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Subtypes of GABAergic Cells in the Inferior Colliculus

Brett R. Schofield, PhD* and

Department of Anatomy and Neurobiology, Northeast Ohio Medical University, 4209 State Route 44, Rootstown, OH 44272, USA

Nichole L. Beebe, PhD

Department of Anatomy and Neurobiology, Northeast Ohio Medical University, 4209 State Route 44, Rootstown, OH 44272, USA

Abstract

The inferior colliculus occupies a central position in ascending and descending auditory pathways. A substantial proportion of its neurons are GABAergic, and these neurons contribute to intracollicular circuits as well as to extrinsic projections to numerous targets. A variety of types of evidence – morphology, physiology, molecular markers – indicate that the GABAergic cells can be divided into at least four subtypes that serve different functions. However, there has yet to emerge a unified scheme for distinguishing these subtypes. The present review discusses these criteria and, where possible, relates the different properties. In contrast to GABAergic cells in cerebral cortex, where subtypes are much more thoroughly characterized, those in the inferior colliculus contribute substantially to numerous long range extrinsic projections. At present, the best characterized subtype is a GABAergic cell with a large soma, dense perisomatic synaptic inputs and a large axon that provides rapid auditory input to the thalamus. This large GABAergic subtype projects to additional targets, and other subtypes also project to the thalamus. The eventual characterization of these subtypes can be expected to reveal multiple functions of these inhibitory cells and the many circuits to which they contribute.

Keywords

GABA; inhibition; perineuronal net; auditory system; cell type

1 Introduction

The inferior colliculus (IC) is widely described as a hub in the central auditory pathways (Winer and Schreiner, 2005). The IC serves as the major source of auditory projections to the thalamus. In addition, the IC provides projections to many brainstem regions, including many lower auditory nuclei as well as the nucleus of brachium of the IC, superior colliculus,

*corresponding author bschofie@neomed.edu. nbbeebe@neomed.edu.

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periaqueductal gray, pontine nuclei and various nuclei of the reticular formation. In contrast to most other sensory pathways, long-range inhibitory projections are quite common in the auditory brainstem, and contribute to the outputs of the IC.

GABAergic cells constitute 20–40% (depending on the species) of the neurons in the IC, with glutamatergic neurons forming the rest of the population (Oliver et al., 1994; Merchán et al., 2005; Ono et al., 2005; Mellott et al., 2014a). GABAergic neurons are distributed throughout the IC, suggesting they play a role in most or all functions attributed to the IC, including relay of information for auditory perception, orientation to sounds, initiation of defensive behaviors and top-down modulation of early auditory processing (Oliver, 2005; Xiong et al., 2015; Schofield and Beebe, 2018). Consistent with this view is the fact that GABAergic IC cells have extensive outputs, including intracollicular projections within and between IC subdivisions, commissural projections from one IC to the other, and extra-collicular projections to numerous targets. Also consistent with a view of multiple functions is evidence that GABAergic IC cells comprise multiple subtypes. The identification of subtypes has proven instrumental in understanding functional roles of GABAergic neurons in areas such as neocortex and hippocampus (e.g., Markram et al., 2004; Lawrence et al., 2008; Rudy et al. 2011; Mesik et al., 2015; Li et al., 2015; Yavorska and Wehr, 2016; Hattori et al., 2017; Wood et al., 2017). In these regions, GABAergic subtypes differ in their axonal projections, intrinsic physiological properties, inputs and various molecular markers. We first review evidence for the existence of subtypes of GABAergic cells in the IC. We then discuss the projections of GABAergic IC cells which, unlike GABAergic cells in the hippocampus and neocortex, contribute substantially to extrinsic projections as well as to local circuits.

2 Evidence for subtypes of GABAergic cells

Given the large population of GABAergic neurons in the IC, it is not surprising that many authors have suggested there are subtypes, or subpopulations of GABAergic cells that serve different functions. The identification of GABAergic neuron subtypes has been quite valuable in neocortex and hippocampus (e.g., Lawrence, 2008; Rudy et al., 2011). A key step has been recognition that subtypes that serve different functions are distinguishable on a wide range of factors, including dendritic morphology, inputs, axonal projections, intrinsic membrane properties and expression of various molecular markers. As we will review, numerous studies have suggested that GABAergic cells in the IC comprise two or more subtypes. However, we have yet to reach a point where different characteristics have converged on a “master plan” identifying functional groups, so the following discussion is organized around categories of information that have been used for identifying subtypes.

