JARO 02: 104–117 (2001)
DOI: 10.1007/s101620010057

JARO
Journal of the Association for Research in Otolaryngology

Responses to Combinations of Tones in the Nuclei of the Lateral Lemniscus

CHRISTINE V. PORTFORS AND JEFFREY J. WENSTRUP

Department of Neurobiology and Pharmacology Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272, USA

Received: 10 July 2000; Accepted: 20 December 2000; Online publication: 4 May 2001

ABSTRACT

Combination-sensitive neurons integrate specific spectral and temporal elements in biologically important sounds, and they may underlie the analysis of biosonar and social vocalizations. Combination-sensitive neurons are found in the forebrain of a variety of vertebrates. In the mustached bat, they also occur in the central nucleus of the inferior colliculus (ICC). However, it is not known where combination-sensitive response properties emerge. To address this question, we used a two-tone paradigm to examine responses of single units to combination stimuli in a brainstem structure, the nuclei of the lateral lemniscus (NLL). We recorded and histologically localized 101 single units in the NLL. The majority (82%) of units had a single excitatory frequency tuning curve. Seven units had two separate excitatory frequency tuning curves but displayed no combinatorial interaction. Twelve units were combination-sensitive. Of these, three units were facilitated by the combination of two separate frequency bands and nine units were inhibited by combinatorial stimuli. The three facilitatory neurons had excitatory responses tuned to the second harmonic constant frequency (CF2, 57–60 kHz) component of the biosonar signal and were facilitated by a second signal within the first harmonic (Hl, 24–30 kHz) of the biosonar call. Most of the inhibitory interactions occurred between signals in the frequency bands associated with the frequency-modulated (FM) components of the biosonar call. The strongest combinatorial effects (facilitatory and inhibitory) were elicited by simultaneous onset of the two signals (i.e., 0 ms delay). All combination-sensitive units were in the intermediate nucleus of the NLL (INLL), which in bats is a hypertrophied structure that projects strongly to combination-sensitive neurons in the ICC. Thus, the combination-sensitive neurons in the INLL may impart their response properties onto ICC neurons. However, the small number of facilitatory combination-sensitive neurons in the NLL suggests that the majority of these combinatorial responses originate in the ICC.

Keywords: lateral lemniscus, combination-sensitive, mustached bat, spectral integration

INTRODUCTION

Acoustic communication in many animals depends on spectrally and temporally complex sounds that provide biologically relevant information to conspecifics. Analysis of these sounds begins at the cochlea, where the complex sounds are separated into narrowband frequency components. This frequency-by-frequency representation is maintained in the auditory nerve and at low levels of the ascending auditory system. In the auditory nerve and cochlear nucleus, spectral and temporal elements of complex sounds are represented by the discharge patterns of neurons in the form of rate-place or temporal-place codes (Young and Sachs 1979; Sachs and Young 1979; Miller and Sachs 1983; Sinex and Geisler 1983; Delgutte and Kiang 1984a, b, c; Blackburn and Sachs 1990). However, by the level of auditory cortex, there is evidence that the encoding and representation of complex sounds is performed by neurons that integrate specific spectral and temporal elements within the complex sounds. In auditory cortex, the neuronal response features suggestive of spectral and temporal integration include multiple

Correspondence to: Dr. Christine V. Portfors, Ph.D • Department of Neurobiology and Pharmacology Northeastern Ohio Universities College of Medicine • 4209 State Route 44 • P.O. Box 95 • Rootstown, OH 44272-0095. Telephone: (330) 325-6651; fax: (330) 325-5916; email: cvp@neoucom.edu

frequency tuning peaks, enhanced sensitivity to noise, and/or facilitatory responses to the combination of two distinctly different frequency elements (Suga et al. 1978; Fuzessery and Feng 1983; Schreiner and Cynader 1984; Margoliash and Fortune 1992; Rauschecker et al. 1995; Sutter and Schreiner 1991; Ohl and Scheich 1997; Rauschecker 1998; Brosch et al. 1999; Fritz 2000; Kadia et al. 2000.

One form of spectral integration present in the forebrain of a variety of vertebrates is performed by *combination-sensitive* neurons. These neurons respond best to combinations of spectrally separate elements in complex sounds when the elements are in an appropriate temporal relationship. Furthermore, combinatorial neurons are selective for particular spectral and temporal combinations of elements within species-specific vocalizations. This selectivity often supports the neurons' abilities to discriminate among social vocalizations (Margoliash and Fortune 1992; Ohlemiller et al. 1996; Esser et al. 1997; Rauschecker et al. 1995; Rauschecker 1998). Moreover, it has been suggested that combination-sensitive neurons underlie the encoding of phonemic elements of speech sounds (Suga 1996; Sussman et al. 1998). Although combination-sensitive response properties have been described in the forebrain of a variety of vertebrates, it is unclear where in the ascending auditory pathway these combinatorial response properties emerge. This study investigates whether combination-sensitive neurons occur in the auditory brainstem of the mustached bat.

There are many types of combination-sensitive neurons in the auditory system of the mustached bat, each of which is characterized by particular frequency tuning and temporal tuning. One type of combination-sensitive neuron that has receive much attention is the FM–FM neuron. The frequency tuning of this neuron corresponds to frequencies contained in the frequency-modulated (FM) components of the mustached bat's multiharmonic biosonar call. FM–FM neurons have excitatory frequency tuning curves in the 48–56 kHz (FM2), the 72–87-kHz (FM3) or the 96–116-kHz (FM4) bands and are facilitated or inhibited by a low-frequency signal within the frequency band of the first harmonic FM (FM1, 24–29 kHz). Facilitatory FM–FM neurons have the characteristic feature that they are selective for delays between the FM1 signal and the higher-frequency signal of 1–20 ms and thus have been implicated in the representation of target distance (Suga and O'Neill 1979; O'Neill and Suga 1982; Suga and Horikawa 1986; Edamatsu and Suga 1993). Inhibitory FM–FM neurons are selective for 0-ms delays but their functional importance is unclear (O'Neill 1985; Portfors and Wenstrup 1999; Wenstrup 1999).

There also are combination-sensitive neurons that are tuned to the constant frequency (CF) components in sonar signals. These neurons have different physiological properties than FM–FM neurons. Their excitatory response to one of the higher harmonic CF signals (CF2, 57–60 kHz; CF3, 88–90 kHz; CF4, 117–120 kHz) is facilitated or inhibited by simultaneous onset of a second signal in the frequency range of the first harmonic (Hl, 24–30 kHz) of the biosonar call. A distinguishing feature of Hl–CF neurons is their temporal tuning. The majority of Hl–CF neurons are selective for delays of 0 ms (simultaneous onset of the two signals), regardless of whether they show facilitatory or inhibitory combinatorial interactions (Suga et al. 1983; Olsen and Suga 1991a; Fitzpatrick et al. 1993; Portfors and Wenstrup 1999; Wenstrup 1999).

FM–FM and Hl–CF neurons are known to occur in the auditory cortex (O'Neill and Suga 1979, 1982; Suga and O'Neill 1979; Suga et al. 1979, 1983; Edamatsu and Suga 1993; Fitzpatrick et al. 1993, 1998), the medial geniculate body (MGB) (Olsen and Suga 1991a, b; Yan and Suga 1996; Wenstrup 1999), and the central nucleus of the inferior colliculus (ICC) (Mittmann and Wenstrup 1995; Yan and Suga 1996; Portfors and Wenstrup 1999, 2000) of the mustached bat. In the ICC, over 75% of the neurons in the 72–87-kHz (FM3) frequency representation are combination-sensitive, with more than half displaying facilitatory interactions and about 25% displaying inhibitory combinatorial interactions (Portfors and Wenstrup 1999). Furthermore combination-sensitive neurons have been found in frequency representations of the ICC that are not involved in sonar analyses, suggesting that these combinatorial response properties may be involved in the analysis of social communication calls (Leroy and Wenstrup 2000).

While it is unknown whether combination-sensitive neurons exist in the auditory brainstem, it is clear that combination sensitivity is a common response property of neurons in the midbrain and forebrain. In this study, we examined responses of single neurons in the nuclei of the lateral lemniscus (NLL) to combination stimuli to examine whether combination-sensitive neurons are present in the auditory brainstem of the mustached bat. We focused on responses in the NLL because its neurons form the largest group of brainstem neurons projecting to the ICC (Wenstrup et al. 1999). Therefore, if the NLL does not contain combination-sensitive neurons, it is more likely that combination-sensitive neurons are created in the ICC.

METHODS

Responses to single tone bursts and combinations of tone bursts were recorded from single units histologically localized to the NLL of seven mustached bats (*Pteronotus parnellii rubiginosa*) captured in Trinidad

and Tobago. All experimental procedures were approved by the Northeastern Ohio Universities College of Medicine Animal Care and Use Committee.

