Response Selectivity for Species-Specific Calls in the Inferior Colliculus of Mexican Free-Tailed Bats is Generated by Inhibition

ACHIM KLUG, ERIC E. BAUER, JOSHUA T. HANSON, LAURA HURLEY, JOHN MEITZEN, AND GEORGE D. POLLAK
Section of Neurobiology, University of Texas, Austin, Texas 78712

Klug, Achim, Eric E. Bauer, Joshua T. Hanson, Laura Hurley, John Meitzen, and George D. Pollak. Response selectivity for species-specific calls in the inferior colliculus of Mexican free-tailed bats is generated by inhibition. *J Neurophysiol* 88: 1941–1954, 2002; 10.1152/jn.00260.2002. Here we show that inhibition shapes diverse responses to species-specific calls in the inferior colliculus (IC) of Mexican free-tailed bats. We presented 10 calls to each neuron of which 8 were social communication and 2 were echolocation calls. We also measured excitatory response regions: the range of tone burst frequencies that evoked discharges at a fixed intensity. The calls evoked highly selective responses in that IC neurons responded to some calls but not others even though those calls swept through their excitatory response regions. By convolving activity in the response regions with the spectrogram of each call, we evaluated whether responses to tone bursts could predict discharge patterns evoked by species-specific calls. The convolutions often predicted responses to calls that evoked no responses and thus were inaccurate. Blocking inhibition at the IC reduced or eliminated selectivity and greatly improved the predictive accuracy of the convolutions. By comparing the responses evoked by two calls with similar spectra, we show that each call evoked a unique spatiotemporal pattern of activity distributed across and within isofrequency contours and that the disparity in the population response was greatly reduced by blocking inhibition. Thus the inhibition evoked by each call can shape a unique pattern of activity in the IC population and that pattern may be important for both the identification of a particular call and for discriminating it from other calls and other signals.

INTRODUCTION

The processing of species-specific communication signals has been the focus of neuroethological studies in both invertebrates and vertebrates for more than three decades. Neuroethological studies on vertebrates are typically conducted on anurans (Fuzessery and Feng 1983; Mudry and Capranica 1987), birds (Bonke 1979; Koppl et al. 2000; Marler and Doupe 2000; Scheich 1977b; Solis and Doupe 1999; Theunissen and Doupe 1998), primates (Glass and Wollberg 1983; Rauschecker and Tian 2000; Wang 2000; Winter and Funckenstein 1973), and bats (Esser et al. 1997; Kanwal et al. 1994; Ohlemiller et al. 1994, 1996), because these animals are highly vocal and their communication calls have been cataloged and studied behaviorally. With few exceptions, these studies have focused on forebrain structures and have largely left lower auditory nuclei unexplored. The inferior colliculus (IC), the principal auditory midbrain nucleus, is one of the lower auditory nuclei that has received little attention in this context. Although the IC has been the subject of numerous neurophysiological studies that employed pure tones, modulated tones, clicks, or noise as stimuli (Aitkin 1985; Carney and Yin 1989; Irvine 1986; Pollak and Park 1995; Yin et al. 1984), there have been only a few reports of how IC neurons respond to species-specific communication signals (Fuzessery and Feng 1983; Scheich 1977a), and none of those studies was conducted on mammals.

This is a significant omission because the IC is a nexus of the auditory system. It is the common target of the projections from many lower auditory nuclei (Brunso-Bechtold et al. 1981; Kelly et al. 1998; Oliver and Huerta 1992; Oliver et al. 1995; Ross et al. 1988; Roth et al. 1978) and is strongly innervated by descending projections from the auditory cortex (Huffman and Henson 1990; Saldana et al. 1996; Winer et al. 1998). It also provides the principal innervation to the medial geniculate body (Clarey 1992; Winer 1992) and thus indirectly to the auditory cortex. In short, the IC processes and integrates almost all acoustically evoked information from lower centers, is influenced by cortical activity, and also determines what form of information is conveyed to the auditory cortex. Consequently, knowing what response transformations occur in the IC has an important bearing on interpretations that assign additional transformations to processing in thalamic and cortical regions.

It was for these reasons that we studied how IC neurons respond to species-specific communication calls in Mexican free-tailed bats. These bats are highly social animals and employ a rich repertoire of spectrally and temporally complex communication calls for a variety of social interactions, including mother-infant interactions, courting, agonistic encounters, and territoriality (Balcombe and McCracken 1992; French and Lollar 2000; Gelfand and McCracken 1986; McCracken 1984). Here we show that each call evokes a wide diversity of responses among the population of IC neurons, and the diversity is shaped by inhibition. In the discussion, we argue that the response diversity creates a unique spatiotemporal pattern of activity evoked by each call and propose that the diverse patterns may be important for both the identification of a particular call and for discriminating it from other calls and other signals.

METHODS

Surgical procedures

Thirty Mexican free-tailed bats, *Tadarida brasiliensis mexicana*, were used in this study. Surgical and pharmacological procedures, electronic equipment, sound generation, and criteria for isolating single neurons are described in detail in previous publications (Bauer et al. 2000; Klug et al. 1999). Prior to surgery, each animal was anesthetized with isoflurane inhalation (IsoFlo, Abbott Labs, North Chicago, IL). The hair on the head was removed with a depilatory, and the head was secured in a head holder with a bite bar. The muscles and skin overlying the skull were reflected, and lidocaine (Abbott Labs) was applied topically to all open wounds. The surface of the skull was cleared of tissue, and a foundation layer of cyanoacrylate and small glass beads was placed on the surface. A small hole was made in the skull over the IC using landmarks visible through the skull. The bat was then transferred to a heated recording chamber, where it was placed in a restraining cushion constructed of foam molded to the animal's body. The restraining cushion was attached to a platform mounted on a custom-made stereotaxic instrument (Schuller et al. 1986). A small metal rod was cemented to the foundation layer on the skull and then attached to a bar mounted on the stereotaxic instrument to ensure a uniform positioning of the head. A ground electrode was placed between the reflected muscle and the skin.

After the animal was fixed in the stereotaxic instrument, the electrode was positioned over the IC while viewed with an operating microscope. The electrode was advanced to a depth of about 300 μm to ensure that recordings were obtained from neurons in the central nucleus of the inferior colliculus. The electrode was subsequently advanced from outside of the experimental chamber with a piezoelectric microdrive (Burleigh 7121W). Recordings were begun after the bats recovered from the anesthetic, and thus all data were obtained from awake animals. The bats typically lay quietly during the remainder of the experiments. If they showed signs of discomfort, doses of the neuroleptic ketamine hydrochloride (Vetamine, Mallinckrodt Veterinary, 1/40 dilution, 0.01 ml injection) were administered. All experimental procedures were in accordance with a protocol approved by the University of Texas Institutional Animal Care Committee.

Electrodes

"Piggyback" multibarrel micropipettes (Havey and Caspary 1980) were used for recordings and iontophoresis of drugs. Multibarrel electrodes were pulled from a five-barrel blank (A-M Systems) and blunted to 15–20 μm. A single barrel pipette was then attached to the five-barrel pipette and glued with cyanoacrylate so that the tip of the single barrel pipette protruded 10–15 μm from the blunted tip of the five-barrel pipette. The single-barrel micropipette was used for recording single-unit activity and was filled with buffered 1 M NaCl and 2% Fast Green (pH 7.4) to enhance the visibility of the electrode. One barrel of the five-barrel pipette was the balancing barrel and was filled with buffered 1 M NaCl and 2% Fast Green. The other barrels were filled with solutions of bicuculline methiodide (Sigma, St. Louis, MO), an antagonist of GABA$_A$ receptors, and with the glycine receptor antagonist, strychnine HCl (both were 10 mM in 0.165 M NaCl, pH 3.0, Sigma). In some experiments, one barrel was also filled with glutamic and aspartic acid (500 mM each in dH$_2$O, pH 9–10, Sigma). Drugs were retained in the electrode with a 15- to 20-nA retention of opposite polarity compared with the ejection current. For bicuculline and strychnine, retention currents were negative and ejection currents were positive, whereas for glutamate and aspartate, retention currents were positive and ejection currents negative. The drug and balancing barrels were connected via silver-silver chloride wires to a six-channel microiontophoresis constant current generator (Medical Systems Neurophore BH-2) that was used to generate and monitor ejection and retention currents. The sum channel was employed to balance current in the drug barrels and reduce current effects. The recording barrel was connected by a silver-silver chloride wire to a Dagan AC amplifier (model 2400).

Acoustic stimuli

Acoustic signals were tone bursts and species-specific calls. The tone bursts were 20 ms in duration with 0.2-ms rise/fall-times that were created by custom-made software and hardware. The species-specific calls consisted of a suite of 10 Mexican free-tailed bat vocalizations (Fig. 2) that were chosen from a much larger library that was previously recorded using a Racal 4-channel ultrasonic tape recorder. Eight of the 10 vocalizations that were chosen from this library were communication signals of various behavioral contexts (social calls, or SC1–8), and the remaining two vocalizations were echolocation calls (EC9–10). These calls were also used in the companion paper to evaluate responses to communication calls in the dorsal nucleus of the lateral lemniscus. The calls were played at 1/8 speed with a Racal 4DS tape recorder and digitized on an Apple computer with SoundEdit software (Macromedia) at a sampling rate of 25 kHz. This was an effective sampling rate of 200 kHz that allowed encoding of sound frequencies of up to 100 kHz, which is above the reported hearing range of this species of bat (Schmidt and Thaller 1994; Vater and Siefer 1995). The calls were then high pass filtered (2 kHz and above) and adjusted so that they were all at the same peak intensity that corresponded to the peak intensity of tone bursts at 70 dB SPL and stored as AIFF files. Thus a library of 10 species-specific sounds was created and stored for later playback through the downloadable arbitrary waveform generator. The playbacks were presented at various intensities by attenuating those signals, usually in steps of 10 dB.

Sound stimuli were delivered through a custom-made earphone (Schuller 1997) whose output was flat, within ± 5 dB, from about 8 to 70 kHz. At the start of each experiment, the earphone was inserted into the pinna contralateral to the recording site. The flexible pinna were folded onto the housing of the microphones and wrapped with Scotch tape. The acoustic cross-talk with this arrangement was at least -40 dB (Wenstrup et al. 1986).

Speaker calibrations were performed in a "free-field" setup where sounds emitted by the speaker were recorded by a 1/4-in microphone (B&K model 4135) and a measuring amplifier (B&K model 2608). The microphone in turn was calibrated with a calibrated signal of known intensity (1,000 Hz, 94 dB tone, B&K sound-level calibrator model 4230).