2.1 Morphology

In the central nucleus of the IC, neurons can be divided into two groups based on dendritic morphology (reviewed by Oliver, 2005; Ito and Malmierca, 2018). Disc-shaped (or flat) cells have dendrites and axons that remain within an iso-frequency lamina, whereas stellate (or less flat) cells have dendrites and axons that cross multiple laminae. Given the relationship of the laminae to tonotopic organization of the central nucleus, the disc-shaped cells have

been associated with narrow frequency tuning and their intralaminar axons with “on-frequency” effects. Stellate cells, in contrast, can integrate inputs from a wider frequency range and through their extra-laminar axons can provide for cross-frequency effects. GABAergic cells of both morphologies have been observed, suggesting that GABAergic IC cells contribute to neuronal responses both within and across frequency bands (rat: Roberts and Ribak, 1987a; gerbil: Roberts and Ribak, 1987b; barn owl: Carr et al., 1989; cat: Oliver et al., 1994; mouse: Ono et al., 2005).

IC regions outside the central nucleus are populated by cells with a variety of dendritic morphologies that are often considered variations on a multipolar (stellate) scheme (Faye Lund and Osen, 1985; Meininger et al., 1986; Malmierca et al., 2011). GABAergic cells in these areas have also been associated with each of the morphologic types (Ono et al., 2005). In other words, use of GABA as a neurotransmitter has been associated with every morphologic type described in the IC.

Soma size has frequently been used to subdivide GABAergic IC cells. The large GABAergic cells of the IC are particularly striking; an early study in guinea pig commented that GABAergic neurons in the IC appear larger than in any other part of the brainstem (Thompson et al., 1985). However, across species, GABAergic IC cells exhibit a wide range of soma sizes (rat: Roberts and Ribak, 1987a; Tongjaroenbuangam et al., 2006; Ito et al., 2009; gerbil: Roberts and Ribak, 1987b; cat: Oliver et al., 1994; chicken and pigeon: Ito and Atoji 2016; guinea pig: Beebe et al., 2016). Most authors recognize small, medium, and large size categories, with minor differences in the type of measurement (soma diameter versus soma profile area), and in where lines are drawn defining categories (Fig. 1). Soma size in general correlates with numerous physiological parameters, and can be related to differences in morphology and function. For example, Roberts and Ribak (1987a) found that cells in their medium soma size category could be either multipolar or bipolar in soma shape, while small and large cells were exclusively multipolar. Not surprisingly, soma size is under genetic influence. The number of GABAergic neurons is increased in the IC of the genetically epilepsy prone rat, but the increase is exclusively within populations of small and medium GABAergic cells (Roberts et al., 1985). As the rest of this review will show, soma size is likely to be an important measure of GABAergic IC cells. Given the morphological differences, it seems reasonable to expect functional differences among IC neurons.

2.2 Physiological properties

Overall, IC neurons, regardless of their transmitter type, can be divided into six types based on intrinsic physiological properties (Sivaramakrishnan and Oliver, 2001; Tan and Borst, 2007; Tan et al., 2007). Less information is available about the intrinsic properties of GABAergic IC cells, but such studies have been facilitated by the creation of transgenic animals that express fluorescent proteins in GABAergic IC cells. Using such an approach, Ono et al. (2005) found four subtypes of GABAergic cells distinguished by intrinsic physiology. Cells were divided into tonic and phasic categories based on their response to a current pulse injected into the soma. Tonic-firing cells were further subdivided into regular sustained and buildup/pauser types based on differences in their firing patterns when stimulated from a hyperpolarized resting potential. Phasic-firing cells were subdivided based

on whether they displayed an after-hyperpolarization or an after-depolarization potential following spiking. Three of the physiological subtypes were heterogeneous with respect to their distribution in the IC, their soma size and dendritic morphology, and the orientation of their dendritic fields. The fourth subtype, comprising transient-firing cells that displayed an after-depolarization potential, were present only in dorsolateral IC, and exclusively had small dendritic fields. Ono et al. (2005) also compared the GABAergic cells to a smaller sample of non-GABAergic (i.e., presumptive glutamatergic) IC cells. The latter cells also exhibited several subtypes; these appeared to be distinguishable from the GABAergic subtypes, but a detailed description was not provided.

