, 1984) and LG neurons are the largest neurons in the IC (rat, Ito et al., 2009; Merchan et al., 2005; cat, Oliver et al., 1994).

To determine the origin(s) of dense excitatory axosomatic ending is important for understanding the function of LG neurons. Since calyx- or endbulb-like synapses have not been found in the IC, the LG neurons are presumed to receive converging inputs from multiple axons (Ito and Oliver, 2012). We have performed several studies that identify the origin of the axosomatic terminals on the LG neurons of rats (Ito et al., 2011; Ito et al., 2015; Ito and Oliver, 2010; Ito and Oliver, 2014).

Since excitatory axosomatic terminals are positive for vesicular glutamate transporter (VGLUT) 2 but not VGLUT1 (Ito et al., 2009), excitatory neurons innervating LG neurons should express the message for VGLUT2 but not VGLUT1. Auditory neurons fitting this description were found in DCN, SOC, INLL, and IC (Fig. 1, Ito et al., 2011). Many neurons that expressed mRNA for VGLUT2 but not VGLUT1 and projected to the IC were found in these nuclei (Ito and Oliver, 2010). For example, DCN fusiform and giant cells expressed only VGLUT2 and projected to the contralateral IC (Fig. 3). In contrast, T-stellate cells in the VCN, that are known to project to the contralateral IC (Cant, 1982), expressed both VGLUT1 and VGLUT2 and are not likely to be a source of axosomatic terminals on the LG neurons. In the SOC, many LSO and MSO cells as well as periolivary neurons projected to the IC and expressed VGLUT2 alone. Many INLL neurons expressed VGLUT2 alone and projected to the ipsilateral IC.

To determine whether axons originating from these nuclei make excitatory axosomatic synapses on LG neurons or not, labeling single axons from an identified origin is necessary. Conventional anterograde tracers (e.g. dextran, PHA-L, etc.) are not ideal for this purpose because (1) these tracers can be taken up by axons passing through injection site, (2) insufficient labeling of a cell body located outside the injection site is common, and (3) background labeling in the injection site makes it difficult to identify cell types of each

labeled cell. Recombinant viral tracers that force infected cells to express fluorescent proteins (e.g. GFP, mRFP, etc., Furuta et al., 2001; Hioki et al., 2010) are ideal for this purpose, because (1) viruses such as Sindbis virus and adeno-associated virus cause little retrograde infection, (2) infected cells are filled with fluorescent proteins in a Golgi-like manner even in the very distal axons (e.g. Ito et al., 2007; Matsuda et al., 2009), and (3) there is no background staining of uninfected tissue. We injected the viral tracers in either DCN, SOC, INLL, or IC (Ito et al., 2015; Ito and Oliver, 2014). In all cases, some labeled axons made varicosities that were positive for VGLUT2 and terminated on the somata of LG neurons. Both local and ascending fibers converged on single LG cell bodies (Fig. 4). The total number of inputs that converge on a single LG cell body will be large (~100) because the number of axosomatic contacts made by a single axon is small (1–7, Fig. 5A–H). The estimated total number of inputs on a single LG cell body was almost proportional to the surface area of the LG cell body (Fig. 5I, J), and this suggested that the dense axosomatic terminals covering the LG somata are a specialization to overcome the low input resistance and high membrane capacitance of the LG neurons in the mouse ICD (Geis and Borst, 2013; summarized in Ono and Ito, 2015). Because of the large number of single axons making axosomatic inputs on one LG neuron, synchronized activation of the excitatory synapses is needed to elicit an action potential in an LG neuron. Although axons from different origins make similar numbers of axosomatic contacts (Fig. 5H), single axons from different sources may not produce the same magnitude of synaptic current. The mean volume of axosomatic terminals from ascending axons is significantly larger than that from local IC axons (Fig. 5G). Since larger terminals contain more synaptic vesicles docked to the active zone (Schikorski and Stevens, 1997) and have a higher release probability (Holderith et al., 2012), it is likely that synapses from ascending fibers may have a higher release probability than those from local axons.

The local inputs to LG neurons suggest a large degree of convergence from local excitatory neurons (Ito and Oliver, 2014). When single neurons are labeled and the dendritic trees and axonal plexuses reconstructed from serial sections (Fig. 6), labeled IC excitatory neurons are found to have flat axonal plexuses. These combine with the flat axonal plexuses from ascending fibers and terminate on postsynaptic dendrites also parallel to the axonal plexuses. This structure is called the fibrodendritic lamina, which is the basis of tonotopicity of the IC (Morest and Oliver, 1984; Oliver, 2005; Oliver and Morest, 1984). This suggests that local IC excitatory neurons contribute to the fibrodendritic laminae, and within them many LG neurons (10–30) receive local axosomatic contacts. These local excitatory neurons will influence LG neurons located in the same plexus within a distance of ~500 μ m. Thus, the fibrodendritic lamina is the field of convergence for both local and ascending inputs.

