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Circuit mechanisms underlying the segregation and integration of parallel processing streams in the inferior colliculus

Abbreviated title: Modularity and local circuit mechanisms of integration

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ABSTRACT

The lateral cortex of the inferior colliculus (LCIC) forms a nexus between diverse multisensory, motor, and neuromodulatory streams. Like other integration hubs, it contains repeated neurochemical motifs with distinct inputs: GABA-rich modules are innervated by somatosensory structures, while auditory inputs to the LCIC target the surrounding extramodular matrix. To investigate potential mechanisms of convergence between these input streams, we used laser photostimulation circuit mapping to interrogate local LCIC circuits in adult mice of both sexes and found that input patterns are highly dependent upon cell type (GABAergic/ non-GABAergic) and location (module/matrix). At the circuit level, these inputs yield a directional flow of local information primarily from the matrix to the modules. Further, the two compartments were found to project to distinct targets in the midbrain and thalamus. These data show that while connectional modularity in the LCIC gives rise to segregated input-output channels, local circuits provide the architecture for integration between these two streams.

SIGNIFICANCE STATEMENT

Modularity is a widespread motif across the brain involving the segregation of structures into discrete sub-regions based on dichotomies in neurochemical expression or connectivity. The inferior colliculus is one such modular structure, containing auditory-recipient matrix regions and GABA-rich modules that are innervated by somatosensory inputs. While modularity suggests *segregation* of processing streams, here we show that

local circuits in the inferior colliculus connect the module and matrix regions, providing an avenue for *integration* of information across compartments.

INTRODUCTION

A number of brain regions can be parcellated at the subnuclear level based on differences in neurochemistry, cytoarchitecture, or connectivity. The most heavily studied of these "modular" structures include the somatosensory barrel cortex and the patch/matrix organization of the striatum (Gerfen, 1992; Petersen, 2007). The inferior colliculus (IC), a midbrain structure that is centrally positioned within the auditory system and thought to serve as an integration hub for acoustic information, also exhibits neurochemical and structural modularity (Casseday, Fremouw, & Covey, 2002). Specifically, the lateral cortex of the IC (LCIC) can be subdivided into modular regions ("modules") characterized by dense staining for glutamic acid decarboxylase-67, parvalbumin, cytochrome oxidase, acetylcholinesterase, and NADPH-diaphorase, and extramodular regions (termed "matrix") that are characterized by heavy calretinin labeling (Chernock, Larue, & Winer, 2004; Dillingham, Gay, Behrooz, & Gabriele, 2017; Lesicko, Hristova, Maigler, & Llano, 2016; Stebbings, Lesicko, & Llano, 2014).

These neurochemical divisions also correlate with differences in connectivity: somatosensory inputs target LCIC modules, while matrix regions receive auditory inputs from the auditory cortex (AC) and central nucleus of the IC (CNIC) (Lesicko et al., 2016). An unresolved paradox exists in this arrangement of multimodal inputs: the apparent anatomical segregation in somatosensory and auditory inputs belies a long history of physiological studies demonstrating multisensory convergence in the LCIC. Early recordings from the cat LCIC discovered single units that respond to both auditory and somatosensory stimuli (L. M. Aitkin, Dickhaus, Schult, & Zimmermann, 1978; L. M. Aitkin, Kenyon, & Philpott, 1981). Other studies that have examined the effect of spinal

trigeminal nucleus or dorsal column stimulation on responses to sound in the LCIC have found that the majority of units respond bimodally (L. M. Aitkin et al., 1978; Jain & Shore, 2006).

Given the evidence for the role of the LCIC in multisensory integration, it is peculiar that the somatosensory and auditory inputs to the LCIC are spatially segregated. This lack of convergence among multisensory inputs to the LCIC suggests that a secondary mechanism of integration must be present: either information from the two senses is integrated in a lower structure that projects to the LCIC, or there is communication between local circuits in module and matrix regions of the LCIC. In the present study, we specifically investigate the latter possibility through functional characterization of the local inputs to LCIC neurons. To further elucidate the extent and potential role of connectional modularity in the LCIC, we also use retrograde tract-tracing to determine whether the compartments of the LCIC send information to distinct targets.

MATERIALS AND METHODS

Animals

Juvenile (postnatal day 11-21) and adult (postnatal day 30-90) GAD67-GFP knock-in mice of both sexes were used for laser photostimulation and anatomy studies, respectively. Knock-in mice were initially obtained from the University of Connecticut and were bred with wild-type Swiss Webster mice to generate hemizygous progeny in which enhanced GFP is under control of the endogenous GAD67 promoter (Ono, Yanagawa, & Koyano, 2005; Tamamaki et al., 2003). Previous work has shown that immunostaining for GABA or GAD colocalizes with GFP in these mice (Gay, Brett, Stinson, & Gabriele, 2018; Tamamaki et al., 2003). Animals were screened for phenotypic evidence of transgene expression between postnatal day 2 and 7. The screening procedure involved illuminating the dorsal surface of the scalp with blue light (the excitation range for GFP) and checking for evidence of green fluorescence in the cortex, midbrain, and cerebellum. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois. Animals were housed in care facilities approved by the American Association for Assessment and Accreditation of Laboratory Animal Care. Every attempt was made to minimize the number of animals used and to reduce suffering at all stages of the study.

Slice preparation

Mice were anesthetized with a mixture of ketamine hydrochloride (100 mg/kg) and xylazine (3 mg/kg) and perfused transcardially with a cold slicing solution containing: 206 mM sucrose, 10 mM $MgCl_2$, 11 mM glucose, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$,

0.5 mM $CaCl_2$, 2.5 mM KCl, and 1 mM kynurenic acid (pH 7.4). The brain was removed and 300 μm-thick coronal tissue slices throughout the IC were obtained using a vibratome. The slices were transferred to an incubation solution (126 mM NaCl, 3 mM $MgCl_2$, 10 mM glucose, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 1 mM $CaCl_2$, 2.5 mM KCl; pH = 7.4) and warmed to 32 degrees C for 1 hour prior to recording.

Electrophysiology

Tissue slices containing the LCIC were transferred to a recording chamber and submerged in an oxygenated artificial cerebrospinal fluid (ACSF) solution containing: 126 mM NaCl, 2 mM $MgCl_2$, 10 mM glucose, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 2 mM CaCl, 2.5 KCl; pH 7.4. Modular and matrix regions of the LCIC were identified through differential expression of GAD67-GFP under blue illumination. Cells were categorized into four groups based on whether they were found in module or matrix regions of the LCIC and whether they were GAD67+ or GAD67-. After identification of cell type, neurons were recorded in either a single or dual whole-cell configuration using Cs-gluconate (117 mM Cs-gluconate, 13 mM CsCl, 1 mM $MgCl_2$, 0.07 mM $CaCl_2$, 0.1 mM EGTA, 10 mM HEPES, 2 mM Na_2-ATP, 0.4 mM Na-GTP) filled pipettes with tip resistances of 4-7 MΩ. Both Alexa Fluor 594 hydrazide (ThermoFisher, #A10438; 7 μM) and biocytin (Sigma-Aldrich, #B4261; 4 mM) were added to the internal solution to aid in morphological reconstruction and post-hoc confirmation of cell location. Data were acquired using a Multiclamp 700B amplifier and Digidata 1440A digitizer at a sampling rate of 20 kHz in pClamp software (Molecular Devices). Cells were held in voltage

clamp at -60 mV and +10 mV to isolate excitatory and inhibitory currents, respectively, and traces were filtered with a 1 kHz Bessel filter to remove noise.