In vivo studies of GABAergic IC cells are few. Ono et al. (2017) recorded neuronal responses to acoustic stimuli and used optogenetic methods to distinguish GABAergic vs. glutamatergic IC cells. Their main finding was that the GABAergic and the glutamatergic populations were indistinguishable on most quantitative measures. The authors show that each group was heterogeneous; i.e., both GABAergic and glutamatergic groups appear to contain subtypes, a conclusion consistent with the in vitro studies. We would suggest that functional distinctions are likely related to the subtypes, and that distinctions between the subtypes could have been obscured by comparing the larger GABAergic and glutamatergic groups. Support for such a proposal comes from another study, in which Geis and Borst (2013) performed in vivo whole-cell recordings of GABAergic cells in the IC of mice. The technology -- in vivo two-photon imaging of IC cells -- limited the observations to cells near the dorsal surface of the IC (mostly in the dorsal cortex). Despite the spatial limitations on their sample, the authors concluded that numerous physiological properties varied in parallel with soma size and that the large cells constitute a distinct physiological subtype. This provides support for the existence of physiological subtypes of GABAergic IC cells, with possible correlation to their morphology.

2.3 Molecular markers of GABAergic IC cells

Molecular markers carry the potential for distinguishing cell types and, in many cases, can provide an experimental handle for selective manipulation of those cells via optogenetic methods. GABAergic cells in neocortex are divided into 3 major subtypes, each with a unique set of characteristics (and a convenient molecular marker). The markers that have proven useful in neocortex and hippocampus have not yet yielded the same insights in the IC. However, a few markers have begun to emerge.

2.3.1 VGLUT2 inputs (and soma size)—Ito et al. (2009) distinguished two subtypes of GABAergic cells in the IC of rats that differ in the prominence of excitatory somatic inputs from presynaptic boutons that contain VGLUT2 (a vesicular glutamate transporter). Dense inputs from VGLUT2 immunopositive boutons are associated with large GABAergic somas (in rat, diameter > 16.5 μm) but are not present on small GABAergic somas (in rat, diameter < 10.7 μm). A distinction based on dense VGLUT2 inputs to large GABAergic somas versus minimal VGLUT2 inputs to small GABAergic somas can be recognized in numerous other species, including mouse (Geis and Borst, 2013), chicken and pigeon (Ito and Atoji, 2016), marmoset (Ito et al., 2016), and guinea pig (Beebe et al., 2016). Quantitative analyses demonstrate that the small cells without VGLUT2 inputs predominate

in all IC subdivisions in guinea pigs and marmosets (Beebe et al., 2016; Ito et al., 2016) and in the dorsal cortex and lateral cortex in rats (the two GABAergic types occur in roughly equal numbers in the central nucleus in rats). Whether the two subtypes get inputs from different sources is not known. The VGLUT2+ inputs appear to arise from several sources, including other IC neurons as well as several subcollicular auditory nuclei (Ito et al., 2016). In theory, some or all of these sources could also provide input to the small GABAergic subtype, although such input would contact dendrites rather than the soma; this issue has yet to be addressed experimentally.

2.3.2 Perineuronal nets (and VGLUT2 inputs, and soma size)—Perineuronal nets (PNs) are aggregates of extracellular matrix that surround GABAergic neurons in many brain areas, including the IC and many other brainstem auditory nuclei (Karetko and Skrangiel-Kramka, 2009; Foster et al., 2014; Sonntag et al., 2015; Beebe and Schofield, 2018). PNs appear to support fast spiking and to play a role in neuronal plasticity and protection against oxidative stress (Beurdeley et al., 2012; Suttkus et al., 2012; Cabungcal et al., 2013; de Vivo et al., 2013). We combined PN staining with VGLUT2 staining to distinguish four subtypes of GABAergic IC cells: cells that are surrounded by both a PN and a perisomatic “ring” of VGLUT2 boutons; cells that had a VGLUT2 ring *or* a PN (but not both), and cells that had neither a VGLUT2 nor a PN (Fig. 2, A-D). The GABAergic subtypes are present in different proportions in the various IC subdivisions. The “GA D-only” subtype, with neither PN nor VGLUT2 ring, is the most numerous subtype in each IC subdivision (“GAD” refers to glutamic acid decarboxylase, a selective marker of GABAergic cells; immunochemical staining with anti-GAD was used to classify the GABAergic cells; Beebe et al., 2016). GABAergic cells with both a PN and a VGLUT2 ring are most prominent in the IC central nucleus, where this subtype constitutes nearly one quarter of the GABAergic cells.

These GABAergic subtypes also differ in their range of soma sizes. (Fig. 2E). The data obtained in guinea pigs matched that from rats in showing that GABAergic cells with VGLUT2 rings (particularly those that were also surrounded by a PN; dark green in Fig. 2E) included almost all of the largest GABAergic cells, and none of the smallest GABAergic cells. However, it is worth noting that even those subtypes that have the greatest difference in average size show considerable overlap in soma size distributions; only the very largest or the very smallest cells can be assigned to a subtype based on size alone. As described above, somatic inputs from VGLUT2+ boutons commonly characterize a subset of IC neurons across species. PNs are also common in the IC across species (Beebe and Schofield, 2018). Thus, staining for VGLUT2 and PNs is likely to allow discrimination of four subtypes of GABAergic neurons across species.

