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Synchrony, connectivity, and functional similarity in auditory midbrain local circuits

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Abstract

The central nucleus of the inferior colliculus (ICC) contains a laminar structure that functions as an organizing substrate of ascending inputs and local processing. While topographic distributions of ICC response parameters within and across laminae have been reported, the functional micro-organization of the ICC is less well understood. For pairs of neighboring ICC neurons, we examined the nature of functional connectivity and receptive field preferences to gain a better understanding of the structure and function of local circuits. By recording from pairs of adjacent neurons and presenting dynamic broad-band stimulation, we estimated functional connectivity and local differences in frequency response areas (FRAs), spectrotemporal receptive fields (STRFs), nonlinear input/output functions, and single spike information. From the cross-covariance functions we identified putative unidirectional as well as bidirectional excitatory/inhibitory interactions. STRFs of neighboring neurons strongly conserve best frequency, and moderately agree in STRF similarity, bandwidth, temporal response type, best modulation frequency, nonlinearity structure, and degree of information processing. Excitatory connectivity was stronger and temporally more precise than for inhibitory connections. Neither connection strength nor degree of synchrony correlated with receptive field parameters. The functional similarity of local pairs of ICC neurons was substantially less than for local pairs in the granular layers of primary auditory cortex (AI). These results imply that while the ICC is an obligatory nexus of ascending information, local neurons are comparatively weakly connected and exhibit considerable receptive field variability, potentially reflecting the heterogeneity of converging inputs to ICC functional zones.

Keywords

inferior colliculus; STRF; information; microcircuits; fine-scale networks

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INTRODUCTION

The central nucleus of the inferior colliculus (ICC) is the main lemniscal station in the auditory midbrain, yet relatively little is known about the functional processing in ICC local networks, which are the main arbiters of information convergence, integration and conversion. Characterizing these networks is necessary because the ICC is an obligatory processing hub: the ascending inputs from several brainstem nuclei converge and interact in the ICC, and the ICC is the main source of input to the auditory thalamus (Adams, 1979, Brunso-Bechtold et al., 1981, Aitkin and Phillips, 1984). The ICC is highly structured; it contains fibrodendritic laminae (Oliver and Morest, 1984, Malmierca et al., 1995) that consist of neurons with different synaptic domains or functional zones associated with various converging brainstem inputs (Loftus et al., 2004, Malmierca et al., 2005, Loftus et al., 2010). Synaptic domains are thought to contain groups of adjacent ICC neurons that share similar patterns of inputs (Loftus et al., 2004, Loftus et al., 2010). Knowledge of the local connectivity and synaptic organization is essential for discovering the principles of intranuclear processing that shape the functional transformation of the converging inputs in different functional zones.

Each fibrodendritic lamina contains neurons that preferentially respond to a restricted frequency range of $\sim 1/3$ octave (Schreiner and Langner, 1997, Malmierca et al., 2008). While each layer responds best to a preferential range of frequencies, the set of layers covers the whole frequency range in tonotopic fashion, so that frequency preference strongly and systematically varies orthogonally to ICC laminae (Merzenich and Reid, 1974). Further, individual ICC laminae contain a range of basic acoustic parameters and response properties, such as bandwidth, latency, and modulation characteristics, with some of them showing topographic organization (Schreiner and Langner, 1988, Langner et al., 2002, Rodriguez et al., 2010, Schnupp et al., 2015). Thus, the intricate functional organization of the ICC provides a prime opportunity for exploring how auditory information is integrated and processed in the auditory midbrain.

The ICC's functional organization is a reflection of the spatial distribution of the anatomical inputs that it receives. Inputs from the medial and lateral superior olive, as well as the cochlear nucleus, preferentially target restricted regions of the ICC (Spangler et al., 1985, Oliver, 1987, Shneiderman and Henkel, 1987, Oliver et al., 1997). Thus, the ICC contains a highly organized global pattern of receptive field parameters and anatomical inputs (Loftus et al., 2010). Despite this, the functional utility of the input patterns has not been recovered. The segregated inputs may be a way for the ICC to organize ascending information before packaging it for subsequent auditory stations. The ICC likely also performs further processing on the inputs by imposing specific computations (Ehret, 1997). These two options are not mutually exclusive, although if the ICC's laminar structure is used merely as a consolidation point, local ICC neurons may remain heterogeneous in their response properties. In contrast, if neighboring ICC neurons share similar processing principles, then this may indicate that ICC neurons can perform new computations that further refine and potentially transform ascending information.

Few studies have targeted the micro-organization of the ICC. When pure tones were presented, neighboring neurons had similar CFs (Syka et al., 1981, Seshagiri and Delgutte, 2007). Yet, other tone response properties, such as bandwidth, threshold, and latency, appeared to be more divergent between local neurons. Complex sound reverse-correlation analysis also found that CF was highly similar between neighboring neurons (Chen et al., 2012). Other acoustic feature preferences, such as temporal and spectral modulation preferences, and receptive field similarity, were less similar, even for neighboring neurons, with spatial distance being a major determinant of receptive field and spike train correlation strength (Chen et al., 2012).

Our goal was to provide a more detailed picture of several aspects of micro-processing in the ICC for very closely spaced neurons while taking into account the type of local interaction. Previous studies did not scrutinize the relationship between STRF parameters and functional connectivity, nor did they assess how different temporal interaction types are related to functional processing. We addressed this by assessing the functional connectivity of neurons recorded from the same electrode contact and by distinguishing between excitatory and inhibitory interactions, as well as their unilateral or bilateral directionality. Based on these distinctions, we compared local differences in tonal responses, spectrotemporal receptive field (STRFs), nonlinear input/output functions, and information processing. By comprehensively analyzing local pairs of ICC neurons, we were able to quantify characteristics of receptive field processing between pairs of neurons from a circuit perspective. Additionally, we compared the ICC results to similar observations in the input layers of the primary auditory cortex (Atencio and Schreiner, 2013) to determine whether joint processing in highly local circuits shares characteristics across different stations in the lemniscal pathway.

EXPERIMENTAL PROCEDURES

Surgical procedures, stimulation, and recording

The experimental procedures used in this study have been previously described (Atencio et al., 2012), and were similar to previous reports (Schreiner and Langner, 1997, Escabi and Schreiner, 2002). Three young female adult cats were given an initial dose of ketamine (22 mg/kg) and acepromazine (0.11 mg/kg), and anesthesia was maintained with pentobarbital sodium (Nembutal, 15-30 mg/kg) during the surgical procedure. The animal's temperature was maintained with a thermostatic heating pad. A custom head holder was used to stabilize the head. Bupivacaine was applied to incision points. Surgery consisted of a tracheotomy, reflection of the soft tissues of the scalp, craniotomy over cortex, and durotomy. The cortex posterior to auditory cortex, and above the inferior colliculus, was then aspirated, which allowed for direct visualization of the inferior colliculus and recording access using a lateral-medial approach (Schreiner and Langner, 1997). The tentorium overlaying the inferior colliculus was not removed, which likely allowed full access to most regions of the ICC, though there may have been possible under-sampling of the caudal ICC. After surgery, the animal was maintained in an areflexive state with a continuous infusion of ketamine/diazepam (2-10 mg/kg/hr ketamine, 0.05-0.2 mg/kg/hr diazepam in lactated Ringer solution). All procedures were in strict accordance with, and were administered under an

experimental protocol approved by, the University of California, San Francisco Committee for Animal Research under protocol AN086113.

With the animal inside a sound-shielded anechoic chamber (IAC, Bronx, NY), stimuli were delivered via a closed speaker system to the ear contralateral to the ICC that was recorded from (electrostatic diaphragms from Stax, Japan). Extracellular recordings were made using multi-channel silicon recording probes, which were provided by the University of Michigan Center for Neural Communication Technology. The probes contained sixteen linearly spaced recording channels, with each channel separated by 150 μm . We only used probes with channel impedances between 4 and 5 M Ω since these impedances allowed us to resolve ICC single units; lower impedances usually did not allow for adequate single unit isolation. Probes were carefully positioned using a microdrive (David Kopf Instruments, Tujunga, CA), and inserted multiple times in each subject (N = 14 in subject 1, N = 27 in subject 2, and N = 19 in subject 3).

Channels that were within the ICC were identified by examining pure tone stimulation responses. The most superficial electrode contacts usually exhibited little tuning, and if tuning was present, it was very diffuse, indicating that the contacts were likely located in the dorsal cortex of the IC. At increasing depths, clear pure tone tuning emerged along the extent of the linear electrode. Manual manipulation of a function generator during experiments allowed pure tone response to be systematically varied, with low frequencies exciting shallower depth channels and higher frequencies exciting deeper channels. After experiments, single unit frequency response areas (FRAs) and spectrotemporal receptive fields (STRFs) were estimated offline. The data from a channel were included in analyses if: (1) The channel contained neurons with clear FRAs and/or STRFs; (2) the FRAs/STRFs of the channel followed the prototypical lateral-medial, low-high characteristic/best frequency progression.

Neural traces were bandpass filtered between 0.6 and 6 kHz and digitally recorded with a Neuralynx Cheetah A/D system at a sampling rate of 18 kHz. The traces were sorted off-line with a Bayesian spike sorting algorithm (Lewicki, 1994, 1998). Only isolated events that exceeded the DC baseline by 5 root-mean-square noise levels were used in the spike sorting procedure (termed spike events). Sorting occurs in two steps: First, template waveforms are estimated from up to 5000 spike events, with each spike event required to be separated by at least 1 ms. This ensures that the resulting templates are derived from temporally separated events. Second, the estimated templates are used to sort the recording trace. For each neuron's spike train, the autocorrelation function was estimated to ensure that fewer than 0.5% fell within a 1 ms refractory period; spike trains that violated this criteria were not analyzed. The spike sorting algorithm also allowed for the proper classification of spikes even if they overlapped with an action potential of another neuron. This is a challenging problem for spike-sorting algorithms, and even tetrode recordings cannot resolve this issue (Franke et al., 2015). In a third step of our approach, which utilizes the procedure of (Lewicki, 1994), an overlap decomposition procedure is performed when a potential spike event cannot be assigned to a well-characterized single unit and may be the result of two spike waveform overlapping in time. To identify a possible overlap event, the algorithm combines the single unit waveforms of the neurons on a channel (N = 2 for a pair). The two

waveforms are summed for different temporal relationships, resulting in a set of composite waveforms. Each member of this set is then compared to the potential overlap event. If there is a significant fit between the recorded summation waveform and a composite waveform in the set, then the spike times of the two contributing units are recorded (Lewicki, 1994). To ensure data quality, we only included data in correlation and STRF analyses if two neurons recorded from a single channel had a significant cross-covariance function (see *Connectivity*).

Stimuli

We used pure tone and broadband noise stimuli to probe ICC neurons. For pure tones, the duration was 50 ms and the inter-stimulus interval was 300 ms. Amplitudes covered a 70 dB range in 5 dB steps (15 amplitudes). Frequencies varied in 0.13 octave steps (45 frequencies), and covered ranges of 0.137-8 kHz, 0.219-12.8 kHz, or 0.343-20 kHz depending on the depth of the recording electrode. Each tone frequency and amplitude combination was presented 5 times in pseudo-random order.