Laser photostimulation

MNI-caged-L-glutamate (Tocris, #1490) was added to recirculating ACSF at a concentration of 150 μM and focally photolysed by a pulsed 355 nm laser (1 ms pulses). The power of the laser beam at slice level was measured and maintained at 3 mW for all experiments. The laser beam was directed into the side port of an Olympus microscope using UV-enhanced aluminum mirrors and a pair of mirror galvanometers and then focused onto the brain slice using a 10X objective. Angles of the galvanometers were computer-controlled using PrairieView software (Prairie Technologies). The Q-switch of the laser and a shutter controlled the timing of the laser pulse for stimulation. The stimulation pattern for input mapping consisted of 200 positions arranged in a 10 x 20 array, with 80 μm between adjacent rows and columns (Fig. 1C). A non-neighbor stimulation paradigm in which sequentially stimulated sites are spatially dispersed was used to prevent local accumulation of uncaged glutamate and desensitization of receptors following repeated stimulation (Shepherd, Pologruto, & Svoboda, 2003). Excitatory and inhibitory maps were repeated 2-3 times each and averaged to ensure consistent results and reduce the effect of spontaneous inputs. To distinguish between direct activation of the recorded cell and synaptic activation of presynaptic partners, the excitatory mapping was repeated in low calcium ACSF (126 mM NaCl, 4 mM $MgCl_2$, 10 mM glucose, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 0.2 mM CaCl, 2.5 KCl; pH = 7.4) to block synaptic activity, and this “direct” input map was subtracted from the original excitatory map to

generate a map containing only excitatory synaptic inputs (Llano & Sherman, 2009; Slater, Yudintsev, Lee, & Llano, 2019) (Fig. 1D).

Excitation profiles

Excitation mapping was performed to determine how photostimulation at specific experimental parameters (3 mW, 1 ms laser pulses) affects the spike output of each of the four cell types of interest. Cells were recorded from in cell-attached mode and a 10X10 grid with 20 μm between adjacent stimulation sites was centered over the soma. For each cell, the mapping was repeated four times and the total number of spikes in the average map was compared to determine if differences in excitability exist between cell types (Fig. S1B,C). The average number of spikes at various distances from the soma was also computed to determine the approximate width of activation for a single laser pulse (Fig. S1D). The spike output for all cells plateaued at distances further than 80 μm, with an average spike output of less than 1 spike per stimulation site (Fig. S1D). This spacing was therefore used for all mapping experiments throughout the rest of the study to maximize the likelihood that each laser stimulus samples distinct populations of cells.

Tracer injection

Mice were anesthetized intraperitoneally with a mixture of ketamine hydrochloride (100 mg/kg) and xylazine (3 mg/kg) and a small hole was drilled in the skull above the structure of interest. A glass micropipette, tip diameter 20-30 μm, was filled with a 2% solution of FG dissolved in acetate buffer (pH 3.3) and lowered into the brain. FG was injected iontophoretically using 5 μA positive current pulses (50% duty cycle) for 10-20

minutes. A 15 μA negative holding current was applied during placement and removal of the pipette to prevent unwanted leakage of the tracer.

Tissue processing and microscopy

After recording, slices containing biocytin-filled cells were fixed overnight in a solution of 4% paraformaldehyde (PFA). Slices were rinsed three times in phosphate-buffered saline (PBS) and transferred to a solution containing 0.3% Triton X-100 and Alexa Fluor 568-conjugated streptavidin (#S-11226, ThermoFisher). To visualize cell morphology, slices were wet-mounted on coverslips and imaged using a Leica SP8 laser scanning confocal microscope and LAS X control software. Mosaic Z-stacks were taken at 20X throughout the extent of the LCIC, collapsed into 2D maximum intensity projections, and tiled into a single image.

Following a 3-7 day survival period, FG-injected animals were anesthetized with a mixture of ketamine hydrochloride (100 mg/kg) and xylazine (3 mg/kg) and perfused transcardially with 4% PFA in PBS. The brain was removed and post-fixed overnight in the PFA solution. After being cryoprotected in an ascending series of sucrose solutions, the brain was embedded and cut into 40 μm thick sections on a freezing sledge microtome. Tissue sections were imaged with a Leica SP8 laser scanning confocal microscope and LAS X control software. FG and GFP were visualized separately using 405 nm and 488 nm excitation laser lines, respectively. Using these settings, images of each fluorophore were captured for each IC tissue section containing retrograde label, and 20X mosaic Z-stacks were taken throughout the entire depth and x-y plane of the IC. The stacks were collapsed into 2D maximum intensity projections and tiled into a single

image using LAS X software. Composite images of the FG and GFP channels were overlaid and Photoshop was used to adjust the color balance and to draw masks around the edge of the tissue to cover the embedding medium. Reconstructions and cell counts were performed using Neurolucida software. The Allen Reference Atlas was used to determine the location of injection sites (Goldowitz, 2010).

Analysis

Custom-written MATLAB scripts were used to quantify laser-driven responses. For a given cell, a trapezoidal integration function was applied to each trace to calculate the inhibitory and excitatory charge in the first 100 ms after laser onset. These values were then converted into heat maps. Images of GAD67-GFP fluorescence were parcellated into modules (high-pixel intensity regions in layer 2 of the LCIC) and matrix regions first using hand-drawn ROIs. These ROIs were then independently confirmed using clustering algorithms in the MATLAB Image Segmenter App. The location of the recorded cell was determined from images of Alexa Fluor 594 hydrazide fluorescence overlaid with the GAD67-GFP images. Finally, heat maps were overlaid onto the fluorescence images, and each stimulation site was categorized as originating from either the module or matrix regions. The border between the LC and the CNIC was estimated by drawing a curved ROI extending orthogonally from the border of layer 2 (delineated as the medial-most edge of the modules) at a distance 1.5X the width of layer 2. This ratio was determined from previous studies that have used histochemical approaches to determine the borders between LC and CNIC (Loftus et al. 2008). All CNIC and off-tissue stimulation sites were removed from the analysis. The charge from responses

originating from each compartment was summed to yield the total inhibitory and excitatory synaptic charge arising from the module and matrix areas. To quantify the balance of input to individual cells from the module and matrix regions, a modularity index was computed:

$$(M\ index = \frac{total\ charge\ from\ region\ containing\ cell\ body - total\ charge\ from\ opposite\ region}{total\ charge}).$$

Cells with a positive index receive more input from the region containing their soma (e.g., a cell located within a module receives more input from the modules than the matrix), cells with a negative index receive more input from the opposite region, and cells with an index near zero receive mixed input. Similarly, the balance of excitatory and inhibitory synaptic input for each cell was calculated using an E:I index:

$$(E{:}I\ index = \frac{total\ excitatory\ charge - total\ inhibitory\ charge}{total\ charge}).$$

Two separate methods, a cross-correlation analysis and a correlation-coefficient analysis, were used to quantify the similarity between maps from simultaneously recorded cells. The cross-correlation analysis was derived from methods previously used to analyze paired recordings (Yoshimura & Callaway, 2005; Zarrinpar & Callaway, 2006), and involved taking a site-by-site cross-correlation of the photostimulation responses for each pair and generating a map of these values (Fig. 5B,C right). The average cross-correlation value of the entire map was computed to compare between different pair types (Fig. 5D). The correlation-coefficient analysis was used to generate correlation coefficients (Fisher z-transformed) between total synaptic input at

corresponding sites for each pair of maps (Fig. 5E). The cross-correlation analysis method generates the largest values for pairs of cells having similar time-courses of synaptic inputs, while the correlation-coefficient analysis will yield largest values for cells with the largest spatial overlap of inputs, yielding complementary information.