Data acquisition and processing

Responses to both tone bursts and the species-specific signals were recorded as peristimulus time (PST) histograms, generated by 20 repetitions of each signal presented pseudorandomly. If a call evoked a spike-count of at least 10% of the maximum spike-count evoked by any 1 of the 10 calls, that call was considered to have evoked a response. Frequencies that recruited neural inhibition were visualized against a background of pharmacologically evoked background activity and measured as gaps in this background rate. Excitatory response regions (ERRs), the range of frequencies capable of evoking discharges at a fixed intensity, were evaluated by presenting tone bursts at frequency intervals of 1.0 kHz.

One of our initial goals was to evaluate how the excitation and inhibition evoked by tones could account for the responses or lack of responses to the various species-specific calls we presented. Given the complex nature of the signals, especially the large FMs that differed from call to call, we found that we could not associate responses or lack of responses with particular features of the stimuli by visual inspections of each cell's tuning and surround inhibition. We turned instead to evaluations of how well the excitatory responses evoked by tones could predict each neuron's responses to the species-specific calls we presented. We did this by convolving two matrices. One

TABLE 1. *Peak and RMS sound pressures for tone bursts and each of the 10 species-specific calls when the attenuator was set at 0 dB*

Signal	Peak Pressure, mPa	RMS Pressure, mPa
Tone burst (20 ms @ 30 kHz)	63.2	44.6
SC1	63.2	18.6
SC2	63.2	17.0
SC3	63.2	17.3
SC4	63.2	17.1
SC5	63.2	21.7
SC6	63.2	20.3
SC7	63.2	15.9
SC8	63.2	16.3
EC9	63.2	9.8
EC10	63.2	13.6

The values represent the maximum intensities that could be presented. RMS, root-mean-square.

matrix was a spectrotemporal representation of one call (a separate matrix was computed for each call) and the other matrix represented the neuron's ERR, derived from the tone-evoked responses presented at a fixed intensity.

Each call was converted into a matrix or spectrogram (Canary 1.2.4, Cornell Bioacoustics Labs), where each grid pixel had a time resolution of 2.56 ms and a frequency resolution of 780 Hz. The time and frequency resolutions of the pixels were chosen due to constrains presented by the software. Each matrix covered times from 0 to 199.68 ms and frequencies from 0 to 81.12 kHz. The values in each pixel of the call matrix were in decibels relative to the peak intensity of 70 dB SPL. This matrix represented the call when presented at 0-dB attenuation. Calls were typically played at lower intensities, and the matrices of those calls were simply attenuated by the same number of decibels as the calls that were played. Intensities in the pixels of the call matrix whose values were below the neuron's BF threshold were set to zero. Because each call had pronounced AM, the root-mean-square (RMS) value of each call differed even though they all had the same peak intensity. The RMS levels of the tone burst and each call are shown in Table 1.

The ERR matrix had the same resolution as the call matrix, 2.56 ms by 780 Hz. The PST histograms from the neuron's ERR were con-
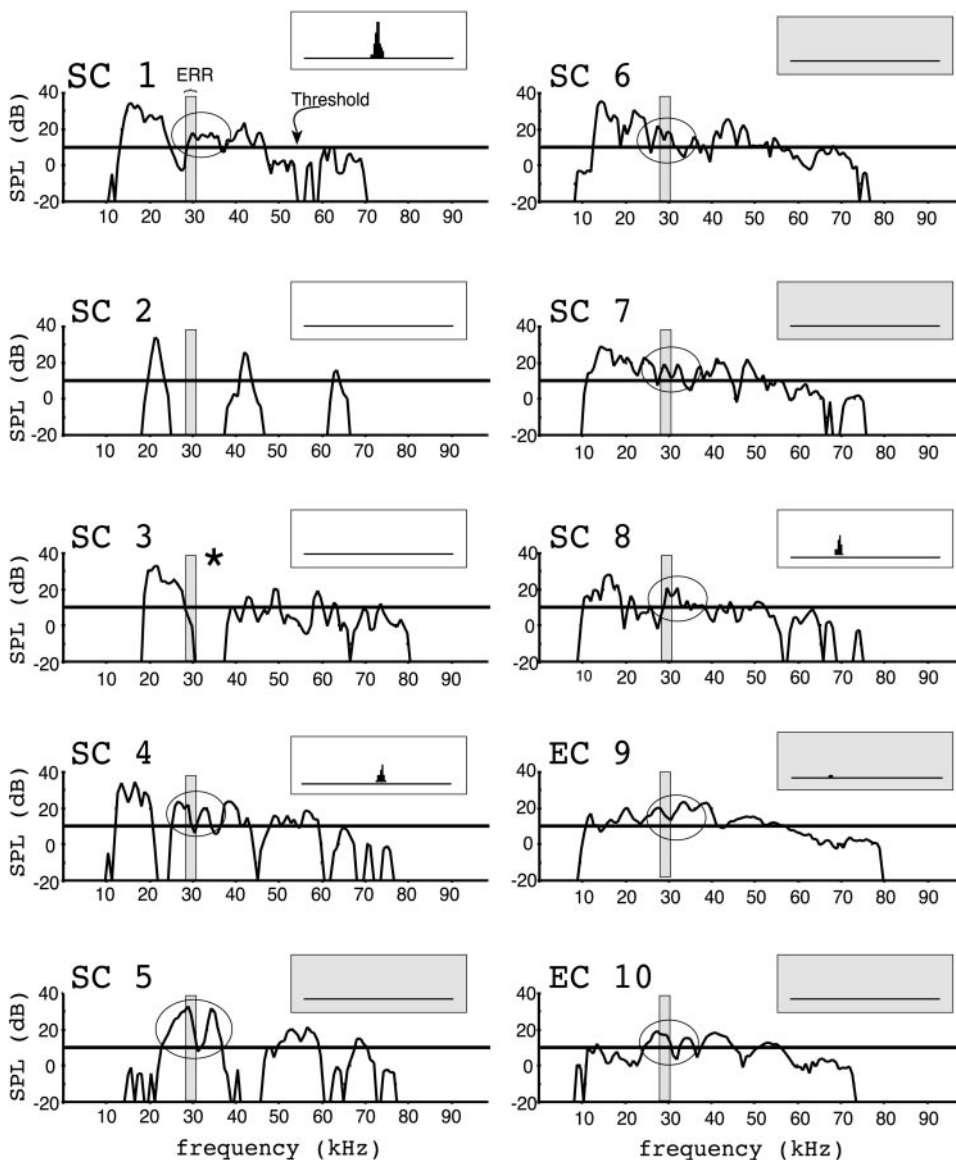

FIG. 1. Power spectra of the 10 species-specific calls and the responses evoked by each call when the calls were played at 30 dB above the best frequency (BF) threshold (10 dB SPL indicated by the solid horizontal lines). The neuron's excitatory response region (ERR), the range of frequencies that evoked responses at that intensity, is indicated by the gray vertical bars. Regions enclosed by circles show suprathreshold energy in frequencies that encroached upon the neuron's ERR. The responses or lack of responses to each of the calls are shown in the *top right portion* of each panel. Call SC2 has no circled region because none of the energy in that signal entered the ERR. For Call SC3, the energy in the frequencies corresponding to the ERR were just at or were below threshold, and SC3 evoked no response. Notice that 6 calls, SC5–7 and EC9–10, all had suprathreshold energy in frequencies that crossed the neuron's ERR, yet none of those calls evoked a response (response panels are gray filled).
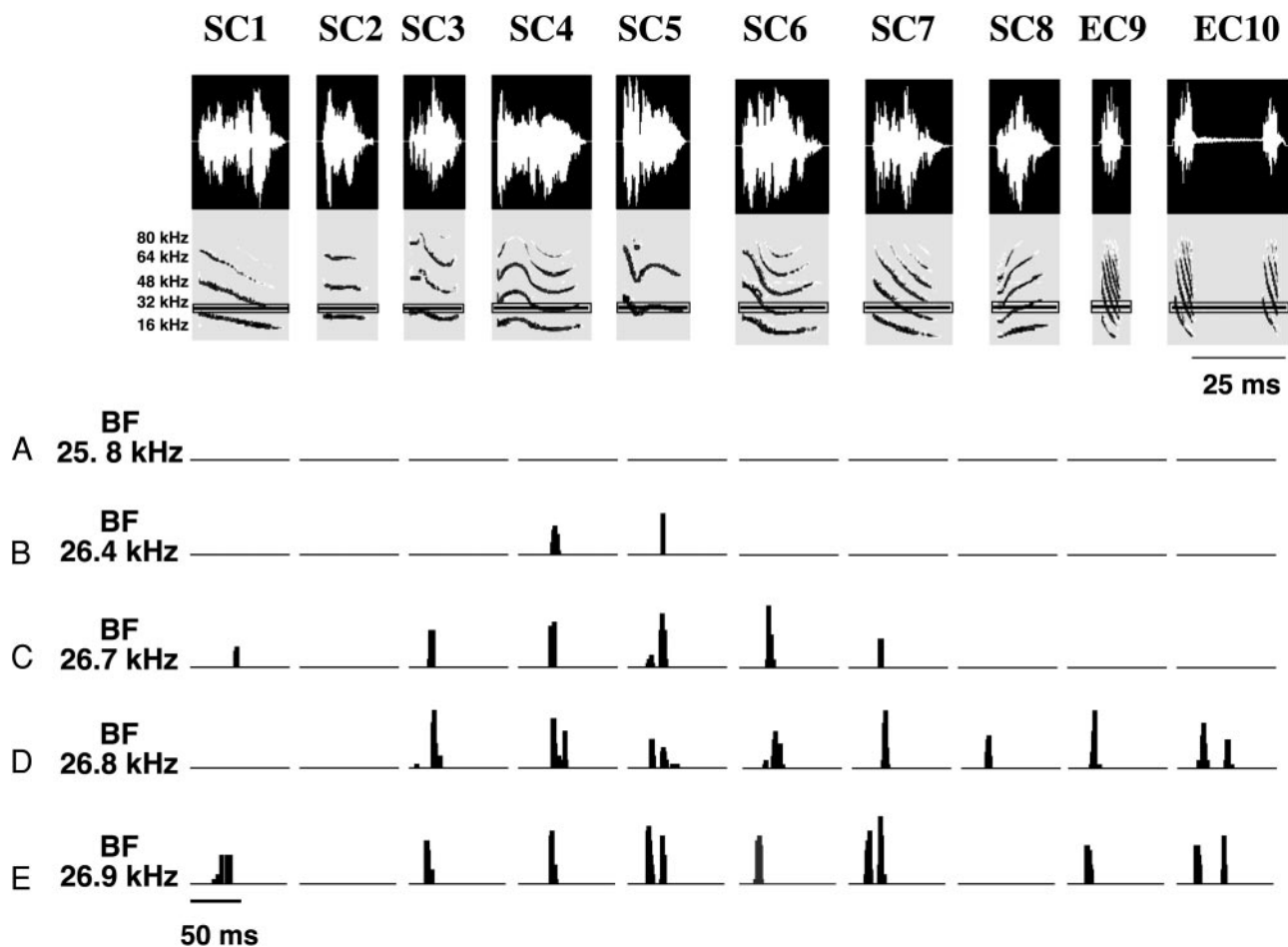
FIG. 2. Different response selectivities displayed by 5 neurons to the suite of 10 species-specific calls presented at the higher intensity, 30–50 dB above each cell's threshold. The BFs of the cells were closely aligned and ranged from 25.8 to 26.9 kHz. The horizontal bars in the sonogram of each call indicate the extent of the response regions of the 5 cells. The thick line in the center illustrates the range of the BFs of the cells. Spectral components of 9 calls swept through the excitatory response regions of all 5 cells, although none of the neurons responded to all 9 calls. None responded to call SC2, which was composed of harmonic stacks of constant frequency components. The lowest constant frequency component was about 20 kHz and did not encroach upon the response areas of any of the neurons. Note the different time scales for the calls and the peristimulus time (PST) histograms of the responses to each call. Signal intensities re threshold for: *neuron A,* 40 dB above threshold; *neuron B,* 50 dB above threshold; *neuron C,* 40 dB above threshold; *neuron D,* 30 dB above threshold; *neuron E,* 40 dB above threshold.

