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Bilateral projections to the thalamus from individual neurons in the inferior colliculus

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Abstract

The medial geniculate body (MG) receives a large input from the ipsilateral inferior colliculus (IC) and a smaller but substantial input from the contralateral IC. Both crossed and uncrossed inputs comprise a large percentage of glutamatergic cells and a smaller percentage of GABAergic cells. We used double labeling with fluorescent retrograde tracers to identify individual IC cells that project bilaterally to the MGs in adult guinea pigs. We also used immunohistochemistry for glutamic acid decarboxylase (GAD) to distinguish GABAergic from glutamatergic cells that project bilaterally to the MG. We found cells in the IC that contained both retrograde tracers, indicating that they project bilaterally. Across cases, the bilaterally projecting cells constituted up to 37% of the cells that project to the ipsilateral MG and up to 73% of the cells that project to the contralateral MG. GABAergic cells averaged 20% of the bilaterally-projecting population. We conclude that a population of IC cells sends branching axonal projections to innervate the MG bilaterally. Most of the neurons in this population are glutamatergic, with a minority that are GABAergic. A mixed projection, with glutamatergic cells outnumbering GABAergic cells, originates from each of the major IC subdivisions (central nucleus, dorsal cortex, lateral cortex). The bilaterally projecting cells are likely to serve functions different from the larger unilateral projections, perhaps synchronizing activity on the two sides of the auditory brain.

Graphical Abstract

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Deposits of fluorescent retrograde tracers revealed individual inferior colliculus cells that send branching axonal projections bilaterally to the medial geniculate bodies. We propose that these bilaterally projecting cells may allow the thalamus to synchronize activity on the two sides of the auditory brain.

Keywords

GABA; inferior colliculus; medial geniculate body; collateral; synchronization;
RRID:AB_2278725; RRID:AB_260754

INTRODUCTION

The auditory system is well known as a highly bilateral system, with crossed and uncrossed projections forming a complex array of circuits among the many auditory nuclei of the brainstem. Most of the ascending brainstem pathways converge in the inferior colliculus (IC), which in turn provides the largest source of ascending auditory projections to the medial geniculate body (MG), the auditory center of the thalamus. Finally, the MG distributes auditory information to the auditory cortex and other telencephalic targets. The thalamocortical (and corticothalamic) projections are almost exclusively ipsilateral, providing a stark contrast with the brainstem pathways (Rouiller, 1997). Interestingly, the tectothalamic projection (from the IC to the MG) has long been known to have a large ipsilateral component as well as a smaller but nonetheless substantial contralateral component (reviewed by Wenstrup, 2005). Thus, each MG receives input from both ICs, and could integrate information from both sides of the brainstem. However, this possibility is often overlooked, with a common claim that the IC provides the last site for subcortical interactions between the two sides of the brain.

We previously described tectothalamic projections in guinea pigs, with an emphasis on GABAergic projections within this pathway (Mellott et al., 2014a). The results demonstrate

a contralateral projection from the IC to the MG, and further demonstrate a GABAergic (presumptively inhibitory) component to this pathway. In the course of those experiments, we discovered individual IC cells that send collateral projections to both MGs. Such branched axonal projections are in a position to serve different functions from unilaterally projecting cells. This study characterizes these bilaterally projecting neurons.

MATERIALS AND METHODS

All procedures were conducted in accordance with the Institutional Animal Care and Use Committee and NIH guidelines. Results are described from five adult pigmented guinea pigs (Elm Hill Labs; Chelmsford, MA, USA) of either sex weighing 389–867 g. Methods for these experiments have been described in detail previously (Mellott et al., 2014a); details are provided primarily for analyses related to the bilateral projections, which were not described previously.

Surgery, perfusion and tissue processing

Each animal was anesthetized with isoflurane (4–5% for induction, 1.75–3% for maintenance; Butler Schein, Dublin, OH, USA) in oxygen and the head positioned in a stereotaxic frame. An incision was made in the scalp and the surrounding skin was injected with 0.25% bupivacaine with epinephrine (1:200,000; Hospira, Inc., Lake Forest, IL, USA), a long-lasting local anesthetic. A surgical drill was used to make a craniotomy over the desired target coordinates. Following the tracer injection, Gelfoam (Harvard Apparatus, Holliston, MA, USA) was placed in the craniotomy site and the scalp was sutured. Ketofen (ketoprofen, 3mg/kg i.m.; Henry Schein, Melville, NY 11747, USA) was administered to provide post-operative analgesia. The animal was monitored until it could walk, eat and drink without difficulty.