Statistical procedures

Summary statistics including the mean, median, standard deviation (SD), standard error of the mean (SEM) and 95% confidence intervals (CI) were computed for all measures. In all cases, statistical outliers were included in subsequent analysis. Shapiro-Wilk tests were used to determine if the data were normally distributed. In cases in which the assumption of normality was violated, Kruskal-Wallis and Dunn's post-hoc testing (with a Holm adjustment for multiple comparisons) were used to compare >2 groups, and Wilcoxon rank-sum tests were used for two group comparisons. Effect sizes for Kruskal-Wallis tests were computed as the eta-squared (η^2) based on the H-statistic. For Wilcoxon rank-sum tests, the effect size r was calculated as the Z statistic divided by the square root of the sample size.

RESULTS

Experimental design

To determine the degree and directionality of communication between cells in modular and matrix regions of the LCIC, we performed whole-cell voltage-clamp recordings and stimulated pre-synaptic partners throughout the LCIC using laser photostimulation of caged glutamate (Fig. 1A,B). Module and matrix regions were visually distinguished

under blue light illumination by their differential GAD67-GFP labeling in tissue slices from a transgenic mouse line. Modules are defined by their high densities of GAD67+ cells and terminals and appear as regions of high intensity fluorescence embedded in the surrounding matrix (Fig. 1A). Both GAD67+ (presumed inhibitory) and GAD67- (presumed excitatory) cells in both regions were recorded from to measure inhibitory and excitatory inputs, respectively (Fig. 1A). For input mapping, a grid of stimulation sites was centered over the LCIC and potential presynaptic partners were stimulated in a non-neighbor fashion (Fig. 1C). Responses were plotted spatially according to the location from which they were generated and converted into heat maps by computing the area under the curve of each response (Fig. 1C). Cells were voltage-clamped at +10 and -60 mV to measure inhibitory and excitatory currents, respectively (Fig. 1D). Excitation profiles were generated for each of the four cell types by recording spike output in cell-attached mode at various distances from the cell body (Fig. 1E). No significant differences were found in spike output between cell types, indicating that the specified laser parameters activate each population similarly (Fig. 1F,G). Two or three maps were averaged for each condition for each cell to account for variability between maps and spontaneous currents (Fig. 2). Excitatory mapping was repeated in low calcium ACSF, and this "direct" input map was subtracted from the original excitatory map to isolate excitatory synaptic inputs (Fig. 2B-D).

Input patterns for cells in matrix regions

Inhibitory input maps were generated for a total of 26 GAD- and 30 GAD+ cells in matrix regions and excitatory input maps were also obtained for 10 and 13 of these

same cells, respectively. Heat maps were overlaid with an image of the GAD67-GFP labeling and a region of interest was drawn around the border of any modules present in the tissue (Figure 3A, left). For matrix GAD- cells, inhibitory inputs predominately arose from stimulation sites in the matrix (Fig. 3A middle left), Most of the excitatory input to these cells arose from direct activation of the recorded cell (Fig. 3A middle right), and only sparse synaptic excitation was observed (Fig. 3A left). Given the relative paucity of excitatory input at the population level compared to inhibitory input (Fig. 3D; p=0.017, Wilcoxon rank-sum test), additional example inhibitory input maps are shown without corresponding excitatory maps (Fig. 3B,C), demonstrating that the pattern of predominately matrix-derived input is conserved. This pattern was striking at the population level for both excitatory and inhibitory inputs, with a 10-fold greater input charge arising from the matrix compared to the modules (Fig. 3E; inhibitory: p=1.7e-06, r=0.62; excitatory: p = 0.00032, r=0.74; Wilcoxon rank-sum tests).

A similar pattern of inhibitory input was seen for GAD+ matrix cells, with virtually all of the inhibitory input arising from the matrix regions of the LCIC (Fig. 3F-H, 3J left; p=3.5e-08, r=0.71; Wilcoxon rank-sum test). Unlike GAD- matrix cells, GAD+ matrix cells received balanced levels of excitatory and inhibitory input overall (Fig. 3F right, 3I; p=0.69, Wilcoxon rank-sum test). The spatial pattern of excitatory inputs, however, is consistent with those of GAD- matrix cells, in that an overwhelming majority of input arises from the matrix regions of the LCIC (Fig. 3F right, 3J right; p=0.0023, r=0.58, Wilcoxon rank-sum test).

Input patterns for cells in modules

Inhibitory input maps were generated for 33 GAD- and 34 GAD+ cells in modules of the LCIC, and excitatory input maps were also obtained for 15 of these same cells for both cell types. Some GAD- cells in modules received predominately clustered inputs from within a module (Fig. 4A), while others received mixed input from both module and matrix regions (Fig. 4B,C). Overall, GAD- cells in modules received more inhibitory than excitatory input (Fig. 4D; p=0.00088, Wilcoxon rank-sum test). Spatial patterns of inhibitory and excitatory input were highly heterogeneous from cell to cell, with some cells receiving more input from modules, some receiving a balance of input from both domains, and some receiving more input from matrix regions (Fig. 4E). At the population level, this heterogeneity culminated in an overall similar level of input from both regions (Fig. 4E; p=0.10, r=0.21 for inhibitory inputs and p=0.37, r=0.17 for excitatory inputs, Wilcoxon rank-sum tests). Input values from the matrix region remained consistent across cells, while the degree of input from the modules exhibited variability (Fig. 4E).

GAD+ cells in modules showed a unique spatial pattern of input: most of the input to these cells arose from sites in the matrix, with very little input coming from the modules, where their cell bodies are located (Fig. 4F-H). Similar to GAD- cells in modules, GAD+ cells in modules received predominately inhibitory input compared to excitatory input (Fig. 4I, p=0.020, Wilcoxon rank-sum test). The spatial pattern of both excitatory and inhibitory inputs for GAD+ cells in modules resembled those of matrix cells, with most of the input arising from the matrix, despite the difference in the compartmental location of the cell body (Fig. 4J; p=8.5e-06, r=0.51 for inhibitory inputs and p=0.0090, r=0.48 for excitatory inputs, Wilcoxon rank-sum tests).