verted into a matrix representation of the spike-counts of tone evoked responses, normalized to the maximum count. To accommodate the 0.78-kHz frequency resolution, set by Canary, the responses obtained at 1.0-kHz increments were interpolated to yield a matrix with a 0.78-kHz resolution. Only data of the ERR at one intensity were used to construct a matrix.

The two matrices, namely the ERR matrix and the matrix of the call's spectrogram, were then convolved in a spreadsheet application (Microsoft Excel 98) using custom-programmed formulas. The resulting convolution matrix was collapsed along the frequency axis into one row of numbers, which represented an envelope of the predicted PST histogram, i.e., the envelope of the predicted firing pattern to that call. Because the envelope generated for each call had a latency, overall shape, and magnitude, we refer to these features as the neuron's "response profile" evoked by that call. Envelopes of PST histograms were calculated for each of the 10 calls and were normalized to the maximum response for any one of the 10 calls. Numbers in the bins that were smaller than 10% of the maximum peak value were set to zero to eliminate noise and to obtain the final form of the predicted spike trains. The envelopes of the PST histograms are referred to as the "predicted responses" throughout the manuscript, where each predicted response has a particular response profile. It is important to

keep in mind that the predicted responses based on the convolutions assume linearity. If a neuron is linear, then the responses in its ERR, obtained with tones, should predict how the cell responds to complex stimuli. If a cell is nonlinear, however, its ERR, measured with simple stimuli, will miss nonlinear relationships among frequencies, and thus the predictions of responses to complex sounds will not resemble the actual responses of that cell to those sounds.

The accuracies of the predicted responses were tested by comparing them to the spike trains of the cell's actual responses evoked by the same calls. The comparisons were based on the correlation coefficient for each predicted and obtained response. To create the envelope of the obtained responses, the values for each bin of the PST histograms were retrieved from the database and exported to MS Excel with the same 2.56-ms time resolution as used for the predicted responses. The obtained responses were normalized to the highest spike count evoked by any of the 10 calls, in the same way as we did for the predictions. Values smaller than 10% of the maximum peak value were set to zero to eliminate noise. The result was the envelope of the PST histogram actually evoked by one call. The envelopes of the predicted and actual spike trains of each call were cross-correlated in a 100-ms window starting 10 ms before stimulus onset. The resulting correlation coefficients (cc) were measures of how well the predicted and actual

responses matched, i.e., how well the neuron's responses to the 10 calls could be predicted based on the responses to tone bursts. Some temporal jitter was allowed in this analysis by allowing the two data sets to shift by plus or minus one time bin, or 2.56 ms, and selecting the shift that produced the highest correlation for each call. This shift was allowed to account for potential distortions due to the conversion of a continuous event into discreet time bins.

Correlating predicted and obtained responses in general provided a quantitative value of the correspondence between the two response profiles, although the temporal resolution was coarse. One reason for the coarse temporal resolution was that the binwidth of each response matrix was relatively wide, 2.56 ms, and thus the fine temporal resolution of the discharge trains evoked by each tone burst was lost. A second reason is that we used responses evoked by 20-ms tones to generate the ERR matrices. Responses evoked by tone bursts of this duration could potentially blur the temporal resolution of the convolution and thus invalidate a comparison between predicted and evoked responses. However, the phasic responses of most IC neurons together with the wide binwidth of the response matrices substantially reduced such blurring. Typically phasic neurons discharged for only a few milliseconds to 20-ms tones. Thus each discharge train typically occupied only two to three bins in each matrix. Even for neurons that fired with a sustained discharge to tones, the highest discharge rates occurred within two to three bins of each matrix and were substantially lower for the remainder of the signal.

To ensure that the tone evoked responses were not unduly smearing the predicted responses, we artificially simulated "impulse" responses in 17 neurons by only using the discharges in the major peak of the tone evoked PST histograms and manually deleting all other responses from ERR matrices in the Excel spreadsheets. We then convolved the simulated "impulse" response with each call and compared (correlated) the predicted impulse response with the response evoked by each call. We then compared, for each neuron, the correlation coefficients of the simulated impulse predicted responses with the correlation coefficients obtained from predictions computed from the ERR matrices of 20-ms tones. The correlation coefficients obtained from convolving the simulated impulse and standard 20-ms tone ERR matrices were very similar, and thus the correlation coefficients themselves were highly correlated with each other. The two values, however, differed slightly. The impulse correlations were typically about 5% higher, and the difference was statistically significant (paired, 2 tailed t-test $P < 0.05$). Thus although the impulse responses yielded a better temporal prediction than did the 20-ms tone responses, the differences were small and therefore marginal.

We also point out that due to the nature of the computations underlying the correlation algorithm, such as an overemphasis on alignment of the peaks of the two profiles without considering their absolute values, some unexpected results were occasionally obtained. Thus in some cases, a worse (or better) correlation value was returned than one would have expected based on a simple visual examination of the predicted and obtained response profiles (e.g., SC1 in Fig. 6). These uncertainties may also have been due, in part, to the coarse temporal grain by which predictions were calculated and by interpolation of discharge patterns in smaller frequency intervals than the intervals used to acquire the raw tuning curve data (e.g., Fig. 5). While the correlations present some inherent uncertainties, they nevertheless provide an overall view of the degree to which responses to tones could predict responses to complex signals.

Evaluations of inhibition

Although we could not directly incorporate inhibition into the covolutions (because we could not obtain a quantitative value of the strength and latency of inhibition at each frequency), we could determine whether inhibition was present, the range of frequencies that evoked it and the general role it played in shaping responses to the signals presented. To evaluate the overall role of inhibition in shaping responses, in many neurons we obtained both the ERR and the responses to the 10 calls before and while inhibition was blocked by the iontophoretic application of bicuculline or bicuculline and strychnine. Thus we recorded a neuron's tuning information before and during blocking inhibition and converted both sets of data into independent ERR matrices. These matrices were then used to calculate the neuron's predicted responses to the vocalizations with inhibition intact and with inhibition eliminated, respectively. The correlation coefficients of predicted and obtained responses acquired before and while inhibition was blocked were also compared.

Iontophoresis of bicuculline and strychnine

After recording a cell's responses to tones and the 10 calls, pharmacological agents were iontophoretically applied, and the responses to the same signals were recorded again. Before evaluating responses while inhibition was blocked by bicuculline or bicuculline and strychnine, we first applied a low ejection current (10 nA) while obtaining rate-level functions. During the application of the blockers, rate-level functions were repeatedly taken until the shape of the function and the maximal spike-count stabilized. The ejection current was then increased and the procedure repeated until the maximal spike-count no longer increased. The final currents ranged from 10 to 60 nA, and it was the final current that we subsequently used. Once responses were stable, the complement of tone bursts and communication calls was presented again, and the same response features were obtained for comparison with those obtained before the application of drugs. The ejection current was then switched off, and the cell was allowed to recover. Recovery was complete when both the shape and maximum spike count of the rate-level function returned to their predrug values. Because recovery times were usually 30–90 min, most neurons were lost before recovery was attained. We allowed ≥45 min before searching for another neuron in those instances.

RESULTS

We monitored the discharges evoked by tones and by communication and echolocation calls from 145 single neurons in the IC of Mexican free-tailed bats. For each neuron, we first determined the frequency to which it was most sensitive, its best frequency (BF). The BFs among our sample ranged from 10 to 47.5 kHz, although most neurons were tuned between 20 and 26 kHz. In most neurons, we also determined the neuron's excitatory response region (ERR), the range of frequencies that evoked discharges when presented at a fixed intensity. Discharges evoked by frequencies in ERRs were obtained at 10–20 dB above BF threshold and at a second intensity, 20–30 dB higher than the first intensity. We next recorded the responses evoked by the 10 natural calls shown in Fig. 2. The calls were all presented at the same peak intensity as the tones used to evaluate the ERRs. The calls were presented at the lower intensity, at 10–20 dB above threshold, to 145 neurons and at both the lower and a higher intensity to 109 of the 145 neurons. Of the 10 calls, 8 (SC1–8) were social communication calls and 2 were echolocation calls (EC9–10). These 10 calls were part of a much larger sample of complex calls composed of multiple harmonics with spectral components that change in both frequency and amplitude throughout the duration of each call. These 10 were selected for use because each had a unique temporal and spectral structure and, as a group, provides a representative sample of the various temporal and spectral features of the communication calls emitted by this

species. Thus some calls are composed of FMs that sweep downward at various sweep rates (e.g., SC1, SC7, EC9–10), other calls are more complex and have both upward and downward FMs (e.g., SC3–6, 8), whereas others have only harmonic stacks of constant frequencies (SC2) or harmonic stacks of very shallow FMs (SC1).

IC neurons responded selectively to natural calls

Almost all IC neurons responded selectively to the 10 calls in that they responded only to some of the calls and not to others, although most of the calls to which they did not respond had suprathreshold energy in their ERRs. We illustrate selectivity with the neuron in Fig. 1 and the five neurons in Fig. 2, where all signals were presented at the higher intensity in both cases. Figure 1 shows the power spectrum of each call as well as the neuron's ERR (shaded bar) and its BF threshold (horizontal line) relative to the peak intensity. The neuron did not respond to call SC2 because none of the energy in that call entered the neuron's ERR, nor did it respond to call SC3, the spectral components of which barely brushed the low-frequency border of the ERR. The other eight calls, however, had energy that entered its ERR, yet the neuron only responded to three of those calls and failed to respond to five others. Thus not only did spectral components of the eight calls enter the neuron's ERR, but those components were above threshold, and yet the neuron responded to only three of those calls.