The fibrodendritic lamina does not receive homogenous inputs, but provides a further level of organization for the IC microcircuit. For example, in cats DCN axons terminate in a more ventral and medial part of the ICC (Oliver, 1984), while SOC axons terminate in a more lateral part (Oliver et al., 1995) (rat data are shown in Fig. 7). The organization of ascending inputs creates synaptic domains within the central nucleus, each of which receives a particular combination of ascending fibers from different brainstem sources (Cant and Benson, 2006; Casseday et al., 2002; Loftus et al., 2010; Oliver, 2005). The laminar local axons from IC excitatory neurons may travel to neighboring synaptic domains aside from its

own. This suggests that an LG neuron may mix auditory information directly from ascending excitatory inputs to its own synaptic domain with indirect inputs from neighboring domains via local collaterals of excitatory neurons. Currently no detailed anatomical information concerning local inhibitory connection to LG neurons or other cell types is available. However, it is clear that there are massive local inhibitory inputs in the IC since almost all IC neurons have local collaterals (Oliver et al., 1991; Wallace et al., 2012). A recent study using glutamate uncaging combined with voltage clamp of mouse ICC neurons demonstrated that ICC neurons receive both excitatory and inhibitory inputs from local neurons which are mainly located within the same laminae (Sturm et al., 2014). Interestingly, after hearing onset, the region from which inhibitory inputs arise is larger than that from which excitatory inputs arise, suggesting that more synaptic domains join in inhibitory inputs on a single ICC neuron than in excitatory inputs.

Our studies suggest that LG neurons integrate auditory information from the lower brainstem nuclei. However, the kind of auditory information integrated by LG neurons is unknown. One can speculate that LG neurons preserve temporal information of synaptic inputs better than some other cell types in the IC. In the auditory system, excitatory axosomatic synapses from multiple neurons are observed in several cell types that preserve timing information. In the cat VCN, globular bushy cells, receive 15–60 excitatory axosomatic inputs (Spirou et al., 2005), and this enhances the phase locking seen in the auditory nerve (Joris and Smith, 2008). Octopus neurons receive hundreds of axosomatic inputs from the auditory nerve and respond best to the onset of the stimulus (Young and Oertel, 2004). This suggests that the synchronous activation of excitatory axosomatic terminals on LG neurons may lead to the generation of short-latency, well-timed responses as compared to other cell types.

LG neurons project to the medial geniculate body (MGB, Fig. 1) (rat, Ito et al., 2009; cat, Winer et al., 1996) where they innervate stellate neurons and some tufted neurons (rat, Bartlett and Smith, 1999). Axons of LG neurons are the largest in the brachium of the IC (cat, Saint Marie et al., 1997), and after electrical stimulation of the brachium, monosynaptic inhibitory postsynaptic potentials are elicited before excitatory postsynaptic potentials in MGB neurons (rat, Peruzzi et al., 1997). This suggests that LG neurons shape the synaptic responses of the MGB.

Like other sensory systems, the auditory pathway in the forebrain is subdivided into lemniscal “core” and non-lemniscal “shell” pathways (Jones, 2007). The former originates from the ICC and terminates in the ventral division of the MGB that projects to the primary auditory cortex. The non-lemniscal shell originates from IC cortices, and terminates in the dorsal division of the MGB and suprageniculate nucleus that project to caudal and rostral areas of the secondary auditory cortex (Te2 and Te3, respectively, in accordance with Herbert et al., 1991). Tectothalamic inhibition from LG neurons may be more important in the “core” auditory pathway, because the ratio of LG neurons to all GABAergic neurons is higher in rat ICC (Ito et al., 2009), and LG neurons mainly terminate in the ventral division of the MGB (guinea pig, Mellott et al., 2014). Inside the MGB, further integration of sound information may occur since some MGB neurons receive ascending inputs from excitatory

and LG neurons that are located in separate areas of the IC (mouse, Lee and Sherman, 2010).

3. Evolutionary aspect of the inhibitory systems that control the MGB

Comparative neuroanatomy of the system may give us some clues about the function of the tectothalamic inhibitory system. A comparative study may clarify the common system that is preserved among evolutionary traits (i.e. clades), and how it will be specialized in each clade, especially in animals with specialized hearing (e.g. echolocation in bats and toothed whales). The former reflects whether it is involved in the more basic functions of auditory processing, and the latter whether the phenotype of the LG neurons is important for specialized hearing.

It appears that there are considerable differences in the organization of the MGB among species. In the MGB of rats, mice, and mustached bats, GABAergic interneurons are very scarce (< 1% of MGB neurons), while in cats, horseshoe bats, and macaque monkeys local inhibitory neurons represent a considerable part of the population (25% or more, Ito et al., 2011; Vater et al., 1992; Winer and Larue, 1996). In mustached bats and rodents, the vast majority of the inhibitory inputs to MGB neurons should come from extrinsic origins. One source is feedback inhibition from the reticular thalamic nucleus, which receives excitatory inputs from layer 6 pyramidal neurons in the auditory cortex and other multimodal cortical areas as well as neurons in the thalamic nuclei including MGB (rat, Kimura et al., 2005; Kimura et al., 2012), and the other source is feedforward inhibition from the LG neurons (Fig. 1). It appears that cats, horseshoe bats, and monkeys add GABAergic interneurons to the MGB while keeping both feedforward and feedback systems. The dendrites of MGB interneurons forms triad synapses with the ascending glutamatergic tectothalamic terminals and the dendrites of MGB principal neurons (Morest, 1971; Morest, 1975). The dendrodendritic synapse between the MGB interneuron and principal neuron may elicit a short-latency inhibition which is comparable to tectothalamic inhibition on the MGB principal neurons. Since rodents are phylogenetically closer to primates than carnivores, the MGB GABAergic interneurons are likely to have evolved several times independently (Winer and Larue, 1996).