340

341 *Pharmacological controls*

342 Application of bath-applied GABAzine blocked inhibitory responses, suggesting
343 that outward currents are mediated by $GABA_A$ receptors (Fig. 5A). We also repeated
344 excitatory input mapping in the presence of GABAzine to 1) determine if inhibition in the
345 LCIC was "masking" excitatory responses and 2) to confirm that our stimulation
346 parameters elicit monosynaptic responses. In either instance, an increase in the overall
347 map area would be expected in the presence of GABAzine. We observed that excitatory
348 input maps remained largely unaltered (Fig. 5B,C). To confirm the validity of the low
349 calcium ACSF technique for segregating direct inputs from excitatory synaptic inputs,
350 input mapping was repeated in TTX for a subset of cells (Fig. 5D,E). Similar input maps
351 were generated using both techniques (Fig. 5D,E), as we have previously validated in
352 other preparations (Slater et al., 2019). Scale = 400 µm.

353

354 *Balance of inhibitory and excitatory input from different regions of the LCIC*

355 For each cell for which both inhibitory and excitatory input data were collected,
356 an E:I index was computed to assess differences in the overall balance of excitation and
357 inhibition at the population level. Each of the four cell types had a negative median E:I
358 index, indicating that they are dominated by inhibition (Fig. 6A). No differences were
359 found in the E:I index across groups at the population level (Fig. 6A; $p=0.50$, Kruskal-
360 Wallis rank-sum test).

361 To further assess differences in the balance of input from the module and matrix
362 regions of the LCIC, a modularity index was calculated for each cell (the normalized

difference in input from the compartment in which the cell body is located and the opposite region). Both cell types in the matrix exhibited high positive modularity indices for both inhibition and excitation, indicating that most of their input arises from matrix regions of the LCIC (Fig. 6B,C). GAD- cells in modules showed evidence of mixed input, with median excitatory and inhibitory modularity indices close to zero (Fig. 6B,C). Module GAD+ cells had negative modularity indices (more pronounced for inhibition than excitation), indicating that most of their input arises from the matrix (Fig. 6B,C). The modularity indices were significantly different between cell types, with the exception of the comparison between GAD- matrix and GAD+ matrix cells for inhibitory input and between GAD- module and GAD+ module cells for excitatory input (Fig. 6B,C; inhibitory: $p=2.2e-16$, $\eta^2=0.78$; excitatory: $p= 1.7e-08$, $\eta^2=0.30$; Kruskal-Wallis rank-sum tests). These findings indicate that there are distinct spatial patterns of input among different cell types in the LCIC.

The local LCIC circuit

The spatial patterns of input derived from the modularity indices for each cell type were used to construct a diagram of the local LCIC circuit (Fig. 6D). Both GAD- (putative excitatory) and GAD+ (putative inhibitory) matrix cells receive excitatory and inhibitory input primarily from the matrix regions of the LCIC (i.e. GAD- and GAD+ matrix cells predominately receive information from other GAD- and GAD+ matrix cells) (Fig. 6D, area shaded in gray). GAD+ cells in modules also receive most of their input from the matrix region (i.e. from GAD- and GAD+ matrix cells), while GAD- cells in modules receive a mixture of input from both domains (i.e. from GAD- and GAD+ cells in both matrix and modules) (Fig. 6D, area shaded in light green). Every cell type

receives substantially more inhibitory than excitatory input (thick vs. thin arrows in Fig. 6D; see Fig. 6A for comparisons between inhibitory and excitatory charge). Taken together, these input patterns give rise to a predominately unidirectional flow of information from the matrix to the modular regions of the LCIC (Fig. 6D, gray arrow).

To determine if individual cells' dendritic fields cross module borders, a subset of cells were filled with biocytin during recording to recover their neuronal morphology and local projection patterns. Both GAD- (Fig. 6E left, white arrow) and GAD+ (Fig. 6E, left green arrow) matrix cells had large and heavily branched dendritic arbors and axons that projected throughout the LCIC, sometimes innervating nearby modules (Fig. 6E right, red arrows). These anatomical data support results from input mapping that show that matrix cells send information into modular regions of the LCIC. GAD+ cells in modules (Fig. 6F left, green arrow) also exhibited heavily branched dendritic arbors that frequently sprawled beyond the borders of their home modules (Fig. 6F right, red arrow). This non-compartmentalized dendritic structure could serve as an anatomical substrate for the matrix-dominated input patterns revealed from photostimulation experiments. Unlike the other LCIC cell types, GAD- matrix cells (Fig. 6F left, white arrow) typically had small dendritic trees that remained within the borders of their home modules (Fig. 6F right, white arrow).

Shared input to neurons in putative LCIC cell classes

Since clear differences were observed in the inhibitory and excitatory input patterns for cells that differed in terms of location (module or matrix) and type (GAD67+ or GAD67), we reasoned that each of these categories of cells comprises a distinct cell class. As such,

we hypothesized that each cell class would share common local input, as measured during dual recordings (Fig. 7A). Therefore, dual recordings were made from 80 cells (40 pairs) that were either matched in terms of location and type (Fig. 7B), differed in only one parameter, or were unmatched in both parameters (Fig. 7C). The degree of input similarity was computed using two methods. Cross-correlations between the detailed time-courses at corresponding stimulation sites were computed, as described in (Yoshimura & Callaway, 2005; Zarrinpar & Callaway, 2006). This method measures the similarity in the time-courses of synaptic inputs to two cells. In addition, the Pearson's correlation coefficient (Fisher z-transformed) using total inward current at corresponding sites for each pair of maps was also computed. This method emphasizes spatial similarity of inputs. These two metrics were compared across each group of pairs (Fig. 7D,E). Using either method, we observed that pairs that were more similar (in terms of cell type and location) had large values for both metrics, while cells that differed in one or both parameters had smaller values for both metrics (Fig. 7D,E). Cross-correlation values were significantly different between different types of pairs ($p=0.015$, $\eta^2=0.21$; Kruskal-Wallis rank-sum test); specifically, pairs in which both the location and cell type were matched had significantly higher cross-correlation values than pairs in which both of these parameters differed ($p=0.0043$, post-hoc Dunn's test with Holm correction). A similar pattern was seen for the correlation-coefficient analysis ($p=0.021$, $\eta^2=0.19$, Kruskal-Wallis test; Same vs. Diff: $p=0.0077$, post-hoc Dunn's test with Holm correction). To determine if the distance between the cells in a pair could account for these differences, we computed the regression coefficient between distance and cross-correlation values and found no relationship (Fig. 7D; $R^2=-0.027$, $p=0.94$). Similarly, no relationship was found

using distance as a predictor for correlation-coefficient values (Fig. 7E; R^2=0.059, p=0.073) These data suggest that residence inside or outside of a module, as well as neurochemical identity, define distinct cell classes in the LCIC and strongly determine local input patterns.

Projections to other regions of the IC arise from the matrix

Given the marked segregation of auditory and somatosensory inputs to the matrix and modular regions, respectively (Lesicko et al., 2016), and the modularity of LCIC microcircuit organization shown above, we hypothesized that outputs from the LCIC would also be organized along the basis of whether cells resided in a module/matrix or expressed GAD67. To test this idea, we examined four outputs of the LCIC: ipsilateral IC, contralateral IC (contraIC), superior colliculus (SC), and the medial division of the medial geniculate body (mMGB) and determined if 1) they were located in the module or matrix region and 2) if they expressed GAD67 (Fig. 8A).