We also presented a broadband (0.5 – 40 kHz, 11 or 21 minutes) dynamic moving ripple (DMR) stimulus (Escabi and Schreiner, 2002, Atencio et al., 2008). The maximum spectral modulation frequency of the DMR was 4 cyc/oct, and the maximum temporal modulation frequency was 5000 cyc/s (Escabi and Schreiner, 2002). The maximum modulation depth of the spectrotemporal envelope was 40 dB. The mean intensity of the DMR was set at 30-50 dB above the average pure tone threshold of acoustically driven recording sites. Last, for some neurons we also presented a short DMR segment that had the same frequency range and modulation characteristics as the longer DMR stimulus. The duration of the short DMR was 12 seconds, and it had 750 ms onset/offset cosine squared ramps, and was presented 150 times.

Connectivity

To analyze the functional connectivity between neurons, we followed standard cross-covariance procedures (Rosenberg et al., 1989, Halliday and Rosenberg, 1999). First, spike trains were obtained by binning the spike times with 0.5 ms resolution. For a single spike train $A(n)$, n is the bin number and $A(n)$ is either 1 (spike) or 0 (no spike). For two spike trains $A(n)$ and $B(n)$, the mean intensities, P_A and P_B , for a sample of duration D bins, are estimated as $P_A = N_A/D$ and $P_B = N_B/D$, where N_A and N_B are the total number of spikes in trains A and B , respectively. For the spike trains in this study, the stimulus duration was either 11 or 21 minutes, giving $D = 1,320,000$ or $2,520,000$ bins.

The cross-correlation function, or correlogram, for spike trains $A(n)$ and $B(n)$ is then estimated as

$$C_{AB}(m) = \sum_{n=0}^{D-m} A(n+m) B(n)$$

From $C_{AB}(m)$ an unbiased estimate of the second order cross-product density, $P_{AB}(m)$, is

$$P_{AB}(m) = \frac{C_{AB}(m)}{\Delta \cdot D}$$

where Δ is the bin size of the spike train, in milliseconds. The cross-covariance function, $Q_{AB}(m)$, is then defined as

$$Q_{AB}(m) = P_{AB}(m) - P_A \cdot P_B$$

Thus, the cross-covariance function is a scaled version of the cross-correlation function, with the mean background activity removed. This allows excitatory and suppressive interactions to be more easily visualized. Cross-covariance values that are approximately zero represent chance coincidences between the two spike trains. Deflections from zero represent how the activity of one neuron influences the firing of the other neuron. Note that

$\lim_{|m| \rightarrow \infty} Q_{AB}(m) = 0$. The cross-covariance function $Q_{AB}(m)$ has an asymptotic distribution from which its variance can be estimated (Halliday and Rosenberg, 1999). Under the assumption of independent Poisson spike trains, the variance of $Q_{AB}(m)$ may be approximated as

$$\text{Var}(Q_{AB}(m)) \cong \frac{P_A \cdot P_B}{\Delta \cdot D}$$

Thus, for two spike trains, upper and lower 99% confidence limits (CL) for $Q_{AB}(m)$ can be set at

$$CL = \pm 3 \left(\frac{P_A \cdot P_B}{\Delta \cdot D} \right)^{\frac{1}{2}}$$

To be included in analyses, a cross-covariance function needed to have two consecutive exceeding the 99% confidence limits. For purely suppressive deflections our criteria were more stringent: purely suppressive interactions needed to have the maximum deflection occur at delays of 2 ms or greater, ensuring that the deflection reflected neural suppression.

To estimate the strength of functional connections, we calculated a normalized Cross-Correlation Coefficient (CCC) (Agmon, 2012). For each correlogram, C_{AB} , the CCC was defined as:

$$CCC = \left[C_{AB}(PD) - \frac{N_A N_B}{D} \right] / \left(\min(N_A, N_B) - \frac{N_A N_B}{D} \right)$$

where PD, the peak delay, is the time at which the peak in the correlogram occurs, $C_{AB}(PD)$ is the number of spikes in the correlogram at the peak delay, and $\min(N_A, N_B)$ is the number of spikes in the shorter spike train. This form of the CCC is impervious to firing rate differences between the spike trains of the two neurons.

Information analysis

To assess the information transmitted by each neuron, we used the single spike information approach developed by Brenner and colleagues (Brenner et al., 2000). To estimate information for a neuron, a short sample from a stimulus distribution is presented multiple times. We used the short DMR stimulus described earlier. The spiking responses to the stimulus are used to calculate the post-stimulus time histogram (PSTH). If the neuron conveys no information about the stimulus, the PSTH, and thus the instantaneous firing rate, is flat and its average value is equivalent to the average firing rate over the course of the stimulus. Deviations from the average firing rate indicate that the neuron conveys information about the stimulus. This approach makes no assumptions regarding the nature of the neural code and focuses on the information conveyed by the single spikes of a neuron. Thus, only two items need to be estimated: the PSTH and the average firing rate.

We estimated single spike information from the instantaneous firing rate (obtained from the PSTH) and the average firing rate according to:

$$I_{spike} = \frac{1}{T} \int_0^T dt \frac{r(t)}{\bar{r}} \log_2 \left[\frac{r(t)}{\bar{r}} \right].$$

Since information values are sensitive to the stimulus duration and bin size in the PSTH, we employed multiple controls. First, we estimated I_{spike} as a function of the temporal bin size of the PSTH. A best fit linear line was fit to the information estimates as a function of bin size, beginning with the largest bin size. Successive bin sizes were added to the bin size values until the information value for the smallest bin size diverged from the best fit line by 20%. This smallest bin size was then used in further analyses. Next, after determining the bin size, the influence of stimulus duration was compensated for by estimating I_{spike} for different durations of the stimulus (10, 20, 30, 40, 50, 60, 70, 80, 90, 92.5, 95, 97.5 and 100%). For each duration (except 100%), 100 information estimates were made. Each estimate was obtained by sampling from the PSTH; since each sample represents a specific amount of time, the number of samples summed to the specified duration. Next, information was estimated for each set of samples using the I_{spike} equation, and the mean of the 100 estimates was taken as the information value for the specified duration. Then, I_{spike} was plotted against the inverse of the percent duration (1/10, 1/20, ..., 1/100). The ordinate intersect of the best fitting line to the data was used as the final estimate for I_{spike} .

Frequency response areas and spectrotemporal receptive fields

Data analysis was carried out in MATLAB (Mathworks, Natick, MA). For frequency response areas (FRAs), the number of spikes for each frequency-level tone stimulus was accumulated, and the resulting matrix was our estimate of the FRA. Boundaries of the FRA were determined by frequency-level combinations that elicited responses that were twice the spontaneous background firing rate. Characteristic frequency (CF), bandwidth (BW), and threshold were obtained from the FRA using standard analytical procedures (Schreiner and Mendelson, 1990). CF was the frequency at which the lowest level produced a driven response (the tip of the FRA). Threshold was the level at which the CF was estimated. BW

was measured at multiple levels (10, 20, 30, and 40 dB) relative to threshold, and was determined by noting the contours of the FRA that exceeded background firing response levels. Spectral tuning was measured using the quality factor Q defined as $Q = CF / BW$.

We evaluated the temporal response profiles of neurons for tones using post-stimulus time histograms (PSTHs). PSTHs were constructed from all pure tone responses. Each temporal response profile was assessed using the Phasic-Tonic Index (PTI):

$$PTI = N_E / (N_B + N_E) = N_E / N_T,$$

where N_B is the number of spikes during the first half of the stimulus, N_E is the number of spikes during the last half of the stimulus, and N_T is the sum of N_B and N_E . For purely phasic neurons, the PTI is 0, while for tonic responses, the PTI is approximately 0.5. To account for transmission time, the analysis window for PTIs began at 4 ms. Thus, for a 50 ms tone, the responses for the first half of the stimulus were obtained from 4-29 ms, and the responses from the last half were obtained from 29-54 ms.

For spectrotemporal receptive fields (STRFs), the spike-triggered average was used to estimate the STRF (Escabi and Schreiner, 2002). STRFs were thresholded so that only significant features ($p < 0.01$) were included in the analysis. The best frequency (BF) was extracted by summing the STRF across time, and then identifying the frequency corresponding to the maximum value. Peak latency was obtained in a similar fashion: the STRF was summed across frequency and then the time at which the maximum occurred was calculated. The BW of each STRF was obtained by first setting all negative values in the STRF to zero. Then, the main excitatory subfield frequency boundaries were estimated as the frequencies at which the STRF magnitude decreased to 10% of the peak STRF value. Spectral tuning was then defined as Q , the quality factor, and calculated according to $Q = BF / BW$. The similarity between STRFs was measured using the Pearson correlation coefficient.

Response precision

Using previously described methodologies, we computed a Response Precision Index (RPI) for each neuron using the relation $RPI = (max(STRF) - min(STRF)) / (r \sqrt{8})$, where $max(STRF)$ and $min(STRF)$ are the maximum and minimum values in the STRF, and r is the average firing rate (Escabi and Schreiner, 2002, Atencio and Schreiner, 2012, Atencio et al., 2012). Dividing by r and the square root of 8 allows the RPI to range from 0 (not precise timing) to 1 (very precise timing). Here precision refers to how well the spikes align to specific parts of the ripple stimulus. If the spikes predominantly align to ripple stimulus values that have large magnitudes, then the RPI will be closer to 1, since the difference between the maximum and minimum will be great. When spikes are not as precisely aligned, the maximum in the STRF will decrease, and thus the RPI will decrease in value.

Nonlinearity analysis

For each STRF, we computed the nonlinear input/output function that related the stimulus to the probability of spike occurrence (Atencio et al., 2008, Atencio and Schreiner, 2012). The following steps were used to calculate the nonlinearities. (1) Each ripple stimulus segment, s , that elicited a spike, was correlated with the STRF by projecting it onto the STRF via the inner product $z = s \cdot \text{STRF}$. These projections, or stimulus-filter similarities, characterize the probability distribution $P(z | \text{spike})$. (2) We then projected a large number of randomly-selected stimulus segments onto the STRF, and formed the prior stimulus distribution, $P(z)$. (3) The mean and standard deviation of $P(z)$, μ and σ , were then calculated. (4) $P(z | \text{spike})$ and $P(z)$ were transformed to units of standard deviation by using $x = (z - \mu) / \sigma$, to obtain the distributions $P(x | \text{spike})$ and $P(x)$. The x values here are not similarity index values since the x values are normalized differently. Because of the transformation, the values of x are now in units of standard deviation (SD) and are called projections. (5) The nonlinearity for the STRF was then computed as $P(\text{spike} | x) = P(\text{spike}) \frac{P(x | \text{spike})}{P(x)}$, where $P(\text{spike})$ is the average firing rate of the neuron. Thus, the nonlinearity describes the likelihood of a spike given the similarity between the STRF and the stimulus. High x values indicate STRF-stimulus correlations that would not be expected from a randomly spiking neuron, while values near 0 would be expected if the neuron fired indiscriminately. Thus, if nonlinearity values increase as the x values increase, then greater similarity between the STRF and the stimulus results in greater firing rates.

To evaluate individual nonlinearities, we took two approaches: non-parametric and parametric. The first approach assessed the structure of the nonlinearity using an asymmetry index (ASI). The ASI is defined as $\text{ASI} = (R - L) / (R + L)$, where R is the sum of the nonlinearity values for similarity values greater than 0 (positive correlations between stimulus and filter), and L is the sum for similarity values less than 0 (negative correlations between stimulus and filter). The ASI varies between -1 and 1, with 1 corresponding to a nonlinearity where the firing rate increases only when the stimulus is highly similar to the STRF.