Surgical procedures

Bats were anesthetized with methoxyflurane inhalation (Metofane, Pitman-Moore, Inc., Mundelein, IL) in combination with sodium pentobarbital (Nembutal 5 mg/kg, Abbott Laboratories, North Chicago, IL) and acepromazine (2 mg/kg; Med-Tech, Inc., Buffalo, NY) injected intraperitoneally. The skull was exposed by reflecting the overlying skin and muscles, and a metal pin was cemented onto the skull with cyanoacrylate to ensure a uniform positioning of the head in the stereotaxic apparatus. A tungsten reference electrode was implanted into the right cerebral cortex and cemented in place, and a small hole (usually less than 0.5 mm) was cut in the skull overlying the cerebellum. We applied a local anesthetic (lidocaine, Elkins-Sinns, Inc., Cherry Hill, NJ) and a topical antibiotic to the open tissue, returned the bat to its cage, and allowed the bat to recover for at least one day prior to electrophysiological recordings.

Acoustic stimulation and single unit recordings

During recording sessions the awake bat was restrained in a custom-made Plexiglas restraining apparatus molded with foam to fit the animal's body. The bats typically remained quiet in the restraining apparatus and showed no signs of pain or discomfort. If the bat struggled or showed signs of discomfort, the recording session was terminated and the bat returned to its cage. The restraining apparatus and stereotaxic device were contained in a heated (27°C) acoustic chamber that was covered inside with polyurethane foam. Sound stimuli were presented by a leaf tweeter speaker placed 10 cm away from the bat at 0° elevation and 25° into the sound field contralateral to the lateral lemniscus under investigation. Acoustic stimulation and data acquisition were controlled from outside the recording chamber by a computer running custom-made applications within the Labview environment (National Instruments Inc., Austin, TX). Two separate tone burst stimuli (3–30-ms duration, 0.5-ms rise time, 4/s) were generated by separate arbitrary waveform generators (Wavetek, model 395, San Diego, CA), shaped with switches (Tucker-Davis Technologies, model SW2, Gainesville, FL), attenuated (Tucker-Davis Technologies, model PA4), added (Tucker-Davis Technologies, model SM3), fed to a power amplifier (Parasound, model HCA-800II, San Francisco, CA), and finally fed to the speaker in the recording chamber. The acoustic system was calibrated several times over the course of the study. A calibrated microphone (Bruel & Kjaer, model 4135, Naerum, Denmark) was placed in the location of the bat's head during recording sessions. There was a smooth, consistent decrease of 2.7 dB per 10 kHz from 10 kHz to 120 kHz. Based on fast Fourier transform analyses, distortion components were not detectable 60 dB below the peak signal level.

Single unit responses were recorded with tracer-filled micropipette electrodes (5–20 MΩ resistance). The electrodes were filled with either dextran-conjugated rhodamine, dextran rhodamine green, biotin dextran amine (Molecular Probes, Eugene, OR), or Fluoro-Gold (Fluorochrome Inc., Engleweed, CO) in 1 M NaCl (or 0.9% NaCl when using Fluoro-Gold). The action potentials were amplified, filtered (bandpass, 500–6000 Hz), and fed to a window discriminator (Frederick Haer, model 74-60-3, Bowdoinham, ME), audiomonitor, and oscilloscope. The pulse output of the window discriminator was digitized at 10 kHz (National Instruments, model NB-MIO-16X) for quantitative data analysis including poststimulus time (PST) histograms, raster displays, and statistics on the neural responses. Each PST histogram was generated from the discharges evoked by 32 presentations of a stimulus at a particular set of stimulus parameters.

The electrode was aimed stereotaxically and advanced using a Kopf hydraulic microdrive. Electrode penetrations into the NLL typically passed through the inferior colliculus. To avoid recording from fibers, we monitored the shape of the waveform and collected only data from units that appeared to be cell bodies on the basis of the biphasic nature of the spike waveform (e.g., Hubel 1960). Potentials from fibers had less complex waveforms, shorter waveform duration, and shorter latencies than cell body potentials. Only data collected from well-isolated single units are described here. By analyzing the shape of the waveform, we are confident that we were recording from only one unit even when excitatory responses were elicited from signals in two separate frequency bands.

Whenever a single unit was isolated, the following tests were performed. We first used audiovisual methods to determine the best frequency (BF, the frequency requiring the lowest intensity to elicit stimulus-locked spikes) and threshold (the lowest intensity required to elicit one or more spikes to each of five consecutive stimuli) of the unit using tone burst stimuli. Tones were presented instead of FM sweeps so that we could generate frequency tuning curves and obtain BF for each excitatory response. These stimuli also allowed us to compare our results with those obtained in the ICC, in which most FM–FM responses can be elicited using combinations of tones as well as FM sweeps (Mittmann and Wenstrup 1995; Portfors and Wenstrup 1999, 2000).

Using a two-tone stimulus paradigm, neurons were then tested for sensitivity to combinations of tones

across the bat's audible range. One tone was set at BF and at 10 dB above threshold; the frequency, intensity, and relative timing of a second tone were varied. If a facilitatory or inhibitory interaction was obtained, the frequencies, intensities, and timing of the two signals were adjusted to obtain the strongest combinatorial effect. Quantitative tests were then performed, using the previously determined stimulus parameters, to assess the strength of the combination-sensitive interaction and its sensitivity to delay between the two interacting signals. Typically, the intensity of the BF tone was 10 dB above threshold and the facilitating or inhibiting second tone was 10 dB above the threshold for facilitation or inhibition. Response magnitude data were collected only for this one combination of frequencies and intensities. Data were collected in the following conditions: no stimulus (to measure spontaneous activity), BF tone alone, facilitating or inhibiting tone alone, and then to the combination of the two signals at different delays. For each stimulus condition, neural responses to 32 presentations were collected. The delay between the two signals that elicited the strongest effect (facilitation or inhibition) was defined as the neuron's best delay. A neuron was considered combination-sensitive if the response to the two tones was 20% more or 20% less than the sum of the responses to the single tones (Portfors and Wenstrup 1999; Wenstrup 1999). The neuron's frequency tuning curves to the single stimuli and the combination stimuli were then obtained. Once combination-sensitive tests were finished, more detailed properties of responses to single tones were determined if time permitted. Rate-level functions were obtained by plotting spike counts evoked by 32 presentations of BF tone bursts at intensities ranging from threshold to 40–50 dB above threshold in 10-dB increments. Response latency was measured at 30 dB above threshold as the time of occurrence of the sharp change in slope of the cumulative PST histogram.

Histological procedures

To confirm that the single unit recordings were from the NLL, we made iontophoretic deposits of tracers. At the end of a penetration, we made the deposits using positive current (+10 μA, 1 min to blow off the tip of the electrode; +5 μA, 5–10 min of intermittent current for the deposit). Two deposits were made for each electrode penetration to aid in reconstructing the electrode track. After 4–6 tracer deposits in the NLL on each side of the brain, the experiment was terminated and the bat perfused. The bat was deeply anesthetized with Nembutal (>60 mg/kg IP). Once nociceptive reflexes were eliminated, the chest cavity was opened and the animal perfused through the heart with phosphate buffer and 4% paraformaldehyde. The brain was dissected and blocked in a consistent plane, inclined about 15° from dorsal and caudal to ventral and rostral (Wenstrup and Grose 1995; Portfors and Wenstrup 2000). Before sectioning, the brain was refrigerated overnight in a 30% sucrose–phosphate buffer solution. Brains were sectioned transversely on a freezing microtome at a thickness of 40 μm. Sections were collected into cold 0.1 M phosphate buffer or phosphate-buffered saline.

To visualize both fluorescent tracers and biotin dextran amine (BDA), every third section was processed by a different protocol. The first two were processed to visualize fluorescence and the third for BDA. The first two series were mounted, cleared, and coverslipped with DPX mounting medium. Fluorescent labeling was viewed with an Olympus BH-2 microscope and the appropriate filter combinations. BDA deposits were visualized using an avidin–biotin–peroxidase procedure (Vector Laboratories, Burlingame, CA). Heavy metal-intensified diaminobenzidine was used as chromogen. The third series was also counterstained with cresyl violet to draw cytoarchitectonic boundaries. We followed the cytoarchitectural boundaries (Fig. 1) described by Zook and Casseday (1982a) for the dorsal nucleus of the lateral lemniscus (DNLL) and the intermediate nucleus of the lateral lemniscus (INLL). The one deviation we made from Zook and Casseday's nomenclature was for the ventral nucleus of the lateral lemniscus (VNLL). We identified the columnar region of the VNLL (VNLLc) as the area containing small spherical cells arranged in a columnar fashion. All other parts of the VNLL were identified as the multipolar region (VNLLm). Similar divisions have been identified in the VNLL of the big brown bat, *Eptesicus fuscus* (Covey and Casseday 1986).