This substantial interspecies difference in local MGB inhibition raises the question of whether neurons responsive for tectothalamic inhibition show interspecies differences or not. The morphology of GABAergic neurons was examined in different clades, i.e. *Rodentia* (rat and mouse, Geis and Borst, 2013; Ito et al., 2009), *Lagomorpha* (rabbit), *Primates* (common marmoset and Japanese monkey), and *Chiroptera* (Japanese house bat). In all species tested, large GABAergic cells were observed in all subdivisions of the IC and had somata and dendrites that were densely covered with VGLUT2-positive terminals (Figs. 2 and 8). We also examined the distribution of LG and SG cells in the IC of the common marmoset. In comparison to the rat (Ito et al., 2009), the spatial distributions of both classes of GABAergic neurons were very similar (Fig. 8). This strongly suggests that the presence of the tectothalamic inhibitory neurons is a common feature of the clade *Boreoeutheria* (Murphy et al., 2001) which includes all the species tested and is widely preserved in mammals. Inside the clade, it is an interesting question whether specialization of LG

neurons occurs in animals with specialized hearing or not. The phylogenic origin of tectothalamic inhibitory neurons remained to be uncovered.

4. Functional organization of local circuit of the IC

Although the anatomical studies described above give insights into the function of the local IC circuit in auditory information processing, they do not show how the local circuit works in real environments. Traditional electrophysiological techniques such as intracellular, juxtacellular, or whole-cell patch clamp recordings are useful for this purpose because they can be combined with anatomical techniques (e.g. Geis and Borst, 2013; Kuwada et al., 1997; Wallace et al., 2012). These methods, however, give us limited information about the ensemble of activities within the local circuit. In contrast, calcium imaging has cellular resolution and enables us to record from many neurons in an area of $\sim 0.3 \text{ mm}^2$, so it is useful for studying the interaction of multiple neurons in the IC. In this section we will introduce a pilot study of simultaneous recordings from multiple nearby IC neurons.

The physiology of neurons in the cortices of the IC is less well understood than that of the neurons in ICC. The ICD consists of several layers (rat, Faye-Lund and Osen, 1985; cat, Morest and Oliver, 1984) each of which receives a distinct combination of inputs and outputs (rat, Herbert et al., 1991; Linke, 1999). Layer 1 receives auditory inputs mainly from the ICC, visual inputs from the retina (rat and macaque monkey, Itaya and Van Hoesen, 1982), and dense descending inputs from the Te2 secondary auditory area that lies ventral and caudal to the primary auditory cortex in the rat (Arnault and Roger, 1990; Roger and Arnault, 1989). Since Te2 is considered multimodal for auditory and visual information (rat, Linke, 1999; Shi and Cassell, 1997), layer 1 of the IC cortex is likely to be involved in multimodal functions. To understand the role of auditory information in the multimodal pathway, it is essential to know what kind of auditory information is coded in layer 1 of the IC cortex. However, there had been no systematic study *in vivo* that showed the activity of layer 1 neurons. This is primarily due to the difficulty in placing electrodes in layer 1 due to its shallow depth ($\sim 100 \text{ }\mu\text{m}$). On the other hand, the mouse layer 1 is relatively flat and exposed since it is on the surface of the brain. This allowed us to use an *in vivo* calcium imaging method for simultaneous recording of sound-evoked activity of many layer 1 cells in mouse ICD (Ito et al., 2014). We obtained sound-evoked responses from 162 cells in 8 mice. In each mouse, the recording site covered most of the surface of the ICD (Fig. 9A). Most neurons (92.6%) showed a strong response to broad-band noise (Fig. 9B, C). Most neurons (142 of 162) also showed a significant response to at least one frequency (pure tone stimuli ranged from 4.5–55 kHz at 75 dB SPL). The majority of neurons had their maximal response to the low frequency end ($< 10 \text{ kHz}$) of the mouse audible range (1–100 kHz, Fay, 1988). There were a variety of frequency response area shapes. These results suggest that broad-band noise and low-frequency sound are important for the multimodal pathway in which layer 1 is involved and that not all neurons process this information in the same way.

In the auditory cortex, *in vivo* calcium imaging studies have shown that neurons located nearby share similar response properties (mouse, Bandyopadhyay et al., 2010; Rothschild et al., 2010), and this may reflect common inputs to the local circuitry. In our data sets, ICD neurons located nearby showed similar calcium responses during repetitive presentation of a

single sound stimulus. To quantify this, we calculated the similarity of the calcium response with a cross correlation of the waveforms of all pairs of recorded cells, and we asked whether the result was related to the separation distance between the pair. From the scatter plot between the similarity and distance of all pairs, linear regression between the similarity and distance was made. Most, but not all, data sets showed a weak negative linear correlation ($R < 0.45$) between similarity and distance, and this suggests that nearby cells share a similar local circuit. However, this conclusion must be tempered since the correlation value depends on several factors such as the stimulus used, recording quality, and the data analysis methods.

The similar response of a local cluster of neurons is a basis for functional organization such as tonotopicity. When the lateral part of layer 1 of the ICD was imaged, most cells had a similar best frequency (~10 kHz, see above), but no clear frequency map was found (Fig. 9E). On the other hand, when the medial part was imaged, cells with a higher best frequency were located more medially, while those with a lower best frequency were located more laterally (Fig. 9D, F) consistent with the presence of tonotopicity at least in a part of layer 1. Since our samples did not cover layer 1 completely, the details of the frequency organization must await future studies.