In the parametric approach, we used a function that has wide theoretical and experimental support (Hansel and van Vreeswijk, 2002, Miller and Troyer, 2002). The function has the following form:

$$f(x | A, \theta, \sigma) = \left[\frac{A(x - \theta)}{2} \right] \left[1 + \text{erf} \left(\frac{x - \theta}{\sigma \sqrt{2}} \right) \right] + \frac{A\sigma}{\sqrt{2\pi}} \exp \left(-\frac{(x - \theta)^2}{2\sigma^2} \right)$$

Here, A is a gain term, θ is the threshold to response, and σ is the transition smoothness in the nonlinearity. $\text{erf}(x)$ is the error function. For analysis, we only used fits that gave normalized mean squared errors less than 0.1. θ and σ are the most significant parameters in the fit: θ determines the threshold, while σ determines how smooth the transition is when the responses become greater than the average firing rate. When σ is 0, the function describes hard rectification.

Statistical comparisons

Throughout this report, we compare functional processing for each member in a pair of neurons. For plotting, there is no principle that guides how to associate a neuron's parameter value with an axis (abscissa/ordinate). Therefore, we used the following procedure: (1) For each pair of neurons, randomly select one value for the abscissa, and the other for the ordinate; (2) Estimate the Pearson's correlation value across all pairs; (3) Repeat steps (1) and (2) 1000 times, and accumulate the correlation values. (4) Estimate the median correlation value from the distribution in (3), and estimate 95% confidence intervals (CIs). In figures, only the first randomization is shown (Fig. 5,6,7,9,10,11,13,14).

To determine the significance of the median correlation value, we implemented another randomization procedure. (1) We combined all parameter values for all pairs of neurons into one distribution (for N pairs this gives 2N parameter values). (2) Randomly choose two values, without replacement, from the parameter distribution in (1). (3) Perform (2) until the number of pairs equals the number for the actual data distribution. (4) For each pair, randomly determine which value in a pair goes with the abscissa/ordinate. (5) Calculate the Pearson's correlation value. (6) Repeat (2-5) 1000 times. (7) Compare the median correlation value from the data to the accumulated random distribution and estimate significance.

For parameter differences, the absolute difference (absolute value of the difference between the parameters) or the octave difference ($\log_2$ ratio between the values) was used. The similarity between STRFs and nonlinearities was measured using the Pearson correlation coefficient. Difference statistics are reported median $\pm$ MAD (median absolute deviation), unless otherwise noted.

We used permutation tests to determine if the parameter differences were non-randomly organized. To determine significance, we used the following procedure: (1) Calculate parameter differences for each pair of ICC neurons, resulting in a data distribution of N differences. (2) Estimate the median of the data difference distribution in (1); (3) Combine the parameter data from the N pairs of neurons, giving 2N parameter values; (4) Randomly sample, without replacement, N values from the combined data set; (5) Assign the remaining N parameter values to a second distribution; (6) Pairwise subtract the two randomly chosen parameter distributions; (7) Calculate the median of the random population differences; (8) Perform the randomization-difference procedure 1000 times; (9) Estimate significance by calculating the number of random difference population medians that were less than the data median; the significance is this number divided by 1000.

We performed factor analysis to determine the covariation between receptive field parameter differences and functional connectivity parameters. Factor analysis was performed using the R software language (<http://www.r-project.org>). For factor analysis, the variables we used were: the cross-correlation coefficient, peak delay, and half width; the differences between BF, Q, latency, firing rate, RPI, nonlinearity asymmetry, threshold, transition; and the correlation between STRFs and nonlinearities. We used the following command in R to estimate the factors: `fit <- factanal(X, M, rotation="varimax")`, where X is a data matrix whose columns represent the variables, and where each row holds the data for one pair of

neurons; M is the number of factors; and rotation indicates that the factors were rotated to maximize the variance. The number of factors was confirmed using the nFactors R library.

RESULTS

Correlation function

To determine putative functional connectivity and the functional similarity between neighboring ICC neurons, we presented ICC neurons with a long, complex, noise-like sound – the dynamic moving ripple (DMR) – and recorded neural responses using a multi-channel recording probe. From the activity at the different electrode contacts we sorted and reconstructed spike trains.

The continuous, noise-like structure of the DMR greatly limited sharp onset/high multi-unit synchrony responses of ICC neurons that are usually seen for shorter, repeated stimuli. This allowed us (a) to robustly identify uncontaminated spiking events and (b) to estimate correlation functions not dominated by sharp, stimulus-driven synchrony. Because the DMR is appropriate for reverse correlation, we were also able to estimate spectrotemporal receptive fields (STRFs), and then compare the spike waveforms, receptive fields, and cross-correlation functions for neurons recorded from the same electrode channel (Atencio and Schreiner, 2013). Well-classified action potential waveforms from the same electrode channel were highly divergent (Fig. 1A,E-H), indicating that they originated from different neurons. Further, the STRFs associated with the two waveforms diverged strongly, consistent with the assumption that they reflect the functional properties of two different neurons. Finally, the peak delay for the cross-covariance function occurred at ~ 0.5 ms, which is too short to allow spikes in a burst from the same neuron to be classified as different waveforms, since the short delay, coupled with spike waveform durations of 1-2 ms, would produce distorted waveforms.

When two adjacent neurons emit spikes in close temporal proximity, they produce events with irregular waveforms resulting from the sum of both spikes (Fig. 1B). The spike-sorting procedure does not classify these irregular events as candidate neurons, since the shape of irregular events changes drastically on a trial-by-trial basis due to their variable relative spike-timing, as reflected in the width of the cross-covariance function (Fig. 1C1,D2-G2). Post-sorting visual inspection confirmed that no model spike waveforms resembled irregular events. The individual irregular events, however, can be assigned to classified neurons in a pair by comparing the sum of the individual, regular spike waveform models (Fig. 1A) to the recording trace (Fig. 1B, Waveform Sum and Recording). The exceptional match between the sum of the spike waveforms for two putative neurons and the irregular event provides strong evidence that the spike-sorting procedure is performing as expected, and effectively rules out other less probable hypotheses. Since spiking events of two neurons with distinct waveforms that occur close in time can be separated this way, functional connectivity can be assessed at millisecond resolution (Fig. 1C).

Even though pairs of neurons were recovered from the same recording channel, the STRFs for the constituent neurons could be divergent (Fig. 1C2,C3), which indicates nearby neurons may have diverse acoustic feature preferences. Also, estimated functional

connectivity for neuron pairs took multiple forms (Fig. 1D2-G2). Connectivity functions displayed facilitative interactions (Fig. 1D2,E2), facilitative and suppressive interactions (Fig. 1F2), and predominantly or purely suppressive interactions (Fig. 1G2). Purely suppressive interactions, however, are difficult to uncover since they require cross-covariance troughs that are clearly removed from 0 ms delay and are highly efficacious to reach statistical significance. Since the number of unequivocal, purely suppressive, unilateral connections was quite small in our sample ($N=15$ or $<3\%$ out of a total of $N=578$), in this report we focused on connectivity functions that have either facilitative interactions or both facilitative and suppressive interactions.

We tested the relationship between STRF overlap - or signal correlation - and cross-covariance function width by correlating the STRFs of pairs of neurons. For each STRF in a pair, a slice through the STRF at the best frequency was extracted (Figs. 2A-D and F-I show examples). The two STRF slices were then correlated, giving the correlation width that is expected based only on receptive field similarity (Fig. 2E, J). The STRF correlation width was greater than the accompanying cross-covariance function width determined from spike-timing. Across the population, STRF correlation widths were approximately three times greater than cross-covariance widths (mean = 2.87, SE = 0.16; $p < 10^{-10}$, Signed-Rank test). While the STRF correlation and connectivity measures are both linear descriptors, they are appropriate measures of comparison; any nonlinear aspects are likely substantially less information bearing. Therefore, the sharp synchrony exhibited by cross-covariance functions is not merely a reflection of stimulus entrainment and STRF overlap, but represents significant aspects of neural functional connectivity and functional interactions.

Correlation parameters

Cross-covariance functions contain features that can be used to deduce the sign and direction of functional connectivity in local networks. Positive deflections denote functional connectivity where the spiking of one neuron facilitates the spiking of the other neuron. Negative deflections indicate that the spiking of one neuron is associated with the suppression of spiking in the other neuron (Fig. 1F,G).

We found 578 pairs that had significant cross-covariance functions (1014 neurons involved in single pairs, and an additional 71 neurons involved in two pairs). Of these, 481 pairs (83.2% of 578 connected pairs) had only an excitatory peak (EP); 82 pairs (14.2%) had both an excitatory peak and a suppressive trough (EP+ST; Fig. 1F); and 15 pairs (2.6%) had only a suppressive trough (ST; Fig. 1G). Due to the small sample of ST temporal interactions, only EP and EP+ST data are displayed in the following figures, and when suppressive peaks are described, they refer to suppressive peaks that were part of EP+ST temporal interactions.

We assessed significant peaks with three measures: (1) the time of the deflection, which is the peak delay (PD); (2) the width of the peak at half the maximum value, which is the half-width (HW); (3) and the magnitude of the peak, which is assessed using the cross-correlation coefficient (CCC).

For pairs of ICC neurons, the distribution of positive peak delays attained a maximum at 1 ms (Fig. 3A; EP: median = 1 ms, MAD = 0.5 ms). Negative troughs also occurred at similar

short delays (ST: median = 1.0 ms, MAD = 0.5 ms), suggestive of monosynaptic interactions. The large percentage of connected pairs with peak delays ≤ 2 ms indicates that many local ICC neurons essentially fire in synchrony.

The temporal dispersion of spikes indicates whether the firing is sharply or broadly correlated. Eighty percent of pairs had a correlation function half-width (HW) < 3 ms (Fig. 3B), suggestive of a small temporal window for concerted firing. Positive peaks were slightly narrower than negative troughs (Fig. 3B; EP: median = 1.5 ms, MAD = 0.5 ms; ST: median = 2.0 ms, MAD = 0.5 ms; EP vs. ST: $p < 0.0001$, rank-sum test).

Cross-correlation coefficients indicated a relatively weak connection strength between local pairs (Fig. 3C). Excitatory interactions (EP: median CCC = 0.027, MAD = 0.012) were approximately twice as strong as suppressive interactions (ST: median CCC = 0.012, MAD = 0.004; $p < 0.0001$, rank-sum test). Thus, though the peak and the half width indicate tightly concerted firing, the proportion of correlated/synchronous spikes in the peak is relatively small.

The connectivity analysis shows that pairs of local neurons are able to fire synchronously, and that the precision of the firing occurs with millisecond resolution. It also demonstrates that nearby ICC neurons are functionally connected or, at a minimum, act as if they are connected.

Information content

We next determined how the concerted firing of ICC neurons is related to information processing. Do synchronously firing ICC neurons have similar information processing characteristics? We addressed this systematically by, first, examining the information coding of single spikes for local neuron pairs (Fig. 4). To examine the information processing of ICC neurons, we used a distribution free approach that allowed us to estimate the temporal resolution and the information capacity of ICC neurons, and determine how similar these aspects are for pairs of local ICC neurons (Fig. 4).