Fluorescent tracers (red fluorescent RetroBeads [“red beads”], Luma-Fluor, Inc., Naples, FL, USA; Fast Blue, EMS-Chemi GmbH, Gross Umstadt, Germany; FluoroGold, FluoroChrome, Inc., Englewood, CO, USA) were deposited in the MG with a Hamilton microsyringe (1 µl; Hamilton, Reno, NV, USA) positioned according to stereotaxic coordinates. Each tracer was injected with a different syringe at 1–4 sites (depending on the diffusibility of the tracer, Schofield, 2008) in order to include as much of the MG as possible while limiting the spread of tracer into neighboring nuclei (Table 1). Five days after surgery, the animal was deeply anesthetized with isoflurane and the brain was fixed by perfusion with 4% buffered paraformaldehyde. The brain was frozen and cut into 40 or 50 µm thick transverse sections collected serially in six sets. One series of sections was divided so that sections through the thalamus could be stained for cytochrome oxidase to identify MG subdivisions (Anderson et al., 2007) and sections through the midbrain could be stained with antibodies to brain nitric oxide synthase (bNOS) (Coote and Rees, 2008). Additional sections were immunostained (see below) then mounted on gelatin-coated slides, dried and coverslipped with DPX (Sigma).

Antibody characterization

Putative GABAergic cells were stained with mouse anti-glutamic acid decarboxylase (GAD) monoclonal antibody (GAD67; #MAB5406 Millipore, RRID:AB_2278725, diluted 1:1000 to 1:100) (Nakamoto et al., 2013; Mellott et al., 2014a). The primary antibody was visualized with 1% biotinylated goat anti-mouse antibody (Vector Laboratories, Burlingame, CA, USA: BA-9200) and streptavidin conjugated to AlexaFluor 488 (AF488, green; Invitrogen, Carlsbad, CA, USA). Selected IC sections were stained with antibodies to bNOS (monoclonal antibody BNOS; #N2280 Sigma, RRID:AB_260754, diluted 1:1000) to identify IC subdivisions. Both primary antibodies have been validated previously in rat and produced staining as seen in previous studies (Xiong et al., 2008; Coote and Rees, 2008).

Data analysis

Retrogradely labeled cells were readily identified. The different tracers were easily distinguished with different filters, so double-labeled cells could be identified with certainty. The use of a different channel for the immunocytochemistry made the GAD immunostain readily visible in tracer-labeled cells, including cells that contained two different retrograde tracers. Labeled cells in the IC were plotted with a NeuroLucida reconstruction system (MBF Bioscience, Williston, VT, USA) attached to a Zeiss Axioplan II microscope (Carl Zeiss MicroImaging, Inc., Thornwood, NY, USA). For each case, every labeled cell was plotted in both left and right IC in two transverse sections. One section went through the center of the IC (along the rostro-caudal axis) to include substantial portions of central (ICc), dorsal (ICd) and lateral IC subdivisions. A second section was chosen through the rostral IC to obtain data from more rostral regions; for analyses, the intercollicular tegmentum was combined with the lateral cortex (i.e., matching the area traditionally identified as the IC external cortex; ICx), and the rostral pole was included with the central nucleus. Results from eight injections (4 RB; 2 FB; 2 FG) that also had robust immunostaining were used for quantitative analysis. We excluded GP632 because the FG injection spread caudally into the superior colliculus, which receives projections from the IC.