RESULTS

Responses to single tones

We recorded from 101 histologically localized single units in the NLL. We recorded 23 units in the DNLL, 54 units in the INLL, 8 units in the VNLLc, and 16 units in the VNLLm. The distribution of best frequencies across all subdivisions of the NLL is shown in Figure 2A. We summed the number of neurons over a frequency bin width of 5 kHz for presentation purposes, keeping the units with BFs corresponding to the second harmonic, constant frequency component (CF2) of the Trinidadian mustached bats sonar signal (57–60 kHz) together. The majority (82%) of neurons in the NLL had a single excitatory frequency tuning curve and their BF is plotted. For units with multiple frequency tuning curves, only the frequency with the

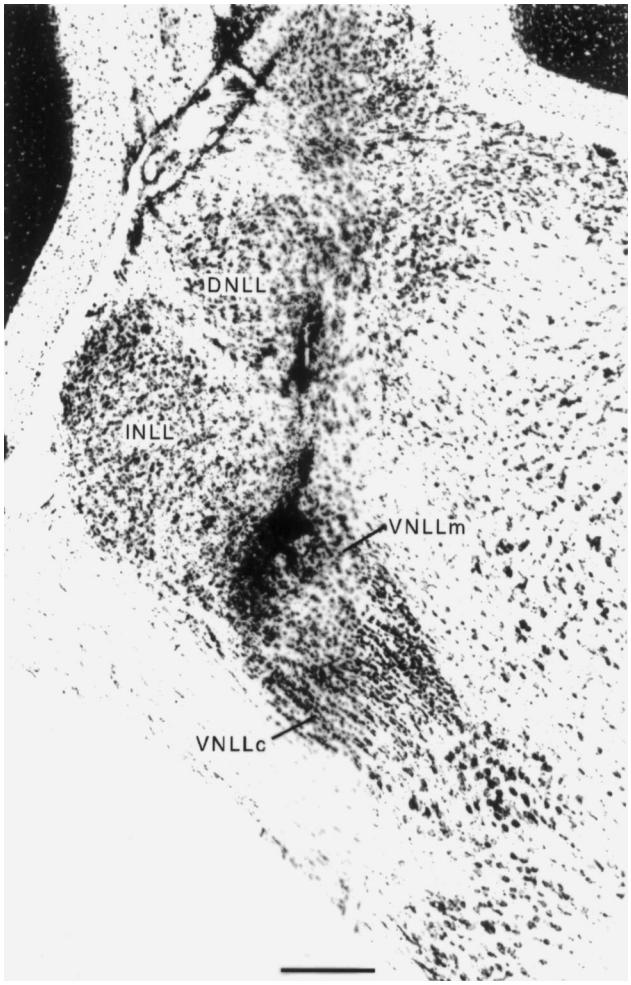

FIG. 1. Photomicrograph of tracer deposits in the NLL. BDA deposits in the dorsal nucleus of the lateral lemniscus (DNLL) and ventral nucleus of the lateral lemniscus (VNLL). Nissl staining illustrates the columnar region of the ventral nucleus of the lateral lemniscus (VNLLc) as the area containing small spherical cells arranged in a columnar fashion. The noncolumnar parts of the VNLL are identified as the multipolar region (VNLLm). Scale bar = 0.25 mm.

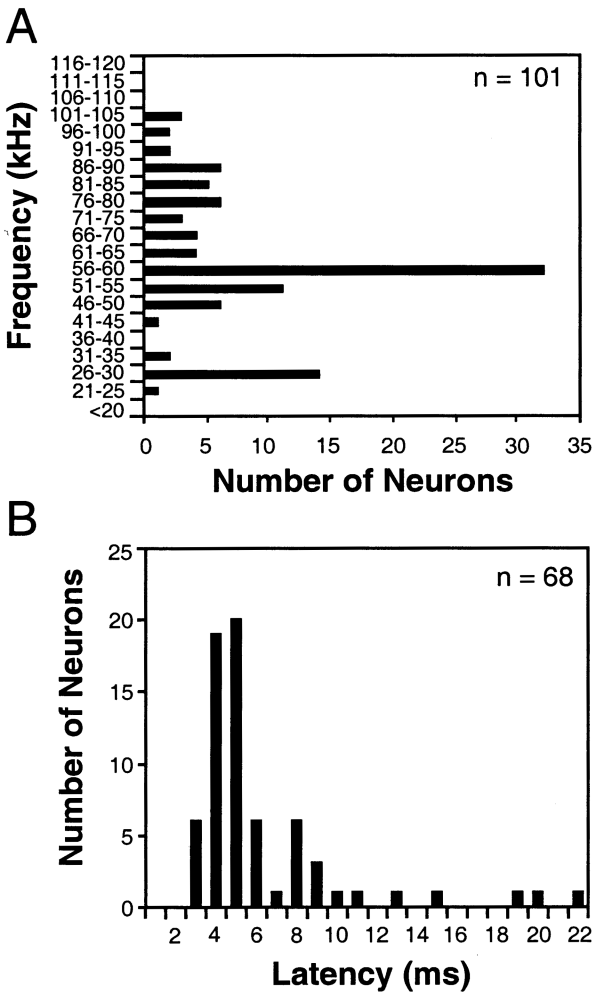

FIG. 2. Distribution of response properties to single tone burst stimuli. **A** Best frequency (BF) distribution. **B** Response latency to the BF tone.

lowest threshold is plotted in Figure 2A. The distribution of BFs in our sample had two peaks. The largest peak was at 56–60 kHz, the frequency band associated with the CF2 sonar component. The second peak in the frequency distribution was at 25–30 kHz. These frequencies correspond to those contained in the fundamental harmonic (Hl) of the bat's biosonar signal. We also examined the distribution of BFs in DNLL, INLL, VNLLm, and VNLLc individually. In the DNLL and INLL, where we have the largest number of neurons, the BFs were broadly distributed with one distribution peak between 56–60 kHz and a second peak at 25–30 kHz. In the VNLLm and VNLLc, all the neurons we recorded had BFs of 56–60 kHz or higher. This is most likely a sampling bias and not an indication that lower frequencies are not represented in the VNLLm and VNLLc.

Response latencies to BF tones, measured at 30 dB above threshold, are shown in Figure 2B. For the 68 units in which we measured latency, the range was between 3 and 22 ms with a mean of 6.1 (SD 3.3) ms. The mean value, however, is skewed by the presence of a few very long latency values. As illustrated in Figure 2B, the majority of latencies were concentrated between 4 and 5 ms. Both the mode and the median of the sample were 5 ms. This distribution of response latency is similar to that found in the big brown bat (Covey and Casseday 1991; Haplea et al. 1994). When we compared the response latencies in the individual subdivisions, we found that while neurons with the shortest latencies were found in all the subdivisions, neurons with the longest latencies were mostly found in INLL. In INLL, the range of latencies was 3–22 ms, while the range of latencies was 4–6 ms in DNLL, 3–10 ms in VNLLm, and 4–13 ms in VNLLc. As we were focusing our study on responses to combinatorial stimuli, we did not assess the variability in latency with

frequency or intensity. In both the mustached bat (O'Neill et al. 1992) and big brown bat (Covey 1993; Covey and Casseday 1991; Haplea et al. 1994), neurons in VNLLc respond with constant latencies, even with large variations in frequency and intensity.

The discharge patterns of neurons in the different subdivisions were similar to those that have been described previously in the big brown bat (Covey 1993; Covey and Casseday 1991; Haplea et al. 1994). The most predictable responses were in the VNLLc, in which all eight neurons responded with an onset discharge pattern. In DNLL, INLL, and VNLLm, responses were not as consistent. In DNLL and INLL, about two-thirds of the neurons responded in a sustained manner (DNLL, 65%; INLL, 62%). In VNLLm, half of the neurons had sustained responses.

Seven single units showed complex frequency tuning to single tone stimuli without any combinatorial facilitatory or inhibitory interaction. These neurons had excitatory responses to two different frequency bands that were separated by an octave or more. Figure 3 illustrates two of these single units. The neuron in Figure 3A had a best frequency of 58.9 kHz and a threshold of 14 dB SPL. It also responded to 28.6 kHz with a threshold of 47 dB SPL. The neuron shown in Figure 3B had a best frequency of 34.5 kHz and a threshold of 18 dB SPL. This unit also responded to 61.3 kHz and had a threshold of 27 dB. The neuron in Figure 3B shows that the two excitatory frequency bands were not limited to frequencies contained within the biosonar signal. Although 61.3 kHz is in the very upper range of the Trinidadian mustached bat's CF2 echoes, 34.5 kHz is definitely outside the frequency range of the biosonar signal (Henson et al. 1987). However, many social vocalizations of the mustached bat contain energy in the 34.5-kHz range (Kanwal et al. 1994; Ohlemiller et al. 1994). The best frequencies of the seven neurons with two excitatory tuning curves are listed in Table 1. At least two of these frequencies (34.5 and 19.8 kHz) are outside of the frequency range of the biosonar signal (Henson et al. 1987). Table 1 also illustrates that units with multiple excitatory frequency tuning curves were recorded in each of the subdivisions of the NLL.