5. Concluding remarks

In this review we described several methods to analyze the local circuitry of the IC and the LG neuron in particular. In the future, it will be desirable to combine these techniques. For example, an injection of a virus that codes for flex-GCaMP into the IC of transgenic mice that express Cre recombinase under a cell-type-specific promoter will cause Golgi-like labeling of a specific cell type with GCaMP, a calcium sensor fluorescent protein. This will enable us to simultaneously analyze the activity and the morphology of a population of a specific neurons. The induction of optogenetic channels will enable us to manipulate a specific subpopulation of local neurons. The optimization of such techniques in the auditory system is needed to break-through the complex neural circuitry of this system.

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References

- Adams JC. Ascending projections to the inferior colliculus. *J Comp Neurol.* 1979; 183(3):519–538. [PubMed: 759446]
- Altschuler RA, Tong L, Holt AG, Oliver DL. Immunolocalization of vesicular glutamate transporters 1 and 2 in the rat inferior colliculus. *Neuroscience.* 2008; 154(1):226–232. [PubMed: 18436385]

- Arnault P, Roger M. Ventral temporal cortex in the rat: connections of secondary auditory areas Te2 and Te3. *J Comp Neurol.* 1990; 302(1):110–123. [PubMed: 1707895]
- Bandyopadhyay S, Shamma SA, Kanold PO. Dichotomy of functional organization in the mouse auditory cortex. *Nat Neurosci.* 2010; 13(3):361–368. [PubMed: 20118924]
- Bartlett EL, Smith PH. Anatomic, intrinsic, and synaptic properties of dorsal and ventral division neurons in rat medial geniculate body. *J Neurophysiol.* 1999; 81(5):1999–2016. [PubMed: 10322042]
- Cant NB. Identification of cell types in the anteroventral cochlear nucleus that project to the inferior colliculus. *Neurosci Lett.* 1982; 32(3):241–246. [PubMed: 7177487]
- 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(5):511–528. [PubMed: 16498677]
- Casseday JH, Ehrlich D, Covey E. Neural tuning for sound duration: role of inhibitory mechanisms in the inferior colliculus. *Science.* 1994; 264(5160):847–850. [PubMed: 8171341]
- Casseday JH, Fremouw T, Covey E. The Inferior Colliculus: A Hub for the Central Auditory System. In: Oertel D, Fay RR, Popper AN, editors *Integrative Functions in the Mammalian Auditory Pathway*. New York: Springer; 2002. 238–318.
- Chernock ML, Larue DT, Winer JA. A periodic network of neurochemical modules in the inferior colliculus. *Hear Res.* 2004; 188(1–2):12–20. [PubMed: 14759566]
- Covey E, Casseday JH. The monaural nuclei of the lateral lemniscus in an echolocating bat: parallel pathways for analyzing temporal features of sound. *J Neurosci.* 1991; 11(11):3456–3470. [PubMed: 1941092]
- Fay RR. *Hearing in Vertebrates: a Psychophysics Databook*. Winnetka, IL: Hill-Fay Associates; 1988.
- Faye-Lund H, Osen KK. Anatomy of the inferior colliculus in rat. *Anat Embryol (Berl).* 1985; 171(1):1–20. [PubMed: 3985354]
- Furuta T, Tomioka R, Taki K, Nakamura K, Tamamaki N, Kaneko T. In vivo transduction of central neurons using recombinant Sindbis virus: Golgi-like labeling of dendrites and axons with membrane-targeted fluorescent proteins. *J Histochem Cytochem.* 2001; 49(12):1497–1508. [PubMed: 11724897]
- Geis HR, Borst JG. Large GABAergic neurons form a distinct subclass within the mouse dorsal cortex of the inferior colliculus with respect to intrinsic properties, synaptic inputs, sound responses, and projections. *J Comp Neurol.* 2013; 521(1):189–202. [PubMed: 22700282]
- Grothe B, Pecka M, McAlpine D. Mechanisms of sound localization in mammals. *Physiol Rev.* 2010; 90(3):983–1012. [PubMed: 20664077]
- Herbert H, Aschoff A, Ostwald J. Topography of projections from the auditory cortex to the inferior colliculus in the rat. *J Comp Neurol.* 1991; 304(1):103–122. [PubMed: 2016407]
- Hioki H, Nakamura H, Ma YF, Konno M, Hayakawa T, Nakamura KC, Fujiyama F, Kaneko T. Vesicular glutamate transporter 3-expressing nonserotonergic projection neurons constitute a subregion in the rat midbrain raphe nuclei. *J Comp Neurol.* 2010; 518(5):668–686. [PubMed: 20034056]
- Holderith N, Lorincz A, Katona G, Rozsa B, Kulik A, Watanabe M, Nusser Z. Release probability of hippocampal glutamatergic terminals scales with the size of the active zone. *Nat Neurosci.* 2012; 15(7):988–997. [PubMed: 22683683]
- Itaya SK, Van Hoesen GW. Retinal innervation of the inferior colliculus in rat and monkey. *Brain Res.* 1982; 233(1):45–52. [PubMed: 7059806]
- Ito T, Bishop DC, Oliver DL. Two classes of GABAergic neurons in the inferior colliculus. *J Neurosci.* 2009; 29(44):13860–13869. [PubMed: 19889997]
- 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(2):316–340. [PubMed: 21165977]
- Ito T, Hioki H, Nakamura K, Tanaka Y, Nakade H, Kaneko T, Iino S, Nojyo Y. Gamma-aminobutyric acid-containing sympathetic preganglionic neurons in rat thoracic spinal cord send their axons to the superior cervical ganglion. *J Comp Neurol.* 2007; 502(1):113–125. [PubMed: 17335042]
- Ito T, Hioki H, Sohn J, Okamoto S, Kaneko T, Iino S, Oliver DL. Convergence of lemniscal and local excitatory inputs on large GABAergic tectothalamic neurons. *J Comp Neurol.* 2015