The subdivisions of the IC are heavily interconnected, and the LCIC projects to both the ipsilateral and contralateral DCIC and CNIC (Coleman & Clerici, 1987). To determine if LCIC cells that project within the ipsilateral colliculus are also predominately distributed in either module or matrix regions, Fluorogold (FG) was injected into LCIC targets within the IC. The injection site was centered in the caudal half of the IC along the border between the DCIC and the CNIC (Fig. 8A left). UV illumination revealed several retrogradely labeled cells in the LCIC (Fig. 8B middle left). Overlay images showed that cells that project to the ipsilateral IC are found almost exclusively (97%) in matrix regions of the LCIC (Fig. 8B,C; Fig. 9E). However, a substantial percentage (33%) of backlabeled cells were found to be GFP-positive, indicating that this pathway is partially GABAergic (Fig. 9E).

To examine the projection pattern to the contraIC, an injection of FG was placed in the ventro-medial and mid-rostro-caudal portion of this structure (Fig. 8A middle-left). Backlabeled cells were found throughout all subdivisions of the IC (Fig. 8D) and were

most concentrated along the same rostro-caudal plane as the injection site (Fig. 8E right). Overlay images revealed that the pattern of outputs to the contraIC mimics the pattern to the ipsilateral IC; the vast majority (98%) of backlabeled cells are found in matrix regions of the LCIC (Fig. 8D,E; Fig. 9E). However, a smaller proportion of backlabeled cells were found to be GFP-positive (4%), indicating that this pathway is predominately non-GABAergic (Fig. 9E).

Inputs to the SC also come from the matrix zone

To examine the distribution pattern of outputs to the SC, FG was injected at a mid-rostro-caudal level of the SC, in both deep and superficial layers (Fig. 8A middle-right). Overlay images of the FG and GAD67-GFP labeling revealed that a) cells projecting to the SC were almost exclusively found in the matrix regions of the LCIC and b) the vast majority of retrogradely-labeled cells were non-GABAergic (Fig. 9A,B). These observations were confirmed with quantification; cell counts revealed that 96% of the retrogradely labeled cells in the LCIC were found in matrix regions, and that 96% were also non-GABAergic (Fig. 9E). Though cells projecting to the SC were found throughout the rostro-caudal extent of the LCIC, they were heavily concentrated in the rostral-most regions of the LCIC (Fig. 9B).

Cells projecting to the mMGB are found in modules

All subdivisions of the IC project heavily to the MGB, and the main thalamic target of the LCIC is the mMGB (Calford & Aitkin, 1983). A small deposit of FG was made in this region to backlabel colliculo-thalamic cells and determine whether their

distribution in the LCIC is patterned (Fig. 8A right). As shown, the injection site appeared largely restricted to the mMGB, with potential spillover into the surrounding paralaminar nuclei (i.e. the suprageniculate nucleus and the posterior intralaminar nucleus) (Goldowitz, 2010). In contrast to cells projecting to the SC and IC, cells projecting to the mMGB were found to form clusters that were largely found within the modules (Fig. 9C,D). Backlabeled cells were found throughout the rostro-caudal extent of the LCIC (Fig. 9D). Cell counts revealed that 86% of these cells were found in modules of the LCIC, while the remaining cells were found in the matrix. Interestingly, of the 404 FG-labeled cells that were identified in the LCIC, none were found to be double-labeled with GFP, indicating that this pathway is strictly non-GABAergic (Fig. 9E).

493 DISCUSSION

494 In the present study, we used a combination of single and paired whole-cell
495 voltage-clamp recordings, laser photostimulation, and tract-tracing to measure functional
496 patterns of integration and segregation in a brain structure containing strong anatomical
497 modularity, the LCIC.

498 Our photostimulation studies suggest that local input patterns for LCIC cells are
499 strongly dependent upon their neurochemical identity (GAD+ or GAD-) and location
500 (module or matrix), with distinct yet highly consistent input phenotypes found for each
501 cell type. Both GAD- and GAD+ matrix cells receive input mainly from the matrix, but
502 relay information to cells in both the module and matrix compartments of the LCIC.
503 Though cells in modules receive input from both domains of the LCIC, they
504 predominately relay it to other cells within the modules (Fig. 6D, Fig. 10). This circuitry
505 gives rise to a directional flow of information predominately from the matrix to module
506 regions of the LCIC. One advantage of such an arrangement could be to allow for
507 independent modulation of auditory and somatosensory inputs while retaining local
508 circuit mechanisms that allow for multimodal integration (see below). These data suggest
509 that anatomical modules in the LCIC serve as guideposts to segregate the massive
510 converging input onto this structure, as further described below.

511

512 *Outputs of the LCIC are associated with distinct extrinsic inputs*

513 We observed that the widely divergent outputs of the LCIC to the SC, mMGB, ipsi- and
514 contralateral IC were nearly entirely determined by whether the cells of origin were
515 found in the matrix or modules. These segregated streams of outputs have previously

been shown to be targeted by distinct inputs. LCIC modules receive input from somatosensory structures, such as the dorsal column nuclei (DCoN) and the primary somatosensory cortex (SScx) (Lesicko et al., 2016) (Fig. 10, purple arrows). Matrix areas of the LCIC, on the other hand, are targeted by auditory structures such as the AC and the CNIC (Lesicko et al., 2016) (Fig. 10, teal arrows). Though these two subregions of the LCIC are segregated on the basis of their neurochemistry and connectivity, they do not form wholly separate processing streams; cells in modular regions of the LCIC receive input from the matrix. These connections could serve to route auditory information into the somatosensory-recipient modules, thereby forming multisensory processing zones. The local connections in the LCIC appear largely unidirectional, in that the matrix regions receive very little input from the modules (Fig. 6B,C). This combination of segregated inputs and outputs and highly-specific local integration could therefore maximize the computational possibilities among parallel streams of information. For example, matrix regions of the LCIC could perform computations related to auditory processing and route it to midbrain targets including the SC and other regions of the IC, while the modules may integrate somatosensory and auditory information and route it to the mMGB. Though we have demonstrated a clear connection between the distribution of cells giving rise to these particular output pathways and the underlying neurochemical modularity present in the LC, it is worth noting that there are a number of additional targets of the LC whose sub-compartment of origin remains unknown, including outputs to additional subdivisions of the auditory thalamus and descending projections to the auditory brainstem (Caicedo & Herbert, 1993; Linke, 1999).

Similarities to patch/matrix compartments of the striatum

While the function of the neurochemical and connectional modularity found in the LCIC remains unknown, studies in other structures with a similar organization can help shed light on the potential advantage of such an arrangement. Modularity is also present in the striatum, with inputs and outputs being segregated according to whether they are found in the acetylcholine-rich "matrix" areas or the opiate receptor-dense "patch" areas (Gerfen, 1984; Graybiel & Ragsdale, 1978; Kincaid & Wilson, 1996). Studies that have investigated whether the two domains are fully segregated have found that the dendrites of retrogradely-filled cells in both compartments are confined to the region containing their cell bodies (Gerfen, 1985). While this finding suggests that the two compartments may form segregated processing streams, additional experiments have shown that intrinsic somatostatin-positive neurons form a bridge between the patch and matrix regions; the cell bodies of these interneurons are found in both regions, but their axons selectively innervate the matrix compartment (Gerfen, 1985). Single somatostatin-immunoreactive cells in the patch compartment send axons to the surrounding matrix, suggesting that these cells provide a unidirectional projection from the patch to the matrix. Interestingly, this organization bears resemblance to the largely directional flow of information from the matrix to module regions demonstrated in the present study (Fig. 6).