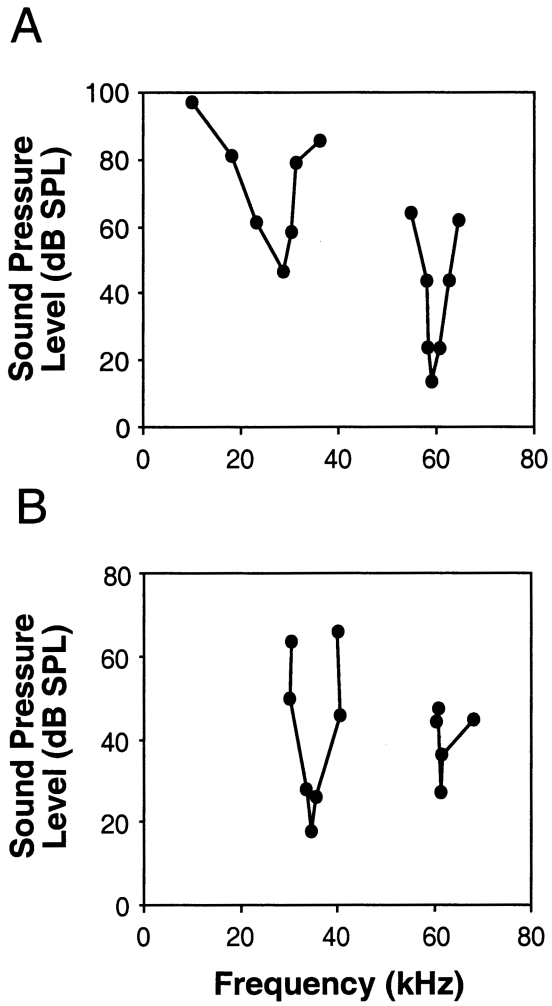

FIG. 3. Excitatory frequency tuning curves of two multiply tuned single units. **A** Both frequency tuning curves of this unit were in the range of the bat's sonar signal. **B** This unit had a BF outside the sonar range, 34.5 kHz, and a second tuning peak at 61.3 kHz.

TABLE 1

Frequency tuning of multiply tuned neurons in the nuclei of the lateral lemniscus

BF (kHz)	*2nd frequency (kHz)*	*Subdivision*
51.4	31.3	INLL
58.1	19.8	DNLL
58.4	25.7	VNLLc
58.9	28.6	DNLL
60.8	30.5	INLL
34.5	61.3	INLL
81.3	25.4	VNLLm

Responses to combination stimuli

Of the 101 single units we recorded in the NLL, 12 responded in a facilitatory or inhibitory manner to combinations of tone bursts (Fig. 4). Three units were facilitated by the combination of two tone bursts and the remaining nine units were inhibited. Figures 5A and B show the frequency tuning and delay sensitivity of one facilitatory combination-sensitive response. This neuron had a BF of 60.6 kHz and threshold of 12 dB SPL. It did not respond to single tones in any other frequency bands, but the neuron's response at BF was facilitated by simultaneous presentation of a low-frequency signal within the Hl frequency band (24–30 kHz). The best low frequency for facilitation was 26 kHz with a threshold of 51 dB SPL. The delay

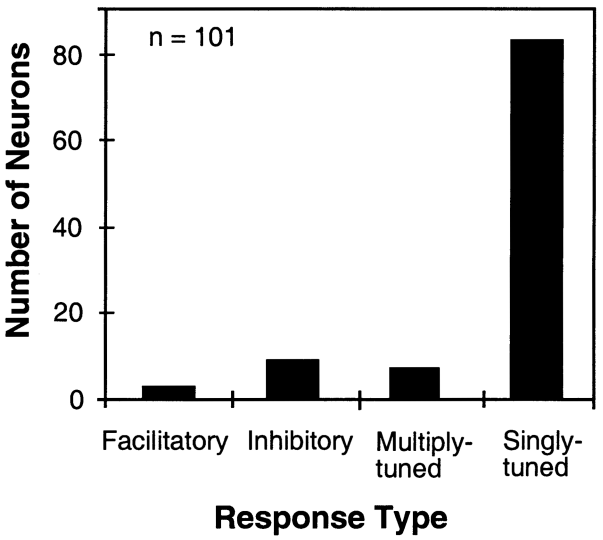

FIG. 4. Types of responses elicited from combinations of tone burst stimuli in the NLL. Singly tuned neurons had only one excitatory tuning curve. Facilitatory responses responded best to the combination of two tone bursts in a specific temporal relationship. The excitatory response of inhibitory neurons was inhibited by the presentation of a second tone burst stimulus. Multiply tuned neurons had two excitatory frequency tuning curves with no combinatorial interaction.

curve in Figure 5B shows that the strongest facilitation occurred when the two tones had simultaneous onset times. The three facilitatory combination-sensitive neurons had similar frequency and temporal response properties. First, they were all tuned to the second harmonic constant frequency (CF2) component of the bat's biosonar signal. Second, all the units were facilitated by a second signal within the Hl (24–30 kHz) frequency band. Third, all the units showed the strongest facilitation with simultaneous presentation of the two sounds. This temporal selectivity is also a characteristic of facilitatory combination-sensitive neurons in the 60-kHz frequency representation of the ICC (Portfors and Wenstrup 1999), MGB (Olsen and Suga 1991a; Wenstrup 1999), and auditory cortex (Suga et al. 1983; Fitzpatrick et al. 1993) in the mustached bat.

Nine single units showed inhibitory combination sensitivity. The excitatory response of these units to BF tones was suppressed by a second tone in the 24–29-kHz frequency range. Figures 5C and D illustrate the frequency tuning and delay sensitivity of an inhibitory combination-sensitive response. This neuron responded best to 55.9 kHz and had a threshold of 45 dB SPL. Its response was suppressed almost 100% by the simultaneous presentation of a 29-kHz sound at an intensity of 50 dB SPL. All of the units except one were tuned to excitatory frequencies contained in the FM component of the bat's biosonar signal. Four units had best frequencies in the 48–56-kHz (FM2) range, three had best high frequencies in the 72–87-kHz (FM3) range, and one had a best high frequency of 104 kHz (FM4). The one neuron tuned to the bat's CF signal had a best high frequency of 59.6 kHz. The best low frequencies that elicited the inhibition were all in the 24–29-kHz range, frequencies contained in the FM1 of the sonar signal. In terms of temporal selectivity of the inhibitory neurons, all were maximally suppressed when the two signals were presented simultaneously. This temporal selectivity is similar to that of inhibitory combination-sensitive neurons in the ICC (Mittmann and Wenstrup 1995; Portfors and Wenstrup 1999) and MGB (Wenstrup 1999).

An interesting finding regarding the distribution of combination-sensitive responses in the NLL was that all 12 combination-sensitive neurons were located in the INLL. We recorded 54 neurons in the INLL. Figure 7 illustrates the percentage of each of the response types. Inhibitory combination-sensitive responses comprised 17% of all the responses in the INLL. This number is not much less than the approximately 25% of inhibitory combination-sensitive neurons found in the ICC.

DISCUSSION

To understand the mechanisms underlying combination-sensitive response properties in the auditory system, it is important to study these responses at their point of creation. Although combination sensitivity is known to be a common property of neurons in the ICC of the mustached bat (Mittmann and Wenstrup 1995; Portfors and Wenstrup 1999), previous studies have not examined any of the lower brainstem nuclei for similar combinatorial neurons. The purpose of this study was to assess whether combination-sensitive neurons are present in the NLL. We focused on the NLL because of the finding that the NLL has the largest number (>50%) of brainstem neurons that project to combination-sensitive neurons in the ICC of the mustached bat (Wenstrup et al. 1999). In particular, the intermediate nucleus of the lateral lemniscus (INLL) and the magnocellular part of the ventral nucleus of the lateral lemniscus (VNLLm) have over 40% of those projecting brainstem neurons.

Responses to single tones in the NLL

In echolocating bats, the nuclei of the lateral lemniscus are hypertrophied and highly differentiated. The four separate cell groups comprising the NLL in bats are easily distinguished from one another on the basis of the arrangement and morphology of their neurons (Zook and Casseday 1982a; Covey and Casseday 1986). The INLL is particularly hypertrophied and forms a conspicuous protrusion on the lateral side of the brainstem. Also prominent is the VNLLc because of its high