- Ito T, Hirose J, Murase K, Ikeda H. Determining auditory-evoked activities from multiple cells in layer 1 of the dorsal cortex of the inferior colliculus of mice by in vivo calcium imaging. *Brain Res.* 2014; 1590:45–55. [PubMed: 25278189]
- Ito T, Oliver DL. Origins of Glutamatergic Terminals in the Inferior Colliculus Identified by Retrograde Transport and Expression of VGLUT1 and VGLUT2 Genes. *Front Neuroanat.* 2010; 4:135. [PubMed: 21048892]
- Ito T, Oliver DL. The basic circuit of the IC: tectothalamic neurons with different patterns of synaptic organization send different messages to the thalamus. *Front Neural Circuits.* 2012; 6:48. [PubMed: 22855671]
- Ito T, Oliver DL. Local and commissural IC neurons make axosomatic inputs on large GABAergic tectothalamic neurons. *J Comp Neurol.* 2014; 522(15):3539–3554. [PubMed: 24796971]
- Jones EG. The medial geniculate complex. *The Thalamus.* 2nd ed.. New York: Cambridge University Press; 2007. 875–923.
- Joris PX, Smith PH. The volley theory and the spherical cell puzzle. *Neuroscience.* 2008; 154(1):65–76. [PubMed: 18424004]
- Kimura A, Donishi T, Okamoto K, Tamai Y. Topography of projections from the primary and non-primary auditory cortical areas to the medial geniculate body and thalamic reticular nucleus in the rat. *Neuroscience.* 2005; 135(4):1325–1342. [PubMed: 16165287]
- Kimura A, Yokoi I, Imbe H, Donishi T, Kaneoke Y. Auditory thalamic reticular nucleus of the rat: anatomical nodes for modulation of auditory and cross-modal sensory processing in the loop connectivity between the cortex and thalamus. *J Comp Neurol.* 2012; 520(7):1457–1480. [PubMed: 22101990]
- Kuo RI, Wu GK. The generation of direction selectivity in the auditory system. *Neuron.* 2012; 73(5):1016–1027. [PubMed: 22405210]
- Kuwada S, Batra R, Yin TC, Oliver DL, Haberly LB, Stanford TR. Intracellular recordings in response to monaural and binaural stimulation of neurons in the inferior colliculus of the cat. *J Neurosci.* 1997; 17(19):7565–7581. [PubMed: 9295401]
- Lee CC, Sherman SM. Topography and physiology of ascending streams in the auditory tectothalamic pathway. *Proc Natl Acad Sci U S A.* 2010; 107(1):372–377. [PubMed: 20018757]
- Linke R. Differential projection patterns of superior and inferior collicular neurons onto posterior paralaminar nuclei of the thalamus surrounding the medial geniculate body in the rat. *Eur J Neurosci.* 1999; 11(1):187–203. [PubMed: 9987023]
- Loftus WC, Bishop DC, Oliver DL. Differential patterns of inputs create functional zones in central nucleus of inferior colliculus. *J Neurosci.* 2010; 30(40):13396–13408. [PubMed: 20926666]
- Malmierca MS, Blackstad TW, Osen KK, Karagulle T, Molowny RL. The central nucleus of the inferior colliculus in rat: a Golgi and computer reconstruction study of neuronal and laminar structure. *J Comp Neurol.* 1993; 333(1):1–27. [PubMed: 7688006]
- Matsuda W, Furuta T, Nakamura KC, Hioki H, Fujiyama F, Arai R, Kaneko T. Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. *J Neurosci.* 2009; 29(2):444–453. [PubMed: 19144844]
- 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.* 2014; 8:17. [PubMed: 24744703]
- Merchan M, Aguilar LA, Lopez-Poveda EA, Malmierca MS. The inferior colliculus of the rat: quantitative immunocytochemical study of GABA and glycine. *Neuroscience.* 2005; 136(3):907–925. [PubMed: 16344160]
- Morest DK. Dendrodendritic synapses of cells that have axons: the fine structure of the Golgi type II cell in the medial geniculate body of the cat. *Z Anat Entwicklungsgesch.* 1971; 133(2):216–246. [PubMed: 4929220]
- Morest DK. Synaptic relationships of Golgi type II cells in the medial geniculate body of the cat. *J Comp Neurol.* 1975; 162(2):157–193. [PubMed: 1150917]
- 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(2):209–236. [PubMed: 6699208]