Implications for multisensory processing

The results of the present study suggest a mechanism by which multisensory convergence could occur within the LCIC. Further studies will be required to determine if single cells

within the modules receive convergent input from both of these sources. It is presently unknown whether the dendrites of cells within module and matrix zones are confined to the region containing their soma, as is the case with other modular structures such as the striatum and the pons (Gerfen, 1985; Schwarz & Thier, 1995). If not, multisensory integration could also arise from direct input to a cell whose dendrites cross the module/matrix boundary. The advantage of having multisensory convergence arise from a local circuit mechanism rather than direct convergence of extrinsic inputs is presently unclear, but may permit independent modulation of each input prior to their convergence.

Potential functional significance of module outputs

The mMGB is the only target of cells residing in modules that has been identified thus far. Similar to the LCIC, this division of the auditory thalamus is known to integrate multisensory inputs, and neurons in this region exhibit broad frequency tuning and large tactile receptive fields (Lindsay M Aitkin, 1973; Bordi & LeDoux, 1994). The mMGB is also interconnected with limbic structures, such as the amygdala, and has been shown to be involved with auditory fear conditioning (LeDoux, Ruggiero, & Reis, 1985; LeDoux, Sakaguchi, & Reis, 1984). Though it has traditionally been thought that the IC provides auditory input to the mMGB, it is possible that the inputs from modules of the LCIC actually provide multisensory information important for executing conditioned fear behaviors (Ledoux, Ruggiero, Forest, Stornetta, & Reis, 1987).

The mMGB is also reciprocally interconnected with all regions of the AC, and it has previously been hypothesized to serve as a site for multisensory integration and/or to modulate the AC based on state of arousal (Rouiller et al., 1989; Jeffery A Winer, 1992).

Inputs from the LCIC to the mMGB could therefore convey somatosensory, auditory, or multisensory cues relevant to the animal's state of arousal. Somatosensory convergence occurs at multiple stations within the auditory system, and has generally been thought to mediate cancellation of self-generated sounds (Wu, Stefanescu, Martel, & Shore, 2014). The potential participation of the LCIC-mMGB-AC circuit in this process is intriguing given that a) non-GABAergic module cells in the LCIC, such as those that project to the mMGB, receive strong module-based inhibition that could be driven by extrinsic somatosensory inputs and b) inhibiting this population of projection neurons could effectively prevent activation of auditory cortical networks and conscious awareness of self-generated noise, given that the mMGB projects widely to all areas of the AC (Rouiller et al., 1989; Jeffery A Winer, 1992).

Potential functional significance of matrix outputs

Cells in matrix regions of the LCIC project to at least two distinct targets: the SC and other regions of the IC. It is presently unknown whether these projection systems are formed by different groups of cells, or if single cells project to both targets. Projections from the LCIC to the SC have long been thought to mediate various acoustico-motor behaviors (Huffman & Henson Jr, 1990). For example, stimulation of the IC causes movement of the pinna and eyes in conjunction with activation of auditory neurons in the SC, and this pathway is thought to mediate additional orienting and escape/defense behaviors (Syka & Straschill, 1970). Connections between the IC and the SC are also thought to be critically involved in pre-pulse inhibition of the acoustic startle reflex (Koch & Schnitzler, 1997). The SC is thought to receive information about auditory

prepulses in an acoustic startle paradigm from the IC and routes this information to the pedunculopontine nucleus (PPT), a brainstem structure that also provides cholinergic input to modular areas of the LCIC (Motts & Schofield, 2009; Schofield, 2010; Swerdlow, Geyer, & Braff, 2001). The PPT then routes this information to the pontine reticular nucleus, where it converges with and influences the primary startle pathway (Davis, Gendelman, Tischler, & Gendelman, 1982).

Not only do matrix regions of the LCIC send input to the CNIC, but they also receive dense inputs from this region (Lesicko et al., 2016). It is therefore possible that some of the LCIC cells that project to the CNIC participate in feedback loops with the lemniscal auditory pathway. In addition to inputs from the CNIC, matrix regions of the LCIC receive descending inputs from the AC (Lesicko et al., 2016). Though descending connections from the AC to the IC predominately terminate in the LCIC and DCIC, their activation has been shown to cause striking shifts in the auditory response properties of cells in the CNIC (Andersen, Snyder, & Merzenich, 1980; Gao & Suga, 2000; Jeffery A. Winer, Larue, Diehl, & Hefti, 1998). Given that direct descending inputs to the CNIC are sparse, it is possible that these changes are mediated through connections from the LCIC to the CNIC (Stebbings et al., 2014).

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763

764 FIGURE LEGENDS

765 Figure 1: Experimental design. A) Experiments were performed in tissue slices from the
766 GAD67-GFP mouse in which modules are visually distinguishable under blue
767 illumination. Cells were voltage-clamped at -60 mV and +10 mV to isolate excitatory and
768 inhibitory inputs, respectively. B) In focal regions of UV laser activation, caged
769 glutamate is converted to active glutamate, thus generating spikes in presynaptic partners
770 and postsynaptic currents in the recorded cell. C) Four groups of cells in the LCIC were
771 recorded from: GAD67+ (putative inhibitory) and GAD67- (putative excitatory) cells
772 from both the modular and matrix regions. A 10X20 grid of stimulation sites was
773 centered over the LCIC and potential presynaptic partners were stimulated in a non-
774 neighbor fashion. Responses were plotted according to the location from which they were
775 generated and converted into heat maps by computing the area under the curve of each
776 response. D) Responses to photostimulation at various holding potentials. Note the
777 presence of inward current at 0 mV, and the absence at +10 mV. E) Left: Example of
778 spikes recorded in cell-attached mode in response to laser photostimulation (1 ms pulses,
779 3 mW) at various locations (10x10 grid, 20 μm between adjacent rows and columns) near
780 the cell body. Right: Spike output 100 ms after laser onset shown as a heat map. The
781 location of the recorded cell is shown in black. The white outline indicates the 20 μm
782 radius used to generate the first bin shown in G. F) The total number of spikes (average
783 of four trials) was computed for each cell and compared across cell types. No statistically
784 significant differences were found across cell types (Kruskal-Wallis test, $p = 0.84$) G)
785 The average number of spikes elicited at various distances from the cell body was
786 calculated and compared for each cell type (GAD- matrix: mean = 77.41, median = 29,