Pairwise information comparison

Do putatively connected neighboring neurons code a similar amount of information, or are information values unrelated? The average firing rate between pairs of neurons was weakly correlated (Fig. 5A; $r = 0.343$, 95% CI: [0.342, 0.347], $p < 0.001$, randomization test), with the difference less than 4 Hz (median difference = 1.72 sp/s; MAD = 1.29 sp/s). The optimal temporal resolution of each neuron was moderately correlated, indicating information processing occurs at similar time-scales for each neuron (Fig. 5B; $r = 0.624$, 95% CI: [0.623, 0.628], $p < 0.001$, randomization test), with the difference usually less than 3 ms (median difference = 2.20 ms; MAD = 1.40 ms). The transmitted information was weakly similar between pairs of connected neurons (Fig. 5C; $r = 0.460$, 95% CI: [0.457, 0.464], $p < 0.001$, randomization test; median difference = 0.61 bits; MAD = 0.44 bits). Finally, using permutation tests across non-neighboring neurons we observed that local neurons had non-randomly organized firing rates, time resolution, and information values compared to non-neighboring pairs; the similarity of these values between neighboring neurons was greater than if neurons had been randomly organized ($p < 0.001$, permutation test).

Thus, nearby neurons in the ICC with correlated firing convey moderately similar amounts of information. The information capacity of pairs of ICC neurons is similar, implying that local functional networks may encode information through similar temporal response patterns. Further, the information bearing temporal response patterns are more similar between local neurons than between non-functionally connected neurons in the ICC.

FRA CF and temporal response profiles

Many studies in central auditory stations use the frequency response area (FRA) to characterize functional properties by assessing stimulus preferences to sparse, tonal stimulation. Since the response properties to narrow-band stimuli may differ significantly from those observed with broad-band stimuli, we also compared ICC pairs under pure tone stimulation. For local pairs of ICC neurons, the structure of FRAs was broadly similar, though the degree of responsiveness could vary considerably between neurons in a pair (Fig. 6C2,D2). Further, the temporal response profile – such as tonic or phasic activity obtained by estimating the PSTH from all tonal stimuli – could be divergent (Fig. 6C1,D1).

The most fundamental parameter that can be ascertained from an FRA is characteristic frequency (CF). When neighboring neurons have similar CFs, the local network contains neurons with similar tonotopic preferences reflecting a locally non-fractured frequency distribution. We found that the CFs for pairs of neurons were highly similar (Fig. 6E; $r = 0.967$, 95% CI: [0.966, 0.968], $p < 0.001$, randomization test). Correspondingly, CF differences between the constituent neurons were small (Fig. 6F; median = 0.13 oct, MAD = 0.13 oct; $N = 540$ pairs). The distribution of CF differences was heavily skewed toward low values. Thus, CF is highly conserved between local pairs of ICC neurons.

Neighboring ICC neurons showed variability in their temporal response type, i.e. a highly phasic neuron could have a neighbor with a response that was highly sustained (e.g., Fig 6C1,D1). The PTIs of pairs of neurons covered a range of possible values (Fig. 6G; $r = 0.439$, 95% CI: [0.438, 0.442], $p < 0.001$, randomization test). Accordingly, we found that local neurons are quite diverse in their temporal response patterns (Fig. 6G,H; median PTI difference = 0.095; MAD = 0.068), i.e., ~50% of pairs having PTI differences less than 20% of the full phasic/tonic range of 0.5. Despite this variability in temporal response type, local pairs of neurons have temporal response profiles that are significantly more similar than estimated for randomly distributed profiles ($p < 0.001$, permutation test). Therefore, CF was highly conserved between pairs of neurons, and the temporal response type of constituent neurons was less variable than for random pairs.

FRA bandwidth and spectral tuning

Analysis of the bandwidth of neighboring ICC neurons at 10 and 40 dB above threshold revealed strong correlations between their FRA bandwidths (Fig. 7A,C). The correlation between bandwidths for neurons in a pair slightly increased from 10 dB to 40 dB above threshold (BW10: $r = 0.719$, 95% CI: [0.716, 0.740], $p < 0.001$; BW40: $r = 0.859$, 95% CI: [0.858, 0.865], $p < 0.001$, randomization test). The octave difference between bandwidths for constituent neurons was less than ~30% (Fig. 7B,D; BW10 difference: median = 0.49 oct, MAD = 0.32 oct; BW40 difference: median = 0.32 oct, MAD = 0.21 oct).

Spectral tuning is a measure of frequency selectivity relative to the CF of a neuron, and is measured through the quality factor, Q ($= BW/CF$). For connected pairs of ICC neurons, the Q10 and Q40 values were weakly correlated, though less than for bandwidth (Fig. 7E, G; Q10: $r = 0.323$, 95% CI: [0.321, 0.332], $p < 0.001$; Q40: $r = 0.272$, 95% CI: [0.222, 0.309], $p = 0.002$; randomization test). Q10 values were more variable, with differences up to 2 octaves (Fig. 7F; Q10 difference: median = 0.48, MAD = 0.33). For Q40, the variability decreased relative to Q10 (Fig. 7H; Q40 difference: median = 0.34, MAD = 0.21). BW10 and Q10 may be more variable since they are measured near threshold, which is subject to spiking variability. In accord with the similarity of bandwidth and spectral tuning, randomization tests revealed that local, connected pairs, versus randomly selected pairs, have ordered parameter values ($p < 0.001$, permutation test). Thus, the bandwidths of neighboring ICC neurons are well correlated, and both bandwidth and Q are more correlated than expected from randomly sampled neuron pairs.

STRF similarity

The STRFs of neurons in a pair could be highly variable, reflecting differences in spectral and temporal STRF extent as well as relative shifts of excitatory and inhibitory subregions (Fig. 1C,E F). Consistent with their varying shapes, STRFs of adjacent neurons were only moderately correlated (Fig. 8A). Over the population, STRF similarity indicated only a moderate agreement between neuron pairs with excitatory and/or inhibitory interactions (All data: median = 0.26, MAD = 0.097; EP: median = 0.26, MAD = 0.095; EP+ST: median = 0.32, MAD = 0.13). However, pairs of STRFs were more similar when the temporal interaction had both an excitatory and suppressive peak, compared to an excitatory peak alone ($p = 0.0002$, rank-sum test), perhaps indicating stronger recurrent connectivity and shared inputs for EP+ST neuron pairs.

To determine if STRF similarity correlated with functional connectivity strength, we compared STRF similarity to the cross-correlation coefficient for each pair of ICC neurons; the two measures were not substantially correlated (Fig. 8B; EP: $r = 0.125$, $p = 0.006$, t-test; ST: $r = 0.196$, $p = 0.078$, t-test). Thus, global receptive field similarity only weakly predicts the correlation strength between adjacent neurons.

STRF firing rate and response precision index

We next examined the response strength and firing precision of ICC neurons to the DMR stimulus. The firing rate of pairs of neurons in response to DMR stimuli was weakly correlated (Fig. 9A; EP: $r = 0.265$, 95% CI: [0.264, 0.268], $p < 0.001$; EP+ST: $r = -0.276$, 95% CI: [-0.282, -0.246], $p = 0.99$, randomization test). Pairs with both excitatory and inhibitory interactions appeared to have higher overall firing rates than for excitatory only interactions. Though there was variability between neighboring neurons firing rates, the differences were usually less than 5 Hz when the correlation function exhibited only a positive peak (Fig. 9B; EP: median = 2.25, MAD = 1.68 Hz). When there was both an excitatory and suppressive correlation trough, the firing rate differences were significantly larger (EP+ST: median = 11.28 Hz, MAD = 7.35; $p < 0.001$, rank-sum test). Firing rate difference weakly predicted the peak cross-correlation coefficient, with a higher predictive value for pairs with suppressive interactions (Fig. 9C; EP: $r = 0.41$, $p < 0.001$; ST: $r = 0.64$, $p <$

0.001; t-test). The correlation was positive, i.e., the larger the firing rate differences, the higher was the connectivity strength (Fig. 9C).

The response precision index (RPI) indicates the degree to which neural firing is locked to the peaks or troughs of the spectrotemporal envelope of the DMR. Values near 1 indicate perfect precision. Local ICC neurons had moderately similar RPIs (Fig. 9D; EP: $r=0.520$, 95% CI: [0.519, 0.523], $p<0.001$; EP+ST: $r=0.509$, 95% CI: [0.505, 0.531], $p<0.001$, randomization test). RPIs differences were usually less than 0.1 and irrespective of the interaction type (Fig. 9E; EP: median = 0.035, MAD = 0.023; EP+ST: median = 0.027, MAD = 0.019), and the distributions were not significantly different ($p = 0.067$, rank-sum test). Finally, RPIs differences were weakly correlated with the cross-correlation coefficient obtained from functional connectivity (Fig. 9F; EP: $r = 0.28$, $p < 0.001$; ST: $r = 0.27$, $p = 0.014$; t-test). Overall, firing rate and RPI are only modestly correlated between local ICC neurons.

Are these receptive field parameters more similar than predicted from randomly selected pairs of neurons? To test this, we extracted individual neuron parameter values, and sampled with replacement from this distribution until we obtained a population that had the same number of pairs as the original data set. We found that firing rate was significantly more clustered for EP interaction types, and less clustered for EP+ST interactions (EP vs Rand: $p < 0.0001$; EP+ST vs. Rand: $p < 0.0001$, rank-sum test). For RPI, both interaction types had distributions that were statistically different from randomly sampled pairs (EP vs. Rand: $p < 0.00001$; EP+ST vs. Rand: $p < 0.00001$, rank-sum test). Therefore, compared to randomly paired neurons, firing rate and RPI are more similar of local pairs with for EP interactions. For local pairs with EP+ST interactions, RPI shows a higher similarity than firing rate. However, both parameters are only moderately predictive of the interaction strength.

STRF best frequency, spectral tuning, and latency

Next, we examined STRF parameters to determine if neighboring neurons had similar basic stimulus preferences. We first examined best frequency (BF), which is the frequency of the excitatory peak in the STRF. For pairs of neurons, BF was highly similar (Fig. 10A; EP: $r = 0.957$, 95% CI: [0.956, 0.958], $p < 0.001$; EP+ST: $r = 0.973$, 95% CI: [0.972, 0.974], $p < 0.001$; randomization test). The BF difference between ~85% of local neurons was 0.2 octaves, independent of interaction type (Fig. 10B; EP: median = 0.10 oct, MAD = 0.08 oct; EP+ST: median = 0.08 oct, MAD = 0.06 oct; EP vs. EP+ST: $p = 0.93$, rank-sum test). Spectral tuning of STRFs (Q) was weakly correlated and showed a higher variability than BF (Fig. 10C; EP: $r = 0.316$, 95% CI: [0.315, 0.319], $p < 0.001$; EP+ST: $r = 0.499$, 95% CI: [0.495, 0.528], $p < 0.001$; randomization test), similar to the finding for FRAs. Only ~10% of neurons in each interaction type had Q differences above 1.2 octaves (Fig. 10D; EP: median = 0.51 oct, MAD = 0.39 oct; EP+ST: median = 0.55 oct, MAD = 0.34 oct; EP vs. EP+ST: $p = 0.87$, rank-sum test). Response latency was moderately correlated for pairs of neurons (Fig. 10E; EP: $r = 0.736$, 95% CI: [0.735, 0.738], $p < 0.001$; EP+ST: $r = 0.640$, 95% CI: [0.637, 0.658], $p < 0.001$; randomization test), indicating that for >80% of ICC neurons in a local pair, the difference in response timing was within <2ms (Fig. 10F; EP: median =

0.50 ms, MAD = 0.50 ms; EP+ST: median = 0.50 ms, MAD = 0.50 ms; EP vs. EP+ST: $p > 0.10$, randomization test).