- Murphy WJ, Eizirik E, O'Brien SJ, Madsen O, Scally M, Douady CJ, Teeling E, Ryder OA, Stanhope MJ, de Jong WW, Springer MS. Resolution of the early placental mammal radiation using Bayesian phylogenetics. *Science*. 2001; 294(5550):2348–2351. [PubMed: 11743200]
- Nakagawa H, Ikeda M, Houtani T, Ueyama T, Baba K, Kondoh A, Yamamoto T, Yamashita T, Sugimoto T. Immunohistochemical evidence for enkephalin and neuropeptide Y in rat inferior colliculus neurons that provide ascending or commissural fibers. *Brain Res*. 1995; 690(2):236–240. [PubMed: 8535842]
- Oliver DL. Dorsal cochlear nucleus projections to the inferior colliculus in the cat: a light and electron microscopic study. *J Comp Neurol*. 1984; 224(2):155–172. [PubMed: 19180810]
- 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(1):24–46. [PubMed: 2445792]
- Oliver DL. Neuronal organization in the inferior colliculus. In: Winer JA, Schreiner CE, editors *The Inferior Colliculus*. New York: Springer; 2005. 69–114. Chapter 2
- 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(1):17–32. [PubMed: 7499562]
- Oliver DL, Kuwada S, Yin TC, Haberly LB, Henkel CK. Dendritic and axonal morphology of HRP-injected neurons in the inferior colliculus of the cat. *J Comp Neurol*. 1991; 303(1):75–100. [PubMed: 2005240]
- Oliver DL, Morest DK. The central nucleus of the inferior colliculus in the cat. *J Comp Neurol*. 1984; 222(2):237–264. [PubMed: 6699209]
- 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(1):27–42. [PubMed: 7909821]
- Ono M, Ito T. Functional organization of the mammalian auditory midbrain. *J Physiol Sci*. 2015
- Ono M, Yanagawa Y, Koyano K. GABAergic neurons in inferior colliculus of the GAD67-GFP knock-in mouse: electrophysiological and morphological properties. *Neurosci Res*. 2005; 51(4):475–492. [PubMed: 15740810]
- Peruzzi D, Bartlett E, Smith PH, Oliver DL. A monosynaptic GABAergic input from the inferior colliculus to the medial geniculate body in rat. *J Neurosci*. 1997; 17(10):3766–3777. [PubMed: 9133396]
- Poon PW, Chen X, Cheung YM. Differences in FM response correlate with morphology of neurons in the rat inferior colliculus. *Exp Brain Res*. 1992; 91(1):94–104. [PubMed: 1301377]
- Roger M, Arnault P. Anatomical study of the connections of the primary auditory area in the rat. *J Comp Neurol*. 1989; 287(3):339–356. [PubMed: 2778109]
- Rothschild G, Nelken I, Mizrahi A. Functional organization and population dynamics in the mouse primary auditory cortex. *Nat Neurosci*. 2010; 13(3):353–360. [PubMed: 20118927]
- Saint Marie RL, Stanforth DA, Jubelier EM. Substrate for rapid feedforward inhibition of the auditory forebrain. *Brain Res*. 1997; 765(1):173–176. [PubMed: 9310410]
- Schikorski T, Stevens CF. Quantitative ultrastructural analysis of hippocampal excitatory synapses. *J Neurosci*. 1997; 17(15):5858–5867. [PubMed: 9221783]
- Shi CJ, Cassell MD. Cortical, thalamic, and amygdaloid projections of rat temporal cortex. *J Comp Neurol*. 1997; 382(2):153–175. [PubMed: 9183686]
- Sivaramakrishnan S, Oliver DL. Distinct K currents result in physiologically distinct cell types in the inferior colliculus of the rat. *J Neurosci*. 2001; 21(8):2861–2877. [PubMed: 11306638]
- Spiro GA, Rager J, Manis PB. Convergence of auditory-nerve fiber projections onto globular bushy cells. *Neuroscience*. 2005; 136(3):843–863. [PubMed: 16344156]
- Sturm J, Nguyen T, Kandler K. Development of intrinsic connectivity in the central nucleus of the mouse inferior colliculus. *J Neurosci*. 2014; 34(45):15032–15046. [PubMed: 25378168]
- Tamamaki N, Yanagawa Y, Tomioka R, Miyazaki J, Obata K, Kaneko T. Green fluorescent protein expression and colocalization with calretinin, parvalbumin, and somatostatin in the GAD67-GFP knock-in mouse. *J Comp Neurol*. 2003; 467(1):60–79. [PubMed: 14574680]

- Vater M, Kossl M, Horn AK. GAD- and GABA-immunoreactivity in the ascending auditory pathway of horseshoe and mustached bats. *J Comp Neurol*. 1992; 325(2):183–206. [PubMed: 1460113]
- Wallace MN, Shackleton TM, Palmer AR. Morphological and physiological characteristics of laminar cells in the central nucleus of the inferior colliculus. *Front Neural Circuits*. 2012; 6:55. [PubMed: 22933991]
- Whitley JM, Henkel CK. Topographical organization of the inferior collicular projection and other connections of the ventral nucleus of the lateral lemniscus in the cat. *J Comp Neurol*. 1984; 229(2):257–270. [PubMed: 6501602]
- Winer JA. Three systems of descending projections to the inferior colliculus. In: Winer JA, Schreiner CE, editors *The inferior colliculus*. New York: Springer; 2005. 231–247.
- Winer JA, Larue DT. Evolution of GABAergic circuitry in the mammalian medial geniculate body. *Proc Natl Acad Sci U S A*. 1996; 93(7):3083–3087. [PubMed: 8610172]
- Winer JA, Saint Marie RL, Larue DT, Oliver DL. GABAergic feedforward projections from the inferior colliculus to the medial geniculate body. *Proc Natl Acad Sci U S A*. 1996; 93(15):8005–8010. [PubMed: 8755593]
- 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]
- Young ED, Oertel D. Cochlear nucleus. In: Shepard GM, editor *The synaptic organization of the brain*. 5th ed.. Oxford: Oxford University Press; 2004. 125–163.