787 SD = 103.73, SEM = 59.89, CI = ±257.67, n = 3 cells; GAD+ matrix: mean = 39.39,
788 median = 33.5, SD = 30.76, SEM = 17.76, CI = ± 76.41, n = 4 cells; GAD- module: mean
789 = 67.42, median = 84.75, SD = 45.31, SEM = 26.16, CI = ± 112.55, n = 3 cells; GAD+
790 module: mean = 48, median = 18.63, SD = 64.72, SEM = 32.36, CI = ±102.98, n = 3
791 cells).
792
793 Figure 2: Examples of replicates of inhibitory and excitatory responses. A) Inhibitory
794 responses for three separate runs. The average maps (as shown in the final panel) were
795 analyzed and used for quantification. Example traces from two stimulation sites (white
796 square = top trace, black square = bottom trace) are shown to the right. B) Total
797 excitatory responses, including both direct activation and synaptic input to the recorded
798 cell. C) Excitatory responses in a low-calcium ACSF which blocks synaptic inputs,
799 giving rise to only direct activation of the recorded cell. Note that the response in the
800 bottom trace from B is no longer present, indicating that it was a synaptic input. D) Maps
801 of the excitatory synaptic inputs were generated by subtracting the direct input maps from
802 the total excitatory maps. Scale = 400 μm.
803
804 Figure 3: Input patterns for matrix cells. A) Example of inhibitory and excitatory inputs
805 to a GAD- matrix cell in layer 3 of the LCIC. Black dot indicates the location of the cell
806 body. White outlines represent the borders of the modules. B) Example of inhibitory
807 inputs to a GAD- matrix cell positioned between two modules in layer 2 of the LCIC. C)
808 Example of inhibitory inputs to a GAD- matrix cell in layer 2 of the LCIC. D) The total
809 inhibitory (mean = 1667.39, median = 1125, SD = 1825.73, SEM = 358.06, CI =

±737.42, n = 26 cells) and excitatory (mean = 470.8, median = 378.5, SD = 358.75, SEM = 113.45, CI = ± 256.63, n = 10 cells) synaptic input to GAD- matrix cells. Note that these cells receive significantly more inhibitory input than excitatory input. E) Inhibitory (matrix: mean = 1454.85, median = 891, SD = 1742.15, SEM = 341.66, CI = ±703.66; modules: mean = 191.34, median = 150, SD = 182.07, SEM = 35.71, CI = ±73.54) and excitatory (matrix: mean = 396.05, median = 331, SD = 300.3, SEM = 94.96, CI = ± 214.82; modules: mean = 57.3, median = 35.9, SD = 63.14, SEM = 19.97, CI = ± 45.16) synaptic input to GAD- matrix cells parcellated by region of origin. GAD- matrix cells receive significantly more inhibitory and excitatory input from the matrix compared to the modular regions of the LCIC. F) Example of inhibitory inputs to a GAD+ matrix cell positioned between two modules in layer 2 of the LCIC. G) Example of inhibitory inputs to a GAD+ matrix cell in layer 3 of the LCIC. H) Example of inhibitory inputs to a GAD+ matrix cell in layer 2 of the LCIC. I) The total inhibitory (mean: 2387.57, median: 1745, SD: 2072.93, SEM: 378.46, CI: ±774.04, n = 30 cells) and excitatory (mean: 3736.15, median: 1450, SD: 6256.96, SEM: 1735.37, CI: ±3781.04, n = 13 cells) synaptic input to GAD+ matrix cells. Note that these cells receive balanced inhibitory and excitatory input. J) Inhibitory (matrix: mean = 2068.63, median = 1450, SD = 1892.51, SEM = 345.52, CI = ±706.67; modules: mean = 300.03, median = 129, SD = 287.07, SEM = 52.41, CI = ±107.19) and excitatory (matrix: mean = 3231.16, median = 1320, SD = 5422.89, SEM = 1504.04, CI = ±3277.02; modules: mean = 456.02, median = 153, SD = 872.48, SEM = 241.98, CI = ±527.24) synaptic input to GAD+ matrix cells parcellated by region of origin. GAD+ matrix cells receive significantly more inhibitory and

832 excitatory input from the matrix compared to the modular regions of the LCIC. Scale =
833 400 μm.
834
835 Figure 4: Input patterns for cells in module regions of the LCIC. A) Example of
836 inhibitory and excitatory inputs to a GAD- cell in a module in the LCIC. Black dot
837 indicates the location of the cell body. White outlines represent the borders of the
838 modules. B) Example of inhibitory inputs to a GAD- cell in a module in layer 2 of the
839 LCIC. C) Example of inhibitory inputs to another GAD- cell in a module in layer 2 of the
840 LCIC. D) The total inhibitory (mean: 1612.06, median: 1390, SD: 1221.26, SEM: 212.59,
841 CI: ±433.04, n = 33 cells) and excitatory (mean: 510.93, median: 580, SD: 251.08, SEM:
842 64.83, CI: ±139.04, n = 15 cells) synaptic input to GAD- cells in modules. Note that these
843 cells receive significantly more inhibitory input than excitatory input. E) Inhibitory
844 (matrix: mean = 596.03, median = 400, SD = 442.75, SEM = 77.07, CI = ±156.99;
845 modules: mean = 1001.76, median = 939, SD = 862.34, SEM = 150.11, CI = ±305.77)
846 and excitatory (matrix: mean = 265.4, median = 245, SD = 132.76, SEM = 34.27, CI =
847 ±73.52; modules: mean = 231.69, median = 166, SD = 189.74, SEM = 48.99, CI =
848 ±105.07) synaptic input to GAD- cells in modules parcellated by region of origin. GAD-
849 cells in modules receive equal input from the matrix and module regions of the LCIC for
850 both inhibitory and excitatory inputs. F) Example of inhibitory inputs to a GAD+ cell in a
851 module in the LCIC. G) Example of inhibitory inputs to a GAD+ cell in a module in
852 layer 2 of the LCIC. H) Example of inhibitory inputs to another GAD+ m cell in a
853 module in layer 2 of the LCIC. I) The total inhibitory (mean: 1398.94, median: 833, SD:
854 1540.33, SEM: 264.16, CI: ±537.44, n = 34 cells) and excitatory (mean: 464.13, median:

315, SD: 423.31, SEM: 109.3, CI: ± 234.42, n = 15 cells) synaptic input to GAD+ cells in modules. Note that these cells receive more inhibitory than excitatory input. J) Inhibitory (matrix: mean = 1144.78, median =624, SD = 1270.96, SEM = 217.97, CI = ±443.46; modules: mean = 231.31, median = 113.9, SD = 297.02, SEM = 50.94, CI = ±103.64) and excitatory (matrix: mean = 281.34, median = 230, SD = 179.2, SEM = 46.27, CI = ±99.24; modules: mean = 165.92, median = 52.9, SD = 261.73, SEM = 67.58, CI = ±144.94) synaptic input to GAD+ cells in modules parcellated by region of origin. GAD+ cells in modules receive significantly more inhibitory and excitatory input from the matrix compared to the module regions of the LCIC. Scale = 400 μm.

Figure 5: Pharmacological controls with GABAzine and TTX. A) Example map showing the effect of 20 μM GABAzine on inhibitory inputs. Note that inhibitory inputs are abolished (right). B) Effect of GABAzine on the total excitatory (including direct activation) input to the same cell shown in A. C) Effect of GABAzine on the excitatory synaptic input of the same cell shown in A and B. Scale = 400 μm. D) Map of direct activation generated in low calcium ACSF (left) and TTX (right). Note the similarity in the distribution of input sites. E) Maps of excitatory synaptic input for each condition from the same cell shown in A. Scale = 400 μm.