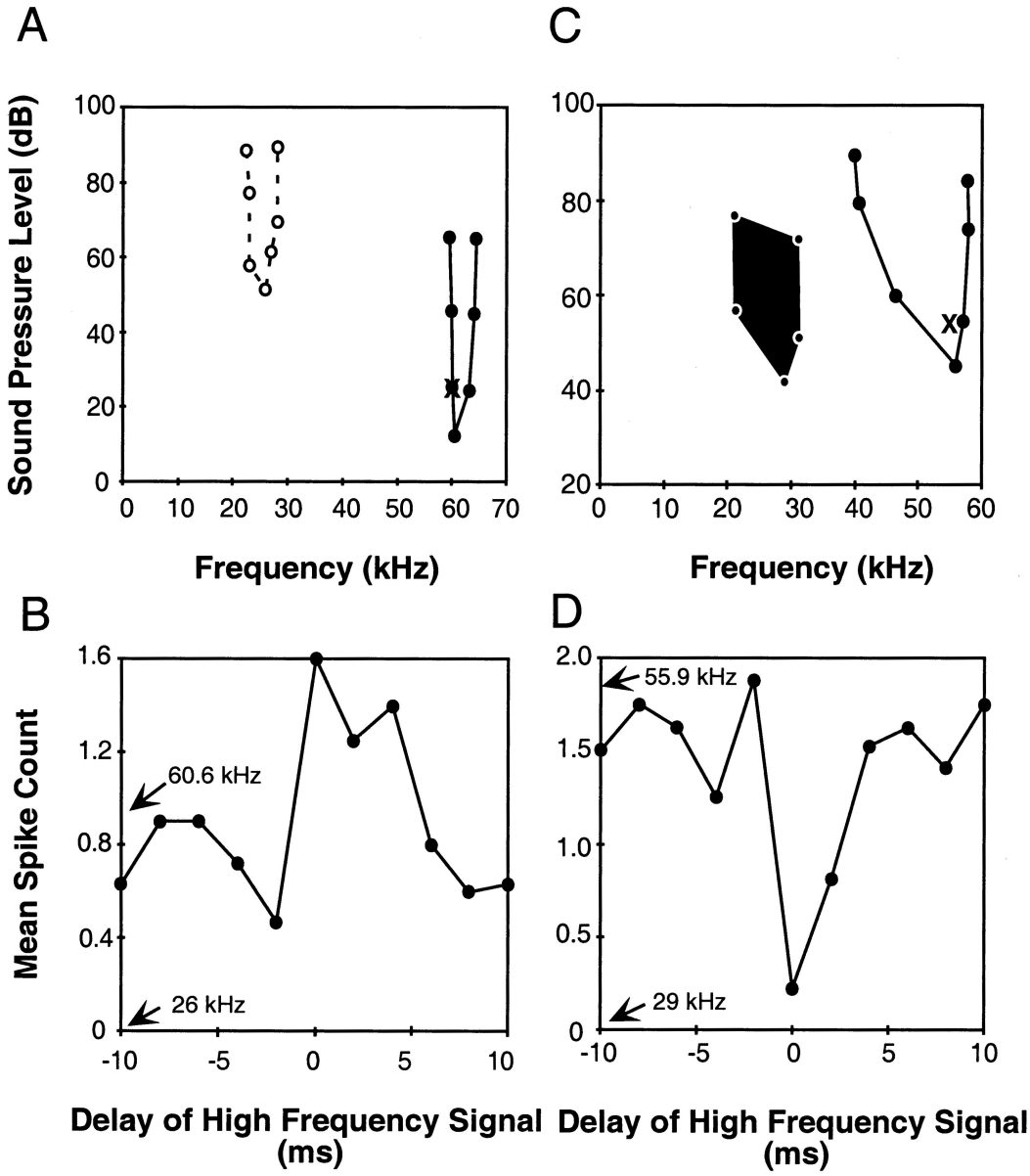

FIG. 5. Characteristic features of combination-sensitive neurons in the NLL. **A** Frequency tuning curves. The solid line is the excitatory tuning curve to the high-frequency signals. This unit did not respond to low-frequency signals presented alone. Dashed line is the facilitatory low-frequency tuning curve. This tuning curve was obtained by holding the high-frequency tone at BF and 10 dB above threshold as illustrated by the X. **B** Delay tuning of the facilitatory combination-sensitive response. The onset of the high-frequency signal was held constant and the onset time of the lower-frequency signal varied. 0 ms indicates simultaneous onset of the two tones. All three facilitatory neurons were most strongly facilitated at simultaneous onset of the two signals (0 ms delay). **C** Frequency tuning of an inhibitory combination-sensitive neuron. To obtain the inhibitory frequency tuning curve, the frequency and amplitude of the BF signal was held constant and the frequencies and amplitudes causing inhibition were documented. **D** Delay tuning of the inhibitory neuron. All 9 inhibitory units were most strongly inhibited at simultaneous onset of the two stimuli (0 ms delay).

packing density of small, spherical-shaped cells and their distribution in columns. Although all echolocating bats studied to date have the same cell populations in INLL and VNLL, the distribution of cell types in VNLL varies among species. In the mustached bat, the VNLLc is the most ventral of the nuclei, but, in the big brown bat, the VNLLc is located between the INLL and the VNLLm so that the VNLLm is located most ventrally (Covey and Casseday 1986). Nonecholocating mammals have the same basic cell types as in the bat INLL and VNLL, but there is a less clear segregation between the INLL and VNLL (Adams 1979). Similar to bats, echolocating dolphins have a hypertrophied INLL and VNLL, and the VNLL is differentiated into columnar and multipolar cell regions (Zook et al. 1988). This similarity between echolocating species

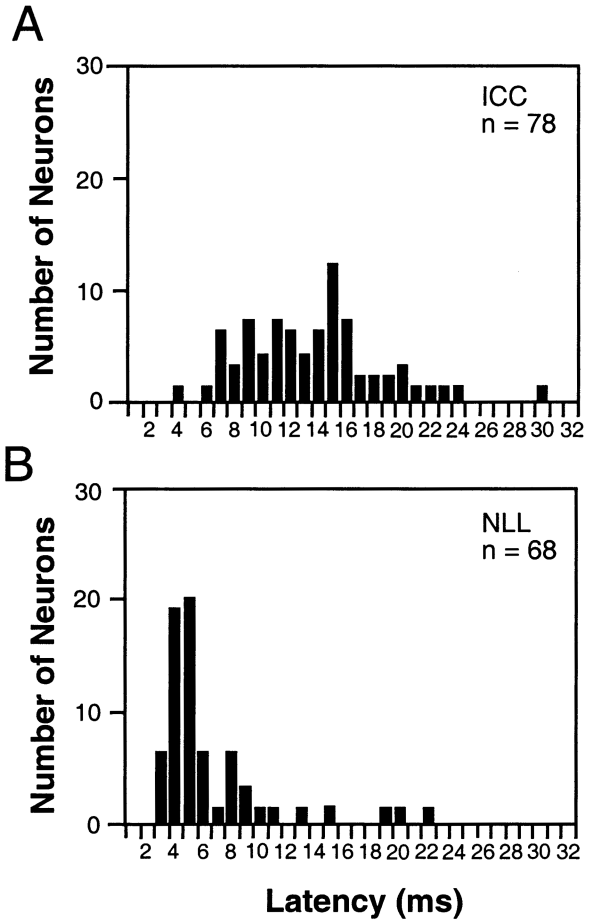

FIG. 6. Comparison of response latencies in the ICC and NLL. **A** *Predicted latencies* of excitation in response to low-frequency signals among delay-tuned, facilitated neurons of the ICC. Predicted values were obtained by subtracting the measured high-frequency latency from the best delay (in ms). See text for justification. **B** *Measured latencies* of NLL neurons in our sample. Although these neurons were tuned to a wide range of best frequencies, the figure indicates the range of latencies that may occur among NLL neurons tuned to low frequencies. Note that relatively few NLL neurons have latencies above 8 ms, which are required in the ICC to create a range of best delays of 1–20 ms.

suggests that much of the neural processing performed by the INLL and VNLL is important for echolocation.

Some of the clearest evidence of specialization related to echolocation has been described in the VNLLc of the big brown bat (Covey and Casseday 1991; Haplea et al. 1994). Neurons in the VNLLc lack spontaneous activity and always respond with only one spike per stimulus. The latency of the spike is precisely locked to the onset of the stimulus and remains constant over large variations in frequency or intensity (Covey and Casseday 1991). These neurons provide an extremely precise marker of the time of onset of a sound and may be important for providing timing markers for specific portions of the bat's biosonar call and the returning echoes. Although we recorded from only a small number of neurons in VNLLc, these neurons had onset responses as have previously been described in the VNLLc of the mustached bat (O'Neill et al. 1992), and the big brown bat (Covey and Casseday 1991; Haplea et al. 1994). As our focus was on testing for combinatorial responses, we did not assess whether neurons in the VNLLc had constant latencies with variations in frequency or intensity.

The discharge patterns and frequency tuning of neurons we recorded in the INLL and VNLL in the mustached bat were similar to responses reported in the big brown bat (Covey and Casseday 1991; Haplea et al. 1994). These neurons showed both sustained and transient responses. Variability in response type is common for the INLL and VNLLm in the big brown bat, horseshoe bat, and cat (Aitkin et al. 1970; Metzner and Radtke–Schuller 1987; Covey and Casseday 1991). About two-thirds of the neurons we recorded in the DNLL and INLL and half the neurons in VNLLm had sustained responses. In the big brown bat, it has been suggested that sustained, nonadapting responses may play a role in marking the duration of specific components in the biosonar signal and returning echoes (Covey 1993). This may also be true in the mustached bat. In the horseshoe bat VNLLm, all the neurons have BFs within the range of the CF component of the biosonar call, suggesting that this nucleus is specialized for processing the CF components of the biosonar call (Metzner and Radtke–Schuller 1987). In the mustached bat VNLLm, we did not find that neurons responded to only frequencies within the CF range. The neurons we recorded had best frequencies of 55 and 60 kHz or higher. Furthermore, some neurons had multiple excitatory frequency tuning curves.

Only one other study has reported multiple excitatory frequency tuning curves in the NLL. Metzner and Radtke–Schuller (1987) found a small number ($n = 12$) of neurons in the DNLL and INLL of the rufous horseshoe bat (*Rhinolophus rouxi*) that had harmonically related excitation maxima. In half of the neurons, the best frequencies were not correlated with any harmonic components of the bat's echolocation signal, suggesting that these neurons may play a role in analyzing other complex sounds. The other half of the neurons had best frequencies within the ranges of the harmonic components in the biosonar signal. There are no published reports on whether combination-sensitive neurons exist in the NLL or ICC of the rufous horseshoe bat. However, the auditory cortex of the rufous horseshoe bat contains combination-sensitive neurons that have response features similar to the HI–CF and FM–FM neurons found in the mustached bat (Schuller et al. 1991). As with the mustached bat, the facilitatory FM–FM neurons in the horseshoe bat show a range of best delays, and these neurons have

also been implicated in the analysis of target distance information (Schuller et al. 1991).