Figure 1.

A schematic diagram of auditory projection to the IC in mammals. Most of projections which do not terminate in the IC are omitted for conciseness. Inhibitory (In) pathways (blue) include both GABAergic and glycinergic projection. Green arrows indicate glutamatergic excitatory (Ex) pathways which mainly terminate on dendrites (axodendritic, AD). Red arrows indicate glutamatergic excitatory (Ex) pathways which terminate on both somata (axosomatic, AS) and dendrites (AD). In the IC, most Ex AS terminals synapse on large GABAergic (LG) neurons. LG neurons are the major source of In ascending projection to

the MGB. The MGB receives feedback inhibition from the reticular thalamic nucleus (Rt). In addition, local inhibition system is present in the MGB in some species (e.g. cat and monkey). The size of arrows indicates relative density of innervation.

Figure 2.

Glutamatergic axosomatic terminals on LG neurons in various mammalian species. A, rat (*Rattus norvegicus*); B, GAD67-GFP mouse (Ono et al., 2005; Tamamaki et al., 2003); C, rabbit (*Oryctolagus cuniculus*); D, common marmoset (*Callithrix jacchus*); E, Japanese monkey (*Macaca fuscata*); F, Japanese house bat (*Pipistrellus abramus*). VGLUT2-immunopositive terminals (green) make dense axodendritic and axosomatic contacts on GAD67-immunopositive (red in A, C-F) or GFP-positive (red in B) LG neurons (asterisks). Smaller GAD67-positive (SG) neurons (“+”) and GAD67-negative cells do not receive VGLUT2 axosomatic terminals. Bar: 20 μ m.

Figure 3.

DCN neurons projecting to the IC express VGLUT2 but not VGLUT1. After an injection of Fluorogold (FG) into the IC, large DCN neurons, presumably fusiform and giant cells, show immunoreactivity for FG (brown). Their cell bodies are positive for VGLUT2 mRNA (dark blue in A), and negative for VGLUT1 mRNA (B). Note that numerous granule cells express VGLUT1 alone. Bar: 40 μ m. From Ito and Oliver (2012).

Figure 4.

Convergence of axons from lower brainstem nuclei and local neurons on a single LG neuron. (A) Injection sites of Sindbis pal-GFP (red) and Sindbis pal-mRFP (green) viruses of case 11–72. Consequently, infected IC neurons expressed GFP while SOC neurons expressed mRFP. A gray circle indicates approximate location of the LG neuron in B–F. (B) Maximal projection of Z-stack images of a putative LG neuron, which had MAP2+ cell body (gray) with dense VGLUT2+ axosomatic endings (blue), receiving axosomatic contact with both GFP+ (red arrows) and mRFP+ terminals (green arrows). The projection image was made

from 23 Z-stack images, in which each image was separated by 0.48 μ m. (C) A single Z-plane image showing axosomatic contact between mRFP+ terminals (green) and the LG cell body (gray). (D) A single Z-plane image showing axosomatic contact between GFP+ terminals (red) and the LG cell body (gray). (E) A high-magnification image of a box in C with orthogonal views of the stack cut at 3 planes parallel to xy- (blue lines), yz- (a red line), and xz-planes (a green line). The terminal positive for both mRFP (green) and VGLUT2 (blue) was indicated by arrows. (F) A high-magnification image of a box in E with orthogonal views of the stack. The terminal positive for both GFP (red) and VGLUT2 (blue) was indicated by arrows. Scale bars: 1 mm (A), 10 μ m (B, C, D), and 1 μ m (E, F). Adapted from Ito et al., (2015) with permission from Wiley.

Figure 5.

Single axons made only a few axosomatic contacts on a single LG neuron. Analyzed on GFP-labeled axons from single nuclei. (A–F) Three-dimensional reconstruction of GFP+ axons (red), GFP+/VGLUT2+ terminals (green), and LG neural cell bodies (blue). LG neurons which received contact with axons from ipsilateral IC (A), contralateral IC (B), ipsilateral SOC (C), contralateral SOC (D), contralateral CN (E), and ipsilateral NLL (F) were shown. Note that terminals from lower nuclei (C–F) are larger than IC excitatory axosomatic terminals (A, B). Scale bars: 10 μm. (G, H) The volume of a single GFP+/

VGLUT2+ terminal which made contact on a single LG cell body (G) and number of axosomatic contacts (H) by a single GFP+ axonal branch originated from the IC, SOC, CN, or NLL. Thick horizontal bars indicate medians. Top and bottom horizontal hinges of boxes indicate 1st and 3rd quartiles, respectively. Circles are the outliers, and whiskers indicate the maximum and minimal values after eliminating outliers. The number of apposed terminals made by a single axon from the different sources was not significantly different ($P = 0.53$, Kruskal-Wallis multiple comparison test). On the other hand, volume of a single terminal that arose from IC neurons was significantly smaller than that from neurons in other nuclei. Asterisks indicate the pair showing significant difference ($P < 0.05$ for *, $0.05 < P < 0.01$ for **, and $0.01 < P < 0.001$ for ***, Kruskal-Wallis multiple comparison test). (I, J) The number of axosomatic inputs was almost proportional to the surface area of LG cell bodies. Estimated number of inputs, which was calculated by dividing total number of VGLUT2+ axosomatic terminals by number of GFP+/VGLUT2+ axosomatic terminals that arose from a single axon (I) or all axons (J) in a Z-stack. The fitting curves were calculated under a generalized linear model. Adapted from Ito et al., (2015) with permission from Wiley.