In some cases the GAD staining extended through the entire section thickness, but more often it extended 5–10 μm from each surface. Sections cut at 40–50 μm thickness typically shrink during dehydration such that the final thickness on slides is 20–30 μm . Thus, our typical protocol could leave a central layer up to 20 μm thick that was unstained. Variation was greater between cases than within a case; we interpret this to reflect subtle variations in the level of fixation that commonly occur with transcardial perfusion. For this reason, analysis was restricted to tissue depths in which the GAD staining worked well. Variation could also occur between sections or between different areas of a given section. The staining depth was assessed with a 63X objective (NA = 1.4) for each subdivision of each section to determine the range of depths (measured from the top surface of the section) where GAD staining was robust. Special attention was then given to focusing on the center of the soma when plotting the symbol for a particular cell. This yielded 2 zones of data (1 associated with each surface), and a central zone that was not stained with GAD. All markers in the unstained zone were excluded from further analyses. This approach provides sufficient resolution in the z plane (section depth) to allow meaningful filtering of the data by depth. After the data were plotted, the X, Y, and Z coordinates of all markers from each subdivision

of each tissue section were exported from Neurolucida to Microsoft Excel and sorted based on the Z coordinate.

Photomicrographs were captured using either a Zeiss AxioImager Z1 microscope and AxioCam HRm or HRc cameras (Zeiss) or a Zeiss AxioPlan 2 microscope and Magnafire camera (Optronics, Goleta, CA, USA). Adobe Photoshop (Adobe Systems) was used to crop images, erase background around tissue sections, to globally adjust intensity levels and to colorize monochrome images.

RESULTS

Injection sites

Figure 1 shows injections in the left and right MG in a representative experiment. On each side, the tracer deposits include large portions of all major MG subdivisions. The resulting distributions of retrogradely labeled cells were similar qualitatively across cases and across tracers. Each injection labeled a large number of cells bilaterally in the IC. These labeled cells were spread across the IC subdivisions, consistent with injection sites that involved all MG subdivisions (Anderson et al., 2007). Additional labeled cells were located in the dorsal and ventral cochlear nuclei, superior olivary complex and areas in and around the lateral lemniscal nuclei, consistent with previous reports (Anderson et al., 2006; Schofield et al., 2014a, b).

Identification of bilaterally-projecting cells

Individual cells that contained both retrograde tracers and thus innervate both left and right MG were readily identifiable with either combination of retrograde tracers (i.e., red beads and FluoroGold or red beads and Fast Blue; Fig. 2). A subset of these cells were GAD⁺ (Fig. 2, green arrowheads), suggesting that these cells are GABAergic. The majority of the double-retrograde cells were GAD⁻ (Fig. 2, white arrowheads). Most of these cells were located near GAD⁺ cells, suggesting that the lack of immunolabel in the GAD⁻ cells cannot be attributed to failure of antibody penetrance. Thus, the majority of the retrogradely labeled cells are likely non-GABAergic.

Quantification of bilateral projections

Across the 8 ICs that were analyzed, we observed 9,396 cells that contained retrograde tracer, of which 476 contained both retrograde tracers (Table 2). The relative size of a population with branching axonal projections is typically described by expressing the double-labeled cells as a percentage. Here, the bilaterally-projecting cells can be expressed as a percentage of the large population that projects ipsilaterally (Table 2, “bilat as % of ipsilateral projection”) or as a percentage of the (much smaller) population that projects contralaterally (Table 2, “bilat as % of contralateral projection”). The bilaterally-projecting cells constitute, on average, 12% of the ipsilaterally-projecting population and 42% of the contralaterally-projecting population. We would argue that a more relevant metric (rather than the average) is the maximum percentage seen, because the nature of retrograde transport is to underestimate collaterals (because neither tracer is likely to label *every* cell in the pathway in any given experiment). These values show that the bilaterally projecting cells

constitute up to 37% of the ipsilateral projection and up to 73% of the contralateral projection (Table 2).

Analysis of individual IC subdivisions indicates that bilaterally-projecting cells are present throughout the IC. Moreover, their prominence is relatively similar across subdivisions, averaging 10–11% of the ipsilaterally-projecting population and 39–46% of the contralaterally-projecting population (Table 3). Looking at the maximum percentages also showed similarities, with the maximum percentages for the ipsilateral projection ranging from 26–33% and the maxima for the contralateral projection ranging from 62–86%. We conclude that the bilaterally projecting cells constitute a significant portion of the ipsilateral projection and a large proportion, likely a majority, of the contralateral projection from each of the IC subdivisions analyzed.