Similar arguments apply to the five neurons in Fig. 2. The BFs of the five neurons were similar and ranged from 25.8 to 26.9 kHz. None of the neurons responded to call SC2 because none of the frequencies in call SC2 entered their ERRs. The other nine calls had spectral components that stimulated ERRs at suprathreshold levels (not shown). The five neurons displayed selectivity because they failed to respond to some of those calls. It should also be noted that while each neuron only responded to a subset of the 10 calls, the particular subset to which each neuron responded was different even though the neurons were tuned to about the same frequency and each call had energy that encroached on their response regions. *Neuron A* in Fig. 2, for example, did not respond to any of the calls whereas *neuron B* only responded to two calls, SC4 and SC5. *Neuron D* responded to eight calls, SC3-SC8 and EC9–10; *neuron E* also responded to eight calls, SC1 and SC3–7, but failed to respond to call SC8, although *neuron D* responded to call SC8.

In general, neurons were more selective at lower intensities than at higher intensities. The distributions of selectivities for the two intensities are shown in Fig. 3. On average, the 145 neurons tested at the lower intensity responded to 3.2 of the 10 calls (Fig. 3*A*). When intensity was increased to 30–50 dB above threshold, many neurons were somewhat less selective and thus responded to a larger number of calls (Fig. 3*B*). On average, the 109 neurons responded to 5.1 calls at the higher intensity. The change in selectivity with intensity was not always straightforward and differed among the population, as shown in Fig. 3*C*. Thus 11 neurons responded to fewer calls at the higher intensity than at the lower intensity. These neurons had nonmonotonic rate level functions, and it appears that higher intensities evoked a stronger inhibition than did lower intensities. The selectivities of 14 neurons were unchanged and

Selectivity to calls at lower intensity

A — n = 145, Average selectivity 3.19 calls
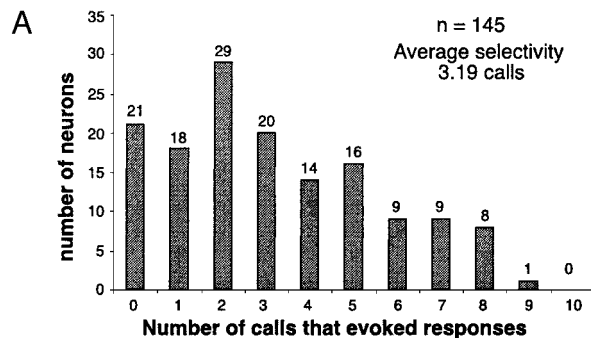
Selectivity to calls at higher intensity

B — n = 109, Average selectivity 5.07 calls
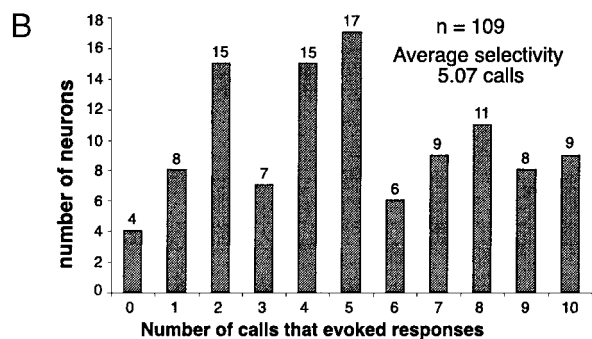
Difference in selectivity to calls when intensity was increased
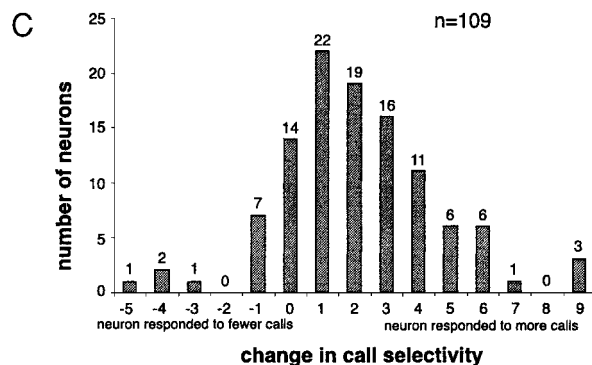
C — n=109

FIG. 3.   Distributions of selectivities to the 10 species-specific calls at the 2 intensities presented (*A* and *B*) and the changes in selectivities with intensity (*C*).

these neurons responded to the same calls at both intensities. However, 83 of 109 neurons responded to more calls at the higher intensity. In these neurons, spectral components of some calls were below threshold at the lower intensity, and thus the cells did not respond to those signals, but at the higher intensity they were suprathreshold. Nevertheless, most IC neurons were still highly selective even at the higher intensity. As we show in the following text, it was inhibition at the IC that in large part determined which signals drove the particular cell and which signals did not.

Inhibition is evoked by a wide range of frequencies

We evaluated the range of frequencies that evoked excitation and inhibition in 16 neurons as shown in the following text and found that inhibition was evoked by a broader frequency range than excitation. Inhibition was evoked by the same frequencies that evoked discharges, that is, by the frequencies in the ERR, but inhibition was also evoked by frequencies that flanked the ERR on either its low- or high-frequency side or on both sides.

The inhibition evoked by frequencies in and surrounding the ERR is illustrated by the neuron in Fig. 4. This neuron, like most others in the IC, had little or no spontaneous activity. To assess inhibition, we evoked background discharges by iontophoretically applying a cocktail of the excitatory neurotransmitters, glutamate and aspartate (glu/asp, 50–80 nA, electrode negative) and visualized inhibition as a stimulus-locked gap in the glu/asp evoked background. In Fig. 4, frequencies ranging from 25 to 27 kHz evoked discharges and comprised the neuron's ERR at 20 dB SPL. Note that each frequency in the ERR evoked both excitation and inhibition as seen by the gaps in the background that followed the discharge bursts. Additionally, inhibition was also evoked by frequencies that flanked the ERR on its high side. These features of inhibition, that the frequencies of the ERR almost always evoked a mixture of excitation and inhibition and that inhibition was evoked by frequencies that flanked the excitatory region, have a significant bearing on the interpretation of the results presented in the following sections.

Roles of inhibition for shaping discharge patterns and generating selectivity for species-specific sounds

In the preceding section, we evoked background activity in collicular neurons to visualize the inhibition evoked by tone bursts. In this section, we describe how we analyzed the impact of inhibition on response selectivity for species-specific calls in 50 neurons. In 44 of the cells, we also obtained their ERRs before and while inhibition was blocked. In these neurons, we could evaluate the degree to which the activity in their ERRs shaped the response latencies, magnitudes, and temporal discharge patterns evoked by the 10 species-specific calls. Here-

after we refer to the latency, response magnitude, and temporal discharge pattern as the neuron's response profile evoked by that signal. To evaluate how inhibition influenced response selectivities and profiles for the species-specific calls, we first recorded the responses evoked by tones and the 10 natural calls and then presented the same tones and calls while inhibitory receptors were blocked by iontophoretically applying bicuculline or bicuculline and strychnine. If inhibition at the IC shapes both the neuron's response profile and its selectivity, then blocking inhibition should change its response profiles and also reduce or eliminate selectivity. Conversely, if the selectivity for natural calls is formed in a lower nucleus, then the IC neuron should respond to the same subset of calls before inhibition was blocked as it did while inhibition was blocked, although response profiles should change because responses to tones are shaped by the inhibition evoked by frequencies in the excitatory response region.

We begin by showing that the activity produced by excitatory innervation alone, in the absence of most or all inhibitory influences, generated relatively nonselective responses that were evoked by most, and in some neurons, all of the 10 calls. We assessed the role of excitation by first blocking inhibition with bicuculline, or with bicuculline and strychnine, and documenting the ERR with tone bursts while inhibition was blocked. These responses to tones were then converted into a matrix of the ERR as described in METHODS. The graphical representations of the ERR matrices from one neuron, obtained before and while inhibition was blocked, are shown in Fig. 5. The ERR matrix obtained while inhibition was blocked was then convolved with the spectrograms from each of the 10 calls. Assuming responses are determined largely by the activity evoked by tonal signals in the neuron's response region, the convolution with each call provides a prediction of whether the neuron should respond to the call and if so, how the neuron should respond in terms of relative response magnitude, latency, and temporal discharge pattern, i.e., it should predict the neuron's discharge profile. We then compared (correlated) the predicted response profiles with the actual responses to those calls obtained while inhibition was blocked.

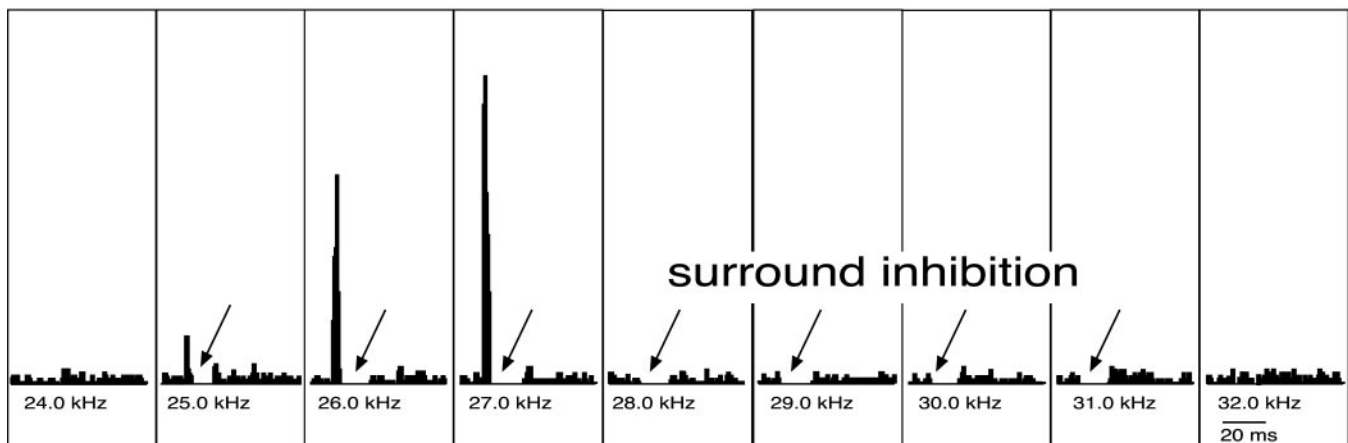In most neurons, blocking inhibition reduced or eliminated

FIG. 4. Excitatory response region and inhibitory surround of an IC neuron. Frequencies from 25 to 27 kHz evoked both an excitatory onset response followed by an inhibition, indicated by the gap in the background activity (arrows). These frequencies defined the excitatory response area. Frequencies from 28 to 31 kHz evoked only inhibition and comprised the inhibitory surround. Background activity was evoked by iontophoretic application of a cocktail of glutamate and aspartate. Sound intensity was fixed at 20 dB SPL (20 dB above threshold).