The horseshoe bat and the mustached bat both use multiharmonic biosonar signals composed of a constant frequency (CF) component followed by a frequency-modulated (FM) sweep to forage in highly cluttered environments. The main difference between the two biosonar signals is in their harmonic composition. The mustached bat's signal contains up to five harmonics (fundamental frequency ~30 kHz), whereas the horseshoe bat's call contains only two harmonics (fundamental frequency ~38 kHz). The spectral interactions of combination-sensitive neurons in the horseshoe bat are the same as those in the mustached bat, in that only combinations of the fundamental harmonic with a higher harmonic elicit facilitated responses. Although mustached bats (New World family Mormoopidae) and horseshoe bats (Old World family Rhinolophidae) are not closely related phylogenetically, these species show a convergence of biosonar signals and the spectral integrative response properties involved in analyzing these signals (Schuller et al. 1991). These bat species may have evolved combinatorial response properties independently or it may be that combination sensitivity is a common neuronal mechanism among a variety of species.

Facilitatory responses to combinations of tones in the NLL

Only three neurons in the NLL exhibited facilitatory combination sensitivity. These facilitatory neurons had two distinctive features: Their best frequencies to tones were in the 60-kHz range, and they were facilitated by simultaneous presentation of a second tone burst in the 26–29-kHz frequency range. These frequency and temporal tuning properties are similar to those of Hl–CF neurons found in the ICC (Portfors and Wenstrup 1999), MGB (Olsen and Suga 1991a; Wenstrup 1999), and auditory cortex (Suga et al. 1979, 1983; Fitzpatrick et al. 1993). Hl–CF neurons are typically selective for delays of 0 ms. Some of these neurons are thought to play a role in analyzing relative velocity, partly because of the relative frequency tuning of the CF signals (Suga et al. 1979, 1983; Olsen and Suga 1991a; Fitzpatrick et al. 1993, 1998). Facilitatory FM–FM neurons, on the other hand, have less sharp frequency tuning and also have a broad range of best delays (Suga and O'Neill 1979; O'Neill and Suga 1979; Edamatsu and Suga 1989; Fitzpatrick et al. 1993, 1998; Portfors and Wenstrup 1999; Wenstrup 1999). We did not find any facilitatory neurons that were either tuned to FM frequencies or tuned to nonzero delays, even though 17, 20, and 7 neurons in our sample had excitatory frequency tuning curves in the FM2, FM3, and FM4 ranges, respectively. This suggests that tuning to nonzero delays may be an emergent property of facilitatory FM–FM neurons in the ICC of the mustached bat.

There is some evidence that delay-tuned neurons occur in the NLL of two different species of echolocating bats. In the big brown bat, where recording sites were localized, delay-tuned neurons were found in the DNLL (Covey 1993). Although delay-tuned neurons have been reported to occur in the NLL of the little brown bat (*Myotis lucifugus*), it is unclear where these are located (Suga and Schlegel 1973). In the big brown bat and the little brown bat, delay-tuned neurons respond to the same FM harmonic in the simulated pulse and echo (Sullivan 1982a, b; Feng et al. 1978; Dear et al. 1993). In contrast to combination-sensitive neurons in the mustached bat, delay-tuned neurons in these other bat species respond in a facilitated manner to the second of two identical sounds (typically downward FM sweeps) when it occurs at a specific time after the first sound. While showing temporal selectivity, delay-tuned neurons in big brown and little brown bats do not show the same spectral integrative properties that are found in combination-sensitive neurons in the mustached bat.

Facilitatory FM–FM neurons in the mustached bat are tuned to delays between 1 and 20 ms. These neurons respond best when the low-frequency signal (emitted pulse) is presented 1–20 ms before the high-frequency signal (returning echo). Recent evidence suggests that delay-tuned neurons in the ICC act as coincidence detectors in that facilitation is generated by the low and high-frequency excitation occurring simultaneously at the delay-tuned neuron (Portfors and Wenstrup 1999). For coincidence to occur, the excitation in response to the low-frequency signal must be neurally delayed to coincide with the acoustically delayed excitation in response to the high-frequency signal. Therefore, the latencies of the excitation in response to the low-frequency signals should show, in general, longer latencies than the response latencies to the higher-frequency signals. This is indeed the case in the ICC. Among delay-tuned neurons that show an excitatory response to the low-frequency signal alone, the response latencies range between 6 and 30 ms, whereas latencies to the high-frequency signals range between 4 and 12 ms (Portfors and Wenstrup 1999). Furthermore, the coincidence hypothesis is supported by the finding that the difference between the low and high-frequency response latencies is highly correlated with the best delay of the delay-tuned neuron.

Presently, it is unclear how the longer response latencies to the low-frequency signals in the ICC are created. The longer latencies may be generated in auditory brainstem nuclei or by the postsynaptic response of the delay-tuned neurons in the ICC. An examination of the latencies in the NLL can provide

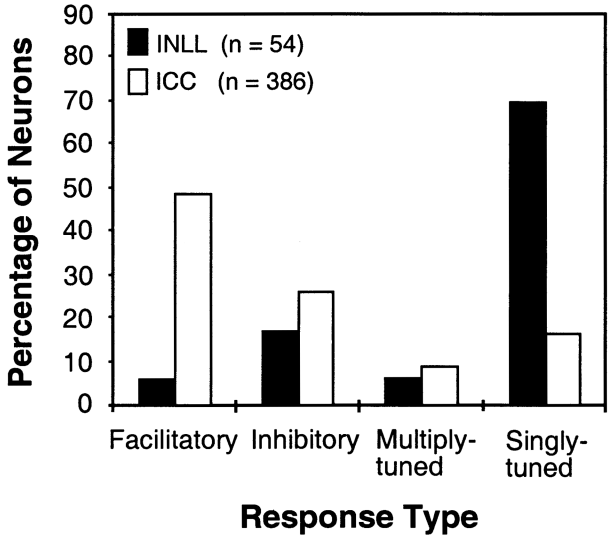

FIG. 7. Comparison of types of responses elicited from combinatorial stimuli in the INLL and ICC. Dark bars show that the majority of responses in the INLL are singly tuned. Open bars indicate that the majority of responses in the ICC are combination-sensitive. The numbers of multiply tuned and inhibitory combination-sensitive neurons are similar in the INLL and ICC. ICC data from Portfors and Wenstrup (1999, 2000) and Leroy and Wenstrup (2000).

an indication of whether the longer latencies to the low-frequency signals are created in the brainstem or in the ICC. Figure 6 compares the predicted low-frequency latencies among delay-tuned ICC neurons with the measured latencies of NLL neurons. We must predict the low-frequency latencies in the ICC because most cells do not respond to low-frequency signals presented alone. Because best delay is highly correlated with the difference between the low-frequency latency and the high-frequency latency (Portfors and Wenstrup 1999), we can predict a neuron's low-frequency latency of excitation based on its best delay and measured high-frequency latency. Figure 6A shows the range of low-frequency latencies predicted for a population of delay-tuned neurons with best delays between 1 and 20 ms. Most latencies are in the range 6–24 ms, extending up to 30 ms. In the NLL, latencies are as long as 22 ms, but nearly all NLL neurons had latencies below 8 ms (Fig. 6B). This comparison suggests that, at least for many of the longer best delay neurons, the excitation from the low-frequency signal must be further delayed in the ICC.

Figure 7 illustrates that there is a dramatic increase in the percentage of facilitatory combination-sensitive neurons between the NLL and ICC, suggesting that most facilitatory responses emerge at the level of the ICC. However, it is unknown whether there are combination-sensitive neurons in other brainstem nuclei, such as the cochlear nucleus, because neurons in these regions have yet to be thoroughly tested with combination stimuli. The NLL receives projections from the anterior ventral cochlear nucleus and the posterior ventral cochlear nucleus but not from the dorsal cochlear nucleus (DCN), which projects directly to the ICC (Adams 1979; Ryugo et al. 1981; Zook and Casseday 1982b, 1987; Ryugo and Willard 1985). Combination-sensitive neurons may occur in the DCN and their projections to the ICC may pass along these combination-sensitive response properties. Therefore, it is necessary to test neurons in the DCN for combination sensitivity to address this issue. However, we have additional recent evidence to suggest that combination-sensitive facilitation is created in the ICC. Iontophoretic application of strychnine (a glycine antagonist) onto facilitatory combination-sensitive neurons in the ICC eliminated or significantly reduced the facilitated response in 100% of the neurons tested (Wenstrup and Leroy 2001). This suggests that a glycinergic input contributes significantly to creating the facilitated responses and that these combination-sensitive responses are created in the ICC.