2.3.3 Calcium binding proteins—The expression of different calcium buffering proteins, especially parvalbumin, has helped differentiate subtypes of GABAergic interneurons in neocortex and hippocampus (Rudy et al., 2011; Kubota, 2014). The three main neuronal calcium buffering proteins (parvalbumin, calbindin, and calretinin) have distinct distributions within the IC: parvalbumin is present throughout the IC, although heaviest in the central nucleus of the IC, and calbindin and calretinin are most prevalent in

the IC cortices (Ouda and Syka, 2012). Given the association between these proteins and GABAergic cells of the cerebral cortex (Markram et al., 2004), surprisingly little research has been done on the relationship of these proteins with GABAergic IC cells. Parvalbumin is associated with most or all GABAergic cells in the central nucleus of the IC (as well as some non-GABAergic cells; Fredrich et al., 2009). There is even less evidence about the association between GABAergic IC cells and calbindin or calretinin, however these markers would presumably be associated primarily with GABAergic cells in cortical regions, given their distribution (Ouda and Syka, 2012).

2.3.4 Expression of modulatory neurotransmitter receptors—In cerebral cortex, expression of the 5HT_{3a} receptor, an ionotropic serotonergic receptor, has helped distinguish a subtype of inhibitory interneuron (Rudy et al., 2011). Although expression of 5HT_{3a} receptors has not been examined directly in GABAergic cells in the IC, there is indirect evidence for 5HT₃-associated activation of GABAergic IC cells (Bohorquez and Hurley, 2009). Metabotropic serotonergic receptors have also been associated with GABAergic IC cells. About 2/3 of GABAergic IC cells are associated with the 5HT_{1a} receptor and about 2/3 are associated with the 5HT_{1b} receptor (Peruzzi and Dut, 2004). The extent to which these two populations overlap is unknown, but about 2/3 of GABAergic IC cells are in apposition to serotonergic fibers, indicating that they may overlap extensively. Overall, this may indicate two subclasses of GABAergic IC cells: those that are modulated by serotonin, and those that are not. GABAergic IC cells can also be differentiated based on expression of three different types of opioid receptors (mu, delta, and kappa), with the majority of GABAergic IC cells expressing mu opioid receptors (Tongjaroenbuangam et al., 2006). This presumably indicates differences in the response to opioids by different GABAergic cells. Additionally, Yigit et al. (2003) showed that acetylcholine can activate GABAergic IC cells by acting on M3 receptors in the IC. Whether this occurs in all GABAergic IC cells is unclear, but it presents the opportunity for possible subdivision of GABAergic IC cells based on their responses to acetylcholine.

3 Projections of GABAergic IC cells

In many brain areas, GABAergic cells are interneurons. In contrast, the auditory brainstem includes a substantial number of long range inhibitory projections, including GABAergic projections from the IC. GABAergic neurons in the IC have axons that branch to provide both local connections (within the IC) and extrinsic projections to a variety of targets (Fig. 3). The intrinsic branches can ramify within a subdivision and/or extend to adjacent subdivisions (González-Hernández et al., 1991). Additional axonal branches can travel extrinsically to innervate the superior colliculus (Appel and Behan, 1990; Mellott et al., 2018), contralateral IC (González-Hernández et al., 1996; Hernández et al 2006;., Nakamoto et al., 2013a) or thalamus (Winer et al., 1996; Saint Marie et al., 1997; Peruzzi et al., 1997; Ito et al., 2009; Mellott et al., 2014a,b; Ito and Atoji, 2016; Clarke and Lee, 2018). Differences in axonal projections reflect distinct functions and can help define GABAergic subtypes, but few data are currently available.

3.1 GABAergic cell types and intrinsic projections

GABAergic IC cells are contacted directly by ascending inputs from lower auditory nuclei, commissural inputs from the contralateral IC and descending inputs from the auditory cortex (Chen et al., 2018). Many of these extrinsic inputs are excitatory, but can inhibit IC cells by activating IC GABAergic neurons which then inhibit their neighbors through local axonal projections (Mitani et al., 1983; Moore et al., 1998; Torterolo et al., 1998; Jen et al., 2001; Nakamoto et al., 2013a,b; Ito and Oliver, 2014; Xiong et al., 2015; Chen et al., 2018). Together, the local axons form a substantial source of inhibitory input to IC neurons and contribute to the selectivity of IC neurons for different aspects of acoustic stimuli (Saldaña and Merchan, 2005; Sivaramakrishnan et al., 2013; Ito and Oliver, 2014; Sturm et al., 2014).