Tone evoked excitatory response region

predrug - inhibition intact
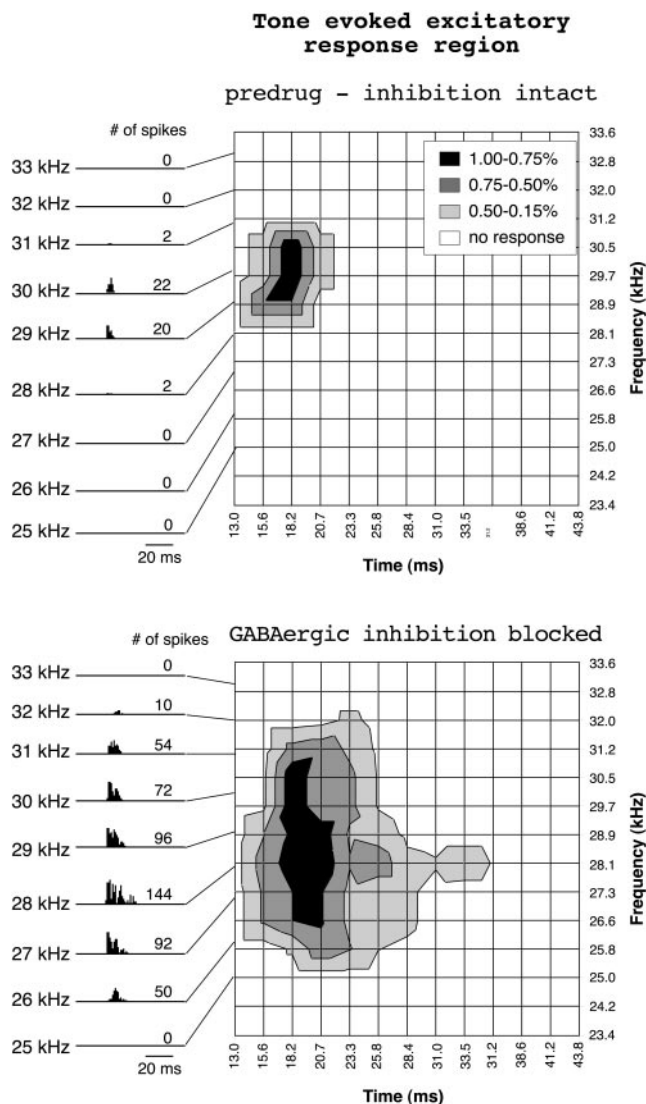

GABAergic inhibition blocked

FIG. 5. ERRs of a neuron before and while inhibition was blocked by bicuculline. Records to the *left* of each EER are post-stimulus time histograms and show the responses to tone burst at the frequencies that were used to generate the ERRs. Discharge rates in each ERR were normalized to highest rate before and while inhibition was blocked. Darker shading indicates higher discharge rate, as shown in pre-drug inset. All tone bursts were 40 dB SPL (30 dB above threshold).

selectivity. An example for responses evoked at the higher intensity is shown in Fig. 6. The graphical representation of this cell's ERR matrix is shown in Fig. 5, and its ERR relative to the power spectrum of each call is shown in Fig. 1. The predicted responses to each call, based on the convolution of the ERR matrix and the spectral-temporal features of each call, are shown in the *left panels,* while the responses actually evoked by each call are shown in the *right panels.* Notice that the response to each call is shown as the envelope of the PST histogram that either was generated by the call or predicted by the convolution. The number in the *right corner* of each panel is the correlation coefficient between the predicted and obtained response envelopes. When GABAergic inhibition was blocked by bicuculline (Fig. 6A), the neuron responded to 9 of the 10 calls; the only call to which the neuron did not respond was call SC2 because no spectral component of call SC2

entered the neuron's ERR, a failure predicted by the convolution. Note also that the convolutions accurately predicted that the neuron would respond to the other nine calls. In addition, the predicted response profiles were in close agreement with the response profiles that were actually evoked by each signal as quantified in the correlation coefficients of the predicted and evoked responses. The overall correlation for the nine calls was 0.83, and correlations of predicted and obtained responses to individual calls ranged from 0.61 to 0.93 (not including the correlation of 1.00 for call SC2 where the convolution predicted no response).

Next we look at the responses to the 10 calls in the same neuron when inhibition was intact (Fig. 6B). The convolutions predicted that the neuron should respond to nine calls because all nine calls had energy within its ERR. However, when inhibition was intact, the neuron responded only to three of the nine calls and failed to respond to six calls for which it was predicted to respond (▦ in Fig. 6B, *right*). Thus the inhibition evoked by six of the calls, calls SC3, SC5–7, and EC9–10, prevented the neuron from responding to those calls, thereby creating its response selectivity.

It is evident from the preceding text that convolutions were far less accurate in predicting whether or not the neuron would respond to each call when inhibition was intact than when it was blocked. The convolutions also appear to predict response profiles much less accurately when inhibition was intact than while it was blocked. The overall correlation of the predicted and obtained response profiles was only 0.32 when inhibition was intact, compared with the overall correlation of 0.83 when inhibition was blocked. The low overall correlation, however, is misleading because its value was skewed by the response failures to six calls that were predicted to have responses. If these calls are neglected and only those three calls for which there were both predicted and obtained responses are considered, the correlations are much higher. For two calls, SC1 and SC8, the correlations were 0.86 and 0.92, respectively. For call SC4, the correlation was only 0.42 because two bursts of discharges were predicted, whereas only one discharge burst was evoked. Thus the overall correlation of predicted and obtained responses for these three calls was 0.73.

Our explanation for these results is that each of the signals evoked varying degrees of inhibition. The signals were complex in that each displayed complex changes in both frequency and amplitude that varied from signal to signal (see Figs. 1 and 2). Some signals recruited inhibitions that completely suppressed discharges that should have been evoked based on the predictions from the neuron's ERR. That the cell did not respond to those sounds implies that strong inhibitions, not factored into the ERR matrix, were overwhelming the excitatory drives (see DISCUSSION). Presumably these inhibitions were strongly evoked by those calls for which responses were predicted but none were evoked (e.g., SC5–7). The responses to other signals (e.g., SC1 and SC8) were dominated by the excitation and inhibition evoked by frequencies in the response region, features that were partially incorporated into ERR matrix. Presumably, the influences of other inhibitions were not evoked or were evoked weakly by these signals. Thus for these signals, the convolutions provided an accurate prediction of how the neuron should respond to each of those signals.
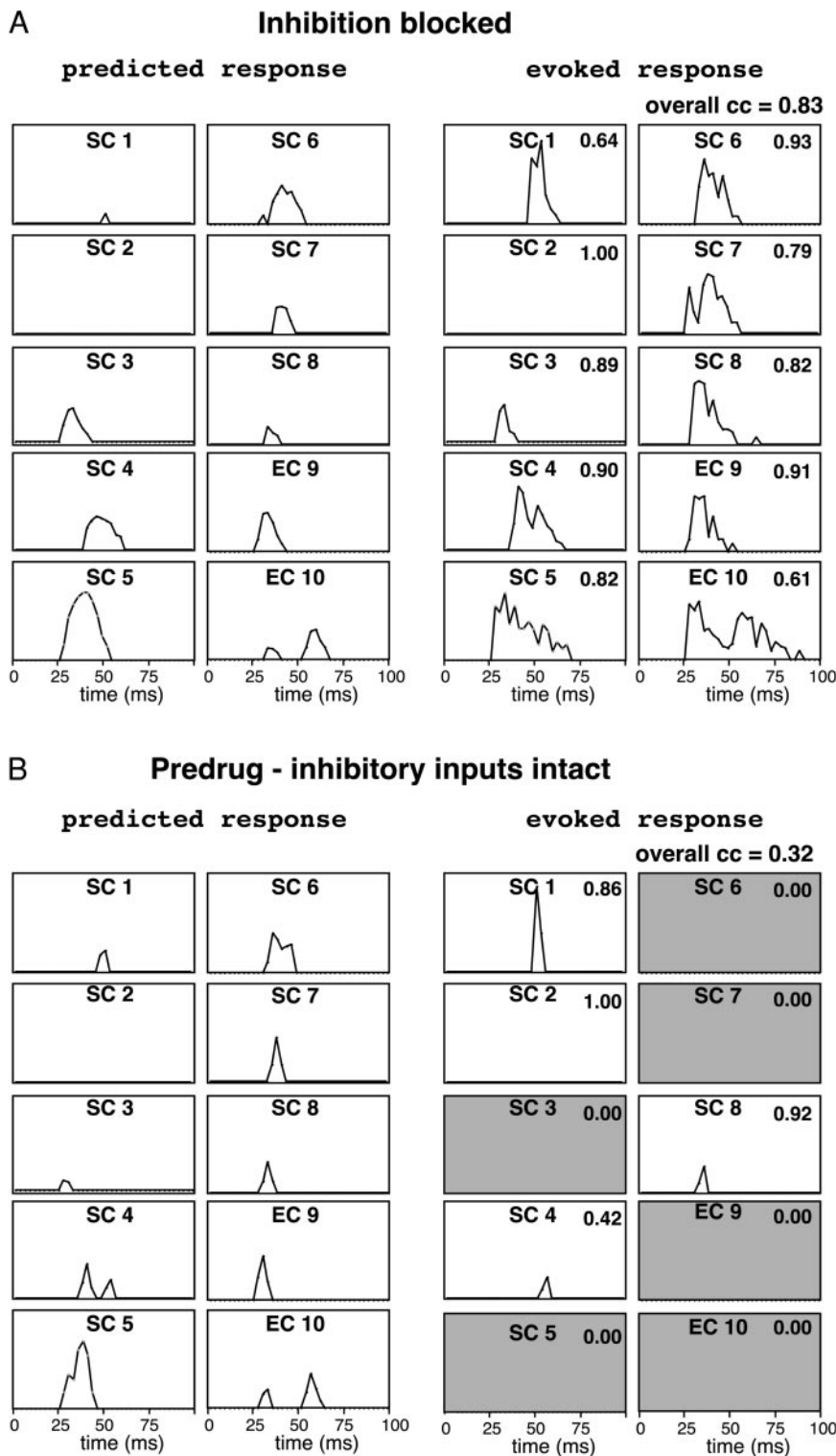
FIG. 6. Envelopes of response profiles for predicted responses and the responses actually evoked by the 10 calls. *A*: predicted and evoked responses while GABAergic inhibition was blocked by bicuculline. Numbers in *right corner* of evoked response panels show the correlation coefficients for predicted and evoked response to each call. *B*: predicted and obtained responses before inhibition was blocked. ▢, calls for which there were predicted responses but no evoked responses. The excitatory response regions of this neuron are shown in Fig. 5. All signals presented at the higher intensity (40 dB SPL) that was 30 dB above threshold.