Figure 6: Cell type-specific input patterns yield a largely unidirectional flow of information in the LCIC microcircuitry. A) E:I indices each of the four cell types (GAD- matrix: mean = -0.22, median = -0.38, SD = 0.54, SEM = 0.18, CI = ±0.42, n = 9 cells; GAD+ matrix: mean = -0.15, median = -0.44, SD = 0.54, SEM = 0.15, CI = ±0.33, n = 13

878 cells; GAD- module: mean = -0.4, median = -0.54, SD = 0.37, SEM = 0.1, CI = ±0.2, n
879 =15 cells; GAD+ module: mean = -0.35, median = -0.6, SD = 0.55, SEM = 0.14, CI =
880 ±0.3, n = 15 cells). Note that the mean and median E:I index is negative for each cell
881 type, indicating that the balance of charge is skewed toward inhibition. B) Modularity
882 indices for the inhibitory inputs to each of the four cell types of interest (GAD- matrix:
883 mean = 0.71, median = 0.72, SD = 0.15, SEM = 0.03, CI = ±0.06, n = 26 cells; GAD+
884 matrix: mean = 0.72, median = 0.74, SD = 0.14, SEM = 0.03, CI = ±0.05, n = 30 cells;
885 GAD- module: mean = 0.12, median = 0.22, SD = 0.39, SEM = 0.07, CI = ±0.14, n = 33
886 cells; GAD+ module: mean = -0.67, median = -0.75, SD = 0.22, SEM = 0.04, CI = ±
887 0.08, n = 34 cells). C) Modularity indices for the excitatory inputs to each of the four cell
888 types of interest (GAD- matrix: mean = 0.77, median = 0.76, SD = 0.13, SEM = 0.04, CI
889 = ±0.09, n = 10 cells; GAD+ matrix: mean = 0.74, median = 0.71, SD = 0.2, SEM = 0.05,
890 CI = ±0.12, n = 13 cells; GAD- module: mean = -0.17, median = -0.2, SD = 0.37, SEM =
891 0.1, CI = ±0.21, n = 15 cells; GAD+ module: mean = -0.46, median = -0.61, SD = 0.29,
892 SEM = 0.08, CI = ±0.16, n = 15 cells). D) Cell-type specific input patterns give rise to a
893 largely unidirectional flow of information from matrix to modular regions of the LCIC.
894 E) Neuronal morphology for a GAD- (white arrow left) and GAD+ (green arrow left)
895 matrix cell. Note the axons terminating in nearby modules (red arrow right). F) Neuronal
896 morphology for a GAD- (white arrow left) and GAD+ (green arrow left) cell located in a
897 module. Note the differences in dendritic arborization, with the GAD+ cell's dendrites
898 extending beyond the borders of the modules (red arrow right) and GAD- cell's dendrites
899 confined to the modules (white arrow right). Scale = 250 μm.
900

Figure 7: Dual recordings. A) Example of a pair of simultaneously recorded cells. B) A pair of cells that is matched in terms of location and type. Note the prominent pattern of clustered positive values in the map of cross-correlations. C) A pair of cells that differs in both location and type. Note the absence of a pattern in the cross-correlation map. D) Left: Average cross-correlation values for each category of pairs (Both same: mean = 0.2, median = 0.2, SD = 0.13, SEM = 0.04, CI = ±0.09, n = 10 pairs; Location different: mean = 0.16, median = 0.12, SD = 0.19, SEM = 0.06; CI = ±0.13, n = 10 pairs; Type different: mean = 0.12, median = 0.09, SD = 0.1, SEM = 0.03, CI = ±0.06, n = 12 pairs; Both different: mean = 0.004, median = 0.05, SD = 0.1, SEM = 0.04; CI = ±0.09, n = 7 pairs). Right: Distance vs. cross-correlation value between simultaneously recorded cells. E) Left: Correlation-coefficient values for each category of pairs (Both same: mean = 0.47, median = 0.52, SD = 0.29, SEM = 0.09, CI = ±0.21, n = 10 pairs; Location different: mean = 0.2, median = 0.16, SD = 0.22, SEM = 0.07; CI = ±0.16, n = 10 pairs; Type different: mean = 0.31, median = 0.17, SD = 0.31, SEM = 0.09, CI = ±0.19, n = 12 pairs; Both different: mean = 0.05, median = 0.01, SD = 0.15, SEM = 0.06, CI = ±0.13, n = 7 pairs). Scale = 400 µm. Right: Distance vs. correlation coefficient value between simultaneously recorded cells.

Figure 8: Cells that project to the ipsilateral IC and contraIC are found in matrix regions of the LCIC. A) FG injection sites. B) FG labeling in the LCIC after an ipsilateral IC injection in a GAD67-GFP mouse. White arrows indicate examples of double-labeled GAD+ and FG+ cells. C) Rostro-caudal distribution of cells in the LCIC that project to

924 other regions of the ipsilateral IC. D) FG labeling in the LCIC after an injection in the
925 contraIC of a GAD67-GFP mouse. E) Rostro-caudal distribution of cells in the LCIC that
926 project to the contraIC. Scale (A-B) = 1000 µm, inset = 250 µm. Scale (C-D) = 500 µm,
927 inset = 250 µm.
928
929 Figure 9: Distribution of cells that project to the SC and mMGB. A) FG labeling in the
930 LCIC after an SC injection in a GAD67-GFP mouse. B) Rostro-caudal distribution of
931 cells in the LCIC that project to the SC. C) FG labeling in the LCIC after an mMGB
932 injection in a GAD67-GFP mouse. D) Rostro-caudal distribution of cells in the LCIC that
933 project to the mMGB. E) Percentage of GABAergic and non-GABAergic cells from each
934 LCIC subregion projecting to various targets. Scale = 500 µm, inset = 250 µm.
935
936 Figure 10: Summary diagram of modularity in the LCIC neurochemistry, extrinsic inputs
937 and outputs, and local circuitry. Left: somatosensory inputs (purple arrows) arising from
938 the DCoN and SScx target modular regions of the LCIC, while auditory inputs (teal
939 arrows) from the ipsilateral IC and contraIC and the AC target the matrix. In addition to
940 GAD67, the modules are enriched in PV, AChE, CO, and NADPH-d, while the matrix
941 region contains calretinin neurons. Right: findings from the current study. Local input
942 patterns are highly cell-type specific, with an overall flow of information from the matrix
943 to modular regions (small green and black arrows). The two compartments of the LCIC
944 project to distinct targets: the modules send a purely excitatory projection to the mMGB
945 (large black arrow). Excitatory cells in the matrix send information to the SC, ipsilateral
946 IC, and contraIC (large black arrows), while inhibitory matrix cells project to the

947 ipsilateral IC (large green arrow). GAD67: glutamic acid decarboxylase-67; PV:
948 parvalbumin; AChE: acetylcholinesterase, CO: cytochrome oxidase; NADPH-d:
949 NADPH-diaphorase; CR: calretinin; AC: auditory cortex; SScx: somatosensory cortex;
950 DCoN: dorsal column nuclei; SC: superior colliculus; mMGB: medial division of the
951 medial geniculate body; CNIC: central nucleus of the inferior colliculus.