While the question has not been addressed directly, the widely held view that most or all IC neurons have local axons would suggest that all subtypes of GABAergic IC cells contribute to local circuits and IC cellular responses (e.g., Oliver et al., 1991; Wallace et al., 2012). Of particular interest will be to identify possible differences in the local axon morphology that correlate with other cellular properties. In the IC central nucleus, GABAergic cells can have disc-shaped or stellate morphology, suggesting that their axons can be confined to an iso-frequency lamina (for the disc-shaped cell) or cross into adjacent laminae (the stellate cell). How these dendritic/axonal morphologies relate to the other markers of GABAergic cell types discussed above remains to be determined.

3.2 GABAergic cell types and extrinsic projections

GABAergic IC neurons project to several extrinsic targets, including the contralateral IC, the superior colliculus and the medial geniculate body (MG) of the thalamus (Fig. 3). In each case, the inhibitory projections parallel those from glutamatergic IC neurons (in that axons of each phenotype terminate in the target nucleus; they may or may not converge on the same target cells). The projections from inhibitory and excitatory IC cells provide an opportunity for various forms of integration in cells targeted by IC axons. For example, some cells in the auditory thalamus receive convergent excitatory and inhibitory IC inputs, whereas other cells receive direct inputs only from excitatory IC cells (Bartlett and Smith, 2002). Smaller populations of GABAergic IC cells project to the superior colliculus or contralateral IC. With the exception of IC projections to the thalamus, there are few data available on the GABAergic subtypes that contribute to these projections. We describe the midbrain projections followed by a more detailed description of projections to the thalamus.

3.2.1 Projections to superior colliculus—Auditory projections to the superior colliculus likely play a role in multiple behaviors, including orienting to and possibly attending to sounds and in coordinating approach or avoidance behaviors (reviewed by Huerta and Harting, 1984; Sparks, 1988; Stein and Meredith, 1993; Mysore and Knudsen, 2011; Comoli et al., 2012; Costa et al., 2016; Savage et al., 2017). These projections arise mainly from the IC lateral cortex, less so from the IC rostral pole and IC dorsal cortex, and minimally from other IC regions (Mellott et al., 2018). These areas of the IC contain the four GABAergic subtypes distinguished by VGLUT2 rings and PNs, and it is possible that all four subtypes project to the superior colliculus. Nonetheless, the “GAD-only” type, lacking a VGLUT2 ring and a PN, are most prominent in the SC-projecting parts of the IC and likely

predominate in this projection (Beebe et al., 2016). This question has yet to be addressed experimentally.

3.2.2 Projections to the contralateral IC—The commissure of the IC allows for interactions between the two ICs. Commissural projections affect the way IC neurons respond to sound frequency and intensity as well as binaural cues, suggesting that the commissure serves a multitude of functions (e.g., Saldaña and Merchan, 2005; Malmierca et al., 2005; Cheng et al., 2013; Orton and Rees, 2014; Orton et al., 2016). GABAergic cells projecting through the commissure have cell bodies located throughout the IC subdivisions and their axons also terminate throughout the IC (Saldaña and Merchan, 2005). Electrical stimulation of the IC commissure leads to inhibition (and excitation) of almost all IC cells, indicating that commissural inhibition is widespread. Reports of the proportion of commissural cells that are GABAergic vary considerably, with values of 10–50% most common (González-Hernández et al., 1991; Fredrich et al 2009;., Nakamoto et al., 2013b). Commissural cells are quite numerous (outnumbering all other output pathways except possibly the tectothalamic pathway; Okayama et al., 2006), so even lower estimates of GABAergic cells could represent a large population.

Molecular markers of GABAergic subtypes have yet to be examined for commissural cells, but morphological characteristics emphasize a great deal of heterogeneity. In fact, the evidence suggests that every IC cell type, including GABAergic and glutamatergic neurons of all sizes and dendritic morphologies, may participate in the commissural pathway (González-Hernández et al., 1991; Okayama et al., 2006). These observations apply to the commissural pathway overall, leaving open the question of whether different commissural axons have different patterns of termination.