Finally, we assume other signals were intermediate in that they most strongly evoked the excitation and inhibition in the response region and also other inhibitions but not strongly enough to completely suppress the cell. These calls, such as call SC4 for the neuron in Fig. 6, evoked responses but the discharge profiles evoked were poorly correlated with the profile predicted by convolving their ERR matrices with the spectral-temporal features of that call.

Population data

The features shown for the neuron in Fig. 6 were also seen in most other neurons, although not all neurons were as selective. As mentioned in the preceding text, in 50 neurons, we obtained responses to the 10 calls before and while inhibition was blocked, and in 44 of these, we also recorded their ERRs. The first feature shown by the neuron in Fig. 6 is that the ability
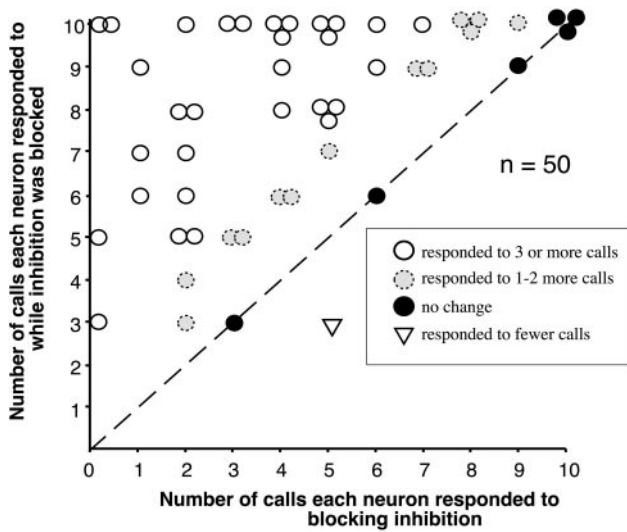
FIG. 7. Scatter plot showing changes in response selectivity when inhibition was blocked. ●, neurons that responded to the same number of calls before and while inhibition was blocked. ○ and ◎, neurons that responded to more calls while inhibition was blocked. ◎, neurons that responded to 1–2 additional calls when inhibition was blocked; ○, neurons that responded to 3–10 additional calls.

of convolutions to predict whether the neuron would respond to each of the calls was poor when inhibition was intact but improved when inhibition was blocked. We evaluated this aspect of predictability in the 44 neurons for which we obtained both excitatory response regions and responses to the 10 calls before and while inhibition was blocked. On average, the convolutions predicted responses to 7.2 calls at the higher intensity, whereas the average number of calls to which the neurons actually responded was only 4.5 calls. When inhibition
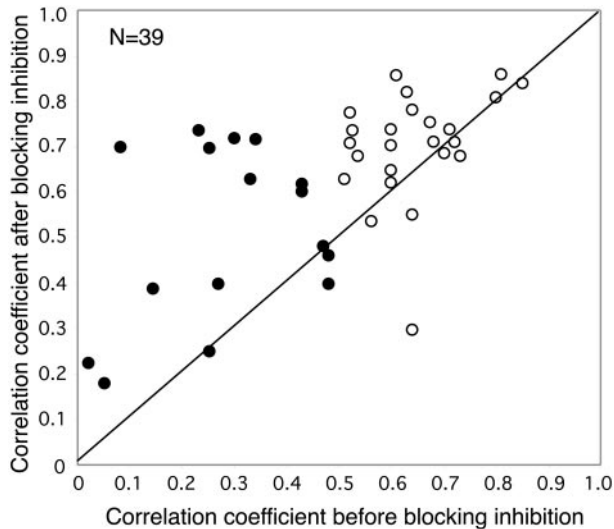

FIG. 8. Scatter plot showing changes in degree to which convolutions predicted response profile before and while inhibition was blocked. Each point represents the overall correlation coefficient for the predicted and evoked responses for 39 neurons before and while inhibition was blocked. The overall coefficients were calculated only for calls for which there were predicted and evoked responses; calls for which responses were predicted but were not evoked were not included in the calculations. ○, neurons in which the correlation was at least 0.5 before blocking inhibition. ● and ○ above the line, neurons that had higher overall correlations while inhibition was blocked than before it was blocked. All points are responses evoked by signals presented at the higher intensity.
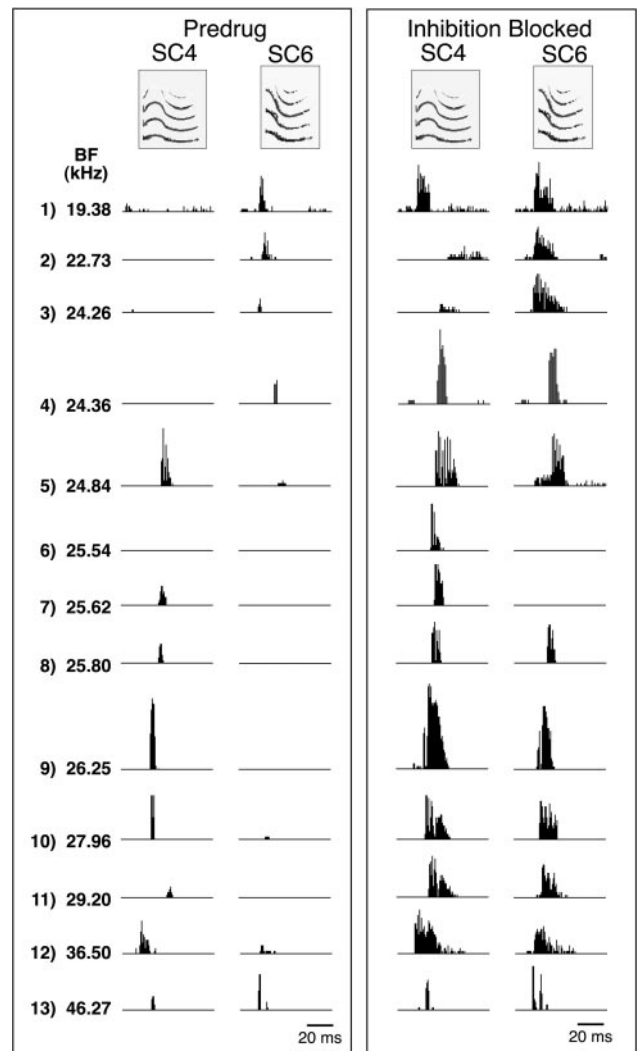

FIG. 9. Responses of 13 neurons to 2 calls, SC4 and SC6, before and while inhibition was blocked. The BFs of the neurons are arranged from low to high, which corresponds to the tonotopic organization of the IC. Both signals were presented to all neurons at the higher intensity, 30–50 dB above threshold. Note that the calls had similar spectrotemporal features but evoked different responses among the population. Before inhibition was blocked, the responses to the 2 calls differed both in the selectivities of the neurons, where 8 responded to 1 call but not the other, or in response profile for the 4 neurons that responded to both calls. Blocking inhibition greatly reduced selectivity and allowed 11 of the 13 neurons to respond to both calls. Additionally the response profiles evoked by the 2 calls were now similar in many neurons.

was blocked, the convolutions predicted an average of 7.8 calls and the neurons then responded to 6.9 calls on average.

A second feature illustrated in Fig. 6 is that the neuron was less selective and thus responded to more calls while inhibition was blocked than when it was intact. Figure 7 shows that a reduction in selectivity when inhibition was blocked was a common feature of the population. Of the 50 neurons from which we recorded responses to the 10 calls at the higher intensity, while inhibition was intact and while it was blocked, 60% (30 of 50) responded to at least three additional calls when inhibition was blocked (○) and 26% (13 of 50) responded to one to two additional signals (◎). Six neurons (12%) responded to the same number of calls (●) and one neuron responded to fewer calls while inhibition was blocked. In general, neurons that responded to only a few calls before

inhibition was blocked responded to a much larger number of calls while inhibition was blocked. Not surprisingly, neurons that originally responded to six or more calls responded to fewer or no additional calls while inhibition was blocked. Thus inhibition at the IC played a prominent role in shaping response selectivity for complex signals, and this was seen in IC neurons that were highly selective as well as in many IC neurons that were less selective.

Finally, Fig. 6 also showed that although the convolutions often predicted a response where none was evoked when inhibition was intact, those profiles that were evoked by the calls had fairly high correlations with those that were predicted. Additionally, when inhibition was blocked the correlations among the predicted and evoked profiles improved, although not to a great degree. As shown in Fig. 8, these were also features seen in many other IC neurons. This figure plots the overall coefficient that was calculated for each of 39 neurons, but only for calls for which there were predicted and evoked responses; calls for which responses were predicted but were not evoked were not included in the calculations (there were 39 neurons because 5 of the 44 did not respond to any call before blocking inhibition and thus a correlation could not be calculated for these). The overall correlations of the predicted and evoked response profiles varied among the population before inhibition was blocked. For most neurons (62%, 24/39), the correlation was at least 0.5 (○), although 38% (15/39) were below 0.5. The average correlation of the predicted and evoked discharge profiles for 39 neurons was 0.49. While inhibition was blocked, the correlations of predicted and obtained profiles increased to an average of 0.60. The largest increases were for the neurons that had a low correlation when inhibition was intact (●), whereas the overall correlations were unchanged or only slightly improved in most neurons (○) because their overall correlations were already fairly high when inhibition was intact.

How complex signals are encoded by the population of IC neurons

The features described in the preceding text suggest that at a given sound level, each call is encoded in the IC by a unique spatiotemporal pattern of activity distributed across and within isofrequency contours. We illustrate the qualitative nature of such patterns in Fig. 9, by considering the responses of 13 neurons evoked by two calls that had very similar spectrotemporal features, calls SC4 and SC6. Both calls were presented to all neurons at the higher intensity, 30–50 dB above threshold. The 13 neurons had increasingly higher BFs and are stacked from dorsal to ventral in accordance with the tonotopic organization of the IC. Eight neurons had BFs from about 22–26 kHz, which is the frequency range overrepresented in the Mexican free-tailed bat's auditory system (Bauer et al. 2000; Vater and Siefer 1995).

Because of response selectivity, only some of the neurons responded to each call. Eight neurons responded to one call but not the other (*neurons 1–4, 7–9,* and *11*), while one neuron (*neuron 6*) did not respond to either call. Four neurons responded to both calls, but the response profiles evoked by each call were different (e.g., *neurons 5* and *12*). In short, there was a pronounced difference in the "population" response to the two signals.