Figure 6.

Spatial distribution of GAD67+ cell bodies receiving axosomatic contact from axons of a single glutamatergic neuron. Reconstructed dendrites (blue) and axons (red) of GFP-labeled neurons in 3 cases that showed single cell labeling with Sindbis pal-GFP virus. Green dots indicate cell bodies of GAD67+ neurons that receive one or more axosomatic contacts. In all cases, axonal plexuses were flat and made axosomatic contacts with GAD67+ presumable LG neurons. In the case 11–13, axon collaterals made two planar plexuses in the ICC and ICD, and one plexus in the ICL. The GAD67+ cells receiving the axosomatic contacts were

located in the dorsal and lateral cortices as well as in the ICC. Gray lines indicate the border of the ICC. Scale bars: 250 μ m. Adapted from Ito and Oliver (2014) with permission from Wiley.

Figure 7.

Distribution of LG neurons (dots) which received axosomatic contacts with GFP+/VGLUT2+ terminals after an injection of Sindbis pal-GFP or AAV pal-GFP viruses. (A) An SOC injection case (13–66). LG neurons receiving contact were found bilaterally. In the ICC, LG neurons receiving contact were located mainly in the lateral part. (B) A CN injection case (10–66). LG neurons receiving contact were mainly found in the ICC of the contralateral side. (C) An NLL injection case (14–16). LG neurons receiving contact were sparsely distributed throughout the IC of the ipsilateral side. Each trace is separated by 240

m. Scale bar: 1 mm. Abbreviations: BIC, brachial nucleus of the IC; ICC, central nucleus of the IC; ICD, dorsal cortex of the IC; ICL, lateral cortex of the IC; ICR, rostral cortex of the IC. From Ito et al., (2015) with permission from Wiley.

Figure 8.

Distribution of IC GABAergic cells is similar between marmoset (A–C) and rat (D–F). Both LG and small GABAergic (SG) neurons are distributed in all IC subdivisions, and LG neurons have larger cell bodies than SG neurons in both species. (A) Distribution of LG (red circles) and SG (blue “x”) neurons in a single confocal optical section of common marmoset (*Callithrix jacchus*) IC. Cells of which nuclei were identifiable were plotted. ICL, lateral cortex of the IC; ICD, dorsal cortex of the IC; ICC, central nucleus of the IC. Bar: 1 mm. (B) Percentage of LG (red) and SG (blue) cells to all GABAergic cells in each subdivision.

Numbers indicate total counted cells (5 sections for each monkey, $N = 2$). (C) Distributions of mean diameter of LG (red) and SG (blue) cells in each subdivision. Thick horizontal bars indicate medians. Top and bottom horizontal hinges of boxes indicate 1st and 3rd quartiles, respectively. Circles are the outliers, and whiskers indicate the maximum and minimal values after eliminating outliers. (D) Distribution of LG (red circles) and SG (blue “x”) cells in a single confocal optical section of rat IC. Cells of which nuclei were identifiable were plotted. Note that the presence of GABA modules (Chernock et al., 2004) in the ICL and ICD. Bar: 1 mm. (E) Percentage of LG (red) and SG (blue) cells to all GABAergic cells in each subdivision. Numbers indicate stereologically counted cells ($N = 4$). (F) Distributions of mean diameter of LG (red) and SG (blue) cells in each subdivision. Rat data were re-analyzed from those used for Ito et al., (2009).

Figure 9.

In vivo calcium imaging of cells in layer 1 of the dorsal cortex of the IC. (A) Approximate injection sites of Oregon Green BAPTA-1 AM (OGB-1); eight injection sites of 8 animals were superimposed on a micrograph of the dorsal view of a fixed mouse brain. Injection sites covered most of the dorsal cortex of IC. The lateral cortex of IC is shown to the right of the numbers. Numbers indicate cases on the right side of the image. In medially located sites (black circles) cells tended to have higher best frequency (BF). In the more lateral sites (gray circles), most cells showed preference to a single frequency. Example sites, which are shown

in D and E, are indicated with thick lines. Bar, 1 mm. (B) Pixels that corresponded to layer 1 cell bodies and responded significantly to at least one type of stimuli in Case 131010. (C) An example of fluorescence changes in response to noise stimuli. The activity of cell #1 in B (an asterisk) is shown. The cell exhibited immediate positive fluorescence changes following exposure of the contralateral ear to broad-band noise (bars). (D) Distribution of best frequencies (BF) in cases 121011 (left) and 131010 (right). Cells with lower BF were located laterally, whereas those with higher BF were located medially; M, medial; L, lateral; R, rostral; C, caudal. (D) Distribution of BF in cases 121015 (left) and 121108 (right). The majority of cells had BF of 7.8 kHz. Cells with other BF were sparsely distributed. Cell 121015#4 did not respond to tones. (E) Presence of frequency gradient in medio-lateral axis in medial part of layer 1 revealed by linear regression between distance of cell bodies from lateral to medial end of image and logarithm of the BF. In cases 121011, 131010, and 140123, the slopes of the fits were significantly larger than zero ($P = 0.001$, < 0.001 , and $= 0.031$, respectively). In the other cases, the slopes were not significantly different from zero ($P > 0.05$), suggesting no clear frequency gradient. Adapted from Ito et al., (2014) with permission from Elsevier.