3.3.3 Projections to the thalamus—The tectothalamic projection is the largest pathway out of the IC and, via subsequent thalamic projections to the forebrain, is the main pathway for auditory perception. GABAergic IC projections to the thalamus have been demonstrated in numerous species (cat: Winer et al., 1996; Saint Marie et al., 1997; rat: Peruzzi et al., 1997; Ito et al., 2009; guinea pig: Mellott et al., 2014a,b; mouse: Clarke and Lee, 2018; chicken and pigeon: Ito and Atoji, 2016). GABAergic tectothalamic cells are located throughout the IC and, as a group, terminate in all the MG subdivisions (Mellott et al., 2014a,b). To a large extent, the relationship between the IC subdivision containing the somas and the MG subdivision targeted by the GABAergic axons parallels the projections from the glutamatergic cells, suggesting that the GABAergic projection sustains (and contributes to) the parallel nature of the tectothalamic pathway overall (Mellott et al., 2014b). This in turn implies that the GABAergic projections to the MG contribute to the full array of functions associated with the tectothalamic pathway.

GABAergic cells constitute about 25–40% of the tectothalamic pathway (depending on species; Oliver et al., 1994; Merchán et al., 2005; Ono et al., 2005; Mellott et al., 2014a). Soma size has provided a particular focal point for many of the studies. In the initial report of GABAergic projections from the IC to the MG, Winer et al. (1996) stated, “There was a wide range in size and shape among double-labeled neuronal somata, which included medium sized, spindle-shaped cells ..., and the largest IC neurons, up to 33 μ m in average

somatic diameter.” (page 8009). Thus, this first report provided data that multiple subtypes of GABAergic IC cells project to the MG. In particular, the medium spindle-shaped cells likely include disc-shaped cells, whereas the largest neurons are almost certainly stellate cells. Interestingly, subsequent work has focused almost exclusively on the largest cells. Saint Marie et al. (1997) stained the brachium of the IC for GABAergic axons. The brachium serves as the main conduit for axons travelling from the IC to the thalamus (note: it also contains other axons, including descending corticocollicular axons). As expected from earlier studies, GABAergic axons in the brachium ranged from small to very large in diameter. Most impressive was that the largest axons were *all* GABAergic (Fig. 4). As the authors point out, this not only suggests a fast inhibitory projection to the MG, but implies that an acoustic stimulus may give rise to inhibition that precedes ascending excitation reaching the thalamus. Not surprisingly, subsequent studies have focused on the largest cells.

The next major advance on this topic came from Ito and Oliver (2009), who related GABAergic subtypes distinguished by size and VGLUT2 somatic inputs to tectothalamic projections (see Section 2.3.1). This initial report showed that both the large GABAergic cells with dense VGLUT2 inputs and small GABAergic cells without such inputs can project to the thalamus. However, the authors concluded that the large GABAergic cells with VGLUT2 inputs were dominant in the tectothalamic pathway, and suggested that the smaller GABAergic cells project to other targets. This emphasis on the large cells, and downplay of the small cells, has since dominated views of the tectothalamic pathway (Ito and Oliver, 2012; Fig. 5).

Interest in the large GABAergic cells centered on the initial suggestions from Winer et al. (1996) that these cells could provide fast inhibition to the thalamus. While electrical stimulation of the brachium supported the view of fast conduction to the thalamus (Peruzzi et al., 1997), it did not address how rapidly an acoustic stimulus could activate the large IC cells. As discussed above (Section 2.2), Geis and Borst (2013) used acoustic stimuli to activate large, medium or small cells in the dorsal cortex of the IC. After intracellular labeling, they followed the axons of four large cells as far as the thalamus, confirming that large cells with rapid responses to acoustic stimuli project to the thalamus. Interestingly, all four axons had branches that also projected to the nucleus of the brachium of the IC (a nucleus usually associated with projections to the superior colliculus and a functional role in orientation to sounds). These results support the idea of fast inhibitory transmission to the auditory thalamus and possibly to other targets by the large GABAergic IC cells.

The question remains, where do small and medium GABAergic cell subtypes project? Beebe (2016) combined retrograde tracing from the MG with GABAergic, PN and VGLUT2 staining in guinea pigs. The results showed that all four subtypes of GABAergic cells distinguishable with these markers project to the MG. Moreover, the “GAD-only” cells, which lack a PN and a VGLUT2 ring, are the smallest GABAergic cells and yet were the most numerous of the GABAergic subtypes that project to the MG (Fig. 6). Do these data conflict with the earlier reports? No; even though the focus has been on the largest cells in the IC or the largest axons in the brachium, the earlier studies support the idea of tectothalamic projections from small GABAergic cells. In fact, Ito et al. (2009) showed that both large and small GABAergic cells project to the MG. The difference may be quantitative

rather than qualitative; they found that the large cells with dense VGLUT2 inputs outnumbered the small cells without such inputs, whereas we found the smaller cells more numerous. Interestingly, the data from the GABAergic axons in the brachium of the IC indicate that small axons, presumably originating from small cells, are particularly numerous in cats (Saint Marie et al., 1997; Fig. 7). The original study emphasized that the largest axons are all GABAergic. When one combines the information from the two graphs (Fig. 4), it becomes clear that the vast majority of the GABAergic axons are near the small end of the distribution (Fig. 7). For example, 75% of the GABAergic axons have a diameter $< 2.0 \mu\text{m}$. We conclude that small GABAergic IC cells may outnumber the large GABAergic cells in the cat tectothalamic pathway, similar to the case in guinea pigs. Further studies will be needed to address the differences cited above and to determine the extent to which the combination of PN and VGLUT2 stains are useful for distinguishing GABAergic subtypes in other species.