GABAergic component of the bilateral projection

Both GAD⁺ and GAD⁻ double-retrograde cells were observed in each IC subdivision. Across cases, GAD⁺ cells averaged 20% (range: 14–28%) of the bilaterally-projecting population within the IC as a whole (Table 4). The GAD⁺ proportion was somewhat more variable in different IC subdivisions, with the lowest value in the ICd and the highest in the ICx (Table 4).

DISCUSSION

This is the first report of bilateral projections to the thalamus from individual IC cells. Bilaterally-projecting cells are present in all the major IC subdivisions. These cells constitute a small proportion of the IC cells that project to the ipsilateral thalamus and a substantial proportion of the IC cells that project to the contralateral thalamus (Figure 3). The bilaterally projecting cells may play a significant role in thalamic function, perhaps coordinating activity on the two sides of the thalamus, that is distinct from the larger IC projection to the ipsilateral MG. Moreover, GABAergic cells make up about 20% of the bilaterally-projecting cells; the majority of the remaining cells are likely to be glutamatergic. It is likely that the GABAergic and non-GABAergic projections serve distinct functions.

Technical Considerations

Technical considerations for GAD staining in these experiments have been discussed previously (Mellott et al., 2014a). In vitro electrical stimulation of the brachium of the IC, which includes the contralateral IC-MG projection and presumably the axons of any bilaterally-projecting IC-MG cell, leads to glutamatergic excitation and GABAergic inhibition in the majority of MG cells (Peruzzi et al., 1997; Saint Marie et al., 1997; Bartlett and Smith, 1999, 2002; Smith et al., 2007; Venkataraman and Bartlett, 2013). Such stimulation does not differentiate between unilaterally, contralaterally or bilaterally projecting IC-MG cells, but the results are consistent with our finding of GAD⁺ cells, and suggests further that most or all of our GAD⁻ cells are glutamatergic (discussed further in Mellott et al., 2014a). The present results indicate that excitatory projections predominate in the bilaterally projecting population, but that GABAergic inhibitory projections are also likely to play a role. The presence of the two phenotypes suggests mechanisms that include

coordinated excitation and inhibition. The recent development of optogenetic methods for selectively activating or inactivating specific circuits may prove particularly beneficial for investigating the functional roles of these relatively small pathways.

Additional technical issues relate to the use of multiple retrograde tracers for identification of collateral projections. The use of large tracer deposits to maximize the retrograde labeling of cells is critical for identification of collateral projections (Schofield et al., 2007; Schofield, 2008). In the present study, some injections spread into adjacent nuclei, but these regions are not known to receive projections from the IC (reviewed by Wenstrup, 2005) and so they do not likely affect our results. Cases in which the deposited tracer spread into the superior colliculus or nucleus of the brachium of the IC were excluded from the analysis.

The use of double retrograde tracing can be expected to underestimate the number of cells with collateral projections because neither tracer deposit is likely to label every cell in the pathway (Schofield et al., 2007; Schofield, 2008; Han et al., 2018). We tried to minimize this limitation by making large injections (often depositing tracer at multiple sites) and by using different combinations of tracers. Variation in the percentage of double-labeled cells between cases may reflect differences in the injection sites and efficacies of the different tracers. The observation of double-labeled cells with different tracer combinations supports the conclusion that collateral projections are generally present.

A related issue concerns the existence (or non-existence) of IC cells that project to the contralateral MG but not to the ipsilateral MG; i.e., a “contralateral-only” projection. All our experiments yielded IC cells that contained the contralateral tracer but not the ipsilateral tracer but given the limits of retrograde tracers, we cannot distinguish between the lack of an ipsilateral projection versus a failure to label some of the cells in that projection. The number of double-retrograde cells as a percentage of the contralaterally-projecting cells ranged from 9–73% for the IC overall (Table 2) and as high as 86% for an individual subdivision (in this instance, the ICx). Given the propensity for double-retrograde tracing to underestimate the prominence of axonal branching (Schofield et al., 2007), it is possible that all the contralaterally-projecting axons are from cells that also project ipsilaterally. In other words, there may not be any IC cells that project to the contralateral MG but not to the ipsilateral MG. Whether or not there is a “contralateral only” projection, the present data suggest that that bilaterally-projecting cells make up a substantial proportion of the contralateral projection.