The difference was less pronounced when inhibition was blocked. Now 11 neurons responded to both calls, whereas only 4 did so when inhibition was intact. Moreover, the temporal discharge patterns evoked by both calls were now similar in many neurons (e.g., *neurons 1, 4,* and *8–12*). Thus although the patterns of population activity evoked by the two calls were not identical, the difference was far less pronounced when inhibition was blocked than when it was intact.

These differences in selectivities and response profiles are also reflected within isofrequency contours. Such differences are obvious both from the selectivities of the five neurons in Fig. 2 as well as from Fig. 9. Consider, for example, *neurons 3–8* in Fig. 9. The BFs of these cells are similar and span a range of about 1.5 kHz. Three of the six neurons responded to call SC4, whereas three responded to SC6. Blocking inhibition allowed all six neurons to respond to call SC4 and four neurons to respond to SC6. In short, pronounced differences in the population responses evoked by the two signals occur both within frequency contours and across contours and those differences are enhanced considerably by inhibition.

DISCUSSION

There are four main findings of this study. The first is that IC neurons display selectivity for complex signals where they respond only to some signals and not to others, even though the signals they fail to respond to have energy that encroaches on the their excitatory response regions. The second is that neurons with the same BF have different degrees of selectivity. The third finding is that almost all calls evoke both excitation and inhibition in each IC cell. The inhibition evoked by some signals completely suppresses excitation and thus is a primary determinant of the neuron's selectivity. In the same neurons, inhibition evoked by other signals does not completely suppress excitation but rather shapes the excitation and thus modifies the response profiles evoked by those calls. The fourth finding is that the preceding features endow the IC population with a spatio-temporal pattern of activity that appears to be unique for each call, suggesting that population patterns represent the particular call that the animal hears.

Contributions of inhibition for shaping response profiles and selectivities

The variety of inhibitions evoked by the species-specific calls we presented were undoubtedly complex. Just the inhibitions evoked by frequencies in the response region almost surely had multiple components. Some portions of the inhibition shaped the discharge trains evoked by the tone bursts (Bauer et al. 2000; Faingold et al. 1991, 1989; Le Beau et al. 1996; Pollak and Park 1993; Vater et al. 1992), and these influences of inhibition were indirectly factored into the matrices constructed from the ERRs. But previous studies have also shown that inhibition evoked by frequencies in the response region can occur before excitation in some IC neurons, and in most neurons, the inhibition persists for tens of milliseconds beyond the end of the signal (Bauer et al. 2000; Casseday et al. 2000; Covey and Casseday 1999; Kuwada et al. 1997; Pollak and Park 1993). Thus multiple types of inhibition may have been evoked by the frequencies in the response

region, each with its own threshold, latency and duration. Similar arguments apply to the inhibitions evoked by surrounding frequencies, which also must have had various thresholds, latencies, durations, and strengths. Surround inhibition often shapes responses to upward and downward sweeping frequency-modulated signals (Fuzessery and Hall 1996). These features of inhibition were not directly factored into the ERR matrices and almost certainly were partially responsible for the low correlations between predicted and obtained responses to some of the calls.

In this study, all of these features of inhibition were evoked by the species-specific signals that were strongly modulated in both amplitude and frequency. Thus not only were frequencies changing throughout the call, but the amplitudes of the spectral components present at any moment were also changing, and the patterns of these modulations differed from call to call. It follows that each call must have evoked different sequences of inhibition, where the response or suppression of response to that call was determined by the timing and strength of the inhibition relative to the timing and strength of the excitation.

Viewed in this way, it is not surprising that the convolutions of the ERRs and spectrograms of each call often predicted responses when none were evoked. The flip side of this view is that it seems remarkable that the convolutions could have accurately predicted the response profile to any call. As mentioned previously, we assume that some calls evoked restricted patterns of excitation and inhibition that were dominated by activity reflected in the neuron's ERR, and it was the response profiles to these calls that were predicted by the convolutions.

Inhibition, however, may also be evoked by other spectral regions that are far removed from frequencies in the excitatory response region and the surround. Potentially, the inhibition evoked by these spectral regions may affect response profiles or selectivities by a more subtle, nonlinear mechanism, called combination sensitivity (Mittmann and Wenstrup 1995; Suga 1992). Combination sensitivity is prevalent in the forebrain and contributes substantially to the response selectivity to complex signals (Doupe 1997; Esser et al. 1997; Fitzpatrick et al. 1993; Kanwal et al. 1994; Margoliash and Fortune 1992; Olsen and Suga 1991; Rauschecker et al. 1997; Sutter and Schreiner 1991). It was previously thought that combination sensitivity was first generated in the forebrain, although recent studies in mustache bat show conclusively that it is an emergent property of the IC (Mittmann and Wenstrup 1995; Portfors and Wenstrup 2001; Wenstrup and Leroy 2001). Combination sensitivity is produced by subthreshold influences from frequencies an octave or more removed from the neuron's BF. It can be demonstrated when two frequencies, the BF and the distant frequency, are presented together in the appropriate temporal sequence. Under these conditions, the distant frequency either suppresses or facilitates the response evoked by the BF. Wenstrup and Leroy (2001) showed that both the facilitative as well as the inhibitory influences of the distant frequency in the IC could be blocked by strychnine, suggesting that combination sensitivity is mediated by glycinergic inhibition. Combination sensitivity in the IC, however, has only been demonstrated in mustache bats, where about half of the IC neurons are combination sensitive (Portfors and Wenstrup 2001). It is unclear whether combination sensitivity also occurs in the IC of other mammals, including Mexican free-tailed bats, because the tests that would reveal it have never been applied in studies of the

IC in other mammals. However, if combination sensitivity is indeed present in some of the IC neurons of Mexican free-tailed bats, then it most likely contributed to the response profiles or selectivities in those cells and would have been eliminated when inhibition was blocked with the cocktail of bicuculline and strychnine that we applied to many cells.

Selectivity sharpens differences in the population response to complex calls

Perhaps the most significant feature of the IC, and one that we now emphasize, is that the subset of calls that evoked responses differed markedly from neuron to neuron, and this was true among neurons tuned to the same frequency or similar frequencies as illustrated in Fig. 2. In other words, the selectivity was remarkably diverse among isofrequency neurons and such diverse selectivity is a feature of particular importance for encoding complex signals. One role that selectivity may play is to accentuate the population response evoked by any given call in and across isofrequency contours. To develop this argument, consider first what the population response in an isofrequency contour would be if IC neurons were not selective, i.e., if most neurons in the contour responded to a given call, as occurred when inhibition was blocked. Because the active neurons in a contour express diverse response profiles, a given call would evoke a particular spatiotemporal pattern across that population. The response profile of each neuron, in turn, would change with the particular call that was heard. Thus even without selectivity, one could imagine that each call would drive most neurons in each contour and generate a population response profile that was different from the population profile evoked by any other call of the same intensity.

Next consider the effect of adding selectivity, where each neuron responds only to some calls and not to others. Selectivity must increase the contrast of the spatiotemporal pattern across the population evoked by any call because it creates regions of inactivity. Thus instead of evoking a near continuous profile of activity across the population of a contour, selectivity imposes regions of inactivity dispersed among active regions, which would be reiterated in each isofrequency contour. Additionally, because IC neurons express different selectivities, the loci of the inactive regions in a contour would shift with each call.

We therefore view selectivity in the IC as a feature that accentuates the population response evoked by a call in a manner similar to the way that lateral inhibition in the somatosensory system enhances the discrimination of stimuli that impinge on neighboring portions of the sensory surface (Gardner and Kandel 2000). There, lateral inhibition suppresses the excitation of neurons between the neuronal populations excited by two or more stimuli, thereby sharpening the spatial clarity of the excited populations. In the IC, selectivity inserts regions of suppressed neurons between regions of active neurons. What is analogous to the lateral inhibition described above is that groups of active IC neurons would be more clearly demarcated from neighboring active groups because the various active groups would be separated by regions of suppression.

Communication calls convey specific types of information and are emitted in specific social situations. It follows that the animal has to recognize each call and discriminate it from other calls and other signals. Presumably then, the auditory system

has to encode each call in some unique fashion. Our results show that in most neurons selectivity at very low intensities was different from selectivity at a higher intensity, but our results did not address the question of whether or not selectivity remains stable over a range of higher intensities. Thus the degree to which the pattern of activity across the population evoked by each communication call remains stable at various higher intensities is unclear. What our results do suggest, however, is that a call having a particular intensity will generate not only a unique population response but also a population response more pronounced than the population response that would be evoked if IC neurons were unselective. It is such unique patterns of activity that the IC then presents to higher regions in the forebrain for further processing and elaborations.

REFERENCES

AITKIN LM. *The Auditory Midbrain: Structure and Function in the Central Auditory Pathway.* Clifton, NJ: Humana Press, 1985.

BALCOMBE JP AND MCCRACKEN GF. Vocal recognition in Mexican free-tailed bats: do pups recognize mothers? *Anim Behav* 43: 79–87, 1992.

BAUER EE, KLUG A, AND POLLAK GD. Features of contralaterally evoked inhibition in the inferior colliculus. *Hear Res* 141: 80–96, 2000.

BONKE D, SCHEICH H, AND LANGNER G. Responses of units in the auditory neostriatum of the guinea fowl (*Numida meleagris*) to species-specific calls and synthetic stimuli. *J Comp Physiol [A]* 132: 243–255, 1979.

BRUNSO-BECHTOLD JK, THOMPSON GC, AND MASTERTON R B. HRP study of the organization of auditory afferents ascending to central nucleus of inferior colliculus in cat. *J Comp Neurol* 197: 705–722, 1981.

CARNEY LH AND YIN TC. Responses of low-frequency cells in the inferior colliculus to interaural time differences of clicks: excitatory and inhibitory components. *J Neurophysiol* 62: 144–161, 1989.

CASSEDAY JH, EHRLICH D, AND COVEY E. Neural measurement of sound duration: control by excitatory-inhibitory interactions in the inferior colliculus. *J Neurophysiol* 84: 1475–1487, 2000.

CLAREY JC, BARONE P, AND IMIG TJ. Physiology of thalamus and cortex. In: *The Mammalian Auditory Pathway: Neurophysiology*, edited by Webster DB, Popper AN, and Fay RR. New York: Springer-Verlag, 1992, p. 232–334.

COVEY E AND CASSEDAY JH. Timing in the auditory system of the bat. *Annu Rev Physiol* 61: 457–476, 1999.

DOUPE AJ. Song- and order-selective neurons in the songbird anterior forebrain and their emergence during vocal development. *J Neurosci* 17: 1147–1167, 1997.

ESSER KH, CONDON CJ, SUGA N, AND KANWAL JS. Syntax processing by auditory cortical neurons in the FM-FM area of the mustached bat *Pteronotus parnellii. Proc Natl Acad Sci USA* 94: 14019–14024, 1997.