4 Where are we now?

In summary, morphological, physiological and molecular characteristics have led to descriptions of up to four subtypes of GABAergic cells in the IC. It remains to be seen whether a combination of the criteria described above reinforce the idea of 4 subtypes or suggest further subdivision (note that work in hippocampus describes 15 GABAergic subtypes; Lawrence, 2008). The identification of additional markers could refine or alter the groups described above. Notably, markers that have proven useful in neocortex and hippocampus (e.g., VIP, parvalbumin, etc.), have not yet yielded the same insights in the IC. Critically, few correlations have yet been found between any of the three major categories of phenotype: intrinsic physiology, molecular markers (intracellular or extracellular) and axonal projection patterns. What characteristics distinguish functionally relevant GABAergic cell types, and how many subtypes are there? Which GABAergic subtypes are involved in which pathways (extrinsic or intrinsic)? Do different GABAergic subtypes get input from different presynaptic sources? Are different subtypes affected differently by neuromodulatory inputs? In neocortex, acetylcholine and other neuromodulators have different effects on different GABAergic subtypes, allowing the subtypes to alter cortical circuit dynamics (Bacci et al., 2005). Of course, one can ask about *any* of the neuronal inputs, but neuromodulatory inputs may be of particular interest given indications that such inputs occur (e.g., ACh to GABAergic cells; Yigit et al., 2003).

5 Conclusions

There are wide-ranging data demonstrating that GABAergic cells in the IC comprise multiple subtypes that almost certainly serve distinct functions. However, we are in the early stages of identifying criteria for distinguishing the subtypes, or even determining how many subtypes exist. Recent progress has focused primarily on the largest GABAergic cells, which appear to provide fast inhibition to the thalamus in response to an acoustic stimulus. Further studies will be needed to characterize additional GABAergic subtypes that project to the MG, and to identify the subtypes that project to other targets of IC projections. Optogenetic methods that allow manipulation of chemically-defined populations are currently limited by our incomplete understanding of GABAergic subtypes. With further classification and

identification of the right markers, transgenic animals and optogenetics should provide excellent tools for testing the functional roles of specific subtypes of GABAergic IC neurons.

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Highlights for Subtypes of GABAergic Cells in the Inferior Colliculus, by Schofield and Beebe

- GABAergic cells in the inferior colliculus can be subdivided by multiple criteria.
- At least 4 subtypes can be distinguished, but a single scheme has yet to emerge.
- GABAergic subtypes have both intrinsic and extrinsic projection targets.
- Best characterized is a large GABAergic subtype that projects to the thalamus.
- Additional GABAergic subtypes are likely to serve multiple functions.

Figure 1.

Summary of previous studies reporting soma sizes of GABAergic IC cells. Each row indicates a previous study that used soma diameter to classify IC cells. Boxes indicate finite ranges, and arrows indicate unbounded classification windows (i.e., < or >). Modified from Beebe et al. (2016), with permission.

Figure 2.

GAD⁺ neurons can be subdivided into four distinct subtypes. **A–D.** Photographs of four subtypes of GAD⁺ neurons in the IC. Each image row shows a single field that was imaged to reveal four markers. The first column shows an overlay of images stained for GAD (glutamic acid decarboxylase, a marker of GABAergic neurons) and perineuronal nets (PNs); the second column shows an overlay of images stained for NeuN (a neuron-specific cell stain) and VGLUT2 (vesicular glutamate transporter type 2); the third column shows an overlay of all four images. Some neurons have both a PN and a ring of VGLUT2⁺ terminals (**GAD–PN–VGLUT2 ring**; arrows in A–C), some neurons only have a PN (**GAD–PN**; open arrowheads in B and C), some neurons only have a ring of VGLUT2⁺ terminals (**GAD–VGLUT2 ring**; double arrow in D), and some neurons lack both a PN and a ring of VGLUT2⁺ terminals (**GAD-only**; arrowheads in A and C). The complete lack of PN staining in D is attributable to a local lack of PNs rather than a staining issue, because other