Functional Implications

The present study provides a new perspective on projections from the IC to the contralateral MG by demonstrating that a portion of this pathway, probably a majority of it, is made up of cells that have bilateral projections to both MGs. The existence of bilaterally projecting cells suggests functional roles that could differ from unilateral projections. Of course, convergence of information from left and right IC could still occur in either MG, but this does not require a bilateral projection from individual axons. A branched axon could provide a uniformly timed signal to both left and right MGs. Geis and Borst (2013) showed that large GABAergic tectothalamic cells provide fast inhibition to the ipsilateral MG, and suggested that this inhibitory signal could serve as a timing mark. Such a timing mark,

provided by bilaterally-projecting GABAergic or glutamatergic tectothalamic cells, may provide a temporal reference for an acoustic event that is being processed by the MGs on both sides.

Another potential function for bilateral axons, not necessarily independent of providing a timing mark, could be to synchronize activity on the two sides of the thalamus. Synchronization could have multiple roles (see discussion in Rockland, 2018). It is not known if the collateral projections described here accomplish synchronous activation (or inhibition) of MG cells, but in the auditory brainstem and elsewhere it has been shown that differences in axon caliber and degree of myelination of collateral branches can contribute to isochronous activation at different axonal termination sites (Seidl, 2014; Seidl and Rubel, 2016). Isochronous activation of left and right MG could have a broad effect on brain state, and could control information flow within the thalamo-cortico-thalamic circuitry. Synchronization between the left and right hemispheres could potentially enhance callosal interactions at the cortical level (possibly binding the activity associated with a single sound source or helping to distinguish sounds that originate from different sources). A particularly interesting area to explore concerns the glutamatergic versus the GABAergic bilateral projections. The two types could drive thalamic cells toward different firing modes, enhancing or suppressing transmission of information by different MG cells.

We observed bilaterally projecting cells in each of the major subdivisions of the IC. These subdivisions give rise to parallel pathways that have different patterns of termination in the MG and are thought to play different roles in auditory function (see discussion in Mellott et al., 2014b). There is widespread agreement that the subdivisions serve different purposes (Calford and Aitkin, 1983; de Ribaupierre, 1997; Edeline et al., 1999; Hu, 2003; Anderson et al., 2007; Abrams et al., 2011). The ICc is part of the lemniscal pathway which is generally credited with carrying the high resolution details for auditory perception (Rouiller, 1997). The ICx is known for multisensory inputs as well as projections to the superior colliculus, for which it is associated with acoustically-driven movements (orienting or avoidance responses) (Aitkin et al., 1978, 1984; Binns et al., 1992). The ICd is least well understood, but its strong inputs from the auditory cortex (and some more recent studies) suggest it may be particularly important for auditory attention and integration of inputs from cortex and lower brainstem (Bledsoe et al., 2003; Syka and Popelár, 1984; Pérez-González et al., 2005; Malmierca et al., 2009; Lumani and Zhang, 2010). Why, then, do each of these subdivisions have cells that project bilaterally to the MG? One possibility is that the bilateral projections serve a singular purpose (e.g., the synchronization or reset functions described above), that is useful throughout the MG for any incoming stimulus. Alternatively, the bilateral projections could serve distinct purposes related to the separate functions of the IC subdivisions. Exactly what such roles could be will await further clarification of the broader functions of the subdivisions. Meanwhile, future efforts to reveal these functions can include a role for bilateral, nearly simultaneous, excitatory and inhibitory projections to the MG.

Finally, the termination pattern of individual tectothalamic axons within each MG is also important, but unknown. Contralateral tectothalamic projections from both GABAergic and glutamatergic cells terminate in each of the three main MG subdivisions (MGd, MGv, MGM; unpublished observations from the same data set described in Mellott et al., 2014b).

It would be of particular interest to identify the termination patterns within each MG for individual bilaterally-projecting axons; e.g., does a single axon terminate in multiple MG subdivisions? Are the termination patterns similar on the two sides of the thalamus?