We compared the paired data to randomly selected difference values, following the procedure used for firing rate and response precision (Fig. 9). We did not resample BF, since randomly sampling across the tonotopic axis would, by definition, lead to greater divergence. We found that Q for local pairs was significantly more tightly clustered than for the random distribution (EP vs Rand: $p < 0.0001$; EP+ST vs. Rand: $p < 0.0001$, rank-sum test). For latency, the distributions were not statistically different (EP vs. Rand: $p = 0.282$; EP+ST vs. Rand: $p = 0.252$, rank-sum test). Therefore, BF and latency are highly matched for connected neurons throughout the ICC, and Q is more tightly matched than expected from randomly paired values.

STRF best temporal and spectral modulation frequency

Temporal and spectral modulations are essential aspects of communication sounds. To determine the modulation processing of ICC neurons, we examined the structure of STRFs. The relationship between excitatory and suppressive STRF subfields indicates the modulation preferences of a neuron (Atencio and Schreiner, 2010). When we examined ICC STRFs, we found that best temporal modulation frequencies (bTMFs) in neuron pairs could often reach >200 Hz, independent of the interaction type (Fig. 11A), and that bTMFs between neurons in a pair were weakly correlated (EP: $r = 0.361$, 95% CI: [0.360, 0.364], $p < 0.001$; EP+ST: $r = 0.279$, 95% CI: [0.276, 0.304], $p < 0.001$; randomization test). For ~40% of pairs, the difference between bTMFs was larger than 45Hz, indicating a significant level of local temporal modulation variance (Fig. 11B; EP: median = 30.2 Hz, MAD = 24.5 Hz; EP+ST: 44.7 Hz, 24.5 Hz). Further, EP pairs had smaller bTMF differences compared to EP+ST pairs ($p = 0.0234$, rank-sum test).

Pairs of neurons had moderately similar best spectral modulation frequencies (bSMF) (Fig. 11D; EP: $r = 0.686$, 95% CI: [0.685, 0.691], $p < 0.001$; EP+ST: $r = 0.664$, 95% CI: [0.661, 0.691], $p < 0.001$; randomization test). Further, the population of differences between bSMFs decayed sharply, indicating substantially similar local bSMF preferences (Fig. 11E; EP: median = 0.097 cyc/oct, MAD = 0.069 cyc/oct; EP+ST: median = 0.052 cyc/oct, MAD = 0.053cyc/oct). Also, the EP and EP+ST bSMF difference distributions were not significantly different ($p = 0.32$, rank-sum test). Finally, when comparing bTMF and bSMF differences to correlation strength, we did not find a significant relationship (Fig 12C,F; bTMF: EP: $r = 0.022$, $p = 0.64$; ST: $r = 0.13$, $p = 0.26$; bSMF: EP: $r = -0.034$, $p = 0.45$; ST: $r = 0.11$, $p = 0.31$; t-tests). Therefore, though neighboring ICC neurons share moderately similar modulation preferences, especially bSMF, this similarity does not reflect the strength of functional connectivity.

Last, we found that both bTMF and bSMF of local pairs were more tightly clustered than expected by chance. EP interaction types had bTMFs that were significantly more similar than randomly selected pairs (EP vs. Rand: $p < 0.00001$; EP+ST vs. Rand: $p = 0.0717$; rank-sum test). Both EP and EP+ST interaction types had significantly similar bSMF values (EP vs. Rand: $p < 0.00001$; EP+ST vs. Rand: $p < 0.00001$; rank-sum test). Thus, bTMF and bSMF are non-randomly distributed in local ICC networks.

Example nonlinearities

STRFs describe the dominant stimulus feature (“what”) preference of neurons. They do not describe the manner (“how”) in which a neuron will respond to those features. The relationship between response strength and STRF/stimulus similarity is captured by the neuron's nonlinearity. We estimated a nonlinearity for each neuron to assess the relationship between the stimulus and the response output.

We found that nonlinearities could be well fit by a parametric curve that was characterized by two main parameters: a threshold, θ , and a transition, σ . High threshold values indicate that a significant match between STRF and stimulus is required before the neuron increases its response rate (Fig. 12A). A low transition value indicates that the nonlinearity exhibits ‘hard’ rectification and little ‘leakage’ noise (Fig. 12B).

STRFs and nonlinearities for a pair of neurons are shown in Fig. 12C-F. The STRFs showed narrow (Fig. 12C) and moderately broad tuning (Fig. 12D). The nonlinearities for both neurons were strongly asymmetric about the vertical axis when projection values equaled zero (Fig. 12E, F). Thus, as the correlation (‘projection’) between the STRF and the stimulus increased (positive projection values), the firing rate response increased. When the stimulus was anti-correlated with the STRF (negative projection values), the response was suppressed below the average firing rate. Curve fits to these nonlinearities showed that they captured the essential shapes of the nonlinearities very well (Fig. 12E,F).

Nonlinearity asymmetry and similarity values

We estimated the asymmetry of nonlinearities, and correlated the nonlinearities of pairs of neurons to determine if their global input-output relationships were similar. The asymmetry index (ASI) ranges between -1 and 1 , with 1 indicating nonlinearities that are highly skewed to positive STRF-stimulus correlation values, and 0 indicates a symmetric nonlinearity. More than 98% of ICC neurons had highly asymmetric nonlinearities (ASI values >0.7), independent of interaction type, and they were weakly correlated between the two neurons (Fig. 13A; EP: $r = 0.140$, 95% CI: $[0.138, 0.145]$, $p = 0.003$; EP+ST: $r = 0.386$, 95% CI: $[0.379, 0.414]$, $p < 0.001$; randomization test). While the differences were small, and decayed rapidly (Fig. 13B; EP: median = 0.037 , MAD = 0.023 ; EP+ST: median = 0.064 , MAD = 0.040), the EP distribution was significantly shifted to lower values (EP vs EP+ST: $p = 0.0001$, rank-sum test). Pairs of neurons with excitatory and suppressive connectivity showed slightly more asymmetric nonlinearities than interactions with only excitatory components.

Nonlinearity similarity for neurons in a connected local pair, which is the correlation between the nonlinearities, was very high (Fig. 13C; EP: median = 0.971 , MAD = 0.020 ; EP +ST: median = 0.974 , MAD = 0.019 ; EP vs. EP+ST: $p = 0.75$, rank-sum test). Thus, the global shape of the nonlinearity was highly similar for nearby ICC neurons, independent of interaction type.

Parametric nonlinearity analysis: threshold and transition

We next assessed the parametric nonlinearity curve fits (see Fig. 12 for examples). The transition parameter indicates the noise in the response. The largest proportion of transition values (sigma) was less than 0.5, indicating that a substantial number of ICC neurons respond in a manner that approximates hard rectification (Fig. 14A; transition median = 0.99, MAD = 0.69).

Threshold values indicate the match that is required between STRF and stimulus for the neuron to respond. High threshold values indicate stimulus feature selectivity. The distribution of thresholds was symmetrical. The main mode of the distribution was centered between 0 and 1 (Fig. 14C; threshold median = 0.31, MAD = 0.64). Values of 1 or greater indicate that relatively high stimulus-STRF correlations are necessary to drive neurons to fire above the mean background rate. Together, these data indicate that ICC neurons require a moderate match between filter and stimulus before an elevated response is delivered, though when this threshold is reached the response increases linearly with increasing amounts of stimulus-filter correlation.

Do nearby neurons in the ICC integrate information in a similar manner? We examined how the threshold and transition parameters of the nonlinearities correlated for neighboring neurons. For threshold, there was large variability between local neurons (Fig. 14D; EP: $r = 0.0649$, 95% CI: [0.0631, 0.0825], $p = 0.058$; EP+ST: $r = 0.124$, 95% CI: [0.0983, 0.1728], $p = 0.060$; randomization test). The threshold differences decayed slowly, indicating that neighboring neurons could have nonlinearities with moderately different thresholds largely independent of interaction types (Fig. 14E; EP: median = 0.73, MAD = 0.54; EP+ST: median = 1.23, MAD = 0.93), though there was more variance between EP+ST pairs (EP vs EP+ST: $p < 0.001$, rank-sum test). Transition values were also diverse for local neurons, though they were mainly confined to values less than 2 (Fig. 14F; EP: $r = 0.207$, 95% CI: [0.205, 0.214], $p < 0.001$; EP+ST: $r = 0.390$, 95% CI: [0.367, 0.468], $p < 0.001$; randomization test). The difference between transition values was skewed toward 0, indicating that many pairs of ICC neurons had similar transition values, also independent of interaction type (Fig. 14G; EP: median = 0.58, MAD = 0.41; EP+ST: median = 0.63, MAD = 0.43; EP vs. EP+ST: $p = 0.13$, rank-sum test).

Last, we found that both nonlinearity threshold and transition were more tightly aligned for local pairs than expected by chance. EP interaction types had thresholds and transition values that were significantly more similar than randomly selected pairs (Fig. 14E,G: EP vs. Rand threshold: $p < 0.00001$; EP vs. Rand transition: $p < 0.00001$; rank-sum test). Alternatively, EP+ST interaction types were less similarly clustered for threshold and more clustered for transition (Fig. 14E,G: EP+ST vs. Rand threshold: $p = 0.0322$; EP+ST vs. Rand transition: $p < 0.0221$; rank-sum test). Thus, nonlinearity threshold and transition are significantly clustered for the majority of ICC neurons.

Relationship between local connectivity and receptive fields

To determine more general relationships between cross-covariance parameters, STRF parameter differences, and nonlinearity parameter differences, we performed a factor

analysis on all paired ICC data. Factor analysis determines if correlations between variables can be accounted for by specific hidden variables.

We found four significant factors for the set of 13 variables ($p < 0.0001$, Chi-Square test, eigenvalues = 2.58, 1.79, 1.45, 1.19). Factor analysis produces loadings, which describe how strongly the factor metric accounts for the parameter of interest. Loadings near 1 indicate that changes in the parameter are well accounted for by changes in the factor. Very few factors had loadings near 1.0, indicating that the different parameters captured by a single factor did not strongly vary together. In order of significance, with only the loadings greater than 0.3, the factors we found were: Factor 1 (firing rate difference: 0.81; nonlinearity transition difference: 0.51; CCC: 0.37; response precision difference: 0.35; nonlinearity asymmetry difference: 0.32); Factor 2 (nonlinearity transition difference: 0.33; nonlinearity similarity: 0.54; nonlinearity threshold difference: 0.93); Factor 3 (latency difference: 0.52; STRF similarity: 0.71; BF difference: 0.39; spectral tuning difference: 0.47); and Factor 4 (co-variance half-width: 0.99). Thus, correlation strength covaried with firing rate, response precision, and nonlinearity asymmetry differences (F1), while other nonlinearity aspects, such as differences in similarity and threshold, were captured by the F2 factor. Pair-wise relationships of global response aspects captured by the STRFs and more specific functional aspects (latency, BF, spectral tuning) were subsumed in a single factor (F3) and, therefore, essentially independent of differences in nonlinearity behavior. Finally, correlation half-width, which is indicative of synchrony, was contained in its own factor (F4). Thus, the degree of functional similarity of neighboring ICC neurons (F3) is neither predictive of connectivity and synchrony (F1, F4), nor of the relationship of other response characteristics such as basic nonlinearity aspects (F2), and response strength (F1).