Inhibitory combination-sensitive neurons in the NLL

Nine percent of the neurons we recorded in the NLL showed inhibitory combination-sensitive responses. However, if we consider only responses in the INLL, where all the combination-sensitive neurons were recorded, the inhibitory combination-sensitive responses comprised 17% of all the responses. The excitatory response at BF of these neurons was inhibited by simultaneous presentation of a second sound in a frequency band at least an octave away from BF. Thus, the inhibitory effects were not due to broadly tuned inhibitory domains near BF. The inhibitory combinatorial responses in the NLL were similar to the inhibitory FM–FM responses that occur in the ICC (Portfors and Wenstrup 1999). The excitatory BFs of the inhibitory combination-sensitive neurons in the NLL were nearly all in the frequency representations related to the bat's FM components in the sonar signal (48–56 or 72–87 kHz), and the frequencies that evoked the inhibition were in the FM1 range.

This type of combinatorial inhibition, which has been documented in the ICC (O'Neill 1985; Portfors and Wenstrup 1999, 2000), the MGB (Wenstrup 1999) and now in the NLL of the mustached bat, is different from most inhibitory response areas previously described in other mammals using two-tone testing paradigms. While many of these tests are performed with two overlapping tones of different frequencies, most inhibitory regions occur close to the excitatory regions around BF. In the cochlear nucleus, inhibition is usually studied with single tones because most neurons are spontaneously active, but Rhode and Greenberg (1994) used a two-tone paradigm and

showed that all the neurons had simple inhibitory response areas immediately higher and lower than BF. This simple "center-surround" inhibitory band structure is also common in the ICC of nonecholocating mammals (e.g., Ehret and Merzenich 1988). Recently, Sutter and colleagues (1999) showed that neurons in the dorsal part of primary auditory cortex of cat have complex inhibitory spectral response fields in which the inhibitory band structures range from a single band to more than four distinct inhibitory regions. Many of the neurons had inhibitory regions that were not near the excitatory BF, similar to the combination-sensitive inhibitory neurons in the mustached bat. Neurons in dorsal primary auditory cortex of cat also show dual frequency tuning (Sutter and Schreiner 1991) and sensitivity for broadband or noise stimuli (Middlebrooks and Zook 1983). All of these response features suggest that neurons in dorsal primary auditory cortex of cat integrate across frequencies and are involved in analyzing spectrally complex sounds. Some neurons in the MGB of cat show complex inhibitory response areas similar to those described in primary auditory cortex (Imig et al. 1997), however it remains to be seen whether complex inhibitory response areas are common in the ICC of cat.

The functional roles of inhibitory combination-sensitive neurons in the NLL or ICC of mustached bat are unclear. Recently, we suggested that inhibitory FM–FM neurons in the ICC may modify facilitatory FM–FM responses in the ICC or MGB (Portfors and Wenstrup 1999). Facilitatory FM–FM neurons in the ICC can be classified into two populations based on their best delay (Olsen and Suga 1991b; Portfors and Wenstrup 1999). These groups are referred to as short best delay neurons and long best delay neurons. Short best delay neurons respond best to delays between 0 and 6 ms, whereas long best delay neurons respond best to delays between 6 and 20 ms. The characteristic difference between the two populations is that long best delay neurons show a strong period of inhibition that precedes the facilitation. This inhibition is strongest at 0 ms best delay. In addition, the time course of the inhibition is similar to the time course seen in inhibitory combination-sensitive neurons both in the NLL and the ICC. It is possible that the inhibitory FM–FM neurons in the NLL provide a dominant excitatory input to long best delay neurons in the ICC. This excitatory input from the inhibitory combination-sensitive neurons would generate a period of inhibition prior to subsequent facilitation at a longer delay. This hypothesis requires testing before the role of inhibitory combination-sensitive neurons will be clear. However, the hypothesis that inhibitory combination-sensitive neurons may modify the response properties of facilitatory FM–FM neurons is made more intriguing by the finding that the percentage of inhibitory combination-sensitive neurons decreases between the ICC and the MGB (Portfors and Wenstrup 2000).

Spectral integrative response properties in the ascending auditory system of other mammals

In the mustached bat auditory cortex, combination sensitivity is a common response property. Although once considered specialized for the analysis of sonar information in this bat, combination-sensitive neurons in the mustached bat's auditory cortex are now known to also be selective for different social vocalizations (Ohlemiller et al. 1996; Esser et al. 1997). Combination sensitivity is also now known to be a common response property in the ICC of the mustached bat. These responses occur throughout the frequency representations involved in sonar analyses (Mittmann and Wenstrup 1995; Portfors and Wenstrup 1999) and also throughout nonsonar frequency representations (Leroy and Wenstrup 2000). Combination sensitivity in ICC frequency bands not related to sonar may provide for neural selectivity among different social vocalizations. Whether neurons in the ICC of the mustached bat show selectivity to social vocalizations remains to be tested. We suggest that combination sensitivity in the ICC may not be specific for the analysis of sonar signals in the mustached bat but, instead, may be a fundamental mechanism underlying spectral integration of complex sounds, including social vocalizations.

That there are only a few facilitatory combination-sensitive responses in the NLL suggests that the majority of combination-sensitive responses originate in the ICC. In other mammalian species, similar combinatorial response properties have been described in secondary cortical areas (Rauschecker et al. 1995; Rauschecker 1998) and there is increasing evidence for spectral integration in mammalian primary cortical areas (Sutter and Schreiner 1991; Wang et al. 1995; Brosch et al. 1999; Fritz 2000; Kadia et al. 2000). These spectral integrative response properties have been described as either emerging in auditory cortex as a result of intracortical projections from tonotopic areas representing diverse frequencies (Kadia et al. 2000) or as primarily emerging in secondary auditory cortex (Rauschecker et al. 1995; Rauschecker 1998). However, it is possible that spectral integrative responses occur in the ICC of mammals other than bats, but this has yet to be fully explored.

ACKNOWLEDGMENTS

We thank C. Grose for helping with histological reconstruction of the recording sites, D. Gans for technical assistance, and the two anonymous reviewers for helpful comments. We are grateful to the government of Trinidad and Tobago for

the permission to collect and export bats. This work was supported by research grant 5 R01 DC-00937 from the National Institute on Deafness and Other Communication Disorders.

REFERENCES

ADAMS JC. Ascending projections to the inferior colliculus. J. Comp. Neurol. 183:519–538, 1979.

AITKIN LM, ANDERSON DJ, BRUGGE JF. Tonotopic organization and discharge characteristics of single neurons in nuclei of the lateral lemniscus of the cat. J. Neurophysiol. 33:421–440, 1970.

BLACKBURN CC, SACHS MB. The representations of the steady state vowel sound /e/ in the discharge patterns of cat anteroventral cochlear nucleus neurons. J. Neurophysiol. 63:1191–1212, 1990.

BROSCH M, SCHULZ A, SCHEICH H. Processing of sound sequences in macaque auditory cortex: response enhancement. J. Neurophysiol. 82:1542–1559, 1999.

COVEY E. Response properties of single units in the dorsal nucleus of the lateral lemniscus and paralemniscal zone of an echolocating bat. J. Neurophysiol. 69:842–859, 1993.

COVEY E, CASSEDAY JH. Connectional basis for frequency representation in the nuclei of the lateral lemniscus of the bat, *Eptesicus fuscus*. J. Neurosci. 6:2926–2940, 1986.

COVEY E, CASSEDAY JH. The monaural nuclei of the lateral lemniscus in an echolocating bat: parallel pathways for analyzing temporal features of sound. J. Neurosci. 11:3456–3470, 1991.

DEAR SP, FRITZ J, HARESIGN T, FERRAGAMO M, SIMMONS JA. Tonotopic and functional organization in the auditory cortex of the big brown bat, *Eptesicus fuscus*. J. Neurophysiol. 70:1988–2009, 1993.

DELGUTTE B, KIANG NYS. Speech coding in the auditory nerve. I. Vowel-like sounds. J. Acoust. Soc. Am. 75:866–878, 1984a.

DELGUTTE B, KIANG NYS. Speech encoding in the auditory nerve. III. Voiceless fricative consonants. J. Acoust. Soc. Am. 75:887–896, 1984b.

DELGUTTE B, KIANG NYS. Speech coding in the auditory nerve. IV. Sounds with consonant-like dynamic characteristics. J. Acoust. Soc. Am. 75:897–907, 1984c.

EDAMATSU H, SUGA N. Differences in response properties of neurons between two delay-tuned areas in the auditory cortex of the mustached bat. J. Neurophysiol. 69:1700–1712, 1993.

EHRET G, MERZENICH MM. Complex sound analysis (frequency resolution, filtering and spectral integration) by single units of the inferior colliculus of the cat. Brain Res. 472:139–163, 1988.

ESSER KH, CONDON CJ, SUGA N, 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.

FENG AS, SIMMONS JA, KICK SA. Echo detection and target-ranging neurons in the auditory system of the bat *Eptesicus fuscus*. Science 202:237–243, 1978.

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

FITZPATRICK DC, SUGA N, OLSEN JF. Distribution of response types across entire hemispheres of the mustached bat's auditory cortex. J. Comp. Neurol. 391:353–365, 1998.

FRITZ J. Spectral integration in the medial belt area of the macaque auditory cortex. Abstr. Assoc. Res. Otolaryngol. 86:301, 2000.

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

HAPLEA S, COVEY E, CASSEDAY JH. Frequency tuning and response latencies at three levels in the brainstem of the echolocating bat, *Eptesicus fuscus*. J. Comp. Physiol. [A] 174:671–683, 1994.