FAINGOLD CL, BOERSMA ANDERSON CA, AND CASPARY DM. Involvement of GABA in acoustically evoked inhibition in inferior colliculus neurons. *Hear Res* 52: 201–216, 1991.

FAINGOLD CL, GEHLBACH G, AND CASPARY DM. On the role of GABA as an inhibitory neurotransmitter in inferior colliculus neurons: iontophoretic studies. *Brain Res* 500: 302–312, 1989.

FITZPATRICK DC, KANWAL JS, BUTMAN JA, AND SUGA N. Combination-sensitive neurons in the primary auditory cortex of the mustached bat. *J Neurosci* 13: 931–940, 1993.

FRENCH B AND LOLLAR A. Communication among Mexican free-tailed bats. *Bats: Bat Conserv Int* 18: 1–4, 2000.

FUZESSERY ZM AND FENG AS. Mating call selectivity in the thalamus and midbrain of the leopard frog (*Rana pippens*): single- and multiunit analyses. *J Comp Physiol [A]*. 150: 333–344, 1983.

FUZESSERY ZM AND HALL JC. Role of GABA in shaping frequency tuning and creating FM sweep selectivity in the inferior colliculus. *J Neurophysiol* 76: 1059–1073, 1996.

GARDNER EP AND KANDEL ER. Touch. In: *Principles of Neuroscience* (4th ed.), edited by Kandel ER, Schwartz JH, and Jessell TM. New York: McGraw-Hill, 2000, p. 451–471.

GELFAND DL AND MCCRACKEN GF. Individual variation in the isolated calls of Mexican free-tailed bat pups. *Anim Behav* 34: 1078–1086, 1986.

GLASS I AND WOLLBERG Z. Auditory cortex responses to sequences of normal and reversed squirrel monkey vocalizations. *Brain Behav Evol* 22: 13–21, 1983.

HAVEY DC AND CASPARY DM. A simple technique for constructing 'piggyback' multibarrel microelectrodes. *Electroenc Clin Neurophys* 48: 249–251, 1980.

HUFFMAN RF AND HENSON OW JR. The descending auditory pathway and acousticomotor systems: connections with the inferior colliculus. *Brain Res Brain Res Rev* 15: 295–323, 1990.

IRVINE DRF. The auditory brain stem. In: *Progress in Sensory Physiology*, edited by Autrum H and Ottoson D. Berlin: Springer-Verlag, 1986.

KANWAL JS, MATSUMURA S, OHLEMILLER K, AND SUGA N. Analysis of acoustic elements and syntax in communication sounds emitted by mustached bats. *J Acoust Soc Am* 96: 1229–1254, 1994.

KELLY JB, LISCUM A, VAN ADEL B, AND ITO M. Projections from the superior olive and lateral lemniscus to tonotopic regions of the rat's inferior colliculus. *Hear Res* 116: 43–54, 1998.

KLUG A, BAUER EE, AND POLLAK GD. Multiple components of ipsilaterally evoked inhibition in the inferior colliculus. *J Neurophysiol* 82: 593–610, 1999.

KOPPL C, MANLEY GA, AND KONISHI M. Auditory processing in birds. *Curr Opin Neurobiol* 10: 474–481, 2000.

KUWADA S, BATRA R, YIN TC, OLIVER DL, HABERLY LB, AND STANFORD TR. Intracellular recordings in response to monaural and binaural stimulation of neurons in the inferior colliculus of the cat. *J Neurosci* 17: 7565–7581, 1997.

LE BEAU FE, REES A, AND MALMIERCA MS. Contribution of GABA- and glycine-mediated inhibition to the monaural temporal response properties of neurons in the inferior colliculus. *J Neurophysiol* 75: 902–919, 1996.

MARGOLIASH D AND FORTUNE ES. Temporal and harmonic combination-sensitive neurons in the zebra finch's HVc. *J Neurosci* 12: 4309–4326, 1992.

MARLER P AND DOUPE AJ. Singing in the brain. *Proc Natl Acad Sci USA* 97: 2965–2967, 2000.

MCCRACKEN GF. Communal nursing in Mexican free-tailed bat maternity colonies. *Science* 223: 1090–1091, 1984.

MITTMANN DH AND WENSTRUP JJ. Combination-sensitive neurons in the inferior colliculus. *Hear Res* 90: 185–191, 1995.

MUDRY KM AND CAPRANICA RR. Correlation between auditory thalamic area evoked responses and species-specific call characteristics. II. H. *Hyla cinerea* (Anura: Hylidae). *J Comp Physiol [A]* 161: 407–416, 1987.

OHLEMILLER KK, KANWAL JS, BUTMAN JA, AND SUGA N. Stimulus design for auditory neuroethology: synthesis and manupulation of complex communication sounds. *Aud Neurosci* 1: 19–38, 1994.

OHLEMILLER KK, KANWAL JS, AND SUGA N. Facilitative responses to species-specific calls in cortical FM-FM neurons of the mustached bat. *Neuroreport* 7: 1749–1755, 1996.

OLIVER DL, BECKIUS GE, AND SHNEIDERMAN A. Axonal projections from the lateral and medial superior olive to the inferior colliculus of the cat: a study using electron microscopic autoradiography. *J Comp Neurol* 360: 17–32, 1995.

OLIVER DL AND HUERTA MF. Inferior and superior colliculi. In: *The Mammalian Auditory Pathway: Neuroanatomy*, edited by Webster DB, Popper AN, and Fay RR. New York: Springer-Verlag, 1992, p. 168–222.

OLSEN JF AND SUGA N. Combination-sensitive neurons in the medial geniculate body of the mustached bat: encoding of relative velocity information. *J Neurophysiol* 65: 1254–1274, 1991.

POLLAK GD AND PARK TJ. The effects of GABAergic inhibition on monaural response properties of neurons in the mustache bat's inferior colliculus. *Hear Res* 65: 99–117, 1993.

POLLAK GD AND PARK TJ. The inferior colliculus. In: *Hearing by Bats, Springer Handbook of Auditory Research*, edited by Popper A and Fay RR. Berlin-Heidelberg: Springer-Verlag, 1995, p. 296–367.

PORTFORS CV AND WENSTRUP JJ. Topographical distribution of delay-tuned responses in the mustached bat inferior colliculus. *Hear Res* 151: 95–105, 2001.

RAUSCHECKER JP AND TIAN B. Mechanisms and streams for processing of "what" and "where" in auditory cortex. *Proc Natl Acad Sci USA* 97: 11800–11806, 2000.

RAUSCHECKER JP, TIAN B, PONS T, AND MISHKIN M. Serial and parallel processing in rhesus monkey auditory cortex. *J Comp Neurol* 382: 89–103, 1997.

ROSS LS, POLLAK GD, AND ZOOK JM. Origin of ascending projections to an isofrequency region of the mustache bat's inferior colliculus. *J Comp Neurol* 270: 488–505, 1988.

ROTH GL, AITKIN LM, ANDERSEN RA, AND MERZENICH MM. Some features of the spatial organization of the central nucleus of the inferior colliculus of the cat. *J Comp Neurol* 182: 661–680, 1978.

SALDANA E, FELICIANO M, AND MUGNAINI E. Distribution of descending projections from primary auditory neocortex to inferior colliculus mimics the topography of intracollicular projections. *J Comp Neurol* 371: 15–40, 1996.

SCHEICH H, LANGNER G, AND KOCH R. Coding of narrow-band and wide-band vocalizations in the auditory midbrain nucleus (MLD) of the Guinea fowl (*Numida melearis*). *J Comp Physiol [A]* 117: 245–265, 1977a.

SCHEICH H, LANGNER G, AND KOCH R. Coding of narrow-band and wide-band vocalizations in the auditory midbrain nucleus (MLD) of the guinea fowl (*Numida meleagris*). *J Comp Physiol [A]* 117: 245–265, 1977b.

SCHMIDT S AND THALLER J. Temporal auditory summation in the echolocating bat *Tadarida brasiliensis*. *Hear Res* 77: 125–134, 1994.

SCHULLER G. A cheap earphone for small animals with good frequency responses in the ultrasonic frequency range. *J Neurosci Methods* 71: 187–190, 1997.

SCHULLER G, RADTKE-SCHULLER S, AND BETZ M. A stereotaxic method for small animals using experimentally determined reference profiles. *J Neurosci Methods* 18: 339–350, 1986.

SOLIS MM AND DOUPE AJ. Contributions of tutor and bird's own song experience to neural selectivity in the songbird anterior forebrain. *J Neurosci* 19: 4559–4584, 1999.

SUGA N. Philosophy and stimulus design for neuroethology of complex-sound processing. *Philos Trans R Soc Lond B Biol Sci* 336: 423–428, 1992.

SUTTER ML AND SCHREINER CE. Physiology and topography of neurons with multipeaked tuning curves in cat primary auditory cortex. *J Neurophysiol* 65: 1207–1226, 1991.

THEUNISSEN FE AND DOUPE AJ. Temporal and spectral sensitivity of complex auditory neurons in the nucleus HVc of male zebra finches. *J Neurosci* 18: 3786–3802, 1998.

VATER M, HABBICHT H, KOSSL M, AND GROTHE B. The functional role of GABA and glycine in monaural and binaural processing in the inferior colliculus of horseshoe bats. *J Comp Physiol [A]* 171: 541–553, 1992.

VATER M AND SIEFER W. The cochlea of *Tadarida brasiliensis*: specialized functional organization in a generalized bat. *Hear Res* 91: 178–195, 1995.

WANG X. On cortical coding of vocal communication sounds in primates. *Proc Natl Acad Sci USA* 97: 11843–11849, 2000.

WENSTRUP JJ AND LEROY SA. Spectral integration in the inferior colliculus: role of glycinergic inhibition in response facilitation. *J Neurosci* 21: RC124, 2001.

WENSTRUP JJ, ROSS LS, AND POLLAK GD. Binaural response organization within a frequency band representation of the inferior colliculus: implications for sound localization. *J Neurosci*: 6: 962–973, 1986.

WINER JA. Functional architecture of the medial geniculate body and primary auditory cortex. In: *The Mammalian Auditory Pathway: Neuroanatomy*, edited by Webster DB, Popper AN, and Fay RR. New York: Springer-Verlag, p. 222–409, 1992.

WINER JA, LARUE DT, DIEHL JJ, AND HEFTI BJ. Auditory cortical projections to the cat inferior colliculus. *J Comp Neurol* 400: 147–174, 1998.

WINTER P AND FUNCKENSTEIN HH. The effect of species-specific vocalization on the discharge of auditory cortical cells in the awake squirrel monkey. *Exp Brain Res* 18: 489–504, 1973.

YIN TC, KUWADA S, AND SUJAKU Y. Interaural time sensitivity of high-frequency neurons in the inferior colliculus. *J Acoust Soc Am* 76: 1401–1410, 1984.