Comparison to auditory cortex

Both, ICC and primary auditory cortex are lemniscal stations that are tonotopically organized but differ in some basic functional aspects. For example, temporal modulation following of cortical neurons is significantly reduced compared to the ICC (Schreiner and Urbas, 1986, Langner and Schreiner, 1988, Joris et al., 2004); the redundancy of information across neurons is lower in auditory cortex (Chechik et al., 2006); and stimulus encoding by cortical neurons is significantly better characterized using multidimensional STRFs than for the majority of ICC neurons (Atencio et al., 2012). To assess whether there are distinct differences in the local functional micro-organization between ICC and AI that may account for those differences, we compared the findings in this study to functional relationships previously obtained for pairs of adjacent neurons in primary auditory cortex (Atencio and Schreiner, 2013). Since fundamental elaborations of auditory information are performed in different cortical layers, we compared ICC local processing only to the local processing in AI granular layers (Atencio and Schreiner, 2013), the first stage of cortical integration.

For every parameter, except nonlinearity asymmetry, there were significant differences between the two stations. The interaction between neighboring cortical neurons, as expressed through cross-covariance analysis, was significantly stronger in cortex (Fig. 15A; $p < 0.001$), with shorter peak delays (Fig. 15B; $p < 0.001$), and narrower correlation half-width

(Fig. 15C; $p < 0.001$). This indicates a stronger and more specific connectivity pattern in the cortex than within collicular laminae.

For several basic receptive field parameters, ICC neighboring neurons showed greater disparity among the paired neurons compared to neurons in local AI sub-networks. The disparity between neighboring ICC neurons was greater for: BF (Fig. 15E; $p < 0.0001$); spectral tuning (Fig. 15F; $p < 0.0001$); firing rate (Fig. 15G; $p < 0.0001$); and nonlinearity similarity (Fig. 15J; $p = 0.0051$). By contrast, the disparity in AI granular layers was greater for: latency (Fig. 15H; $p < 0.0001$) and response precision (Fig. 15I; $p = 0.0109$). STRF similarity between pairs of local neurons was much higher for cortical neurons (Fig. 15D; $p < 0.001$). We found no ICC to AI difference for nonlinearity asymmetry (Fig. 15K; $p = 0.164$; all comparisons assessed using KS-tests). Thus, the overall picture is that in the ICC neighboring neurons with correlated activity are more diverse in their receptive fields and nonlinearities compared to those in the granular layers of auditory cortex. By contrast, temporal spiking aspects, such as response latency and precision, were more diverse for local cortical neurons.

The greater functional connectivity strength and sharper synchrony in AI may, perhaps, account for the higher functional similarity for nearby cortical neurons, as indicated by their higher STRF similarity and the lower differences in BF, spectral tuning, and firing rate, as compared to ICC pairs.

DISCUSSION

Our goal was to characterize in detail some of the micro-processing characteristics in cat ICC for closely spaced neurons that showed different types of correlated activity. By taking into account the functional connectivity of neurons recorded from the same electrode contact and by distinguishing between excitatory and inhibitory interactions, we were able to illuminate several aspects of local processing. The spatial separation of recorded neurons was likely $< 100 \mu\text{m}$ given that their action potentials were recorded from the same electrode contact and were not present on the neighboring contacts $150 \mu\text{m}$ away. Therefore, the recorded neuronal pairs have a high probability of being within the same fibrodendritic laminae, which are on average separated by $\sim 175 \mu\text{m}$ (Schreiner and Langner, 1997). This is supported by the observation that BF differences for $\sim 85\%$ of the recorded pairs were ~ 0.2 octaves - less than the average BF difference between neighboring laminae of ~ 0.28 octaves in cats (Schreiner and Langner, 1997). These conclusions are also in agreement with the observation that neurons separated by $\sim 150 \mu\text{m}$ along the tonotopic axis likely belong to different fibrodendritic laminae and have BF differences > 0.28 octaves (Chen et al., 2012).

Our analysis of putative interactions between local pairs of ICC neurons within a fibrodendritic lamina showed both excitatory and inhibitory components. But, the sources of these excitatory and inhibitory inputs are not clear. Unfortunately, little is known about the intrinsic anatomical connectivity between different cell types in ICC. Although most ICC neurons have local axon collaterals that parallel the fibrodendritic laminae, the number and strength of the synapses between pairs of neurons may be small (Oliver, 2005, Ito and Oliver, 2014; Oliver, personal communication). Since the extrinsic projections to the ICC

contain both excitatory and inhibitory inputs, it is not clear whether local inhibitory connections are separable from the ascending inhibitory inputs to the ICC. While direct excitatory inputs from cochlear nucleus are monosynaptic, the inputs from cochlear nucleus via other brainstem sources have at least one synaptic delay (see (Schofield, 2005)). Thus, extrinsic excitatory and inhibitory inputs may merge, or arrive at slightly different times at adjacent neurons, and may account for some correlograms that show both excitatory and inhibitory troughs. This might occur without the presence of direct connections between the local pair of neurons. Future studies are required to accurately interpret the observed synchronous activity of adjacent collicular neurons in a framework of both intrinsic and extrinsic connectivity.

Functionally, for neighboring neurons we found that correlation strength covaried with differences in firing rate, response precision, and nonlinearity asymmetry, with functional connectivity strength increasing as these parameters became more similar. Global response aspects (STRF similarity) and more specific functional aspects (latency, BF, spectral tuning) varied together, and therefore were essentially independent of differences in nonlinearity behavior. Finally, correlation half-width, which is indicative of the degree of synchrony, did not covary with other response parameters. Thus, functional similarity does not predict connectivity or synchrony, and it does not predict other response characteristics, such as basic nonlinearity aspects and response strength.

Paired neuron spike-sorting and alternative explanations

Paired recordings from neighboring neurons are challenging to acquire with current spike-sorting techniques. It is especially difficult when the goal is examining sharply synchronous responses. The main difficulty is resolving overlapping spike waveforms, which is an unresolved problem with few viable approaches (Pillow et al., 2013, Franke et al., 2015). Our approach used an established algorithm that allowed us to first recover two spike waveform templates, based on a large number of well-separated spiking events, and then use these templates to decompose the relatively small numbers of overlapping spike events (Lewicki, 1994). The results of our spike-sorting procedure, combined with functional assessments of the recovered neurons, provide an implicit and explicit validation of our approach.

The consistency of our approach can be appreciated by considering alternative explanations. In our approach, after templates were estimated, they were assigned to different neurons, and then used to process the recording trace and determine spike times for each neuron. There are two alternatives that may be distinguished. In the first case, the waveforms that we recovered included one that looked like a standard action potential template, while the other resembled an overlap event. In this case, the irregular overlap event does not come from two neurons, but is thought to originate from slow recovery from sodium channel inactivation and depolarization block, and results in a distorted template shape (Sivaramakrishnan and Oliver, 2001). The other case is that the two recovered templates come from the same neuron, but correspond to spikes during bursting episodes.

All explanations must be consistent with our data, which exhibited sharp (widths of ~2 ms) and synchronous (peak delays of 0.5-2 ms) functional connectivity, and revealed that STRFs

from the same recording contact could have divergent shapes. The first case assumes that one of the recovered templates resembles an overlap event (which we term a “distorted” template) and does not result from the “collision” of two templates. However, this is implausible, since in this case the overlap events would need to be stereotyped enough to be clustered and classified as the same underlying template. We note, though, that overlapping events could often be strongly variable, making it unlikely that such events would be classified as a single template (Fig. 1B). Second, if an overlapping event was classified as a template, then it would have a spike train associated with it. This spike train would then be cross-correlated with the spike train associated with the other recovered template. However, we found that peak delays were often 0.5-1 ms, meaning that if both templates came from the same neuron the unit would fire in its own refractory period, which is unlikely. Third, since the distorted template occurs over ~2 ms, sharp synchrony would not be recovered since the non-distorted template would not overlap with the distorted template. Fourth, if the templates came from the same neuron the STRFs would be similar; however, our data shows that they are often dissimilar (Fig. 1D). Last, upon examination we never saw templates that resembled overlap events.

The other case is that the two templates came from the same neuron as a result of bursting. Since peak delays were short, the burst frequency would have to be ~500-1000 Hz, which is exceptionally fast. Such high burst rates are seen in neurons with specialized mechanisms, and in these cases the spike waveform shape is often conserved (Gray and McCormick, 1996, Ritzau-Jost et al., 2014). Second, if the spike trains came from the same neuron, the STRFs would again only be slightly different, since the DMR stimulus parameters change only slowly over tens of milliseconds; however, we found that STRFs were often strongly divergent (Fig. 1D). This stands in contrast to our earlier study in auditory cortex (Shih et al., 2011), and previous work in visual cortex (Reich et al., 2000), which described similar STRFs for short inter-spike intervals (ISIs). Since short ISIs corresponded to similar STRFs, this supports the conclusion that we obtained distinct pairs of single units. Third, spike waveform models were obtained from large numbers of isolated spiking events, and not from irregularly shaped events. In no case did a recovered model waveform resemble an irregularly shaped event. Finally, bursts can be ruled out by considering template shapes. Complete templates last from 1 to 2 ms. To estimate two clean, non-distorted, templates from bursts, the spikes in the burst would need to be consistently separated by at least 3 ms. However, connectivity peak delays were 0.5-2 ms, indicating that the overlapping events could only be decomposed using templates from distinct neurons. Therefore, our data is implicitly and explicitly consistent, and we conclude that the simplest explanation is correct: our study reports on pairs of different neurons recorded from the same electrode channel.

Synchrony and local circuits

Neighboring, potentially connected ICC neurons are characterized by weakly synchronous firing. This synchrony does not result from receptive field overlap, since the temporal duration of excitatory STRF subfields occurs over a longer period of time than the synchrony peak delay and half-width parameters (Fig. 2).

Putative monosynaptic functional connectivity has been found in multiple auditory systems. Monosynaptic connectivity between layers in the auditory cortex occurs at peak delays of 1-4 ms and is commonly found, as it is in the visual cortex (Alonso and Martinez, 1998). Transmission times between thalamus and cortex also occur between 1-4 ms (Miller et al., 2001b). Hippocampal recordings further show delays of only a few milliseconds (Fujisawa et al., 2008). In the lower auditory system, cross-correlation studies have revealed short transmission times. Reports in the cochlear nucleus have found peak delays of less than 2 ms (Voigt and Young, 1990). Further, auditory nerve to cochlear nucleus functional connectivity also occurs within 2 ms (Young and Sachs, 2008). Therefore, our reported delays are well integrated with previous connectivity studies.