Further insights could be gained with information about the identity of the MG targets (cell types as well as their location in particular MG subdivisions). The presence of the branching axons may facilitate selective labeling with viral vectors for optogenetic analysis (e.g., Zingg et al., 2017). At the same time, the presence of these axons will have to be acknowledged in any optogenetic labeling of the IC-MG pathway; e.g., injecting a vector into the right IC and then activating axons with an optical probe in the right MG could be expected to activate the branched IC-MG/MG axons and thus lead to transmitter release in the left MG as well as the right MG. Given the importance of temporal features in auditory processing, and the high degree to which auditory stimuli are represented bilaterally in the brain, it is perhaps not surprising that there are opportunities for bilateral interactions at *every* level of the ascending auditory pathways. Further information on the IC cells that provide the bilateral projections as well as the MG cells that receive these inputs is needed before we can understand both the unilateral and bilateral projections from the IC to the MG.

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Figure 1.

Injection sites of retrograde tracers typically involved all major subdivisions of the MG. Photomicrographs show a representative case with large deposits of red beads in the left medial geniculate body (MG) (a) and FluoroGold in the right MG (b) in a single animal (GP636). Each row shows a series of transverse sections arranged from caudal to rostral. D – dorsal; L – lateral; LG – lateral geniculate nucleus; LP – lateral posterior nucleus; M – medial; MGd – dorsal division of the MG; MGm – medial division of the MG; s – shell of the MG; MGsg – supragenulate division of the MG; MGv – ventral division of the MG; PP/PIN – peripeduncular nucleus and posterior intralaminar nucleus.

Figure 2.

Bilaterally projecting IC cells could be GAD⁻ (white arrowheads) or GAD⁺ (green arrowheads). Each row shows a single region of the IC with cells labeled by a tracer injected into one MG (left column: FluoroGold or Fast Blue) and/or a second tracer injected into the other MG (center column: red beads) and immunostained with anti-GAD (right column, green). Arrowheads identify cells labeled by both retrograde tracers, whereas arrows identify cells labeled by a single retrograde tracer. For both symbols, white indicates GAD⁻ cells and green indicates GAD⁺ cells. Scale bar = 20 μ m. Cells are from GP 636 left IC (a) or right IC (b-d) and GP 640 right IC (e).

Figure 3.

Bilaterally-projecting tectothalamic cells represent a small proportion of the IC cells that project to the ipsilateral MG and a larger proportion of IC cells that project to the contralateral MG. The largest population projects to the ipsilateral MG and does not project to the contralateral MG (red circles). A much smaller population projects to the contralateral MG and includes a substantial number of cells that project bilaterally (purple circle). There may be a third, small population that projects to the contralateral MG and not to the ipsilateral MG (blue circle). Numbers show the maximum values of bilaterally projecting cells as a percentage of cells that project to one or the other MG. Each pathway - ipsilateral, contralateral and bilateral - arises from both GABAergic and non-GABAergic (i.e., glutamatergic) neurons (not shown).

Table 1.

A list of the tracers injected in the left (L) and right (R) MG in each case along with injection volumes. FB - Fast Blue (5% aqueous); FG - FluoroGold (4% aqueous); RB - red RetroBeads (undiluted). Total Volume: total volume of tracer injected.

Case	Side	Tracer	# of deposit sites	Total Volume (µl)
GP632	L	RB	1	0.2
GP632	R	FG	1	0.15
GP633	L	RB	2	0.4
GP633	R	FG	1	0.08
GP636	L	RB	2	0.4
GP636	R	FG	1	0.05
GP638	L	RB	2	0.5
GP638	R	FB	1	0.08
GP640	L	RB	4	0.4
GP640	R	FB	1	0.08

Table 2.

Number and percentages of IC cells that were single- or double-labeled by tracers injected into the left and right MGs. **# to Ipsilateral MG** - number of cells that contained only the tracer that was injected into the ipsilateral MG; **# to Contralateral MG** - number of cells that contained only the tracer that was injected into the contralateral MG; **# with bilateral projections** - number of cells that contained both retrograde tracers; **Bilat as % of ipsilateral projection** = column 5/(column 3 + column 5)+100; **Bilat as % of contralateral projection** = column 6/(column 3 + column 6)+100. Values are given for 8 ICs separately, with total number of cells as well as the average/case and the maximum (Max) value across cases.