photographs (not shown here) of PN-surrounded cells were collected from the same section, and a small amount of WFA labeling of the extracellular matrix is present in the background. Scale bar, 20 μm . **E.** The four subtypes of GAD^+ neurons differ in soma profile area. Frequency distributions of soma profile area for each of the GAD^+ subtypes, as well as GAD^- neurons. Vertical red lines indicate a size classification (small, $<105 \mu\text{m}^2$; medium, $105\text{--}318 \mu\text{m}^2$; large, $>318 \mu\text{m}^2$) based on the distributions of soma sizes of the subtypes. The analysis is based on 28,607 IC neurons. From Beebe et al. (2016), with permission.

Figure 3.

Schematic summary of the output pathways from the inferior colliculus (IC). Red arrows indicate pathways in which GABAergic cells contribute substantially (along with glutamatergic cells). Red arrows within the IC indicate connections between (straight arrows) and within (circular arrows) IC subdivisions. Black dashed arrows show pathways originating from glutamatergic cells. CN – cochlear nucleus; MG – medial geniculate nucleus; NBIC – nucleus of the brachium of the inferior colliculus; NLL - nuclei of the lateral lemniscus and surrounding regions (horizontal cell group; sagulum); PAG – periaqueductal gray; Pn – pontine nuclei; RF - reticular formation (e.g., ventrolateral tegmental nucleus, lateral paragigantocellular nucleus, caudal pontine reticular formation); SC – superior colliculus; SOC – superior olivary complex.

Figure 4.

A: pooled distribution of axon size in the 30 sampled frames of the brachium of the IC. Larger axons ($> 2 \mu\text{m}$) were many fewer than smaller axons. B: proportions of axons that were GABA immunoreactive, based on axon size. Larger axons were much more likely to be immunoreactive than smaller axons. BIC – brachium of the inferior colliculus. GABA-IR – GABA-immunoreactive. From Saint Marie et al. (1997), with permission.

Figure 5.

A schematic diagram of the basic IC circuit. Large GABAergic neurons (1) receive strong excitatory inputs on their somata, send their axons to the MGB, and presumably inhibit tufted or stellate neurons in the medial geniculate body (MGB). Small GABAergic neurons (2) do not target MGB. Glutamatergic neurons (3, 4) project to the MGB but lack the dense VGLUT2 axosomatic inputs. Glutamatergic neurons with small terminals (3) co-innervate tufted or stellate neurons with Large GABAergic neurons. Other tufted neurons are innervated by glutamatergic neurons with large terminals (4) and do not receive inputs from Large GABAergic neurons. Small GABAergic and glutamatergic neurons receive most of their excitatory inputs on their dendrites. Red puncta indicate excitatory glutamatergic terminals. Blue puncta indicate inhibitory (GABAergic and glycinergic) terminals. From Ito and Oliver (2012), with permission.

Figure 6.

The four types of GABAergic neurons in the IC-MG projection differ in soma size and spatial distribution. A. Box and whisker plot showing the range and median soma profile areas of non-GAD (yellow), GAD-only (light blue), GAD-VGLUT2 ring (dark blue), GAD-PN (light green), and GAD-PN-VGLUT2 ring (dark green) IC-MG cells. On average, GAD-only cells have the smallest somata, GAD-PN cells have larger somata, and GAD-PN-VGLUT2 ring cells have the largest somata. Note that the y-axis of the box plot is logarithmically scaled. Sample sizes: non-GAD = 1951 cells; GAD-only = 145 cells; GAD-VGLUT2 ring = 3 cells; GAD-PN = 51 cells; GAD-PN-VGLUT2 ring = 11 cells. B. Composite plot of all the GAD+ IC-MG projecting cells in this study (cells from two sections each from six cases). Light blue markers indicate GAD-only cells, dark blue rings indicate GAD-VGLUT2 ring cells, light green markers indicate GAD-PN cells, and dark green markers indicate GAD-PN-VGLUT2 ring cells. GAD-only cells are relatively more dense along the lateral and dorsal edges of the IC, while GAD+ cells surrounded by PNs and/or VGLUT2 rings are relatively more dense in deeper regions of the IC (ICc and IClc layer III). ICc- central nucleus of the IC, ICd- dorsal cortex of the IC, IClc I, II, III- layers I, II, and III of the lateral cortex of the IC. From Beebe (2016), with permission.

Figure 7.

Distribution of axon diameters of GABAergic cells in the brachium of the inferior colliculus of the cat. Values were calculated from Saint Marie et al., (1997; data shown in present Fig. 4).