ICC Processing

Nearby ICC neurons were characterized by similar best frequency preferences. They have moderate agreement in frequency integration, but less agreement in the overall distribution of spectrotemporal feature preferences and other response characteristics such as firing rate, temporal response profile, and their nonlinearity (or stimulus/response function). This picture of a functionally less rigorous local ICC organization is surprising, since local regions of the ICC receive a restricted and fairly specific mixture of inputs from preceding auditory centers (see Oliver 2005).

Does the ICC merely collect and feed information to ensuing auditory stations? If collection and grouping were the main goals, then little synchrony would be expected, since correlations would be mainly based on stimulus entrainment, and not on neural ensemble coding. Our synchrony analysis, however, shows clearly that nearby neurons may fire in a highly concerted manner (see Fig. 1B,D,F). The synchronous spikes likely indicate specific stimulus moments that carry significant information with consequences for either local processing or on the efficacy of information transmission to the next stations. Even though differences between receptive field parameters show moderate breadth for nearby neurons, the differences are significantly smaller than expected from random sampling. Thus, synchronous firing by pairs of neurons is accompanied by significantly organized acoustic preferences. This suggests that synchronous firing is a way to group collections of neurons that will transmit information with higher fidelity than would be possible in a simple single neuron code. Therefore, the ICC appears to be not merely a consolidation point; instead, ensembles of neurons, of which a pair is the simplest, are a means by which the ICC processes and conveys information in addition to the coding performed by individual neurons in isolation.

Permutation testing showed that local pairs of ICC neurons are functionally organized and not merely randomly arranged. Best frequency shows local consistency, as are spectral tuning, response latency, temporal response type, temporal and spectral modulation processing, and the structure of nonlinearities. Information content and the temporal resolution at which local ICC pairs perform information coding, are also marginally non-random. These parameters capture the degree to which ICC local circuits have similar receptive field preferences.

Even though these parameters are non-randomly distributed among pairs of neighboring ICC neurons, they do not all covary. Factor analysis showed that many of the parameter relationships vary independently of the others. Therefore, in any given region, while BF may be similar, other parameters can independently vary. Since some topographic functional organizations have been reported for the ICC, such as for latency, bandwidth, and modulation processing, this indicates that local parameter relationships and interaction types add further dimensions to ICC information processing. While we cannot confirm that the observed relationships are based on anatomically verifiable connections between the recorded neurons, the observed synchrony between local pairs strongly suggest that both neurons are involved in related processing activity. Their close proximity does indicate that they are participating in a functionally defined microcircuit.

Temporal interactions and functional processing

The observation of excitatory-only and excitatory/suppressive relationships of the firing in local pairs, in contrast to local pairs with no precise temporal relationship at all, adds to the notion that local input diversity and putative local circuits do influence collicular processing. Functional differences between the two clearly identifiable interactions types, such as a higher STRF similarity for pairs with EP+ST and higher nonlinearity similarity for pairs with EP only, uncovers some functional specificity of these different relationships. Yet, the strength of either type of interactions is not strongly predicted by functional similarity of the analyzed pairs, with the exception of firing rate.

It appears, then, that global distributions of response parameters within and across fibrodendritic laminae are augmented by local receptive field heterogeneity and response synchrony indicative of a functional role of local circuits in the ICC.

Comparison to previous studies

Two previous studies performed pioneering work on paired ICC neurons (Seshagiri and Delgutte, 2007, Chen et al., 2012). In the first, Seshagiri and Delgutte (2007) used single tetrode recordings in the cat to assess receptive differences between neighboring neurons. They found that the median BF difference between neurons in a pair was 0.16 octaves, consistent with our FRA results of 0.13 octaves for pure tones, likely placing the difference between the studies in the margin of error.

They also found that the correlation between CFs for neighboring neurons was 0.89 (Seshagiri and Delgutte, 2007). Their ordering of CF values was most likely random. Our randomization procedure yielded a correlation value of 0.967 for neighboring neurons, indicating that for both approaches the correlation between neighboring neuron CFs is high.

The bandwidth measures of Seshagiri and Delgutte (2007) diverged slightly from our approach. They determined bandwidths to be 0.24 octaves at approximately 5-10 dB above threshold, which is a stimulation level subject to sharp tuning and more variable spiking. When we re-calculated bandwidth differences in the same manner as Seshagiri and Delgutte (2007), we found that the median bandwidth 10 dB above threshold was 0.27 octaves, implying consistency between the studies.

Temporal response profiles of ICC neurons were also examined by Seshagiri and Delgutte (2007). They parsed pure tone PSTHs into chopper, pauser, onset, on-sustained, on-chopper, and sustained classes. Units with onset characteristics had a tendency to be members of pairs, but the overall impression was that temporal response profiles showed no systematic match between neighboring neurons. In our framework, onset, on-sustained, and on-chopper response types would have low PTI values, while the other types would have values near 0.5. Their observation diverges from our results, which showed that neighboring neurons had response types that were non-randomly organized. However, the metric used by Seshagiri and Delgutte (2007) to assess temporal response properties was categorical, while ours was continuous. Therefore, in the main, these results show that in the ICC, using pure tones, CFs are highly similar, bandwidths are slightly divergent, and temporal response types can be similar between neighboring neurons.

In a second report on ICC neighboring neurons, Chen et al. (2012) presented DMRs to cats while recording from the ICC using multiple tetrodes. Some analyses used recordings that spanned across ICC laminae, while others assessed receptive field similarity at the location of a single tetrode, most closely matching our data collected from a single electrode contact and potentially from the same lamina.

Chen et al. (2012) found that ~60% of ICC neuron pairs from a single tetrode had BFs within 0.1 octaves compatible with our findings and supporting the assumption that both neurons were members of the same lamina.

Chen et al. (2012) also examined temporal modulation processing and receptive field similarity. The range and upper bTMF values that they found were similar to our reported estimates. By contrast, receptive field similarity was different between their report and ours. They found a mean receptive field similarity of ~0.5, which is much higher than the mean value of 0.28 that we recovered. The reasons for this discrepancy are unclear, though they may be due to methodological differences, including spatial sampling procedures, and signal-to-noise ratios of the different electrodes.

Correlation analyses were also performed by Chen et al. (2012). Their goal was to compare receptive field correlations to stimulus driven correlations; thus, they wished to identify the amount of spiking correlation that is due to stimulus drive alone. Therefore, to estimate the spike train cross-covariance for two neurons and two stimulus presentations, they used the spike train of one neuron to the first stimulus, and the spike train of the other neuron to the second stimulus presentation. This approach assesses stimulus driven correlation, and is similar to the shift predictor procedure used in classical correlation analysis (Atencio and Schreiner, 2010a). In contrast, we estimated correlation functions using the spike trains from neighboring neurons in response to the same stimulus presentation. Our approach was aimed at neural functional connectivity, with an eye to determine the correlation parameters of nearby neurons that are likely due to synaptic connectivity. By comparing STRF correlation to connectivity, we were able to determine how stimulus driven activity compared to putative neural connectivity. This is also similar to assessing signal correlations (Cohen and Kohn, 2011), which are measured by correlating responses to the same stimulus, and may be approximated by correlating STRFs (Chen et al., 2012). In either situation, we found that the

STRF correlation was significantly broader than neural correlation, indicating the importance of measuring connectivity in local ICC circuits.

Therefore, in total, our findings for pure tone and dynamic stimulation are consistent with previous reports. Our findings significantly expand the notion of modest local functional similarity to a broader range of functional properties and, importantly, to distinct types of putative connectivities. Neurons with excitatory and suppressive temporal interactions had more similar STRFs than those with only excitatory correlation features. Alternatively, neurons involved with only excitatory interactions had more similar firing rates, while those with both excitatory and suppressive interactions were less similar, even when compared to randomly distributed values, indicating a strong heterogeneity for these neuron pairs. Also, the nonlinearity thresholds for neurons in excitatory interactions were the most similar, indicating that local neurons that show facilitated spiking have similar sensitivity to spectrotemporal features. Finally, we found that the degree of parametric similarity within local pairs of neurons is not a strong predictor of type or strength of neural synchrony, which is potential evidence that synchrony reflects broader network activity that goes beyond simple receptive field overlap.

We note that future reports that incorporate binaural stimulation would be helpful in delineating the function of local ICC circuits. We focused on monaural stimulation, though the ICC contains input zones that segregate according to projections from preceding stations (Oliver, 2005), such as the dorsal cochlear nucleus (DCN), lateral superior olive (LSO), and medial superior olive (MSO) (Oliver, 1984a, Oliver et al., 1997, Oliver et al., 2003, Loftus et al., 2004). The MSO and LSO, in particular, perform specialized binaural processing, and therefore further separating local pairs of neurons in these input zones may reveal similarities in local circuit processing that were masked in our data.

Relation to auditory cortex

Compared to the auditory cortex, receptive field processing in ICC local circuits appeared more heterogeneous. This holds for basic parameters, such as characteristic frequency, and for more complex measures, such as receptive field similarity and nonlinearities. The receptive field variability between nearby neurons is a consequence of the wiring properties of ICC networks and is dominated by properties of multiple converging inputs rather than local operations. Thus, though distinct regions of the ICC receive topographically precise inputs, this does not result in homogeneous processing in these regions. In contrast, auditory cortex receives strongly tonotopically organized inputs from the medial geniculate body, and these mainly terminate in granular layers. Since granular layers in AI contain neurons with very similar receptive fields, this implies that tonotopic organization is sharpened as information proceeds from the midbrain to the cortex. The extensive local networks of local cortical processing, both through feed-forward and feedback connections and multiple circuits specific for different types of interneurons, likely contribute to the higher local receptive field congruity. In addition, cortical neurons are more susceptible to plasticity effects than auditory midbrain neurons (Popescu and Polley, 2010), another feature that can contribute to increased similarity of neighboring AI neurons.

These considerations may point to different goals for each station. In the ICC, local circuit processing may simply organize the tonotopic auditory information from previous centers with more limited functional transformations, leaving intact the heterogeneous nature of ascending inputs. In contrast, AI receives information that has been combined, and local circuits perform essential refinements and transformations, such as establishing modulation preferences (Miller et al., 2001a), increasing representational robustness to background-noise (Rabinowitz et al., 2013), and creating multi-dimensional receptive fields (Atencio et al., 2008, 2009). In this regard, recurrent local networks in AI may be implementing increasingly complex representations, as evidenced by the different nature of STRFs throughout AI layers (Atencio et al., 2009, 2012). Thus, in distinction to the ICC, AI layers perform two discernible tasks: they increase stimulus specificity and they increase processing complexity (Sharpee et al., 2011).

Our present report and our earlier study in auditory cortex did not address the role of the MGB in local circuit processing. Our finding of heterogeneous ICC processing and more homogeneous AI local circuit processing does not take the thalamus into account. One possibility is that the MGB merely relays information from the ICC to AI, and then AI recurrent processing decreases the heterogeneity of functional inputs. However, the ICC to MGB anatomical projection substantially differs from the projection from MGB to AI: while thalamocortical projections are exclusively excitatory, tectothalamic projections may be both excitatory and inhibitory (Oliver, 1984b, Winer et al., 1996, Peruzzi et al., 1997, Oliver et al., 1999). Thus, 10-30% of the neurons in local ICC circuits that project to the MGB may have their functional inputs inverted (Winer et al., 1996), which may mitigate the heterogeneity of ICC inputs. Therefore, we cannot rule out that some of the local processing changes that occur from the ICC to AI may be accomplished in the MGB.