Case	Side	# to Ipsilateral MG	# to Contralateral MG	# with bilateral projections	Bilat as % of ipsilateral projection	Bilat as % of contralateral projection
GP633	L IC	2456 (RB)	63 (FG)	84	3%	57%
	R IC	473 (FG)	289 (RB)	30	6%	9%
GP636	L IC	2038 (RB)	107 (FG)	94	5%	47%
	R IC	1123 (FG)	237 (RB)	47	4%	17%
GP638	L IC	1899 (RB)	19 (FB)	18	1%	49%
	R IC	218 (FB)	260 (RB)	53	24%	17%
GP640	L IC	371 (RB)	22 (FB)	59	16%	73%
	R IC	246 (FB)	51 (RB)	91	37%	64%
	Total	8824	1048	476		
	Average	1103	131	60	12%	42%
	Max	2456	289	94	37%	73%

Table 3.

Numbers and percentages of cells projecting ipsilaterally, contralaterally or bilaterally to the medial geniculate (MG) from subdivisions of the inferior colliculus. The number of bilaterally-projecting cells expressed as a proportion of ipsilateral or contralateral projections is similar in the major IC subdivisions, including the ICc (A), the ICd (B) and the ICx (C). Conventions as in Table 2.

A. ICc n = 5,029 cells						
Case	Side	# to Ipsilateral MG	# to Contralateral MG	# with bilateral projections	Bilat as % of ipsilateral projection	Bilat as % of contralateral projection
GP633	L	1124	16	37	3%	70%
	R	155	158	11	7%	7%
GP636	L	1331	42	49	4%	54%
	R	579	122	27	4%	18%
GP638	L	809	5	6	1%	55%
	R	103	105	17	14%	14%
GP640	L	136	7	32	19%	82%
	R	103	16	39	27%	71%
	Total	4340	471	218		
	Average	543	59	27	10%	46%
	Max	1331	158	49	27%	82%
B. ICd n = 2,419 cells						
Case	Side	# to Ipsilateral MG	# to Contralateral MG	# with bilateral projections	Bilat as % of ipsilateral projection	Bilat as % of contralateral projection
GP633	L	651	20	25	4%	56%
	R	121	74	10	8%	12%
GP636	L	360	15	24	6%	62%
	R	73	73	10	12%	12%
GP638	L	440	10	9	2%	47%
	R	71	74	25	26%	25%
GP640	L	176	13	15	8%	54%
	R	81	28	21	21%	43%
	Total	1973	307	139		
	Average	247	38	17	11%	39%
	Max	651	74	25	26%	62%
C. ICx n = 2,900 cells						
Case	Side	# to Ipsilateral MG	# to Contralateral MG	# with bilateral projections	Bilat as % of ipsilateral projection	Bilat as % of contralateral projection
GP633	L	681	27	22	3%	45%
	R	197	57	9	4%	14%
GP636	L	347	50	21	6%	30%
	R	471	42	10	2%	19%
GP638	L	650	4	3	0%	43%
	R	44	81	11	20%	12%

GP640	L	59	2	12	17%	86%
	R	62	7	31	33%	82%
	Total	2511	270	119		
	Average	314	34	15	11%	41%
	Max	681	81	31	33%	86%

Table 4.

Numbers and percentages of bilaterally-projecting cells that are immunoreactive for GAD (GAD⁺). **bilat/GAD-neg**: bilaterally-projecting cells (cells that contained both retrograde tracers) that were GAD-immunonegative. **bilat/GAD⁺**: bilaterally-projecting cells that contained both retrograde tracers and were also GAD-immunopositive. # cells –number of cells of indicated type totaled across the 3 quantified ICs; **average** ± sd: average ± standard deviation per IC analyzed. IC: totals for IC overall; ICc, ICx, ICd – data for central, external and dorsal divisions of the IC, respectively.

		bilat/GAD-neg	bilat/GAD ⁺	% of bilat that are GAD ⁺
IC	# cells	385	91	
	average ± sd	48 ± 24	11 ± 6	20% ± 6%
ICc	# cells	177	41	
	average	22 ± 12	5 ± 3	19% ± 6%
ICx	# cells	104	35	
	average	13 ± 6	4 ± 2	26% ± 9%
ICd	# cells	104	15	
	average	13 ± 8	2 ± 2	12% ± 10%