HENSON OD, BISHOP A, KEATING A, KOBLER J, HENSON M, WILSON B, HANSEN R. Biosonar imaging of insects by *Pteronotus p. parnellii*, the mustached bat. Natl. Georgraphic Res. 3:82–101, 1987.

HUBEL DH. Single unit activity in lateral geniculate body and optic tract of unrestrained cats. J. Physiol. (Lond.) 150:91–104, 1960.

IMIG TJ, POIRIER P, IRONS WW, SAMSON FK. Monaural spectral contrast mechanism for neural sensitivity to sound direction in the medial geniculate body of the cat. J. Neurophysiol. 78:2754–2771, 1997.

KADIA S, SNIDER R, WANG X. Influence of stimulus components placed outside classical receptive field reveals harmonic structure of the auditory cortex. Abstr. Assoc. Res. Otolaryngol. 14:54, 2000.

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

LEROY S, WENSTRUP JJ. Spectral integration in the inferior colliculus of the mustached bat. J. Neurosci. 20:8533–8541, 2000.

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

METZNER W, RADTKE–SCHULLER S. The nuclei of the lateral lemniscus in the rufous horseshoe bat, *Rhinolophus rouxi*. J. Comp. Physiol. 160:395–411, 1987.

MIDDLEBROOKS JC, ZOOK JM. Intrinsic organization of the cat's medial geniculate body identified by projections to binaural response-specific bands in the primary auditory cortex. J. Neurosci. 3:203–224, 1983.

MILLER MI, SACHS MB. Representation of stop consonants in the discharge patterns of auditory-nerve fibers. J. Acoust. Soc. Am. 74:502–517, 1983.

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

O'NEILL WE. Responses to pure tones and linear FM components of the CF–FM biosonar signal by single units in the inferior colliculus of the mustached bat. J. Comp. Physiol. 157:797–815, 1985.

O'NEILL WE, SUGA N. Target range sensitive neurons in the auditory cortex of the mustached bat. Science 203:69–73, 1979.

O'NEILL WE, SUGA N. Encoding of target range and its representation in the auditory cortex of the mustached bat. J. Neurosci. 2:17–31, 1982.

O'NEILL WE, HOLT JR, GORDON M. Responses of neurons in the intermediate and ventral nuclei of the lateral lemniscus of the mustached bat to sinusoidal and pseudorandom amplitude modulations. Abstr. Assoc. Res. Otolaryngol. 15:140, 1992.

OHL FW, SCHEICH H. Orderly cortical representation of vowels based on formant interaction. Proc. Natl. Acad. Sci. USA 94:9440–9444, 1997.

OHLEMILLER KK, KANWAL JS, BUTMAN JA, SUGA N. Stimulus design for auditory neuroethology: synthesis and manipulation of complex communication sounds. Aud. Neurosci. 1:19–37, 1994.

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

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

OLSEN JF, SUGA N. Combination-sensitive neurons in the medial geniculate body of the mustached bat: encoding of target range information. J. Neurophysiol. 65:1275–1296, 1991b.

PORTFORS CV, WENSTRUP JJ. Delay-tuned neurons in the inferior colliculus of the mustached bat: implications for analyses of target distance. J. Neurophysiol. 82:1326–1338, 1999.

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

RAUSCHECKER JP. Cortical processing of complex sounds. Curr. Opin. Neurol. 8:516–521, 1998.

RAUSCHECKER JP, TIAN B, HAUSER M. Processing of complex sounds in the macaque nonprimary auditory cortex. Science 268:111–114, 1995.

RHODE WS, GREENBERG S. Lateral suppression and inhibition in the cochlear nucleus of the cat. J. Neurophysiol. 71:493–514, 1994.

RYUGO DK, WILLARD FH. The dorsal cochlear nucleus of the mouse: a light microscopic analysis of neurons that project to the inferior colliculus. J. Comp. Neurol. 242:381–396, 1985.

RYUGO DK, WILLARD FH, FEKETE DM. Differential afferent projections to the inferior colliculus from the cochlear nucleus in the albino mouse. Brain Res. 210:342–349, 1981.

SACHS MB, YOUNG ED. Encoding of steady-state vowels in the auditory nerve: representation in terms of discharge rate. J. Acoust. Soc. Am. 66:470–479, 1979.

SCHREINER CE, CYNADER MS. Basic functional organization of second auditory cortical field (AII) of the cat. J. Neurophysiol. 51:1284–1305, 1984.

SCHULLER G, O'NEILL WE, RADTKE–SCHULLER S. Facilitation and delay sensitivity of auditory cortex neurons in CF–FM bats, *Rhinolophus rouxi* and *Pteronotus p. parnellii*. Eur. J. Neurosci. 3:1165–1181, 1991.

SINEX DG, GEISLER CD. Responses of auditory-nerve fibers to consonant–vowel syllables. J. Acoust. Soc. Am. 73:602–615, 1983.

SUGA N. Basic acoustic patterns and neural mechanisms shared by humans and animals for auditory perception: a neuroethologists's view. In: Ainsworth M, Greenberg S, (eds) Auditory Basis of Speech Perception. Keele University Staffordshire, UK, 1996, pp 31–38.

SUGA N, HORIKAWA J. Multiple time axes for representation of echo delays in the auditory cortex of the mustached bat. J. Neurophysiol. 55:776–805, 1986.

SUGA N, O'NEILL WE. Neural axis representing target range in the auditory cortex of the mustache bat. Science 206:351–353, 1979.

SUGA N, SCHLEGEL P. Coding and processing in the auditory systems of FM-signal-producing bats. J. Acoust. Soc. Am. 54:174–190, 1973.

SUGA N, O'NEILL WE, MANABE T. Cortical neurons sensitive to combinations of information-bearing elements of biosonar signals in the mustached bat. Science 200:778–781, 1978.

SUGA N, O'NEILL WE, MANABE T. Harmonic-sensitive neurons in the auditory cortex of the mustached bat. Science 203:270–274, 1979.

SUGA N, O'NEILL WE, KUJIRAI K, MANABE T. Specificity of combination-sensitive neurons for processing of complex biosonar signals in auditory cortex of the mustached bat. J. Neurophysiol. 49:1573–1626, 1983.

SULLIVAN WE. Neuronal representation of target distance in auditory cortex of the echolocating bat *Myotis lucifugus*. J. Neurophysiol. 48:1011–1032, 1982a.

SULLIVAN WE. Possible neuronal mechanisms of target distance coding in auditory system of echolocating bat *Myotis lucifugus*. J. Neurophysiol. 48:1033–1047, 1982b.

SUSSMAN HM, FRUTCHER D, HILBERT J, SIROSH J. Linear correlates in the speech signal: the orderly output constraint. Behav. Brain Sci. 21:241–299, 1998.

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

SUTTER ML, SCHREINER CE, MCLEAN M, O'CONNOR KN, LOFTUS WC. Organization of inhibitory frequency receptive fields in cat primary auditory cortex. J. Neurophysiol. 82:2358–2371, 1999.

WANG X, MERZENICH MM, BEITEL R, SCHREINER CS. Representation of a species-specific vocalization in the primary auditory cortex of the common marmoset: temporal and spectral characteristics. J. Neurophysiol. 74:2685–2706, 1995.

WENSTRUP JJ. Frequency organization and responses to complex sounds in the medial geniculate body of the mustached bat. J. Neurophysiol. 82:2528–2544, 1999.

WENSTRUP JJ, GROSE CD. Inputs to combination-sensitive neurons in the medial geniculate body of the mustached bat: the missing fundamental. J. Neurosci. 15:4693–4711, 1995.

WENSTRUP JJ, LEROY SA. Spectral integration in the inferior colliculus: role of glycinergic inhibition in response facilitation. J. Neurosci. 21:RC124:1–6, 2001.

WENSTRUP JJ, MITTMANN DH, GROSE CD. Inputs to combination-sensitive neurons of the inferior colliculus. J. Comp. Neurol. 409:509–528, 1999.

YAN J, SUGA N. The midbrain creates and the thalamus sharpens echo-delay tuning for the cortical representation of target-distance information in the mustached bat. Hear. Res. 93:102–110, 1996.

YOUNG ED, SACHS MB. Representation of steady-state vowels in the temporal aspects of the discharge patterns of populations of auditory nerve fibers. J. Acoust. Soc. Am. 66:1381–1403, 1979.

ZOOK JM, CASSEDAY JH. Cytoarchitecture of auditory system in lower brainstem of the mustached bat, *Pteronotus parnellii*. J. Comp. Neurol. 207:1–13, 1982a.

ZOOK JM, CASSEDAY JH. Origin of ascending projections to inferior colliculus in the mustached bat, *Pteronotus parnellii*. J. Comp. Neurol. 207:14–28, 1982b.

ZOOK JM, JACOBS MS, GLEZER I, MORGANE PJ. Some comparative aspects of auditory brainstem cytoarchitecture in echolocating mammals: speculations on the morphological basis of time-domain signal processing. Nachtigall PE, Moore PWB, Animal Sonar: Processes and Performance. Plenum, New York, 1988, pp 311–316.