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Highlights

Synchronous firing was discovered between neurons in local midbrain circuits

Synchrony was characterized by excitatory and suppressive temporal interactions

Local neurons displayed similar information encoding and nonlinear processing

Neurons diverged in receptive fields, temporal responses, and modulation processing.

Compared to the auditory cortex, ICC circuits were more functionally heterogeneous

Figure 1.

Spike waveforms, spectrotemporal receptive fields (STRFs), and cross covariance functions. (A) Recovered waveforms from spike sorting and associated recording traces. (B) Example waveforms for two neurons recorded from the same recording contact in response to dynamic moving ripple stimulation. Spiking may occur closely in time, leading to irregular waveform shapes (Waveform Sum). The shapes may still be used to determine the spike times of the single neurons by comparing the shape to the summed waveform of the individual spikes (Gray: recording trace sample, 7 ms duration; violet: waveform sum). (C1)

Functional connectivity assessed using cross-covariance functions shows significant temporal interactions at short delays for the neurons in (B). The peak near 0 ms delay indicates that overlapping events were recovered and that the neurons were differentiated. Red lines indicate 99% confidence intervals. (C2,C3) STRFs for the neurons in (A). (D-H) Example same-contact neuron pairs. Each row shows one pair of neurons. Functional connectivity may be strictly facilitative (D,E), facilitative and suppressive (F), or purely suppressive (G).

Figure 2.

STRF correlation and functional connectivity. (A,B) STRFs for a pair of neurons recorded from the same electrode channel. Dashed line indicates slice through the peak value in the STRF. (C,D) STRF slices for the two neurons in (A,B). Amplitudes are scaled to maximum values. (E) Correlation of STRF slices in (C,D) compared to spike train cross-covariance function. Amplitudes are scaled to maximum values. (F-J) Additional example pair of neurons.

Figure 3.

Functional connectivity parameters for pairs of ICC neurons. (A) Delay at which the Excitatory Peak (EP) or Suppressive Trough (ST) occurred. The time at which the EP or ST occurred is the Peak delay. (B) Half-width is the width of the cross-covariance function at half the peak/trough height. EPs were narrower than STs. (C) Cross-correlation coefficient is the normalized magnitude of the peak/trough in the cross-covariance function. The EP distribution is shifted to higher values, indicating that EPs were stronger than STs.

Figure 4.

Information analysis for one pair of ICC neurons. Each column holds data for one of the neurons in the pair. (A1,2) Raster plots of responses to a short dynamic moving ripple (DMR) stimulus (Duration: $T = 12$ seconds). (B1,2) Post-stimulus time histogram (PSTH) from raster plots in (A). (C1,2) Information versus time bin size. Gray line indicates linear fit to data. The point at which the line deviated from the data by 20% was the time bin size used for further information analyses. (D1,2) Information versus the inverse of the stimulus duration, T . Information estimates were calculated for different durations; except for the

total stimulus duration, 100 estimates were made through random sampling. (E1,2) Mean information values from (D1,2) for each stimulus duration. (F1,2) Standard Deviation of the information estimates in (D1,2).

Figure 5.

Pairwise information analysis. (A) Average firing rate over the 12 second DMR stimulus. Each point represents one pair of neurons ($r = 0.343$, $p < 0.001$, $N = 393$, randomization test). (B) Time bin size from information analysis for each neuron in a pair ($r = 0.624$, $p < 0.001$, randomization test). (C) Information for each pair of neurons ($r = 0.460$, $p < 0.001$, randomization test).

Figure 6.

Frequency response areas (FRAs) and parameters for ICC neurons. (A) Tone stimulus and recording trace for a pair of neurons. (B) Recording trace from (A) over the interval 5-20 ms, showing three identified action potentials. (C1,2) PSTH and FRA for Neuron 1 in (A). (D1,2) PSTH and FRA for Neuron 2 in (A,B). Second example pair of neurons. (E) Comparison of characteristic frequency (CF) for each ICC pair of neurons ($r = 0.967$, $p < 0.001$, $N = 540$, randomization test). The higher CF value for each neuron was placed on the abscissa. (F) Distribution of CF differences across all ICC pairs. (G) Phasic-tonic index

(PTI) for each pair of ICC neurons ($r = 0.439$, $p < 0.001$, $N = 540$, randomization test). PTIs were estimated from the pure-tone PSTHs. PTIs near 0 indicate phasic responding neurons (initial peak in PSTH); values near 0.5 indicate tonically responding neurons (approximately flat PSTH). The larger value was placed on the abscissa. (H) PTI differences across all ICC pairs.

Figure 7.

Pairwise FRA bandwidth (BW) and spectral tuning (Q). (A) BW 10 dB above threshold for ICC neuron pairs ($r = 0.719$, $p < 0.001$, $N = 540$, randomization test). (B) BW10 differences for each pair. (C) BW40 pairwise comparison ($r = 0.859$, $p < 0.001$, $N = 436$, randomization test). (D) BW40 differences. (E) Comparison between spectral tuning 10 dB above threshold (Q10; $r = 0.323$, $p < 0.001$, $N = 540$, randomization test). (F) Q10 differences. (G) Q40 comparison ($r = 0.272$, $p < 0.001$, $N = 436$, randomization test). (H) Q40 differences. Bandwidth and Q differences are in octaves.

Figure 8.

STRF similarity for ICC pairs of neurons classified according to correlation interaction. (A) STRF similarity for ICC pairs grouped according to cross-covariance function structure. Cross-covariance functions were analyzed and classified if they had only an Excitatory Peak (EP; $N = 481$), or if they had both an Excitatory Peak and a Suppressive Trough (EP+ST; $N = 82$). (B) Comparison between cross-correlation coefficient (CCC) and STRF similarity. The CCC was calculated from the EP or ST in each cross-covariance function. EP: $r = 0.125$, $p = 0.006$, t-test; ST: $r = 0.196$, $p = 0.078$, t-test.

Figure 9.

Pairwise STRF firing rate and response precision comparisons grouped according to cross-covariance type. (A) Firing rate over the course of the DMR stimulus (total number of spikes divided by stimulus time). Pairs were grouped according to whether their cross-covariance function had an excitatory peak (EP), or both an excitatory peak and a suppressive trough (EP+ST). EP: $r = 0.265$, $p < 0.0001$, $N = 481$; EP+ST: $r = -0.276$, $p = 0.99$, $N = 82$; randomization tests). (B) Difference between firing rates for neurons in ICC pairs. *Rand* indicates differences for pairs of randomly sampled firing rates. (C) Cross-correlation coefficient (CCC) for the EPs and STs compared to firing rate difference. EP: $r = 0.41$, $p < 0.001$; ST: $r = 0.64$, $p < 0.001$; t-test. (D) Response precision index (RPI) for each pair of ICC neurons. EP: $r = 0.520$, $p < 0.001$; EP+ST: $r = 0.509$, $p < 0.001$, randomization test. (E) RPI differences. (F) CCC against RPI difference. EP: $r = 0.28$, $p < 0.001$; ST: $r = 0.27$, $p = 0.014$; t-test.

Figure 10.

STRF best frequency, spectral tuning, and latency for EPv and EP+ST cross-covariance interaction types. (A) Pairwise comparison of STRF best frequency (BF). $r=0.957$, $p<0.001$, randomization test. EP: $N = 481$; EP+ST: $N = 82$. (B) BF differences. (C) Spectral tuning (Q) comparison. $r = 0.316$, $p < 0.001$, randomization test. (D) Spectral tuning differences. *Rand* indicates differences for pairs of randomly sampled firing rates. (E) STRF Latency pairwise comparison. $r = 0.736$, $p < 0.001$, randomization test. (F) Latency differences.

Figure 11.

STRF best temporal and spectral modulation frequency for EP and EP+ST cross-covariance interaction types. (A) Pairwise comparison of STRF best temporal modulation frequency (bTMF). $r = 0.361$, $p < 0.001$, randomization test. (B) bTMF differences. *Rand* indicates differences for pairs of randomly sampled firing rates. (C) Cross-correlation coefficient (CCC) versus bTMF difference. EP: $r = 0.022$, $p = 0.64$, $N = 481$; ST: $r = 0.13$, $p = 0.26$, $N = 82$; (D) Comparison of STRF best spectral modulation frequency (bSMF). $r = 0.686$, $p < 0.001$, randomization test. (E) bSMF differences. (F) CCC versus bSMF difference. EP: $r = -0.034$, $p = 0.45$; ST: $r = 0.11$, $p = 0.31$; t-tests.

Figure 12.

Nonlinearity analysis for ICC pairs. (A,B) Schematic nonlinearity shapes. Shapes produced using parametric curve (see Methods). (A) When there is no transition noise in the nonlinearity, the nonlinearity sharply rectifies the input to produce an output spike rate. (B) Nonlinearities may also contain transition noise near response threshold. (C-F) Example STRFs and nonlinearities for a pair of ICC neurons. (C,E) STRF and nonlinearity for one neuron in the pair. (D,F) STRF and nonlinearity for the second neuron in the pair. Data points were fit with a parametric curve (examples shown in (A), (B)).

Figure 13.

Nonlinearity asymmetry and similarity for ICC pairs grouped according to EP and EP+ST cross-covariance interaction type. (A) Pairwise asymmetry index (ASI) values across ICC pairs. ASI describes symmetry about the projection = 0 vertical axis. ASIs near 1 indicate highly asymmetric nonlinearities, where firing rate response increases for positive correlations between stimuli and STRF. The majority of pairs of neurons each had asymmetric nonlinearities, regardless of cross-covariance interaction. (B) Differences

between ASIs for each pair. (C) Nonlinearity similarity (or correlation) between each nonlinearity for neurons in a pair. Pairs of ICC neurons had similar nonlinearity shapes.

Figure 14.

Parametric analysis of nonlinearities. Comparison between nonlinearity threshold (theta) and transition (sigma). (A) Transition (sigma) values for ICC nonlinearities for all recorded neurons. Values near 0 indicate that the neuron responded in a rectified manner. 1/3 of ICC have nonlinearities that indicate hard-rectification (transition ~ 0). (B) Comparison between nonlinearity transition (sigma) and threshold (theta). (C) Threshold values for all ICC nonlinearities indicate that most neurons require small STRF-stimulus correlations before they vigorously respond. (D) Pairwise nonlinearity threshold (theta) comparison. Thresholds were grouped according to cross-covariance function EP and EP+ST interaction type. (E)

Pairwise threshold differences. *Rand* indicates differences for pairs of randomly sampled firing rates. (F) Pairwise transition (sigma) comparison. (G) Pairwise transition differences.

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Figure 15.

Comparison between ICC and primary auditory cortex (AI) granular layer neuron pairs. Population comparison between parameter differences for pairs of ICC neurons, and pairs in cat AI granular layers (Atencio and Schreiner, 2013). All figure insets show Median $\pm$ MAD. (A) Cross-correlation coefficient. (B) Cross-covariance function peak delay. (C) Cross-covariance function half-width. (D) STRF similarity between pairs of neurons. (E) STRF Best frequency (BF) difference cumulative distribution function. (F) STRF Spectral tuning (Q) difference. (G) Firing rate difference. (H) STRF peak latency difference. (I) Response precision difference. (J) Nonlinearity similarity. (K) Nonlinearity asymmetry index (ASI) difference. All comparisons assessed with KS-tests.